Chapter 8
Rational Design of Therapeutics Targeting the BCL-2 Family

Are Some Cancer Cells Primed for Death but Waiting for a Final Push?

Victoria Del Gaizo Moore and Anthony Letai*

Abstract A mechanism for circumventing apoptosis prevalent in many cancer cells is the overexpression of antiapoptotic BCL-2 family members. Upregulated expression of BCL-2 may be required to permit ongoing death signaling without a cellular response. Therefore, antagonizing BCL-2 function may cause death in many cancer cells. The selection for expression of BCL-2 or other antiapoptotic proteins during oncogenesis may derive from these proteins' ability to bind and sequester proapoptotic BH3-only proteins. This situation may be advantageous from a therapeutic viewpoint because cancer cells may be distinguished from normal cells by being primed with death signals. There are several strategies currently under investigation that may lead to improved treatment of many cancers by taking advantage of these differences.

Keywords apoptosis, BCL-2, BH3, therapeutics, peptide

1 The BCL-2 Family of Proteins

The BCL-2 family of proteins plays a critical role in controlling death via the intrinsic, or mitochondrial, programmed cell death pathway. BCL-2, the namesake of the family, was identified at the breakpoint of the t(14;18) translocation common to follicular lymphoma (1–3). More than 85% of follicular lymphomas contain a chromosomal translocation involving the fusion of the *bcl-2* gene at 18q21 to the immunoglobulin heavy chain locus on 14q32 (4). This translocation places the *BCL-2* gene under the control of the immunoglobulin heavy chain elements. Thus, overexpression of BCL-2 protein is driven in B-cells possessing the t(14;18). BCL-2 was credentialed as an oncogene when it was shown that overexpression was linked to the

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induction of lymphoma in mice (5, 6). Until the discovery of BCL-2, only oncogenes that increased cell proliferation, like *myc, ras*, and *src* had been described. BCL-2's discovery and characterization opened a new class of oncogenes: inhibitors of cell death. The last 20 years have seen the discovery of a family of proteins related to BCL-2 by structural homology and by participation in control over the mitochondrial apoptotic pathway.

BCL-2 proteins largely interact at the mitochondria, the nexus of events that irreversibly commit a cell to programmed cell death via the intrinsic pathway. Some BCL-2 proteins are localized to the mitochondria even during normal cellular conditions while many have other subcellular locations. For example, BAK resides as a monomer at the mitochondrial outer membrane as well as the endoplamic reticulum (7). Prior to activation, BAX exists as a monomer, either in the cytosol or loosely attached to the mitochondrial outer membrane. When activated, however, BAX undergoes alkali-stable insertion into the mitochondrial membrane. BCL-2 itself is found not only at mitochondria, but also at the endoplasmic reticulum where it is implicated in calcium homeostasis (8). Many BCL-2 family members have identified roles outside of control of apoptosis, and it is likely that BCL-2 family members are important in other aspects of cellular homeostasis. The extra-apoptotic functions of BCL-2 family members remain an area of active investigation (9–11).

BCL-2 family members can be divided into three broad groups: antiapoptotic, multidomain proapoptotic, and BH3-only proapoptotic proteins (Fig. 8.1). Antiapoptotic proteins include BCL-2, MCL-1, BCL-X_L, BCL-w, and BFL-1, all of which have the ability to oppose cell death. These antiapoptotic proteins possess sequence homology in four alpha-helical BCL-2 homology or BH regions. Multidomain proapoptotic proteins, including BAX and BAK, promote the progression of cell death and share homology in the BH1–3 regions. BH3-only proapoptotic proteins also promote cell death but, as their name implies, have only a BH3 domain in common. The BH3 domains contain an amphipathic α-helix that is necessary for the proapoptotic function of BH3-only proteins. However, this pro-death function requires interaction with multidomain BAX or BAK (12–14).

