BCL-2 Family Proteins: Critical Checkpoints of Apoptotic Cell Death

Nika N. Danial

Abstract Apoptosis is a morphologically distinct form of programmed cell death essential for normal development and tissue homeostasis. Aberrant regulation of this pathway is linked to multiple human diseases, including cancer, autoimmunity, neurodegenerative disorders, and diabetes. The BCL-2 family of proteins constitutes a critical control point in apoptosis residing immediately upstream of irreversible cellular damage, where family members control the release of apoptogenic factors from mitochondria. The cardinal member of this family, BCL-2, was originally discovered as the defining oncogene in follicular lymphomas, located at one reciprocal breakpoint of the t(14;18) (q32;q21) chromosomal translocation. Since this original discovery, remarkable efforts marshaled by many investigators around the world have advanced our knowledge of the basic biology, molecular mechanisms, and therapeutic targets in the apoptotic pathway. This review highlights findings from many laboratories that have helped uncover some of the critical control points in apoptosis. The emerging picture is that of an intricate cellular machinery orchestrated by tightly regulated molecular interactions and conformational changes within BCL-2 family proteins that ultimately govern the cellular commitment to apoptotic death.

Apoptosis is a conserved genetic and biochemical pathway whose basic tenets are present in all metazoans (1, 2). In mammals, the execution of this pathway is governed by two molecular programs which ultimately lead to the activation of select members of the caspase (cysteinyl aspartate–specific protease) family. Subsequently, cleavage of key cellular substrates ensues, leading to cell demise. The two molecular programs are known as the extrinsic pathway operating downstream of death receptors, such as Fas and the tumor necrosis factor receptor family, and the intrinsic pathway, which is activated by a diverse array of stress signals. The identification of cytochrome *c* as an apoptogenic factor released from mitochondria marked a pivotal breakthrough in uncovering the importance of this organelle in the intrinsic pathway of apoptosis (3). The "point of no return" in this pathway is defined by mitochondrial outer membrane permeabilization (MOMP), which leads to the release of cytochrome *c* (4). BCL-2 family proteins regulate MOMP and thereby determine the cellular commitment to apoptosis. This review is limited in scope to the intrinsic pathway and its regulation by BCL-2 family of proteins. In particular, recent advances in understanding the interplay between distinct members of the BCL-2 family and the molecular mechanisms underlying their regulation of MOMP are described. Broader overview of the BCL-2 family can be found in reviews published elsewhere (1, 2). Likewise, the role of the intrinsic pathway and apoptosis in the larger context of mechanisms of cell death are the subject of another review in this issue of *CCR Focus* (5).

Execution of the Apoptotic Program

Caspases are present as inactive zymogens that are activated during apoptosis (6). Of note, not all caspase family members participate in apoptosis. For example, caspase-1 and caspase-11 are predominantly involved in the processing of proinflammatory cytokines (interleukins 1 and 18). Comprehensive descriptions of caspases and their diverse cellular functions have been covered in other recent reviews (7–10). The following section briefly highlights the mechanisms underlying caspase activation during apoptosis.

Depending on their mode of activation, caspases are categorized as either initiator or effector caspases (11). Initiators such as caspase-8 and caspase-9 are apical caspases that are activated on binding to specialized molecular platforms that are assembled through selective protein-protein interactions. Activated initiator caspases can then cleave and activate effector caspases, such as caspase-3 and caspase-7, through trans-proteolytic processing (12). The molecular platforms in charge of initiator caspase activation are formed through interactions involving distinct protein folds or binding cassettes, of which three have been structurally characterized: death domains, death effector domains, and caspase recruitment domains (13). For example, the extrinsic pathway, which operates downstream of death receptors, such as Fas and the

Author's Affiliation: Department of Pathology, Harvard Medical School and Department of Cancer Biology, Dana-Farber Cancer Institute, Boston, Massachusetts
Received 6/29/07; revised 9/13/07; accepted 10/4/07.
Grant support: Burroughs Wellcome Fund Career Award in Biomedical Sciences and NCI grant KO1CA10659 (N.N. Danial).
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
Note: This review is dedicated to the memory of Stanley J. Korsmeyer, an extraordinary mentor, whose endless enthusiasm for scientific discovery, tireless efforts, scientific rigor, genuine leadership, and exceptional vision continue to inspire us.
Requests for reprints: Nika N. Danial, Department of Cancer Biology, Dana-Farber Cancer Institute, 44 Binney Street, Smith 756, Boston, MA 02115. Phone: 617-632-6436; E-mail: nika_danial@dfci.harvard.edu.

doi:10.1158/1078-0432.CCR-07-1598

tumor necrosis factor receptor family, leads to the recruitment of a death-inducing signaling complex (DISC) on ligand binding (ref. 14; Fig. 1A). This complex, in turn, recruits and activates caspase-8. In the intrinsic pathway, the molecular platform in charge of caspase activation is the apoptosome, a wheel-like heptameric structure (ref. 15; Fig. 1B). Apoptosome is composed of apoptotic protease–activating factor-1 (APAF-1), caspase-9, and cytochrome *c*, a component of the mitochondrial electron transport chain that is released during apoptosis (3). Several models for activation of initiator caspase-8 and caspase-9 have been proposed (12, 16, 17). According to the induced-proximity model, initiator caspases dimerize as their local concentration is increased during the assembly of DISC or apoptosome (18–20).

The BCL-2 Family of Cell Death Regulators

The BCL-2 family consists of both antiapoptotic and proapoptotic proteins, which share sequence homology within conserved regions known as BCL-2 homology (BH) domains (Fig. 2). BH domains correspond to α helical segments that dictate structure and function. All antiapoptotic members, such as BCL-2 and BCL-X_L, and a subset of proapoptotic family members, such as BAX and BAK, are multidomain proteins sharing sequence homology within three to four BH domains. The BH3-only subset of proapoptotic molecules, including BAD, BID, BIM, NOXA, BIK, HRK, and PUMA, show sequence homology only within a single α helical segment, the BH3 domain, which is also known as the minimal death domain required for binding to multidomain BCL-2 family members (21). The ability of BCL-2 proteins to selectively bind each other is integral to their function. The BH1, BH2, and BH3 domains of the antiapoptotic proteins form a hydrophobic groove that binds to the hydrophobic face of the amphipathic α-helical BH3 domain from a proapoptotic binding partner (22–27). The hydrophobic cleft formed by BH1-3 may be further stabilized by the BH4 domain (28). The unstructured loop between the BH3 and BH4 domains in BCL-2 and BCL-X_L is subject to phosphorylation, leading to inactivation of their survival function (29–31). Other posttranslational regulation mechanisms converging on this domain include caspase-mediated proteolytic cleavage, which leads to the removal of BH4, rendering these proteins proapoptotic (32, 33). In MCL-1, several amino acids between BH3 and BH4 domains are subject to posttranslational modification by ubiquitinylation (34), some of which are in close proximity of a GSK-3 phosphorylation site (35). Both of these posttranslational modifications have been shown to regulate the stability of the MCL-1 protein (34, 35). Mutational studies indicate that the BH4 domain is required for the function of antiapoptotic BCL-2 proteins (36–38). In addition, the BH4 domain may link BCL-2 to other signaling pathways through its interaction with multiple different proteins, including the phosphatase calcineurin (39), the Raf-1 kinase (40) and the mitochondrial chaperone FKB38 (41). A role for the BH4 domain in regulating nuclear factor-κB has also been reported (42, 43). In addition to BH domains, several BCL-2 family members possess a transmembrane domain and can localize to the subcellular membranes, including the mitochondrial outer membrane, endoplasmic reticulum (ER) and nuclear membranes (Fig. 2).

