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REVIEW

Bcl-2 family proteins and cancer

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***BCL-2* was the first anti-death gene discovered, a milestone with far reaching implications for tumor biology. Multiple members of the human Bcl-2 family of apoptosis-regulating proteins have been identified, including six antiapoptotic, three structurally similar proapoptotic proteins and several structurally diverse proapoptotic interacting proteins that operate as upstream agonists or antagonists. These proteins, in turn, are regulated through myriad post-translational modifications and interactions with other proteins. Bcl-2-family proteins regulate all major types of cell death, including apoptosis, necrosis and autophagy, thus operating as nodal points at the convergence of multiple pathways with broad relevance to oncology. Experimental therapies targeting Bcl-2-family mRNAs or proteins are currently in clinical testing, raising hopes that a new class of anticancer drugs may soon be available.**
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Introduction

The average adult human produces and in parallel eradicates ~60 billion cells daily, with new cells formed by cell division and old cells eliminated principally by apoptosis, thus striking a balance under normal circumstances. The ability to control cell numbers at both the points of entry and exit allows flexibility to more rapidly respond to stress, injury and physiological cues. However, it also creates a liability in terms of neoplasia, as genes that normally suppress or induce physiological cell death often become dysregulated in cancers, with defective cell death mechanisms now recognized as one of the six hallmarks of cancer (Hanahan and Weinberg, 2000). Bcl-2-family proteins play central roles in cell death regulation and are capable of regulating diverse cell death mechanisms that encompass apoptosis, necrosis and autophagy (Cory *et al*., 2003; Levine and Kroemer, 2008; Reed, 2008). Alterations in their expression and function contribute to the pathogenesis and progression of human cancers, thus providing targets for drug discovery that are currently being explored in human clinical trials.

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Altered expression of *BCL-2*-family genes in cancer

Abundant examples exist where the regulation of genes encoding either antiapoptotic or proapoptotic Bcl-2-family proteins is altered in cancers. In fact, the founding member of the gene family, *BCL-2*, was discovered because of its involvement in *t*(14;18) chromosomal translocations observed in non-Hodgkin's lymphomas (Tsujimoto *et al*., 1985). In *t*(14;18) translocations, the *BCL-2* gene from chromosome 18 becomes fused with the immunoglobulin heavy-chain locus on chromosome 14, bringing the juxtaposed *BCL-2* gene under the control of the immunoglobulin heavy-chain enhancer, and thereby dysregulating *BCL-2* gene expression at a transcriptional level. In addition to chromosomal translocations as a mechanism for activation of the *BCL-2* gene in human malignancies, *BCL-2* gene amplification is also found in non-Hodgkin's lymphomas and small cell lung cancers (Ikegaki *et al*., 1994; Monni *et al*., 1997). Besides changes to *BCL-2* gene structure or copy number, many additional mechanisms contribute to elevated gene expression, which is estimated to occur in perhaps as many as half of all human cancers. Among the contributing mechanisms are (a) loss of endogenous microRNAs (miRs) that normally repress *BCL-2* gene expression (Cimmino *et al*., 2005), which has been documented in chronic lymphocytic leukemia, where the genes encoding miR15 and miR16 become deleted or inactivated by mutations in >70% of these leukemia, and (b) gene hypomethylation, implying altered epigenetic regulation of *BCL-2* in some malignancies (Hanada *et al*., 1993). Altered expression of other antiapoptotic members of the *BCL-2* gene family has also been documented in human cancers and leukemias (see for example, Tron *et al*., 1995; Krajewski *et al*., 1995a, b; Brousset *et al*., 1996; Khanna *et al*., 1996; Krajewska *et al*., 1996a, 2008; Yip *et al*., 2006), although no somatic mutations have been discovered in these genes to date. Loss of miR-29, which represses antiapoptotic family member *MCL-1*, can occur in chronic lymphocytic leukemia and colon cancers (Calin *et al*., 2005; Cummins *et al*., 2006; Mott *et al*., 2007), suggesting at least one responsible mechanism for elevated expression of relatives of *BCL-2*.

