Restoring the switch for cancer cell death: Targeting the apoptosis signaling pathway

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Purpose. The relevance of apoptosis to cancer development and pharmacologic agents that target this pathway in selected malignancies are described.

Summary. Apoptosis is a tightly regulated biological process mediated by both proapoptotic (i.e., prodeath) and antiapoptotic (i.e., prosurvival) proteins. While apoptosis represents a well-established effector mechanism induced by conventional chemotherapy in many malignancies, the development of apoptosis-based targeted therapy is relatively new. The pharmacologic restoration of apoptotic functions, either by blocking the action of antiapoptotic proteins/regulators (e.g., through investigational therapies such as inhibitors of apoptosis proteins, SMAC [second mitochondria-derived activator of caspases] mimetics, MDM2 [murine double minute 2] antagonists) or by inducing apoptosis (e.g., through investigational agonistic monoclonal antibodies or fusion proteins), holds robust potential for cancer pharmacotherapy. Notably, BH domain 3 (BH3) mimetics, a new class of small molecules that block the action antiapoptotic proteins, are touted a success for apoptosis-based targeted therapy. Venetoclax, a synthetic peptide that belongs to this class of BH3 mimetics, is currently approved by the Food and Drug Administration for the treatment of relapsed/refractory chronic lymphocytic leukemia in patients with 17p deletion as a single agent. This agent has been increasingly used either alone or as part of combination therapy for diverse hematologic malignancies in clinical trials.

Conclusion. Advances in the understanding of molecular mechanisms of apoptosis have given rise to more-refined targeted therapies for diverse malignancies, with the goal to improve survival outcome while sparing treatment-related toxicities.

Keywords: apoptosis, BH3 mimetics, IAP, MDM2 inhibitors, SMAC inhibitors, venetoclax

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First coined in 1878 and then borrowed by Kerr et al.[1] in 1972, the term *apoptosis* describes the active process that mediates the removal of aged, damaged, and unnecessary cells by evolutionarily conserved and genetically controlled (i.e., programmed) biochemical pathways.[2] It is an important mechanism for maintaining homeostasis in multicellular organisms and ensuring the survival of long-lived cells and proper turnover of short-lived cells (e.g., progenitor blood cells).[3] This highly regulated process is necessary, because too much cell death can contribute to degenerative diseases and too little may cause autoimmune diseases and malignancies.[4] Impaired apoptosis has been implicated in the pathogenesis of autoimmune diseases and malignancies.[5] There is now an increased understanding about the role of apoptosis in tumorigenesis and the emerging role of targeted therapies in the apoptosis signaling pathway.

This article describes the current understanding of apoptosis and its

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relevance to cancer development. Pharmacologic agents targeting the apoptotic pathways and their therapeutic potential in selected malignancies are also described.

Principles of targeting apoptotic pathways for cancer therapy

Apoptosis is a tightly regulated biological process mediated by both proapoptotic (i.e., prodeath) and antiapoptotic (i.e., prosurvival) proteins. Many components in the apoptotic pathways play important roles in innate and adaptive immunities, reflecting the notion that altruistic cell death may be an effective defense mechanism against pathogens in multicellular organisms. The hematopoietic system is very sensitive to dysregulation in the apoptosis pathway due to the high turnover of these cells. An inability to respond to apoptotic stimuli leads to the accumulation of defective cells and even tumorigenesis. Conversely, overexpression of antiapoptotic proteins in cancer cells leads to a shift in the balance between antiapoptotic and proapoptotic proteins, resulting in the inhibition of the normal apoptotic pathway.[6] Mechanistically, apoptosis-based targeted therapy for malignancies aims to (1) inhibit cell death inhibitors or antiapoptotic pathways that are upregulated in tumors and (2) stimulate the apoptotic pathway or its components to induce tumor cell death.[7]

