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Deciphering the molecular mechanisms of apoptosis: recent advances in controlled cell death pathways

Vajihe Saedi Marghmaleki[1*], Hamid Azizi-Malekabadi[2]

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[1] *Neuroscience Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.*

[2] *Department of Medical BasicSciences,Isf.C.,Islamic Azad University, Isfahan, Iran.*

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***Corresponding author**
Vajihe.saedi@yahoo.com

ABSTRACT

Apoptosis is a genetically encoded and meticulously controlled cellular self-destruction program, fundamental to embryogenesis, tissue equilibrium, immune function, and the removal of compromised or dangerous cells. Unlike necrosis, apoptosis unfolds via a coordinated molecular cascade that maintains the plasma membrane's integrity and avoids inciting inflammation. Mechanistically, apoptosis is driven by a family of cysteine proteases called caspases. Their activation occurs primarily through two routes: the extrinsic pathway, triggered by death receptor activation, and the intrinsic pathway, regulated by mitochondrial events. A key regulatory checkpoint of the intrinsic pathway is mitochondrial outer membrane permeabilization (MOMP), a process controlled by interactions among members of the B-cell lymphoma-2 (Bcl-2) protein family. In parallel, the inhibitor of apoptosis (IAP) proteins provides an additional regulatory layer by restraining caspase activity downstream of mitochondrial signaling. Dysregulation of these apoptotic networks is a defining feature of numerous human diseases, including cancer, autoimmune disorders, neurodegeneration, and ischemic injury. Advances in understanding apoptotic control mechanisms have facilitated the development of targeted therapeutic strategies, such as BH3-mimetics and SMAC mimetics, aimed at modulating cell death susceptibility in disease-specific contexts. This review synthesizes current insights into the molecular architecture of apoptosis, highlighting key regulatory checkpoints, pathway integration, and emerging therapeutic opportunities.

Keywords: Mitochondrial outer membrane permeabilization (MOMP), Death receptors, BH3-mimetics, SMAC mimetics, Efferocytosis.

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Introduction

The term apoptosis was first introduced in 1972 by Kerr, Wyllie, and Currie to describe a distinct, genetically controlled mode of cell death characterized by unique morphological features and a non-inflammatory outcome (Kerr et al., 1972). This foundational work established apoptosis as a fundamentally different process from necrosis and laid the groundwork for subsequent investigations into its role in development, tissue maintenance, and disease. Apoptosis is now recognized as an evolutionarily conserved cellular program that enables multicellular organisms to eliminate superfluous, damaged, or potentially dangerous cells in a controlled manner.

The execution of apoptosis at the molecular level is driven by a sequential proteolytic cascade carried out by caspases. These enzymes are activated through two principal signaling pathways: the extrinsic pathway, initiated when ligands activate cell surface death receptors, and the intrinsic pathway, activated in response to internal stress signals like oxidative stress, growth factor deprivation, or DNA damage. Control of the intrinsic apoptotic pathway hinges on the Bcl-2 protein family, the master regulator of mitochondrial outer membrane permeabilization (MOMP). Concurrently, the Inhibitor of Apoptosis (IAP) proteins establish a crucial secondary checkpoint through direct caspase inhibition. Cellular fate is decided by the dynamic equilibrium between these pro-survival and pro-death factors (Kalkavan and Green, 2018, Singh et al., 2019).

A fundamental physiological function of apoptosis is to sculpt tissues during embryogenesis and to maintain tissue equilibrium in adults through the elimination of superfluous or impaired cells (Fuchs and Steller, 2015). In the immune system, apoptotic mechanisms are essential for eliminating virus-infected cells and for maintaining self-tolerance through the clonal deletion of autoreactive lymphocytes (Nagata, 2018).