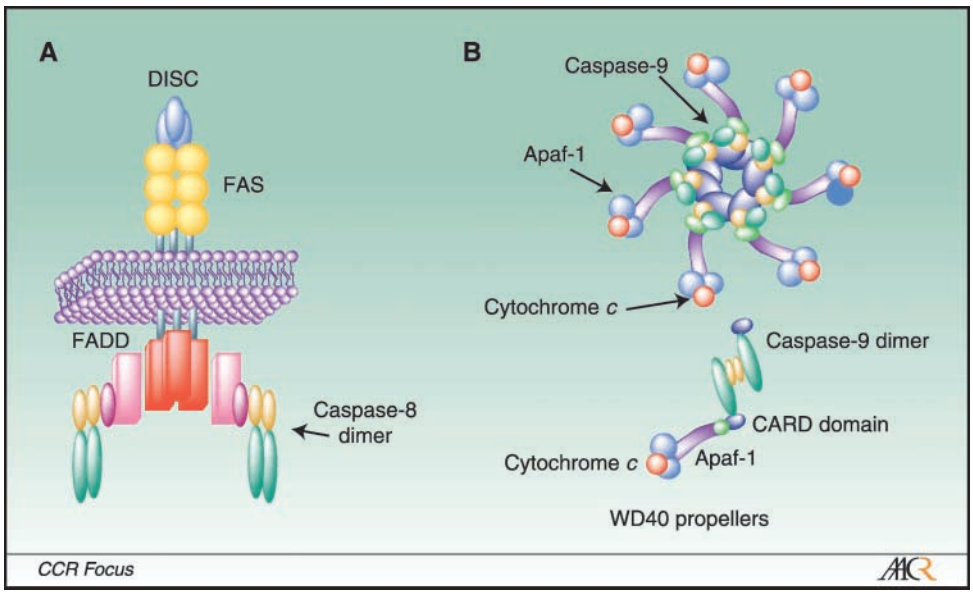

Fig. 1. Multiprotein complexes that constitute the platforms for caspase activation in the extrinsic (*A*) and intrinsic (*B*) pathways of apoptosis. *A,* the DISC is assembled on engagement of death receptors, such as Fas and tumor necrosis factor receptor family. Protein interaction domains (*blocks,* death domains; *ovals,* death effector domains) mediate the associations between death receptor, caspase-8, and adaptor protein FADD. *B,* the three-dimensional structure of the apoptosome resembles a seven-spoked disc (15), with procaspase-9 molecules bound at the hub extending above one surface, and APAF-1 adaptors aligned as spokes, presenting caspase recruitment domain (*CARD*; *mint green*), which interact with the caspase recruitment domain of procaspase-9 (*dark blue*) at the hub. At the rim of the spokes, the WD40 domains of APAF-1 (*blue*) bind cytochrome *c* (*red*), adopting a propeller-like protein conformation. Bottom, detailed composition of the WD40 propellers. Binding of initiator caspase-8 (*A*) and caspase-9 (*B*) to the DISC and apoptosome, respectively, results in their activation.

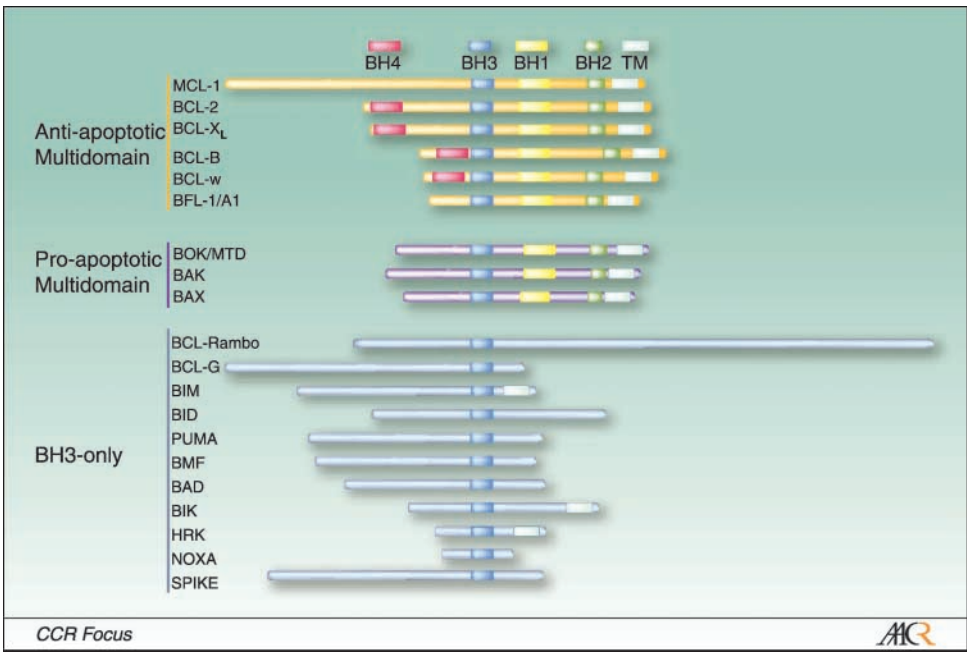

Fig. 2. Classification of BCL-2 family according to conserved domains.

The original characterization and subsequent study of the distinct BCL-2 family members provided an early indication that their function is linked to cancer. The founding member of this family, *BCL2*, was discovered as the defining oncogene in follicular lymphomas (44–46). Cells transduced with *BCL2* remained viable for extended periods in the absence of growth factors (47). Transgenic mice bearing a *BCL-2-Ig* minigene recapitulating the t(14:18) chromosomal translocation displayed B-cell follicular hyperplasia and over time progressed to diffuse large B-cell lymphoma (48). BCL-2 expression specifically blocked the morphologic features of apoptosis, including the plasma membrane blebbing, nuclear condensation, and DNA cleavage. Subsequent studies showed that BCL-2 expression was also required for tumor maintenance (49). Importantly, with the discovery of BCL-2, there emerged a new category of oncogenes, regulators of cell death, which unlike other oncogenes known at that time, did not promote proliferation but rather actively blocked apoptosis (1).

Alterations in the expression of other BCL-2 proteins have also been reported in cancer. Mutations in *BAX* coding region are found in ~50% of colorectal and gastric cancers (50). Furthermore, somatic missense mutations in the *BAD* and *BIK* genes have been identified in colon cancers and peripheral B cell lymphomas, respectively (51, 52). Loss of heterozygosity for *BIM* in mantle cell lymphoma (53) and *HRK* in glioblastoma (54) are but a few examples suggesting that certain proapoptotic members of the BCL-2 family may function as tumor suppressors. Unexpected findings have also suggested that beyond regulating apoptosis, select BCL-2 proteins may have alternate functions in other homeostatic pathways, including glucose metabolism (55), cell cycle checkpoint downstream of DNA damage (56, 57), and regulation of mitochondrial morphology (58).

A combination of genetic approaches, biochemical experiments, and pharmacologic studies has begun to unravel the molecular mechanisms underlying the function of BCL-2 family proteins. The BH3-only proapoptotic proteins are sentinels that sense apoptotic signals and communicate with the multidomain antiapoptotic and proapoptotic molecules to shift the balance of proapoptotic and antiapoptotic proteins towards death (Fig. 3).

BAX and BAK: gateway to the mitochondrial pathway of apoptosis. Cells that are doubly deficient in the two multidomain proapoptotic BAX and BAK fail to release cytochrome *c* and are resistant to all apoptotic stimuli that activate the intrinsic pathway, implicating these molecules as the requisite gateway to the mitochondrial apoptotic machinery (59, 60).

Activation of BAX and BAK during apoptosis involves multiple conformational changes that are accompanied by their mitochondrial intramembranous homo-oligomerization. The mechanisms leading to this conformational change, however, are distinct for each of these proteins. BAX is a soluble monomeric protein in the cytosol or is peripherally attached to mitochondrial membrane that inserts into the mitochondrial outer membrane (MOM) on receipt of a death stimulus (61, 62), whereas BAK is a mitochondria-resident protein. The three-dimensional structure of inactive BAX has shown that its COOH-terminal tail, which is required for its insertion into the MOM, is folded back into the BAX hydrophobic cleft formed by the BH1, BH2, and BH3 domains (63). Soon after induction of apoptosis, cytosolic BAX undergoes a conformational change that releases the COOH-terminal tail allowing BAX docking to mitochondria and exposing an NH_2-terminal epitope. Additional mutagenesis and structural characterization proposed that BAX monomers insert multiple helices (α5 and

