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A Deep Dive into the Hidden Power of Programmed Cell Death: Unlocking Therapeutic Potential with Antibodies

ProSci brings more than 25 years of antibody expertise in developing high quality single domain Variable Heavy domain of Heavy chain (VHH), recombinant, monoclonal, and polyclonal antibodies. In addition, our custom antibody services provide the flexibility to tailor antibody development and production for almost any need. This white paper provides examples of the use of ProSci antibodies for the study of programmed cell death.

Many life forms rely on a process known as programmed cell death. Programmed cell death is essential for the proper functioning of our bodies, and research on this topic has shown us how to better understand and control it.

Many studies that utilized ProSci antibodies have been published in high-impact journals. In the paper published in the journal Immunity (2013) titled "The TACI receptor regulates T-cell-independent marginal zone B cell responses through innate activation-induced cell death," Figgett et al. reported their findings that TLR4 activation by LPS induces MZ B cell death via TACI-dependent upregulation of Fas and FasL expression, which is impaired in Tnfrsf13b mice. The ProSci Bcl-s antibody was used in this study for western blot analysis[1]. Mohmood et al. published a paper in the journal Nature Communication in 2021 titled "β-actin dependent chromatin remodeling mediates compartment level changes in 3D genome architecture." In this paper, the researchers studied the role of ß-actin in chromatin accessibility and gene expression. This study used the ProSci GAPDH antibody as a control for western blot [2]. In the paper published in the journal EMBO (2004) by Gottfried et al. titled "The mitochondrial ARTS protein promotes apoptosis through targeting XIAP," the authors investigated the role of ARTS in promoting apoptosis through its targeting of XIAP and finds that ARTS can bind directly to XIAP. The ProSci ARTS/ARTSmGTP/H5 were used for this study for western blot analysis[3].

Overall, these publications have had a major impact on the broader understanding of programmed cell death and how it can be utilized for medical purposes. This knowledge has been used to develop new treatments for cancer and other diseases as well as to better understand how our bodies work and how diseases can be prevented or treated more effectively.

Overview

The purpose of studying apoptosis, a specific type of programmed cell death, is to enhance our understanding of its potential uses in treating diseases like cancer. Given its substantial role in the pathogenesis of various diseases, grasping the core mechanisms of apoptosis is critically important. For instance, in cancer scenarios, the balance between cell division and cell death becomes disrupted, leading to cells no longer receiving signals to initiate apoptosis [4]. Chemotherapy drugs and radiation are commonly used treatments that induce programmed cell death or apoptosis in cancer cells.

However, a problem with these treatments is their inability to differentiate between healthy and damaged cells, resulting in healthy cells also being killed during treatment [5], [6]. To address this issue, scientists are actively working on developing new medications that can reactivate cellular sensors and initiate the process of apoptosis. The goal is to develop more efficient treatments that can minimize collateral damage to healthy cells.

Research is also underway to deepen our comprehension of necroptosis, an alternative form of programmed cell death, and its effects on the body's reactions to damage and infection [7]. Another fascinating avenue of inquiry revolves around autophagy, yet another variant of programmed cell death. Its possible contributions towards the creation of new treatments for age-associated disorders like Alzheimer's and Parkinson's disease are currently under scrutiny. Intriguingly, it's been observed that faulty autophagy mechanisms are a recurring theme among neurodegenerative diseases, a group that also encompasses Alzheimer's and Parkinson's disease [8], [9].

In the field of programmed cell death research, antibodies are recognized as indispensable tools. These tools allow scientists to identify and quantify proteins implicated in programmed cell death, along with exploring the related routes and mechanisms.

Furthermore, their role is of utmost importance in determining the impact of various drugs or therapies on programmed cell death. Through the utilization of antibodies, scientists can acquire valuable knowledge regarding the mechanisms underlying programmed cell death, as well as investigate potential approaches to modulate or suppress these mechanisms. Consequently, this offers potential avenues for the management of diseases.