The pathological relevance of apoptosis is underscored by the consequences of its dysregulation. Defective apoptosis is a defining feature of cancer, driving both tumor advancement and resistance to treatment. Conversely, neurodegenerative disorders and ischemic injury are often linked to the aberrant overactivation of apoptotic pathways (Carneiro and El-Deiry, 2020). As a result, the molecular machinery governing apoptosis has emerged as a major focus of therapeutic intervention, with strategies aimed at selectively restoring or restraining cell death depending on disease context.

Forms of Programmed Cell Death

While apoptosis is the most widely studied form of regulated cell death (RCD), it is now understood to be one of several distinct pathways. The Nomenclature Committee on Cell Death (NCCD) has established rigorous morphological and biochemical criteria to classify different types of RCD (Galluzzi et al., 2018). The hallmark morphological changes of apoptosis encompass a sequence of events: the cell undergoes shrinkage, its chromatin condenses, the nucleus fragments, and the plasma membrane blebs, ultimately resulting in the production of apoptotic bodies. These bodies are swiftly phagocytosed by neighboring cells or professional phagocytes without eliciting an inflammatory response (D'arcy, 2019). Other major forms of RCD include necroptosis, a caspase-independent inflammatory death, and pyroptosis, an inflammatory form of death mediated by gasdermin proteins, often in response to pathogens (Galluzzi et al., 2018). This review will focus specifically on the molecular mechanisms of classical apoptosis.

Apoptosis versus Necrosis: A Fundamental Dichotomy

A critical distinction in cell biology is that between apoptosis (a genetically regulated suicide program) and necrosis (an accidental death caused by severe damage). Necrosis has long been considered an unregulated, accidental process resulting from severe physicochemical insult or injury. The event is marked by cellular swelling (oncosis), the breaching of the plasma membrane, and efflux of intracellular material into the extracellular space, which invariably triggers a potent inflammatory response and can cause secondary tissue damage (Gong et al., 2019).

In stark contrast, apoptosis is an active, energy-dependent process that occurs under strict molecular control. The morphological hallmarks of apoptosis, as previously described, ensure the neat packaging of cellular contents for silent disposal (D'arcy, 2019). The display of "eat-me" signals, including phosphatidylserine on the cell surface's outer leaflet, allows for the efficient recognition and uptake of apoptotic cells by phagocytic cells. This efficient clearance mechanism is the primary reason apoptosis does not incite inflammation, preserving tissue integrity (Doran et al., 2020). This fundamental dichotomy underscores the vital role of apoptosis in maintaining metabolic and immune homeostasis (Fig.1).

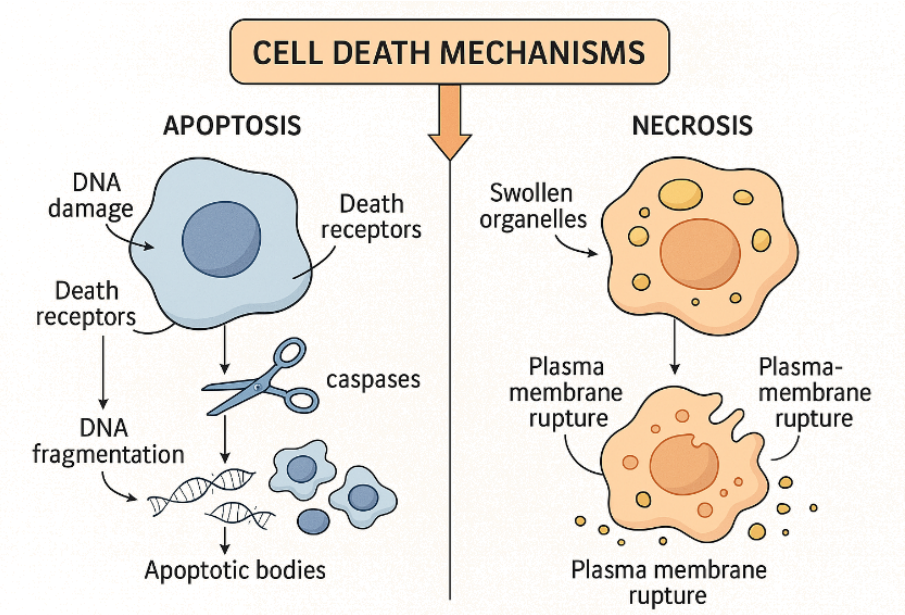

Figure 1: Difference between the stages of necrosis and programmed cell death.