Defects in the expression of proapoptotic members of the *BCL-2* family also occur in cancer, resulting in loss of the tumor suppressor function of these killer genes. The best documented is *BAX*, where homozygous deletions or inactivating mutations have been identified, particularly in cancers that arise with microsatellite instability because of defective DNA mismatch repair (Rampino *et al*., 1997; Meijerink *et al*., 1998). In this regard, the human *BAX* gene contains a homopolymeric stretch of eight guanosine residues in the sense strand that is a target for frame-shift mutations. Defective expression of proapoptotic *BCL-2*-family genes also occurs in the setting of loss of p53 function. Among the direct targets of the p53 transcription factor are *BAX, BID, PUMA* and *NOXA* (Miyashita *et al*., 1994; Miyashita and Reed, 1995; Oda *et al*., 2000; Sax *et al*., 2002; Yu *et al*., 2003), thus demonstrating strong connections between genome surveillance by p53 and cell death genes of the *BCL-2* family. More recently, cytosolic interactions of p53 protein with pro- and antiapoptotic Bcl-2-family proteins have been observed, directly modulating the bioactivities of the p21-Bax and p26-Bcl-2 proteins, and suggesting that p53 regulates the Bcl-2 family at both transcriptional and post-transcriptional levels (Chipuk *et al*., 2004; Deng *et al*., 2006). The activities of additional proapoptotic members of the Bcl-2 family are also suppressed through post-translational modifications. For example, proapoptotic protein BAD is phosphorylated by Akt (PKB) and other protein kinases known to be hyperactive in cancers, resulting in its sequestration by 14-3-3 (Zha *et al*., 1996).

Bcl-2 and resistance to chemotherapy

Overexpression of the Bcl-2 and related antiapoptotic proteins has been demonstrated to inhibit cell death induced by many stimuli, including growth factor deprivation, hypoxia and oxidative stress. However, it is the ability of antiapoptotic Bcl-2-family proteins to suppress cell death induced by cytotoxic anticancer drugs that makes these proteins particularly interesting as potential targets for cancer drug discovery. Regardless of the primary mode of action, whether single or double-strand DNA breaks, whether microtubule depolymerization or aggregation, whether nuclear hormone receptor activation (glucocorticoid receptor) or inhibition (estrogen and androgen receptors), essentially all traditional anticancer drugs appear to depend in large measure on Bcl-2/Bax-dependent mechanisms for killing cancer cells (reviewed by Debatin *et al*., 2002; Reed, 2008). Thus, Bcl-2 operates at a distal point in a conserved cell death pathway utilized by most anticancer drugs, constituting a form of intrinsic chemoresistance, distinct from the previously identified mechanisms involving drug efflux, drug metabolism, drug inactivation and related mechanisms. This observation presumably explains why expression of a variety of Bcl-2-family proteins has been shown to be of prognostic significance for many types of cancer and leukemia treated by chemotherapy (see for example, Yunis *et al*., 1989; Tang *et al*., 1994; Hermine *et al*., 1996; Hill *et al*., 1996; Krajewska *et al*., 1996a, b, 2008; Gascoyne *et al*., 1997; Pedersen *et al*., 2002).

Cellular functions of Bcl-2-family proteins: guardians of organellar cell life and death

Core members of the Bcl-2 family share structural similarity with the pore-forming domains of bacterial toxins, emphasizing the relevance of these proteins to membrane biology (reviewed by Schendel *et al*., 1998). Several Bcl-2 family proteins insert into intracellular membranes, particularly membranes of mitochondria and endoplasmic reticulum (ER), operating as guardians of these organelles.