Molecular mechanisms of apoptosis

Figure 1 provides an overview of the signaling pathways that mediate apoptosis. Morphological changes during apoptosis can be observed microscopically when the nuclear DNA fragments and the nucleus and cytoplasm condense (pyknosis) and break the cell into smaller membrane-bound structures (i.e., "apoptotic bodies"), which are subsequently engulfed by neighboring cells and degraded by lysosomal enzymes.[3,4] The actual molecular events behind these morphological changes are far more complex. Apoptosis is precipitated by the death or stress signals (e.g., genomic instability, metastasis, oncogene activation) that result in the sequential activation of a family of intracellular proteases or zymogens, collectively known as caspases (due to the presence of **c**ysteine in the active site of the enzyme which breaks down the substrate at **asp**artic acid residue), in 2 distinct pathways—the extrinsic and intrinsic pathways.[8]

KEY POINTS

- Apoptosis is a form of regulated cell death characterized by the activation of caspases; impaired apoptosis may lead to malignancy.
- Overexpression of the antiapoptotic regulators (e.g., some proteins of the BCL-2 family) may contribute to the development of malignancy.
- Promising targeted therapies for apoptosis include those that block the prosurvival regulators or induce or restore the prodeath pathway.

The human genome encodes 11 caspases, which are stored intracellularly as inactive zymogens to prevent untimely cell death. Once active, initiator or apical caspases (e.g., caspase-8, caspase-9) can process and activate downstream effector or executioner caspases (e.g., caspase-3, caspase-6, caspase-7), which in turn cleave several hundred proteins. This amplification of events is made possible by the presence of long prodomains in initiator caspases (i.e., procaspases) that allow the recruitment of other scaffold or adapter proteins; the physical proximity of these caspases results in conformational changes of these caspases, promoting their own activation and locking these caspases in the active state.[9] Although this "proximity-induced dimerization" model seems to be operative in both the extrinsic and intrinsic pathways of apoptosis, as described below, its validity remains to be established experimentally.

Extrinsic pathway

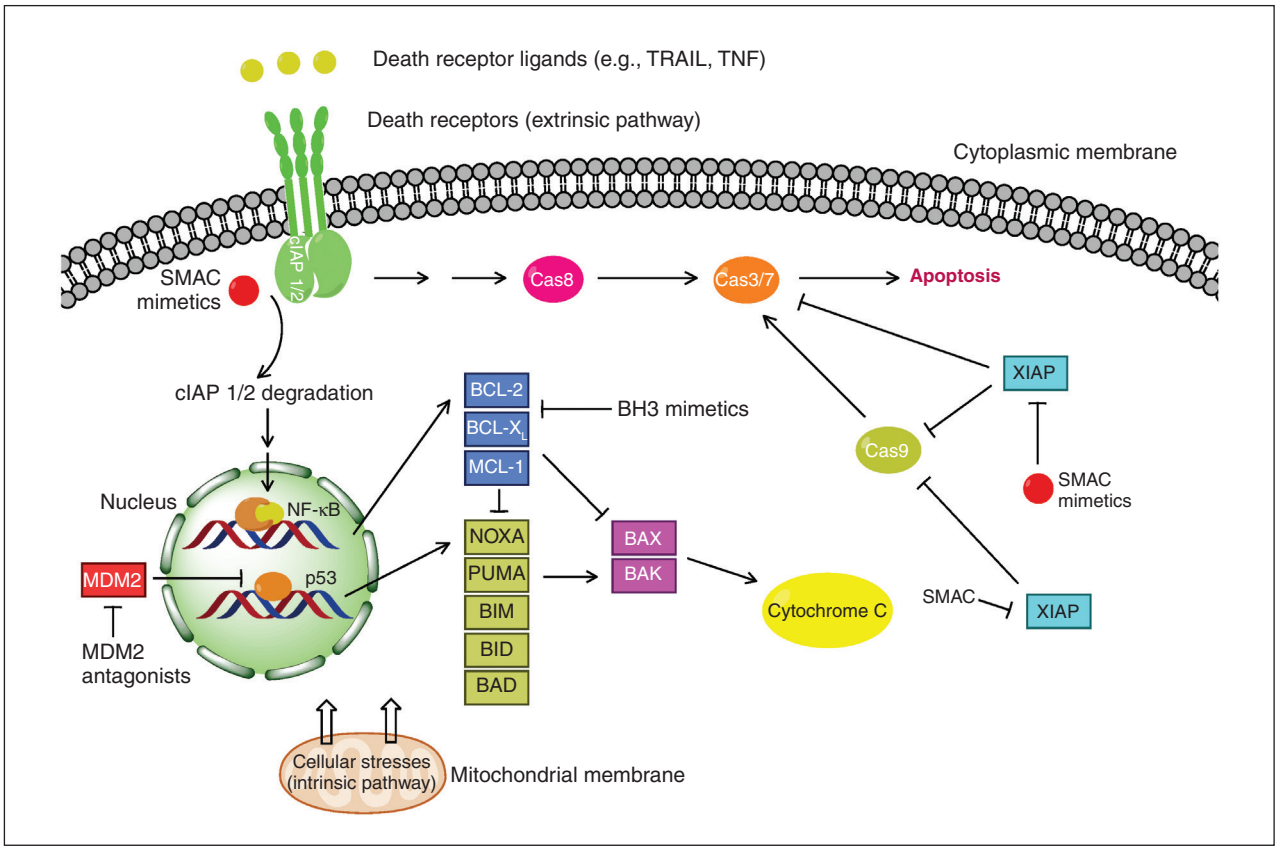
Binding of an extracellular death ligand to its cognate transmembrane death receptor induces receptor-mediated apoptosis. Death ligands are typically glycoproteins that belong to the tumor necrosis factor (TNF) family, which includes TNF-α, TNF-related apoptosis-inducing ligand (TRAIL), and the Fas ligand (a ligand that belongs to an effector molecule of the cytotoxic T lymphocytes).[9,10]

