This is an extensive, up to date review of apoptosis in cancer biology and potential treatments derived from such understanding. It is written by one of Stan's research trainees and good friend, Dr. Emily Cheng. She was given the Distinguished Investigator Award at Washington University in 2010. Her accomplishments and a brief professional biography are included.

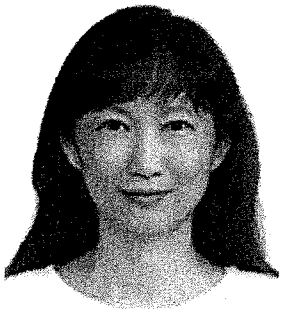
The review documents the vastly greater understanding of apoptosis - cell death- as compared to 2005, the year of Stan's untimely death. On pages 310-316 there is a discussion of cancer treatment with the drug Venetoclax, an inactivator of Bcl-2. With it inactivated, cells will die. So Stan's work has and will continue to have great influence in developing cancer treatments.

WashU Medicine

Emily Cheng, MD, PhD

Distinguished Investigator Award

Distinguished Faculty Awards, 2010



Emily Cheng, MD, PhD, assistant professor of medicine and of pathology and immunology, is recognized as an extraordinarily talented young scientist in the area of apoptosis.

Cheng earned her medical degree from Taipei Medical University and her PhD from Johns Hopkins University. As a graduate student, Cheng made several fundamental discoveries regarding the mechanisms by which BCL-2 family proteins regulate apoptosis. Her thesis yielded three first-authored papers in *Nature*, *Science*, and *PNAS*, as well as two co-first-authored papers in other leading journals.

After completing her clinical training in anatomic pathology, Cheng joined the lab of the late Stanley J. Korsmeyer, MD, a renowned cancer biologist, at Harvard Medical School. She demonstrated that the critical step of cell death commitment converges on BAX/BAK activation, established a model in which anti-apoptotic BCL-2 family proteins inhibit apoptosis by sequestering BH3-only molecules, and identified a novel participant in apoptosis, VDAC2, as a negative regulator of BAK. Cheng joined the Washington University faculty in 2004 and remains at the forefront of cell death research. Studies from her laboratory (1) subdivided the BH3-only molecules into BAX/BAK "activator" or BCL-2/BCL-XL/MCL-1 "inactivator" subgroups and established a unifying, hierarchical regulatory schema among BCL-2 subfamilies, (2) defined DNA damage-induced programmed

necrotic death, (3) demonstrated a VDAC2-BAK rheostat in controlling thymocyte survival and negative selection, and (4) proposed a stepwise activation model of BAX/BAK in the initiation of mitochondrial apoptosis.

Cheng's research accomplishments have been recognized throughout her career. As a graduate student, she received the 20th Young Investigator Award at Johns Hopkins; as a postdoctoral fellow, she received the HHMI Physician Scientist Award and the NCI Howard Temin Award; and as a junior faculty member, she is a Searle Scholar, an American Cancer Society Scholar, and a member of the American Society for Clinical Investigation.

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ANNUAL REVIEWS

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Annual Review of Pathology: Mechanisms of Disease

Apoptosis in Cancer Biology and Therapy

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Keywords

apoptosis, cancer, BCL-2 family, BH3 mimetics, targeted cancer therapy, immunogenic cell death

Abstract

Since its inception, the study of apoptosis has been intricately linked to the field of cancer. The term apoptosis was coined more than five decades ago following its identification in both healthy tissues and malignant neoplasms. The subsequent elucidation of its molecular mechanisms has significantly enhanced our understanding of how cancer cells hijack physiological processes to evade cell death. Moreover, it has shed light on the pathways through which most anticancer therapeutics induce tumor cell death, including targeted therapy and immunotherapy. These mechanistic studies have paved the way for the development of therapeutics directly targeting either pro- or antiapoptotic proteins. Notably, the US Food and Drug Administration (FDA) approved the BCL-2 inhibitor venetoclax in 2016, with additional agents currently undergoing clinical trials. Recent research has brought to the forefront both the anti- and proinflammatory effects of individual apoptotic pathways. This underscores the ongoing imperative to deepen our comprehension of apoptosis, particularly as we navigate the evolving landscape of immunotherapy.

INTRODUCTION

Apoptosis was initially characterized in 1972, on the basis of the morphological characteristics observed in dying cells during embryogenesis, as well as in response to both physiological and pathological stimuli (1). This process is also integral to cell turnover in numerous healthy adult tissues and, notably, in malignant neoplasms. Kerr et al. (1) proposed that apoptosis represents an active, inherently programmed form of cell death, with crucial roles not only in maintaining tissue homeostasis but also in influencing both tumor growth and therapeutically induced tumor regression. More than five decades later, subsequent research has consistently validated and affirmed the accuracy of their groundbreaking idea.

In the decades following that pioneering study, significant progress has been made in unraveling the molecular mechanisms governing apoptosis. Two distinct pathways, intrinsic and extrinsic, converge to activate caspases—a group of proteases responsible for cleaving hundreds, or perhaps thousands, of distinct target proteins and generating the characteristic morphological features initially identified by Kerr et al. The discovery of genes intricately involved in apoptosis has further fortified its link to cancer. BCL-2, the founding member of the protein family that controls the intrinsic pathway, was initially cloned from the t(14;18) translocation observed in follicular lymphoma (2–4). This breakthrough identified a novel class of oncogenes that counterintuitively prevent apoptosis rather than promote proliferation (5). Notably, evasion of apoptosis was among the original hallmarks of cancer (6). Subsequently, numerous additional mechanisms have emerged, illustrating how oncogenes and tumor suppressors intricately interact with apoptotic pathways, thereby mediating the processes of oncogenesis and tumor evolution.

Apoptosis not only contributes to the pathogenesis of cancer but also holds a pivotal role in the effectiveness of cancer therapeutics. Many anticancer agents induce this form of programmed cell death (PCD), including newer treatments such as targeted therapy. In 2016, the US Food and Drug Administration (FDA) even granted approval to venetoclax, a small molecule specifically designed to target protein–protein interactions within the BCL-2 family. Furthermore, apoptosis plays a crucial role in the mechanisms of various immunotherapies currently in clinical use. Cytotoxic lymphocytes, including cytotoxic T cells and natural killer (NK) cells activated or employed in contemporary immunotherapeutic approaches, eliminate their targets through the activation of apoptosis. Although apoptosis is traditionally considered immunologically silent, recent studies have shed light on novel mechanisms by which apoptotic pathways can trigger inflammation. These findings suggest potential therapeutic combinations for future investigations.

This review provides an overview of the molecular pathways regulating apoptosis and delves into the intricate mechanisms by which cancer cells successfully evade apoptosis during oncogenesis. Furthermore, it elaborates on the pivotal role of apoptosis in determining the effectiveness of cancer treatment, with a specific emphasis on targeted therapy and immunotherapy. Lastly, the review explores the anti- and proinflammatory effects linked to individual apoptotic pathways, which may have important implications for modulating the immunotherapy of cancer.

MOLECULAR MECHANISMS OF APOPTOSIS

Apoptosis is activated through two distinct pathways, intrinsic and extrinsic, outlined in detail below. Both pathways converge in the activation of a group of cysteine proteases known as caspases, which can be categorized based on their function (7). Ultimately, apoptosis is executed by effector caspases, caspase-3, -6, and -7. These caspases cleave hundreds of intracellular substrate proteins upon activation, leading to cellular fragmentation into apoptotic bodies and eventual demise. Many targets of effector caspases have been identified through proteomics and compiled in open-access databases (8, 9). Initiator caspases, specifically caspase-9 in the intrinsic pathway

and caspase-8 and -10 in the extrinsic pathway, activate effector caspases. The final group of caspases, caspase-1, -4, -5, and -11, are categorized as inflammatory caspases and can induce a form of inflammatory PCD known as pyroptosis, which is discussed in more detail below.

The Intrinsic Apoptotic Pathway

The intrinsic pathway is regulated by the BCL-2 family of proteins (10–12), which can be categorized into three subfamilies that interact within an interconnected hierarchy (13) (**Figure 1**). The first subfamily comprises multidomain, antiapoptotic members, including the founding member BCL-2, as well as BCL-X_L, MCL-1, BCL-W, and BCL2A1/BFL-1. The second subfamily consists of multidomain, proapoptotic members BAX and BAK. Within the multidomain groups, the four BCL-2 homology domains (BH1–4) are conserved. The third and final subgroup, the BH3-only molecules (BH3s), shares only the BH3 domain, as its name suggests. BH3s act as death sentinels that relay upstream apoptotic signals to initiate apoptosis by either activating BAX/BAK directly (activator BH3s) or inactivating BCL-2/BCL-X_L/MCL-1 (inactivator or sensitizer BH3s). Among BH3s, BID, BIM, PUMA, and NOXA are considered activator BH3s that directly interact with and induce the stepwise structural reorganization of BAX and BAK (**Figure 1**). Notably, only the BH3 peptides derived from BID and BIM, but not from PUMA and NOXA, can consistently recapitulate the full-length BH3-only proteins in activating BAX and BAK (14–17). Thus, early classification of BH3s based on the activity of BH3 peptides has inherent limitations. A wide variety of significant cellular stressors activate the BH3s through different mechanisms to trigger intrinsic apoptosis. BID undergoes activation through proteolytic cleavage in its unstructured loop by caspase-8, resulting in the generation of the active form truncated BID (tBID). This process enables mitochondrial translocation and exposure of its BH3 domain. BIM is transcriptionally upregulated and dephosphorylated to prevent ubiquitination in response to endoplasmic reticulum (ER) stress and deprivation of growth factors. Similarly, PUMA and NOXA are activated through transcriptional upregulation, downstream of the p53-mediated DNA damage response.

