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Review

BCL-2 in the crosshairs: tipping the balance of life and death

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Abstract

The discovery of B-cell lymphoma-2 (BCL-2) over 20 years ago revealed a new paradigm in cancer biology: the development and persistence of cancer can be driven by molecular roadblocks along the natural pathway to cell death. The subsequent identification of an expansive family of BCL-2 proteins provoked an intensive investigation of the interplay among these critical regulators of cell death. What emerged was a compelling tale of guardians and executioners, each participating in a molecular choreography that dictates cell fate. Ten years into the BCL-2 era, structural details defined how certain BCL-2 family proteins interact, and molecular targeting of the BCL-2 family has since become a pharmacological quest. Although many facets of BCL-2 family death signaling remain a mechanistic mystery, small molecules and peptides that effectively target BCL-2 are eliminating the roadblock to cell death, raising hopes for a medical breakthrough in cancer and other diseases of deregulated apoptosis.
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Abbreviations: BCL-2, B-cell lymphoma-2; BH, BCL-2 homology; NMR, nuclear magnetic resonance; SAR, structure-activity relationship; RCM, ring-closing metathesis; SAHB, stabilized alpha-helices of BCL-2 domains; ILOE, interligand nuclear Overhauser effect

Introduction

First identified at the chromosomal breakpoint of t(14;18)(q32;q21) lymphomas, B-cell lymphoma-2 (BCL-2) is the founding member of a family of proteins that regulate cell death.[1–3] Gene rearrangement places *BCL-2* under the transcriptional control of the immunoglobulin heavy chain locus, resulting in high-level BCL-2 expression and pathologic cell survival.[4,5] The oncogenic activity of BCL-2 derives from its ability to block cell death following a wide variety of stimuli.[6–8] Transgenic mice bearing a *BCL-2*-Ig minigene initially displayed a polyclonal follicular lymphoproliferation that selectively expanded a small resting IgM/IgD B-cell population.[4,9] These recirculating B cells accumulated because of an extended survival rather than increased proliferation. Despite a fourfold increase in resting B cell counts, *BCL-2*-Ig mice were initially quite healthy. However, over time these transgenics progressed from an indolent follicular hyperplasia to a diffuse large cell, and often immunoblastic, lymphoma.[10] The long latency period and progression from polyclonal hyperplasia to monoclonal high-grade malignancy implicated secondary genetic abnormalities in BCL-2-driven lymphomagenesis. Indeed, approximately half of the high-grade tumors possessed a *c-myc* translocation involving an immunoglobulin heavy-chain locus.[10] These tumor cells complemented an inherent survival advantage (*bcl-2*) with a gene that promotes proliferation (*c-myc*). By preventing the apoptotic demise of activated lymphocytes, BCL-2 enabled the acquisition of additional genetic aberrations and the emergence of monoclonal neoplasms. Doubly transgenic mice engineered to overexpress both BCL-2 and c-myc displayed synergistic tumorigenesis.[11] When leukemic mice with doubly deregulated BCL-2 and c-myc were conditionally induced to cease BCL-2 expression, tumor regression was observed, confirming a role for BCL-2 in tumor maintenance.[12] Thus, the discovery of BCL-2 established the new paradigm in cancer biology that prolonging cell survival by evasion of apoptosis can both initiate and sustain cancer.

The BCL-2 family has expanded significantly and now includes both pro- and antiapoptotic proteins, which form a complex network of checks and balances that regulate cell fate.[13,14] Disrupting the balance imposed by the BCL-2 family can lead to a host of human conditions that are characterized by excessive cellular demise, such as in neurodegenerative disease[15] or relentless cellular survival, such as in cancer[16] (Figure 1). BCL-2 proteins are defined both by their structure[17] and function (Figure 2). The survival proteins such as BCL-2 and BCL-X_L share three to four conserved BCL-2 homology (BH1-4) domains, and are thus termed 'multidomain anti-apoptotic' members. The executioner proteins such as BAX and BAK share three conserved domains (BH1-3) and are known as 'multidomain proapoptotic' proteins. A subgroup of proapoptotic proteins only displays conservation in the third BH domain. These 'BH3-only' members function as death sentinels that are situated throughout the cell, poised to transmit signals of cellular injury through multidomain

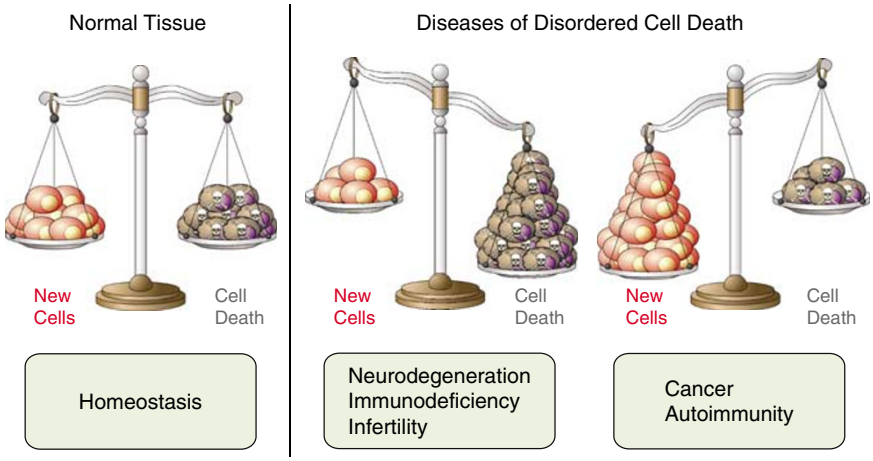

Figure 1 Apoptosis maintains tissue homeostasis by balancing cellular life and death. Deregulated apoptotic pathways disrupt the balance, resulting in diseases of premature cell loss or unrelenting cell survival