This review provides a comprehensive synthesis of recent advances in elucidating the molecular mechanisms of apoptosis. Its objective is to critically analyze the core regulatory checkpoints, most notably the Bcl-2 protein family, caspases, and inhibitor of apoptosis (IAP) proteins, and delineate the intricate signaling cascades of the intrinsic (mitochondrial) and extrinsic (death receptor) pathways. A particular focus is placed on the mechanistic crosstalk between these pathways and the subsequent execution events at the mitochondrial and nuclear levels. Furthermore, this work evaluates the translational potential of modulating apoptosis, critically assessing emerging therapeutic strategies, such as BH3-mimetics and SMAC mimetics, alongside the persistent challenges in achieving selective targeting in pathologies like cancer and neurodegenerative diseases. The following sections systematically detail this molecular framework, concluding with a forward-looking analysis of the therapeutic landscape and unresolved questions in the field.

The Core Molecular Machinery of Apoptosis

The genetic and molecular underpinnings of apoptosis were first elucidated through seminal studies in the nematode *Caenorhabditis elegans*, which identified key genes such as *"ced-3"* and *"ced-4"* that are vital for developmental cell death, and *"ced-9"* that acts as a survival regulator (Horvitz, 2003). The subsequent discovery that Bcl-2 is the mammalian homolog of CED-9 established an evolutionary conservation of the apoptotic machinery and provided a critical link to oncogenesis (Strasser and Vaux, 2018). The execution of apoptosis in mammals relies on two cores, interconnected signaling cascades. One is the extrinsic (death receptor) pathway, which extracellular ligand-receptor interactions activate. The other is the intrinsic (mitochondrial) pathway, mobilized by intracellular stressors like growth factor

deprivation, DNA damage, or oxidative stress (Singh et al., 2019). A third pathway, initiated by cytotoxic T-cells via granzyme B, directly activates the executioner phase of apoptosis (Cullen and Martin, 2015).

The Bcl-2 Protein Family: Guardians of Mitochondrial Integrity

Mitochondrial outer membrane permeabilization (MOMP)—a critical step in the intrinsic apoptotic pathway—is regulated by the Bcl-2 protein family, which functions as its key regulatory determinant (Kalkavan and Green, 2018). Based on the number of Bcl-2 homology (BH) domains (up to four), the family is divided into three functional groups. The first group comprises anti-apoptotic proteins such as Bcl-2, Bcl-xL, and MCL-1. These proteins, equipped with all four BH domains, protect mitochondria by capturing and sequestering proteins that would otherwise trigger apoptosis. MOMP is directly mediated by the oligomeric pore-forming activity of pro-apoptotic effector proteins (e.g., BAX, BAK) following their activation. These proteins contain multiple BH domains.

Proteins belonging to the BH3-only class (e.g., BIM, BID, PUMA, BAD) monitor cellular stress. Their role in promoting MOMP involves two distinct actions: directly activating BAX/BAK or counteracting the inhibitory function of anti-apoptotic proteins (Chipuk et al., 2010). The cell's fate is determined by the complex interactions and stoichiometric balance between these factions, a concept described as the "rheostat" model (Kale et al., 2018). Notably, many viruses have evolved homologs of anti-apoptotic Bcl-2 proteins (e.g., vBcl-2 in Kaposi's sarcoma-associated herpesvirus) to subvert host cell death and promote viral persistence (Fig.2) (Kvansakul and Hinds, 2015).