Through a multitude of investigations, researchers are gaining a better understanding of the complex function of programmed cell death that maintains healthy tissue and facilitates favorable survival outcomes.

In this white paper, we will take a closer look at the various types of programmed cell death and how each can be applied to the advancement of medical and scientific research as well as innovative patient therapies.

Exploring Programmed Cell Death: A New Frontier in Disease Treatment

Cell death is a critical biological process that results from activating an intracellular program brought on by various stimuli, such as cellular stress or DNA damage. It typically manifests via two primary mechanisms: apoptosis, initiated by internal signals, and necrosis, which can be triggered by excessive external stimuli.

Apoptosis, often called "programmed cell death," is an innate response of a cell to certain stimuli, predominantly damage or disease. This is a tightly controlled process characterized by morphological transformations and enzymatic biochemical reactions that remove the cell from the body with minimal disturbance to the surrounding tissue [10, 11]. This process is vital in maintaining proper tissue homeostasis. Disruptions in the apoptotic process can lead to various human ailments, such as autoimmune diseases, neurodegenerative disorders, ischemic damage, and cancer [12].

Conversely, necrosis is commonly characterized as a form of cell death that is not under precise biological regulation. This process typically arises from severe damage to the cellular membrane, leading to the leakage of cellular contents. This leakage often initiates an inflammatory reaction that causes damage to surrounding tissues[10, 11]. Necrosis can coincide with apoptosis, depending on various factors, such as the intensity and duration of the triggering stimulus and the availability of specific enzymes called caspases [12].

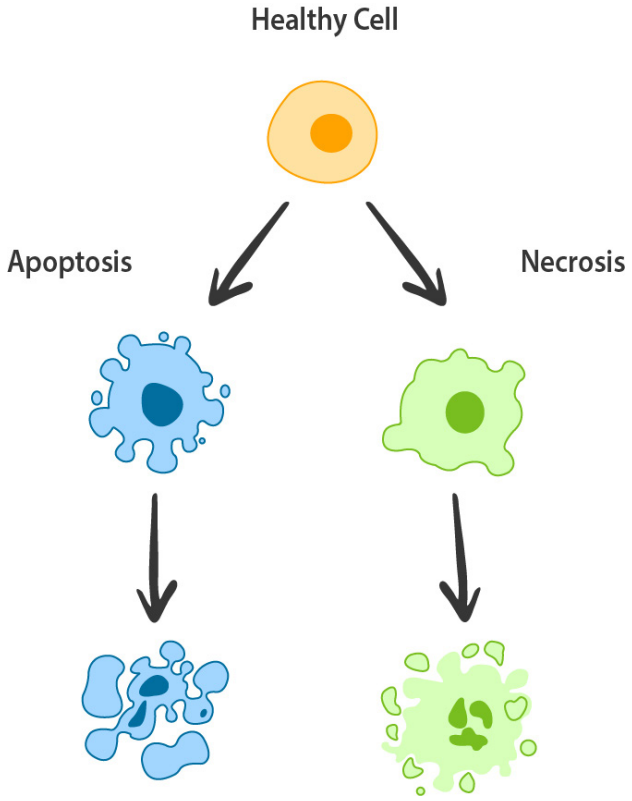

Fig. 1 Cell death manifests via two primary mechanisms: apoptosis and necrosis.

Programmed cell death exists in various distinctive forms, including autophagy, necroptosis, ferroptosis, pyroptosis, parthanatos, and erebosis, a recently uncovered mechanism specific to Drosophila. Unique characteristics and biological functions differentiate the various modes of cell death. Diverse forms of cell death may either promote cell survival or lead to the death of a cell, depending on the environment and signals that trigger the process. Understanding the diverse mechanisms and interactions for these types of cell death is crucial in creating innovative treatment approaches for cancer and other diseases.

