

Bcl-2 family-regulated apoptosis in health and disease

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Abstract: Apoptotic cell death is essential for embryonic development, tissue homeostasis, and a well-functioning immune system, with aberrant apoptosis contributing to numerous disease conditions. Inadequate cell death is a major contributing factor to tumorigenesis, while excess cell death contributes to neurodegeneration and autoimmune disease. The major pathway of apoptotic cell death, the mitochondrial pathway, is controlled by the Bcl-2 family of proteins. The members of this family, more than 17 in humans, share significant sequence and structural homology, and fulfil either prosurvival or proapoptotic roles. Specific interactions between these functionally polar proteins, and their relative expression levels, govern the susceptibility of each cell to toxic insults. Here we review the current understanding on how apoptotic cell death is controlled by this important protein family. We also discuss how excessive or insufficient cell death can contribute to disease, and how targeting the Bcl-2 family offers novel therapeutic opportunities.

Keywords: apoptosis, Bcl-2, cancer, cytochrome *c*, mitochondria

Bcl-2 family

Identification of Bcl-2 (B-cell lymphoma-2) over 20 years ago as a result of its upregulation in follicular B-cell lymphoma,¹ and the seminal finding that this proto-oncogene inhibits apoptosis rather than promotes proliferation,² instigated an explosion of interest in the role of this protein, and its soon-to-be discovered relatives, in cell survival. At least 17 human Bcl-2 proteins have now been identified, each characterized by up to four regions of sequence homology, ie, the Bcl-2 homology (BH) domains. The members are categorized into three functional groups (Figure 1).³ The first group, the prosurvival proteins, Bcl-2, Bcl-x_L, Mcl-1, Bcl-w, A1/Bfl-1, and Bcl-B/Bcl2L10, generally contain all four BH domains and are responsible for protecting cells from apoptotic stimuli.

The second group comprises the BH3-only proteins, Bid, Bim, Puma, Noxa, Bad, Bmf, Hrk, and Bik, whose sequence homology with other members is restricted to the BH3 domain, as their name suggests. The BH3-only proteins are activated in response to diverse cellular stresses including DNA damage, growth factor deprivation, and endoplasmic reticulum stress, to initiate apoptosis. Activation of the BH3-only proteins may involve transcriptional upregulation, as observed for p53-dependent expression of Noxa and Puma following DNA damage.⁴ Alternatively, BH3-only proteins may be post-translationally modified. For example, while Bim and Bmf are normally sequestered by interacting with components of the cytoskeleton, following a death stimulus Bim and Bmf phosphorylation disrupts these interactions and allows interaction with other Bcl-2

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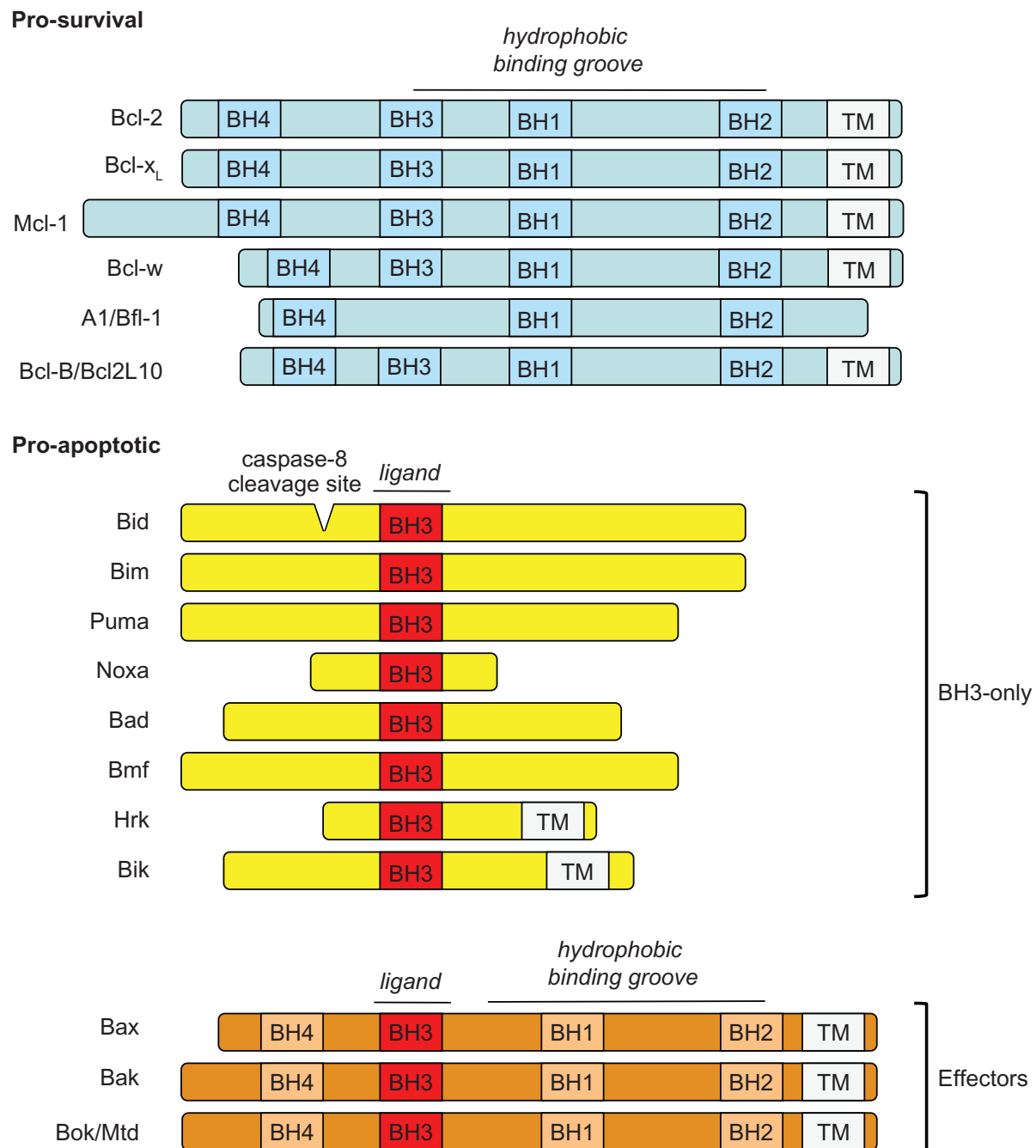


Figure 1 The Bcl-2 family of apoptotic regulators.