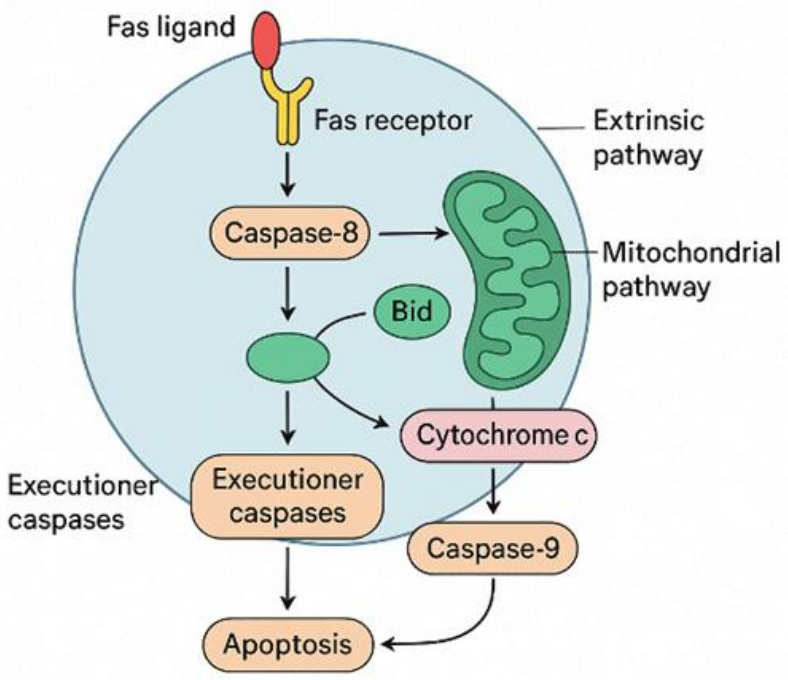

Figure 2. The receptor and mitochondrial pathways of programmed cell death.

Caspases: The Orchestrators of Cellular Demolition

Caspases (cysteine-aspartic proteases) are the primary effectors of apoptosis, cleaving hundreds of cellular substrates after aspartic acid residues (Julien and Wells, 2017). They are synthesized as inactive zymogens (procaspases) consisting of a pro-domain, a large subunit, and a small subunit. Caspase activation follows a

proteolytic hierarchy, dividing them into two structural and functional classes (Julien and Wells, 2017). Initiator caspases (e.g., -8, -9, -10) feature long pro-domains that direct them to specific signaling platforms like the DISC or Apoptosome. Clustered at these sites, they autoactivate and then proteolytically ignite the downstream cascade (Bock and Tait, 2020).

Executioner caspases (e.g., -3, -6, -7) have short pro-domains. Once activated, they mediate the terminal phase of apoptosis by cleaving a wide array of cellular targets—including structural elements, DNA repair machinery, and regulatory proteins—to produce the defining morphological changes of cell death (Bock and Tait, 2020, McIlwain et al., 2013).

Endogenous Caspase Inhibitors: The IAP Family

To prevent inadvertent cell death, caspase activity is tightly regulated by endogenous inhibitors, most notably the Inhibitor of Apoptosis (IAP) proteins (Dubrez et al., 2013). Key mammalian IAPs include XIAP, cIAP1, and cIAP2. XIAP is a potent direct inhibitor of caspase-3, -7, and -9 via its Baculovirus IAP Repeat (BIR) domains (Gyrd-Hansen and Meier, 2010). Other IAPs, such as cIAP1/2, primarily function as regulators of inflammatory signaling via their RING domains, which confer E3 ubiquitin ligase activity. The mitochondrial protein SMAC/Diablo is released upon MOMP and counteracts IAP-mediated inhibition by binding to IAPs and displacing caspases, thereby promoting apoptosis (Lalaoui and Vaux, 2018).