At baseline, BAX and BAK exist in healthy cells as inactive monomers. The detailed molecular mechanism by which BH3-only molecules activate BAX/BAK is outlined in a prior review (11). In brief, the transient binding of activator BH3s to BAX/BAK induces a stepwise, bimodal activation of BAX and BAK. An essential intermediate step in BAX/BAK activation involves the exposure of the BH3 domain, allowing the BH3-exposed BAX/BAK monomer to bind to the hydrophobic dimerization groove of another BAX/BAK molecule. This process results in the formation of dimers and subsequent homo-oligomers. Both modes of activation are inhibited by antiapoptotic BCL-2, BCL-X_L, and MCL-1. BCL-2 and BCL-X_L can sequester BID, BIM, and PUMA, while MCL-1 can sequester BID, BIM, PUMA, and NOXA, preventing their activation of BAX/BAK and serving as the frontline protection. The interaction between antiapoptotic BCL-2 members and activator BH3s leads to mutual inhibition. Inactivator BH3s, including BAD, BMF, BIK, and HRK, prevent BCL-2 and BCL-X_L from sequestering BID, BIM, and PUMA. Meanwhile, NOXA prevents MCL-1 from sequestering BID, BIM, and PUMA. NOXA is unique among BH3s as it not only activates BAX and BAK directly but also inactivates MCL-1. However, it is important to note that NOXA exhibits lower potency compared with BID, BIM, and PUMA in inducing apoptosis due to its inability to inhibit BCL-2 and BCL-X_L. BCL-2, BCL-X_L, and MCL-1 can also sequester partially activated BH3-exposed BAX and BAK monomers, providing a fail-safe mechanism or a second line of defense. BCL-X_L and MCL-1 can sequester both BH3-exposed BAX and BAK monomers, whereas BCL-2 can sequester only BAX. Notably, BAX/BAK can autoactivate in the absence of all activator BH3s following the downregulation or inactivation of all

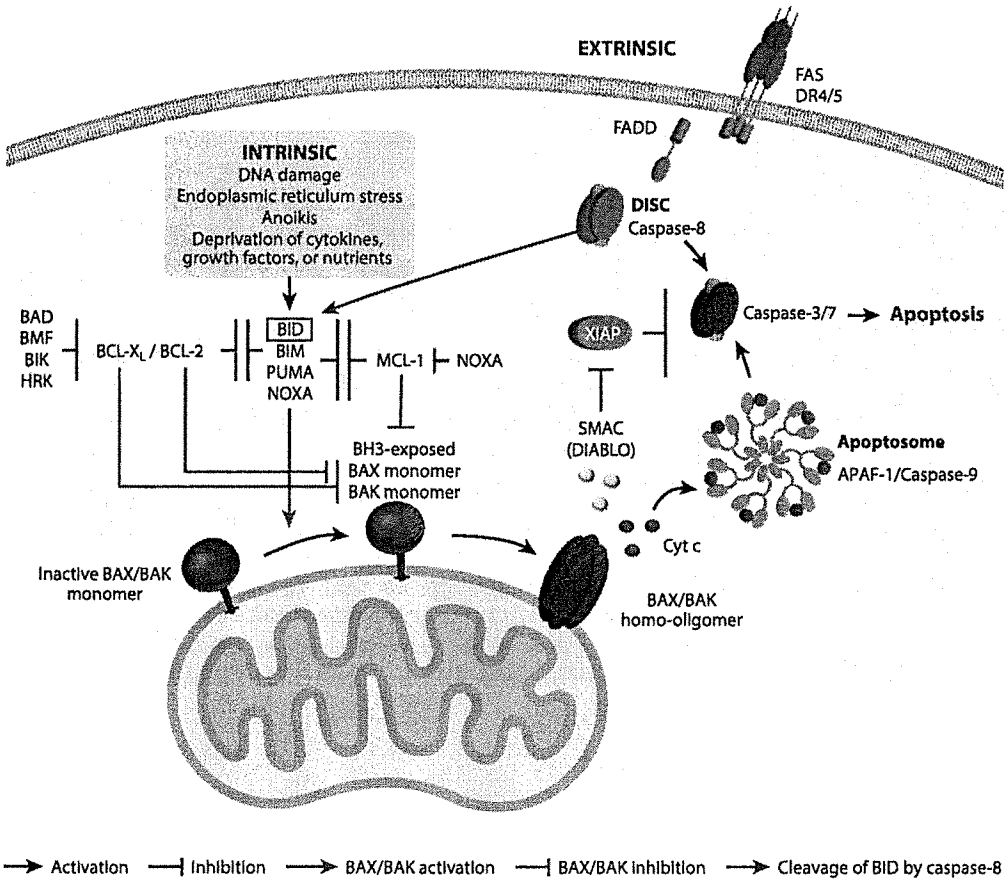

Figure 1

The intrinsic and extrinsic pathways of apoptosis. The intrinsic pathway can be initiated by transcriptional or posttranslational activation of BH3-only molecules (BH3s) in response to diverse death stimuli. Activator BH3s, including BID, BIM, PUMA, and NOXA, bind transiently to BAX and BAK to induce a stepwise, bimodal activation of BAX and BAK. An important intermediate step in BAX/BAK activation involves the exposure of the BH3 domain, allowing the BH3-exposed BAX/BAK monomer to bind to the hydrophobic dimerization groove of another BAX/BAK molecule to form dimers and then homo-oligomers. Both modes of activation can be inhibited by antiapoptotic BCL-2 members. BCL-2 and BCL-X_L can sequester BID, BIM, and PUMA, while MCL-1 can sequester BID, BIM, PUMA, and NOXA, preventing their activation of BAX/BAK and serving as frontline protection. BCL-X_L and MCL-1 can also sequester BH3-exposed BAX and BAK monomers, whereas BCL-2 can only sequester BAX, providing a fail-safe mechanism or a second line of defense. BAD, BMF, BIK, and HRK, can displace BID/BIM/PUMA from BCL-2 and BCL-X_L to activate BAX/BAK indirectly, while NOXA can perform a similar function for MCL-1. The homo-oligomerization of BAX and BAK results in mitochondrial outer member permeabilization (MOMP) and the release of cytochrome c (cyt c) and SMAC from the mitochondrial intermembrane space to the cytosol. Upon binding to cyt c and dATP, APAF-1 oligomerizes into a heptameric complex known as the apoptosome, resulting in the recruitment and activation of caspase-9 and subsequent activation of effector caspase-3/7. The extrinsic pathway is triggered upon binding of death ligands FasL/CD95L and tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) to their respective death receptors Fas/CD95 and DR4/5, resulting in the recruitment of FADD (Fas-associated death domain). FADD then dimerizes with caspase-8 to form the death-inducing signaling complex (DISC) and promotes the autoactivation of caspase-8. In type I cells with low expression of the caspase inhibitor XIAP, death receptor mediated caspase-8 activation is sufficient to activate effector caspase-3/7. In type II cells with high expression of XIAP, effector caspase activation requires a mitochondrial amplification loop to alleviate XIAP-mediated caspase inhibition through mitochondrial release of SMAC. Caspase-8 mediated proteolytic cleavage of cytosolic BID into truncated BID (tBID) activates BAX and BAK-dependent MOMP, connecting the extrinsic pathway to the intrinsic mitochondrial apoptosis pathway.

three antiapoptotic BCL-2 proteins, although this process occurs with slower kinetics than in the presence of activator BH3s (13).

The homo-oligomerization of activated BAX/BAK within the mitochondrial outer membrane (MOM) initiates mitochondrial outer membrane permeabilization (MOMP), resulting in the release of SMAC and cytochrome c from the mitochondrial intermembrane space into the cytosol. SMAC inhibits the caspase-3/7/9 inhibitor XIAP while cytochrome c binds to APAF-1. The binding of cytochrome c to APAF-1 induces conformational changes that enable the exchange of ADP for dATP or ATP and free the caspase recruitment domain, facilitating its binding to caspase-9 (18). While still complexed with cytochrome c, APAF-1 undergoes heptamerization after hydrolysis of dATP or ATP, forming the pinwheel-shaped apoptosome. This structure serves as a scaffold upon which caspase-9 autodimerizes and becomes activated. Activated caspase-9 then proceeds to cleave the effector caspases, ultimately triggering cellular demise.

The Extrinsic Apoptotic Pathway

In the extrinsic pathway of apoptosis, the binding of extracellular death ligands to death receptor complexes on the cell surface triggers caspase-8 activation (**Figure 1**). These death ligands, primarily produced by cytotoxic lymphocytes, include FAS ligand (or CD95L), tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), and TNF-α, which bind to their respective death receptors, FAS (or CD95), DR4/5, and TNFR1 (19). Intracellular signaling through death receptor complexes following ligand binding is covered in depth elsewhere (20). Briefly, ligand engagement with FAS and DR4/5 leads to the formation of the death-inducing signaling complex (DISC), which at a minimum consists of the proteins Fas-associated death domain (FADD) and caspase-8. Signaling through TNFR1 is more complex and occurs through the formation of multiple protein complexes with additional adaptors, which can lead to the activation of nuclear factor kappa B (NF-κB), apoptosis, or necroptosis. Caspase-8 is ultimately recruited to complex II, whose formation is augmented by certain conditions, including depletion of cellular inhibitor of apoptosis proteins 1 and 2 (cIAP1/2). Upon recruitment to DISC or complex II, caspase-8 dimerizes and is activated.

