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The Bcl-2-regulated apoptosis switch: mechanism and therapeutic potential

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Abstract

Apoptosis is essential for tissue homeostasis, particularly in the hematopoietic compartment, where its impairment can elicit neoplastic or autoimmune diseases. Whether stressed cells live or die is largely determined by interplay between opposing members of the Bcl-2 protein family. Bcl-2 and its closest homologs promote cell survival, but two other factions promote apoptosis. The BH3-only proteins sense and relay stress signals, but commitment to apoptosis requires Bax or Bak. The BH3-only proteins appear to activate Bax and Bak indirectly, by engaging and neutralizing their pro-survival relatives, which otherwise constrain Bax and Bak from permeabilizing mitochondria. The Bcl-2 family may also regulate autophagy and mitochondrial fission/fusion. Its pro-survival members are attractive therapeutic targets in cancer and perhaps autoimmunity and viral infections.

Introduction

Programmed cell death plays critical roles in both development and tissue homeostasis. Indeed, in the immune system, apoptosis shapes the immune repertoire and refines and terminates immune responses [1*]. Since impaired apoptosis can lead to either autoimmunity or malignancy, there is intense interest in uncovering its control mechanisms and exploring the therapeutic options they provide.

Apoptosis is precipitated by sequential activation of cysteine proteases of the caspase family, in two distinct but converging pathways [2,3]. The *extrinsic pathway* activates Caspase-8 (and Caspase-10 in humans) when ligand-mediated trimerisation of ‘death receptors’ of the Tumor Necrosis Factor family on the plasma membrane recruits the adaptor protein FADD and the caspase into multi-protein complexes. The *intrinsic* pathway (also termed the ‘mitochondrial’ or ‘stress’ pathway) activates Caspase-9 on the scaffold protein Apaf-1 when cytochrome c is released from damaged mitochondria in response to diverse stresses, including cytokine deprivation and DNA damage. These initiator caspases can cleave and activate the effector caspases (-3, -6 and -7) that mediate cellular demolition by cleaving multiple critical cellular proteins.

The intrinsic pathway is controlled by the Bcl-2 protein family, which constitutes a tri-partite regulatory cassette [4*]. Bcl-2 itself, the oncoprotein discovered via the chromosome translocation that hallmarks human follicular lymphoma, inhibits apoptosis, as do its close homologs Bcl-x_L, Bcl-w, Mcl-1, A1 and (in humans) Bcl-B. In contrast, two other sub-groups promote apoptosis: one comprises Bax and Bak (and the little-studied Bok), which also

resemble Bcl-2, particularly in three "BH" (Bcl-2 Homology) domains, whereas the other group, which includes Bim, Bad, Bid, Bik, Bmf, Puma, Noxa and Hrk, shares only the BH3 domain. When activated by stress signals, these 'BH3-only' proteins insert that domain, an amphipathic α helix, into a hydrophobic groove on the pro-survival relatives [5]. This coupling primes the cell for apoptosis, but commitment requires activation of Bax and/or Bak [6,7], which then form oligomers on intracellular membranes, including the mitochondrial outer membrane, and thereby perturb their integrity.

In reviewing the role of the Bcl-2 family in apoptosis, we will focus primarily on two areas receiving great attention: the vexed issue of how interactions between the warring Bcl-2 factions commit the cell to apoptosis and the exciting prospect of targeting this family as a new approach to the therapy of cancer and perhaps autoimmune and virally-induced diseases. We also briefly discuss recent evidence that the family also influences autophagy and mitochondrial morphology. Further background on the Bcl-2 family and apoptosis is provided by recent reviews [1*,4*,8,9*], including the accompanying ones.

Flipping the life-death switch

Activated BH3-only proteins were thought to bind indiscriminately to all their pro-survival counterparts until quantitative studies revealed marked differences [10**]. Bim, Puma and tBid (the activated, truncated form of Bid) do bind avidly to all the pro-survival proteins, but the others associate only with subsets [10**]. For example, Noxa engaged only Mcl-1 and A1, and Bad only Bcl-2, Bcl-x_L and Bcl-w. Importantly, the promiscuous binders killed much more potently than the selective binders, but the combination of the complementary binders Noxa and Bad readily induced cell death [10**]. These findings indicate that efficient apoptosis requires neutralization of multiple pro-survival proteins.

The BH3-only proteins clearly act upstream of Bax and Bak, because they cannot induce apoptosis in cells lacking both Bax and Bak [6,7]. Their role in the activation of Bax and Bak, however, is highly controversial. In the *direct activation* model (Figure 1A), a sub-group of BH3-only proteins, termed *activators*, are proposed to bind directly to Bax and Bak to promote their activation [11,12*,13,14*,15,16*]. The putative activators include Bim and tBid [11, 12*,13,14*,15], and perhaps also Puma [16*,17], although that has been disputed [12*,13, 18]. In this model, the remaining BH3-only proteins, termed *sensitisers*, function by binding to the pro-survival proteins and freeing any bound Bim or tBid to directly activate Bax and Bak.

The *indirect activation* model (Figure 1B) proposes instead that all BH3-only proteins function solely by binding to their pro-survival relatives, thereby preventing those guardians of cell survival from inhibiting Bax and Bak [10**,19**,20**,21]. In this model, Bim, tBid and Puma are the most potent BH3-only proteins simply because they can engage all the pro-survival proteins [10**].

Several recent findings strongly challenge the *direct activation* model [20**]. Firstly, no Bak co-immunoprecipitated with any BH3-only protein. Secondly, no Bax bound detectably to the physiologically relevant forms of Bim (Bim_{EL} and Bim_L), and none co-immunoprecipitated with Bim in dying cells. Thirdly, although tBid and a minor Bim isoform (Bim_S) bound Bax weakly, tBid and Bim_S bearing BH3 mutations that prevented binding to Bax but retained normal binding to pro-survival proteins remained just as potent as killers. Most tellingly, in response to several apoptotic stimuli, or enforced co-expression of Noxa and Bad, cells lacking both Bid and Bim died as readily as wild-type cells, and even down-regulation of Puma by RNAi in these cells did not impair apoptosis driven by several stimuli [20**]. Thus, none of the putative activator BH3-only proteins appears to be essential for apoptosis.