Mitochondria

The central pathway involved in daily ('normal') programmed cell death in most tissues involves mitochondria, energy-producing organelles that play critical roles in both cell life and death (Green and Kroemer, 2004). Several Bcl-2-family proteins, both antiapoptotic and proapoptotic, have C-terminal transmembrane domains that insert in the outer membrane of mitochondria. Proapoptotic Bcl-2-family proteins such as Bax and Bak induce mitochondrial outer membrane permeabilization (MOMP), causing the release of caspase-activating proteins and other cell death mediators, whereas antiapoptotic proteins such as Bcl-2 serve as guardians of the outer membrane and preserve its integrity by opposing Bax and Bak (Figure 1a). Induction of MOMP correlates with oligomerization of Bax and/or Bak in the outer mitochondrial membrane, which is opposed by antiapoptotic proteins such as Bcl-2 and Bcl-X_L (Eskes *et al*., 2000; Wei *et al*., 2000; Chipuk and Green, 2008). In fact, these observations have been reconstituted in synthetic liposomes, showing that Bax can be induced to oligomerize, permeabilizing liposomes *in vitro*, through a Bcl-X_L-inhibitable mechanism (Kuwana *et al*., 2002). Thus, Bax and structurally related proapoptotic proteins (Bak; Bok) are hypothesized to form proteinacous or lipidic pores upon oligomerization in mitochondrial membranes, alone or in conjunction with resident mitochondrial proteins, thereby allowing escape of various apoptogenic proteins from these organelles (reviewed by Reed, 2006; Chipuk and Green, 2008). Gene ablation studies in mice have demonstrated that either Bax or Bak is necessary for MOMP, establishing these pore-forming proapoptotic proteins as the distal elements controlling permeability of mitochondrial membranes (Wei *et al*., 2001), and thus the governors of life/decisions in the mammalian mitochondrial pathway for cell death.

Mitochondria induce apoptosis by releasing proteins that participate in caspase activation (for example, cytochrome *c*) and that neutralize endogenous inhibitors of caspases (for example, SMAC; OMI/Htra2, which inhibit IAPs), as well as unleashing several mediators of

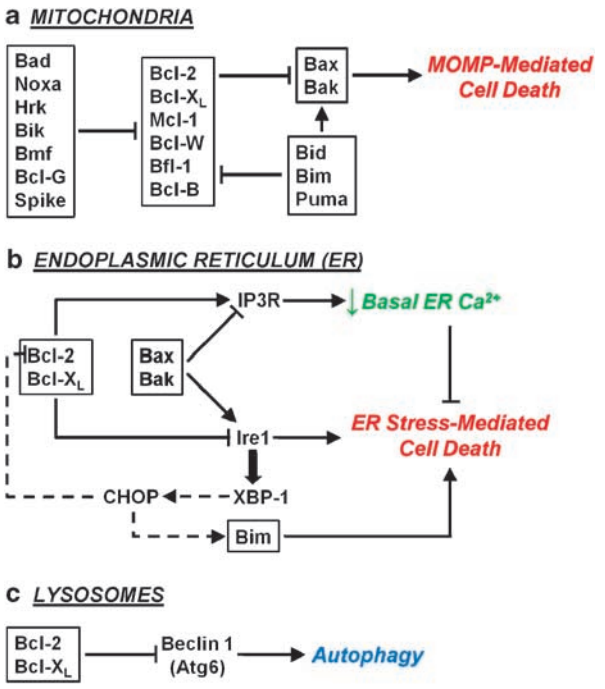

Figure 1 Cellular phenotypes of Bcl-2-family proteins. Bcl-2 family proteins (*in boxes*) insert into membranes of mitochondria (**a**) and endoplasmic reticulum (ER) (**b**), promoting life (*green*) or death (*red*) cellular events. Bcl-2-family proteins do not insert directly into membranes of lysosomes (**c**), but do affect lysosomal-related processes such as autophagy (*blue*). See text for details. Dotted lines represent transcriptional regulation, thick lines (for example, between Ire1 and XBP-1) represent mRNA splicing alterations. Note that some Bcl-2-family proteins are not included here, such as Bok and Bcl-rambo.

caspase-independent necrotic cell death (Reed, 2002). For example, MOMP releases several proteins that contribute to non-apoptotic cell death, including DNase, endonuclease G and AIF, a flavoprotein reported to enter the nucleus and promote genome destruction (Penninger and Kroemer, 2003). MOMP also results secondarily in elaboration of reactive oxygen species, causing lipid peroxidation and membrane damage, which impair normal ion-homeostasis, causing cellular swelling and plasma membrane rupture, as well as rupture of lysosomes and release of hydrolytic enzymes that destroy proteins, nucleic acids and lipids.