Upon cellular stress such as oncogene activation, uncontrolled proliferation, DNA damage, or growth factor withdrawal, BH3-only proteins become functionally upregulated via transcriptional or posttranslational means (15, 16). Proapoptotic BH3-only proteins may be further categorized as "activators" or "sensitizers" (17)

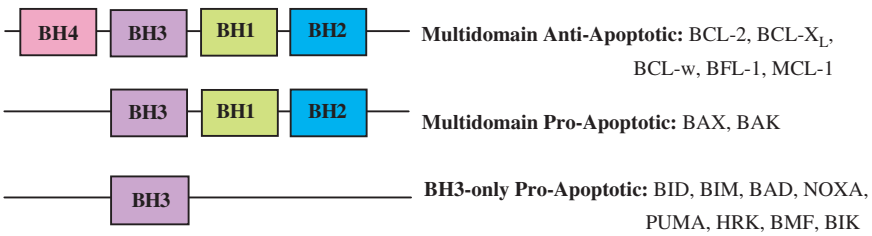

Fig. 8.1 Three classes of the BCL-2 family of proteins. BH3 domains are coded by color

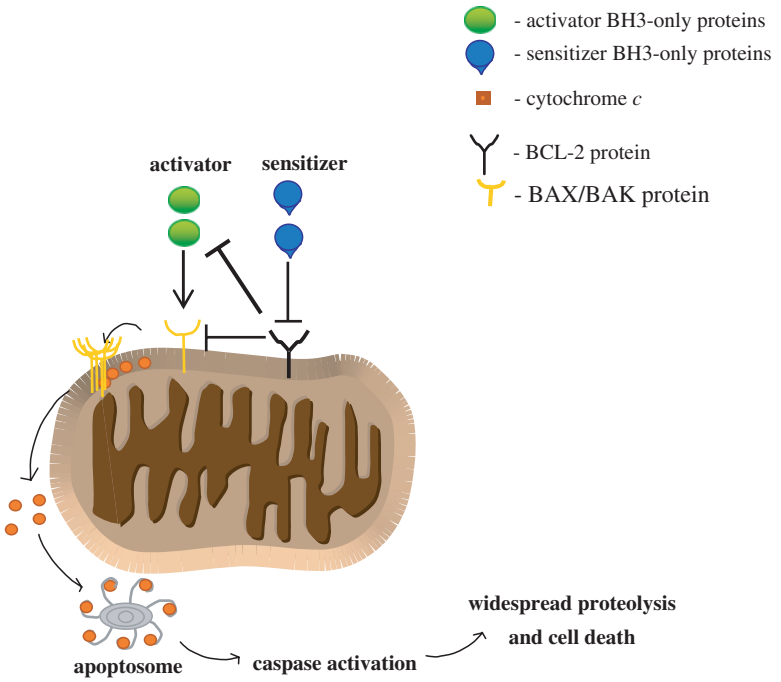

Fig. 8.2 BCL-2 family "activators" vs "sensitizer." BH3 domain-only activators, such as BID or BIM, interact with BAX or BAK to induce their activation, leading to MOMP, caspase activation, and apoptosis. BCL-2 may also bind and sequester BID or BIM, preventing activation of BAX or BAK. Sensitizers binding to BCL-2 may either block activators from binding or displace them from BCL-2

(Fig. 8.2). "Activator" BH3-only proteins, such as BID or BIM, interact with BAX or BAK, inducing an allosteric change. Subsequently, activated BAX or BAK can oligomerize. Oligomerized BAX or BAK, perhaps in complex with other proteins, induce mitochondria outer membrane permeablization (MOMP) (14, 18–22). Permeablization allows certain mitochondrial factors such as cytochrome *c*, Smac/Diablo, and AIF, to be released into the cytosol (23–28). Once in the cytosol, cytochrome *c* forms a holoenzyme complex with caspase-9 and APAF-1, called the apoptosome, which cleaves procaspase-3, into an active protease (29). Widespread proteolysis ensues, leading to cellular dysfunction and death. Consequently, MOMP can be considered the step at which commitment to cell death occcurs. Notably, there are recent studies that suggest that a key proapoptotic function of p53 is mediated by its ability to act as an activator (30–33).