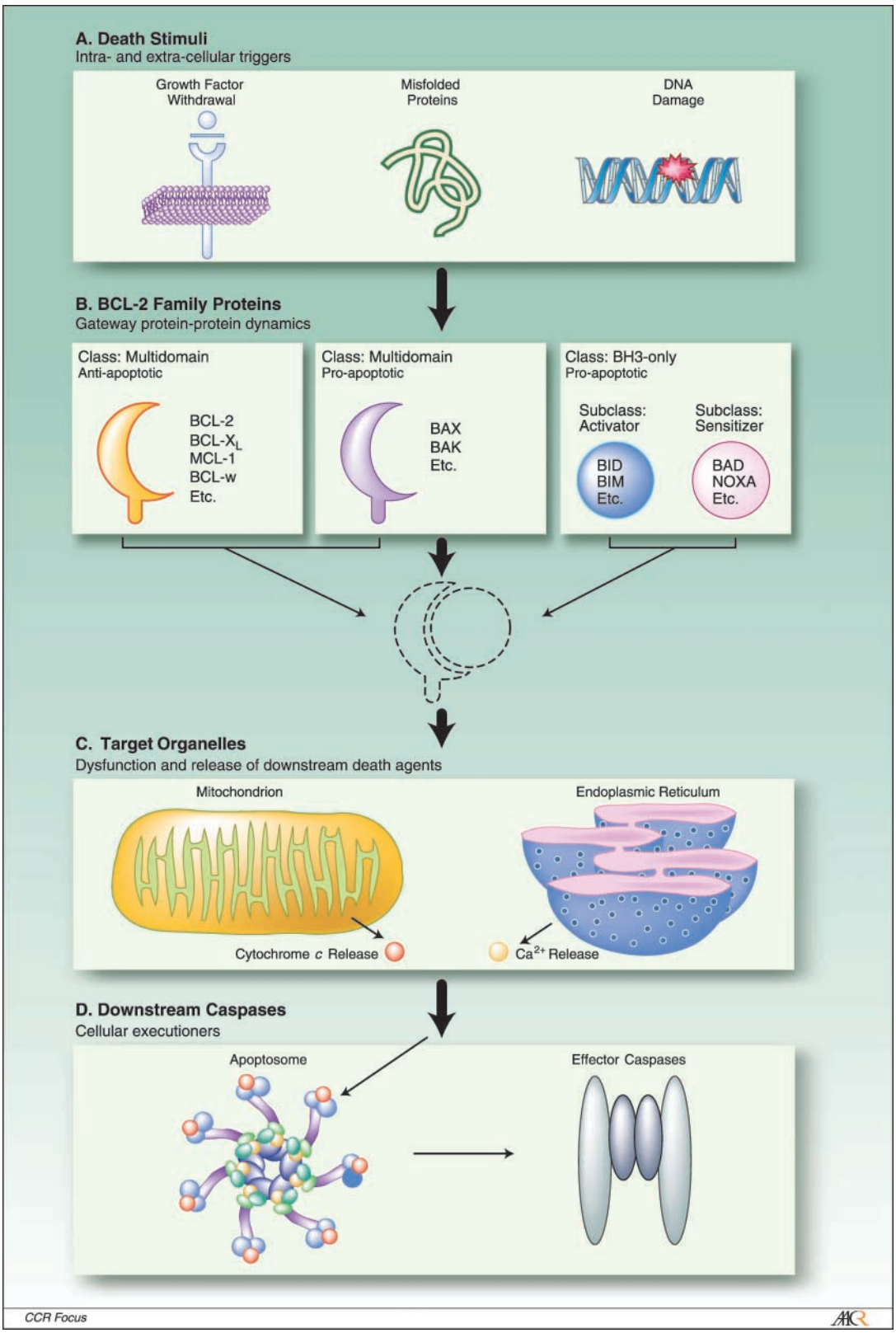

Fig. 3. Schematic representation of the intrinsic apoptotic pathway demonstrating a tiered process from sensing stress signals to organellar dysfunction. Stress signals from a variety of insults are sensed by BH3-only proapoptotic proteins and communicated to multidomain proapoptotic and antiapoptotic BCL-2 proteins. The functional interplay of the proteins ultimately results in the activation of BAX and BAK at target organelles such as mitochondria and ER, which participate in apoptosis by releasing apoptogenic factors and Ca^{2+}, respectively.

α6) into the MOM in addition to its COOH-terminal tail (64). Membrane-integrated monomers subsequently oligomerize to form pores in a manner that is dependent on the exposed NH_2-terminal epitope (64). Multiple conformer-specific binding partners of BAX have been identified and proposed to regulate BAX translocation, insertion, or oligomerization (65). Among these, roles for both antiapoptotic BCL-2 proteins and BH3-only proapoptotic molecules have been proposed (discussed later). A growing body of evidence also shows that, in addition to protein-protein interactions, protein-lipid interactions influence BAX conformation and its ability to permeabilize the MOM. Notably, permeabilization of synthetic liposomes by BAX requires cardiolipin, a phospholipid enriched at the contact sites, where mitochondrial outer and inner membranes meet (66).

Unlike BAX, BAK monomers are integrated into the MOM before the induction of apoptosis. The intramembranous oligomerization of these monomers is inhibited by voltage-dependent anion channel 2, a MOM protein that binds BAK and stabilizes its monomeric conformation (67). Select BH3-only proteins can bind and displace voltage-dependent anion channel 2 from BAK to allow the conformational changes necessary for BAK activation (68). An alternative model has proposed that oligomerization and activation of BAK is specifically inhibited in healthy cells by its interaction with two select members of the prosurvival proteins, i.e., MCL-1 and BCL-X_L (69). These distinct mechanisms impinging on BAK activation may not be mutually exclusive; rather, they may serve to inhibit distinct stages of BAK conformational change and oligomerization.

Mitochondrial intramembranous homo-oligomerization of BAX and BAK is a prime candidate mechanism of MOMP and release of cytochrome *c* (4). Several studies suggest that membrane-activated conformers of BAX or BAK can subsequently activate other latent BAX or BAK molecules through an autoactivation mechanism, serving to amplify the signal leading to MOMP (70, 71). The intramembranous oligomerization of multidomain BCL-2 proteins is consistent with their structural similarities to model pore-forming proteins such as the diphtheria toxin T domain and bacterial colchicines (24). Indeed, structural and biophysical studies using synthetic lipid bilayers and vesicles support the intrinsic pore-forming capacity for several BCL-2 family proteins, including BAX, BCL-2, and BCL-X_L (72–75). Whether these higher order structures possess channel activity *in vivo* awaits further experimentation. Recent findings suggest that only the BAX-activated membrane conformers could form larger oligomers consistent with channels of sufficient diameter to allow the efflux of cytochrome *c* (76, 77). Antiapoptotic BCL-2 and BCL-X_L block channel formation by BAX (72, 76, 77). It is currently unclear whether cytochrome *c* release occurs through a distinct BAX/BAK pore, a hybrid pore with other mitochondria-resident proteins, or some other global mechanism of membrane permeabilization. Alternative mechanisms for pore formation by BAX may also exist. For example, BAX has been reported to decrease the stability of planar lipid bilayers by decreasing linear tension within the membrane, resulting in hydrophilic pores within the lipid membrane itself (78).

In addition to their role at the mitochondria, BCL-2 family proteins affect Ca^{2+} dynamics at the ER, which in turn, could influence the threshold for apoptosis (79, 80). The ER Ca^{2+} dynamics directly affect the function of mitochondria as these organelles are in close proximity and mitochondria take up Ca^{2+} released by the ER (81). Supra physiologic levels of Ca^{2+} can prompt the opening of a mitochondrial inner-membrane large conductance channel known as the permeability transition pore, which can eventually cause the swelling and rupture of mitochondria (82). Cells overexpressing BCL-2 or those deficient for both BAX and BAK show lower levels of ER Ca^{2+} and consequently lower Ca^{2+} entry into the mitochondrion (83, 84). Lower ER Ca^{2+} content in these cells is associated with higher rate of ER Ca^{2+} leak (79, 85). Consequently, Ca^{2+} mobilizing death stimuli, including C2-ceramide, arachidonic acid, and oxidative stress specifically require the function of BAX and BAK at the ER (84).

BH3-only proapoptotic proteins: apoptotic sentinels upstream of BAX and BAK. BH3-only molecules, including BAD, BID, BIM, NOXA, and PUMA are upstream sentinels that selectively respond to proximal death and survival signals, and require BAX/BAK to induce death (Fig. 3; refs. 60, 86). Genetic loss-of-function models together with biochemical studies point to an emerging paradigm for BH3-only proteins, which consists of latent lethality requiring the transcription or posttranslational modifications for activation in a tissue-restricted and signal-specific manner. For example, cytosolic BID is activated on cleavage by caspase-8 (tBID; refs. 87, 88). On the other hand, BAD's proapoptotic activity is regulated through phosphorylation (89, 90). Other BH3 proteins interact with distinct extramitochondrial targets. For example, BIM is localized to the microtubule dynein motor complex by binding to the dynein light chain 1, and BMF associates with dynein light chain 2 in the myosin V actin motor complex (91). Lastly, the activation of NOXA and PUMA is under direct transcriptional regulation by p53, a finding that is consistent with their roles as specialized death sentinels during DNA damage (92–94). Thus, the large number of BH3-only members is indicative of specialization, rather than redundancy. The unique localizations, protein associations, and mechanisms of activation for these individual BH3-only proteins suggest that each acts as a sentinel for distinct damage signals, thereby increasing the range of input for stress signals, including DNA damage, growth factor and glucose withdrawal, unfolded proteins and hypoxia.