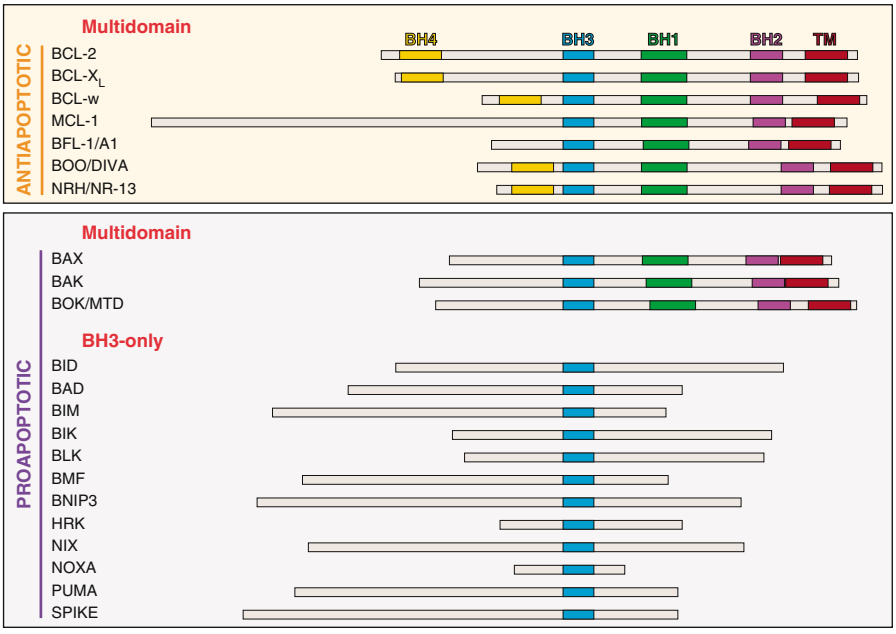

Figure 2 BCL-2 family proteins are structurally defined by their BCL-2 homology domains (BH domains) and functionally categorized by their ability to inhibit or activate cell death. Human BCL-2 family proteins are drawn to scale based upon polypeptide length and aligned by their BH3 domains

members. A variety of physiological death signals, as well as pathological cellular insults, trigger the genetically programmed pathway of apoptosis[13,18–40] (Figure 3). Depending upon the nature of apoptotic stimuli and the cellular context, a BH3-only protein's death signal will either be neutralized by antiapoptotic proteins or delivered, directly or indirectly, to the mitochondrial executioners BAX and BAK. When activated, these proapoptotic multidomain members induce permeabilization of the outer mitochondrial membrane, enabling released mitochondrial factors to activate caspases, which irreversibly execute the death program.

The network of interactions among BCL-2 family members is complex and remains a focus of intensive investigation. Diverse cellular signaling pathways engage the apoptotic program by launching particular BH3-only proteins[41,42] (Figure 3). For example, activation of death receptors Fas and tumor necrosis factor receptor 1 triggers caspase-8-induced cleavage of BH3-only protein BID.[43,44] The amino terminus of truncated BID (tBID) becomes myristolated, which facilitates its mitochondrial targeting and subsequent transmission of a plasma membrane death signal to multidomain BCL-2 family members at the mitochondria.[45] In the DNA-damage response, nuclear p53 induces gene transcription of BH3-only PUMA,[46] which in turn displaces cytoplasmic p53 from BCL-X_L, enabling direct protein interaction-based activation of BAX by p53.[47,48] Just as distinct signaling pathways employ specific BH3-only proteins, BH3-only proteins display sequence-dependent specificity for their target multidomain antiapoptotic proteins.[12,49] The ability of antiapoptotic proteins to form heterodimers with multidomain