The Bcl-2 family of proteins are functionally categorized into three groups: i) pro-survival; ii) pro-apoptotic BH3-only; and iii) pro-apoptotic effector proteins. Protein sequences are characterized by BH (Bcl-2 homology) domains 1–4. Note that while the BH4 domain as initially defined¹³⁸ is absent in Bak, Bax, Mcl-1, and A1, the BH4 domain described more recently⁷³ appears to be present in most multidomain proteins. The indicated BH3 ligand and hydrophobic binding groove regions mediate homo- and heterotypic “BH3:groove” interactions between family members. Many Bcl-2 members also have a C-terminal transmembrane (TM) domain. Note also that Bok/Mtd is included as an effector protein based on sequence homology to Bak and Bax, but a similar pro-apoptotic function is yet to be verified.

family members to initiate apoptosis.^{5,6} Another activation mechanism is the cleavage of Bid by caspase-8 to generate the activated truncated form, tBid.

The third group, Bax, Bak, and Bok/Mtd, are again multidomain proteins, and contain all four BH domains as do the pro-survival proteins, but they are pro-apoptotic, with

their activation downstream of BH3-only proteins ultimately responsible for the demise of the cell. Bak and Bax activation and function are discussed below.

Recently, novel putative members of the Bcl-2 family have been identified with differing degrees of sequence homology. For example, Bcl2L12,⁷ Bfk,⁸ and Bcl-G/Bcl2L14⁹ all contain

BH domains 2 and 3 only. However, whereas Bcl2L12 is a prosurvival protein, Bflk and Bcl-G are proapoptotic.^{8–10} Further, Bcl-Rambo/Bcl2L13 is a multidomain proapoptotic protein containing BH domains 1–4, seemingly homologous to Bak and Bax.¹¹ However, in contrast with Bak and Bax, the proapoptotic potential of Bcl-Rambo is independent of its BH domains.¹¹ Therefore, there is currently considerable interest in identifying novel Bcl-2 homologs and elucidating their precise roles in regulating apoptosis.

It is clear that multiple and specific interactions between the three groups of Bcl-2 proteins coordinate the apoptotic response. Considerable effort has been made to decipher these interactions, and so understand the susceptibility of each cell to apoptotic stimuli.

Prosurvival Bcl-2 proteins: Guardians at the mitochondrial gate

A number of mechanisms were initially proposed for how Bcl-2 and its prosurvival relatives protect cells from stress. Originally it was argued that Bcl-2 acted as a free-radical scavenger and suppressed the effect of damaging reactive oxygen species generated by mitochondrial respiration.¹² Consistent with this, mice deficient for Bcl-2 developed polycystic kidney disease and cerebellar neuron loss,¹³ which can be attributed to oxidative stress. Further, Bcl-2 expression could protect cells from oxidant-induced death.¹² Subsequently, Bcl-2 was proposed to block the “permeability transition pore” in the mitochondrial inner membrane, and so indirectly block the associated production of oxygen species. However, studies have since questioned the importance of the antioxidant effects of Bcl-2 for its prosurvival function,¹⁴ and the role of the permeability transition pore in apoptosis,¹⁵ suggesting that Bcl-2 protects cells from apoptosis principally via other mechanisms.

The early evidence that Bcl-2 protected from oxidative stress provided the first hint that Bcl-2 function was associated with mitochondrial function.¹² Then in 1994, Newmeyer et al reported that mitochondria were necessary for apoptotic changes in a cell-free system, and that Bcl-2 could block the effect of mitochondria.¹⁶ That group and the Wang group then showed that Bcl-2 actually acts by preserving the integrity of the mitochondrial outer membrane.^{17,18} Maintaining mitochondrial integrity serves not only to conserve mitochondria as the powerhouses of the cell, but also to prevent proapoptotic proteins such as cytochrome *c* and Smac/DIABLO from being released into the cytoplasm

where they rapidly activate a family of proteases, the caspases (Figure 2).^{19–21} Once activated, caspases cleave a plethora of intracellular proteins to package the cell in readiness for its “silent” noninflammatory removal by resident phagocytes.

While the Bcl-2 family regulates the mitochondrial or “intrinsic” apoptotic pathway, it does not regulate the death receptor or “extrinsic” pathway, at least in most cell types. In the death receptor pathway, apoptotic signalling is initiated by ligation of cell surface receptors for Fas (CD95/Apo-1) ligand, tumor necrosis factor (TNF)- α , and TRAIL (TNF-related apoptosis-inducing ligand). This activates caspase-8, which in turn directly activates downstream caspases, without the need for mitochondria or the Bcl-2 proteins.¹⁴ However, in certain cells (termed Type II cells), including hepatocytes and pancreatic β -cells the death receptor pathway does recruit mitochondria and the Bcl-2 family via caspase-8 cleavage of the BH3-only protein Bid to generate tBid (Figure 2).^{22–24} Consequently, the Bcl-2 family also plays a role in immune-mediated conditions such as diabetes (see below).