Mouse genetic studies also favor the *indirect activation* model. Mice lacking both Bim and Bid [20**], or both Bim and Puma [22], appear normal, and their cells remain normally sensitive to certain apoptotic signals. On the *direct activation* model, the absence of these *activators* should have blocked apoptosis and promoted severe developmental abnormalities, as in mice lacking both Bax and Bak [23].

Some viral Bcl-2 homologs, which may have been selected for resistance to inhibition by BH3-only regulators, also act by binding to and restraining Bax and Bak [24], further supporting the *indirect activation* model. Remarkably, M11L of myxoma virus, an anti-apoptotic protein lacking sequence homology to Bcl-2, has an extremely similar 3D fold [25*], and structure-based mutagenesis suggested that its critical binding targets were Bax and Bak rather than BH3-only proteins.

Collectively, these findings demonstrate that apoptosis does not rely upon direct activation of Bax or Bak by any known BH3-only protein (Figure 1A). Nevertheless, direct activation might occur in some circumstances. Protein-free liposomes can be lysed by cooperation of Bax with tBid [26] or with BH3 peptides from Bim or Bid at high (eg >10 micromolar) concentrations [12*], or at much lower levels if these peptides are chemically 'stapled' into an α helix and/or artificially targeted to membranes [14*,15]. Perhaps direct binding by Bim or tBid contributes to an amplification step in apoptosis.

Death by default

In the *indirect activation* model, apoptosis is the default pathway and the Bcl-2 pro-survival proteins function mainly by constraining activation of Bax and/or Bak (Figure 2). The diverse phenotypes observed in mice on inactivation of individual pro-survival genes (see [4*] and the review by Opferman in this issue) presumably indicate that specific pro-survival proteins are the dominant guards on Bax/Bak in different cell types. For example, a Bcl-x_L/Bak switch primarily controls the lifespan of anucleate platelets [27**]. Platelets exposed to a BH3 mimic that inactivates Bcl-x_L (see below) underwent apoptosis, and the reduced platelet level found in *bcl-x* heterozygotes or mice bearing labile hypomorphic Bcl-x_L mutants was ameliorated by concomitant Bak deficiency. The inference is that Bcl-x_L is rate limiting for platelet survival, and once normal turnover drops its level below the threshold for constraining Bak, the unleashed Bak drives apoptosis [27**].

Inhibition of Bax and Bak by pro-survival Bcl-2 proteins relies, in part, upon direct association [19**,20**]. In healthy cells, Bak can bind to either Mcl-1 [28] or Bcl-x_L (albeit, unexpectedly, not Bcl-2), and this interaction appears to require the Bak BH3 domain [19**]. To initiate apoptosis, BH3-only proteins seem to displace Bak from these pro-survival proteins. Importantly, Bak could drive cell death if and only if both Mcl-1 and Bcl-x_L were inactivated by, for example, Noxa plus Bad [19**]. Bax also appears to be constrained in part by direct interaction with pro-survival proteins [20**]. The significance of their association has long been questioned, because it is often detectable only in non-ionic detergents, which change the conformation of Bax, perhaps mimicking its activation [29]. Recent killing assays, however, have established that neutralisation of multiple pro-survival proteins, including Bcl-2, by BH3-only proteins is necessary and apparently sufficient for Bax activation (Figure 2) [20**].

How do the pro-survival proteins restrain Bax and Bak, given that the known structures of Bax [30] and Bak [31] have the BH3 domain buried? One proposal is that, in healthy cells, a small proportion of Bax and Bak (see below) is in a 'primed' state, with its BH3 domain exposed but restrained by binding to pro-survival proteins [19**] (Figure 2). Any primed Bax is likely to reside on the mitochondrial membrane, because the cytosolic form is monomeric [32]. Rather than pre-existing, the primed conformers of Bax and Bak may instead form early in apoptotic

signalling, either spontaneously or in response to an independent cytotoxic signal [20**]. For example, phosphorylation of Bax may regulate its translocation to the membrane [33,34].

The pro-survival proteins may also constrain Bax and Bak by a second, unknown mechanism not requiring their association via the Bax/Bak BH3 domain. BH3 mutants of Bak and Bax have been identified that cannot bind detectably to their pro-survival relatives but are still functional upon cytotoxic stimulation [16*] (J Fletcher, DSC Huang and JMA, unpublished results).

The prospect of BH3 mimetic drugs

Since most tumors have defects in the p53 pathway or over-express a Bcl-2 homolog, interest is surging in the potential of anti-cancer drugs that, like the BH3 domain, bind one or more Bcl-2 homologs and trigger apoptosis [4*,9*]. Such 'BH3 mimetics' should be more effective than anti-tumor agents acting far upstream. Furthermore, BH3 selectivity [5,10**] heralds the prospect of specifically targeting the Bcl-2 homolog(s) required to maintain a particular tumor type, sparing more of the normal cells. Such drugs may also be applicable to certain autoimmune diseases and perhaps to infections with viruses that express responsive vBcl-2 homologs.

Although few drugs are peptides, BH3 peptides constrained as an α-helix by a chemical staple [14*,35], or by inclusion of unnatural amino acids [36,37], may have potential. The ability of a stapled Bid BH3 peptide to activate Bax, at least *in vitro* [14*], may mean that Bax (or Bak) could also be targeted, although that should kill more normal cells than a compound engaging selected pro-survival proteins.