While antiapoptotic proteins like BCL-2 and MCL-1 have been shown to directly interact with multidomain BAX and BAK, their interaction with BH3-only proteins may be more important to their antiapoptotic function (13, 34). The BH1–3 domains of BCL-2 form a hydrophobic cleft where the BH3 domain of multidomain and BH3-only proteins can bind. BCL-2 binding of BID or BIM causes

sequestration of these activator proteins, thereby preventing interaction and activation of BAX and BAK and thereby preventing MOMP (13, 17). Not all BH3-only proteins, however, are able to activate BAX or BAK. BH3-only proteins that do not activate BAX or BAK, including BAD, BIK, BMF, NOXA, and PUMA, we classify as "sensitizers" (17, 35). In contrast to activators that can activate BAX and BAK, these BH3 domains exert their proapoptotic function by binding to antiapoptotic BCL-2 proteins. In so doing, they compete with the binding of activators, either preventing activator binding, or displacing activators from BCL-2. In the presence of sensitizers, displaced activator BH3-only proteins are freed from antiapoptotic proteins to activate BAX and BAK and induce MOMP (17, 35, 36). While antiapoptotic proteins apparently share the common function of inhibiting apoptosis by sequestering activator BH3-only proteins, their binding pockets are nonetheless distinct. This is most clearly shown by the fact that each antiapoptotic protein has a distinct pattern of interaction with the range of sensitizer BH3 domains (35–37).

In addition to the intrinsic or mitochondrial pathway, apoptosis also can be initiated through the death receptor-mediated, or extrinsic, pathway. The extrinsic pathway is triggered when ligands, such as TNF, Fas ligand, or TRAIL, are bound by cell surface death receptors that cause changes in the intracellular domains of these receptors, resulting in assembly of a so-called death-inducing signaling complex (DISC) reviewed in (16). Activation of the initiator caspase-8 activation results, leading to activation of downstream effector caspases. In some systems, linkage to the intrinsic apoptotic pathway is accomplished by caspase-8 cleavage of the activator BH3-only protein BID, which can then trigger BAX or BAK oligomerization and MOMP (38, 39). Even though initiation of the intrinsic and extrinsic pathways is different, both converge at the activation of downstream effector caspase-3 and caspase-7.

2 The Link Between BCL-2 and Cancer

While elevated BCL-2 levels as a result of the t(14;18) translocation involving the *BCL-2* gene occurs in 80–90% of follicular non-Hodgkins lymphomas, aberrant expression of antiapoptotic expression has been implicated in many other cancers (4, 40, 41). 20–55% of diffuse large cell lymphomas have elevated BCL-2, either due to t(14;18) translocations, gene amplification, or other mechanisms, which may correlate with decreased patient survival (42–44). Many other cancers exhibit high levels of BCL-2 protein in the absence of a t(14;18); the mechanism of upregulated BCL-2 remains obscure in most of these instances. Examples include 70% of breast cancer (45, 46), 30–60% prostate cancer (47), and 90% of colorectal cancer cases (41, 48, 49). Chronic lymphoid leukemia (CLL) is largely considered a disease of failed apoptosis (50–52), but usually not due to t(14;18) (53). Nonetheless, the majority of CLL cells express high levels of BCL-2 (54). Recently, a more common chromosomal aberration, deletion, or translocation of 13q14.3, was implicated in

elevated BCL-2 in CLL (55). Changes affecting region 13q14.3 downregulated two microRNAs (miRNA) *mir-15A* and *mir-16-1*, and occurred in >50% of all CLL cases. miRNAs are a class of genes involved in tumorigenesis that produce short, single-stranded RNAs that bind to specific mRNA sequences and either prevent the translation of the mRNA or hasten degradation of the mRNA, thereby lowering the levels of the corresponding protein (56, 57). Expression of *mir-15A* and *mir-16-1* inversely correlates to BCL-2 expression in CLL samples and both negatively regulate BCL-2 levels (58, 59).