Cross-talk of the mitochondrial pathway with other cell death pathways probably occurs through a variety of mechanisms. A prominent example utilized by TNF-family death receptors involves caspase-mediated cleavage and activation of Bid, which then targets mitochondrial membranes where it binds Bcl-2/Bax-family proteins and modulates their activities (Korsmeyer *et al*., 2000). Integration of mitochondria into cell death pathways initiated from other organelles such as ER and lysosomes has also been documented (Ferri and Kroemer, 2001; Jaattela *et al*., 2004; Xu *et al*., 2005; Kroemer *et al*., 2007), thus placing mitochondria-associated Bcl-2-family proteins at a downstream point of convergence of many cell death pathways.

Endoplasmic reticulum (ER)

During times of cellular stress, the ER can initiate molecular events that lead to either caspase-dependent or caspase-independent cell death (reviewed by Xu *et al*., 2005). In this regard, the accumulation of unfolded proteins in the lumen of the ER ('ER stress') triggers an evolutionarily conserved series of signaling events, termed the unfolded protein response (reviewed by Kim *et al*., 2006; Ron and Walter, 2007). ER stress is induced by several stimuli of relevance to tumor biology, including hypoxia, oxidative stress and nutrient insufficiency, and markers of ER stress have been identified in the centers of tumors with insufficient vasculature supply (Blais *et al*., 2004; Daneshmand *et al*., 2007; Dong *et al*., 2008; Zheng *et al*., 2008). Factors that perturb normal Ca^{2+} homeostasis in the ER also cause accumulation of unfolded proteins, presumably due to the Ca^{2+} dependence of many ER chaperones, including Grp78 (Hsp70 homolog), Grp94 (Hsp90 homolog) and Calreticulin. Overexpression of antiapoptotic Bcl-2-family proteins (for example, Bcl-2; Bcl-X_L) has been shown to protect cells against cell death induced by ER stress, whereas proapoptotic Bcl-2-family proteins (for example, Bax; Bak) are required (Scorrano *et al*., 2003; Thomenius and Distelhorst, 2003) (Figure 1b). Precisely how Bcl-2-family proteins regulate ER-initiated cell death mechanisms is unclear. Effects of Bcl-2-family proteins on ER Ca^{2+} regulation are among the likely contributors. In this regard, Bcl-2 and Bcl-X_L reduce basal Ca^{2+} concentrations in the ER, apparently through effects on inositol-3-phosphate receptors (IP3Rs)—second messenger-gated Ca^{2+} channels (He *et al*., 1997; Kuo *et al*., 1998; Pinton *et al*., 2000; Li *et al*., 2002). The ability of Bcl-X_L to reduce resting ER Ca^{2+} concentrations is dependent on BI-1, a cytoprotective ER protein that associates with Bcl-2 and Bcl-X_L (Xu and Reed, 1998; Xu *et al*., 2008). Bax and Bak have opposing effects on ER Ca^{2+} concentrations.

The full implications of these changes in ER Ca^{2+} regulation by Bcl-2/Bax-family proteins are not entirely understood, but myriad Ca^{2+}-dependent cell death mechanisms exist that may be of relevance to circumstances where cellular stress causes the ER to dump Ca^{2+} into the cytosol, including (a) Ca^{2+}-induced mitochondrial membrane permeability transition, releasing apoptogenic proteins from mitochondria and stimulating reactive oxygen species production (Bernardi *et al*., 2006); (b) Ca^{2+}-sensitive mitochondrial fission protein Drp-1 (Szabadkai *et al*., 2004), which has been implicated in Bax-induced release of cytochrome *c* from mitochondria; (c) Ca^{2+}-sensitive phosphatases such as calcineurin, (PPase-B), which regulates activity of proapoptotic Bcl-2-family protein BAD (Wang *et al*., 1999) and which dephosphorylates NFAT-family transcription factors, allowing entry into the nucleus and transactivation of proapoptotic genes encoding Fas-ligand and Nur77/TR3 (Youn *et al*., 1999); (d) Ca^{2+}-dependent phospholipases and phospholipid scramblases, the latter of which have been suggested to transfer cardiolipin from the inner to the outer membrane of mitochondria (a signal for targeted