TNF-α, 1 of the first characterized members of the TNF family, is known for its transient expression in immune cells.[10] There are 2 main receptors for TNF-α: TNF receptor 1, which contains an intracellular death domain capable of activating the extrinsic pathway through caspases, and TNF receptor 2, which lacks the death domain but can induce cell proliferation through the nuclear factor κB (NF-κB) activation.[11]

The TRAIL (or APO2L) ligand transduces the signals intracellularly through different death receptors: DR4 (TRAILR-1), DR5 (TRAILR-2), DcR1 (TRAILR-3), and DcR2 (TRAILR-4).[12] The death receptors DR4 (TRAILR-1) and DR5 (TRAILR-2) possess highly conserved motifs of death domain responsible for transducing death signals. Other receptors such as DcR1 and DcR2 are decoy receptors that bind ligands but lack a functional death domain and therefore are unable to transduce apoptotic signals.[12]

The Fas receptor/Fas ligand system has been postulated to play an important role in lymphocyte homeostasis. Causative mutations have been identified in the *FAS* human gene (CD95) in patients with autoimmune lymphoproliferative syndrome, also known as Canale-Smith syndrome.[13] Many hematologic malignancies that express the Fas ligand are also susceptible to Fas ligand-induced apopto-

Figure 1. Simplified schematic illustration of sites of putative targeted therapies in the extrinsic and intrinsic pathways for apoptosis. The extrinsic pathway is initiated at the cell membrane when there is engagement of death receptors with their cognate ligands (e.g., TRAIL, TNF). The intrinsic pathway can be activated by diverse stimuli such as growth factor deprivation, chemotherapy agents that lead to increased cellular stresses, loss of integrity of the mitochondrial outer membrane and release of cytochrome c and SMAC into the cytosol. Members of the BCL-2 are anti-apoptotic and counteract the activity of BAX and BAK. BH3-only proteins (and BH3 mimetics) are pro-apoptotic. They stimulate apoptosis either by directly interacting with BAX or BAK or by binding to the BCL-2 protein. BCL-2 = B-cell lymphoma 2, BH3 mimetics = BCL-2 homology domain 3 mimetics, IAP = inhibitor of apoptosis protein, MDM2 = murine double-minute 2, SMAC = second mitochondrial-derived activator of caspase, XIAP = X-linked inhibitor of apoptosis protein.



sis.[14] However, the significance of the Fas expression and its tumor biology remains controversial.