Small organic BH3 mimics appear more promising. A structure-based approach led to ABT-737, which binds strongly (low nM affinity) to Bcl-2, Bcl-x_L and Bcl-w but not to Mcl-1 or A1 [38**]. ABT-737 proved cytotoxic as a single agent for many samples of B lymphoid tumors and chronic lymphocytic leukemia (CLL), as well as small cell lung cancer cell lines. Significantly, it induced stable regression of lung cancer in mouse xenografts, with minimal side effects [38**]. It also killed AML cells but not normal hematopoietic progenitor cells *in vitro* and delayed leukemia in xenografts without collateral damage [39].

Unlike six other putative BH3 mimetics, ABT-737 behaved in the mechanism-based fashion, since it spared cells lacking both Bax and Bak [40*]. Notably, cells with high levels of Mcl-1, which the drug does not bind, proved much more refractory than those with little Mcl-1 (compare Figure 3A and B) [39,40*,41,42*,43], and the less frequently expressed A1 also promoted some resistance [40*]. However, down regulation of the short-lived Mcl-1 by different strategies, some potentially clinically applicable, conferred sensitivity [39,40*,41, 42*,43]. Interestingly, the drug readily overcame high levels of Bcl-2 [40*]. Indeed, abundant Bcl-2 may actually render neoplasms such as CLL *more* sensitive, by sequestering an elevated level of a potent killer like Bim – presumably induced by oncogenic stress - that the drug can release (Figure 3C) [44]. Hence, ABT-737 should be effective on its own in tumors with low Mcl-1 and A1, even in the face of high Bcl-2, and may prove more widely efficacious if combined with agents that prevent Mcl-1 synthesis, promote its degradation or inactivate it.

BH3 mimetics may also prove to be important adjuvants in conventional therapy. ABT-737 and the Bcl-x_L-specific A-385358 [45] markedly sensitised cells to diverse chemotherapy agents, in part due to Mcl-1 degradation [38**,39,40*,41,42,46,47*]. For example, Bim and Bad mediate the killing of CML cells by imatinib, and co-treatment with ABT-737 might augment responses in some patients with refractory disease [47*]. Thus, a BH3 mimetic might allow lower doses of a conventional agent or more durable responses, unless substantial adverse effects arise. Phase I clinical trials have begun for an orally available ABT-737 derivative, and

Phase II trials for the less well-characterized GeminX broad-spectrum BH3 mimetic GX15-070 [48].

Links of the Bcl-2 family to autophagy and mitochondrial morphology

Autophagy is an ancient mechanism by which starved cells produce energy and stave off death by gradually targeting their organelles and cytoplasmic elements to lysosomes for digestion. Excessive self-cannibalisation may represent a second form of programmed cell death [49*, 50]. Significantly, Bcl-2 and Bcl-x_L associate with the evolutionarily conserved autophagy inducer Beclin-1, a haplo-insufficient tumor suppressor [51], and inhibit autophagy [52**]. The inhibition may require Bcl-2 localized on the endoplasmic reticulum (ER) [52**,53]. Notably, a BH3 domain within Beclin-1 mediates their association [54].

Since the Beclin-1:Bcl-x_L association is of low affinity (1–2 μM versus low nM for BH3-only proteins) [54], presumably the BH3-only proteins induced by stress (or some BH3 mimetic agents) can displace Beclin-1, rendering the cell susceptible to autophagy as well as apoptosis (Figure 4). Curiously, if apoptosis is blocked, e.g. by the absence of Bax and Bak, the autophagic response to nutrient/cytokine deprivation is prolonged, albeit not indefinite, survival [49*,52**], whereas that to cytotoxic compounds (e.g. etoposide) is primarily autophagic cell death [55**] (Figure 4). Therefore, manipulating autophagy may well affect therapy, but whether its promotion or its inhibition [56] will be more effective remains to be established.

Independent of its apoptotic role, the Bcl-2 family seems to influence mitochondrial shape, which reflects the balance between continual fission and fusion processes [57]. Paradoxically, however, whereas either loss of both Bax and Bak or expression of pro-survival relatives curtails apoptosis, the former promotes fission [58] and the latter fusion [59,60]. In any case, most evidence suggests that the mitochondrial fission machinery is not required for mitochondrial permeabilization or apoptosis [57,61].

Conundrums and controversies

Surprisingly, most Bax molecules probably translocate from the cytosol to membranes *after* caspases are activated [62*,63,64]. Bax translocation and cytochrome c release were slower in cells lacking both Caspase-3 and -7, implicating a proteolytic amplification loop [62*]. Conceivably, these activated caspases augment the initial response by cleaving Bcl-2 pro-survival proteins or by generating tBid from full-length Bid. In any case, such findings imply that commitment to apoptosis requires only a small proportion of Bax, even in the absence of Bak [63].

Several Bcl-2 family members, including Bcl-2 and Bak, reside not only on mitochondria but also on the ER/nuclear envelope, where they may regulate cytosolic Ca^{2+} levels. Association of Bcl-2/Bcl-x_L with the inositol triphosphate receptor type 1 (InsP3R) reportedly increases leakage of Ca^{2+} ions into the cytosol [65,66]. Bax and Bak may also modulate the ER unfolded protein response by binding to the cytosolic domain of the inositol-requiring endoribonuclease 1alpha (IRE1alpha) [67]. Whether apoptotic signals from the ER must be routed through the mitochondria is not certain.

Bid loss primarily affects the extrinsic pathway, but conflicting recent reports suggested that Bid either countered or enhanced apoptosis due to replicative stress or genotoxic damage [68,69], Contrary to those reports, Bid appears to be dispensable for both DNA damage-induced apoptosis and cell cycle arrest [70].

Concluding remarks

Both biochemical and genetic evidence now strongly supports the hypothesis that the BH3-only proteins trigger apoptosis primarily by engaging their pro-survival relatives (Figure 1B), freeing Bax and Bak (Figure 2) [10**,19**,20**,21]. Nevertheless, *in vitro* data from liposome disruption [12*,14*] and from some mutant proteins [16*], argue that certain BH3-only proteins, such as tBid, can directly activate Bax/Bak (Figure 1A). Hence, direct activation may still have a role, perhaps in amplifying the apoptotic signal once mitochondrial permeation has commenced. Precisely how the pro-survival proteins restrain Bax and Bak remains unclear, as more evidence is needed for the presumptive 'primed' Bax/Bak (Figure 2), and to clarify the mechanism of the 'association-independent' constraint hypothesized above.