insertion of proapoptotic Bcl-2-family proteins Bid and Bax into mitochondrial membranes) (Lutter *et al*., 2000; Kuwana *et al*., 2002; McMillin and Dowhan, 2002); (e) Ca^{2+}-sensitive isoforms of nitric oxide synthase, thereby generating reactive nitrogen species and contributing to oxidative stress (reviewed by Orrenius *et al*., 2003; Benhar *et al*., 2006); (g) Ca^{2+}-binding protein TCTP (fortilin), a putative modulator of antiapoptotic Bcl-2/Bax-family proteins such as Mcl-1 (Liu *et al*., 2005); (h) Ca^{2+}/calmodulin-induced activation of the peptidyl proylyl isomerase, FKBP38, which binds to Bcl-2 and induces apoptosis (Edlich *et al*., 2005); (i) calpain-family cysteine proteases, Ca^{2+}-dependent proteases implicated in many pathological cell death scenarios and whose substrates include Bax and Bid (which are activated) (Wood *et al*., 1998; Wood and Newcomb, 1999; Chen *et al*., 2001), Bcl-2 and Bcl-X_L (which are inhibited), several caspases, and autophagy protein Atg5, which binds and inhibits Bcl-X_L upon cleavage (reviewed by Breckenridge *et al*., 2003; Yousefi *et al*., 2006); and (j) death-associated kinase (DAP kinase) and its close relative DRP-1, which contain calmodulin-binding domains (reviewed by Shohat *et al*., 2002), where DAP kinase can induce either apoptosis or autophagy (reviewed by Bialik and Kimchi, 2004). Moreover, Ca^{2+}-mediated activation of protein kinase C-theta (PKC-Θ) and of calmodulin-dependent kinase kinase-β induce autophagy in the context of ER stress (Hoyer-Hansen *et al*., 2007; Sakaki *et al*., 2008), having further implications for cell life and death mechanisms of relevance to circumstances where ER stress plays a role.

In addition to regulating ER Ca^{2+} homeostasis, Bcl-2-family proteins directly regulate Ire1, one of the three principal ER signal-transducing proteins involved in unfolded protein response (reviewed by Xu *et al*., 2005; Kim *et al*., 2006; Ron and Walter, 2007). Ire1 is a transmembrane ER resident protein that oligomerizes upon release by ER chaperones when unfolded proteins accumulate. Ire1 possesses an intrinsic serine/threonine kinase domain and triggers activation of a kinase pathway that includes Ask1, JNK and p38 MAPK. In addition, Ire1 has an endoribonuclease domain that processes mRNAs encoding XBP-1, a transcription factor involved in inducing expression of downstream transcription factor genes (for example, CHOP) that control the expression of *BCL-2*-family genes, such as *BIM* and *BCL-2*, to promote cell death. It has been reported that Bax and Bak directly bind and activate Ire1, whereas antiapoptotic Bcl-2 family proteins oppose this effect (McCullough *et al*., 2001; Hetz *et al*., 2006; Puthalakath *et al*., 2007) (Figure 1b).

Lysosomes

Lysosomes participate in cell life and death in at least two contexts—necrosis and autophagy. Rupture or deterioration of lysosomal membranes is a hallmark of necrotic cell death, and although Bcl-2 family proteins do not appear to insert into lysosomal membranes to an appreciable degree, they have been reported to suppress downstream sequeli of lysosomal enzyme release into the cytosol, particularly as pertains to effects on mitochondria (reviewed by Ferri and Kroemer, 2001; Kroemer *et al*., 2007). Lysosomes are also critically involved in autophagy, where autophagic vesicles fuse with lysosomes to accomplish the degradation of the contents for purposes of generating fuel for sustaining energy. In this regard, antiapoptotic Bcl-2 family proteins are well known for their ability to prolong survival of growth factor-dependent cells when deprived of the obligate growth factors. In fact, the antiapoptotic function of Bcl-2 was first elucidated in gene transfection studies where interleukin-3 (IL-3)-dependent murine hematopoietic cells were shown to cease division but to survive for prolonged periods in the absence of IL-3 when Bcl-2 was constitutively overexpressed (Vaux *et al*., 1988). Growth factor deprivation can also lead to nutrient deprivation, because expression of cell surface glucose transporters and amino-acid transporters depends on them. Prolonged nutrient deprivation invokes autophagy, an evolutionarily conserved response for catabolizing macromolecules and organelles, thereby generating substrates for ATP production (Edinger and Thompson, 2004; Lum *et al*., 2005). Autophagy is initially induced to prolong cell survival, but when taken to extremes, it may cause cell death. Indeed, circumstances have been identified where autophagy is induced and where Bcl-2 or Bcl-X_L either suppress or promote cell death, through mechanisms requiring components of the autophagic machinery (Baehrecke, 2005; Meier and Vousden, 2007). In this regard, Bcl-2 and Bcl-X_L suppress autophagy by binding the protein beclin 1 (Atg6), an essential component of the mammalian autophagy system that marks autophagic vesicles for fusion with lysosomes (Shimizu *et al*., 2004; Pattingre *et al*., 2005) (Figure 1c). Beclin 1 has been touted as a tumor suppressor gene, becoming haploinsufficient in some types of human cancers (Aita *et al*., 1999; Liang *et al*., 1999). Interestingly, protein-targeting studies suggest that the anti-autophagic function of Bcl-2 is conveyed from the ER (Pattingre *et al*., 2005).