The mechanism by which caspase-8 activation leads to apoptosis is determined by the cell type. In type I cells, such as T lymphocytes that express low levels of the caspase inhibitor XIAP, death receptor–mediated caspase-8 activation is sufficient to activate effector caspase-3/7. However, the majority of cells are type II cells that express higher levels of XIAP, which inhibits caspase-3/7 upon cleavage by caspase-8 (21). To induce apoptosis in type II cells, caspase-8 cleaves the BH3-only molecule BID to generate tBID, initiating activation of BAX/BAK-dependent MOMP and the subsequent release of SMAC to alleviate XIAP-mediated caspase inhibition (22, 23).

APOPTOSIS AND TUMORIGENESIS

The impairment of apoptosis plays a central role in cancer development. Many oncogenes that drive cell division, including MYC, E1A, E2F1, and cyclin D, also activate apoptosis, forming an intrinsic tumor suppressor checkpoint (24, 25). Consequently, evading apoptosis becomes imperative for the successful oncogenic transformation and initiation of cancer. Moreover, resistance to apoptosis not only fosters tumor progression by enabling the survival of genetically unstable cells but also provides a shield against hypoxia and oxidative stress as the tumor mass expands. This resistance allows neoplastic cells to persist even in the absence of external survival factors and nutrients. Furthermore, the inhibition of apoptosis can facilitate metastasis by granting cells the ability to invade areas where normal cells would typically undergo programmed cell death. Additionally, it permits epithelial cells to survive without attachment to the extracellular

matrix, thereby avoiding anoikis, a form of cell death triggered by the lack of proper cell–matrix interactions.

While cell proliferation and apoptosis are diametrically opposed cellular fates, they are intricately coupled to serve as a cellular fail-safe mechanism to limit the consequences of aberrant mitogenic signaling (24, 25). Oncogenes such as MYC and E2F1 induce not only genes required for cell cycle progression but also the expression of ARF. ARF binds to MDM2 and thereby blocks its interaction with p53, allowing the latter to accumulate and transactivate proapoptotic targets, including *PUMA*, *NOXA*, *BID*, *BAX* (in humans but not rodents), and *FAS* (26–31). In addition, E2F1 can directly induce the expression of proapoptotic *BIM*, *PUMA*, *NOXA*, and *APAF1* (32, 33). Consequently, aberrant proliferation signals can induce apoptosis through both p53-dependent and -independent pathways. These pathways converge on activator BH3s, activating BAX/BAK-dependent mitochondrial apoptosis (**Figure 2*a***). Thus, the inhibition of apoptosis is crucial for the successful oncogenic transformation and initiation of cancer. One of the most vital strategies employed by cancer cells to evade oncogene-induced apoptosis involves the upregulation of antiapoptotic BCL-2 members.

The *BCL2* gene was identified at the breakpoint of the t(14;18)(q32;q21) translocation (2–4), which is present in 80% of follicular lymphomas and 20% of diffuse large B cell lymphomas. This translocation places the *BCL2* gene under the control of the immunoglobulin heavy chain locus, leading to the overexpression of BCL-2. In vivo evidence from *Bcl-2* transgenic mice supports the notion that BCL-2 overexpression plays a pivotal role in oncogenesis (34, 35). When the *Bcl-2* transgene was expressed in B lymphocytes, mice exhibited polyclonal follicular hyperplasia, with some cases progressing to high-grade monoclonal lymphoma. The slow progression from polyclonal hyperplasia to monoclonal malignancy indicates the presence of secondary genetic abnormalities. The retention of deleterious mutations, which would have otherwise led to cell death, is facilitated by the inhibition of apoptosis. Lymphomas originating from *Bcl-2-Ig* transgenic mice often exhibit a common second hit: the translocation of the *Myc* oncogene (35). The overexpression of BCL-2 impedes MYC-induced apoptosis, thereby allowing the transformation by MYC to proceed. The synergy between these two distinct classes of oncogenes results in more potent transformation and tumor initiation. Along the same line, genetic ablation of *Bim*, *Puma*, or *Bax* promotes MYC-induced lymphomagenesis in mice (36–38), supporting the tumor suppressor function of proapoptotic BCL-2 members. In stark contrast, APAF-1 and caspase-9 do not act as tumor suppressors in MYC-induced lymphomagenesis, probably because BAX/BAK-dependent, caspase-independent cell death still proceeds in the absence of the apoptosome (39, 40).

Overexpression of BCL-2 can also occur secondary to the loss of an inhibitory microRNA, as reported following *miR*-15/16 deletion in chronic lymphocytic leukemia (CLL) (41). The genomic regions containing BCL-X_L and MCL-1 undergo somatic amplification in 10–15% of human cancers (42). Cancer cells containing amplifications of *MCL1* and *BCL2L1* (the gene encoding BCL-X_L) often depend on the expression of these genes for survival, supporting their roles as antiapoptotic oncogenes. Growth factors and oncogenic kinases can inhibit apoptosis by either upregulating/stabilizing the antiapoptotic BCL-2 members or downregulating/degrading the proapoptotic BH3s, as discussed in detail below. Additionally, microRNA-mediated suppression of BIM has been reported in multiple tumor types. Inhibition through the *miR*-17-92 cluster decreases levels of *BIM* transcripts (43) and is associated with lymphomagenesis based on patient data (44). Another microRNA, *miR*-25, has been shown to decrease BIM expression through blocking its translation in human gastric adenocarcinoma (45). Epigenetic silencing of BIM and PUMA has been reported in Burkitt lymphoma, and gene deletions at the BIM locus have been shown in mantle cell lymphoma (46).

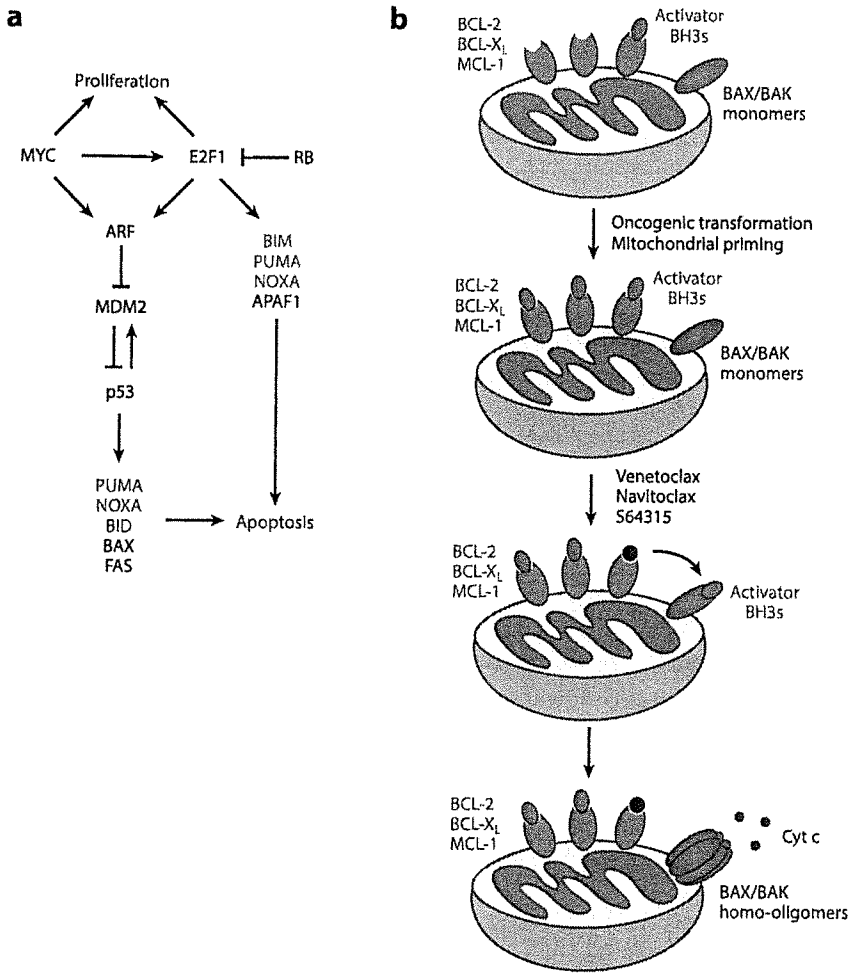

Figure 2

Oncogenic transformation induces mitochondrial priming of cancer cells that are poised to undergo apoptosis in response to BH3 mimetics. (*a*) Oncogenes such as MYC and E2F1 not only induce genes required for cell cycle progression but also activate apoptosis, forming an intrinsic tumor suppressor checkpoint to limit the consequences of aberrant mitogenic signaling. MYC and E2F1 induce apoptosis through both p53-dependent and -independent pathways. These pathways converge on activator BH3-only molecules (BH3s), which in turn activate BAX/BAK-dependent mitochondrial apoptosis. Therefore, the inhibition of apoptosis is crucial for the successful oncogenic transformation and initiation of cancer. One common strategy employed by cancer cells to evade oncogene-induced apoptosis involves the upregulation of antiapoptotic BCL-2 members. (*b*) Oncogenic transformation induces activator BH3s that are sequestrated by antiapoptotic BCL-2, BCL-X_L, and MCL-1 as inert complexes. Consequently, many cancer cells are primed to undergo apoptosis upon the administration of BAD and NOXA mimetics. Navitoclax, a BAD mimetic, can displace activator BH3s from BCL-2/BCL-X_L to activate BAX/BAK indirectly. Venetoclax and S64315 can perform a similar function on BCL-2 and MCL-1, respectively.

p53 was the first tumor suppressor gene identified to be linked to apoptosis. Mutations in *TP53* (the gene encoding p53) occur in approximately 50% of human tumors and are frequently associated with advanced tumor stages and poor patient prognosis (47). This transcription factor is activated in response to various cellular stresses, including oncogene activation, DNA damage,

hypoxia, and telomere malfunction (47). DNA damage triggers p53 activation through the phosphorylation of its amino-terminal region by ATM, ATR, CHK1, or CHK2, disrupting the binding of p53 to MDM2. Acetylation is another modification that can influence the transcriptional activities of p53 (48). Notably, *TP53* mutations typically occur in later stages of tumorigenesis, suggesting that the loss of p53 may contribute to disease progression (25). This occurrence might be linked to the selective advantage of cells acquiring *TP53* mutations under conditions such as hypoxia or significant telomere erosion. p53 is known to suppress oncogenesis through various mechanisms, not solely by activating apoptosis but also by inducing cell cycle arrest, senescence, DNA repair, and ferroptosis (49–51).