Given that Bcl-2 proteins control apoptosis by regulating permeabilization of the mitochondrial outer membrane, it is not surprising that several members locate to this membrane. They do so via a hydrophobic C-terminal transmembrane (TM) domain that targets them principally to mitochondria. An association with the mitochondrial outer membrane is not always constitutive, because certain Bcl-2 proteins require apoptotic signalling to drive them there. For example, Bax is actually cytosolic in the majority of healthy cells, but translocates to the mitochondrial outer membrane during cell death.²⁵ This contrasts with its structural and functional homolog, Bak, which is resident at mitochondria before a death stimulus. BH3-only and prosurvival proteins are also often distributed between the cytosol and mitochondria in healthy cells, with at least some members (Bim, Bcl-x_L and Bcl-w) reported to translocate to membranes during apoptosis.^{5,26,27} Bcl-2, Bak, and Bax have also been detected at the endoplasmic reticulum membrane, although a proposed role in regulating Ca²⁺ homeostasis in the endoplasmic reticulum is contentious.^{28–31}

Interactions between Bcl-2 members determine cell fate

In response to an apoptotic stimulus, the balance of prosurvival and proapoptotic Bcl-2 proteins, and the specific interactions between them, determine the proapoptotic activity of Bak and Bax.^{32–34} Activation of Bak and Bax

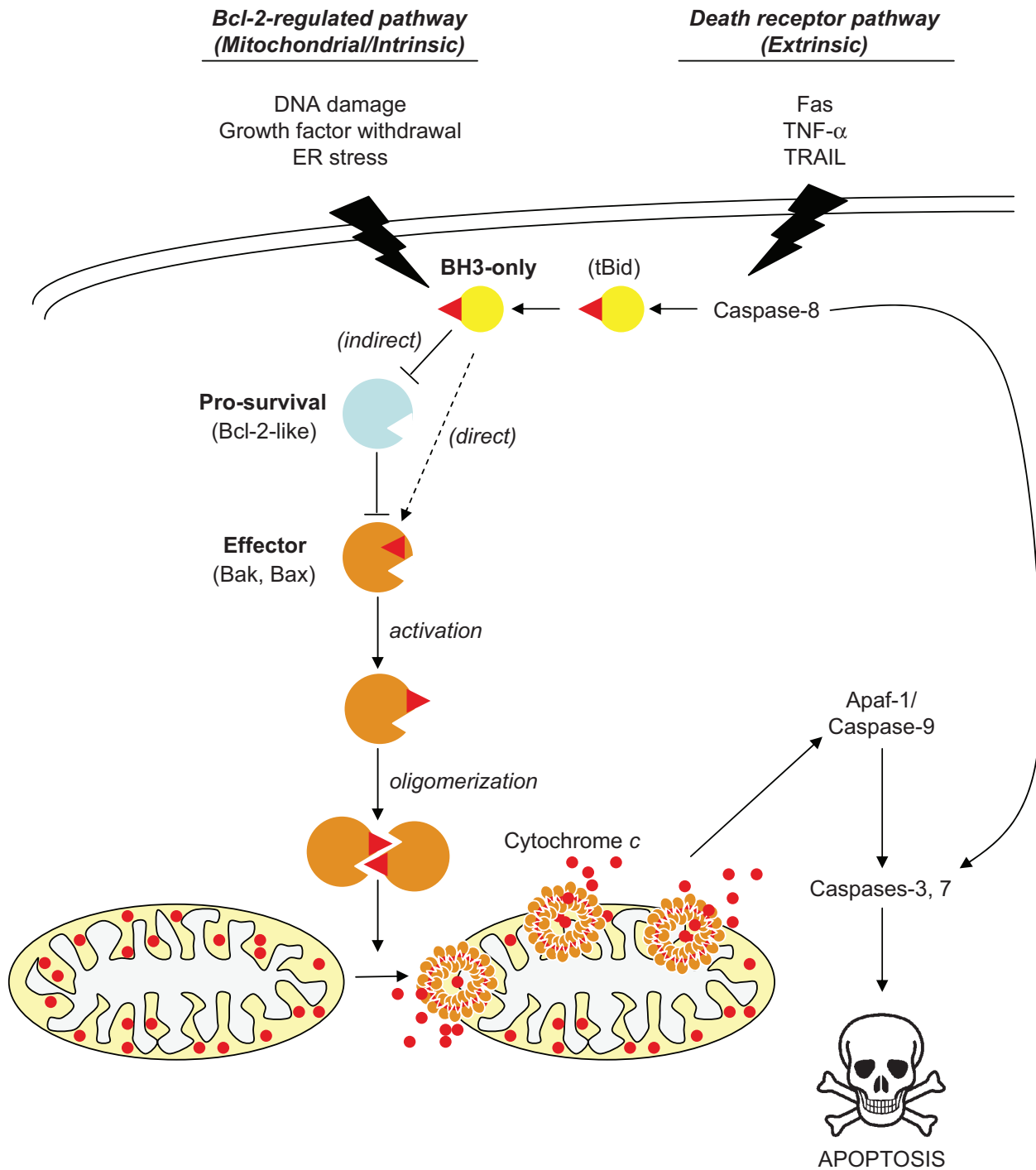


Figure 2 The pathways to apoptotic cell death.

In the Bcl-2-regulated pathway (also called the mitochondrial or intrinsic pathway) of apoptosis, a range of stimuli triggers the activation of BH3-only proteins, which then bind via BH3:groove interactions to specific prosurvival proteins. Certain BH3-only proteins (eg, tBid and Bim) may also directly bind and activate the effector Bcl-2 proteins Bak and Bax (broken arrow) to expose their BH3 domain (red triangle). If prosurvival proteins are occupied by BH3-only proteins, activated Bak and Bax are free to homo-oligomerize via a BH3:groove interaction. The Bak and Bax oligomers then form pores that permeabilize the mitochondrial outer membrane leading to the release of several proapoptotic proteins including cytochrome c. Once in the cytosol, cytochrome c binds to the adaptor protein Apaf-1 to activate the downstream caspases that cleave multiple cellular proteins during apoptosis. In the death receptor pathway (also called extrinsic pathway), the Fas, TNF- α , and TRAIL ligands bind to their cell surface receptors to activate caspase-8. Activated caspase-8 then directly activates the downstream caspases, which in most cells is sufficient to cause cell death. However, certain cells (Type II cells) also require caspase-8 to cleave Bid (tBid), which recruits the mitochondrial arsenal of proapoptotic proteins to fully activate the downstream caspases.

involves major conformation change and self-association into large oligomers that permeabilize the mitochondrial outer membrane, potentially by forming a proteinaceous pore, thereby killing the cell (Figure 2).³⁵ Thus, the BH3-only proteins are charged with instigating Bak and Bax homo-oligomerization, whilst the prosurvival Bcl-2 proteins are charged with preventing it.