Bcl-2-family protein interaction networks: dueling dimers

Bcl-2 family proteins contain up to four conserved Bcl-2 homology (BH) domains (Figure 2). All antiapoptotic members of the human Bcl-2 family contain BH1, BH2, BH3 and BH4, including Bcl-2, Bcl-X_L, Mcl-1, Bcl-W, Bfl-1 and Bcl-B (Figure 2). Mammalian proapoptotic proteins Bax, Bak and Bok contain BH1, BH2 and BH3 (but not BH4) and have been termed 'multi-domain' proapoptotic proteins (reviewed by Reed, 2006) (Figure 2). Where elucidated to date, the antiapoptotic and multi-domain proapoptotic Bcl-2 family proteins have been shown to share the same basic three-dimensional protein fold, comprised of a bundle of amphipathic α-helices surrounding a core pair of hydrophobic α-helices, bearing striking similarity to the pore-forming domains of bacterial toxins such as diphtheria toxin and the colicins (reviewed by Schendel *et al*., 1998).

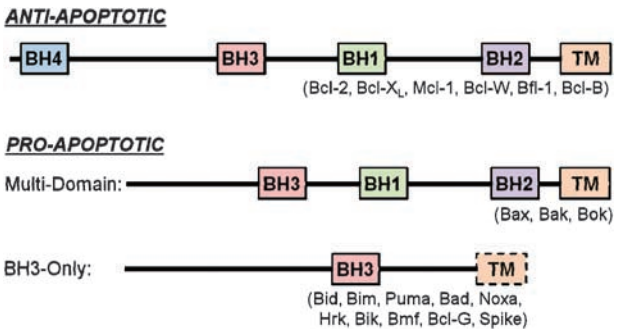

Figure 2 Comparisons of domain structures of Bcl-2-family proteins. Bcl-2-family proteins have at least one of four Bcl-2 homology (BH) domains (BH1, BH2, BH3 or BH4), and typically also possess a transmembrane domain (TM). Antiapoptotic Bcl-2-family members contain all four BH domains. Proapoptotic Bcl-2-family members can be separated into 'multi-domain' or 'BH3-only' proteins. Some BH3-only proteins do not have a TM (dotted line). Note that some BH3-only proteins have a limited set of BH domains (for example, Bcl-G has BH2 and BH3).

Bcl-2-family proteins dimerize with each other through BH3 domain-dependent interactions (Chittenden *et al*., 1995). Many proapoptotic proteins share sequence homology only in the BH3 domain ('BH3-only' proteins), including Bid, Bim, Hrk, Bik, Puma, Noxa, Bcl-G_S, Bmf and possibly Spike (reviewed by Strasser, 2005) (Figure 2). These BH3-only proteins uniformly operate as antagonists of antiapoptotic Bcl-2-family proteins in a BH3-dependent manner that correlates with BH3-dependent binding. However, some BH3-only proteins, namely Bid, Bim and Puma, have dual functions as both antagonists of antiapoptotic Bcl-2 family members and as agonists of proapoptotic multi-domain proteins Bax and Bak (reviewed by Korsmeyer *et al*., 2000) (Figure 1a).

