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Apoptosis: From Oncogenesis to Oncotherapy

Kalpataru Halder

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Department of Molecular Biology & Biotechnology, Brahmananda Keshab Chandra College, 111/2 B.T Road, Kolkata-108, West Bengal, India

E-mail/Orcid Id:

KH, ✉ kalpataruhalder@outlook.com, ⓘ https://orcid.org/0009-0007-8832-8092

Abstract: Cell death is critical in maintaining the balance between cell proliferation and elimination in all living organisms. Among the different modalities of regulated cell death, apoptosis remains the most extensively studied and interesting pathway for targeted carcinogenesis therapy. Dysfunctions in apoptotic pathways contribute to the development and progression of cancer, and targeting these pathways is essential for effective cancer therapy. This review provides an overview of different types of apoptotic pathways and their significance in the development and progression of cancer. We also discuss the present oncotherapy strategies targeting different cell death pathways and mechanisms and the challenges associated with apoptosis-based therapies. This review highlights the need for the development of 3-D cellular models to study the interaction between tumor cells and their microenvironment, reduced in-vivo toxicity, and increased specificity for certain drugs targeting p53 or inhibitor of apoptosis proteins (IAPs). Overall, this review provides a comprehensive understanding of the significance of apoptosis in oncogenesis and oncotherapy and the potential of targeting apoptotic pathways for effective cancer treatment.

Introduction

Cell death is the fundamental process that maintains the balance between proliferation and elimination of cells in all living organisms. It represents a vital role in early embryonic development and morphogenetic balance and is essential in pathophysiological conditions, including cancer, AIDS, and neurodegenerative diseases, for removing diseased cells (Bertheloot et al., 2021). The foremost modalities of the intricate and intertwined system of cell death are- the non-lytic apoptotic pathway and the non-apoptotic pathways of autophagic cell death, pyroptosis, and necroptosis (Newton et al., 2024), commonly called as PANoptosis (Bedoui et al., 2020). Each pathway has unique mechanisms and outcomes, yet they all share similar key players and characteristics, and comprehending the commonalities and distinctions among various cell death mechanisms is essential during the pathologic evaluations to facilitate focused approaches for modifying these pathways for potential therapeutic advantages (Ketelut-Carneiro and Fitzgerald,

2022). Pyroptosis is a regulated cell death pathway that is inflammatory and is triggered by inflammasome sensors (Aglietti et al., 2016). Necroptosis, another inflammatory and necrotic cell death pathway, is stimulated when the extracellular ligands bind several death receptors on the cellular surface (Park et al., 2023), while autophagy regulates the selective recycling of organelles and molecules within the cell by the formation of autophagosomes (Fan and Zong, 2013). Apart from these non-apoptotic inflammatory pathways, ferroptosis, necrosis mediated by MPTP or Mitochondrial permeability transition pore, Parthanatos, alkaliptosis, NETosis, lysosome-dependent cell death (LCD), oxeiptosis and cuproptosis are also important regulated cell death modalities essential to the efficacy of cancer treatment (Tong et al., 2022a).

Cell death is an act of balancing that, if left unchecked, may result in tissue damage, eventually contributing to the spread of the infection. Hence, the main mechanisms of cell death are tightly regulated to

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prevent this. Disruptions in various cell death processes are hallmarks of the complexities of cancer and targeting cell death, particularly apoptosis and autophagy, is pivotal in effective cancer therapy. The majority of the clinically used anti-cancer drugs utilize the functional apoptotic pathway, thus, any kind of dysfunction leads to uncontrolled cell proliferation, growth, and spread of cancer, ultimately resulting in the acquisition of therapeutic resistance (Pistritto et al., 2016; Das et al., 2021; Boga and Bisgin, 2022; Madhu et al., 2022, 2023).

As early as 1970, apoptosis was shown to be connected to the abolition of unnecessary malignant cells and tumor growth. Hence, reduction in apoptosis and circumventing its strict surveillance is essential for malignant cell progression and this can be achieved by several mechanisms such as an imbalance creating underexpression of proteins belonging to the pro-apoptotic family, and over-expression of proteins of the anti-apoptotic family, a disruption in the signaling pathway mediated by death receptor and reduction of caspase activity (Wong, 2011).

Two essential characteristics of malignancy include uncontrolled cell proliferation and inhibition of controlled cell death; thus, targeting different pathways of cell death has always been a gold standard in anticancer strategies. In recent years, several types of cell death mechanisms have been discovered and identified, along with the underlying signaling pathways and included as pharmaceutical targets e.g., CQ, shikonin, sphingosine analog FTY720 or fingolimod, metformin, polyphyllin VI, Sorafenib- all these anti-cancer agents and chemotherapeutic drugs such as actinomycin-D, doxorubicin, topotecan, bleomycin target different non-apoptotic cell death modalities and a lot of promising molecules targeting these novel pathways and combination therapies are in the pipeline (Tong et al., 2022b).

However, apoptosis remains the most interesting vehicle for targeted carcinogenesis therapy due to the abundance of literature and multiple targetable defects along the pathway. The new-age cancer drugs are developed by targeting one of the following mechanisms (Wong, 2011) -Inhibition of proteins and genes of anti-apoptotic family, development of inhibitors of apoptosis proteins (IAPs) antagonists or caspase-based therapy. Nonetheless, there are still challenges with the therapies based on apoptosis that need to be resolved in the future. E.g., need for 3-D cellular models to study the interaction between tumor cells and their microenvironment, reduced in-vivo toxicity and increased specificity for certain drugs targeting p53 or IAP etc.

In this review, we attempt to discuss the important players and signaling pathways involved in apoptosis and how it is interconnected with other cell death mechanisms. Our goal is to summarize the most recent treatment strategies that target apoptosis, the existing resistance mechanisms, and the strategies that can be used to overcome them for successful clinical translation.

Mechanisms of Apoptosis

A series of energy-intensive molecular processes comprise the intricate and highly selective process of apoptosis. According to present studies, there are three primary pathways of apoptosis: the mitochondrial or intrinsic pathway, the perforin/granzyme mediated pathway, and the extrinsic pathway induced by death receptors (Elmore, 2007). Growing evidence suggests that these routes are interconnected and key players in one pathway have the ability to impact the mediators in the other. Apoptosis can be commenced by either of the pathways, converging at the execution terminal by the recruitment of effector caspases (Kashyap et al., 2021). This leads to fragmentation of DNA, breakdown of cellular proteome, protein cross-linking, apoptotic body formation, synthesis of ligands to bind to phagocytic cell receptors, and, subsequently, engulfment mediated by phagocytic cells such as Langerhans cells, macrophages and dendritic cells. (Thapa et al., 2022).