The Intrinsic and Extrinsic Pathways of Apoptosis

Apoptosis can occur via two main pathways: the extrinsic and intrinsic pathways (Fig 2). The extrinsic pathway of apoptosis refers to the process through which cells activate programmed cell death in response to external stimuli, such as signals originating from adjacent cells or the immune system. This pathway's activation is initiated by binding specific ligands to death receptors located on the cell surface, including but not limited to tumor necrosis factor (TNF) or Fas ligand [13]. Based on the triggering stimulus and nature of the components involved, at least two apoptotic pathways can be differentiated: one involving receptor systems and one triggered by cytotoxic stress [14].

The extrinsic apoptosis pathway can be initiated through the death receptors, such as Fas, TNF receptor (TNFR), and TNF-related apoptosis-inducing ligand (TRAIL) receptor. The binding of these receptors to their ligands activates caspases, leading to apoptotic cell death [13], [14]. Cytotoxic stress can also induce apoptosis. Chemotherapeutic drugs can induce apoptosis and the upregulation of death ligands or their receptors. Downstream events following cytotoxic stress-induced DNA damage and the signaling pathways that lead to the induction of apoptosis may be either dependent or independent of death receptor signaling [15].

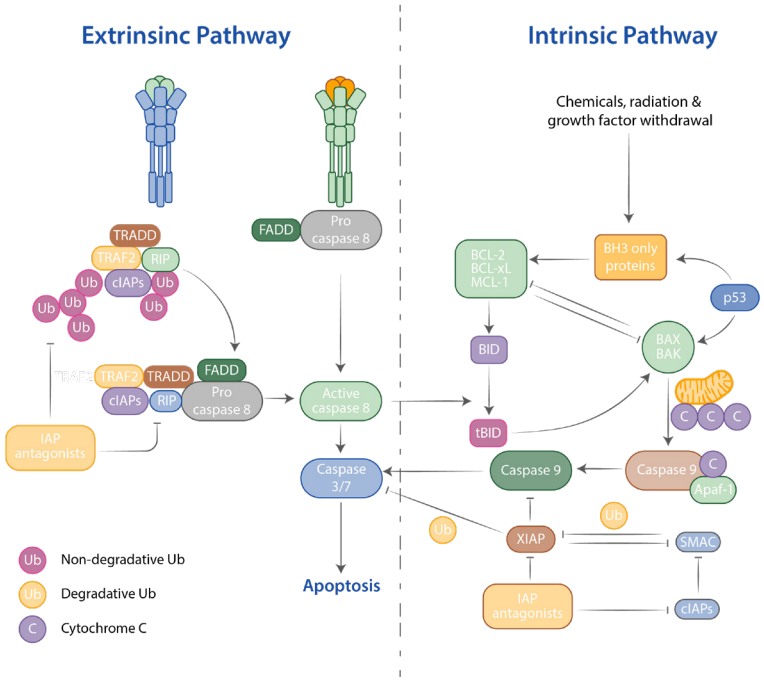

Fig. 2 Apoptosis occurs via the extrinsic and intrinsic pathways

The intrinsic apoptosis pathway, also called the mitochondrial pathway, is triggered by various forms of cellular stress, such as DNA damage, oxidative stress, and growth factor deprivation. The pathway is regulated by the BCL-2 protein family, which includes members that exhibit both pro-apoptotic and anti-apoptotic properties. In response to cellular stress, pro-apoptotic proteins, such as Bax and Bak, are activated and undergo oligomerization. This process leads to the formation of pores in the outer mitochondrial membrane.

This process leads to the release of cytochrome c from the mitochondria and its subsequent translocation to the cytosol. Cytochrome c protein binds with Apaf-1, a cytoplasmic protein that initiates apoptosis. This binding results in the formation of an oligomeric apoptosome, then triggering the activation of caspases, which are a family of protease enzymes that play a central role in regulating cell death and inflammation. Apoptosome specifically activates caspase-9, and that, in turn, initiates the activation of downstream effector caspases, such as caspase-3, which leads to the execution phase of apoptosis [14],[15].