Pre-clinical studies with BH3-mimetics, particularly ABT-737, suggest that these new agents will prove valuable additions to the oncologist's armamentarium (Figure 3). Eventually, we envision a panel of BH3 mimetics that target different Bcl-2 homologs or combinations thereof. It remains unclear whether strategies targeting multiple or individual family members will prove most efficacious, but both may well find important niches. The possibility that BH3 mimetics may also trigger autophagy (Figure 4) raises new questions and prospects.

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References

* of special interest

** of outstanding interest

1*. Strasser A. The role of BH3-only proteins in the immune system. Nat Rev Immunol 2005;5:189–200. [PubMed: 15719025]This recent review clearly outlines the physiological roles of the Bcl-2 family and apoptosis, particularly in the immune system.

2. Adams JM. Ways of dying: multiple pathways to apoptosis. Genes Dev 2003;17:2481–2495. [PubMed: 14561771]

3. Shi Y. Mechanical aspects of apoptosome assembly. Curr Opin Cell Biol 2006;18:677–684. [PubMed: 17046227]

4*. Adams JM, Cory S. The Bcl-2 apoptotic switch in cancer development and therapy. Oncogene 2007;26:1324–1337. [PubMed: 17322918]This recent review focuses on the mechanisms by which the Bcl-2 family control apoptosis and their impact on cancer development and therapy.

5. Hinds MG, Day CL. Regulation of apoptosis: uncovering the binding determinants. Curr Opin Struct Biol 2005;15:690–699. [PubMed: 16263267]

6. Cheng EH, Wei MC, Weiler S, Flavell RA, Mak TW, Lindsten T, Korsmeyer SJ. BCL-2, BCL-x_L sequester BH3 domain-only molecules preventing BAX- and BAK-mediated mitochondrial apoptosis. Mol Cell 2001;8:705–711. [PubMed: 11583631]

7. Zong WX, Lindsten T, Ross AJ, MacGregor GR, Thompson CB. BH3-only proteins that bind pro-survival Bcl-2 family members fail to induce apoptosis in the absence of Bax and Bak. Genes Dev 2001;15:1481–1486. [PubMed: 11410528]

8. Danial NN, Korsmeyer SJ. Cell death: critical control points. Cell 2004;116:205–219. [PubMed: 14744432]

9*. Fesik SW. Promoting apoptosis as a strategy for cancer drug discovery. Nat Rev Cancer 2005;5:876–885. [PubMed: 16239906]This review outlines different approaches for targeting the apoptotic machinery in cancer therapy.

10**. Chen L, Willis SN, Wei A, Smith BJ, Fletcher JI, Hinds MG, Colman PM, Day CL, Adams JM, Huang DCS. Differential targeting of pro-survival Bcl-2 proteins by their BH3-only ligands allows complementary apoptotic function. Mol Cell 2005;17:393–403. [PubMed: 15694340]This study revealed the selectivity of BH3 domains in binding the pro-survival proteins (see also ref 12, 13) and demonstrated that apoptosis requires neutralization of multiple Bcl-2 homologs.

11. 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;2:183–192. [PubMed: 12242151]

12*. Kuwana T, Bouchier-Hayes L, Chipuk JE, Bonzon C, Sullivan BA, Green DR, Newmeyer DD. BH3 Domains of BH3-Only Proteins Differentially Regulate Bax-Mediated Mitochondrial Membrane Permeabilization Both Directly and Indirectly. Mol Cell 2005;17:525–535. [PubMed: 15721256] This work provided evidence for direct activation of Bax by showing that certain BH3 peptides can act together with Bax to lyse protein-free liposomes (see also ref 11, 13, 14*).

13. Certo M, Moore Vdel G, Nishino M, Wei G, Korsmeyer S, Armstrong SA, Letai A. Mitochondria primed by death signals determine cellular addiction to antiapoptotic BCL-2 family members. Cancer Cell 2006;9:351–365. [PubMed: 16697956]

14*. Walensky LD, Pitter K, Morash J, Oh KJ, Barbuto S, Fisher J, Smith E, Verdine GL, Korsmeyer SJ. A Stapled BID BH3 Helix Directly Binds and Activates BAX. Mol Cell 2006;24:199–210. [PubMed: 17052454]The first quantitative data supporting the idea that certain BH3 peptides, if constrained into an a-helix, may be able to active Bax directly (see also 15).

15. 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;281:36999–37008. [PubMed: 16987815]

16*. Kim H, Rafiuddin-Shah M, Tu HC, Jeffers JR, Zambetti GP, Hsieh JJ, Cheng EH. Hierarchical regulation of mitochondrion-dependent apoptosis by BCL-2 subfamilies. Nat Cell Biol 2006;8:1348–1358. [PubMed: 17115033]This study provided evidence that sequestration by pro-survival proteins in itself is insufficient to account for the regulation of Bax and Bak and reported further data indicating that Bak is inhibited by the VDAC2, a pore-forming protein in the mitochondrial outer membrane.

17. Cartron PF, Gallenne T, Bougras G, Gautier F, Manero F, Vusio P, Meflah K, Vallette FM, Juin P. The first alpha helix of Bax plays a necessary role in its ligand-induced activation by the BH3-only proteins Bid and PUMA. Mol Cell 2004;16:807–818. [PubMed: 15574335]

18. Ming L, Wang P, Bank A, Yu J, Zhang L. PUMA Dissociates Bax and Bcl-XL to Induce Apoptosis in Colon Cancer Cells. J Biol Chem 2006;281:16034–16042. [PubMed: 16608847]

19**. Willis SN, Chen L, Dewson G, Wei A, Naik E, Fletcher JI, Adams JM, Huang DC. Pro-apoptotic Bak is sequestered by Mc1-1 and Bcl-xL, but not Bcl-2, until displaced by BH3-only proteins. Genes Dev 2005;19:1294–1305. [PubMed: 15901672]This study showed that Mcl-1 and Bcl-x_L can bind Bak in healthy cells (see also 28) and that their neutralization by BH3-only proteins is necessary and apparently sufficient to induce Bak-mediated apoptosis (see also ref 20** and 21).