The extrinsic pathway is initiated when a death ligand (e.g., TRAIL, TNF-α) is bound to its death receptor (DR4/DR5 or TNF receptor 1, respectively), resulting in the formation of a ligand–receptor complex known as DISC (death-inducing signaling complex). Within the complex, an adapter molecule known as FADD (Fas-associated death domain protein) is recruited, followed by the activation of the initiator caspase-8, which in turn activates caspase-3 and caspase-7, leading to propagation of the caspase cascade that culminates in cell death.[8]

In addition, caspase-8 cleaves other intracellular substrates such as BID (a proapoptotic protein). Truncated BID then translocates back to the mitochondrial membrane to stimulate cytochrome C release to initiate the intrinsic pathway. Thus, diverse molecular connections exist between different apoptotic pathways.

Therapies targeting the extrinsic pathway

TNF-α is a pleiotropic cytokine with antitumor effects in vitro and in vivo.[15] Severe and even fatal inflammatory responses have been documented during the early use of TNF-α as systemic therapy.[16] It possesses a wide range of biological activities, such as the regulation of inflammatory responses, cell proliferation, cell differentiation, and apoptosis.[15] It is also a potent inducer in other signaling pathways. Study shows that the binding of TNF to its principal receptor, TNF receptor 1, can trigger at least 3 different signaling pathways.[17] These pathways involve overlapping and distinct protein complexes, underpinning their biological functions

in both host defense and immune regulation.[18] Increased expression of decoy receptors is associated with the resistance to TRAIL. In certain lymphomas and leukemias, the expression of TRAIL and death receptors can be induced by proteasome inhibitors or other agents.[19] Thus, combination therapy that acts on different parts of the apoptotic pathways is a promising option for the treatment of these malignancies.

Furthermore, despite the concern about proinflammatory cytokines and hepatotoxicity associated with the use of TNF agonists in early studies, the extrinsic pathway has 2 key features that make it an attractive therapeutic target for cancer treatment: (1) other than the on-target toxicities, there is minimal off-target toxicity and (2) the pathway can induce apoptosis regardless of p53 mutation status.[20] Currently, targeted therapies for the extrinsic pathway refer to the development of agents that can (1) exert their antitumor effect through death receptor-mediated (e.g., TNF-α) apoptosis, (2) stimulate death receptors by agonistic monoclonal antibodies (e.g., mapatumumab, tigatuzumab, lexatumumab), and (3) use fusion proteins that possess death ligands or are part of the death receptor domain to induce apoptosis.[21-25] Clinical trials of these investigational agents demonstrated acceptable tolerance and modest efficacy. However, no death receptor agonist has advanced to Phase III clinical trials or has received regulatory approval. Studies using some agonistic monoclonal antibodies in combination with other agents did not show improved clinical benefit.[26,27]

Intrinsic pathway

Developmentally, the intrinsic pathway (also known as the mitochondrial pathway) is of more ancestral origin than the extrinsic pathway. Diverse cytotoxic stimuli and intracellular damage engage the intrinsic pathway and converge to the mitochondrial outer membrane for activation of the common pathway.[4]