Extrinsic Pathway

The extrinsic apoptotic pathway involves cell receptors belonging to the tumor necrosis factor TNFRs superfamily. These receptors have a homologous intracellular death domain (DD) composed of 80 residues. The most studied members of this family are FasR/FasL and TNF-α/TNFR1 receptor-ligand complexes, but there are others such as UNC5A-D netrin receptors, TRAIL/TRAILR (TNF related apoptosis-inducing ligand/Receptor), and Apo3L/DR3. When the corresponding ligands activate these receptors., they undergo a series of changes and recruit pro-apoptotic adaptor protein containing Death effector Domain (DED) such as FADD protein (Fas-Associated Death Domain), which in turn sequesters the initiator pro-caspase-8/10 and forms the death-inducing signaling complex (DISC). This process generates an activation loop that is auto-catalytic, and downstream of DISC formation, cell killing is commenced by the activity of effector caspases-3, 6, and 7. In some cases, an additional amplification step induced by caspase-8 and engagement of the intrinsic pathway becomes necessary. For TNFR, death domain-associated protein TRADD (Tumor Necrosis Factor Receptor-1-Associated Death Domain) is recruited upon

receptor activation, which forms complex I by involving TRAF and RIP. TRAF2 can also recruit apoptosis inhibitory proteins c-IAP 1/2. The suppression of c-IAP 1/2 leads to the blocking of RIP ubiquitination, freeing the TRADD-RIP complex from the receptor. When the complex reaches the cytoplasm, it binds to FADD, leading to the recruitment of procaspase 8 and triggering the activation of successive executioners (Degterev et al., 2003; Green & Llambi, 2015; Redza-Dutordoir & Averill-Bates, 2016). In addition to death receptors, growth factors also affect apoptosis through the PI3K-Akt signaling pathway. These factors activate PI3K by binding to growth factor receptors. Activated PI3K pathway results in Akt activation. As a pro-apoptotic member of the Bcl2 family (B-Cell Leukemia-2 family) and regulator of BAD (Bcl2-Antagonist of Cell Death), Akt has a crucial function in regulating the process of mitochondrial apoptosis. Because PKC (Protein Kinase-C) activates p90RSKs (Ribosomal-S6 Kinases), which inhibit BAD, PKC may potentially be important for apoptosis (Courtney et al., 2010).

Immune cells that are cytotoxic, such as NK cells or cytotoxic T lymphocytes ((CTLs), rely on the perforin/granzyme pathway for their function. These cells play a vital role in removing abnormal tumorous or virally-infected cells, which is why apoptosis is essential to them. Once the cytotoxic cells detect their target, they release cytotoxic components by inducing exocytosis mediated by the secretory lysosome. Granzymes A and B are the serine proteases that can enter the cytoplasm of the target cell when perforin creates breaches in the membrane. Granzyme A initiates the nicking of DNA without the help of caspases. It starts the production of DNAase NM23-H1 by breaking down the SET-nucleosome protein complex that normally suppresses the NM23-H1 gene. Granzyme B, on the other hand, starts apoptosis in different ways. Firstly, it can selectively cleave procaspase 3 at aspartate residues, which activates procaspase 10 and causes the executioner caspases to become active. This cascade ultimately results in the destruction of ICAD - an inhibitor of caspase-activated DNAase. Secondly, granzyme B is also able to also utilize the intrinsic pathway of apoptosis to induce the execution phase directly. In this way, granzyme B generates tBid from Bid and induces release of cytochrome c. (Elmore, 2007).

Intrinsic Pathway

The intrinsic pathway is stimulated by several intracellular stress conditions, including deprivation of growth factors, absence of cytokines and hormones, DNA damage, generation of reactive oxygen species, radiation stress, toxic components, hypoxic condition, viral infection, hyperthermia,oncogenes etc (Lee et al., 2023). The sequence of events that occur in this pathway are as follows- different pro-apoptotic proteins are released when the intrinsic route is activated by stress, and these proteins then counteract anti-apoptotic proteins., impacting the permeability of mitochondrial outer membrane (MOMP), leading to the apoptosome complex formation and executor caspase-mediated cell death (Czabotar & Garcia-Saez, 2023). In the caspase-dependent intrinsic mitochondrial pathway, major pro-apoptotic proteins are cytochrome c, Second Mitochondria-Derived Activator of Caspase (Smac) /DIABLO, and the High Temperature Requirement Protein-A2 (HTRA2)/Omi serine protease. They result in apoptosome complex formation by recruiting Apaf-1 and procaspase-9. In the caspase-independent pathway, AIF, endonuclease G, and CAD (Caspase-Activated DNAse) are the main pro-apoptotic proteins (Elmore, 2007). Proteins of the Bcl-2 family are responsible for controlling the mitochondrial apoptotic processes.

Crosstalk between Intrinsic and Extrinsic Pathways

At the effector caspase activation stage by the initiator caspases 8 and 9 or the execution phase, all apoptotic pathways combine. Among other activities, the effector caspases (caspase-3, 6, and 7) break hundreds of cellular proteins and can make phosphatidylserine visible on the membrane's outer layer of a dying cell and initiate internucleosomal fragmentation of DNA. Moreover, they induce the rearrangement of the actin cytoskeleton, which results in membrane blebbing and promotes the development of apoptotic bodies. Together, these caspase-induced mechanisms work to accelerate the disintegration of the cell. Both the apoptotic pathways, intrinsic and extrinsic, undergo severe dysregulation during cancer, which increases cell survival under chemotherapy and radiation treatment.