How BH3-only proteins initiate Bak and Bax activation is debated, but is proposed to be by two seemingly distinct mechanisms, ie, the “direct” and “indirect” models of Bak and Bax activation.^{36,37} Briefly, the direct model posits that a subclass of the BH3-only proteins, termed “activators” (Bim, tBid, and Puma), bind directly to Bak and Bax to cause their activation. The role of prosurvival proteins in this model is to sequester the “activators”. The role of other BH3-only proteins, termed “sensitizers” (for example, Noxa and Bad) is to bind to prosurvival proteins and displace the “activators”.³⁸ Concerns with this model include the difficulty in detecting binding of activator BH3-only proteins to Bak and Bax, although this interaction may only be transient. In addition, if direct activators are required for Bak and Bax activation, removing these direct activators should have the same effect as removing both Bak and Bax, ie, complete insensitivity to the majority of cell death stimuli.³⁹ However, this is not the case in genetically altered mice and cells.⁴⁰

The indirect model argues that activation of Bak and Bax does not require this interaction with BH3-only proteins. Rather, Bak and Bax are initially in a “primed” form and sequestered by prosurvival proteins. Then, in response to apoptotic stimuli, activated BH3-only proteins bind to prosurvival proteins, displacing “primed” Bak and Bax.^{40,41} The displaced Bak and Bax may then autoactivate the inactive pool and together permeabilize the mitochondrial outer membrane.⁴² In the indirect model, however, the initial activating stimulus responsible for “priming” Bak and Bax has not been defined, and only minor levels of Bak and Bax appear bound to prosurvival proteins in healthy cells.^{37,43} Irrespective of the exact means by which Bak and Bax become activated, it is clear that the balance of prosurvival (Bcl-2-like) and proapoptotic (BH3-only and effector) proteins is the key to this activation, and thus in governing susceptibility of a cell to toxic insult.

Structure of Bcl-2 proteins

Bcl-2-like fold

The molecular basis of the interactions between the Bcl-2 proteins centers around the ubiquitous BH3 domain (Figure 1) and its ability to bind to a hydrophobic surface groove in the

multidomain proteins (Figures 1 and 3). Significant insight into these “BH3:groove” interactions has been gained from the molecular structures of the Bcl-2 proteins, in particular of BH3-only ligands bound to the groove of prosurvival proteins (Figure 3). Nuclear magnetic resonance or x-ray structures of most prosurvival proteins are now available either as monomers (including Bcl-x_L,⁴⁴ Bcl-2,⁴⁵ Mcl-1,⁴⁶ Bcl-w,⁴⁷ and A1⁴⁸) or complexed with BH3 peptides derived from other Bcl-2 members (Figure 3A).^{49–51}

Structures of nonactivated Bax⁵² and Bak⁵³ are now also available and, despite their proapoptotic function, exhibit a similar overall fold to that of the prosurvival proteins (Figure 3B). Thus, all multidomain members (prosurvival proteins as well as Bax and Bak) are globular proteins consisting of two core hydrophobic α -helices shrouded by six or seven amphipathic α -helices.⁵⁴ The hydrophobic C-terminal TM domain (Figure 1), responsible for targeting the multidomain homologs to intracellular membranes, is missing from most of the structures (except cytosolic Bax, Figure 3C). Thus, the structure of the TM of most members is not known, nor how the TM orientates each protein at the membrane surface.

In contrast with the multidomain members, the BH3-only proteins appear intrinsically unstructured, perhaps to allow the BH3 domain to adopt the optimal helical structure upon binding to a hydrophobic groove and so improve binding affinity.⁵⁵ Bid is an exception, because its structure is similar to that of the multidomain proteins (ie, a Bcl-2-like fold) despite the absence of BH domains 1, 2, and 4.^{56,57} However, upon apoptotic signaling and cleavage by caspase-8 to tBid, it also becomes less structured, which may facilitate binding to a hydrophobic groove.⁵⁸

BH3:groove interactions

The distinct hydrophobic groove on the surface of all prosurvival proteins, has been shown by biochemical and structural studies to be the docking site for the BH3 domains of proapoptotic BH3-only proteins (Figure 3A), and potentially also the BH3 domains of the activated or “primed” forms of Bak and Bax.^{46,50,51} The hydrophobic groove is not a promiscuous receptor for all BH3 domains, because prosurvival protein binding of BH3 ligands is highly selective.⁵⁹ For example Mcl-1 and A1 bind Noxa avidly, but do not bind Bad. Conversely, Bcl-2, Bcl-x_L, and Bcl-w bind Bad, but not Noxa. However, all prosurvival proteins bind Bim and Puma with high affinity. As a result, these specific BH3:groove interactions provide an exquisitely regulated mechanism by which prosurvival proteins sequester their proapoptotic relatives and so block apoptosis.