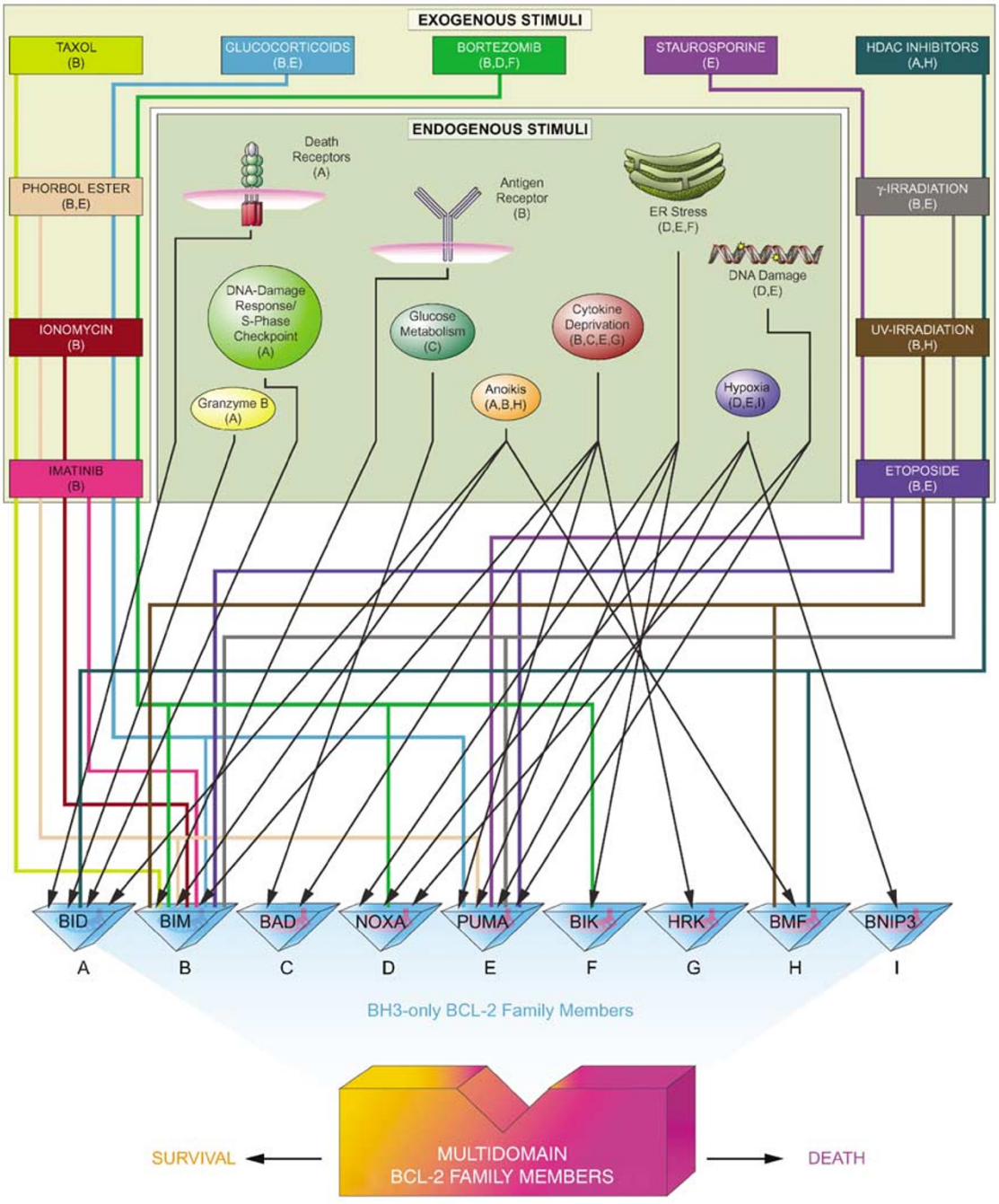

Figure 3 Cytotoxic signals activate the apoptotic program through diverse pathways, recruiting distinct BH3-only members to engage downstream multidomain BCL-2 family proteins

proapoptotic proteins suggests that neutralizing competition plays an important role in their suppression of cell death.[50–56] BH3-only proteins can promote apoptosis by antagonizing antiapoptotic proteins, and thereby relieve antiapoptotic inhibition of BAX/BAK. Such BH3-only proteins (e.g. BAD) have been termed 'sensitizers'[57] or 'derepressors'.[58] Alternatively, select BH3-only proteins such as BID and BIM may also trigger BAX/BAK directly,[22,58–62] and are therefore termed 'activators'.[57] In addition to these internal family-based interactions, BCL-2 proteins associate with a host of other cellular proteins[31,47,63–71] and have emerging roles in diverse physiologic pathways including glucose metabolism[20] and the DNA-damage response.[24,40]

BCL-2 Family Form and Function

A pivotal milestone in the apoptosis field was achieved in 1996 when the first X-ray and nuclear magnetic resonance (NMR) structure of a BCL-2 family protein was reported[72] (Figure 4a).

The architecture of BCL-X_L consists of eight α-helices, two of which form a central hydrophobic core reminiscent of the membrane insertion domains of pore-forming Diphtheria toxin and colicins.[72] This structural analogy led to experimental confirmation that BCL-2 family members can mediate pore formation in liposomal and mitochondrial systems,[73–76] an activity that is dependent upon core helices 5 and 6.[76–78] Another critical architectural feature of BCL-X_L was identified on its protein surface, a hydrophobic groove formed at the apex by the confluence of BH1, 2, and 3 domains and at the base by α-helices 3 and 4. The structure of a BAK BH3 peptide in complex with BCL-X_L revealed that the hydrophobic groove was indeed the contact site for proapoptotic binding[53] (Figure 4b). Thus, the protein interaction that accounts for BCL-2 family member homo- and heteroassociations,[50,54,56] and believed to regulate pore formation, was explicitly