Structural studies have shown that the BH3 peptide is an amphipathic α-helix of ~16–20 amino-acids length that binds a hydrophobic pocket on the surface of antiapoptotic Bcl-2-family proteins, thus revealing the structural basis for antagonism of pro- and antiapoptotic members of the family and setting the stage for subsequent drug discovery strategies based on mimicking BH3 peptides with chemical compounds that bind the same pocket (Muchmore *et al*., 1996; Sattler *et al*., 1997; Oltersdorf *et al*., 2005). Several chemical inhibitors of antiapoptotic Bcl-2-family proteins based on mimicking BH3 peptides have been described (Wang *et al*., 2000a, b; Degterev *et al*., 2001; Enyedy *et al*., 2001; Tzung *et al*., 2001; Kutzki *et al*., 2002; Leone *et al*., 2003; Becattini *et al*., 2004; Pellecchia and Reed, 2004; Walensky *et al*., 2004; Oltersdorf *et al*., 2005; Mohan *et al*., 2007; Nguyen *et al*., 2007; Kitada *et al*., 2008), and at least three of these agents are currently being tested in human clinical trials for cancer (reviewed by Reed and Pellecchia, 2005). The structural basis for activation of Bax and Bak by BH3 peptides has not yet been reported. Agonistic BH3 peptides induce Bax and Bak oligomerization in mitochondrial membranes and in synthetic liposomes, and thus chemicals that mimic their actions could also provide another path forward towards therapeutics (Letai *et al*., 2002). A variety of post-translational modifications and protein interactions have been reported to control the availability of BH3-only proteins for dimerization with other Bcl-2-family members. Thus, compounds that target the relevant signal-transduction pathways might also find utility as activators of BH3-only proteins. Indeed, this notion embodies the observation that longer isoforms of the proapoptotic Bim protein associate through the dynein light chain with microtubules, requiring release to translocate to intracellular membranes and dimerize with other members of the Bcl-2 family (Puthalakath *et al*., 1999), hence, suggesting a probable role for Bim in the cytotoxic mechanisms of antimicrotubule drugs.

Phenotypic conversion of Bcl-2

Bcl-2 is best known for its ability to suppress apoptosis, but a variety of studies have suggested that it may undergo conversion from protector to killer under some circumstances. For example, proteolytic removal of N-terminal sequences by caspase-mediated cleavage reverses the phenotype of Bcl-2 (Cheng *et al*., 1997). Interestingly, mutations in translocated *BCL-2* alleles have been identified in human lymphomas that ablate the asparatic acid residue required for caspase cleavage (Tanaka *et al*., 1992), suggesting that some tumors may evolve strategies for avoiding Bcl-2 phenotype reversal. In addition, the orphan nuclear receptor Nur77 can be induced to translocate from nucleus to cytosol, binding Bcl-2, and inducing a conformational change in Bcl-2 that probably mimics what happens during caspase cleavage, exposing the normally buried BH3 domain of Bcl-2 and causing it to function as a proapoptotic protein (Lin *et al*., 2004). A potentially similar mechanism was identified for Bcl-X_L, showing that lipid modifications of K-Ras can promote its association with Bcl-X_L on mitochondria and induce apoptosis (Bivona *et al*., 2006). Thus, depending on with what proteins Bcl-2 and Bcl-X_L interact, their phenotypes can be converted from anti- to proapoptotic, revealing an additional level of complexity to these proteins that has clear therapeutic implications for malignancies that overexpress these Bcl-2-family proteins. Perhaps phenotypic conversion underlies the recent discovery of compounds that show superior cytotoxic activity in Bcl-2/Bcl-X_L overexpressing cells compared to control cells (Schwartz *et al*., 2007), as well as providing a potential explanation for why high levels of Bcl-2 expression are sometimes associated with better patient prognosis (see for example, Silvestrini *et al*., 1994).

Bcl-2-family proteins—at the center of the action

While much research has focused on homo- and heterodimerization among Bcl-2-family proteins, many if not most of these proteins have other interaction partners that regulate their activity and that link them to a wide variety of cellular pathways, giving the impression

that Bcl-2-family proteins operate as critical nodes in complex networks to integrate information and make ultimate life/death decisions. For the more extensively studied members of the family, such as Bcl-2, Bcl-X_L and Bax, an impressive list of potential interacting proteins has accumulated (reviewed by Reed, 2008). The structural basis for most of these non-familial protein interactions has not been elucidated, and their roles in physiology are not always well understood, but the extensive repertoire of partners suggests that diverse signaling, developmental and metabolic pathways converge on Bcl-2-family members.