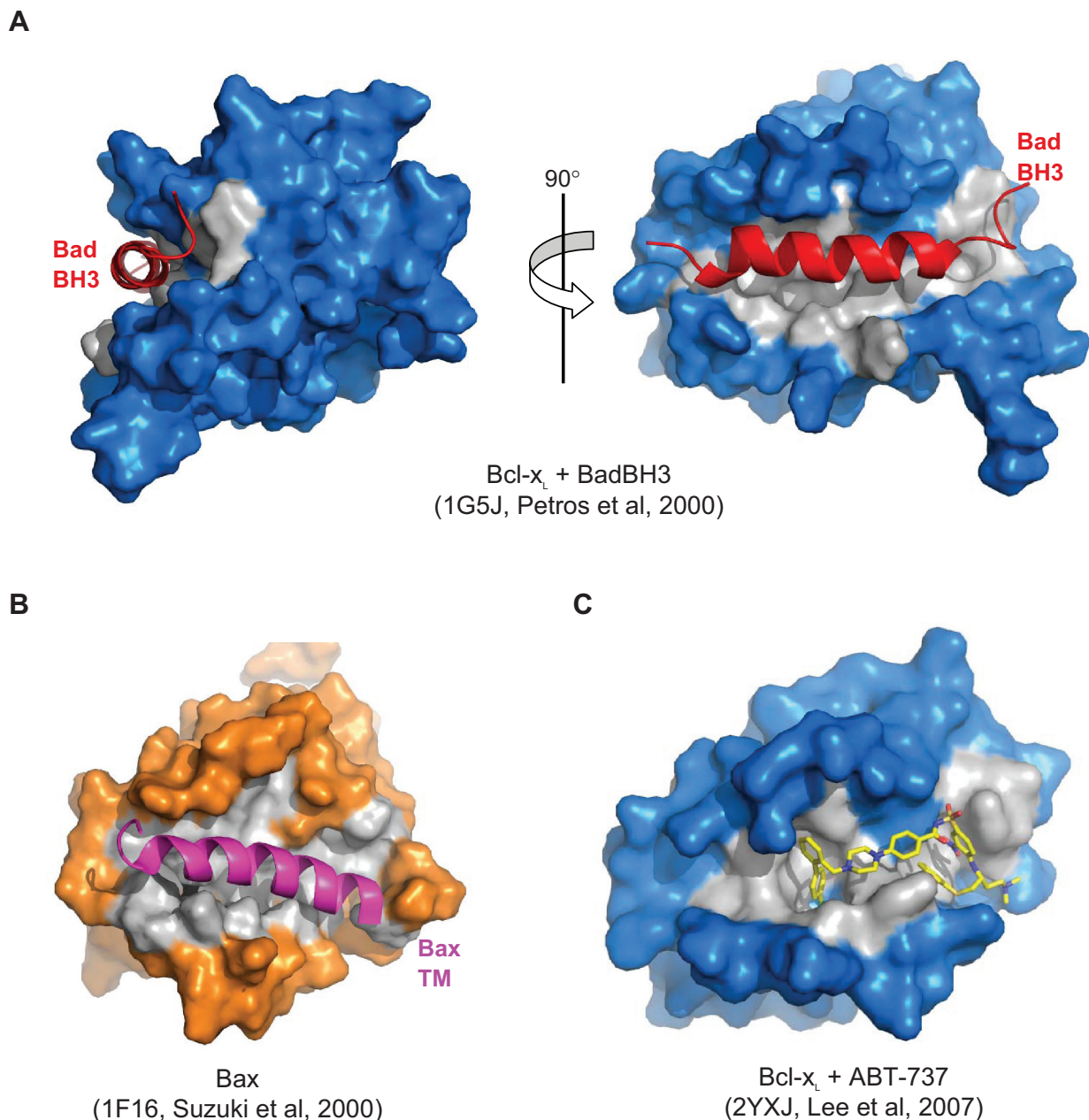


Figure 3 Structural evidence that the hydrophobic surface groove of Bcl-2 proteins can bind BH3 ligands and transmembrane domains.

A) Structural representation of Bcl-x_L complexed with a peptide derived from the Bad BH3 domain. The fold of Bcl-x_L (and other prosurvival Bcl-2 proteins) creates a hydrophobic surface groove (grey) that acts as a receptor for the BH3 domain of Bad (red helix) and other proapoptotic proteins.⁵¹ **B)** The Bax hydrophobic groove is occupied by its TM domain. The structure of cytosolic nonactivated Bax displays a similar fold to that of its prosurvival relatives, including a hydrophobic surface groove (grey). Binding of the Bax transmembrane domain (pink) to that groove facilitates the cytosolic locale of Bax prior to apoptosis.⁵² **C)** A BH3 mimetic also binds to the prosurvival hydrophobic groove. The structure of a small molecule mimetic of Bad, ABT-737 (yellow), bound to the hydrophobic groove of Bcl-x_L (grey) is analogous to that observed for the Bad BH3 peptide bound to Bcl-x_L shown in A.⁹⁷ Images were generated in Pymol using the indicated RCSB Protein Data Bank files (1G5J, 1F16, 2YXJ).

Both Bak and Bax have a hydrophobic surface groove, indicating it may also be an important interaction site for the proapoptotic members. Indeed, while the structures of activated Bak and Bax are not yet available, our recent biochemical studies show that a BH3:groove interface forms between two activated Bak or Bax molecules (GD and RK, unpublished

data).⁶⁰ That is, the Bak and Bax activation process involves exposure of their BH3 domain, with this domain then able to bind to the groove in another molecule to form homodimers. This BH3:groove interaction is required to kill the cell.^{60,61}

There is now structural information regarding binding of BH3-only ligands to Bax. As mentioned above, this

type of interaction may initiate activation and conformation change of Bak and Bax as proposed by the “direct” activation model. One might speculate that such an interaction would occur via a BH3:groove interaction, based on the interactions between other members. However, this was not the case because the Bim BH3 peptide bound to a site distinct from the Bax groove.⁶² Nevertheless, very recent mutagenesis studies indicate that interaction at this alternative site might precede a necessary BH3:groove interaction.⁶³ Hence, it remains possible that BH3:groove interactions underlie all binding between Bcl-2 family members, as we have speculated.³⁷ Clearly, further studies, including structures of full-length BH3-only proteins bound to Bak and Bax, preferably as the membrane-bound forms, are needed to clarify this important step in apoptotic cell death.