Regulation of Apoptosis

The apoptosis regulation contains many different molecular pathways and signaling cascades which decide whether the cell should live or die. Caspases, Bcl-2 family proteins and a number of other factors at higher levels regulate the start and completion points for apoptosis tightly. Many areas in development, cancer or neurodegenerative diseases among others have great implications in understanding this complex process where such fine controls are necessary.
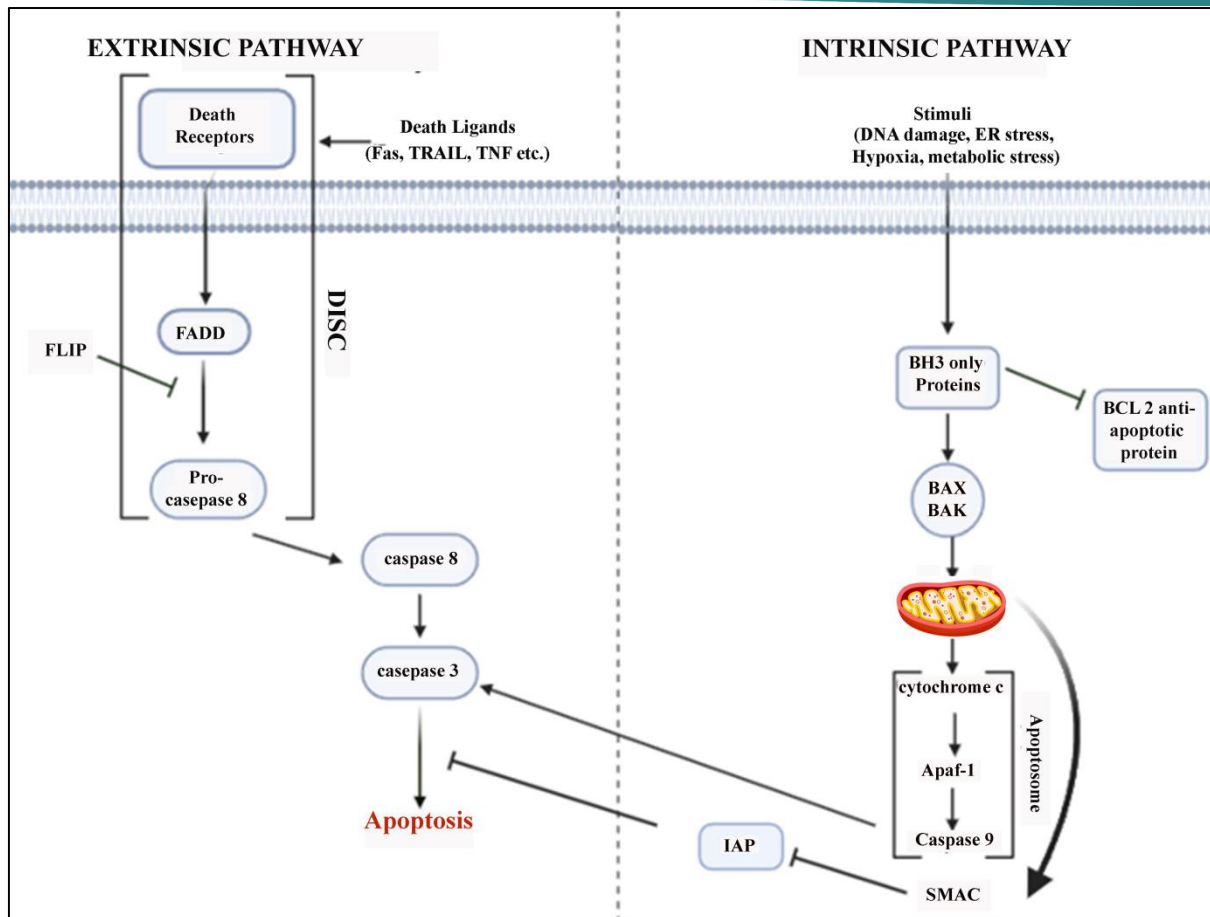
Figure 1. Schematic representation illustrating the dynamic interplay and crosstalk between the extrinsic (death receptor-mediated) and intrinsic (mitochondrial-mediated) pathways of apoptosis. The extrinsic pathway is triggered by the binding of death ligands (e.g., Fas ligand, TNF-α) to their respective death receptors (e.g., Fas receptor, TNF receptor), leading to the activation of caspase-8 and subsequent cleavage of effector caspases (e.g., caspase-3). Meanwhile, the intrinsic pathway can be triggered by various cellular stresses (e.g., DNA damage, growth factor deprivation), resulting in mitochondrial outer membrane permeabilization (MOMP), release of pro-apoptotic factors (e.g., cytochrome c), and activation of caspase-9. Notably, crosstalk between these pathways exists, e.g., caspase-9 activation in the intrinsic pathway can also cleave and activate caspase-8, further propagating apoptosis. This intricate interplay between extrinsic and intrinsic pathways highlights the complexity of apoptotic regulation and its significance in various physiological and pathological processes.