Expression of other antiapoptotic proteins has been detected in many cancers, including BFL-1 in diffuse large-cell lymphoma (60), MCL-1 in myeloma (61), and BCL-X_L in lung adenocarcinoma (62). Both BCL-2 and MCL-1 have been implicated as important contributors to melanoma development and maintenance (63–65). The oncogenic Epstein-Barr virus (EBV) and human herpes virus-8 (HHV-8; also known as Kaposi sarcoma herpes virus) encode BCL-2 homologs that oppose cell death from multiple stimuli, analogous to BCL-2 (66, 67). EBV has been implicated in the causation of HIV-related lymphoma, Burkitt lymphoma, nasopharyngeal carcinoma, and posttransplantation lymphomas, and HHV-8 in the causation of Kaposi sarcoma, Castleman disease, and body cavity lymphomas. The evolutionary selection for BCL-2 homologs in these viruses suggests that blocking the intrinsic pathway to programmed cell death is important in viral infection, and perhaps also for oncogenesis.

Multiple myeloma (MM) cells have been shown to express BCL-2, BCL-X_L, and MCL-1. Clinical and in vitro data suggest important roles for these proteins in MM cell survival as well as clinical resistance to therapy (68, 69). Despite the lack of chromosomal translocations, protein expression of each of these antiapoptotic proteins has been observed in clinical isolates (68–70). Antisense oligonucleotides (ASO) have been used with MM cells to determine if BCL-2, BCL-X_L. or MCL-1 expression is critical for the survival of these cancer cells, with mixed results (61, 71).

It has been hypothesized that oncogenesis requires an apoptotic defect (72, 73). One apparent strategy for apoptotic escape exploited by certain cancer cells is the overexpression of antiapoptotic BCL-2 family members. These proteins can bind and sequester activator BH3-only death signals likely initiated by cancer phenotypes including genomic instability, oncogene activation, and inappropriate cell contact. Therefore, in cancer cells that adopt such a strategy it seems likely that much of the antiapoptotic proteins will be "primed" with activator BH3-only proteins (Fig. 8.3). Primed cells are rendered exquisitely sensitive to mimetics of the sensitizer BH3 domains, which function as selective antagonists of BCL-2 and other antiapoptotic proteins (17, 35, 74) (Fig. 8.4). Certain, though probably not all, normal tissues may lack this priming, as they do not violate the rules of normal cellular behavior that provoke death signals in many cancer cells. Thus, the possibility exists of targeted intervention to exploit the therapeutic window between "primed" cancer cells and "unprimed" normal cells by antagonizing BCL-2 family antiapoptotic protein function.

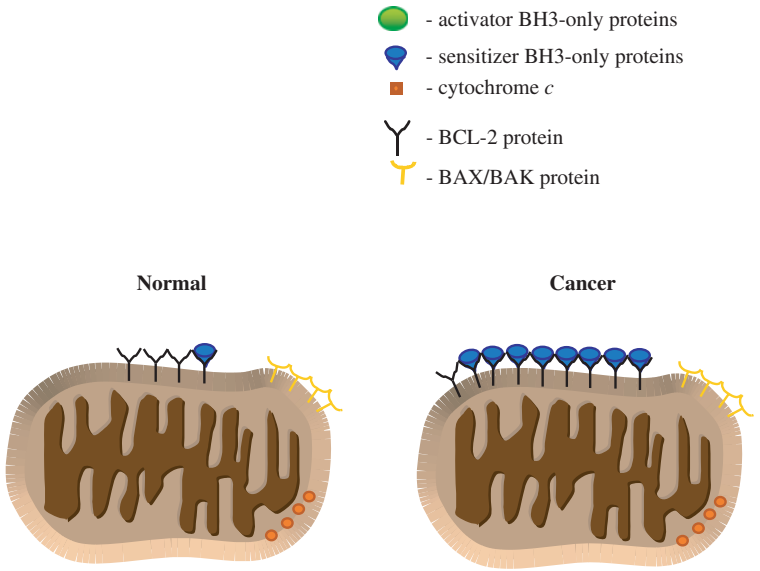

Fig. 8.3 Idealized cartoon representation of a normal mitochondrion compared to a cancer mitochondrion. Though they express more BCL-2 than the normal mitochondrion, the cancer mitochondrion has less antiapoptotic reserve due to significant priming by activator BH3-only proteins