Proapoptotic activity of BH3-only proteins is associated with exposure of the hydrophobic face of their BH3 helix, enabling it to interact with the hydrophobic groove of multidomain dimerization partners. Extensive binding studies using peptides derived from the BH3 domain of BH3-only molecules have assessed the affinities and selectivity of their interactions with multidomain BCL-2 proteins (95–97). These studies have given rise to two current models of how upstream BH3-only molecules trigger the activation of BAX and BAK to induce MOMP (Fig. 4). Experimental evidence for both models has been presented using mutational analysis, loss-of-function models, and *in vitro* studies with isolated mitochondria.

Indirect activation model. The indirect activation model surmises that the principal function of antiapoptotic BCL-2,

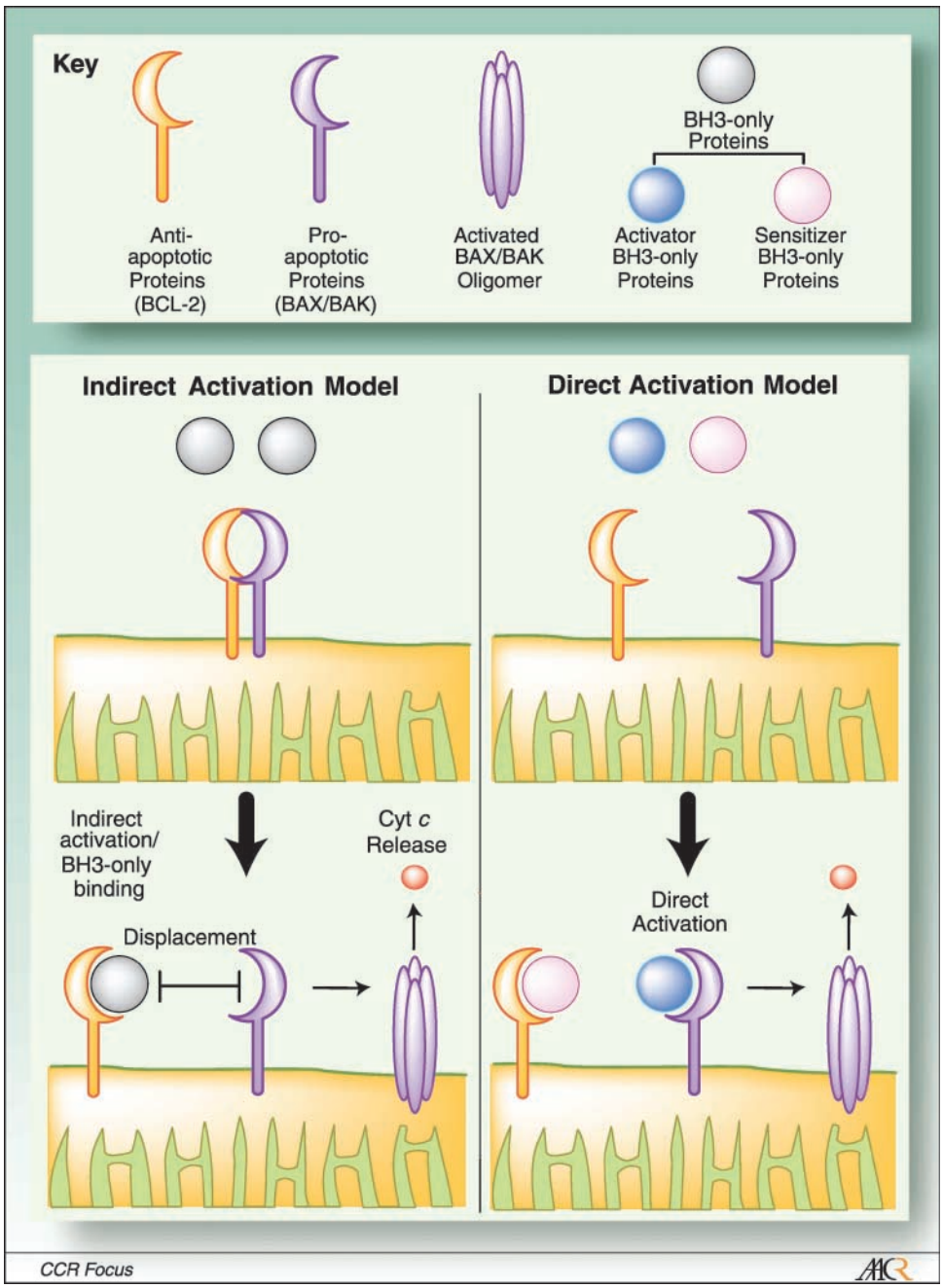

Fig. 4. Models for the activation of BAX and BAK downstream of BH3-only proteins. In the indirect activation model, the primary function of antiapoptotic proteins is to bind and inhibit proapoptotic BAX or BAK. BH3-only molecules bind antiapoptotic proteins to displace them from BAX or BAK. In the direct activation model, BH3-only proteins are functionally subdivided into activators and sensitizers. The primary function of antiapoptotic BCL-2 proteins is to bind and sequester both the activator and sensitizer subclasses. Activation of BAX and BAK requires their direct association with activator BH3-only proteins. However, normally these activators are bound by antiapoptotic proteins. BAX and BAK activation ensues when the number of activator molecules exceeds the neutralizing capacity of antiapoptotic proteins. Sensitizer BH3-only proteins do not activate BAX and BAK directly, but lower the threshold for apoptosis by occupying antiapoptotic members and releasing activators to trigger BAX and BAK oligomerization.

$BCL\text{-}X_L$ and MCL-1 is to inhibit BAX and BAK (ref. 1; Fig. 4). These inhibitory interactions between antiapoptotic and proapoptotic multidomain molecules seem to be selective. For example, in certain cell types, MCL-1 and $BCL\text{-}X_L$, but not BCL-2 directly bind and inhibit BAK (69). BH3-only proteins initiate apoptosis primarily by binding these prosurvival molecules and thereby neutralizing their inhibitory effect on BAX and BAK (Fig. 4; refs. 69, 95, 98, 99). BAX and BAK are then displaced from antiapoptotic molecules to undergo conformational changes required for their activation. Consistent with a displacement reaction, biochemical studies have shown that on NOXA overexpression, the BAK-MCL-1 protein complexes diminish whereas binding of MCL-1 and NOXA increases (69). This model places the BH3-only proteins upstream of antiapoptotic molecules, which are in turn upstream of BAX and BAK. Two categories of BH3 domains exist. One class includes BID, BIM, and PUMA which bind all antiapoptotic molecules and are more potent in activating apoptosis (95). The other category includes NOXA and BAD, which engage only a select group of antiapoptotic proteins and their combined activation is required to kill cells in which MCL-1 and $BCL\text{-}X_L$ are the relevant antiapoptotic molecules (95). Accordingly, a NOXA mutant designed to bind $BCL\text{-}X_L$ in addition to MCL-1 was sufficient to induce apoptosis in the

absence of BAD (69). In this model, commitment to apoptosis is ensured when all antiapoptotic BCL-2 proteins are neutralized by BH3-only molecules (95, 99). As such, NOXA and BAD together proved sufficient to kill cells with the combined inactivation of BIM and BID (99).

Direct activation model. The direct activation model proposes that the two categories of BH3 domain are activator BH3-only proteins, which bind both antiapoptotic and proapoptotic multidomain partners and sensitizer (97), also referred to as derepressor (96) or inactivator (68) BH3-only proteins, which show high affinity binding solely to antiapoptotic partners such as BCL-2/BCL-X_L/MCL-1. Activators like BID and BIM can directly bind and induce the oligomerization of BAX/BAK leading to MOMP (refs. 68, 96, 97, 100; Fig. 4). Of note, two reports have proposed that PUMA is also an activator BH3-only molecule analogous to BID and BIM (68, 101). Furthermore, the possibility that additional activator proteins may exist has been put forward (102). According to the direct activation model, the primary function of antiapoptotic BCL-2 family proteins is to sequester BH3-only molecules (86). The balance is shifted towards apoptosis when cellular levels of activator molecules exceed the neutralizing capacity of antiapoptotic members. Sensitizer BH3-only proteins lower the threshold of apoptosis by occupying the binding pocket of antiapoptotic molecules, and allowing activator BH3-only proteins to engage BAX/BAK to induce MOMP, thus shifting the rheostatic balance of multidomain antiapoptotic BCL-2/BCL-X_L/MCL-1 and proapoptotic BAX/BAK towards apoptosis.