Bcl-2 Family Proteins

BCL-2 family proteins are key modulators of mitochondrial apoptosis. (Galluzzi et al., 2018). In terms of evolution, this family of proteins and their functions are old, with instances spanning a wide range of metazoans. There are around 20 canonical members in the family, each having one to four BCL-2 homology domains (BH domains) ranging from one to four and opposing roles (Popgeorgiev et al., 2020). These are brief sequence homology areas that BCL-2 family protein share despite having different roles. This family is classified into three subgroups based on BH domain initiators' composition, organization, and functionality or BH3-only proteins, guardians, and executioners. Initiators are the ones to respond first to given cellular stress (such as BIM, PUMA and BID) and execute their duties by both blocking the actions of guardian family members (BCL-2, BCL-W, BCL-XL, MCL1, BCL2A1) and stimulating the executioners (BAK, BAX, and BOK). Activated BAX and BAK gather at certain locations on the mitochondrial outer membrane (MOM) and form oligomers upon conformational changes. This is followed by the formation of membrane pores, which causes MOMP. Proteins of the BCL-2 family induce MOMP through a complicated network of communications that controls apoptosis. Different affinities characterize the interactions amongst members of the same family, which occur in the cytosol and at the membranes of cells where these proteins are concentrated, predominantly at the mitochondria and endoplasmic reticulum (Letai et al., 2002).
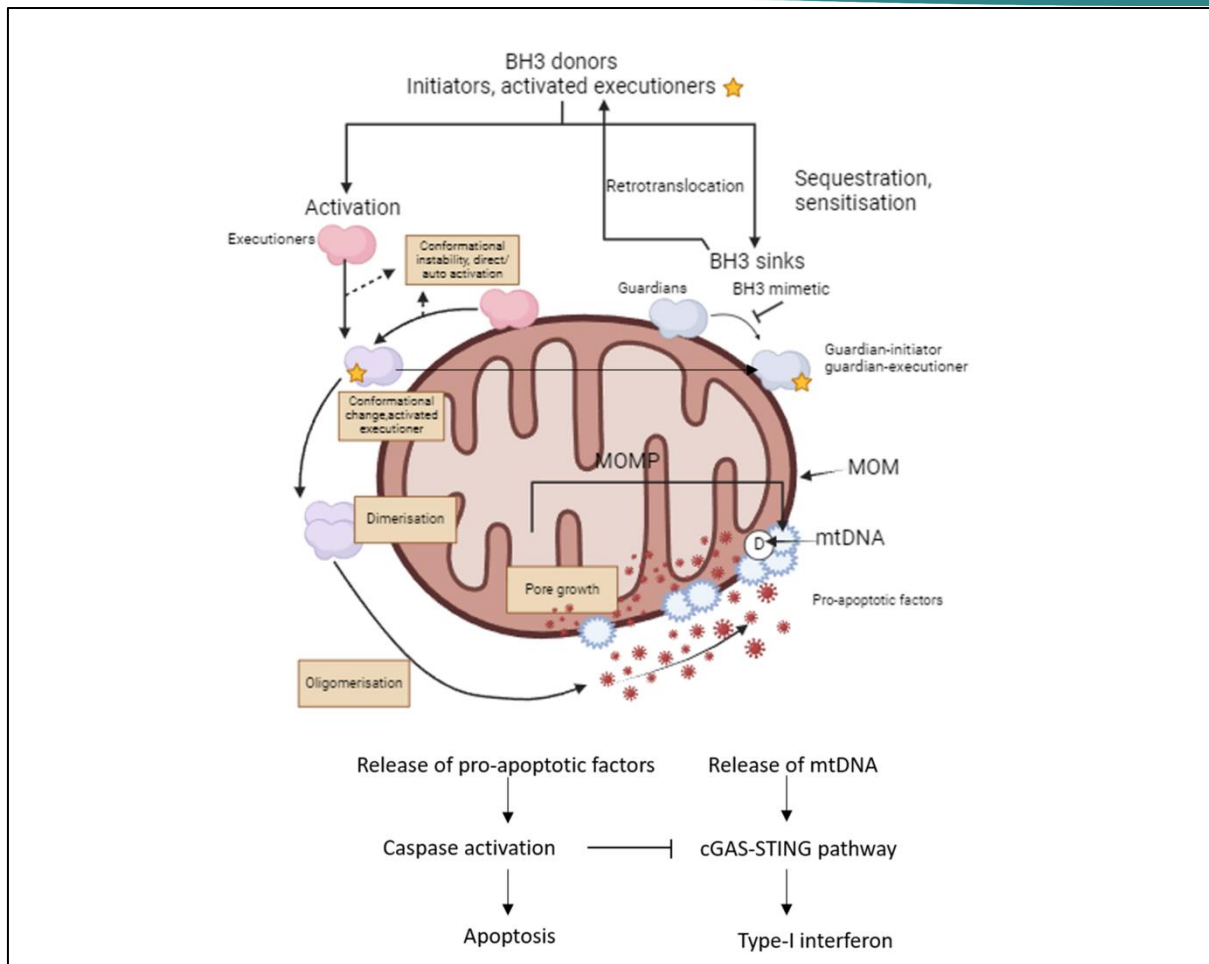
Figure 2. Diagram showing mitochondrial control of Apoptosis; how mitochondria play a critical role in regulating apoptosis, the process of programmed cell death that is essential for maintaining tissue balance and eliminating damaged cells. When apoptosis is initiated, various signals trigger pro-apoptotic pathways that lead to the permeabilization of the outer membrane of mitochondria (MOMP). This process is facilitated by proteins like Bax and Bak, which form pores in the outer membrane of mitochondria. As a result of MOMP, cytochrome c is released into the cytosol, where it interacts with Apaf-1 to form a complex that activates initiator caspase-9. Activated caspase-9 then triggers a cascade of caspase activation, leading to the execution of apoptosis. At the same time, anti-apoptotic Bcl-2 family proteins like Bcl-2 and Bcl-XL prevent MOMP and maintain mitochondrial integrity, countering pro-apoptotic signals. Understanding the complex interplay between pro- and anti-apoptotic factors at the mitochondria provides valuable insights into regulating cell fate decisions.

Caspases and their Activation

Cysteine Aspartyl-specific Proteases, or "caspases," are the proteases that induce apoptosis. These enzymes belonging to the intracellular family of cysteine proteases attack the aspartic acid (Asp) residues on their substrates and cleave them. Usually, caspases need to be activated and dimerized for their functioning. They are produced as inactive proenzymes called zymogens or pro-caspases, which need to be processed proteolytically at specific Asp residues. Because they need to be cleaved at Asp residues to become active and perform the same function on their substrates, their actions can converge in a signaling cascade to autoactivate one another and themselves. Caspases can act as initiators or effectors in the apoptotic pathway. There are most likely many

mechanisms for activating caspases, while specifics are still lacking for some of them. Most important of effector caspases, caspase-3, is stimulated by activated caspase-8 (initiator) via two different mechanisms. The first mechanism involves Caspase-8 cleaving Bcl2 Interacting Protein BID. The c-terminal fragment of this protein then moves to the mitochondria and releases cytochrome-C there. To activate Caspase-9, released cytochrome-C binds to dATP, Procaspase-9, and APAF1, also called Apoptotic Protease Activating Factor-1. Caspase-3 is also stimulated by Caspase-9 cleaving Procaspase-3 in an autoactivation loop. An alternative mechanism involves Caspase-8 directly cleaving Procaspase-3 and activating it. DNA fragmentation factor ICAD (Inhibitor of Caspase-Activated DNase) is cleaved by Caspase-3 into a

heterodimeric form that includes cleaved ICAD and CAD. When cleaved ICAD separates from CAD, CAD oligomerizes and exhibits DNase activity. As a result, the internucleosomal breakage of DNA is caused, a characteristic of apoptosis (Farghadani et al., 2021; Raj et al., 2020).

Role of Mitochondria in Apoptosis

Mitochondria plays an important role in activating of apoptosis. The disintegration of the MOM or mitochondrial outer membrane subsequent to the formation of pores driven by members of the executioner family is a critical event in mitochondria-induced apoptosis. As a result of the breakdown of membrane, pro-apoptotic substances are released into the cytoplasm, which activates caspase and triggers apoptosis (Czabotar and Garcia-Saez, 2023). As the pores expand, mitochondrial DNA (mtDNA) is released into the cytosol. When caspases are suppressed (e.g., during viral infection), mtDNA can trigger the cGAS-STING cascade and generate type I interferons. Proteosomal degradation and regulation by non-family members, positioning by translocation and retrotranslocation, and an intricate web of interactions amongst family members of BCL-2 all regulate the process. The consequence of these interactions dictates whether executioners will be triggered, resulting in MOMP. Apoptosis is initiated when the number of BH3 donors, which can be active or non-active initiators or executioners, is sufficient to exceed the guardians' ability to sequester them. The BH3 domains containing initiators or active executioners trigger additional executioner molecules by changing the conformation of the BCL-2 fold. This process is linked to prominent membrane integration, oligomerization, and generation of pores (McArthur et al., 2018; Riley et al., 2018).

Apoptosis and Oncogenesis

Apoptosis, the indispensable morphologic process that PCD-affected cells undergo, is carried out by the caspase family of intracellular proteases. In contrast to inadvertent cell deaths brought on by injury and cerebral infarction, these physiological deaths result in the breakdown of cells into membrane-enclosed entities that are removed by nearby cells by phagocytosis without causing tissue damage or inflammation.