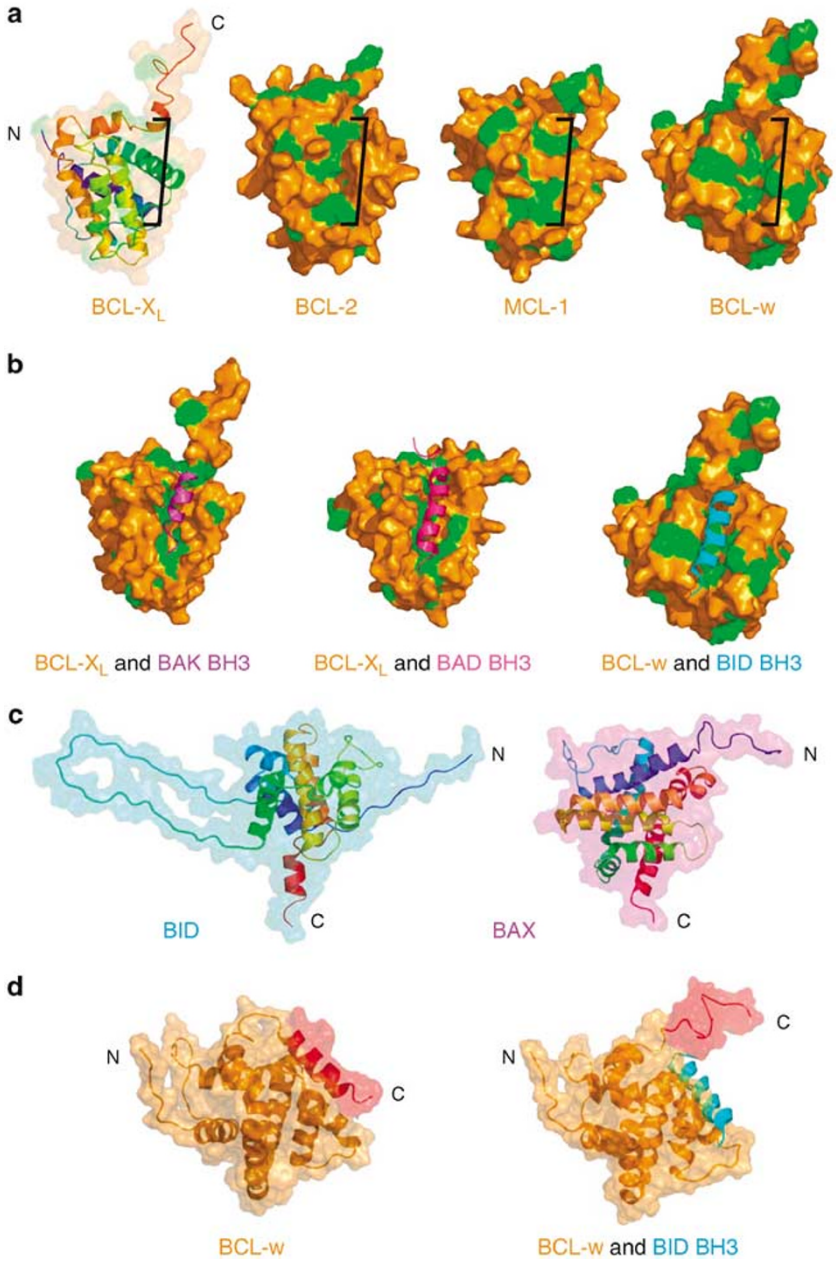

Figure 4 The structures of antiapoptotic BCL-2 family members (**a**) and their BH3 peptide complexes (**b**) revealed a multidomain hydrophobic groove that serves as the critical binding interface for α-helical BH3 domains. Hydrophobic residues are indicated in green and BH3-binding grooves bracketed in black. Despite their opposing functions, pro- and antiapoptotic multidomain members share overall structural similarities, including two hydrophobic core α-helices surrounded by 6–7 amphipathic α-helices, a flexible N-terminal loop, a surface hydrophobic groove, and a C-terminal transmembrane domain (**a**, **c**). BH3-only BID contains an extended amino terminus that contains the cleavage site for caspase-8, which triggers BID activation upon death receptor engagement (**c**). BAX and BCL-w contain a structurally defined C-terminal ninth helix (α9) that may regulate access to the BH3-binding groove (**c**, **d**). A comparison of BCL-w structures with and without BID BH3 peptide binding demonstrates how α9 (red) overlies the binding pocket until displaced by the BH3 helix (blue), which targets the hydrophobic groove (**d**)

defined. The subsequent NMR structures of BCL-2,[79] a BAD BH3 peptide/BCL-X_L complex,[80] BCL-w[81–83] and MCL-1[84] highlighted the three-dimensional theme of an antiapoptotic hydrophobic groove fashioned to engage the BH3 death helix of proapoptotic members (Figure 4b). Discrete differences in the amino-acid composition among antiapoptotic grooves and BH3 ligands dictate the specificity of apoptotic-binding partners.[49,57,79,80,84] With an essential rule of engagement structurally defined, the mechanics of BCL-2 family interactions came into focus.

On the proapoptotic side, NMR structures of BH3-only BID[85,86] and multidomain proapoptotic BAX[52] disclosed striking architectural similarities between the proponents and opponents of cell death (Figure 4c). BID and BAX likewise possess two central core helices that are surrounded by 6 or 7 amphipathic helices, respectively. The amino-terminal portions of BID and BAX contain unstructured loops (Figure 4c), as do select antiapoptotic proteins such as BCL-2 and BCL-X_L. This loop region has distinctive lengths and primary sequences among BID, BAX, and the antiapoptotics BCL-2 and BCL-X_L, and is believed to regulate their apoptotic functions. For example, phosphorylation within the loop region of BCL-2 differentially modulates its antiapoptotic activity depending upon the cellular context,[87,88] and caspase-mediated cleavage at the loop can actually transform BCL-2 into a proapoptotic protein.[64] Caspase-8-mediated cleavage of BID within the unstructured loop results in exposure of the BH3 helix of tBID,[85,86] which is targeted to the mitochondria for apoptosis induction.[43] The amino terminus of BAX has been implicated in BH3 ligand binding,[59] intracellular localization,[89,90] and negative regulation.[91,92] Calpain-mediated cleavage of BAX just prior to its relatively short unstructured loop generates a truncated form with enhanced apoptogenic activity,[93–95] which may reflect its functional conversion to a BH3-only-type protein.[96]