A few examples exist where Bcl-2 family proteins serve as regulators of other types of proteins, rather than the converse, where the interacting proteins regulate the activity of the Bcl-2-family members. For example, proapoptotic protein BAD has been reported to associate with a protein complex containing hexose kinase and regulate glucose-stimulated insulin secretion by pancreatic β cells (Danial *et al*., 2008). In addition, Bcl-2 and Bcl-X_L bind to and suppresses NLR-family protein NLRP1 (NALP1), an activator of inflammatory caspases involved in proteolytic processing and maturation of pro-IL-1β , pro-IL-18 and pro-IL-33 (Bruey *et al*., 2007). The aforementioned interaction of Bcl-2 and Bcl-X_L with autophagy protein beclin 1 (Liang *et al*., 1998) is another example of an effector function achieved through interactions with non-homologous proteins, in this case linking Bcl-2 to suppression of autophagy (Pattingre *et al*., 2005). Thus, Bcl-2 family proteins appear to regulate aspects of metabolism, innate immunity and autophagy, in addition to their central roles in cell life and death.

Current and future therapeutic opportunities

The fruits of over three decades of research on Bcl-2-family proteins have yielded new strategies for therapeutic intervention, some of which have advanced to clinical testing in humans (Table 1). The most advanced of these candidate therapeutics is a nuclease-resistant phosphorothioate antisense oligonucleotide targeting Bcl-2 mRNA (oblimersen sodium), which has shown promising activity for chronic lymphocytic leukemia and malignant melanoma in randomized phase III trials (Reed *et al*., 1990; Klasa *et al*., 2002; Bedikian *et al*., 2006; O'Brien *et al*., 2007). Results for myeloma, AML and hormone refractory prostate cancer however were not encouraging, perhaps due to overexpression of additional antiapoptotic members of the family besides Bcl-2 in these malignancies. Several small-molecule chemical antagonists of antiapoptotic Bcl-2-family proteins have been described that bind the same pocket occupied by proapoptotic BH3 domains (reviewed by Reed and Pellecchia, 2005). These compounds have different potencies and variable spectra of activity against the six antiapoptotic members. Compounds that appear to convert Bcl-2, Bcl-X_L or their relatives from protectors to killers have been identified, including molecules that directly bind these proteins (Manion *et al*., 2004; Schwartz *et al*., 2007) and others that trigger Nur77 translocation from nucleus to cytosol, where it can bind Bcl-2, Bfl-1 and Bcl-B (Han *et al*., 2006; Luciano *et al*., 2007). Compounds targeting the non-BH3 site on Bcl-2 and Bcl-B possibly where Nur77 binds have also been recently reported, providing a firm starting point for drug optimization (Yip *et al*., 2008). Altogether, these activities raise hope that a new class of anticancer drugs may soon be available based on targeting Bcl-2-family proteins.

Table 1 Summary of candidate therapeutics targeting Bcl-2 family proteins

Active agent	*Sponsoring company*	*Stage*
Genasense (oblimersen sodium; *antisense*)	Genta	Phase III
ABT-737/ABT-263	Abbott Laboratories	Phase I/II
Epigallocatechin gallate	Mayo Clinic	Phase I/II
Gossypol	Ascenta/NCI	Phase I/II
GX-15-070 (obatoclax)	Gemin X	Phase I/II
Polyphenon E	Mayo Clinic (Mitsui Norin)	Phase I
Antimycin A3	University of Washington	Preclinical
Apogossypol	Burnham Institute for Medical Research/NCI	Preclinical
Apogossypolone	University of Michigan	Preclinical
BH3I-1/BH3I-2	Harvard University	Preclinical
Compound 6	University of Michigan	Preclinical
CPM-1285 analogs	Raylight Chemokine Pharmaceuticals	Preclinical
HA14-1 analogs	Raylight Chemokine Pharmaceuticals	Preclinical
SAHBs	Harvard University	Preclinical
Terphenyl derivative	Yale University	Preclinical
Theaflavin	Burnham Institute for Medical Research	Preclinical

Abbreviation: NCI, National Cancer Institute.

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