Dysregulation of Apoptosis in Tumor Cells

Cell life span can be prolonged by defects in the PCD processes, which can lead to malignant cell proliferation independent of cell division. Carcinogenesis is aided by the unbalanced production of pro-apoptotic and anti-apoptotic proteins, leading to a decreased apoptosis rate in malignant cells. It has been possible to identify several antagonists of the caspase-mediated apoptotic pathways as well as occurrences of their expression or function being dysregulated in malignancies (Kitada et al., 2002; Kesavan et al., 2023; Kulkarni et al., 2023; Das et al., 2024; Halder, 2024). The antagonists of the intrinsic route are as follows: (i) anti-apoptotic proteins that belong to Bcl-2 family. Their elevated concentrations prevent cytochrome c and other apoptogenic proteins from leaving mitochondria; (ii) Akt kinase (PKB) that leads to phosphorylation and inactivation of Caspase-9 and BAD; (iii) TUCAN, a CARD-protein that attaches and retains pro-Caspase-9; and (iv) Suppressors of IAP-family, which attaches and effectively prevents Caspases-3, 7, and 9 (Krawiec et al., 2022). Similarly, several inhibitors of the Extrinsic Pathway have been discovered, such as BAR, c-FLIP, Bap31, and several other proteins containing DED. These proteins compete to bind to the pro-Caspases or adapter proteins that take part in TNFR-mediated signaling. The activity of multiple pathways, including PI3K/Akt and MAPK pathway, can increase c-FLIP expression and inhibit death receptor-induced apoptosis. Additionally, by engaging and inhibiting the downstream effector caspases, the elevated levels of IAPs also block the extrinsic pathway. Smac/Diablo and HtrA2/Omi are other IAP antagonists that have been identified to function at another level of modulation in mammals. Generally, mitochondria sequester these proteins. They bind IAPs upon release, enabling the caspases to function as effectors.

miRNAs, which do not code for proteins and control gene expression after transcription, can silence target mRNAs, and Their deregulation has been connected to a number of cancer types. These miRNAs can act as tumor suppressors or oncogenes. By targeting different mRNAs, miRNAs can work as either pro-apoptotic or anti-apoptotic regulators that are associated with apoptotic signaling pathways. For instance, miR-15/ miR-16 targeting the Bcl-2 protein has an anti-apoptotic function. In many cancers, miRNA-21 is the most commonly upregulated anti-apoptotic miRNA that targets genes such as PCD4, TPM1, and PTEN to modulate apoptosis. Like mi-RNAs, long non-coding RNAs (lncRNAs) are often dysregulated in cancer cells and can sensitize cells to apoptotic treatments (Thapa et al., 2022).
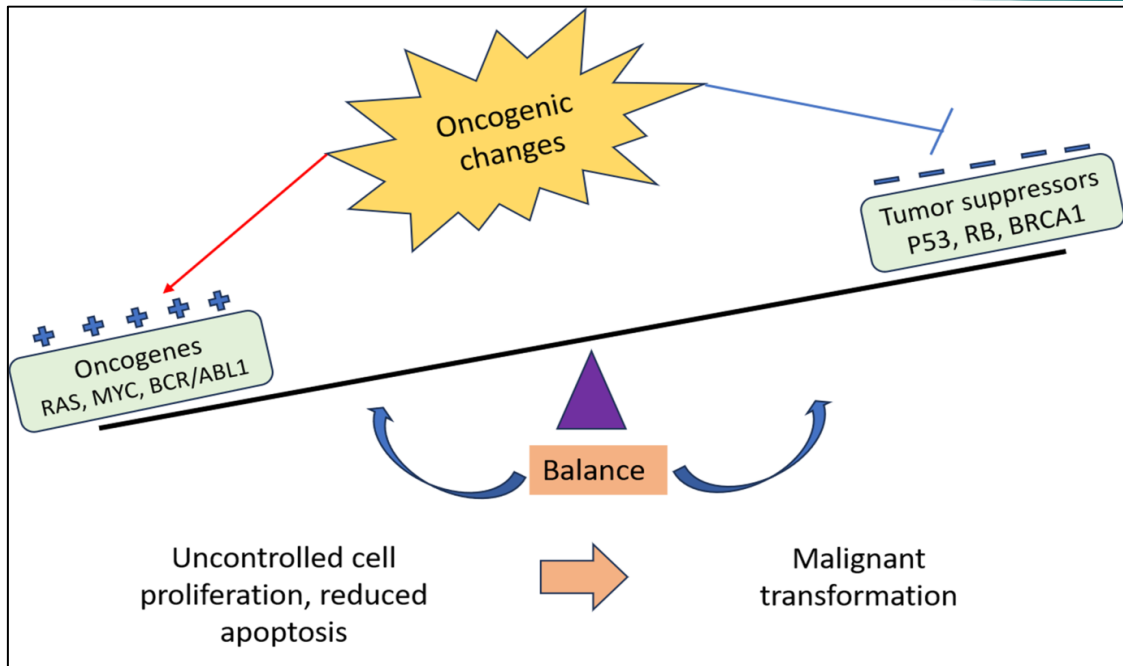
Figure 3. Cancer, tumor suppressors, and oncogenes. Mutations in oncogenes or tumor suppressor genes are caused by genomic instability brought on by various agents, including viruses, cytotoxic medications, and radioactive substances. This instability prolongs the unstable genome, simultaneously stimulating cell proliferation and inhibiting apoptosis, leading to cellular transformation and malignancy

Tumor Suppressor Genes and Apoptosis

Every normal cell has both oncogenes and tumor-suppressive genes. Tumor-suppressive genes are crucial for regulating the cell's growth and differentiation and preventing the formation of cancer. There is a large collection of tumor-suppressor genes, all of which share one critical function: they safeguard the organism from developing neoplasia. Retinoblastoma 1 protein (RB1) and P53 are among the genes that act as tumor suppressors. Apoptosis, the regulated cell death process, is regulated by several genes that can either stimulate or inhibit it. Examples of genes that activate apoptosis include the proteins of MYC and BAX proto-oncogenes, P53 tumor-suppressor gene, and the E2F transcription factor (Kontomanolis et al., 2020). Mutations found in the p53 gene's evolutionarily conserved codons are frequent in various forms of human cancer. The mutational range of p53 depends on the location of the cancer and varies among organs such as the colon, breast, lung, liver, esophagus, reticuloendothelial tissues, brain, or hemopoietic tissues. We can learn more about the origins of these different types of cancers and the function of particular regions of p53 by examining these mutations. Transitions are more common in colon, brain, and lymphoid malignancies, while G dominates liver and lung cancers: C to T: A substitutions. Esophageal carcinomas have more frequent A: T base pair mutations than other solid tumors. CpG dinucleotide mutational hot spots are where most of the transitions in lymphomas,