Evidence in support of the direct association of BAX and BAK with full-length activator BH3-only proteins (68, 101) or peptides derived from their BH3 domain has recently been presented (97, 100, 101). This association is likely transient, however, in that once induced to oligomerize, BAX and BAK no longer remain in association with activator molecules (76). This observation is consistent with a hit-and-run scenario (103), in which few molecules of activator proteins are sufficient to catalyze the initial activation of BAX and BAK.

Detailed mutagenesis studies that used differential binding mutants of BH3-only proteins defective in binding either the antiapoptotic or proapoptotic multidomain partners (68, 104, 105) or mutants of multidomain BCL-2 proteins with differential binding capacity to BH3-only molecules (68) have provided further support for the direct activation model. For example, a BID BH3 mutant (M97A, D98A; ref. 21), incapable of binding antiapoptotic molecules, activated BAX to release cytochrome *c* and induce apoptosis (68, 104, 105), suggesting that the primary mechanism by which BH3-only molecules induce apoptosis does not require their association with BCL-2, MCL-1, or BCL-X_L. Consistent with these findings, neutralization of all antiapoptotic members by NOXA and BAD was insufficient to kill cells in which none of the proposed activator BH3-only proteins (BIM, BID, and PUMA) was available to directly activate BAX and BAK (68).

The indirect and direct activation models may not be mutually exclusive despite the seemingly contradictory nature of the data endorsing one versus the other. Points of convergence between the two may exist. For example, the indirect activation model suggests that the BAX or BAK molecules exist as two conformers within cells; primed with their BH3 domain exposed and unprimed with their BH3 domain hidden (1). The prosurvival BCL-2, BCL-X_L, and MCL-1 bind the primed conformer, as the BH3 domain of BAX and BAK is required for this interaction (69). The priming signal in charge of the shift between these two conformers, however, has not been defined. One possible mechanism for this priming event is the activator BH3-only molecules, as put forward by the direct activation model.

Recent independent studies assessed the conformational changes and binding interactions that occur between the multidomain antiapoptotic and proapoptotic molecules within the mitochondrial membrane and presented evidence in support of a different model for BAX and BAK permeabilization of the MOM (76, 77). This model termed "embedded together" incorporates some aspects of both the indirect and direct activation models and emphasizes that interactions among different BCL-2 proteins involve both cytosolic and membrane conformers of select family members, each of which is subjected to distinct regulatory mechanisms, including binding affinities, on/off rates, and association with membrane lipids and/or other binding proteins (106). The ultimate effect of these interactions is to govern the intramembranous oligomerization of BAX/BAK. According to this model, BH3-only proteins bind and activate both antiapoptotic and proapoptotic multidomain molecules. This activation entails conformational changes that result in the insertion of multiple membrane spanning helices. For example, on activation by tBID, both BCL-2 and BAX undergo conformational changes that lead to the insertion of their helices 5 and 6 into the MOM (64, 76). These activated, membrane-inserted conformers of BCL-2 are themselves defective in oligomerization but can irreversibly bind membrane-embedded BAX and prevent its subsequent oligomerization (76). On tonic activation of BH3-only molecules, the number of inserted BAX conformers capable of oligomerization may exceed those that are inhibited by BCL-2. This release from inhibition, in addition to the ability of activated BAX to autoactivate other latent BAX molecules, triggers the activation of a sufficient number of BAX molecules to induce MOMP. The mechanistic details of these interactions are primarily defined for tBID, BCL-2, and BAX (71, 76). Whether BAK, MCL-1, and BCL-X_L are regulated through analogous mechanisms is not fully known. Although this model awaits the assessment of binding affinities of membrane-associated conformers of BCL-2 family proteins, it integrates elements from both direct and indirect activation models described above. Before insertion of their helices 5 and 6, antiapoptotic proteins can bind and sequester BH3-only proteins, whereas after membrane insertion and conformational change, they exert their inhibitory effects on BAX/BAK, as suggested by the indirect activation model. The membrane conformers of BAX and BAK that are inhibited by activated antiapoptotic molecules are initially generated by direct binding to select BH3-only proteins consistent with the direct activation model.

Therapeutic Implications

Defects in apoptosis are common, perhaps universal to cancer (107). Furthermore, aberrant apoptotic response in

cancer cells is associated with resistance to chemotherapy. In this section, we briefly highlight some of the strategies pursued to inhibit the antiapoptotic BCL-2 family proteins in cancer. In keeping with the scope of this review, we limit our discussion to BH3 mimetic compounds. For a more in-depth review of the drug discovery strategies and therapeutic targets in apoptosis, the reader is referred to accompanying articles by Verdine and Walensky, and Nathan and Benz in this issue of *CCR Focus* (108, 109) and to other recent comprehensive reviews elsewhere (110, 111). Additionally, an accompanying article by Rixe and Fojo provides a critical overview of therapies that induce cell death in comparison to those that block the proliferation of cancer cells (112).

The findings summarized in the previous sections suggest that compounds capable of occupying the hydrophobic pocket of antiapoptotic BCL-2 molecules may mimic the function of BH3-only molecules to lower the threshold for apoptosis. Indeed, several BH3 mimetic compounds have been reported to block the function of antiapoptotic molecules. ABT-737 is a BAD BH3 mimetic that binds and inhibits BCL-2, BCL-X_L, and BCL-w to induce apoptosis in malignant cells but not normal counterparts (113). This compound has shown promising single-agent efficacy in several types of cancer lines and tumor models, including small cell lung carcinoma, lymphoma and leukemia (113). Studies have indicated that resistance to ABT-737 can be associated with overexpression of MCL-1, an antiapoptotic BCL-2 family member that this BH3 mimetic does not target (114–116). Recently, a small molecule pleiotropic BH3 mimetic, TW-37, was designed with high affinity to MCL-1 in addition to BCL-2 and BCL-X_L (117). TW-37 has been shown to work synergistically with mitogen-activated protein kinase inhibitors to selectively kill melanoma cells through a mechanism involving reactive oxygen species–mediated regulation of p53 (118). In addition, preclinical studies suggest that the combination of TW-37 and CHOP (cyclophosphamide-doxorubicin-vincristine-prednisone) may be promising in the treatment of diffuse large B cell lymphoma (119). Another BH3-mimetic compound, Gossypol has shown efficacy in chronic lymphocytic leukemia (120). GX15-070 is a small molecule that binds to Bcl-2, Bcl-w, Bcl-X_L, and Mcl-1, and is cytotoxic in chronic lymphocytic leukemia, mantle cell lymphoma, breast and non–small cell lung cancer cells (121–125). Other BH3 mimetic compounds include BH3-derived peptides that contain an all-hydrocarbon chain crosslink engineered using an olefin metathesis chemical synthesis strategy (126). These stabilized alpha-helices of BCL-2 domains or SAHB compounds are cell permeable, protease resistance and show selective high-affinity binding to multidomain BCL-2 family members. The prototype compound in this category, BID SAHB, was shown to kill leukemia cells both *in vitro* and *in vivo* (126). BID and BIM SAHB compounds directly bind and activate BAX to release cytochrome *c* (100).

Other approaches to inhibit the antiapoptotic BCL-2 family members in cancer include antisense strategies. Genasense or oblimersen (127) is an antisense oligonucleotide against BCL-2, which is currently in clinical trials. Molecules that specifically inhibit the production of both BCL-2 and BCL-X_L are also being considered (128).

Future Directions

Despite the remarkable progress made in uncovering the molecular underpinnings of apoptotic cell death, many unanswered questions remain. Understanding how the membrane-bound conformers of BCL-2 proteins function will allow the identification of additional control points in the apoptotic pathway. Similarly, the structural and molecular details of the interactions between different family members is far from complete, especially given the differential affinities and selectivity of these associations. Furthermore, although within each subfamily of BCL-2 proteins, general functional similarities exist, important differences in subcellular localization among various members and their selective preferred binding partners argue that additional regulatory mechanisms are yet to be discovered. Lastly, much remains to be learned about the cross-talk between apoptosis and other cellular physiologic pathways and alternate roles for different BCL-2 proteins beyond apoptosis are just beginning to be examined.