The p53 Tumor Suppressor: Integrating DNA Damage with Apoptosis

The p53 protein is a critical tumor suppressor and a master regulator of the cellular response to stress, particularly DNA damage. Activation of p53 leads to one of two outcomes. It may enforce cell cycle arrest, creating a window for DNA repair. Alternatively, if the damage is beyond repair, it can activate the apoptotic pathway (Aubrey et al., 2018). It does so primarily by transcriptionally activating pro-apoptotic target genes, including the BH3-only proteins *PUMA and NOXA,* which directly engage the mitochondrial apoptotic pathway, and *BAX* (Kruiswijk et al., 2015). The critical role of p53 in tumor suppression is highlighted by the fact that many viruses, such as Human Papillomavirus (HPV) with its E6 oncoprotein, have evolved mechanisms to degrade or inactivate p53 to prevent apoptosis in infected cells (Kastenhuber and Lowe, 2017).

The Role of Calcium Signaling

Intracellular calcium ions (Ca^{2+}) act as important secondary messengers in apoptosis. Dysregulation of Ca^{2+} homeostasis, often involving excessive release from the endoplasmic reticulum (ER), can promote apoptosis through multiple mechanisms. A rise in cytosolic Ca^{2+} levels exert multiple pro-apoptotic effects. It can stimulate proteases such as calpains, alter the function of *Bcl-2* family proteins, and promote mitochondrial membrane permeabilization. This latter event results in cytochrome c release, thereby amplifying the death signal. (Kerkhofs et al., 2018).

The Principal Pathways of Apoptotic Execution

Initiation of the two primary apoptotic pathways relies on distinct triggers. Intracellular stressors activate the intrinsic (mitochondrial) pathway, whereas the extrinsic (death receptor) pathway is engaged by ligands outside the cell. Despite their different initiation mechanisms, both pathways ultimately converge to activate a cascade of executioner caspases, which orchestrate the systematic dismantling of the cell through the cleavage of hundreds of cellular substrates (Carneiro and El-Deiry, 2020).

The Intrinsic (Mitochondrial) Pathway

The intrinsic apoptotic pathway becomes engaged following significant intracellular distress, such as DNA damage, oxidative stress, lack of growth factors, or stress within the endoplasmic reticulum (ER). The Bcl-2 protein family acts as the central processor for these distress signals, with its control over mitochondrial outer membrane permeabilization (MOMP) determining the cell's fate (Kalkavan and Green, 2018). As outlined earlier, when pro-apoptotic signals dominate, the effector proteins BAX and BAK undergo oligomerization to create pores in the mitochondrial outer membrane. This crucial

step, MOMP, causes the efflux of key pro-apoptotic factors—including cytochrome c and SMAC/Diablo—from the space between mitochondrial membranes into the cell cytoplasm (Kalkavan and Green, 2018, Lalaoui and Vaux, 2018).

Once in the cytosol, cytochrome c interacts with Apaf-1, prompting this factor to assemble into a multi-subunit, wheel-shaped complex termed the apoptosome (Dorstyn et al., 2018). This apoptosome serves as a platform to recruit and activate the initiator protease procaspase-9. Active caspase-9 then propagates the death signal by proteolytically processing and turning on the downstream executioner caspases, primarily caspase-3 and -7 (Dorstyn et al., 2018). In a parallel pro-apoptotic mechanism, the released SMAC/Diablo protein works to counteract the inhibitory effects of IAPs on caspases, thereby further facilitating cell death (Lalaoui and Vaux, 2018).

The Extrinsic (Death Receptor) Pathway

Activation of the extrinsic apoptotic pathway commences when death ligands from the extracellular environment engage their matching death receptors on the cell surface. These receptors are part of the tumor necrosis factor (TNF) receptor family, with notable examples being CD95 (Fas), TRAIL-R1/DR4, TRAIL-R2/DR5, and TNFR1 (Jan, 2019). Following ligand-receptor engagement—such as the binding of FasL to CD95—the receptors assemble into trimers. This oligomerization allows them to bring together specific intracellular adapter proteins through interactions between homologous Death Domains (DDs). In the case of CD95, the primary adapter recruited is FADD (Fas-Associated protein with Death Domain). FADD contains an additional interaction module called a Death Effector Domain (DED). Via this DED, FADD binds to and recruits the inactive zymogen form of the initiator protease, procaspase-8 (and procaspase-10 in humans) (Ashkenazi and Salvesen, 2014). The resulting assembly of the receptor, FADD, and procaspase-8 forms a critical signaling hub known as the Death-Inducing Signaling Complex (DISC). It is within the confines of the DISC that procaspase-8 molecules are brought into proximity, leading to their autoactivation and the production of enzymatically active caspase-8 (Kesavardhana et al., 2020).