3 Therapeutic Strategies Targeting Antiapoptotic BCL-2 Family Members

Efforts have begun to target the expression of antiapoptotic BCL-2 family members. One strategy is to downregulate antiapoptotic genes by ASO. An 18-mer phosphorothioated oligonucleotide directed against the first six codons of the human BCL-2 open reading frame, called Oblimersen or Genasense, was introduced by Genta, Inc. and has advanced through clinical trials (75, 76). Side effects have been tolerable, generally limited to thrombocytopenia, fatigue, back pain, weight loss, and dehydration (77). However, efficacy has been difficult to demonstrate. For example, treatment of metastatic melanoma with dacarbazine and oblimersen in a randomized phase III study showed no significant benefit in overall survival compared with dacarbazine alone (274 vs 238 days, $P = 0.18$). Even though significant benefit in progression free survival was observed (74 vs 49 days, $P = 0.0003$), overall survival was the primary end point, thus an FDA panel declared that clinical benefit was not demonstrated. In a phase III trial of myeloma, oblimersen plus high-dose dexamethasone was compared with dexamethasone alone; this trial also failed to meet its primary end point, time to disease progression. Furthermore, response to oblimersen in another myeloma trial did

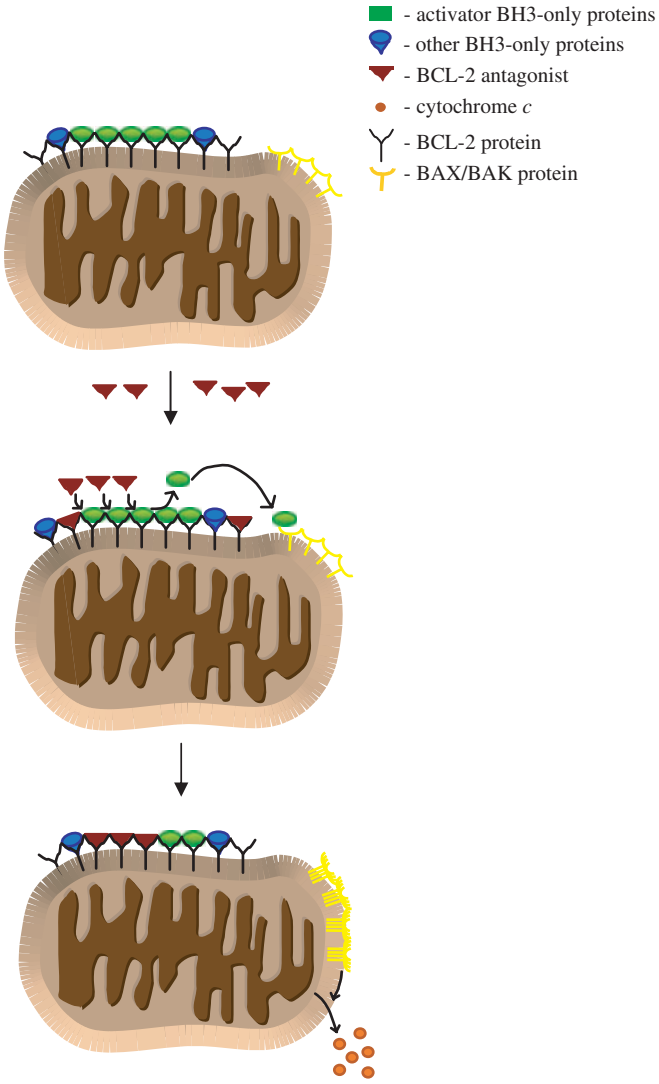

Fig. 8.4 Model of BCL-2 antagonist inducing death in a "primed" cancer mitochondrion. Cancer mitochondria have activator proteins like BIM sequestered by BCL-2 on the outer membrane. Upon addition of a BCL-2 antagonist, BIM is displaced and BCL-2 becomes occupied by the antagonist. Freed BIM then interacts with BAX or BAK, causing oligomerization and leading to cytochrome c release, MOMP, and apoptosis