BCL-2 (B-cell lymphoma) family proteins (24 in humans) are the central regulators of the intrinsic pathway. They all contain conserved sequence motifs, referred to as the BCL-2 homology (BH) domains, and can be categorized into 3 major groups. The first group comprises the proapoptotic BH3-only proteins (e.g., BAD, BIM, NOXA), so named due to the presence of only 1 BH domain (BH domain 3 [BH3]). BH3-only proteins act as molecular sentinels that link the environmental stimuli to the intrinsic pathway when activated. The second group is composed of the proapoptotic multidomain proteins (e.g., BAX, BAK), known as effectors. Each BAX or BAK molecule contains 3 BH domains (BH1, BH2, and BH3). Once activated, these molecules disrupt the mitochondrial outer membrane, leading to the release of cytochrome C, downstream caspase activation, and eventual cell death. The third group of BCL-2 proteins are the antiapoptotic proteins (e.g., BCL-2, BCL-X_L, MCL-1). Each of these proteins contains 4 BH domains and serves to protect cells by sequestering their proapoptotic counterparts.[28,29] The key to inducing apoptosis is an elevated level of BH3-only proteins (e.g., activation of BID, BAD, and BIM) or inactivation of 1 of its antiapoptotic BCL-2 proteins.

BCL-2 is a transmembrane protein and an important member of the BCL-2 family proteins. Its overexpression in cells of hematopoietic lineage gives rise to excessive expansion of cells in the lymphoid and myeloid lineages that are refractory to diverse cytotoxic stimuli. Together with other prosurvival genes (e.g., *BCL-X_L*), *BCL-2* and the genes that encode these proteins are known as oncogenes. Conversely, members of the proapoptotic subfamilies (e.g., BAX, BAK) are known as tumor suppressors.[4]

Therapies targeting the intrinsic signaling pathway

The role of BCL-2 in the survival of lymphoid malignancies is well established.[30] BCL-X_L is another antiapoptotic protein associated with drug resistance and disease progression in hematologic malignancies.[31] Particularly, chronic lymphocytic leukemia (CLL) is a clonal mature B-cell malignancy characterized by high levels of BCL-2 expression.[32] It is associated with the loss of microRNAs (i.e., noncoding RNAs that regulate gene expression) that are implicated in apoptosis.[33] Researchers have found that BIM, a BH3-only proapoptotic protein, is sequestered by BCL-2.[34] These observations led to the exploration of BCL-2 inhibition as a potential therapeutic strategy.

BH3 mimetics

BH3 mimetics are a new class of small molecules that antagonize the activity of BCL-2 prosurvival proteins (e.g., BCL-2, BCL-X_L, BCL-w) in tumor cells by mimicking the action of the proapoptotic BH3-only proteins. Molecules that mimic the BH3 motif of BH3-only proteins, known as BH3 mimetics, bind to BCL-2 and displace these prosurvival proteins, which then induces BAX and BAK oligomerization (formation of dimers) in the mitochondrial membrane, thereby resulting in apoptosis of the tumor cells.[35,36]

The first oral BH3 mimetic, navitoclax, which binds and inhibits BCL-2 and BCL-X_L, proved to be active against relapsed CLL, with partial responses observed in approximately 35% of patients.[37] However, in clinical trials, navitoclax was associated with dose-limiting thrombocytopenia because it induces platelet death due to the BCL-X_L inhibition.[38] Consequently, venetoclax (Venclexta, AbbVie and Genentech), a novel BCL-2 inhibitor that is 200 times less active in targeting BCL-X_L, was developed to reduce the on-target platelet toxicity.[38]

Venetoclax

Based on an overall response rate of 79% and an equivalent response rate in patients with 17p deletion (which is associated with resistance to conventional chemotherapy) and those without this chromosomal ab-

normality, venetoclax gained Food and Drug Administration (FDA) approval for the treatment of relapsed/refractory CLL in patients with 17p deletion in 2016.[39] Furthermore, there was no difference in response rate based on patient age or number of previous treatments. The median progression-free survival time was 25 months (range, 17–30 months) in the dose-escalation group (n = 56). Recently, based on the results of another Phase Ib trial, venetoclax in combination with rituximab gained breakthrough designation status by FDA for the treatment of relapsed/refractory CLL.[40] In patients whose disease progressed after therapy with ibrutinib and idelalisib, the median time that patients were on venetoclax was 31.9 weeks (range, 0.6–52.9 weeks) and 23.7 weeks (range, 5.4–52.9 weeks), respectively.[41]