The initiation mechanism for TNF-R1 is more elaborate. Ligand binding to this receptor results in the recruitment of a different adapter protein, TRADD (TNF Receptor-Associated Death Domain). TRADD subsequently functions as a versatile platform. It can recruit FADD to direct the signal towards apoptosis, or it can assemble other proteins such as RIPK1 to channel the signal into activating the NF-κB pathway, which promotes inflammation (Van Opdenbosch and Lamkanfi, 2019).

Pathway Integration and Cross-Talk

The extrinsic and intrinsic apoptotic pathways function in a connected network, engaging in essential molecular dialogue to potentiate the cell death signal. A pivotal integrator of this cross-talk is the protein BID, a member of the Bcl-2 family. Caspase-8, activated within the DISC, proteolytically cleaves full-length BID residing in the cytosol. This cleavage generates a truncated, highly active fragment known as tBID. The tBID fragment subsequently migrates to the mitochondria. There, it directly stimulates the activation of the pro-apoptotic effector proteins BAX and BAK. This action effectively recruits the intrinsic apoptotic machinery, leading to a powerful amplification of caspase activity via the subsequent events of MOMP and apoptosome assembly (Delbridge et al., 2016). This integrative mechanism is especially vital in so-called Type II cells, where an effective apoptotic response depends on this reinforced mitochondrial feedback loop.

Viral Modulation of Death Receptor Pathways

Viruses have evolved sophisticated mechanisms to subvert death receptor-mediated apoptosis. A prominent example is the cellular and v-FLIP (viral-FLICE-inhibitory protein) proteins. Recruitment of c-FLIP to the DISC is facilitated by its structural mimicry of procaspase-8, a similarity conferred by the presence of homologous DED domains. However, it lacks catalytic activity. By binding

to FADD and procaspase-8, c-FLIP disrupts the formation of a functional DISC and inhibits caspase-8 activation (Guerrache and Micheau, 2024).

Several viruses encode v-FLIPs (e.g., K13 in HHV-8) to exploit this inhibitory mechanism. Other viral proteins, such as the adenovirus E1B-19K and the HIV Tat protein, can modulate the expression or function of death receptors and their ligands, thereby tipping the balance toward cell survival and viral persistence (Guerrache and Micheau, 2024).

The Intrinsic Pathway: Mitochondrial Regulation and Nuclear Execution

Mitochondrial Control of Apoptosis

In the intrinsic apoptotic pathway, mitochondria are cast in the role of the ultimate executioners, with the point of no return being mediated through mitochondrial outer membrane permeabilization (MOMP) (Kalkavan and Green, 2018). The principal mediators of this event are the pro-apoptotic Bcl-2 family proteins BAX and BAK. In viable cells, BAX is maintained in an inactive conformation primarily in the cytosol, whereas BAK is constitutively embedded within the mitochondrial outer membrane. Activation by specific "activator" BH3-only proteins triggers the translocation of BAX to mitochondria and induces extensive conformational changes in both effectors. These changes drive their homo-oligomerization and the formation of proteolipid pores, resulting in the compromise of mitochondrial membrane integrity (Meng et al., 2021, Czabotar et al., 2014).

MOMP causes the irreversible release of key pro-apoptotic factors from the mitochondrial intermembrane space into the cytosol. These include cytochrome c, which initiates apoptosome formation; SMAC/Diablo, which neutralizes the inhibitory function of IAP proteins; and the caspase-independent factors Apoptosis-Inducing Factor (AIF) and Endonuclease G, which are translocated to the nucleus to mediate chromatin condensation and DNA fragmentation, respectively (Lalaoui and Vaux, 2018, Dorstyn et al., 2018).