20**. Willis SN, Fletcher JI, Kaufmann T, van Delft MF, Chen L, Czabotar PE, Ierino H, Lee EF, Fairlie WD, Bouillet P, et al. Apoptosis initiated when BH3 ligands engage multiple Bcl-2 homologs, not Bax or Bak. Science 2007;315:856–859. [PubMed: 17289999]In further support of the indirect activation model, neutralization of multiple pro-survival proteins was shown to be sufficient to activate Bax as well as Bak for apoptosis and, contrary to the direct activation model, none of the three proposed BH3-only activators (Bim, tBid or Puma) was required to trigger apoptosis via Bax or Bak (see also ref 19** and 21).

21. Uren RT, Dewson G, Chen L, Adams JM, Huang DCS, Kluck RM. Mitochondrial permeabilization relies on BH3 ligands engaging multiple pro-survival Bcl-2 relatives rather than Bak. Journal of Cell Biology. In Press

22. Erlacher M, Laabi V, Manzl C, Bock G, Tzankov A, Haecker G, Strasser A, Villunger A. Puma cooperates with Bim, the rate-limiting BH3-only protein in cell death during lymphocyte

development, in apoptosis induction. Journal of Experimental Medicine 2006;203:2939–2951. [PubMed: 17178918]

23. Lindsten T, Ross AJ, King A, Zong W, Rathmell JC, Shiels HA, Ulrich E, Waymire KG, Mahar P, Frauwirth K, 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;6:1389–1399. [PubMed: 11163212]
24. White E. Mechanisms of apoptosis regulation by viral oncogenes in infection and tumorigenesis. Cell Death Differ 2006;13:1371–1377. [PubMed: 16676007]
25*. Kvansakul M, Van Delft MF, Lee EF, Gulbis JM, Fairlie WD, Huang DCS, Colman PM. A structural viral mimic of pro-survival Bcl-2: a pivotal role for sequestering pro-apoptotic Bax and Bak. Molecular Cell 2007;25:933–942. [PubMed: 17386268]The pro-survival function of a viral structural mimic of Bcl-2 is shown to rely upon binding Bax and Bak rather than BH3-only proteins.
26. Kuwana T, Mackey MR, Perkins G, Ellisman MH, Latterich M, Schneiter R, Green DR, Newmeyer DD. Bid, Bax, and lipids cooperate to form supramolecular openings in the outer mitochondrial membrane. Cell 2002;111:331–342. [PubMed: 12419244]
27**. Mason KD, Carpinelli MR, Fletcher JI, Collinge JE, Hilton AA, Ellis S, Kelly PN, Ekert PG, Metcalf D, Roberts AW, et al. Programmed anuclear cell death delimits platelet life span. Cell 2007;128:1035–1036. [PubMed: 17382877]This study reveals that even anucleate platelets undergo apoptosis and that their demise is controlled primarily by Bcl-x_L and Bak, supporting death by default.
28. Cuconati A, Mukherjee C, Perez D, White E. DNA damage response and MCL-1 destruction initiate apoptosis in adenovirus-infected cells. Genes Dev 2003;17:2922–2932. [PubMed: 14633975]
29. Hsu YT, Youle RJ. Nonionic detergents induce dimerization among members of the Bcl-2 family. Journal of Biological Chemistry 1997;272:13829–13834. [PubMed: 9153240]
30. Suzuki M, Youle RJ, Tjandra N. Structure of Bax: coregulation of dimer formation and intracellular localization. Cell 2000;103:645–654. [PubMed: 11106734]
31. Moldoveanu T, Liu Q, Tocilj A, Watson MH, Shore G, Gehring K. The x-ray structure of a BAK homodimer reveals an inhibitory zinc binding site. Molecular Cell 2006;24:677–688. [PubMed: 17157251]
32. Hsu Y-T, Youle RJ. Bax in murine thymus is a soluble monomeric protein that displays differential detergent-induced conformations. Journal of Biological Chemistry 1998;273:10777–10783. [PubMed: 9553144]
33. Kim BJ, Ryu SW, Song BJ. JNK- and p38 kinase-mediated phosphorylation of Bax leads to its activation, mitochondrial translocation and to apoptosis of human hepatoma HepG2 cells. J Biol Chem 2006;281:21256–21265. [PubMed: 16709574]
34. Gardai SJ, Hildeman DA, Frankel SK, Whitlock BB, Frasch SC, Borregaard N, Marrack P, Bratton DL, Henson PM. Phosphorylation of Bax Ser184 by Akt regulates its activity and apoptosis in neutrophils. J Biol Chem 2004;279:21085–21095. [PubMed: 14766748]
35. Walensky LD, Kung AL, Escher I, Malia TJ, Barbuto S, Wright RD, Wagner G, Verdine GL, Korsmeyer SJ. Activation of Apoptosis in Vivo by a Hydrocarbon-Stapled BH3 Helix. Science 2004;305:1466–1470. [PubMed: 15353804]
36. Sadowsky JD, Schmitt MA, Lee HS, Umezawa N, Wang S, Tomita Y, Gellman SH. Chimeric (alpha/beta + alpha)-peptide ligands for the BH3-recognition cleft of Bcl-XL: critical role of the molecular scaffold in protein surface recognition. J Am Chem Soc 2005;127:11966–11968. [PubMed: 16117535]
37. Sadowsky JD, Fairlie WD, Hadley EB, Lee HS, Umezawa N, Nikolovska-Coleska Z, Wang S, Huang DC, Tomita Y, Gellman SH. (alpha/beta+alpha)-peptide antagonists of BH3 domain/Bcl-x(L) recognition: toward general strategies for foldamer-based inhibition of protein-protein interactions. Journal of the American Chemical Society 2007;129:139–154. [PubMed: 17199293]
38**. Oltersdorf T, Elmore SW, Shoemaker AR, Armstrong RC, Augeri DJ, Belli BA, Bruncko M, Deckwerth TL, Dinges J, Hajduk PJ, et al. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. Nature 2005;435:677–681. [PubMed: 15902208]This study describing the initial findings with ABT-737 illustrates the therapeutic potential of this new class of potential anti-cancer agents.