Multidomain pro- and antiapoptotic proteins contain C-terminal transmembrane domains that insert into the mitochondrial outer membrane. BH3-only BID lacks this C-terminal domain and interacts with lipid membrane with its helices parallel to the surface rather than by transmembrane insertion.[97,98] Whereas the constructs for the BCL-X_L, BCL-2, and MCL-1 structures lack the C-terminal transmembrane region, proapoptotic BAX and antiapoptotic BCL-w have a structurally defined ninth α-helix (α9) at the C-terminus[52,82,83] (Figure 4c and d). These α9 helices fold back into the hydrophobic groove in an orientation opposite to that delineated for the interactions of BAK and BAD BH3 peptides with BCL-X_L. The C-terminal helix effectively blocks both access to the groove and exposure of the BH3 domain and C-terminal hydrophobic residues (Figure 4d). Indeed, binding of BH3 peptides to the hydrophobic groove of BCL-w is impaired by α-9, as reflected by decreased BH3 peptide affinity for full-length *versus* C-terminally truncated BCL-w.[82,83] The distinctive C-terminal structure likely accounts for the cytosolic disposition of BAX and BCL-w by optimizing solubility until triggered to undergo a conformational change, which releases α9 for membrane insertion.[52,82,83,99] By obstructing the protein interaction site, α9 may also contribute to maintaining BAX and BCL-w in the monomeric form. Of note, α9 of BCL-w is less hydrophobic and more mobile than that of BAX by NMR, suggesting that the activation criteria for BAX α9 disengagement and resultant BAX translocation are stringent by design. Consistent with the need for tight structural control over BAX activation, numerous proteins in addition to BCL-2 family antiapoptotics have been identified that bind and inhibit BAX or BAK.[63,64,66,67,69]

The published structures of BCL-X_L, BCL-2, BID, BAX, BCL-w, MCL-1, and several of their complexes with BH3 peptides have provided tremendous insights into the functional roles of BCL-2 family members and the protein interactions that enable their apoptotic activities. The explicit mechanics of how BCL-2 family members regulate mitochondrial pore formation, and how select BH3-only members may engage proapoptotic multidomain proteins, remain active areas of investigation. Structural studies that evaluate BCL-2 family activities and interactions in the lipid environment continue to provide new details regarding the complexity of BCL-2 family conformational changes that occur during mitochondrial apoptosis induction.[97,98,100–102] Most importantly, structural delineation of the helical folds responsible for forming both a multidomain groove and its BH3 ligand established a rational means for targeting apoptosis by chemical design.

Getting into the Groove

The fields of chemical genetics and developmental therapeutics share the mission of identifying small molecules that directly and specifically alter protein function so that physiologic activities can be investigated and manipulated on a conditional basis in real-time. The objective of generating small molecules to selectively target apoptotic protein interactions and specifically manipulate their corresponding pathways *in vivo* has been challenging due to the size and complexity of the intracellular protein-binding interface. However, virtual and small molecule screens, in addition to peptidomimetic, secondary structure reinforcement, and NMR-based strategies, have yielded a diverse group of compounds that target the BCL-2 family hydrophobic groove (Figures 5 and 6, Table 1). The development of these compounds as biological tools and clinical candidates promises to deepen our understanding of BCL-2 family biology and deliver a new era of treatments to patients suffering from oncologic, neurodegenerative, autoimmune, and a host of other diseases characterized by an imbalance between cell survival and death.

The Hunt for Small Molecules

One of the earlier strategies that successfully identified BCL-2 inhibitors involved computer-based screening of small molecule databases for structures that matched likely binding sites on the surface of BCL-2. This approach yielded HA14–1 ($IC_{50} = 9\,\mu$M for competing BAK BH3/BCL-2 interaction),[103] several micromolar affinity hits from the National Cancer Institute 3D database including Compound 6,[104] and YC137 ($IC_{50} = 1.3\,\mu$M for competing BID BH3/BCL-2 interaction)[105] (Figure 5a–c). Antimycin A_3 emerged as a BCL-2 groove binder ($K_d = 0.82\,\mu$M) upon screening mitochondrial respira-