not correlate with reduced BCL-2 protein levels, which provokes the question of whether oblimersen has significant off-target activity. In general, ASO has been a somewhat disappointing strategy for targeting BCL-2. The cellular effects of the lowering of BCL-2 levels by antisense oligonucelotides may not only provoke

undesirable coregulation of other BCL-2 family members but decreasing the mRNA is likely very different from functional antagonism of the protein (78). Furthermore, BCL-2 protein levels tend to be in the 10–50% range, which is unlikely to have a widespread cellular effect. Finally, oblimersen contains 2 CpG dinucleotides which may well produce many off-target effects on the immune system (78). While some of these off-target effects may be beneficial, others may well limit its maximum tolerated dose.

4 Delivery of Therapeutic Compounds into Cells

Delivery of drugs and therapeutic compounds is limited by the ability to penetrate the cell membrane. Compounds cross membranes either by passive processes or by mechanisms involving active participation of membrane components. In general, water, small hydrophilic molecules, and molecules <200 Da (79) passively diffuse through membranes. Therefore, most drugs need to be either small and water soluble, or polar enough for absorption into the body yet lipophilic enough to promote passage through the nonpolar lipid bilayer (80). This narrow range of physical characteristics limits the success of many compounds. Additionally, the degree of ionization of the compound, the circulation to the site of absorption, and its concentration can affect a compound's ability to reach its site of action. Even if a compound is able to circumvent passive passage across membranes by interacting with membrane receptors, there are still stringent criteria that must be met. No matter how a drug enters cell, once inside its effects can be terminated by metabolism or excretion. An additional difficulty is that the compound not only has to cross into cells rapidly and efficiently, but it then needs to make its way through the cellular milieu, which is full of proteases and other proteins, and eventually travel to the desired subcellular location to be effective.

While peptides based on sensitizer BH3-domains have been demonstrated to function as selective inhibitors of antiapoptotic proteins, unmodified BH3 domain peptides are cell impermeant (81). One strategy to augment cell entry is use of protein transduction domains (PTD) (82). PTDs are generally small (~10–20 amino acids) peptide sequences enriched for positively charged amino acids that rapidly and efficiently cross cell membranes. When fused to larger molecules, they have been shown to transport into cells a wide variety of cargo along with such large proteins (83), liposomes (84), and even metallic beads (85). The transduction process is not receptor mediated and is temperature independent, making it unlikely that endocytosis or transporter mechanisms are involved (86–88); however, the exact mechanism is not known.

To facilitate cell internalization, BH3 peptides have been linked with PTD such as a poly-D-arginine or Antennapedia internalization sequence tags (17, 26, 89, 90). N-terminal poly-D- arginine octomer (r8) linkage to BH3 peptides from BAD or BID have been shown to kill a human leukemia cell line that expresses BCL-2, while r8BIDBH3 double point mutant did not. Furthermore, r8BADBH3

peptide caused no apoptosis on its own but when added with the r8BIDBH3 peptide increased apoptosis, suggesting that the moiety did indeed facilitate internalization and that an intact BH3 domain was necessary for killing (17). In a separate study, a 27-amino acid peptide derived from the BH3 domain of BAD was linked to decanoic acid (26).