39. Konopleva M, Contractor R, Tsao T, Samudio I, Ruvolo PP, Kitada S, Deng X, Zhai D, Shi Y-X, Sneed T, et al. Mechanisms of apoptosis sensitivity and resistance to the BH3 mimetic ABT-737 in acute myeloid leukemia. Cancer Cell 2006;10:375–388. [PubMed: 17097560]

40*. van Delft MF, Wei AH, Mason KD, Vandenberg CJ, Chen L, Czabotar PE, Willis SN, Scott CL, Day CL, Cory S, 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;10:389–399. [PubMed: 17097561]This work explored the mechanism of action of ABT-737, showed that it is cytotoxic only in cells with minimal Mcl-1 (or A1) and described ways of downregulating Mcl-1 to enhance its efficacy (see also 38, 39, 41 and 42*).

41. Tahir SK, Yang X, Anderson MG, Morgan-Lappe SE, Sarthy AV, Chen J, Warner RB, Ng SC, Fesik SW, Elmore SW, et al. Influence of Bcl-2 family members on the cellular response of small-cell lung cancer cell lines to ABT-737. Cancer Research 2007;67:1176–1183. [PubMed: 17283153]

42*. Chen S, Dai Y, Harada H, Dent P, Grant S. Mcl-1 down-regulation potentiates ABT-737 lethality by cooperatively inducing Bak activation and Bax translocation. Cancer Res 2007;67:782–791. [PubMed: 17234790]Like ref 40*, this study explored how ABT-737 works and ways of enhancing its activity in tumor cells that have substantial Mcl-1.

43. Lin X, Morgan-Lappe S, Huang X, Li L, Zakula DM, Vernetti LA, Fesik SW, Shen Y. 'Seed' analysis of off-target siRNAs reveals an essential role of Mcl-1 in resistance to the small-molecule Bcl-2/Bcl-X(L) inhibitor ABT-737. Oncogene. In Press

44. Del Gaizo Moore V, Brown JR, Certo M, Love TM, Novina CD, Letai A. Chronic lymphocytic leukemia requires BCL2 to sequester prodeath BIM, explaining sensitivity to BCL2 antagonist ABT-737. J Clin Invest 2007;117:112–121. [PubMed: 17200714]

45. Shoemaker AR, Oleksijew A, Bauch J, Belli BA, Borre T, Bruncko M, Deckwirth T, Frost DJ, Jarvis K, Joseph MK, et al. A Small-Molecule Inhibitor of Bcl-XL Potentiates the Activity of Cytotoxic Drugs In vitro and In vivo. Cancer Res 2006;66:8731–8739. [PubMed: 16951189]

46. Chauhan D, Velankar M, Brahmandam M, Hideshima T, Podar K, Richardson P, Schlossman R, Ghobrial I, Raje N, Munshi N, et al. A novel Bcl-2/Bcl-X(L)/Bcl-w inhibitor ABT-737 as therapy in multiple myeloma. Oncogene. In Press

47*. Kuroda J, Puthalakath H, Cragg MS, Kelly PN, Bouillet P, Huang DC, Kimura S, Ottmann OG, Druker BJ, Villunger A, et al. Bim and Bad mediate imatinib-induced killing of Bcr/Abl+ leukemic cells, and resistance due to their loss is overcome by a BH3 mimetic. Proc Natl Acad Sci U S A 2006;103:14907–14912. [PubMed: 16997913]The cytotoxic activity of imatinib was shown to rely on Bim and Bad but their activity could be replaced by the Bad-like ABT-737, suggesting that co-treatment with imatinib and the BH3 mimetic might enhance efficacy, particularly in patients refractory to imatinib alone.

48. 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. In Press

49*. Lum JJ, Bauer DE, Kong M, Harris MH, Li C, Lindsten T, Thompson CB. Growth factor regulation of autophagy and cell survival in the absence of apoptosis. Cell 2005;120:237–248. [PubMed: 15680329]Hematopoietic cells lacking Bax and Bak, when deprived of cytokine, are shown to survive for an extended period by undergoing autophagy but eventually to succumb to non-apoptotic death.

50. Tsujimoto Y, Shimizu S. Another way to die: autophagic programmed cell death. Cell Death Differ 2005;12 (Suppl 2):1528–1534. [PubMed: 16247500]

51. Qu X, Yu J, Bhagat G, Furuya N, Hibshoosh H, Troxel A, Rosen J, Eskelinen EL, Mizushima N, Ohsumi Y, et al. Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. J Clin Invest 2003;112:1809–1820. [PubMed: 14638851]

52**. Pattingre S, Tassa A, Qu X, Garuti R, Liang XH, Mizushima N, Packer M, Schneider MD, Levine B. Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. Cell 2005;122:927–939. [PubMed: 16179260]This study provided evidence that the Bcl-2 family can control autophagy via the interaction of Bcl-2 or Bcl-x_L with the autophagy regulator Beclin-1.