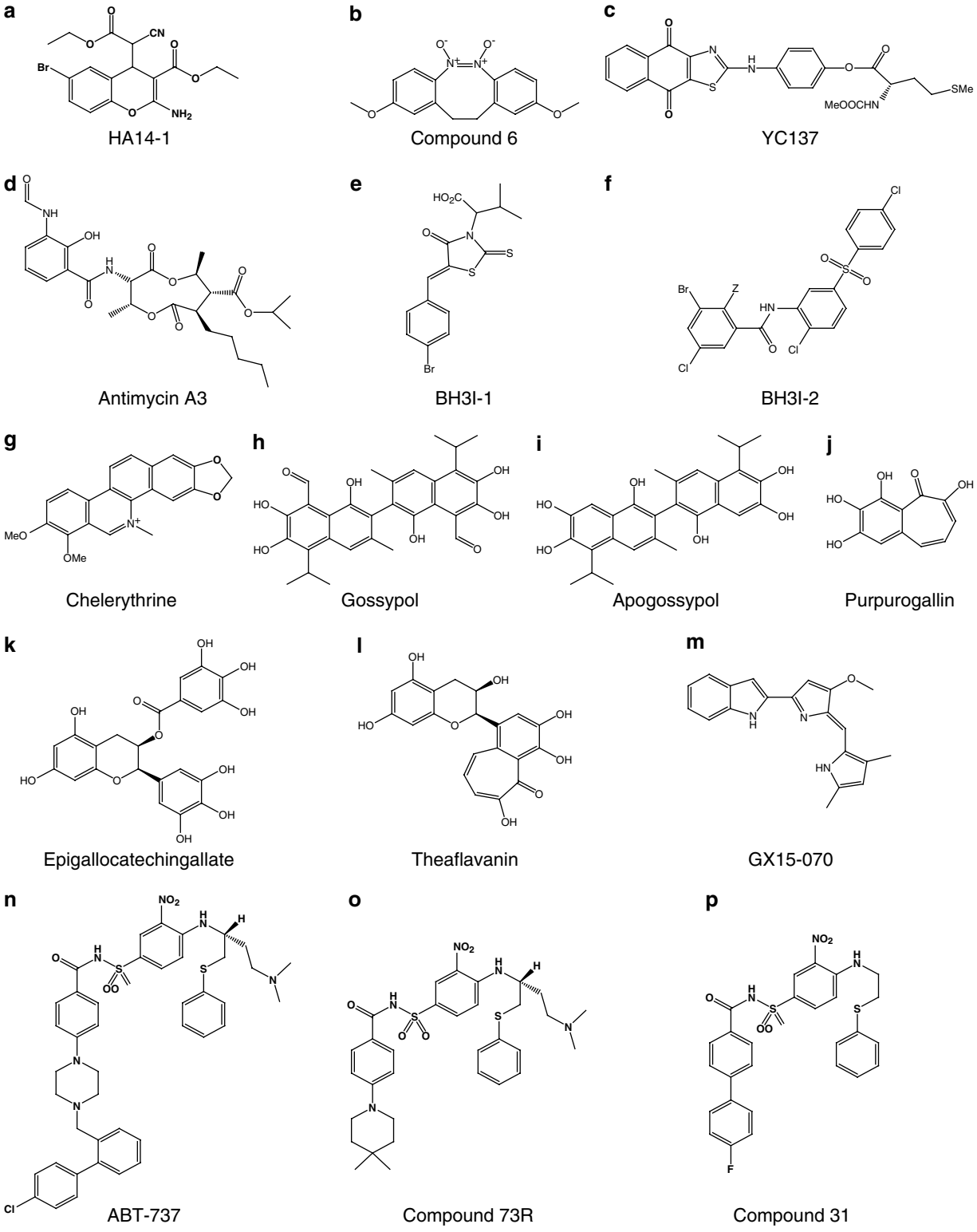

Figure 5 Virtual and biochemical small molecule screening, NMR-based methodologies, and parallel synthesis have yielded a heterogeneous group of antiapoptotic groove binders

tion inhibitors for proapoptotic activity in hepatocyte cell lines with graded expression of BCL-X_L[106,107] (Figure 5d). A BAK BH3/BCL-X_L competitive binding assay-based screen identified two small molecule inhibitors, BH3I-1 and BH3I-2, with K_is in the 2–16 μM range[108] (Figure 5e–f). Similar binding assay strategies were used to screen natural product libraries, identifying chelerythrine ($IC_{50} = 1.5\,\mu$M, BAK BH3/BCL-X_L competition),[109] gossypol ($IC_{50} = 0.5\,\mu$M, BAD BH3/BCL-X_L

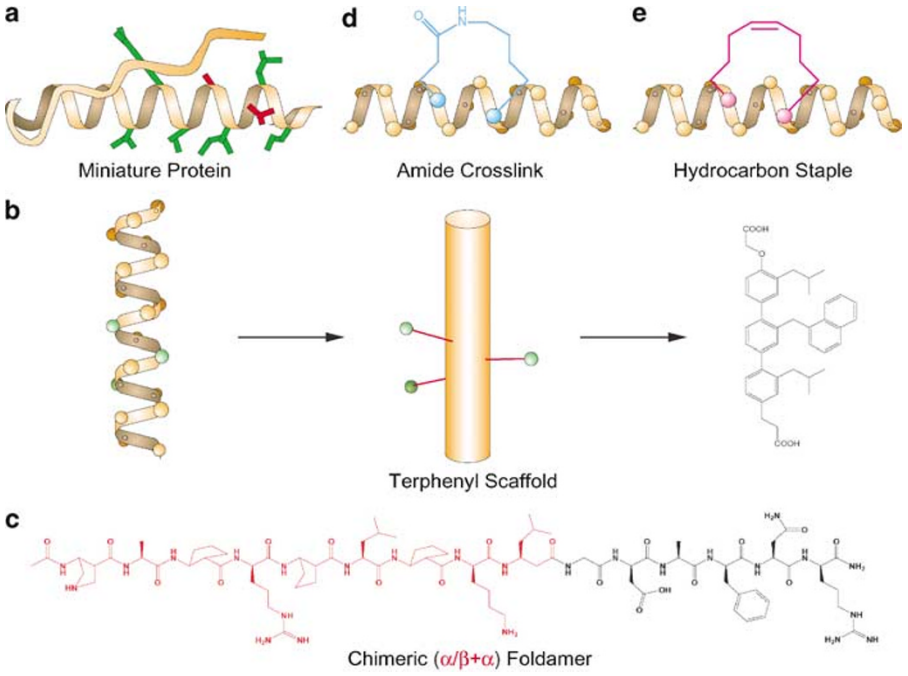

Figure 6 Strategies to reinforce BH3 peptide α-helicity or chemically simulate key projections of the BH3 helix on a synthetic scaffold have generated peptidic and peptidomimetic compounds for BCL-2 family targeting