As the basic biology of apoptosis is being unraveled, rational targets for the development of a new generation of therapies are being identified. The discovery of BCL-2 in follicular lymphoma, followed by the characterization of other family members, their mode of action and generation of mimetic compounds that can disrupt their interactions constitute a success story that attests to the power and benefits of basic research in understanding and targeting cancer.

Acknowledgments

The author thanks Eric Smith for editorial assistance and the creation of figures, and extends an apology to the investigators whose works were not acknowledged because of space constraints.

References

1. Adams JM, Cory S. The Bcl-2 apoptotic switch in cancer development and therapy. Oncogene 2007; 26:1324–37.
2. Danial NN, Korsmeyer SJ. Cell death: critical control points. Cell 2004;116:205–19.
3. Li P, Nijhawan D, Budihardjo I, et al. Cytochrome c and dATP-dependent formation of Apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell 1997;91:479–89.
4. Chipuk JE, Bouchier-Hayes L, Green DR. Mitochondrial outer membrane permeabilization during apoptosis: the innocent bystander scenario. Cell Death Differ 2006;13:1396–402.
5. Amaravadi RK, Thompson CB. The roles of therapy-induced autophagy and necrosis in cancer treatment. Clin Cancer Res 2007;13:7271–9.
6. Nicholson DW. Caspase structure, proteolytic substrates, and function during apoptotic cell death. Cell Death Differ 1999;6:1028–42.
7. Cornelis S, Kersse K, Festjens N, Lamkanfi M, Vandenabeele P. Inflammatory caspases: targets for novel therapies. Curr Pharm Des 2007;13:367–85.
8. Kuranaga E, Miura M. Nonapoptotic functions of caspases: caspases as regulatory molecules for immunity and cell-fate determination. Trends Cell Biol 2007;17: 135–44.
9. Mariathasan S, Monack DM. Inflammasome adaptors and sensors: intracellular regulators of infection and inflammation. Nat Rev 2007;7:31–40.
10. Tinel A, Tschopp J. The PIDDosome, a protein

complex implicated in activation of caspase-2 in response to genotoxic stress. Science 2004;304:843–6.
11. Shi Y. Mechanisms of caspase activation and inhibition during apoptosis. Mol Cell 2002;9:459–70.
12. Riedl SJ, Salvesen GS. The apoptosome: signalling platform of cell death. Nat Rev Mol Cell Biol 2007;8:405–13.
13. Fesik SW. Insights into programmed cell death through structural biology. Cell 2000;103:273–82.
14. Peter ME, Krammer PH. The CD95(APO-1/Fas) DISC and beyond. Cell Death Differ 2003;10:26–35.
15. Acehan D, Jiang X, Morgan DG, Heuser JE, Wang X, Akey CW. Three-dimensional structure of the apoptosome: implications for assembly, procaspase-9 binding, and activation. Mol Cell 2002;9:423–32.
16. Bao Q, Shi Y. Apoptosome: a platform for the activation of initiator caspases. Cell Death Differ 2007;14:56–65.
17. Salvesen GS, Dixit VM. Caspase activation: the induced-proximity model. Proc Natl Acad Sci U S A 1999;96:10964–7.
18. Boatright KM, Renatus M, Scott FL, et al. A unified model for apical caspase activation. Mol Cell 2003;11:529–41.
19. Donepudi M, Mac Sweeney A, Briand C, Grutter MG. Insights into the regulatory mechanism for caspase-8 activation. Mol Cell 2003;11:543–9.
20. Renatus M, Stennicke HR, Scott FL, Liddington RC, Salvesen GS. Dimer formation drives the activation of the cell death protease caspase 9. Proc Natl Acad Sci U S A 2001;98:14250–5.
21. Wang K, Yin XM, Chao DT, Milliman CL, Korsmeyer SJ. BID: a novel BH3 domain-only death agonist. Genes Dev 1996;10:2859–69.
22. Day CL, Chen L, Richardson SJ, Harrison PJ, Huang DC, Hinds MG. Solution structure of prosurvival Mcl-1 and characterization of its binding by proapoptotic BH3-only ligands. J Biol Chem 2005;280:4738–44.
23. Hinds MG, Lackmann M, Skea GL, Harrison PJ, Huang DC, Day CL. The structure of Bcl-w reveals a role for the C-terminal residues in modulating biological activity. EMBO J 2003;22:1497–507.
24. Muchmore SW, Sattler M, Liang H, et al. X-ray and NMR structure of human Bcl-xL, an inhibitor of programmed cell death. Nature 1996;381:335–41.
25. Petros AM, Medek A, Nettesheim DG, et al. Solution structure of the antiapoptotic protein bcl-2. Proc Natl Acad Sci U S A 2001;98:3012–7.
26. Petros AM, Nettesheim DG, Wang Y, et al. Rationale for Bcl-xL/Bad peptide complex formation from structure, mutagenesis, and biophysical studies. Protein Sci 2000;9:2528–34.
27. Sattler M, Liang H, Nettesheim D, et al. Structure of Bcl-xL-Bak peptide complex: recognition between regulators of apoptosis. Science 1997;275:983–6.
28. Wang Y, Cao R, Liu D, et al. Oligomerization of BH4-truncated Bcl-x(L) in solution. Biochem Biophys Res Commun 2007;361:1006–11.
29. Chang BS, Minn AJ, Muchmore SW, Fesik SW, Thompson CB. Identification of a novel regulatory domain in Bcl-X(L) and Bcl-2. EMBO J 1997;16:968–77.
30. Haldar S, Basu A, Croce CM. Serine-70 is one of the critical sites for drug-induced Bcl2 phosphorylation in cancer cells. Cancer Res 1998;58:1609–15.
31. Yamamoto K, Ichijo H, Korsmeyer SJ. BCL-2 is phosphorylated and inactivated by an ASK1/Jun N-terminal protein kinase pathway normally activated at G(2)/M. Mol Cell Biol 1999;19:8469–78.
32. Cheng EH, Kirsch DG, Clem RJ, et al. Conversion of Bcl-2 to a Bax-like death effector by caspases. Science 1997;278:1966–8.
33. Clem RJ, Cheng EH, Karp CL, et al. Modulation of cell death by Bcl-XL through caspase interaction. Proc Natl Acad Sci U S A 1998;95:554–9.
34. Zhong Q, Gao W, Du F, Wang X. Mule/ARF-BP1, a BH3-only E3 ubiquitin ligase, catalyzes the polyubiquitination of Mcl-1 and regulates apoptosis. Cell 2005;121:1085–95.
35. Maurer U, Charvet C, Wagman AS, Dejardin E, Green DR. Glycogen synthase kinase-3 regulates mitochondrial outer membrane permeabilization and apoptosis by destabilization of MCL-1. Mol Cell 2006;21:749–60.
36. Borner C, Martinou I, Mattmann C, et al. The protein bcl-2α does not require membrane attachment, but two conserved domains to suppress apoptosis. J Cell Biol 1994;126:1059–68.
37. Hanada M, Aime-Sempe C, Sato T, Reed JC. Structure-function analysis of Bcl-2 protein. Identification of conserved domains important for homodimerization with Bcl-2 and heterodimerization with Bax. J Biol Chem 1995;270:11962–9.
38. Huang DC, O'Reilly LA, Strasser A, Cory S. The anti-apoptosis function of Bcl-2 can be genetically separated from its inhibitory effect on cell cycle entry. EMBO J 1997;16:4628–38.
39. Shibasaki F, Kondo E, Akagi T, McKeon F. Suppression of signalling through transcription factor NF-AT by interactions between calcineurin and Bcl-2. Nature 1997;386:728–31.
40. Wang HG, Rapp UR, Reed JC. Bcl-2 targets the protein kinase Raf-1 to mitochondria. Cell 1996;87:629–38.
41. Portier BP, Taglialatela G. Bcl-2 localized at the nuclear compartment induces apoptosis after transient overexpression. J Biol Chem 2006;281:40493–502.
42. de Moissac D, Zheng H, Kirshenbaum LA. Linkage of the BH4 domain of Bcl-2 and the nuclear factor κB signaling pathway for suppression of apoptosis. J Biol Chem 1999;274:29505–9.
43. Grimm S, Bauer MK, Baeuerle PA, Schulze-Osthoff K. Bcl-2 down-regulates the activity of transcription factor NF-κB induced upon apoptosis. J Cell Biol 1996;134:13–23.
44. Bakhshi A, Jensen JP, Goldman P, et al. Cloning the chromosomal breakpoint of t(14;18) human lymphomas: clustering around JH on chromosome 14 and near a transcriptional unit on 18. Cell 1985;41:899–906.
45. Cleary ML, Sklar J. Nucleotide sequence of a t(14;18) chromosomal breakpoint in follicular lymphoma and demonstration of a breakpoint-cluster region near a transcriptionally active locus on chromosome 18. Proc Natl Acad Sci U S A 1985;82:7439–43.
46. Tsujimoto Y, Cossman J, Jaffe E, Croce CM. Involvement of the bcl-2 gene in human follicular lymphoma. Science 1985;228:1440–3.
47. Vaux DL, Cory S, Adams JM. Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. Nature 1988;335:440–2.
48. McDonnell TJ, Korsmeyer SJ. Progression from lymphoid hyperplasia to high-grade malignant lymphoma in mice transgenic for the t(14; 18). Nature 1991;349:254–6.
49. Letai A, Sorcinelli MD, Beard C, Korsmeyer SJ. Antiapoptotic BCL-2 is required for maintenance of a model leukemia. Cancer Cell 2004;6:241–9.
50. Miquel C, Borrini F, Grandjouan S, et al. Role of bax mutations in apoptosis in colorectal cancers with microsatellite instability. Am J Clin Pathol 2005;123:562–70.
51. Arena V, Martini M, Luongo M, Capelli A, Larocca LM. Mutations of the BIK gene in human peripheral B-cell lymphomas. Genes Chromosomes Cancer 2003;38:91–6.
52. Lee JW, Soung YH, Kim SY, et al. Inactivating mutations of proapoptotic Bad gene in human colon cancers. Carcinogenesis 2004;25:1371–6.
53. Tagawa H, Karnan S, Suzuki R, et al. Genome-wide array-based CGH for mantle cell lymphoma: identification of homozygous deletions of the proapoptotic gene BIM. Oncogene 2005;24:1348–58.
54. Nakamura M, Ishida E, Shimada K, Nakase H, Sakaki T, Konishi N. Frequent HRK inactivation associated with low apoptotic index in secondary glioblastomas. Acta Neuropathol (Berl) 2005;110:402–10.
55. Danial NN, Gramm CF, Scorrano L, et al. BAD and glucokinase reside in a mitochondrial complex that integrates glycolysis and apoptosis. Nature 2003;424:952–6.
56. Kamer I, Sarig R, Zaltsman Y, et al. Proapoptotic BID is an ATM effector in the DNA-damage response. Cell 2005;122:593–603.
57. Zinkel SS, Hurov KE, Ong C, Abtahi FM, Gross A, Korsmeyer SJ. A role for proapoptotic BID in the DNA-damage response. Cell 2005;122:579–91.
58. Karbowski M, Norris KL, Cleland MM, Jeong SY, Youle RJ. Role of Bax and Bak in mitochondrial morphogenesis. Nature 2006;443:658–62.
59. Lindsten T, Ross AJ, King A, et al. The combined functions of proapoptotic Bcl-2 family members bak and bax are essential for normal development of multiple tissues. Mol Cell 2000;6:1389–99.
60. Wei MC, Zong WX, Cheng EH, et al. Proapoptotic BAX and BAK: a requisite gateway to mitochondrial dysfunction and death. Science 2001;292:727–30.
61. Goping IS, Gross A, Lavoie JN, et al. Regulated targeting of BAX to mitochondria. J Cell Biol 1998;143:207–15.
62. Wolter KG, Hsu YT, Smith CL, Nechushtan A, Xi XG, Youle RJ. Movement of Bax from the cytosol to mitochondria during apoptosis. J Cell Biol 1997;139:1281–92.
63. Suzuki M, Youle RJ, Tjandra N. Structure of Bax: coregulation of dimer formation and intracellular localization. Cell 2000;103:645–54.
64. Annis MG, Soucie EL, Dlugosz PJ, et al. Bax forms multispanning monomers that oligomerize to permeabilize membranes during apoptosis. EMBO J 2005;24:2096–103.
65. Lucken-Ardjomande S, Martinou JC. Newcomers in the process of mitochondrial permeabilization. J Cell Sci 2005;118:473–83.
66. Kuwana T, Mackey MR, Perkins G, et al. Bid, Bax, and lipids cooperate to form supramolecular openings in the outer mitochondrial membrane. Cell 2002;111:331–42.
67. Cheng EH, Sheiko TV, Fisher JK, Craigen WJ, Korsmeyer SJ. VDAC2 inhibits BAK activation and mitochondrial apoptosis. Science 2003;301:513–7.
68. Kim H, Rafiuddin-Shah M, Tu HC, et al. Hierarchical regulation of mitochondrion-dependent apoptosis by BCL-2 subfamilies. Nat Cell Biol 2006;8:1348–58.
69. Willis SN, Chen L, Dewson G, et al. Proapoptotic Bak is sequestered by Mcl-1 and Bcl-xL, but not Bcl-2, until displaced by BH3-only proteins. Genes Dev 2005;19:1294–305.
70. Ruffolo SC, Shore GC. BCL-2 selectively interacts with the BID-induced open conformer of BAK, inhibiting BAK auto-oligomerization. J Biol Chem 2003;278:25039–45.
71. Tan C, Dlugosz PJ, Peng J, et al. Auto-activation of the apoptosis protein Bax increases mitochondrial membrane permeability and is inhibited by Bcl-2. J Biol Chem 2006;281:14764–75.
72. Antonsson B, Conti F, Ciavatta A, et al. Inhibition of Bax channel-forming activity by Bcl-2. Science 1997;277:370–2.
73. Minn AJ, Velez P, Schendel SL, et al. Bcl-x(L) forms an ion channel in synthetic lipid membranes. Nature 1997;385:353–7.
74. Saito M, Korsmeyer SJ, Schlesinger PH. BAX-dependent transport of cytochrome c reconstituted in pure liposomes. Nat Cell Biol 2000;2:553–5.
75. Schendel SL, Xie Z, Montal MO, Matsuyama S, Montal M, Reed JC. Channel formation by antiapoptotic protein Bcl-2. Proc Natl Acad Sci U S A 1997;94:5113–8.