leukemias, brain tumors, and colorectal carcinomas occur. In the case of breast, lung, and esophageal cancers, G to T transversions happen at various codons. When patients from areas where hepatitis B and aflatoxin B1 are cancer risk factors develop liver cancers, the majority of the mutations occur at one codon 249 nucleotide. These variations could be a reflection of the roles that endogenous and external variables play in the development of human cancer. In 5-10% of AML cases, TP53 mutation arises spontaneously, and in 30-40% of cases, it results from therapy, indicating a poor prognosisThe majority of TP53 mutations result in missense mutations, which modify the DNA-binding domain's amino acid sequence (exons 5-8 code for this domain). Interestingly, individuals with a TP53 mutation have fewer mutations in other genes related to myeloid cells, such as genes involved in splicing, epigenetic modifications, and signaling. However, regardless of the mutation status of TP53, many cases of AML are associated with dysfunctional p53, which is likely caused by changes to proteins that regulate p53, leading to the disruption of apoptosis (Krawiec et al., 2022; Quintás-Cardama et al., 2017; Wang and Sallman, 2022).

Oncogenes and Apoptosis Resistance

The development, proliferation, and survival processes necessary for regular cellular homeostasis are regulated by proto-oncogenes. Cancer cells exploit these processes to obtain advantages over their non-neoplastic

equivalents. When a cell undergoes malignant transformation, it must bypass the antineoplastic countermeasures that usually keep proto-oncogenes in check. The benefits of an oncogene, such as enhanced proliferation, can then be used by tumor cells without the adverse consequences of overriding fail-safe mechanisms. For instance, cooperating lesions that suppress apoptosis, such as p53 loss of function or BCL2 overexpression, are required to enhance MYC-driven carcinogenesis when an oncogene like c-MYC stimulates both cell proliferation and death. On the other hand, while BCL2 may be able to stop apoptosis, it does not have much inherent mitogenic activity. For this reason, cellular transformation requires collaborating oncogenes that promote cell division, such RAS and MYC. There are various ways in which oncogenes can be activated, such as increasing the expression of a healthy gene product, producing a mutant protein with modified function or stability, or altering the position or recruitment of a functional gene product by interaction with a binding partner that is mutant or has aberrant expression. Different signaling pathways can be activated due to the increased expression of oncoproteins or mutations that lead to abnormal protein function. For instance, RTK (receptor tyrosine kinase) or GPCR (G-protein coupled receptor) activity independent of ligand interaction can be triggered by gain-of-function mutations in receptor proteins. In a similar way, Mutations that promote PI3K and RAS activation can also cause pathway activation without ligand interaction, which can enhance the responses to receptor ligation upstream. Moreover, Chromosome translocations can produce fusion proteins, such as BCR-ABL, that have carcinogenic enzymatic activity. These oncogenic signaling molecules share a common feature of stimulating cellular proliferation and simultaneously inhibiting apoptosis (Shortt & Johnstone, 2012; Thapa et al., 2022).

Apoptosis-Based Cancer Therapies

Cancer therapies that are based on apoptosis use the natural process of programmed cell death to kill off malignant cells and leave healthy ones alone. While chemotherapy and other traditional treatments can damage healthy tissues, such therapies target certain molecular pathways that stop working correctly in cancer cells. For example, one strategy involves small molecule inhibitors blocking anti-apoptotic proteins like Bcl-2; another approach activates pro-apoptotic pathways with targeted therapy or immunotherapy. Therefore, by taking advantage of weaknesses inherent in cancer cells, these methods represent new hope towards better treatment outcomes for patients through greater efficacy and specificity of action against malignant neoplasms and the ability to overcome drug resistance.

Targeting Apoptotic Pathways

Focusing on the mechanism that inhibits apoptosis is crucial for treating aberrant malignant development and metastasis and avoiding treatment failure. Therefore, the players of the apoptotic process are of utmost significance in cancer treatment. ability of the proteins belonging to the Bcl-2 family to mediate pro-apoptotic and anti-apoptotic functions at the level of mitochondria makes them a desirable target for research on drug development. Similarly, the key component in the planning and executing apoptotic cell death, caspases, has also drawn interest in developing anticancer drugs. One effective treatment approach for cancer is to target the Bcl-2 protein family. Bim expression can be modified to control the apoptotic response of tumor cells. Treatment for cancer may potentially benefit from direct binding and stimulation of Bax. BH3 mimetics can selectively kill cancer cells. Peptide-based inhibitors targeting protein interactions among Bcl-2 family members can control intracellular protein-protein interactions. Antisense constructs, such as genasense, have demonstrated potential in preliminary studies, but clinical trials with melanoma patients have been disappointing. Another approach targets Bcl-xL, often showing overexpression in human tumors. Since many human malignancies co-overexpress Bcl-2 and Bcl-xL, downregulating these proteins simultaneously may maximize the effects of an antisense-based treatment.

Caspases play a crucial role in regulating apoptosis and different approaches have been investigated to upregulate caspase activity in tumors. One strategy is to use demethylation substances, which encourage the demethylation of the caspase promoter. Examples of these agents include decitabine, azacytidine, and synthetic analogs of nucleosides. Small compounds that specifically target and activate caspase have also been designed. Caspase-3, -8, and -9 are potential targets in cancer therapy, and researchers have identified numerous molecules to modulate their expression. In addition to directly targeting caspases, scientists have concentrated on identifying anticancer drugs that target apoptotic proteins (IAP) inhibitors to indirectly increase caspase activation. Several IAP inhibitors, including Smac mimetics and Birinapant, are being examined in phase I and II clinical studies. (Baig et al., 2016; Boice & Bouchier-Hayes, 2020; Daniele et al., 2018; Harada & Grant, 2012; Pistritto et al., 2016).

Small Molecule Inhibitors of Anti-Apoptotic Proteins

A highly specific Bcl-2 inhibitor, Venetoclax is presently used to treat acute lymphocytic leukemia, chronic lymphocytic leukemia, and multiple myeloma T-cell prolymphocytic leukemia. It has been licensed for standard clinical practice. Venetoclax belongs to a class of anticancer drugs known as BH3-mimetics, which reproduce the functions of proteins that contain BH3 domains. Navitoclax (ABT-263) was discovered next. It exhibited potential treatment properties for cancer, and when used in combination with tyrosine kinase inhibitors or MEK, it proved efficient in fighting solid cancers (Kang and Reynolds, 2009).