Decanoic acid allows cell permeablization by a different mechanism than PTDs, which may involve activation of phospholipase C which causes intracellular stores of calcium to be released followed by contraction of calmodulin-dependent actin filaments (91). The BAD-decanoic acid compound, called, cpm-1285, but not a peptide bearing a point mutation at a residue necessary for BH3 function, induced apoptosis in a BCL-2-expressing human myeloid leukemia line, HL-60. Furthermore, immunodeficient mice injected with HL-60 cells survived longer when treated with cpm-1285. However, these studies do not conclusively demonstrate the mechanism of action of the peptide derivatives, and the cytotoxic effects could be independent of direct interaction with BCL-2 family members. Such off-target toxicity was demonstrated by Schimmer and coworkers where linking the BH3 domain of BAD to the Antennapedia internalization sequence had considerable off-target toxicity (89). Their compound was toxic to a wide variety of cells, including yeast, wherein BCL-2 family members have yet to be identified. Others have demonstrated that BH3 peptides derived from BAX and BCL-2 linked to an Antennapedia internalization sequence induce MOMP and apoptosis, but overexpression of either BCL-2 or BCL-X_L did not rescue the cells from apoptosis (92). All of these effects may be due to a nonspecific membrane disruption rather than to interaction with the BCL-2 family pathway. For example, the Antennapedia internalization sequence is mainly a positively charged amphipathic α-helix that could interact and disrupt the negatively charged mitochondrial membranes independent of BCL-2 family protein interaction, in a manner similar to certain natural antibiotics (93–95). Therefore, nonspecific killing due to intrinsic biophysical properties of these internalization moieties make interpretation of cell killing by linked, some tagged BH3 peptides difficult. Further pharmaceutical development of such molecules would require considerable attention to reducing this toxicity.

The α-helix of BH3 peptides is vital for their function, but in aqueous solution the α-helical conformation can be less than 25%. Attempts have therefore been made to improve peptide function by stabilizing α-helicity. Small improvements have been gained by grafting a BAK BH3 domain to a helix-stabilizing miniprotein (96) or synthesizing BH3 peptide analogs with covalent molecular bridges, which improved affinity for BCL-2 or stabilized the α-helical conformation (97). Perhaps the most striking example of the potential of α-helix stabilization was provided by a BID BH3 peptide stabilized by an all-hydrocarbon "staple" (81). Not only did this modification enhance α-helicity, but it also increased affinity for BCL-2, cell entry, protease resistance, as well as leukemia cell line toxicity in vitro and in vivo. Mice bearing leukemia cell line xenografts demonstrated statistically significant survival improvement after 6 days and normal tissues appeared unaffected as measured by histological analysis. Since the molecule was modeled after a BID BH3 domain previously shown to be an activator (17), the compound was able to directly induce

cytochrome *c* release in a BAK-dependent fashion in vitro. Even though the compound did not behave as a selective BCL-2 antagonist but rather an activator, it was still able to exploit an apparent therapeutic window between the tumor xenograft and the normal tissues. It remains to be seen whether an analogous sensitizer BH3-based compounds would provide an even greater therapeutic window.

5 Cell-Permeant Small Molecules

Cell-permeant small molecules that bind to antiapoptotic BCL-2 family members have been identified through structure-based computer screening. One molecule isolated was able to displace the BAK BH3 peptide from BCL-2 with an IC_{50} of 1–14 μM. Since the *K*d for the BAK BH3 peptide is approximately 200 nM, it is reasonable to surmise that the *K*d for binding of these molecules to BCL-2 may be significantly higher. Another molecule identified was toxic to four cell lines tested at concentrations of 10–20 μM and toxicity correlated with BCL-2 expression levels (98, 99). Screens of chemical libraries have also been used. Out of 16,320 screened, Degterev et al. identified two molecules that disrupt a BCL-X_L/BAK BH3 complex, both which had toxicity in the 10–90 μM range in a leukemia cell line (100). A screen of a library of natural products allowed the isolation of Tetrocarcin A, which is derived from *Actinomyces*, identified for its ability to counteract BCL-2 protection of anti-Fas/cycloheximide-treated HeLa cells at concentrations in the micromolar range (101). Antimycin A, an antimicrobial agent with antitumor properties in experimental systems and a known inhibitor of electron transport at mitochondrial respiratory chain complex III, was identified from a screen for inhibitors of mitochondrial respiration in mammalian cells (102). Further characterization demonstrated that antimycin A interacts with BCL-2 and BCL-X_L, and that increasing cellular levels of BCL-X_L correlated with increasing toxicity. Nuclear magnetic resonance (NMR) spectroscopy used to investigate natural products found certain polyphenols from green tea extracts were able to bind to BCL-X_L (103). In addition, these compounds displaced a BH3 domain from BCL-X_L and BCL-2 in the submicromolar range. Another screen of a small library of natural products identified two molecules, purpurogallin and gossypol, both of which resemble human BAD and inhibit binding of a BH3 domain to BCL-X_L (104). While chemical modification of purpurogallin did not lower the IC_{50} of peptide displacement of the parental compound, a racemic mixture of the (+) and (−) isomers of gossypol displaced the BH3 peptide with an IC_{50} of 0.5 μM. Molecular modeling suggested that removal of two aldehyde groups from gossypol might reduce steric hindrance in binding the hydrophobic pocket of BCL-X_L, however this modification actually decreased the binding to BCL-2 family members (105).