53. Hoyer-Hansen M, Bastholm L, Szyniarowski P, Campanella M, Szabadkai G, Farkas T, Bianchi K, Fehrenbacher N, Elling F, Rizzuto R, et al. Control of macroautophagy by calcium, calmodulin-dependent kinase kinase-beta, and Bcl-2. Mol Cell 2007;25:193–205. [PubMed: 17244528]

54. Oberstein A, Jeffrey P, Shi Y. Crystal structure of the BCL-XL-beclin 1 peptide complex: Beclin 1 is a novel BH3-only protein. J Biol Chem. In Press

55**. Shimizu S, Kanaseki T, Mizushima N, Mizuta T, Arakawa-Kobayashi S, Thompson CB, Tsujimoto Y. Role of Bcl-2 family proteins in a non-apoptotic programmed cell death dependent on autophagy genes. Nat Cell Biol 2004;6:1221–1228. [PubMed: 15558033]Whereas most studies suggest that Bcl-2 inhibits autophagy, this study reported that, in the presence of cytotoxic drugs, Bcl-2 instead stimulated autophagic cell death.

56. Amaravadi RK, Yu D, Lum JJ, Bui T, Christophorou MA, Evan GI, Thomas-Tikhonenko A, Thompson CB. Autophagy inhibition enhances therapy-induced apoptosis in a Myc-induced model of lymphoma. J Clin Invest 2007;117:326–336. [PubMed: 17235397]

57. Arnoult D. Mitochondrial fragmentation in apoptosis. Trends Cell Biol 2007;17:6–12. [PubMed: 17116393]

58. Karbowski M, Norris KL, Cleland MM, Jeong SY, Youle RJ. Role of Bax and Bak in mitochondrial morphogenesis. Nature 2006;443:658–652. [PubMed: 17035996]

59. Delivani P, Adrain C, Taylor RC, Duriez PJ, Martin SJ. Role for CED-9 and Egl-1 as regulators of mitochondrial fission and fusion dynamics. Mol Cell 2006;21:761–773. [PubMed: 16543146]

60. Jagasia R, Grote P, Westermann B, Conradt B. DRP-1-mediated mitochondrial fragmentation during EGL-1-induced cell death in C. elegans Nature 2005;433:754–760.

61. Parone PA, James DI, Da Cruz S, Mattenberger Y, Donze O, Barja F, Martinou JC. Inhibiting the mitochondrial fission machinery does not prevent bax/bak-dependent apoptosis. Mol Cell Biol 2006;26:7397–7408. [PubMed: 17015472]

62*. Lakhani SA, Masud A, Kuida K, Porter GA Jr, Booth CJ, Mehal WZ, Inayat I, Flavell RA. Caspases 3 and 7: key mediators of mitochondrial events of apoptosis. Science 2006;311:847–851. [PubMed: 16469926]This study of cells lacking both the major effector caspases unexpectedly revealed that the majority of Bax may translocate to the mitochondrial membrane *after* caspase activation (see also ref 63 and 64), suggesting that a positive feedback loop operates.

63. Zhu Y, Liu X, Hildeman D, Peyerl FW, White J, Kushnir E, Kappler J, Marrack P. Bax does not have to adopt its final form to drive T cell death. J Exp Med 2006;203:1147–1152. [PubMed: 16651384]

64. Kepp O, Rajalingam K, Kimmig S, Rudel T. Bak and Bax are non-redundant during infection- and DNA damage-induced apoptosis. Embo J 2007;26:825–834. [PubMed: 17235284]

65. Oakes SA, Scorrano L, Opferman JT, Bassik MC, Nishino M, Pozzan T, Korsmeyer SJ. 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;102:105–110. [PubMed: 15613488]

66. White C, Li C, Yang J, Petrenko NB, Madesh M, Thompson CB, Foskett JK. The endoplasmic reticulum gateway to apoptosis by Bcl-X(L) modulation of the InsP3R. Nat Cell Biol 2005;7:1021–1028. [PubMed: 16179951]

67. Hetz C, Bernasconi P, Fisher J, Lee AH, Bassik MC, Antonsson B, Brandt GS, Iwakoshi NN, Schinzel A, Glimcher LH, et al. Proapoptotic BAX and BAK modulate the unfolded protein response by a direct interaction with IRE1alpha. Science 2006;312:572–576. [PubMed: 16645094]

68. Kamer I, Sarig R, Zaltsman Y, Niv H, Oberkovitz G, Regev L, Haimovich G, Lerenthal Y, Marcellus RC, Gross A. Proapoptotic BID Is an ATM Effector in the DNA-Damage Response. Cell 2005;122:593–603. [PubMed: 16122426]

69. Zinkel SS, Hurov KE, Ong C, Abtahi FM, Gross A, Korsmeyer SJ. A Role for Proapoptotic BID in the DNA-Damage Response. Cell 2005;122:579–591. [PubMed: 16122425]

70. Kaufmann T, Tai L, Ekert PG, Huang DCS, Norris F, Dixit VM, Strasser A. The Pro-Apoptotic BH3-only protein Bid is dispensable for DNA damage and replicative stress-induced apoptosis and cell cycle arrest. Cell. In Press