TARGETING THE BCL-2 FAMILY FOR CANCER THERAPY

Oncogenic transformation induces activator BH3s that are sequestrated by antiapoptotic BCL-2, BCL-X_L, and MCL-1 as inert complexes. Consequently, many cancer cells are inherently primed to undergo apoptosis upon the administration of BAD and NOXA mimetics. These two BH3 mimetics displace BID/BIM/PUMA from BCL-2/BCL-X_L and MCL-1, respectively, thereby activating the apoptotic gateway BAX/BAK (**Figure 2*b***). Cells with highly primed mitochondria are also more sensitive to chemotherapy that can induce BH3s through p53. Since most normal cells lack excessive BID/BIM/PUMA readily complexed with antiapoptotic BCL-2 members, they are expected to be less susceptible to BAD or NOXA mimetics and chemotherapy. Notable exceptions include cells of the hematopoietic system and intestine, which exhibit a higher degree of mitochondrial priming than other tissues. The elevated mitochondrial priming in cancer cells compared with most normal cells helps explain the therapeutic index for conventional chemotherapy in most cancers. However, when cancer cells are subjected to chemotherapy, there is a selection for reduced sensitivity to apoptosis, which likely contributes significantly to the pan-resistant phenotype for different chemotherapies observed in many relapsed tumors. BH3 profiling was developed as a method to assess mitochondrial priming by utilizing synthetic BH3 domain peptides from individual BH3 proteins to trigger MOMP (52).

The development of BH3 mimetics for cancer therapy has proven to be successful, marking a significant achievement as the first group of drugs effectively targeting a protein–protein interaction (46). This success reached a clinical milestone in 2016 with the FDA approval of the BCL-2 inhibitor venetoclax (ABT-199) for CLL with chromosome 17p deletion (53). Several other molecules are currently in clinical trials, and their progress, alongside that of venetoclax, is discussed in this subsection. Additionally, there is ongoing development of chemical activators targeting the proapoptotic BAX. Among them, BTSA1 induces BAX activation by binding to an allosteric site in the N-terminus and has demonstrated preclinical efficacy in acute myelocytic leukemia xenografts (54). Another agent, CYD-2-11, targets a binding pocket near the S184 phosphorylation site and has shown effectiveness as a single-agent therapy in xenograft models of small cell lung cancer and non–small cell lung cancer (NSCLC) (55). However, none of the BAX activators have entered clinical trials at this point.

BH3 Mimetics Targeting BCL-2 and BCL-X_L

Rigorous structure-based screening efforts culminated in the development of the first on-target specific BH3 mimetic targeting the BCL-2 family, ABT-737 (56). Both ABT-737 and its orally bioavailable analog, ABT-263 (navitoclax), function as BAD mimetics, binding and inhibiting BCL-2, BCL-X_L, and BCL-W but not MCL-1 or BCL2A1/BFL-1 (56–60). Despite navitoclax demonstrating promising clinical activity, it led to dose-dependent rapid thrombocytopenia as an on-target effect of BCL-X_L inhibition (61, 62). This prompted the development of ABT-199

(venetoclax), a platelet-sparing, selective BCL-2 inhibitor (63). Venetoclax has exhibited remarkable therapeutic efficacy in relapsed/refractory CLL with an overall response rate of 79% (53). Consequently, it received FDA approval for treating CLL patients with 17p deletion, and, later, approval was extended to include all CLL and small lymphocytic lymphoma as monotherapy or in combination with anti-CD20 treatment following a phase III trial (64). Additionally, the first-line use of venetoclax in combination with hypomethylating agents was approved for acute myeloid leukemia (AML), on the basis of two phase III trials (65, 66). Venetoclax is currently undergoing further evaluation in trials, both as monotherapy and in combination with other agents, for a diverse range of hematologic malignancies and solid tumors.

While navitoclax continues to undergo clinical trials for various hematologic and solid cancers, it has not yet received FDA approval for any malignancy. In addition to inducing thrombocytopenia, another therapeutic limitation of navitoclax is its inability to disrupt the BCL-X_L/BAK interaction (67). Navitoclax is likely to be ineffective in treating BCL-X_L-addicted cancers with low expression of activator BH3s such as BIM. Additionally, it may face challenges in cancers where there is an overabundance of BCL-X_L. The success of venetoclax may be attributed to the inability of BCL-2 to sequester BH3-exposed BAK (13). Notably, navitoclax has been identified as a senolytic agent that rejuvenates aged hematopoietic stem cells in mice (68). BH3-mimetics specific for BCL-X_L have also been developed using structure-based drug design (SBDD), including WEHI-539 and its analogs A-1155463 and A-1331852 (69–72). Among these, A-1331852 stands out as the most potent and orally bioavailable inhibitor of BCL-X_L (72) and has been utilized in antibody-drug conjugates including ABBV-155 (mirzotamab clezutoclax) and ABBV-637 (73).

In a recent study, a BCL-X_L proteolysis-targeting chimera (PROTAC) degrader, DT2216, was engineered to promote BCL-X_L degradation by targeting it to the von Hippel–Lindau (VHL) E3 ubiquitin ligase (74). DT2216 demonstrated efficacy in a xenograft model of T cell acute lymphocytic leukemia but induced less thrombocytopenia compared with navitoclax, which was attributed to low VHL expression in platelets. A phase I dose-escalation trial has recently been concluded in relapsed/refractory malignancies, with results pending. Of note, the FDA has granted fast-track designation to DT2216 for the treatment of adult patients with relapsed or refractory peripheral T cell lymphoma and cutaneous T cell lymphoma.

Mechanisms of Resistance to Venetoclax

As is typical with many targeted therapies, acquired resistance to venetoclax often emerges, and various mechanisms contributing to this resistance have been extensively discussed in recent reviews (75, 76). In CLL, genetic resistance may arise through mutations in the BCL-2 family, namely *BCL2* and *BAX*. A notable *BCL2* mutation, G101V, has been observed with varying frequencies in multiple CLL cohorts treated with venetoclax (77, 78), resulting in a 180-fold reduction in the binding affinity of venetoclax to BCL-2 (79). Additional mutations in *BCL2* in venetoclax-resistant patients have also been described, putatively affecting drug affinity (80, 81). Loss of *BCL2* amplicons has also been found to decrease therapeutic efficacy in CLL (82).

While mutations in the BCL-2 family have not been directly linked to decreased venetoclax efficacy in AML, mutations in other genes commonly implicated in cancer, including *TP53*, *KRAS*, and *FLT3*, are associated with both primary and adaptive resistance (83, 84). Unsurprisingly, the upregulation of MCL-1, BCL-X_L, and BCL2A1 stands out as one of the most recurrent determinants of venetoclax resistance in AML (85, 86). The upregulation of BCL-X_L and MCL-1 can also be secondary to mutations in various genes, including *KRAS*, *PTPN11*, and components of the SWI-SNF complex (85, 87). Furthermore, global transcriptional remodeling has been identified as another avenue for developing resistance to venetoclax, likely due to elevated MCL-1 levels (82).

The tumor microenvironment (TME) is recognized as a key factor influencing the response of CLL to venetoclax. Notably, CLL cells obtained from peripheral blood exhibit susceptibility to apoptosis induction, whereas those from the bone marrow display resistance (88). Coculturing peripheral blood CLL cells with bone marrow–derived stromal cells is sufficient to decrease susceptibility to apoptosis, underscoring the significant role played by the TME in cell death responses. The specific mechanisms underlying TME-mediated resistance are still being elucidated, but some pathways have been identified. For instance, the activation of CD40 signaling has been shown to induce venetoclax resistance in vitro through JAK-STAT signaling (89, 90). Notably, the combination of venetoclax with an anti-CD20 antibody was found to overcome this resistance effectively (89).

Therapeutic Targeting of MCL-1

The development of MCL-1 inhibitors has presented significant challenges, primarily attributed to the shallow and less flexible BH3-binding groove of MCL-1 (46). The first specific MCL-1 inhibitor exhibiting robust intracellular activity and preclinical efficacy in multiple myeloma xenografts, S63485, was discovered in 2016 through a nuclear magnetic resonance–based drug fragment screen, followed by SBDD (91). Subsequently, several MCL-1 inhibitors have progressed to early-phase clinical trials for hematologic malignancies. Notable examples include the S63485 analog S64315 (also known as MIK 665), ABBV-467, AZD5991, AMG-176, and PRT1419 (92). As of now, ongoing trials have not resulted in FDA approval for any of these agents. Notably, cardiotoxicity emerged as an on-target side effect (93), leading to the termination of the phase I, dose-escalation study of ABBV-467 and AMG-397 (92). Strategies to mitigate or minimize this side effect will be crucial for enabling widespread clinical use. It is noteworthy that most, if not all, of these MCL-1 inhibitors stabilize the MCL-1 protein, whereas NOXA mediates MCL-1 degradation. This raises the question of whether current MCL-1 inhibitors are authentic NOXA mimetics.