Key Proteins in the Mechanism of Programmed Cell Death

Programmed cell death involves several key proteins in its regulatory pathways. These proteins play a pivotal role in the initiation, execution, and regulation of apoptosis, coordinating cellular events leading to controlled cell death. The intricate balance between pro-apoptotic and anti-apoptotic proteins ensures proper cell function and organismal health.

Caspases: Caspases, short for cysteine-aspartic proteases, are a family of proteins that play vital roles in programmed cell death, proliferation, and inflammation [16]. These proteins are synthesized as inactive precursors and are activated by specific stimuli or signals that initiate a cascade of events and lead to their activation [17]. Once activated, they can inactivate or activate substrates, triggering a cascade of signaling events that can lead to an imbalance between cell survival and cell death [18], [19], [20].

Caspase activation and function is a complex process regulated by numerous signals and pathways within cells ranging from interactions with death receptors on the cell surface to internal cellular stress signals and immune responses to interventions from therapeutic treatments [21].

In mammals, three main routes activate the caspases leading to apoptosis:

1. Extrinsic or Death Receptor route: Death ligands (including FasL, TNF-alpha, or TRAIL) bind to specific death receptors on the cell surface to initiate this route. Initiator caspases, including caspase-2, caspase-8, caspase-9, and caspase-10, are recruited and activated through a series of intracellular events. These events lead to apoptosis by triggering the activation of executioner caspases, namely caspase-3, caspase-6, and caspase-7 [21].

2. Intrinsic or Mitochondrial route: Intracellular stress signals, including DNA damage, oxidative stress, or growth factor deprivation, initiate this route. These signals alter the mitochondrial membrane potential, leading to the release of cytochrome c into the cytoplasm. Released cytochrome c associates with Apoptotic protease activating factor 1 (Apaf-1) and pro-caspase-9 to form the apoptosome, activating caspase-9. Caspase-9 activation triggers the executioner caspases, which in turn triggers apoptosis [21], [22].

3. The cytotoxic lymphocyte initiated Granzyme B route: Immune responses cause the activation of this pathway. Perforin and granzymes are released into target cells by cytotoxic T cells and natural killer (NK) cells. Caspases can be directly activated by Granzyme B, resulting in apoptosis [21].

Granzyme B (GrB): A serine protease found in natural killer cells and cytotoxic T cells activates caspases to induce apoptosis in target cells. GrB can directly cleave and activate caspase-3 or other effector caspases. It can also indirectly activate caspase cascade through Bid, a pro-apoptotic member of the Bcl-2 family. This leads to mitochondrial damage and activation of caspase-9, activating effector caspases like caspase-3 [23].

Granzyme A (GzmA): Granzyme A is a structurally related serine protease essential for natural killer cell-mediated and perf-facilitated tumor control. It induces caspase-independent mitochondrial damage, a required first step for apoptosis, and is also responsible for nuclear lamins and histone H1 cleavage.

Granzyme A can target extracellular and intracellular proteins, such as cytokines, matrix proteins, thrombin receptors, and mitochondrial inner membrane potential. Additionally, it has been reported to generate single-stranded DNA nicks rather than the oligonucleosomal DNA fragments typical of granzyme B-induced apoptosis. The role of granzyme A in tumor clearance is controversial, but it is a critical player in cytotoxicity and cell death [16].

Perforin: Perforin is a protein that plays a role in inducing apoptosis in target cells. It is released by cytotoxic T lymphocytes and natural killer cells and forms pores in the target cell membrane, allowing granzymes to enter the cell and initiate apoptosis. Perforin is vital for cytotoxic effector function and is indispensable in granzyme-mediated apoptosis [24].

Bcl-2 family: The Bcl-2 family is a protein group that controls cell death. The presence of conserved sequence motifs characterizes these proteins called Bcl-2 homology motifs and a transmembrane region that forms the interaction sites and determines the intracellular location.