Small interfering RNAs (siRNAs) have the ability to inhibit Bcl-2 family members, which can lead to apoptosis and slow the development of tumors. For example, leukemia cells that had Mcl-1 downregulated by siRNA experienced considerable apoptosis. Numerous microRNAs, including miR-195, miR-24-2, and miR-365-2, have been linked to controlling Bcl-2 expression and function as negative regulators of the Bcl-2 family, indicating their potential for therapeutic applications. One of the most important proteins in controlling apoptosis is Mcl-1, a dominant factor in drug resistance in various human cancers. Several inhibitors have been developed to target Mcl-1, which show promise in preclinical and clinical studies. One such inhibitor, S63845, has been demonstrated to cause apoptosis in vitro in SCLC cell lines at concentrations between 23nM and 78 nM and to reduce tumor volumes in xenograft models. When used in combination with navitoclax In S63845-resistant xenograft models, S63845 had synergistic effects while decreasing the viability of SCLC cells. Because of its non-apoptotic function in DNA repair, Mcl-1 is a desirable therapeutic target in lung cancer. By blocking Mcl-1-mediated HR DNA repair, a small-molecule inhibitor (MI-233) can sensitize cancer cells to replication stress brought on by therapy. In lung cancer models, MI-233 exhibits robust synergism when combined with hydroxyurea or olaparib. Additionally, in cell types like ERα + breast cancer cells, targeted Mcl-1 suppression by RNA interference promotes caspase-mediated cell death. When used with ABT-263, a particular Mcl-1 inhibitor called VU661013 stimulates tumor cell death and results in a synergistic decrease in tumor volume. Over the past few years, numerous Mcl-1 inhibitors have been created., However, targeting Mcl-1 specifically poses a significant challenge due to the huge hydrophobic BH3 interacting region that is exposed to the surface. An increasingly significant mode of action for alternative pharmacological classes, such as deubiquitination inhibitors and CDK9 inhibitors, is indirect targeting of Mcl-1 (Caenepeel et al., 2018; Ocker et al., 2005; Singh and Lim, 2022).

Smac mimetics (SMs) are a specific type of apoptotic inducers. BMT-062789 is a recently developed XIAP inhibitor that has been shown to be successful in combating certain lymphoma cell lines. It has been discovered that this molecule, a heterodimeric mimic of the second mitochondrial activator of caspases (SMAC), inhibits the binding domains of XIAP for both caspase 9 and caspase 3/7. BMT-062789 can cause apoptosis in cell line models Raji 4RH and RL 4RH, which are rituximab-resistant when combined with etoposide. ASTX660 is an additional orally accessible, non-peptidomimetic antagonist of cellular IAP1 (cIAP1) and XIAP. This chemical preferentially binds to and hinders the activity of XIAP and cIAP1, and it may have pro-apoptotic and antineoplastic properties (Hu et al., 2003; Pennati et al., 2007; Sun et al., 2010).

Pharmacologically activating caspases through small chemical activators is also a potential approach for eliminating cancer cells and can potentially overcome drug resistance. One prominent agent for inducing caspase is apoptin, derived from the chicken anemia virus, which causes apoptosis specific to tumors while sparing healthy cells. Therefore, apoptin is believed to be a highly specific therapeutic agent for treating tumors. Apoptin is still preclinical testing (Rohn & Noteborn, 2004).

Biologics and Targeted Therapy

Apoptosis can occur through cellular death receptors, like those belonging to the TNF receptor superfamily. While TNF and CD95L death ligands are effective at inducing cell death in tumor cells, their use has resulted in severe systemic toxicity in humans. However, TRAIL has demonstrated potential as a cancer treatment since it targets tumor cells exclusively and is not toxic in preclinical safety studies. The effectiveness of TRAIL agonists as anticancer medicines is being investigated in a number of clinical and preclinical research. TRAIL-mediated apoptosis avoids Bcl-2-like protein overexpression and is, therefore, independent of p53 (Lu et al., 2011). Several approaches are being employed to resolve issues with the original prototype caspase inhibitors' peptide structure, such as modifying the active warhead. This critical part of a caspase inhibitor decides whether caspase inhibition is reversible and may have negative side effects. Examples of inhibitors developed include nonpeptidic isatin sulfonamides, anilinoquinazolines, and Q-VD-OPH. In cancer treatment, caspase activators can be used to trigger the

Table 1. Various molecules and their interactions with apoptotic pathways are crucial for understanding targeted therapies in cancer.

Name of the molecule	Target of action	Reference
Sodium butyrate, fenretinide, depsipetide, flavipirodo, ABT-263, GX15-070, HA14-1, gossypol, Navitoclax, APG-1252, AZD4320, S44563, Venetoclax, S55746	inhibitors of BCL-2 family	(Kang & Reynolds, 2009)
AMG 176, S64315, AZD5991, UMI-77, A-1210477, AM-8621	MCL-1	(Caenepeel et al., 2018; Singh & Lim, 2022; Tron et al., 2018)
PhiKan083 (A24275), CP-31398	p53	(Boeckler et al., 2008; Rippin et al., 2002)
Hsp90 inhibitors, Cyclin-dependent kinase inhibitors, SMAC mimetics 2 and 3 Cyclopeptide, SM-164	IAP	(Lu et al., 2011; Pennati et al., 2007; Sun et al., 2010)
Small molecule caspase activators	Caspase	(Philchenkov et al., 2004)
Name of the molecule	**Target /Mechanism**	**Reference**
siRNA	Bcl family anti-apoptotic proteins	(Ocker et al., 2005)
siRNA, Antisense oligonucleotides	XIAP	(Hu et al., 2003; Ohnishi et al., 2006)
Apoptin, Immunocaspase-3	Caspase	(Li et al., 2007; Rohn & Noteborn, 2004)
Name of the molecule/Therapy	**Target /Mechanism**	**Reference**
Nutlins, MI-219, Tenovins	p53	(Lain et al., 2008; Shangary et al., 2008; Shangary & Wang, 2009)
ABT-737, ATF4, ATF3, NOXA,	BH3 mimetics	(Oltersdorf et al., 2005)

death machinery. Numerous approaches, including as chimeric proteins and inducible caspases, are being developed to target caspase activation in malignant cells. Small, cell-permeable drugs can activate cellular caspases and reduce the caspase activation threshold. RGD peptides can activate caspase-3, and they are now being used as antithrombotic medications in clinical settings (Philchenkov et al., 2004).