In addition to specific inhibitors for MCL-1, several agents have been identified that reduce MCL-1 activity indirectly. These agents include inhibitors of global transcription such as anthracyclines and CDK9 inhibitors as well as protein translation inhibitors such as puromycin and anisomycin (67, 94). As both MCL-1 transcripts and protein typically have short half-lives, blocking transcription or translation quickly depletes cellular MCL-1. Given their inhibitory effects on MCL-1, multiple CDK9 inhibitors are currently in clinical trials in combination with other cancer therapies for solid and hematologic malignancies. These include flavopiridol (alvocidib) and its oral analog TP-1287, AZD4573, and voruciclib.

Predicting Cancer Dependence on Antiapoptotic BCL-2 Proteins

In the era of precision medicine, it is paramount to identify and select patients who will respond to each specific inhibitor of individual antiapoptotic BCL-2 proteins. In general, most cancers can be divided into BCL-2-addicted cancers, BCL-X_L-addicted cancers, MCL-1-addicted cancers, or cancers that are nonaddicted to any single antiapoptotic BCL-2 member. Although cancers can also be addicted to BCL-W and BCL2A1, they are less common than those addicted to BCL-2/BCL-X_L/MCL-1 due to their restricted expressions. For cancers dependent on a single antiapoptotic BCL-2 member for survival, genetic or pharmacological inhibition of that specific member is usually sufficient to induce apoptosis. However, an exception arises with BCL-X_L-addicted cancers. While they undergo apoptosis upon genetic inaction, pharmacological inhibition of BCL-X_L may not induce apoptosis if such cancers express little BID/BIM/PUMA but highly express BCL-X_L, as described above (67).

BH3 profiling was initially established to evaluate apoptotic sensitivity or mitochondrial priming of cancer cells by measuring whether BH3 domain peptides induce MOMP. As some BH3 peptides display preferential binding to different antiapoptotic BCL-2 members, this technique has also been employed to determine the selective dependence of cancers on antiapoptotic BCL-2 proteins. Given the current availability of clinical-grade, specific inhibitors of BCL-2, BCL-X_L, and MCL-1, the usage of BH3 peptides in BH3 profiling will probably be supplanted by BH3 mimetics. The clinical application of BH3 profiling in guiding the selection of BH3 mimetics for patients faces logistic limitations. For example, the accessibility to patient-derived tumor cells, crucial for effective profiling, poses a greater challenge in solid tumors compared with hematological malignancies.

It has been reported that cancer addiction to antiapoptotic BCL-2 family proteins can be predicted on the basis of the relative protein expression ratio, rather than mRNA expression, among BCL-2, BCL-X_L, and MCL-1 (67). Accordingly, evaluating the expressions of antiapoptotic BCL-2 family proteins in patient tumors through quantitative proteomics or quantitative immunohistochemistry could serve as an alternative means of guiding the selection of BH3 mimetics tailored to each patient. This strategy could be further enhanced by leveraging computational systems biology modeling, as demonstrated successfully in predicting responses to BH3 mimetics in diffuse large B cell lymphoma (95).

APOPTOSIS AND TARGETED CANCER THERAPY

The majority of cancer treatments exert their antitumor effects through activation of apoptosis. This review focuses largely on the mechanisms by which targeted therapy and immunotherapy induce apoptosis. It is noteworthy that conventional chemotherapy and radiation therapy also induce cancer cell apoptosis, and their specific mechanisms are thoroughly discussed elsewhere (96, 97).

Oncogenic Kinase Inhibitors and the BCL-2 Family

The identification of individual oncogenes and the concurrent development of targeted therapies tailored to specific driver mutations have transformed cancer therapy, establishing the groundwork for precision cancer medicine (98). It is now evident that the initiation of apoptosis plays a pivotal role in the success of targeted cancer therapy (99). The majority of currently approved or clinically trialed targeted therapies act as inhibitors of kinase signaling cascades that regulate the BCL-2 family proteins to affect apoptosis (**Figure 3*a***). Activating mutations of *EGFR* and amplification of *HER2* trigger the activation of the RAS-RAF-MEK-ERK and the PI3K-AKT-mTOR signaling cascades. Phosphorylation of BIM by ERK at serine 69 targets BIM for βTrCP-mediated ubiquitination and subsequent proteasomal degradation (100–102). AKT-mediated phosphorylation of FOXO1/3 prevents its nuclear translocation and transactivation of PUMA (102, 103). Therefore, both BIM and PUMA have been identified as key apoptotic effectors in response to the administration of EGFR and HER2 inhibitors in *EGFR*-mutant lung cancer and *HER2*-amplified breast cancer, respectively (99, 102, 103). Moreover, targeted therapies leading to ERK inhibition consistently elevate BIM protein levels, while those inducing AKT inhibition typically result in increased PUMA levels (99, 102, 103). The role of BIM and/or PUMA as apoptotic effectors of targeted therapeutics has also been reported in chronic myelogenous leukemia with *BCR-ABL* mutations, *KRAS*-mutant and *ALK*-positive NSCLC, *BRAF*-mutant melanoma and colorectal cancers, and *PIK3CA*-mutant breast cancers (99). FOXO-mediated regulation of BIM transcription has also been observed in neurons and hematopoietic cells.

Oncogenic kinases also play a crucial role in upregulating antiapoptotic MCL-1 to suppress apoptosis (**Figure 3*a***). ERK, for instance, has been reported to phosphorylate and thereby stabilize

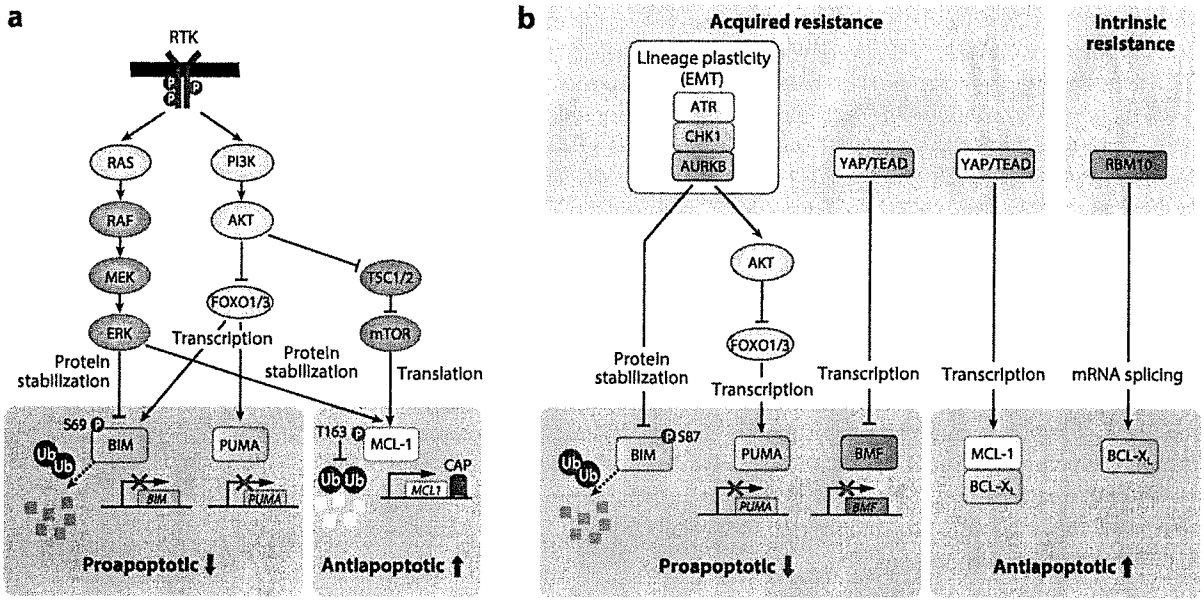

Figure 3

Molecular pathways interconnecting the oncogenic kinase signaling cascades and the BCL-2 family proteins and their implications in the therapeutic efficacy and resistance to targeted therapies. (*a*) Cross talk between the oncogenic kinase signaling cascades and the BCL-2 family proteins. Oncogenic drivers, such as *EGFR* mutations and *HER2* amplifications, activate the RAS-RAF-MEK-ERK and PI3K-AKT-mTOR signaling cascades. ERK phosphorylates BIM at S69, targeting BIM for ubiquitination and degradation. AKT-mediated phosphorylation of FOXO1/3 prevents its nuclear translocation and transactivation of PUMA and sometimes BIM. ERK can also stabilize MCL-1 through T163 phosphorylation, while mTOR enhances cap-dependent translation of MCL-1. Overall, activation of receptor tyrosine kinase (RTK) signaling suppresses apoptosis through downregulation of proapoptotic BIM and PUMA and upregulation of antiapoptotic MCL-1. Conversely, targeted therapies leading to ERK inhibition consistently elevate BIM protein levels, while those inhibiting AKT typically result in increased PUMA levels, leading to apoptosis induction. (*b*) Resistance to targeted therapies caused by alterations of the BCL-2 family proteins. Acquired resistance to EGFR tyrosine kinase inhibitors (TKIs) caused by epithelial–mesenchymal transition (EMT) activates the ATR-CHK1-AURKB signaling cascade, in which AURKB phosphorylates BIM at S87, targeting BIM for ubiquitination and degradation. Additionally, AURKB can activate AKT, suppressing FOXO1/3-mediated transactivation of PUMA. YAP/TEAD-mediated transcriptional reprogramming confers acquired resistance to EGFR or ALK TKIs by suppressing proapoptotic BMF expression or inducing the expression of antiapoptotic MCL-1 and BCL-X_L. Inactivating mutations of RBM10 decrease the ratio of proapoptotic *BCL-X*$_S$ to antiapoptotic *BCL-X*$_L$ isoforms of *BCL-X* mRNA, thus conferring resistance to EGFR TKIs.