The Bcl-2 family comprises both pro-survival and pro-apoptotic members. Pro-survival members of the family, such as Bcl-2 and Bcl-xL, interact with pro-apoptotic members, such as Bax and Bak, to prevent the release of cytochrome c from the mitochondria. This inhibition of cytochrome c release inhibits apoptosome assembly and the subsequent activation of caspases, thus preventing apoptosis [25], [26].

The pro-apoptotic member of proteins contains a BH3 motif, which binds to pro-survival Bcl-2 proteins and prevents them from inhibiting apoptosis. This binding is necessary for the activation of the caspase. In addition, pro-apoptotic Bcl-2 proteins can also bind to autophagy regulators, such as Beclin-1, to induce autophagy, another type of programmed cell death. Viruses have also taken advantage of the Bcl-2 family by encoding their own Bcl-2 proteins, some of which act as apoptosis inhibitors [26].

Fas/FasL: Fas and Fas ligands are crucial in regulating apoptosis. The Fas receptor, also known as CD95 and tumor necrosis factor receptor superfamily member, is located on the surface of various types of cells. It interacts with its corresponding ligand, FasL (also called FasL/CD95L), to initiate a signal transduction pathway that leads to apoptosis. The interaction between FasL and the Fas receptor initiates a series of events that lead to the recruitment and activation of apoptosis-initiating proteases, such as caspase-8 and caspase-10. These proteases subsequently induce apoptosis through various molecules [27].

Inhibitors of Apoptosis Proteins (IAPs): IAPs act as modulators of apoptosis by inhibiting the activity of caspases. This makes them essential in determining cell survival and death, therefore suggesting potential for therapeutic manipulation [25]. Overexpression of IAP proteins is often seen in cancers, making them potential targets for therapeutic intervention [28].

Various Types of Programmed Cell Death

Over the last few decades, various regulated cell death pathways have been identified, each relying on a different subset of proteins for their activation and execution. Apoptosis, necroptosis, and pyroptosis are the most-understood forms of cell death. Still, other pathways are being discovered: autophagy-dependent cell death (ADCD), ferroptosis, parthanatos, and, more recently, a Drosophila-specific cell death mechanism called erebosis.

These different forms of cell death are interconnected and share components with other pathways. Studying cell death pathways and their interconnectivity is important for understanding the physiological and mechanistic aspects of these signaling pathways, leading to better disease treatments caused by deregulated or dysfunctional cell death signaling.

Necroptosis: Necroptosis is a type of controlled cell death resembling necrosis, which causes cells to expand and discharge cytoplasmic material [7]. The receptor-interacting protein kinases (RIPKs) RIPK1, RIPK3, and Mixed Lineage Kinase Domain-Like Protein (MLKL) are all activated during necroptosis, which is caspase-independent [29].

Various factors, including cancer, viral infections, and tissue damage, can bring it on. It is characterized by cell swelling, membrane rupture, and the release of intracellular contents, including damage-associated molecular patterns (DAMPs) that can draw immune cells.

Necroptosis has been demonstrated to affect cancer prognosis; some researchers suggest that it suppresses tumor growth, while others claim it encourages tumor growth. Additionally, the production of cytokines during necroptosis might cause inflammation and further encourage tumor growth [29].

Pyroptosis: Pyroptosis differs from other cell death types. It is characterized by cell swelling, pore formation on cell membranes, rupture and bubbling of plasma membranes, moderate chromatin condensation, gasdermin (GSDM) cleavage, formation of inflammasomes, and IL-18 and IL-1β release [30].

Intracellular pathogens primarily trigger pyroptosis and facilitate rapid clearance of various infections by removing intracellular replication niches and boosting the host's defense response [31]. It is regulated by inflammasome-associated caspases, such as CASP1, CASP4, CASP5, and CASP11 (mouse), while some caspases associated with apoptosis, such as CASP3 and CASP8 also play a role in pyroptosis [27]. It is a crucial mechanism in protecting the host against intracellular pathogens and contributes to antitumor immunity. The induction of pyroptosis can induce potent antitumor activity [29].