Point mutations cause P53 to become inactive in over 50% of human cancers; several tactics have been employed to target this protein. Restoring the mutant p53's normal functioning is one viable strategy. To restore p53's activity, synthetic compounds and synthetic peptides derived from its C-terminus have been produced. Interfering with its negative regulator Mdm2 binding is another technique. Roche's discovery of "nutlins" is the most recent advancement in this field of medicine. Wildtype p53 can be reintroduced into p53-deficient cancer cells to decrease tumor development, and clinical trials are presently being conducted to assess the intratumoral injection administration of wildtype p53-expressing adenovirus. Gedanamycin's targeting of heat shock factor (Hsp)-90 may be an approach to encourage the proteasome to once again break down mutant p53. Kosan Biochemicals has started a phase 2 monotherapy study for metastatic melanoma patients. The susceptibility of the surrounding normal tissues to the administered genotoxic stress limits the use of chemotherapy and radiation (Lain et al., 2008; Shangary & Wang, 2009).

Challenges and Future Directions
Overcoming Resistance to Apoptosis

More recent studies have provided a deeper comprehension of the importance of apoptotic factors and their potential as therapeutic targets. This understanding has resulted in creating more focused anticancer medications and moving toward medications that cause

apoptosis and target cancer cells precisely instead of traditional cytotoxic approaches. One promising development has been using BH3 mimetics, small molecules that target proteins of Bcl-2 family and are being examined in clinical studies now. These inhibitors have shown the potential to promote apoptosis in tumor cell and overcome chemoresistance selectively. However, more effective inhibitors are still being developed, which offer increased clinical effectiveness and specificity for individual Bcl-2 proteins. In addition to targeting proteins of Bcl-2 family, caspase-targeted therapies are also being developed. These include gene therapy approaches and small molecules that inhibit caspase inhibitors. These therapies have the potential to directly and selectively target individual caspases, triggering their activity and potentially enhancing the effectiveness of conventional cancer therapy. Combining these approaches with recognized treatments may also help resolve previous strategies' limitations and improve their efficacy. Overall, apoptosis-targeted therapies are an exciting area of research that holds great promise for the future of oncology (Farghadani et al., 2021).

E.g., A novel strategy for eradicating resistance to conventional BCL-2 inhibitors involves turning on other antiapoptotic BCL-2 family members, including as MCL-1 and BCL-XL. Navitoclax and a number of BCL-XL and MCL-1 monoselective inhibitors are promising options for anticancer medications. Navitoclax's substantial thrombocytopenia limits its usage in AML. A unique and safe targeted anticancer therapy approach is called PROTACs. An inventive method for triggering apoptotic activation, eradicating chemotherapy-induced senescent leukemia cells, and correcting the SnCs phenotype in AML cells is PROTAC 753B. Patients with relapsed or resistant AML are being studied in the NIMBLE trial with AZD0466, another medication. This drug-dendrimer combination is made up of a pegylated poly-L-lysine dendrimer and a dual inhibitor of BCL2/XL covalently coupled to it. Nowadays, Venetoclax is used in conjunction with other medications such as rituximab, azacitidine/decitabine, ibrutinib, and bortezomib/dexamethasone against a range of hematological cancers for improved efficacy (He et al., 2020; Jia et al., 2021; Konopleva et al., 2021).

Combination Therapies for Enhanced Apoptosis

Inducing apoptosis in AML and lowering MCL-1 protein levels have demonstrated the benefits of CDK9 inhibitors. Clinical trials are being conducted to evaluate the efficacy of CDK9 inhibitors in conjunction with other therapies, including dinaciclib, CYC065, AZD4573, and alvocidib (Cidado et al., 2020; Zeidner et al., 2015,

2021). Eprenetapopt and idasanutlin are two promising drugs being investigated for the management of MDS and AML. Eprenetapopt can induce apoptosis in cancer cells with TP53 mutation, while idasanutlin can reactivate p53 function. However, both drugs have limitations in their efficacy. Combination therapies, such as eprenetapopt with azacitidine or idasanutlin with venetoclax, are being explored to improve treatment outcomes (Fujihara et al., 2022; García-Cano et al., 2020). Inhibitors that target histone deacetylases and BET have demonstrated anticancer activity by inducing apoptosis and showed excellent cooperation with inhibitors of BCL-2. The chromatin remodeling process is affected by HDAC inhibitors, which promote apoptosis through different mechanisms. Combined with venetoclax, a BET inhibitor called ABBV-075 has shown positive outcomes for patients with cutaneous T cell lymphoma (CTCL) (Kim et al., 2018). Geldanamycin, ganatespib and onalespib are inhibitors that target chaperones responsible for stabilizing proteins in cancer cells, such as hsp90. Lastly, modulating epigenetic alterations, interfering with specific proteins, and targeting the activity of chaperones are all potentil strategies for developing therapeutic techniques in the field of oncology (Bussenius et al., 2012; Neckers and Workman, 2012).

Emerging Approaches in Apoptosis Research

Significant advancements in the study of apoptosis have been accomplished by utilizing genetically engineered mice with conditional alleles for tumor suppressor and oncogene genes. These models provide us with an important new understanding of the intricate processes behind programmed cell death and help us understand the roles of specific genes in apoptotic pathways. Researchers are also exploring different animal models beyond mice to study apoptosis and broaden our understanding of how it works in different species. Cancer research receives a lot of funding for diagnostic and therapeutic projects. Animal models are essential for successful clinical implementation since they resemble human characteristics. However, creating comprehensive models is challenging and expensive. Advanced models like humanized mice are critical in immunotherapy research, but obtaining large cohorts is difficult. As a result, more targeted funding is needed to support advanced preclinical investigations. Research efforts so far have extended into clinical trials, where novel therapies targeting apoptotic pathways are being rigorously evaluated for their efficacy and safety. These holds promise for developing innovative treatments for numerous diseases, including cancer (Onaciu et al., 2020; Walrath et al., 2010).

Conclusion

New medications have been developed as a result of the discovery of genes and gene products that regulate apoptosis throughout the past few decades. Because apoptosis is controlled at several molecular levels, a wide range of potential targets and treatment approaches exist. IAPs are a viable option for anticancer treatments and caspase inhibitors are a proven therapeutic approach. Nonetheless, several drugs created to regulate apoptosis are still in the preclinical phases because of selectivity, effectiveness, or drug resistance issues. There are also many important unanswered concerns. Several medications are being tested in clinical trials to treat various malignancies, either as monotherapy or in combination with other therapies. However, because several routes can interfere with each other and cancer resistance might develop, it is challenging to modify apoptotic pathways using specific medicines. Reasonable combination methods are possibly the key to overcoming these resistance mechanisms and achieving clinical success. When considering cancer treatment, targeting apoptotic pathways may prove to be an effective approach in clinical settings.

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Conflict of Interest

The author declares that there is no conflict of interest.

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