76. Dlugosz PJ, Billen LP, Annis MG, et al. Bcl-2 changes conformation to inhibit Bax oligomerization. EMBO J 2006;25:2287–96.

77. Peng J, Tan C, Roberts GJ, et al. tBid elicits a conformational alteration in membrane-bound Bcl-2 such that it inhibits Bax pore formation. J Biol Chem 2006; 281:35802–11.

78. Basanez G, Nechushtan A, Drozhinin O, et al. Bax, but not Bcl-xL, decreases the lifetime of planar phospholipid bilayer membranes at subnanomolar concentrations. Proc Natl Acad Sci U S A 1999;96:5492–7.

79. Pinton P, Rizzuto R. Bcl-2 and Ca2+ homeostasis in the endoplasmic reticulum. Cell Death Differ 2006;13: 1409–18.

80. Rong Y, Distelhorst CW. Bcl-2 protein family members: versatile regulators of calcium signaling in cell survival and apoptosis. Annu Rev Physiol. In press 2007.

81. Rizzuto R, Pozzan T. Microdomains of intracellular Ca2+: molecular determinants and functional consequences. Physiol Rev 2006;86:369–408.

82. Szalai G, Krishnamurthy R, Hajnoczky G. Apoptosis driven by IP(3)-linked mitochondrial calcium signals. EMBO J 1999;18:6349–61.

83. Lam M, Dubyak G, Chen L, Nunez G, Miesfeld RL, Distelhorst CW. Evidence that BCL-2 represses apoptosis by regulating endoplasmic reticulum-associated Ca2+ fluxes. Proc Natl Acad Sci U S A 1994;91: 6569–73.

84. Scorrano L, Oakes SA, Opferman JT, et al. BAX and BAK regulation of endoplasmic reticulum Ca2+: a control point for apoptosis. Science 2003;300:135–9.

85. Oakes SA, Scorrano L, Opferman JT, et al. Proapoptotic BAX and BAK regulate the type 1 inositol trisphosphate receptor and calcium leak from the endoplasmic reticulum. Proc Natl Acad Sci U S A 2005;102:105–10.

86. Cheng EH, Wei MC, Weiler S, et al. BCL-2, BCL-X(L) sequester BH3 domain-only molecules preventing BAX- and BAK-mediated mitochondrial apoptosis. Mol Cell 2001;8:705–11.