Table 1 Compounds in development for targeting the BCL-2 family *in vivo*

Compound	Class	Mechanism	Academic institution/ company	Developmental stage
Genasense	Antisense oligonucleotide	Antiapoptotic mRNA downregulation (BCL-2)	Genta	Clinical
HA14-1 analogs	Small molecule	Antiapoptotic inhibition	Raylight Chemokine Pharmaceuticals	Preclinical
Compound 6	Small molecule	Antiapoptotic inhibition	University of Michigan	Preclinical
Antimycin A3	Small molecule	Antiapoptotic inhibition	University of Washington	Preclinical
BH3Is	Small molecule	Antiapoptotic inhibition	Harvard University	Preclinical
AT101: (–) Gossypol	Small molecule	Antiapoptotic inhibition	Ascenta Therapeutics	Clinical
Apogossypol	Small molecule	Antiapoptotic inhibition	The Burnham Institute	Preclinical
Theaflavanin	Small molecule	Antiapoptotic inhibition	The Burnham Institute	Preclinical
Polyphenol E	Small molecule	Antiapoptotic inhibition	Mayo Clinic	Preclinical
GX15-070	Small molecule	Antiapoptotic inhibition	Gemin X	Clinical
ABT-737	Small molecule	Antiapoptotic inhibition	Abbott Laboratories/Pfizer (Idun)	Preclinical
IFI-983L, IFI-194	Small molecule	Antiapoptotic inhibition	Infinity Pharmaceuticals/ Novartis	Preclinical
CPM-1285 analogs	Lipidated peptide	Antiapoptotic inhibition	Raylight Chemokine Pharmaceuticals	Preclinical
Terphenyl derivative	Peptidomimetic	Antiapoptotic inhibition	Yale University	Preclinical
SAHBs	Stapled peptide	Antiapoptotic inhibition	Dana-Farber Cancer Institute/Harvard University	Preclinical
4-Phenylsulfanyl-phenylamine derivatives	Small molecule	Proapoptotic inhibition (BID)	The Burnham Institute	Preclinical
3,6-Dibromocarbazole piperazine derivatives of 2-propanol	Small molecule	Proapoptotic inhibition (BAX)	Serono	Preclinical
Humanin peptides	Peptide	Proapoptotic inhibition (BAX)	The Burnham Institute	Preclinical
Ku70 peptides	Peptide	Proapoptotic inhibition (BAX)	The Blood Center of South Eastern Wisconsin	Preclinical

competition),[110,111] and purpurogallin ($IC_{50} = 2.2\,\mu M$, BAD BH3/BCL-X_L competition)[110] as BCL-X_L groove binders (Figure 5g–j). Natural polyphenols found in extracts of green tea (e.g. epigallocatechingallate) and black tea (e.g. theaflavanin) compete with BAD BH3 for BCL-X_L and BCL-2 binding with K_is in the 120–1230 nanomolar range[112] (Figure 5k and l). A polypyrrole derivative identified from a natural compound library screen was developed to yield GX15-070, which binds to BCL-X_L, BCL-w, and MCL-1 in the 500 nM range[113,114] (Figure 5m).

A major hurdle of small molecule library screening approaches, whether virtual or biochemical, is the chemical optimization required to achieve high-potency target-binding activity from hits that are typically in the micromolar range. An alternative strategy developed by Fesik and co-workers[115] circumvents this shortcoming of library screening by chemically linking ligands that bind to adjacent sites within a target interface, effectively converting relatively low affinity interactors into conjoined high-affinity compounds. The method is called structure–activity relationships (SAR) by NMR, reflecting that compound optimization is derived from SAR determined by NMR. One of the seminal fruits of this strategy is ABT-737, a small molecule that binds to BCL-2, BCL-X_L, and BCL-w at subnanomolar affinity and demonstrates potent antitumor activity *in vitro* and *in vivo*[116] (Figure 5n). The SAR by NMR approach has recently been combined with parallel synthesis to generate optimized BCL-X_L inhibitors[117,118] (Figure 5o and p).

Rebuilding the BH3 Helix

The nanomolar-binding affinities and native selectivities of BH3 peptides for their antiapoptotic targets prompted the development of derivatized peptides for BCL-2 family targeting. In order to overcome cell impermeability, cell penetrating peptides were synthesized by tagging the BH3 domain with moieties that facilitate uptake, including Antennapedia[119–121] and poly-D-arginine[57] amino-acid sequences, and fatty acids such as decanoic acid.[122] Despite their cell permeability, several drawbacks of these derivatized peptides include loss of α-helical structure, protease sensitivity, low cellular potency, and in certain circumstances apoptosis induction independent of BCL-2 targeting.[119]

Short peptides taken out of context from a protein typically lack native three-dimensional structure, and therefore, may fail to exhibit biologic functionality at physiologic doses. As the α-helix participates in a wide variety of intermolecular biological recognition events, a major focus of modern organic chemistry is the development of synthetic strategies to mimic or stabilize the architecture of biologically active structures for both basic research and medicinal purposes (Figure 6). Schepartz and co-workers[123,124] developed a protein grafting strategy in which bioactive α-helical residues, such as BAK BH3 peptide, are inserted into a stable protein scaffold, and then subjected to rounds of phage display to evolve high-affinity ligands with preferences for BCL-2 or BCL-X_L (Figure 6a). In lieu of the natural amide backbone of α-helical peptides, synthetic scaffolds have been developed that effectively present critical amino acid residues to the antiapoptotic groove. For example, constructing compounds using terphenyl,[125,126] terpyridine,[127] or terephthalamide[128] scaffolds that project essential BH3 motifs yielded groove binders with micromolar and nanomolar K_ds (Figure 6b). Gellman and co-workers[129] developed BAK BH3 'foldamers' that are peptidic compounds built from oligomers with defined folding propensities. Chimeric peptides that juxtapose native BAK BH3 sequence with alternating α- and β-peptide foldamer sequence can also present critical groove-binding motifs, resulting in compounds with nanomolar-binding affinities for BCL-X_L(Figure 6c).