Of note, in the dose-escalation group, tumor lysis syndrome (TLS) was noted in 10 (18%) of 56 patients, resulting in 2 fatal events. Subsequently, a risk-mitigation strategy was implemented in the expansion group, whereby patients were started on a venetoclax dose of 20 mg, which was then gradually increased. Intensive monitoring and TLS prophylaxis were introduced. The rate of laboratory-confirmed TLS decreased to 6% in 66 patients. The most common adverse effects (of any grade) associated with venetoclax were neutropenia, diarrhea, nausea, anemia, upper respiratory infection, thrombocytopenia, and fatigue. The risk of Richter transformation (i.e., malignant transformation of CLL into a more-aggressive lymphoma) was 10% (29 of 289) within the first 6 months of treatment, possibly reflecting the transformation rate of heavily pretreated patients or the transformation rate in patients who already had Richter transformation when treatment was initiated.[41]

Clinical trials of venetoclax, either alone or in combination with other drugs, are now underway for a diverse range of hematologic malignancies, with the goals of deepening response, minimizing drug resistance, and reducing overlapping toxicities. Of note, cancer cells often overexpress antiapoptotic BCL-2 family proteins, which sequester the apoptotic BIM and PUMA. Thus, they are "primed" to undergo apoptosis when high-affinity BH3 mimetics are present.[34,42] This rationale of priming may help explain the unique sensitivity of CLL cells to BH3 mimetics. Similarly, up to 80% of BAK cells in acute myeloid leukemia (AML) cells are oligomerized and bound to antiapoptotic BCL-2 family proteins in absence of exogenous death stimuli, indicating a high level of priming may correlate with sensitivity to BH3 mimetics.[43]

There are challenges that exist regarding the future application of BH3 mimetics. One challenge is that there is currently no biomarker that can predict the response to BH3 mimetics, which helps to individualize treatment options. Results of a recent study suggested that *HOX* gene expression may be implicated as a predictive biomarker for sensitivity to venetoclax in patients with AML, though its clinical validity has yet to be established.[44] In addition, researchers have theorized that BH3 profiling may be a predictive biomarker based on the observation that BCL-X_L/BAK complexes predict a patient's sensitivity to navitoclax, whereas MCL-1/BAK complexes predict a patient's sensitivity to another investigational agent, A1210477.[43] Further studies are warranted regarding BH3 profiling. Another challenge is that functional redundancies between BCL-2 family proteins may confer acquired resistance to another agent when 1 agent has already failed. Mutations of *BCL-2* or *BAX* have been linked to acquired resistance.[45]

MDM2 inhibitors

A key modulator of apoptosis is the tumor suppressor protein, p53. Inactivation of p53, either through *TP53* mutations or p53 modulators, such as MDM2 (murine double minute 2) protein, occurs in many malignancies. The defective p53 results in evasion of apoptosis by tumor cells, contributing to cancer development.[46,47] MDM2 is a negative regulator of p53 and functions as an oncogene. Therapeutic design of inhibitors targeting the MDM2 protein, or agents to induce the wild-type p53 expression and activity, may restore p53-mediated apoptosis of tumor cells.