TM:groove interactions

While the TM domain is absent in most structures of Bcl-2 proteins, the Bax structure is of the full-length protein and shows the hydrophobic TM sequestered within its hydrophobic groove (Figure 3B).^{47,52} The structure of Bcl-w also shows a C-terminal helix bound to the hydrophobic groove, although a more C-terminal hydrophobic region (probable TM) had been truncated. In Bax, the TM:groove conformation supports its cytosolic localization in healthy cells. In response to an apoptotic stimulus, Bax (and Bcl-w) translocates to mitochondria with the TM moving from the groove to insert across the mitochondrial outer membrane.^{26,34} Therefore, the hydrophobic groove not only governs interactions between Bcl-2 family members, but may also govern subcellular distribution of these proteins.

Conformation change during apoptosis

Given that biochemical studies suggest that both proapoptotic and prosurvival proteins undergo major conformation changes during apoptosis, most of the structures currently available may only reflect the conformation prior to apoptosis. Furthermore, biochemical evidence suggests that some Bcl-2 proteins adopt markedly different conformations once associated with the lipid environment of the mitochondrial outer membrane. Bak and Bax conformation change during apoptosis includes exposure of N-terminal epitopes and the BH3 domain (the binding motif) on their way to adopting their “activated” conformation.^{34,60,61,64} In addition, Bax inserts two hydrophobic regions (α -helices 5 and 6) into the membrane to adopt a multispanning membrane

topology.^{65,66} The enhanced membrane integration of Bax may perturb the mitochondrial outer membrane analogous to the mechanism by which pore-forming bacterial toxins porate a lipid bilayer.⁶⁷ Intriguingly, Bcl-2 has also been shown to insert α -helices 5 and 6 into the mitochondrial outer membrane,⁴⁶ with this multispanning conformer of Bcl-2 acting as a dominant-negative form of Bax by binding to the multispanning Bax conformer and preventing its oligomerization into large complexes.⁶⁸ Elegant nuclear magnetic resonance studies of Bcl-2 proteins in lipid membrane environments also suggest that both prosurvival and proapoptotic proteins insert their hydrophobic core helices into membranes.^{69–72}

In summary, biochemical and structural analyses are starting to paint a picture of the postapoptotic, membrane-integrated forms of the Bcl-2 proteins. However, molecular structures of the activated and oligomerized conformers of Bak and Bax are still eagerly anticipated, along with their undoubted insight as to how these crucial proteins damage mitochondria.

A non-apoptotic role for Bcl-2 proteins

Despite limited amino acid sequence homology, a number of viral Bcl-2 homologues adopt a remarkably similar protein structure to Bcl-2 (eg, F1L,⁷³ M11L,⁷⁴ and BHRF1⁷⁵). Moreover, several of these function as orthologs of prosurvival proteins in that they bind BH3-only proteins or Bak and Bax to block apoptosis and so sustain the viral infection.^{76,77} Thus, one might speculate that attenuated viruses could be used therapeutically to block apoptosis, and that compounds that target Bcl-2 proteins could be used to counter pathogenic infections. It is also interesting to note that two vaccinia virus proteins that adopt a Bcl-2-like fold were recently shown to inhibit NF- κ B activation, and so prevent the inflammatory response against the virus.⁷⁸ This raises the intriguing possibility that mammalian Bcl-2-like proteins may have a function distinct from their role in apoptosis.

Bcl-2-regulated apoptosis important in development

The critical role of Bcl-2 and its relatives in development and tissue homeostasis is apparent from mice in which one or both copies of the relevant gene have been deleted. Mice lacking both copies of the Bcl-2 gene (*bcl-2*^{-/-}) die in utero.¹³ These *bcl-2*^{-/-} mice die *in utero* because of defects in the development of the hematopoietic system.⁷⁹ Deletion

of Mcl-1 resulted in failure of the early embryo to implant. However, conditional deletion of Mcl-1 in T- and B-cells indicated that this gene is critical for the development and maintenance of a functioning immune system.⁸⁰ These studies highlight the importance of the prosurvival proteins in maintaining cell survival during development.

Certain cells are destined to die during development to allow tissue remodelling. Thus, deleting proapoptotic genes also results in developmental abnormalities, most marked in the central nervous system. For example, neurodevelopmental defects develop in *caspase-9*^{81,82} and *apaf-1*⁸³ knock-out mice, and in mice expressing a mutant of cytochrome *c* that can not activate caspases.⁸⁴ In terms of the Bcl-2 family, mice that lack both Bak and Bax die perinatally because of severe craniofacial and neuronal developmental defects.⁸⁵ It is also noteworthy that mice deficient in either Bak or Bax have relatively few abnormalities,^{85,86} indicating that either Bak or Bax is generally able to mediate apoptosis. These studies highlight both the importance of apoptosis in the developing embryo, and the need for either Bak or Bax for apoptosis.

With at least eight BH3-only proteins in mammals, deletion of any one BH3-only protein has less profound effects on development, consistent with a degree of redundancy in their roles as sensors of intracellular stress. The exception is Bim, which is particularly important for development, because a significant proportion (approximately 40%) of *bim*^{-/-} mice die *in utero*, and those that survive are plagued by expansion of both lymphoid and myeloid compartments and by the development of autoimmune disease.⁸⁷ In summary, the Bcl-2 proteins play a critical role during development, both in protecting cells from the rigors of division and differentiation and in removing cells to allow tissue remodelling.