87. Li H, Zhu H, Xu CJ, Yuan J. Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. Cell 1998;94:491–501.

88. Luo X, Budihardjo I, Zou H, Slaughter C, Wang X. Bid, a Bcl2 interacting protein, mediates cytochrome c release from mitochondria in response to activation of cell surface death receptors. Cell 1998;94:481–90.

89. Datta SR, Katsov A, Hu L, et al. 14-3-3 proteins and survival kinases cooperate to inactivate BAD by BH3 domain phosphorylation. Mol Cell 2000;6:41–51.

90. Zha J, Harada H, Yang E, Jockel J, Korsmeyer SJ. Serine phosphorylation of death agonist BAD in response to survival factor results in binding to 14-3-3 not BCL-X(L). Cell 1996;87:619–28.

91. Huang DC, Strasser A. BH3-only proteins—essential initiators of apoptotic cell death. Cell 2000; 103:839–42.

92. Nakano K, Vousden KH. PUMA, a novel proapoptotic gene, is induced by p53. Mol Cell 2001;7:683–94.

93. Oda E, Ohki R, Murasawa H, et al. Noxa, a BH3-only member of the Bcl-2 family and candidate mediator of p53-induced apoptosis. Science 2000;288:1053–8.

94. Yu J, Zhang L, Hwang PM, Kinzler KW, Vogelstein B. PUMA induces the rapid apoptosis of colorectal cancer cells. Mol Cell 2001;7:673–82.

95. Chen L, Willis SN, Wei A, et al. Differential targeting of prosurvival Bcl-2 proteins by their BH3-only ligands allows complementary apoptotic function. Mol Cell 2005;17:393–403.

96. Kuwana T, Bouchier-Hayes L, Chipuk JE, et al. BH3 domains of BH3-only proteins differentially regulate Bax-mediated mitochondrial membrane permeabilization both directly and indirectly. Mol Cell 2005;17: 525–35.

97. Letai A, Bassik M, Walensky L, Sorcinelli M, Weiler S, Korsmeyer S. Distinct BH3 domains either sensitize or activate mitochondrial apoptosis, serving as prototype cancer therapeutics. Cancer Cell 2002;2:183.

98. Uren RT, Dewson G, Chen L, et al. Mitochondrial permeabilization relies on BH3 ligands engaging multiple prosurvival Bcl-2 relatives, not Bak. J Cell Biol 2007;177:277–87.

99. Willis SN, Fletcher JI, Kaufmann T, et al. Apoptosis initiated when BH3 ligands engage multiple Bcl-2 homologs, not Bax or Bak. Science 2007;315:856–9.

100. Walensky LD, Pitter K, Morash J, et al. A stapled BID BH3 helix directly binds and activates BAX. Mol Cell 2006;24:199–210.

101. Cartron PF, Gallenne T, Bougras G, et al. The first α helix of Bax plays a necessary role in its ligand-induced activation by the BH3-only proteins Bid and PUMA. Mol Cell 2004;16:807–18.

102. Chipuk JE, Bouchier-Hayes L, Kuwana T, Newmeyer DD, Green DR. PUMA couples the nuclear and cytoplasmic proapoptotic function of p53. Science 2005;309:1732–5.

103. Wei MC, Lindsten T, Mootha VK, et al. tBID, a membrane-targeted death ligand, oligomerizes BAK to release cytochrome c. Genes Dev 2000;14:2060–71.

104. Desagher S, Osen-Sand A, Nichols A, et al. Bid-induced conformational change of Bax is responsible for mitochondrial cytochrome c release during apoptosis. J Cell Biol 1999;144:891–901.

105. Oh KJ, Barbuto S, Pitter K, Morash J, Walensky LD, Korsmeyer SJ. A membrane-targeted BID BCL-2 homology 3 peptide is sufficient for high potency activation of BAX *in vitro*. J Biol Chem 2006;281: 36999–7008.

106. Leber B, Lin J, Andrews DW. Embedded together: the life and death consequences of interaction of the Bcl-2 family with membranes. Apoptosis 2007;12: 897–911.

107. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000;100:57–70.

108. Benz EJ, Jr., Nathan DG, Amaravadi RK, Danial NN. Targeting the cell death-survival equation. Clin Cancer Res 2007;13:7250–3.

109. Verdine GL, Walensky LD. The challenge of drugging undruggable targets in cancer: lessons learned from targeting BCL-2 family members. Clin Cancer Res 2007;13:7264–70.

110. Fesik SW. Promoting apoptosis as a strategy for cancer drug discovery. Nat Rev Cancer 2005;5:876–85.

111. Walensky LD. BCL-2 in the crosshairs: tipping the balance of life and death. Cell Death Differ 2006;13: 1339–50.

112. Rixe O, Fojo T. Is cell death a critical end point for anticancer therapies or is cytostasis sufficient? Clin Cancer Res 2007;13:7280–9.

113. Oltersdorf T, Elmore SW, Shoemaker AR, et al. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. Nature 2005;435:677–81.

114. Konopleva M, Contractor R, Tsao T, et al. Mechanisms of apoptosis sensitivity and resistance to the BH3 mimetic ABT-737 in acute myeloid leukemia. Cancer Cell 2006;10:375–88.

115. Lin X, Morgan-Lappe S, Huang X, et al. 'Seed' analysis of off-target siRNAs reveals an essential role of Mcl-1 in resistance to the small-molecule Bcl-2/Bcl-XL inhibitor ABT-737. Oncogene 2007;26:3972–9.

116. van Delft MF, Wei AH, Mason KD, et al. The BH3 mimetic ABT-737 targets selective Bcl-2 proteins and efficiently induces apoptosis via Bak/Bax if Mcl-1 is neutralized. Cancer Cell 2006;10:389–99.

117. Wang G, Nikolovska-Coleska Z, Yang CY, et al. Structure-based design of potent small-molecule inhibitors of anti-apoptotic Bcl-2 proteins. J Med Chem 2006;49:6139–42.

118. Verhaegen M, Bauer JA, Martin de la Vega C, et al. A novel BH3 mimetic reveals a mitogen-activated protein kinase-dependent mechanism of melanoma cell death controlled by p53 and reactive oxygen species. Cancer Res 2006;66:11348–59.

119. Mohammad RM, Goustin AS, Aboukameel A, et al. Preclinical studies of TW-37, a new nonpeptidic small-molecule inhibitor of Bcl-2, in diffuse large cell lymphoma xenograft model reveal drug action on both Bcl-2 and Mcl-1. Clin Cancer Res 2007;13: 2226–35.

120. Becattini B, Kitada S, Leone M, et al. Rational design and real time, in-cell detection of the proapoptotic activity of a novel compound targeting Bcl-X(L). Chem Biol 2004;11:389–95.

121. Campas C, Cosialls AM, Barragan M, et al. Bcl-2 inhibitors induce apoptosis in chronic lymphocytic leukemia cells. Exp Hematol 2006;34:1663–9.

122. Li J, Viallet J, Haura EB. A small molecule pan-Bcl-2 family inhibitor, GX15-070, induces apoptosis and enhances cisplatin-induced apoptosis in non-small cell lung cancer cells. Cancer Chemother Pharmacol. In press 2007.

123. Perez-Galan P, Roue G, Villamor N, Campo E, Colomer D. The BH3-mimetic GX15-070 synergizes with bortezomib in mantle cell lymphoma by enhancing Noxa-mediated activation of Bak. Blood 2007; 109:4441–9.

124. Shore GC, Viallet J. Modulating the bcl-2 family of apoptosis suppressors for potential therapeutic benefit in cancer. Hematology Am Soc Hematol Educ Program 2005;1:226–30.

125. Witters LM, Witkoski A, Planas-Silva MD, Berger M, Viallet J, Lipton A. Synergistic inhibition of breast cancer cell lines with a dual inhibitor of EGFR-HER-2/neu and a Bcl-2 inhibitor. Oncol Rep 2007;17:465–9.

126. Walensky LD, Kung AL, Escher I, et al. Activation of apoptosis *in vivo* by a hydrocarbon-stapled BH3 helix. Science 2004;305:1466–70.

127. Klasa RJ, Gillum AM, Klem RE, Frankel SR. Oblimersen Bcl-2 antisense: facilitating apoptosis in anticancer treatment. Antisense Nucleic Acid Drug Dev 2002;12:193–213.

128. Gautschi O, Tschopp S, Olie RA, et al. Activity of a novel bcl-2/bcl-xL-bispecific antisense oligonucleotide against tumors of diverse histologic origins. J Natl Cancer Inst 2001;93:463–71.