MCL-1 (104), while mTOR enhances cap-dependent translation of MCL-1 (105). Consequently, the suppression of PI3K–mTORC1 signaling leads to a reduction in MCL-1 protein levels (99). Conversely, the reactivation of PI3K–mTORC1 signaling during EGFR tyrosine kinase inhibitor (TKI) treatment promotes MCL-1 upregulation, contributing to acquired resistance in *EGFR*-mutant NSCLC (106). In summary, there exists a crucial interconnection between the receptor tyrosine kinase (RTK) signaling pathways and the BCL-2 family proteins, governing apoptotic responses to targeted therapies across various cancers.

The BCL-2 Family and Resistance to Targeted Therapies

While targeted therapies often yield higher rates of initial responses compared with conventional chemotherapies, the emergence of acquired resistance is nearly unavoidable. Recent advancements in clinical sequencing have shed light on the genetic resistance mechanisms to targeted therapies, particularly in *EGFR*-mutant NSCLC patients. Notably, approximately half of the patients treated

with first-generation EGFR TKIs, such as gefitinib and erlotinib, develop the secondary *EGFR* mutation T790M. Studies conducted previously have shown that acquired resistance to EGFR TKIs arises from the selection of preexisting resistant clones and the evolution of drug-tolerant persisters (DTPs). These DTPs, employing adaptive mechanisms, manage to survive treatment, enabling cancer cells to evade apoptosis and persist (107, 108). Over time, DTPs can develop resistance through both mutational and nonmutational mechanisms.

Currently, the standard-of-care treatment for *EGFR*-mutant NSCLC patients is the third-generation EGFR TKI, osimertinib, targeting T790M. However, most patients still develop resistance to osimertinib, and the mechanisms behind this resistance exhibit greater heterogeneity compared with those associated with first-generation EGFR TKIs. In the majority of EGFR-mutant NSCLC patients, resistance manifests following a marked initial response to EGFR TKIs, leading to a stable minimal residual disease and the subsequent development of drug-resistant tumors. This recurring pattern suggests that EGFR TKIs may fall short in eradicating all tumor cells. Therefore, adopting upfront combination therapies aimed at enhancing apoptosis could potentially eradicate cancer cells and mitigate the emergence of drug resistance. It is noteworthy that sublethal stress can induce MOMP in some but not all mitochondria, an effect called minority MOMP. Minority MOMP leads to limited caspase activation, which is insufficient to trigger cell death but can cleave ICAD [inhibitor of caspase-activated DNase (CAD)] to activate CAD, resulting in DNA damage and genome instability (109).

It has been reported that tumors evolving from DTPs through nonmutational resistance mechanisms exhibit a diminished apoptotic response to osimertinib (**Figure 3*b***). Notably, the BCL-2/BCL-X_L inhibitor navitoclax was found to restore sensitivity to EGFR TKIs both in vitro and in vivo (108). Two recent studies have identified a synthetic lethal interaction between EGFR TKIs and aurora kinase inhibitors in *EGFR*-mutant lung cancer (102, 110). Shah et al. (110) demonstrated that aurora kinase A (AURKA) activation confers resistance to EGFR TKIs and that the AURKA inhibitor MLN8237 enhances osimertinib-induced apoptosis through BIM upregulation. On the other hand, Tanaka et al. (102) identified aurora kinase B (AURKB) inhibitors as potent enhancers of osimertinib-induced apoptosis through high-throughput drug screening. BIM is found to be phosphorylated by AURKB at serine 87, mediating its binding to βTrCP1 and degradation via $SCF^{\beta TrCP}$. Consequently, genetic and chemical inhibition of AURKB reduces BIM S87 phosphorylation, leading to BIM stabilization. Additionally, AURKB inhibition enhances osimertinib-mediated inhibition of AKT and thereby FOXO1/3 phosphorylation, resulting in FOXO1/3-mediated induction of PUMA. Interestingly, osimertinib resistance caused by epithelial–mesenchymal transition activates the ATR-CHK1-AURKB signaling cascade, resulting in heightened sensitivity to corresponding kinase inhibitors through the activation of BIM-mediated mitotic catastrophe (102).

In addition to mechanisms involving BIM and PUMA, Kurppa et al. (111) demonstrated that DTP cells, upon osimertinib treatment, undergo YAP/TEAD-mediated transcriptional reprogramming to evade apoptosis by suppressing proapoptotic BMF. YAP/TEAD-mediated reprogramming also promotes evasion of apoptosis by enhancing BCL-X_L and MCL-1 in *ALK*-translocated lung cancer treated with ALK inhibitors (112). More recently, the modulation of mRNA splicing has been found to impact the expression of different isoforms of BCL-2 family proteins, thereby altering the apoptotic response to targeted therapies. For instance, RBM10 was reported to modulate the alternative splicing of *BCL-X* mRNA (113). Genetic inactivation of *RBM10* reduced EGFR TKI-mediated apoptosis by decreasing the ratio of proapoptotic *BCL-X_S* to antiapoptotic *BCL-X_L* isoforms of *BCL-X* mRNA. Indeed, inactivating mutations of *RBM10* co-occur with *EGFR* mutations in a subset of NSCLC patients and serve as a biomarker of poor response to EGFR TKIs in the clinic. Collectively, these findings have important implications

for the development of upfront treatment strategies aimed at preventing resistance by enhancing apoptosis rather than intercepting acquired resistance mechanisms to targeted therapies (**Figure 3*b***).

APOPTOSIS AND CANCER IMMUNOTHERAPY

Immunotherapeutic agents have revolutionized cancer treatment over the past decade, gaining FDA approvals for treatment of a broad spectrum of solid and hematologic malignancies, with some already established as first-line therapies. Notably, the immunotherapeutic agents that have demonstrated the greatest clinical success are those enhancing the activation of cytotoxic lymphocytes or utilizing cytotoxic lymphocytes to induce apoptosis in cancer cells. This section delves into the mechanisms through which cytotoxic lymphocytes effectively eliminate cancer cells.

Three major categories of immunotherapeutic agents are currently undergoing clinical trials. The most extensively researched and utilized class of immunotherapy is immune checkpoint blockade (ICB) antibodies, particularly those targeting PD-1/PD-L1 and CTLA-4 (114). Since 2011, ICBs targeting all three checkpoint molecules have received numerous FDA approvals in diverse cancer types, both as stand-alone treatments and in combination with targeted therapeutics or chemotherapy (114). Another category comprises bispecific T cell engagers (BiTEs) (115), engineered by combining a T cell–targeting domain with a tumor antigen-specific domain. BiTEs facilitate tumor cell destruction by modulating T cell activity in close proximity to their target. Blinatumomab, one such BiTE, received FDA approval for B cell acute lymphoblastic leukemia in 2018, while others are in clinical trials. The final major class is chimeric antigen receptor (CAR) T cells (116). While the former groups stimulate the activity of endogenous lymphocytes, CAR T cells are engineered ex vivo and then transfused into cancer patients. CD19-specific CAR T cells received FDA approval for acute lymphocytic leukemia in 2017, and ongoing trials are investigating both CD19-specific CAR T cells and CARs targeting various antigens in hematologic and solid malignancies.

Contemporary immunotherapies focus on modulating cytotoxic lymphocyte activation to eradicate cancer cells, leveraging mechanisms established years prior to the introduction of these novel agents. A pivotal aspect of lymphocyte-mediated target cell elimination involves the induction of apoptosis. Cytotoxic lymphocytes employ two major mechanisms for inducing apoptosis. The first mechanism involves the secretion of prodeath ligands, such as FasL (117), which can activate the extrinsic pathway through death receptors on target cells. The second mechanism operates through the release of cytotoxic granzymes from lymphocytes directly into a target cell via perforin pores. The molecular mechanisms of perforin pore formation are described in depth in another review (118). Notably, the cytotoxic effects of granzymes are perforin-dependent. Granzymes cleave numerous intracellular targets, with granzyme B being the most potent apoptotic effector. Granzyme B induces apoptosis in human target cells by cleaving BID (119) and activating the intrinsic pathway. Interestingly, murine granzyme B is believed to induce programmed cell death through the direct cleavage of apoptotic caspases (120), revealing an intriguing divergence in effector function to achieve the same fate for the target cell.

Despite the seemingly straightforward mechanisms of cytotoxic lymphocyte–mediated target cell killing, a single contact between a cytotoxic lymphocyte and a cancer cell in the tumor immune microenvironment, is typically insufficient to induce apoptosis. Instead, most contacts result in sublethal damage, which can accumulate to lethal levels through subsequent interactions over time. For instance, Halle et al. (121) observed that the likelihood of target cell death after a single cytotoxic cell contact was approximately 15%; this probability increased to more than 80% after three or more contacts. Using different experimental models, Weigelin et al. (122) also

demonstrated that apoptotic events often followed multiple cytotoxic T cell contacts. Many additional factors also modulate efficacy, including the presence of tumor neoantigens (123) and various properties of the tumor immune microenvironment, which are detailed elsewhere (124), and broadly regulate the quantity and activation of cytotoxic lymphocytes within the tumor.