The assembled apoptosome serves as a platform for the recruitment and auto-activation of procaspase-9. According to the prevailing model, caspase-9 activation is achieved through proximity-induced dimerization within this complex, forming an active holoenzyme. This active holoenzyme is then responsible for the proteolytic cleavage and activation of the primary executioner caspases, caspase-3 and caspase-7 (Dorstyn et al., 2018).

Nuclear Events in Apoptosis

The executioner caspases orchestrate the systematic dismantling of the nucleus, a hallmark of apoptosis. This process involves critical alterations in nucleocytoplasmic transport, where caspases play a leading role (Kopeina et al., 2018). Key nuclear events include the inactivation of DNA repair machinery via caspase-3-mediated cleavage of PARP-1, chromatin condensation facilitated by caspase-6-mediated cleavage of nuclear lamins, and DNA fragmentation. The subcellular relocation of caspases to the nucleus is a key regulatory step for these events (Prokhorova et al., 2018). DNA fragmentation is primarily mediated by CAD (Caspase-Activated DNase), which is liberated from its inhibitor ICAD upon cleavage by caspase-3, leading to the characteristic internucleosomal DNA ladder.

Cytoskeletal Reorganization and Phagocytic Clearance

The structural integrity of the cell is dismantled through caspase-mediated cleavage of key cytoskeletal and adhesion proteins, including actin, vimentin, and cadherins (Julien and Wells, 2017). Consequently, cell-substrate adhesion is lost, the membrane undergoes blebbing, and apoptotic bodies are formed. A crucial phagocytic "eat-me" signal is generated by the translocation of phosphatidylserine to the outer plasma membrane leaflet. This externalization is enabled by scramblase activation and flippase inhibition, both of which are mediated by caspase activity (Segawa and Nagata, 2015).

The apoptotic process culminates in the prompt removal of the deceased cell. Following fragmentation into membrane-bound apoptotic bodies, these bodies are rapidly phagocytosed by adjacent cells or specialized phagocytes such as macrophages. This process, known as

efferocytosis, occurs without the release of pro-inflammatory cellular contents, thereby preventing an immune response and maintaining tissue homeostasis (Poon et al., 2014, Doran et al., 2020).

Discussion

The molecular landscape of apoptosis, as detailed in this review, reveals an intricate and highly regulated system central to life and death decisions in metazoans. While the canonical pathways (intrinsic and extrinsic) and their core components (Bcl-2 family, caspases, IAPs) are well-established, recent research has shifted towards understanding their context-dependent regulation, crosstalk with other biological processes, and therapeutic exploitation amidst emerging challenges.

A critical comparison of the intrinsic and extrinsic pathways highlights their complementary roles and adaptive significance. The intrinsic pathway acts as a sensor for intracellular well-being, responding to metabolic stress, DNA damage, and oncogenic signals. In contrast, the extrinsic pathway serves as an executioner for immune surveillance and tissue remodeling. The discovery of the BID-mediated cross-talk bridged these pathways, illustrating an elegant fail-safe mechanism to ensure efficient elimination of compromised cells, particularly in cancer and viral infection (Kalkavan and Green, 2018, Singh et al., 2019). Recent findings challenge the simplicity of the "rheostat" model, suggesting that dynamic localization, post-translational modifications (e.g., phosphorylation, ubiquitination), and non-canonical interactions within the Bcl-2 family add layers of regulation that are cell-type and stimulus-specific (Kale et al., 2018).