An important strategic breakthrough in stabilizing natural α-helices derived from installing a covalent bond between amino acids in an attempt to 'lock' the peptide structure into place.[130–134] The incorporation of lactam bridges into the BAK BH3 peptide increased α-helical content from 14% to as high as 78%[135] (Figure 6d). However, stabilization methods that incorporate polar or labile crosslinks may not address peptide shortcomings of instability and cell impermeability *in vivo*. Grubbs and co-workers[133] generated a covalent crosslink between *O*-allyl serine residues on adjacent turns of an α-helix using ruthenium-catalyzed ring closing metathesis (RCM), which employs a metal catalyst to form a covalent bond between non-natural amino acid residues containing terminal double bonds or 'olefins'. This novel chemical approach was successful in generating a covalent hydrocarbon crosslink, but little to no enhancement of peptide α-helicity was observed. Subsequently, Verdine and co-workers[134] developed an alternate 'olefin metathesis'-based approach, which employed α,α-disubstituted non-natural amino acids containing alkyl tethers (Figure 6e). By experimenting with alternative placement of these non-natural amino acids along the α-helix, in addition to varying stereochemistry and alkyl tether length, the chemical features required to dramatically stabilize a model helical peptide using an all-hydrocarbon chain crosslink were defined.

Stabilizing the helical form of biologically active peptides is expected to favor target binding by facilitating structural preorganization.[136] Furthermore, helix formation buries the polar amide backbone, which should increase resistance to proteolytic cleavage and decrease the barrier to cell penetration. In our first biological application of this chemical approach, we developed and tested Stabilized Alpha-Helices of Bcl-2 domains, or 'SaHBs', modeled after BID BH3.[137] An all-hydrocarbon staple inserted into the BID BH3 peptide sequence successfully (1) restored and stabilized α-helical structure, (2) enhanced peptide half-life, (3) improved binding potency, and (4) conferred cellular permeability such that the genetic pathway of apoptosis could be reactivated in cancer cells *in vitro* and *in vivo*. Thus, synthetic approaches such as 'hydrocarbon stapling' that reinforce native peptide sequences offer an alternative strategy for studying and manipulating protein interactions, and provide prototypes for novel therapeutics designed to target aberrant signaling pathways in human disease. The appeal of this strategy includes retaining the complexity, potency, and specificity of natural bioactive peptide sequences, the short timeframe for compound development, and the potential for broad applicability in targeting cell surface, intracellular, and organelle-based protein interactions.

Neutralizing the Proapoptotics

Small molecules that target apoptotic protein interfaces other than the multidomain antiapoptotic groove have also been identified. Using the strategy named SAR by interligand nuclear Overhauser effect (ILOEs), of Reed and co-workers[138] designed a panel of 4-phenylsulfanyl-phenylamine compounds that bind a deep groove on the surface of BID with micromolar affinity. The molecules inhibited tBID-induced mitochondrial SMAC release, caspase-3 activation, and cell

death in the 20–100 μM range. 3,6-Dibromocarbazole piperazine derivatives of 2-propanol were developed as the first small molecule modulators of BAX-induced mitochondrial and liposomal release activity.[139] Subsequently, a small molecule screen using a BAX-induced liposomal release assay identified two additional BAX channel blockers.[140] The Bax channel inhibitors Bci1 and Bci2 prevented cytochrome *c* release from mitochondria and protected cells from apoptosis *in vitro* at micromolar dosing. Bcis were neuroprotective in an *in vivo* model of transient brain ischemia. Peptides derived from humanin[63] and Ku70[141] proteins also interact with BAX and inhibit its activation. Thus, in addition to targeting the antiapoptotic groove to stimulate cell death, small molecule and peptide-based approaches to inhibiting proapoptotic proteins may lead to the development of cytoprotective therapeutics.

Toward a Therapeutic Reality

From a 20-year multidisciplinary dissection of BCL-2 family interactions and pathways has emerged the promise of novel therapeutics to treat human disease. More than a dozen small molecules and peptidic compounds are currently in preclinical and clinical development for targeting the structurally defined multidomain antiapoptotic groove[113,142] (Table 1). Preclinical development of small molecule and peptide inhibitors of proapoptotic BCL-2 family proteins is also underway. A BCL-2 antisense therapeutic,[143–146] Genasense, is in Phase III clinical testing. For the clinicians and scientists who discovered BCL-2 and for those who have subsequently dedicated their lives' work to elucidating and targeting the BCL-2 family of proteins, successful translation of these efforts into the clinic in the form of FDA-approved medicines will be a crowning achievement.

Conflict of Interest

LDW is an advisor to Renegade Therapeutics, an emerging biopharmaceutical company developing next-generation targeted therapeutics.

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