Since the discovery of the first MDM2 inhibitor, nutlin-3a, many investigational agents that target MDM2 have been developed.[48] These inhibitors, also known as MDM2 mimetics, are small molecules that bind to MDM2 by mimicking key amino acid residues engaged by p53. RG7112, the most developed agent in this class of compounds, has been evaluated in a Phase I trial in patients with relapsed/refractory acute and chronic leukemias.[49] Off-target effects of MDM2 inhibitors generally include those of hematologic toxicities and interference with MDM2's role in DNA repair.[50] At present, the predictive biomarker associated with the selection of MTM2 mimetics remains to be elucidated, reflecting the notion that the p53 mutational status that is measured clinically may not be an accurate representation of functional p53 activity.[49]

Common pathway

Both extrinsic and intrinsic pathways converge in a common pathway that ultimately leads to apoptosis by inducing the executioner or effector caspases (i.e., caspase-3 and caspase-7). Mitochondria induce apoptosis by releasing cytochrome C from the intermembrane space into the cytosol, a critical molecular event in the intrinsic pathway that triggers the assembly of a multiprotein caspase-activating complex (i.e., apoptosome), which in turn activates caspase-3. Subsequent caspase activation results in widespread proteolysis and eventual cell death.[28]

Therapies targeting the common pathway

Inhibitors of apoptosis proteins. Because unscheduled activation of the apoptotic pathway could be deleterious for normal cells, mech-

anisms are in place to regulate apoptosis. For example, inhibition of the caspase activity and hence apoptosis can be achieved by a family of 8 proteins collectively known as the inhibitors of apoptosis proteins (IAPs). IAPs were first described in baculoviruses, where these proteins demonstrated inhibition of apoptosis of host cells after viral infection.[51] Several distinct mammalian IAPs including XIAP (x-linked IAP) and cellular IAPs (e.g., cIAP1, cIAP2) have since been identified.[52] The functional unit of each IAP is the baculoviral IAP repeat (BIR) domain. Most mammalian IAPs have more than 1 BIR domain, in which different BIR domains perform distinct functions. Collectively, IAPs function as endogenous inhibitors of caspases in both intrinsic and extrinsic pathways by keeping the apoptotic pathway in check.[52] During apoptosis, IAP-mediated inhibition of caspases is effectively antagonized by a family of proteins that share an IAP-binding tetrapeptide motif.[49] The founding member of this family of proteins is a mitochondrial protein known as second mitochondria-derived activator of caspases (SMAC).[53] SMAC is also known as direct IAP binding protein with low pI (DIABLO).[54] SMAC (or DIABLO) binds to the conserved IAP domains, thereby relieving the inhibition of caspases, which leads to the apoptotic cascade.[52] Of note, cells can also die by nonapoptotic routes, suggesting that mitochondria can control cell death pathways through caspase-dependent and caspase-independent pathways.[55]

Overexpression of IAPs has been observed in many solid and hematologic malignancies.[56] For example, in multiple myeloma, deletion of the *IAP1* gene resulted in activation of the noncanonical NF-κB pathway (a pathway responsible for adaptive immune responses whereas the classical/canonical pathway is responsible for acute inflammatory responses), which led to upregulated expression of antiapoptotic proteins, raising the possibility that IAPs could be promising targets for therapeutic interventions.[57] In addition, the finding that the IAP-binding motif of SMAC contains a conserved tetrapeptide sequence led to the design of synthetic peptides that mimic this tetrapeptide motif (so-called SMAC mimetics) as a mechanism to induce apoptosis in cancer cells.

SMAC mimetics. SMAC proteins form homodimers under physiological conditions. Bivalent SMAC mimetics (i.e., investigational agents such as BV6, birinapant) can be developed from 2 monomeric components linked together by a chemical bridge to enhance their binding to IAP proteins. Divalent SMAC mimetics generally possess a higher binding affinity, higher potency, and better anticancer activity than their monovalent counterparts (e.g., LCL161).[58,59] In addition, because the death receptor depends on the functional autocrine/paracrine signaling induced by SMAC mimetics, which are generally weak in their cytotoxic activity, combination therapy with SMAC mimetics and chemotherapy or radiation therapy can be used to augment the apoptotic responses of SMAC mimetics.[60]