Dysregulated apoptosis contributes to multiple diseases

As well as a pivotal role during development, Bcl-2 proteins are essential for homeostasis in mature tissues, such that defects in expression levels or activation status contribute to a range of pathologies. Before the discovery of Bcl-2, the original description of apoptosis as a programmed form of cell death distinct from necrosis also reported a major role for this cell death in both physiologic and pathologic conditions.⁸⁸ Pathologies included those in which apoptosis is blocked, eg, in cancer, and those in which apoptosis is excessive or inappropriate, eg, in neurodegeneration, autoimmunity, and ischemia. Now, with our increasing understanding of how the Bcl-2 proteins regulate apoptosis,

we have the exciting opportunity to target Bcl-2-regulated apoptosis for therapeutic benefit.

Cancer

Inhibition of apoptosis is a hallmark of tumorigenesis, at least in part because cells with DNA damage fail to be deleted.⁸⁹ The first example was the finding of Bcl-2 upregulation due to t(14:18) chromosomal translocation in follicular B-cell lymphoma. Since then, over-expressed Bcl-2 has been implicated in a number of other cancers, including chronic lymphocytic leukemia, acute lymphoblastic leukemia, small cell lung cancer, prostate cancer and non-Hodgkin's lymphoma. Causes of Bcl-2 overexpression include chromosomal translocation, DNA hypermethylation, and downregulation of the microRNAs that target Bcl-2. Mcl-1 overexpression is also associated with hematopoietic malignancies, such as multiple myeloma and chronic myeloid leukemia, and with solid tumors, such as pancreatic cancer and melanoma. High levels of prosurvival proteins are often associated with aggressive malignancies and with resistance to chemotherapy. In mice, overexpression of prosurvival Bcl-2 proteins alone does not efficiently drive tumorigenesis, but it does synergize with other oncogenic transformations such as overexpression of *myc* or mutations in p53.⁹⁰

Although either Bak or Bax is necessary for apoptosis, compared with overexpression of the prosurvival proteins, relatively few cancers are associated with Bak or Bax downregulation or loss-of-function mutations. This is presumably because of the expression of both Bak and Bax in most cells, and their functional overlap. For instance, failure to undergo apoptosis would require loss of, or mutation in, both alleles of Bak and both alleles of Bax. Even so, low levels of Bak or Bax, or perhaps more pertinently a low ratio of proapoptotic protein to prosurvival protein, can correlate with increased incidence, poor prognosis, or resistance to chemotherapy in certain cases of colorectal cancer, melanoma, and chronic lymphocytic leukemia.⁹¹⁻⁹⁴

Recently developed therapeutics that specifically target the Bcl-2 protein family have considerable potential for the treatment of neoplastic disorders.⁹⁵ For example, Abbott Laboratories used a structure-based approach to design small molecules that can enter cells and mimic BH3-only proteins in their ability to bind to the prosurvival Bcl-2 proteins. The first of these BH3-mimetics, ABT-737, mimics Bad because it binds with high affinity to Bcl-x_L, Bcl-2, and Bcl-w, but not to Mcl-1 or A1 (Figure 3C),^{96,97} and can specifically kill cancer cells whose survival is reliant upon the targeted prosurvival proteins.^{96,98} ABT-737, and its orally

available analog ABT-263, have shown considerable promise in preclinical studies in mice, either as a single agent or in combination with common therapeutics.^{99–102} ABT-263 is currently in Phase I clinical trials for the treatment of hematologic malignancies including chronic lymphocytic leukemia, acute lymphoblastic leukemia, and multiple myeloma.

Neurodegeneration

Mature neurons are exquisitely sensitive to stress such as hypoxia and, because they are not readily replaced, protecting these cells is crucial to stave off cell loss and neural deficits. Prosurvival proteins are important for this protection. For example, in mice lacking Bcl-2 the nervous system develops essentially normally, but there is a progressive loss of peripheral neurons after birth.¹⁰³ In addition, overexpression of Bcl-2 in neurons was found to protect from neuronal loss and limit infarct size in a mouse model of cerebral ischemia.¹⁰⁴ The Bcl-2 family is also implicated in chronic neurodegenerative conditions, because Bax inhibition prevented neuronal death in a mouse model of Parkinson's disease.¹⁰⁵ Together these findings highlight the importance of the Bcl-2 family and apoptosis in regulating neuronal cell survival following both acute and chronic injury, and suggest that targeting Bcl-2-regulated apoptosis may provide a valuable therapy for neuronal injury and a range of neurodegenerative diseases.^{106,107} Clearly, chronic inhibition of apoptosis as a means to prevent neurodegeneration may have the complication of encouraging tumors. However, short-term inhibition of apoptosis, for example immediately following ischemia, may afford sufficient protection from the initial hypoxic insult to ensure long-term neuronal cell survival.

Cardiovascular disease

Terminally differentiated cardiac myocytes are not replaced once lost. Depletion of the myocardium as a result of apoptosis contributes to a number of cardiovascular disorders, and, as in the nervous system, the Bcl-2 family is central in both causing and preventing this loss.¹⁰⁸ An imbalance in the ratio of prosurvival to proapoptotic Bcl-2 proteins appears causal in cardiovascular disease, including ischemic heart disease. In addition, Bcl-2 overexpression in mice protected against ischemia-reperfusion injury of cardiac myocytes, and blocked apoptosis of these cells in culture.^{109,110} Bcl-2 may also protect the heart from subthreshold injury given that acute ischemia-reperfusion preconditioning in mice was shown to inhibit myocyte apoptosis by a mechanism involving upregulation of

Bcl-2.¹¹¹ Cardiac damage seems to hold a central role for Bax, because cardiac myocyte apoptosis and consequent infarct size was markedly reduced in *bax*^{-/-} mice.¹¹² Therefore, preventing loss of cardiac cells by inhibiting apoptosis may have significant therapeutic potential in the treatment of various cardiovascular disorders.^{108,113}