Therapeutic targeting of apoptosis, particularly in oncology, has seen both remarkable successes and sobering challenges. The clinical success of venetoclax (ABT-199), a specific Bcl-2 inhibitor, in hematological malignancies validates the concept of "reactivating" apoptosis (Carneiro and El-Deiry, 2020). However, resistance frequently emerges through upregulation of other anti-apoptotic proteins like MCL-1 or Bcl-xL, highlighting the need for combination therapies or pan-Bcl-2 inhibitors. Similarly, SMAC mimetics showed promise in preclinical models by antagonizing IAPs, but their efficacy in solid tumors has been limited, partly due to compensatory activation of NF-κB signaling (Lalaoui and Vaux, 2018). A major contemporary challenge is the selective induction of apoptosis in cancer cells while sparing normal tissues. Strategies exploiting synthetic lethality (e.g., combining PARP inhibitors with Bcl-2 inhibition in BRCA-mutant cancers) or targeting apoptotic priming represent promising avenues informed by recent mechanistic studies (Singh et al., 2019).

Furthermore, apoptosis does not occur in isolation. Its interplay with other forms of regulated cell death (RCD), such as necroptosis and ferroptosis, forms a complex network where inhibition of one pathway can shunt cell death to another. This has profound implications for therapy; for instance, caspase inhibition in some contexts can lead to necroptosis, an inflammatory death that might enhance anti-tumor immunity (Galluzzi et al., 2018). Recent experimental studies using CRISPR screens and chemical biology are mapping these dependencies, offering blueprints for rational combination therapies. Another emerging frontier is the role of apoptosis in shaping the tumor immune microenvironment. Immunogenic cell death (ICD), a variant of apoptosis accompanied by the release of danger signals, can stimulate anti-tumor immunity, suggesting that coupling apoptosis inducers with immunotherapies could yield synergistic effects (Carneiro and El-Deiry, 2020).

In conclusion, while the core mechanics of apoptosis are deciphered, the field is now grappling with its complexity in vivo. Future progress will depend on integrating structural biology insights with systems-level approaches in relevant disease models to overcome drug resistance, improve specificity, and harness the immunogenic potential of apoptotic cell death.

Conclusion and Future Perspectives

In conclusion, the molecular deciphering of apoptosis has provided unparalleled insights into a fundamental

biological process with direct relevance to human health and disease. The intricate balance within the apoptotic machinery, governed by the Bcl-2 family rheostat, the caspase cascade, and IAP-mediated inhibition, presents a double-edged sword for therapeutic intervention. The successful clinical development of BH3-mimetics like venetoclax (a Bcl-2 inhibitor) for hematological malignancies validates the strategy of reactivating apoptosis in cancer (Kale et al., 2018). Concurrently, efforts to develop caspase inhibitors and IAP mimetics aim to protect neurons in stroke and neurodegenerative conditions.

Looking forward, future research must address several key challenges to fully harness the therapeutic potential of apoptosis modulation. These include improving the specificity of agents to minimize on-target toxicity, developing effective drug delivery systems to target tissues or tumor microenvironments, and devising strategies to overcome inherent and acquired resistance to apoptosis-inducing drugs. Additionally, the growing understanding of how apoptotic cell death shapes the immunogenic landscape—for instance, through the release of damage-associated molecular patterns (DAMPs) in certain contexts—opens new frontiers for rational combination therapies, pairing apoptosis inducers with immunomodulatory agents (Carneiro and El-Deiry, 2020).

Future perspectives should also focus on several emerging areas: First, the systems-level analysis of apoptotic networks in patient-derived samples will be crucial for identifying predictive biomarkers of response and resistance. Second, exploring the role of non-canonical apoptosis and its initiators (e.g., caspase-2, -4, -5) in specific pathological contexts may reveal novel targets. Third, advancing the chemical biology toolbox—such as with proteolysis-targeting chimeras (PROTACs) to degrade anti-apoptotic proteins—offers a promising strategy to achieve deeper and more sustained inhibition. Finally, understanding the temporal and spatial control of apoptosis *in vivo*, perhaps through advanced imaging techniques, will be key to translating mechanistic knowledge into precise therapies. As our molecular understanding continues to deepen, so too will our capacity to precisely manipulate this critical pathway for therapeutic benefit across a wide spectrum of human diseases.

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Conflicts of interest

There are no conflicts of interest.

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