Of interest, because the TNF ligand/receptor pathway is associated with SMAC mimetics in an NF-κB–dependent manner, a common adverse effect manifested by SMAC mimetics (except birinapant, which does not possess the classical NF-κB–mediated proinflammatory activity) is the cytokine release syndrome, due to the release of inflammatory cytokines such as TNF-α and interferon.[61] In fact, cytokine release syndrome was found to be the dose-limiting toxicity in the clinical trial with the SMAC mimetic LCL161.[61]

In clinical trials, SMAC mimetics have been used in combination with various signal transduction inhibitors. For example, histone deacetylase inhibitors have been shown to synergize with SMAC mimetics to trigger leukemic cell death in patients with AML.[62,63] In addition, hypomethylating agents such as azacitidine and decitabine added to SMAC mimetics could also sensitize leukemic blast cells in patients with AML.[64] At present, several SMAC mimetics are being evaluated in early clinical trials. None has received regulatory approval.

Discussion

Much of what we know about apoptosis primarily centers on the results of investigations on 3 models (*Caenorhabditis elegans* [a roundworm], *Drosophila,* and mammals).[20] Research performed in recent years has revealed that the apoptotic pathway achieves its tight control through a cell- or tissue-specific regulatory network of multiple protein–protein interactions. For instance, proapoptotic BH3-only proteins BIM and BID can bind and inhibit the function of all antiapoptotic BCL-2 family proteins, whereas BAD only binds and inhibits BCL-2, BCL-X_L, and BCL-w.[65] This type of regulation allows apoptosis to be fine-tuned by allowing the threshold to undergo apoptosis to vary in each type of tissue. A central point in understanding the role of apoptosis in cancer development is that the lack of death alone does not make the cancer cell "omnipotent"; rather, cancer cells must activate growth (i.e., proliferation) and invasion (i.e., metastasis) programs and/or other survival signals (e.g., epigenetic modulation), which are usually the targets of conventional cytotoxic chemotherapy and newer targeted therapies. Although conventional cytotoxic chemotherapy can indirectly induce apoptosis in cancer cells (due to stress stimuli), they may also disable the apoptosis pathway by making cancer cells intrinsically defective in initiating apoptosis, thus promoting resistance to conventional antineoplastic agents.[66] The pharmacologic restoration of apoptotic functions, either by blocking the action of antiapoptotic proteins/signals (e.g., IAP inhibitors, SMAC mimetics, BH3 mimetics, MDM2 antagonists) or by inducing apoptosis (e.g., agonistic monoclonal antibodies, fusion proteins), holds robust potential for use in cancer therapy.

At present, therapeutic strategies, especially the development of chemical mimetics of BH3-only proteins, which bypass the genetic abnormalities of a tumor (e.g., p53) and restore the BCL-2-regulated apoptotic switch, are avidly sought. With an increasing number of clinical trials juxtaposing venetoclax (and other novel BH3 mimetics) either as monotherapy or part of combination therapy with many different classes of therapeutic agents, there is ample opportunity to identify newer therapies for patients with cancer, as the targets of the apoptosis-signaling pathway may represent novel therapeutic strategies.

Conversely, manipulating apoptosis in cancer therapy is analogous to holding "a double-edged sword." While there may be many putative targets for drug therapy, the on- and/or off-target dose-limiting toxicities must also be considered, as our knowledge of this complex signaling pathway is constantly evolving. For instance, TRAIL, a member of the TNF family, had been studied for its role in apoptosis in some Phase I/II trials. Yet the concern for significant hepatotoxicity due to proinflammatory or stimulatory effects of the TNF pathway remains, underlying the rationale that a suitable apoptosis inducer must also be selective to spare normal tissues. TRAILR-2 and caspase-8 have been shown to be putative predictive biomarkers for response to the TRAIL mimetics due to their increased expressions in these extrinsic death pathways.[67] However, further clinical trials are warranted to validate this hypothesis.

Conclusion

Advances in the understanding of the molecular mechanisms of apoptosis have given rise to more-refined targeted therapies for diverse malignancies, with the goal of improving survival outcome while sparing treatment-related toxicities.

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