Asthma

Asthma is characterized by infiltration of eosinophils into the airways resulting in bronchial hyperresponsiveness. The accumulation and persistence of these granulocytes in the asthmatic lung results from several factors, including selective adhesion and migration, and also prolonged survival conferred by proinflammatory growth factors such as interleukin-5, interleukin-3, and granulocyte macrophage colony-stimulating factor.^{114,115} Consistent with this, in a mouse model of asthma, prolonging eosinophil survival increased disease severity, and shortening survival decreased the severity.¹¹⁶ These changes in eosinophil survival appear linked to the Bcl-2 protein family, because cytokine-driven eosinophil survival involves Bcl-2 upregulation and inhibition of Bax.^{117,118} Furthermore, increased Bcl-2 expression in sputum eosinophils is correlated with increased severity of childhood and acute asthma.^{119,120}

Steroid therapies, commonly used in the treatment of asthma, induce apoptosis and clearance of airway eosinophils.^{115,121} Furthermore, in clinical and preclinical studies cytokine-receptor antagonists and cyclin-dependent kinase (CDK) inhibitors helped resolve airway inflammation in asthma at least partly by inducing apoptosis of airway eosinophils.^{121,122} The CDK inhibitor, R-roscovitine, induced eosinophil apoptosis in culture by downregulating Mcl-1.¹²² Thus, more direct pharmacologic manipulation of Bcl-2 proteins to induce eosinophil apoptosis may provide an effective therapy. It is worth noting that actively driving airway eosinophil apoptosis is likely to have two beneficial effects.¹²³ Firstly, it may tip the eosinophil from a necrotic form of death to an apoptotic death, and thereby limit the release of its arsenal of histotoxic mediators. Secondly, upon phagocytosis of apoptotic cells, resident macrophages release anti-inflammatory cytokines such as transforming growth factor (TGF)- β that may dampen the immune response.

Autoimmune disease

Dysregulated apoptosis has been implicated in the etiology of various autoimmune diseases including rheumatoid arthritis (RA), Type 1 diabetes, systemic lupus erythematosus, Sjogren's syndrome, and Crohn's disease. Autoimmunity can

arise because of too much cell death. For example, autoimmune Type 1 diabetes involves excessive apoptosis of pancreatic β -cells induced by proinflammatory cytokines such as IL-1 β , TNF- α and IFN(interferon)- γ derived from infiltrating cytotoxic T lymphocytes.¹²⁴ Bcl-2 family members are implicated in several instances, for example, the cytokine-induced β -cell apoptosis involved the BH3-only protein Bad and the effector protein Bax, whilst Bcl-2 overexpression prevented this β -cell death.^{125,126} Interestingly, excessive apoptosis may also contribute to the reduction in β -cell function and number observed in Type 2 diabetes, because increased β -cell apoptosis is induced by stresses including fatty acids and high blood glucose levels, and hyperglycemia-induced β -cell apoptosis relies on the Bcl-2-regulated machinery.¹²⁷ Therefore inhibition of β -cell apoptosis may be therapeutically valuable in the treatment of both autoimmune and non-autoimmune diabetes.

However, insufficient apoptosis can also contribute to autoimmunity. For example, deficient cell death of autoreactive lymphocytes is implicated in joint inflammation with RA. RA is characterized by expansion of lymphocytes, macrophages, and synovial fibroblasts in the RA joint, leading to local inflammation, proliferation of the synovial membrane, and extensive damage to surrounding soft tissue. Increased expression of Bcl-2, Mcl-1, and Bcl-x_L may be the cause of this cell expansion and associated synovial hyperplasia.^{128–130} Therefore topical treatment of an RA joint with apoptosis-inducing agents such as BH3-mimetics has been proposed as a means to initiate apoptosis of resident inflammatory cells and resolve disease.^{131,132}

Sepsis

The septic shock and associated multiorgan failure that can occur during chronic infection has been largely attributed to the proinflammatory cytokine storm following excessive induction of the innate immune response. However, somewhat paradoxically, patients with sepsis exhibit lymphopenia as a result of increased apoptosis of B-cells and CD4⁺ T-cells, and apoptosis of antigen-presenting dendritic cells.^{133,134} The net result is an impaired adaptive immune response and failure to resist opportunistic secondary infections. Post mortem examination revealed reduced Bcl-2 expression specifically in patients that died of sepsis. In addition, mice transgenic for Bcl-2 in T- and B-cells are completely protected from sepsis-induced lymphocyte apoptosis, leading to greater survival of the mice.¹³⁵ Therefore, blockade of Bcl-2-regulated apoptosis during sepsis may be an effective alternative to the standard, but

often ineffective, anticytokine therapies such as interleukin-1 and TNF α - antagonists.^{136,137}

Conclusions

Bcl-2 family members are critical determinants of cell survival in essentially all cells, both during development and in mature differentiated cells. They are the major regulators of stress-induced apoptosis, and in certain cases also regulate the apoptosis induced by death receptors. The current understanding of each of the Bcl-2 family members, their interactions, and final activation of Bak and Bax to permeabilize mitochondria, is now allowing the development of agents that target different steps in the pathway. Success in this strategy promises the ability to treat a range of pathologies, including the removal of harmful cells such as autoreactive lymphocytes or those with damaged DNA. A prominent example is the successful preclinical and Phase I clinical trials of small-molecule BH3-mimetics in certain cancers.

Our ongoing challenges include obtaining a better understanding of the molecular control of apoptosis and so improve the specificity and efficacy of agents that target Bcl-2 proteins. It also seems feasible to develop small molecules that specifically block apoptosis. Clearly, it is crucial to develop means of targeting specific cells, be they transformed lymphocytes in a lymphoma, persistent granulocytes in an asthmatic airway, or β -cells in a glucose-bathed pancreas. In some cases, acute administration may effectively initiate or block apoptosis in the target cell with limited effect on other cells. Targeting would also be improved by the development of a simple method of profiling the Bcl-2 proteins in each cell type, including in each cancer.

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