Single-agent immunotherapy often proves insufficient to induce significant or lasting cytotoxic lymphocyte–mediated responses. Therefore, numerous regimens combining immunotherapy with other established cancer therapeutics have been assessed in past and ongoing trials, yielding improved clinical responses (114). Several recent preclinical studies have indicated that combining immunotherapy with the BCL-2 family inhibitors is a promising therapeutic strategy. One such study demonstrated that venetoclax improved CAR T therapy in a lymphoma model (125), consistent with the notion that tumor resistance to apoptosis contributes to resistance to CAR T therapy (126). To mitigate the toxicity of venetoclax in CAR T cells, venetoclax-resistant CAR T cells were generated by overexpressing the F104L mutant of BCL-2, which fails to bind venetoclax. This modified CAR T therapy synergized with venetoclax in multiple lymphoma xenograft models. Moreover, higher levels of BCL-2 in the T cells from apheresis products are associated with improved clinical results of CART19. Another study showed that genetic or pharmacological inhibition of BCL-2 using venetoclax activated dendritic cells (DCs) to enhance antigen presentation, which in turn led to improved tumor control by adoptively transferred DCs that further synergized with PD-1 blockade (127). Notably, venetoclax also activated human DCs. In addition, pharmacological inhibition of BCL-2 using venetoclax or MCL-1 using S63845 is shown to augment NK cell–based immunotherapy if tumor cells are dependent on BCL-2 or MCL-1 for survival (128). In summary, the BCL-2 family plays an important role in both tumor cells and immune cells, impacting the outcome of cancer immunotherapy.

APOPTOSIS AND INFLAMMATION

Traditionally, apoptosis has been regarded as immunologically silent. As billions of cells undergo apoptosis daily as part of physiological turnover (129), logically, this form of cell death must remain largely noninflammatory to avoid activation of significant autoimmunity. However, studies over the past two decades have highlighted numerous mechanisms and signaling pathways by which agents that induce apoptosis also trigger inflammation and even immunological memory. As the myriad interactions between cancer cells and the immune system are further elucidated, understanding how apoptotic pathways mediate immune responses becomes increasingly crucial.

Mechanisms by Which Apoptosis Limits Inflammation

Apoptotic caspases play a crucial role in mitigating potentially inflammatory signals from dying cells (129). They promote apoptotic cell clearance by recruiting phagocytes such as macrophages and dendritic cells, in part through the cleavage of the cell membrane–bound pannexin-1 channel (130). The proteolysis of pannexin-1 releases ATP and UTP, which serve as find-me signals to attract phagocytes (**Figure 4*a***). Effector caspases have also been shown to cleave and mature additional potential find-me signals, including lysophosphatidylcholine and sphingosine-1-phosphate; however, their ability to recruit phagocytes has been demonstrated only in vitro (129, 131). Future studies will likely elucidate their role in vivo and may identify novel prophagocytic signals induced by caspase activation.

Caspases have also been shown to modulate the release of and attenuate inflammation from damage-associated molecular patterns (DAMPs), including HMGB1 and IL-33 (129) (**Figure 4*b***). Furthermore, a recent study employed metabolomics to identify additional molecules released by caspase-cleaved pannexin-1 channels, including spermidine, GMP, and IMP (132) (**Figure 4*c***).

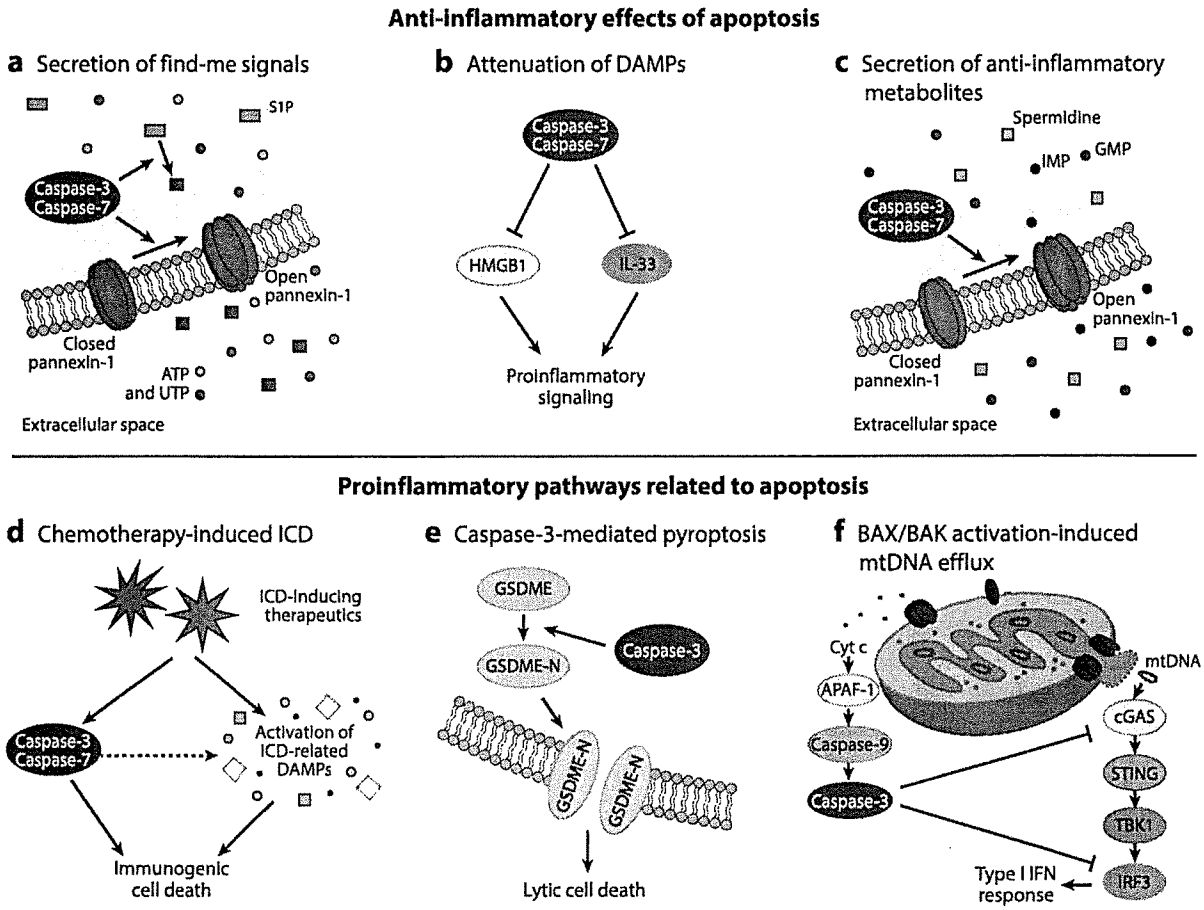

Figure 4

Anti-inflammatory effects of apoptotic caspase and proinflammatory pathways related to apoptosis. (*a–c*) Apoptotic caspases attenuate the inflammatory outcomes of apoptosis through a variety of mechanisms. (*a*) Caspases promote dying cell phagocytosis through find-me signals, including ATP and UTP, helping eliminate potential sources of inflammation quickly. They also cleave other potential find-me signals such as sphingosine-1-phosphate (S1P). (*b*) Caspase activation suppresses inflammatory signaling by regulating the release of damage-associated molecular patterns (DAMPs), including HMGB1 and IL-33. (*c*) Apoptotic caspases contribute to the release of anti-inflammatory metabolites, including spermidine, GMP, and IMP, which improve inflammation in a murine model of arthritis. (*d–f*) Several pathways related to apoptosis have proinflammatory or immunogenic effects. (*d*) Anticancer therapeutic agents, including certain chemotherapies, radiation, and some targeted therapies, are known to induce immunogenic cell death (ICD) by generating immunogenic DAMPs. (*e*) Caspase-3 can trigger pyroptosis, a lytic form of cell death, through the cleavage of gasdermin E (GSDME). The N-terminal fragment of cleaved GSDME (GSDME-N) can oligomerize, leading to the formation of pores in the plasma membrane. Caspase-3-mediated pyroptosis has been shown to suppress solid tumor growth. (*f*) After cytochrome c (cyt c) efflux, BAX/BAK coalesces to form macropores, through which the mitochondrial inner membrane (MIM) herniates. This results in the loss of MIM integrity and exposure of mitochondrial DNA (mtDNA) to the cytosol. In the presence of caspases, transient mtDNA efflux occurs near cell death without activating the type I interferon (IFN) response due to caspase-mediated cleavage of cGAS and IRF3. However, in the absence of caspases, sustained mtDNA release triggers the cGAS/STING pathway and type I IFN response.

These compounds were found to dampen inflammation in a murine model of arthritis, providing further evidence that caspase-mediated signaling facilitates the resolution of potentially inflammatory stimuli. The immunostimulatory and immunoinhibitory roles of phagocytes and other myeloid cells within the tumor immune microenvironment are described elsewhere (133) and remain under active investigation.

Caspase-3 has been reported to cleave cGAS and IRF3, thereby attenuating the type I interferon (IFN) response triggered by mitochondrial DNA (mtDNA) efflux to the cytosol during apoptosis (**Figure 4*f***) (134). Additionally, caspase-3 can cleave MAVS, along with IRF3 cleavage, leading to restrained IFN production caused by RNA virus (134).