Autophagy-dependent cell death (ADCD): Autophagy-dependent cell death (ADCD) is a form of caspase-independent programmed cell death. It's characterized by an over-activation of autophagy, a cellular degradation process used by eukaryotic cells for maintaining homeostasis and managing lipid metabolism [29].

This over-activation leads to a high degree of self-digestion of cellular material in autolysosomes, moving past the threshold of cell survival24. Autophagy substrates (DAMPs) and pathogen-associated molecular pattern (PAMPs) molecules trigger innate immunity whose elimination through autophagy is necessary for immune homeostasis to protect the cells from exposed membranes and other organelles [29]. ADCD occurs during the response of certain cancer cells to specific drugs or natural compounds, showing its relevance in biological and pathological contexts [29], [32].

Ferroptosis: Ferroptosis is regulated cell death dependent on iron and excessive lipid peroxidation, leading to oxidative membrane damage and ruptured plasma membranes. It is distinct from other forms of cell death, regulated by multiple metabolic events, including redox balance, iron handling, and mitochondrial activity [33].

Iron accumulation and lipid peroxidation are the two fundamental processes that trigger ferroptosis [27]. In combination with immunotherapy, targeted therapies (inducers or inhibitors) against ferroptosis may exert potent antitumor activity, even in tumors resistant to immune checkpoint inhibitors [29].

Parthanatos: Parthanatos is a form of programmed cell death initiated by PAR polymer, a component of Poly-ADP-ribose polymerase-1 (PARP-1) and is distinct from other cell death processes such as necrosis and apoptosis. It is caused by the accumulation of Poly (ADP ribose) (PAR) and the nuclear translocation of apoptosis-inducing factor (AIF) from mitochondria. Parthanatos' main mechanism of action is the production of PAR polymer, which leads to NAD+ depletion and the release of apoptosis-inducing factor (AIF), ultimately resulting in cell death.

Other proposed mechanisms of action of parthanatos include mitochondrial permeability transition (PT) and the loss of mitochondrial membrane potential. Understanding parthanatos will help in expanding therapeutic options for PARP-1-related diseases [34].

Erebosis: Erebosis is a newly identified type of cell death discovered in the gut enterocytes of adult Drosophila, or fruit flies. This process differs from known cell death pathways, such as apoptosis, necrosis, or autophagy. It is characterized by the loss of the cytoskeleton, cell adhesion, and organelles. Enterocytes surrounding the erebotic cells often protrude towards them, and old differentiated cells are believed to typically die by apoptosis. Further investigation will be conducted to determine the molecular mechanisms by which erebosis occurs and its role in gut tissue homeostasis [35].

Conclusion

Exploring the Research on Programmed Cell Death

The exploration of programmed cell death holds profound potential for advancing science and medicine. This fundamental biological event can shed light on diverse domains, including cancer therapy, stem cell research, regenerative medicine, understanding disease mechanisms, drug development, and fundamental biological understanding. Broadly, the course of programmed cell death research promises to enrich our knowledge about the intricacies of cellular life and death, thereby creating opportunities for innovative disease therapies.

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Capases

Cat #	Product
1128	Caspase-10 Antibody
2071	Caspase-9 Antibody
PM-2015	Apaf-1 Antibody [2E10]

BCL-2

Cat #	Product
3343	BAD Antibody
3335	Bcl-2 Antibody
3351	Bax Antibody

Ferroptosis

Cat #	Product
14-907	GPX4 Antibody
13-240	NRF2 Antibody
16-557	HSPB1 Antibody, KO Validated

Serine Proteases

Cat #	Product
18-708	GZMB Antibody
22-069	GZMA Antibody
13-029	Perforin Antibody

Pyroptosis

Cat #	Product
5447	NALP3 Antibody
16-910	GSDMD Antibody
3463	Caspase-1 Antibody

Parthantos

Cat #	Product
13-327	PARP1 Antibody, KO Validated
2267	AIF Antibody
15-262	MIF Antibody

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