Small molecules that enter cells and bind the hydrophobic pocket of BCL-2 analogously to sensitizer BH3 peptides are currently in clinical development. The biotechnology company Gemin X has isolated a compound (GX01) that has been reported to bind BCL-2 and BCL-X_L and displace BH3 domains from their binding

pockets (106). GX01 was identified from a high-throughput screen of chemical libraries and is in phase I clinical trials in both chronic lymphocytic leukemia (at the University of California, San Diego [UCSD]) and solid tumors (at Georgetown University). Ascenta Therapeutics has an orally administered gossypol derivative in an ongoing phase I cancer trial.

Using a strategy of combining high-throughput screening with interactive modulation of chemical structure based on NMR, Abbott Laboratories has developed compounds reported to displace BH3 domains from BCL-2, BCL-X_L, and BCL-w with an IC_{50} of not more than 1 nM (74). One lead molecule, ABT-737, is a BAD-like sensitizer that can antagonize BCL-2 protection but cannot directly cause activation of BAX/BAK. ABT-737 was reported to have significant activity in primary CLL cells and mouse xenograft models of lung cancer and lymphoma. When injected into mice ABT-737 was well tolerated with minimal side effects in non-cancerous tissues except for a reduction in platelets and lymphocytes. Furthermore, ABT-737 enhanced the cytotoxicity of paclitaxel against a cancerous cell line where single-agent activity was not achieved. Other preclinical studies have shown that the toxicity of ABT-737 is due to selective antagonism of BCL-2 in cells that require BCL-2 for survival (35). Given its high affinity for BCL-2, the data that support its function via its designed mechanism, and its effectiveness across several different cancer types in vitro, ABT-737 seems to be a promising lead compound, although clinical trials are yet to begin.

6 Conclusions

Our current understanding of the mechanisms by which BCL-2 family members control commitment to cell death gives good theoretical backing to strategies aimed at manipulating this system for clinical benefit. Certain cancers in which antiapoptotic BCL-2 is overexpressed and activator BH3-only proteins are upregulated may be "primed" for death, needing only a modest, targeted biochemical nudge for final execution of apoptosis. Small molecules designed to antagonize BCL-2 and related antiapoptotic proteins appear to be useful tools to generate this targeted signal. As the binding clefts among proteins like BCL-2, MCL-1, and BFL-1 are demonstrably distinct, it may be possible to design molecules which selectively antagonize individual proteins. Whether such "narrow spectrum" antagonists will be better cancer therapeutics than "broad spectrum" antagonists that might target the entire antiapoptotic group remains to be seen. Experimental evidence suggests that the state of protein–protein interactions among BCL-2 family members within cancer cells is different from those within normal cells. Therefore, even if an antagonizing compound entered all cells, induction of apoptosis might selectively be triggered within cancer cells. The promise of these molecules as anticancer therapeutics will soon be tested as clinical trials of compounds targeting BCL-2 are currently underway. It is exciting to witness the emergence of a potentially new class of anticancer drugs, those specifically designed to unleash the latent apoptotic potential within cancer cells.

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