Immunogenic Cell Death in Cancer Therapy

Recent research, starting in the mid-2000s, has challenged the notion that apoptosis is nonimmunogenic. Certain apoptosis-inducing chemotherapeutic agents, including doxorubicin, oxaliplatin, and paclitaxel, have been shown to stimulate immunological memory. Any form of cell death that triggers an adaptive immune response leading to memory generation is classified as immunogenic cell death (ICD) (135). A set of DAMPs has since been identified (135) that characterizes ICD downstream of cytotoxic agents (see **Figure 4*d***) and may aid in predicting the immunogenicity of a particular form of cell death. These markers include the exposure of calreticulin and other ER chaperones on the plasma membrane in the context of the integrated stress response, HMGB1 release, IFN-β-mediated induction of CXCL10, secretion of ATP, and release of annexin A1 (ANXA1). The specific roles of each marker in ICD are extensively covered in other reviews (135). ICD provides robust immunostimulatory signals to DCs for their maturation and functional licensing, enabling mature DCs to cross-prime antigen-specific T lymphocytes.

Apart from chemotherapy, radiation therapy (and certain targeted therapeutics such as cabozantinib, cetuximab, and crizotinib) can also trigger ICD. Therapeutic agents capable of inducing immunological memory are promising candidates for combination use with current immunotherapies. Indeed, induction therapy with ICD-inducing chemotherapeutics has been shown to enhance the efficacy of anti-PD-L1 or anti-PD-1 therapy in patients with metastatic triple-negative breast cancer (136, 137).

Apoptotic Caspases as Inducers of Pyroptosis

Recent studies have identified several scenarios by which activation of pathways that typically result in noninflammatory apoptosis can instead lead to significant inflammation. One such mechanism involves pyroptosis, a form of inflammatory PCD initially characterized as a host-defense mechanism within innate immune cells. In pyroptosis, microbial stimuli induce the formation of multiprotein inflammasome complexes (138). Inflammatory caspases, including caspase-1, -4, -5, and -11, then dimerize and activate within these complexes. Once active, inflammatory caspases cleave gasdermin D (GSDMD), allowing its N-terminal domain to homo-oligomerize and form pores in the plasma membrane. This results in plasma membrane permeabilization, inflammatory cytokine release, cell swelling, and lytic cell death (139–141).

Over the past few years, however, studies have shown that apoptotic caspases can also induce this inflammatory form of PCD. In macrophages, caspase-8 was found to cleave GSDMD to trigger pyroptosis downstream of *Yersinia*-mediated inhibition of TAK1 or IκB kinase (142, 143). As many tissue types express GSDMD (144), whether other stimuli can trigger caspase-8-mediated pyroptosis and the potential applications of this form of inflammatory PCD in the field of cancer will likely be assessed in future studies.

An additional mechanism of pyroptosis was reported in 2017, where gasdermin E (GSDME) was cleaved by caspase-3, freeing its N-terminal fragment to oligomerize and permeabilize the plasma membrane (145) (**Figure 4*e***). Notably, the study found that agents traditionally associated with apoptosis induction, including chemotherapeutics, can activate pyroptosis if GSDME is expressed and cleaved. Thus, cleavage of GSDME by effector caspases may control the switch between noninflammatory apoptosis to inflammatory pyroptosis. Interestingly, overexpression of

GSDME was shown to suppress tumor growth in immunocompetent but not immune-deficient mice, and this tumor suppression was associated with increased intratumoral cytotoxic lymphocyte activation (146). Furthermore, granzymes B and M were reported to cleave GSDME in target tumor cells, suggesting that cleavage of GSDME by granzymes may convert granzyme-mediated, noninflammatory killing of tumor cells to inflammatory cell death that further enhances immune elimination of tumor cells.

Given its immune-mediated tumor suppression activity, it is not surprising that GSDME is frequently mutated or downregulated through promoter methylation across various cancer types, including breast, gastric, and colorectal cancers (146, 147). As discussed in prior sections, many cancer therapies trigger apoptotic caspase-mediated cell death, making GSDME-mediated pyroptosis a potential avenue to augment immune responses to therapy. While a recent study showed that GSDME expression suppressed tumor growth in a manner that requires cytotoxic lymphocytes (146), it did not assess whether GSDME-mediated pyroptosis was a form of ICD capable of inducing immunological memory formation. Further, targeting this form of inflammatory PCD to augment cancer therapy will likely require expressing or reexpressing functional GSDME in tumors, potentially through demethylating agents.

BAX/BAK-Dependent, Caspase-Independent Cell Death

Inducing intrinsic apoptosis in the absence of apoptosome components or in the presence of caspase inhibitors leads to caspase-independent cell death. In this form, cells die through mitochondrial dysfunction at a slower rate than during the caspase-dependent counterpart (40). While the precise mechanism by which these cells die remains uncertain, it may be due to electron transport chain dysfunction, which can lead to both impaired cellular respiration and impaired cell division secondary to the depletion of aspartate in the pyrimidine synthesis pathway. As loss of mitochondrial function inhibits a wide range of pathways important for survival, the exact manner of death may also be specific to the individual cell lineage. Notably, cell death of interdigital webs in mice can occur through a BAX/BAK-dependent, caspase-independent manner, while displaying morphological features of necrosis rather than apoptosis (148). Given that apoptosis is defined by the characteristic ultrastructural features that are dependent on caspases, this form of cell death is not considered apoptosis.

Despite ongoing investigations into the exact mechanism of such type of cell death, the robust cell-intrinsic inflammation induced by BAX/BAK-dependent, caspase-independent cell death has been firmly established. Two studies published side by side in 2014 unveiled a robust type I IFN response in the absence of apoptotic caspases, one conducted in a model of viral infection (149) and the other in hematopoietic stem cells (150). Both studies showed that IFN-β induction in their respective models relied on mtDNA-mediated activation of the cGAS-STING pathway. As previously mentioned, IFN-β serves as a hallmark of ICD (135). Moreover, independent studies have underscored the immunostimulatory roles of mtDNA and cGAS/STING activation (151–153). Therefore, activation of BAX/BAK in the absence of caspases produces robust cell-intrinsic inflammation. Nonetheless, it remains unknown whether this type of cell type can induce immunological memory.

The mechanism by which mtDNA was released from the mitochondrial matrix was elucidated using advanced microscopy techniques in 2018 (154). These imaging studies revealed that cytochrome c exits from BAX/BAK pores that are too small to be resolved by super-resolution microscopy. After cytochrome c efflux, BAX/BAK coalesces to form macropores in the MOM, through which the mitochondrial inner membrane (MIM) herniates and then loses membrane integrity, resulting in mtDNA efflux. mtDNA can then be exposed to cytosolic DNA sensors such

as cGAS (**Figure 4*f***). Intriguingly, only a minority of herniated MIM appears to lose membrane integrity, and most TFAM-bound mtDNA remains inside the mitochondria during such processes (154). Of note, apoptotic caspases do not prevent mtDNA efflux but attenuate the inflammatory response to mtDNA through cleavage of cGAS and IRF3 (134).

As many anticancer agents trigger intrinsic apoptosis, activation of mtDNA-mediated inflammation may be a promising therapeutic strategy to augment the immune response to cancer treatment. Notably, *APAF1* is frequently silenced by methylation in melanoma, and various mechanisms inactivating the apoptosome have been reported in different cancer types (155), suggesting that some tumors may be prone to mtDNA-mediated inflammation following BAX/BAK activation. It has been reported that combining radiation therapy with the pan-caspase inhibitor emricasan sensitizes colorectal cancer to anti-PD-L1 blockade through mtDNA-cGAS-STING signaling in mice (156). Future studies will continue to elucidate the immune response to BAX/BAK-dependent, caspase-independent cell death and explore its potential to enhance cancer therapy.

CONCLUDING REMARKS

Over the past five decades, significant strides have been made in comprehending the molecular mechanisms underlying apoptosis and their implications in oncogenesis and cancer treatment. The elucidation of how apoptosis operates at a molecular level has not only enhanced our insight into how cancer cells evade apoptosis during tumorigenesis but also catalyzed the development of BH3 mimetics, a pioneering class of drugs targeting protein–protein interactions. The FDA's approval of venetoclax, the first BH3 mimetic, to treat CLL in 2016 and its subsequent indications in diverse malignancies, alongside the ongoing clinical exploration of other BH3 mimetics, underscore the importance of decades-long research efforts in unraveling the molecular underpinnings of apoptosis.

The past 25 years have witnessed significant therapeutic advancements in cancer treatment, marked by the emergence of small-molecule targeted therapy followed closely by the inauguration of the immunotherapy era. Both targeted therapy and immunotherapy, alongside conventional chemotherapy and radiation therapy, exert their antitumor effects by triggering apoptosis. Recent research has revealed that apoptotic pathways can elicit either anti- or proinflammatory responses in a context-dependent manner. As we delve deeper into the impact of tumor–immune interactions on therapeutic outcomes, deciphering the influence of cancer cell death, particularly apoptosis, on the tumor immune microenvironment becomes paramount. Moreover, as combinations of targeted therapy or chemotherapy with immunotherapy progress through clinical trials, it becomes imperative to grasp the effects of these treatments on various immune cell populations, along with the accompanying proapoptotic and inflammatory consequences.

In the coming decades, we anticipate additional clinical approvals for various agents that trigger apoptosis, including those directly targeting this process. With the ever-expanding array of therapeutic options, simultaneous advancements in biomarker identification and personalized medicine technologies are invaluable for customizing the optimal treatment regimen for each individual patient. As research and clinical trials in these areas progress, not only will our understanding of apoptosis's role in cancer deepen but also patient outcomes will continue to improve as we advance toward effective treatments for all types of cancer.

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