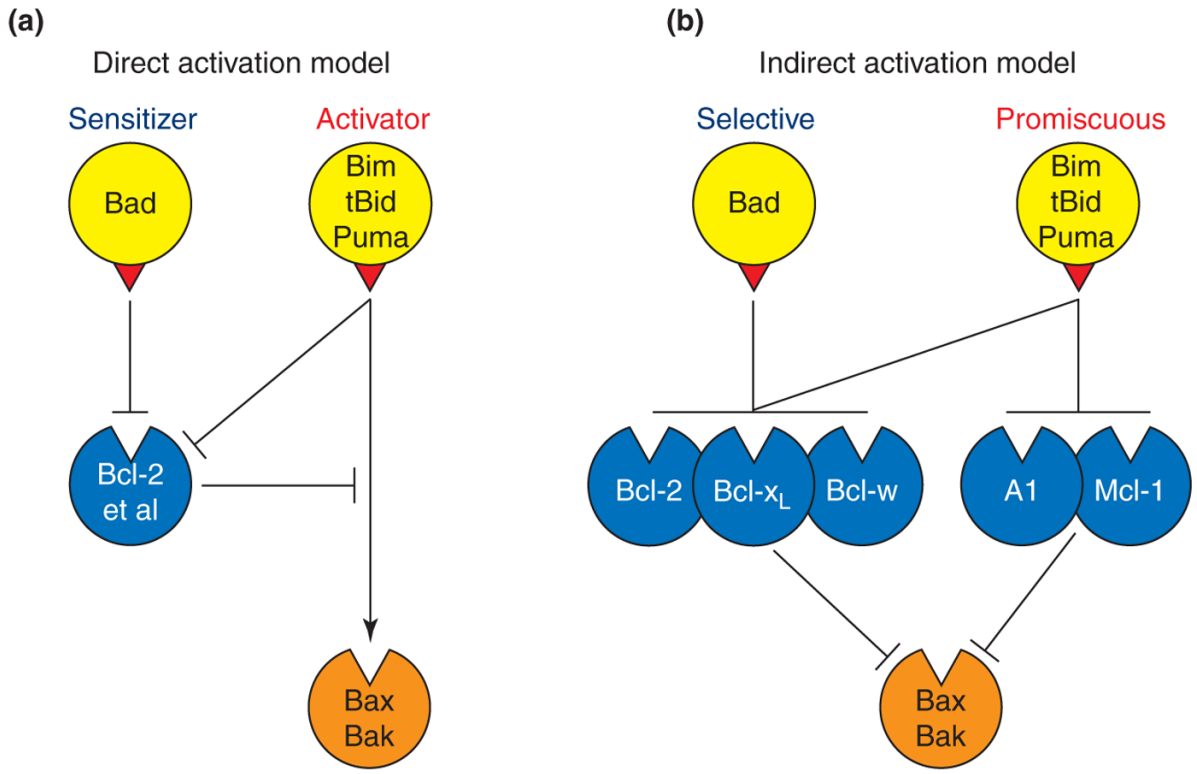

Figure 1.

Two models for how BH3-only proteins activate Bax and Bak. (**A**) In the *direct activation* model [11], the indicated *activator* BH3-only proteins, via their BH3 domain (red triangle), directly engage Bax and Bak and activate them, whereas *sensitizer* BH3-only proteins (e.g. Bad or Noxa), which can only bind the pro-survival proteins, serve only to displace *activators* from the pro-survival proteins. (**B**) In the *indirect activation* model [10**,19**, 20**] the BH3-only proteins only bind the pro-survival proteins. Because the promiscuous binders (Bim, tBid, Puma) can neutralise all pro-survival proteins, each can readily trigger Bax/Bak activation, whereas any selective binder (eg. Bad) must be co-expressed with a complementary binder (e.g. Noxa) to do so.

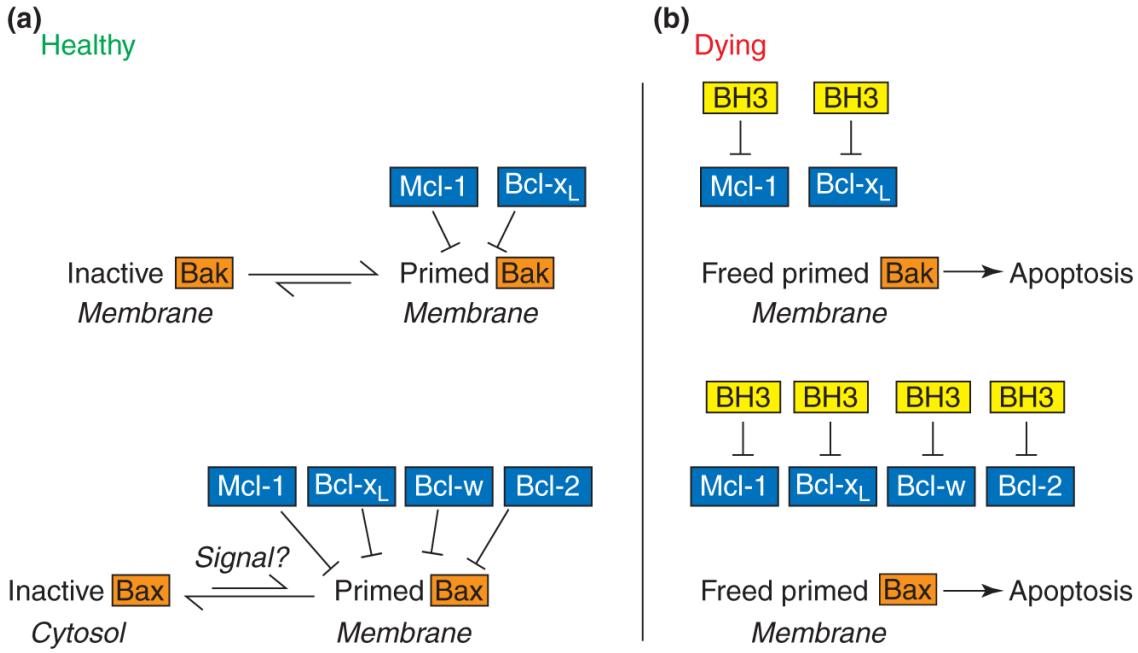

Figure 2.

Model for constraint of Bak (top) and Bax (bottom) by pro-survival family members [19**, 20**,21]. (**A**) In unperturbed cells or early in apoptotic signalling, a small proportion of Bak and Bax molecules is proposed to acquire a 'primed' conformation in which their BH3 domains are accessible but engaged by the indicated pro-survival proteins (and perhaps also A1). The priming might require an independent undetermined signal, particularly to recruit Bax from the cytosol. (**B**) In apoptotic cells, once BH3-only proteins ('BH3'), or a BH3 mimic, have engaged the appropriate pro-survival proteins, the freed primed Bak or Bax can nucleate the oligomerization thought to permeabilize the outer mitochondrial membrane and thereby commit the cell to apoptosis. The pro-survival proteins also appear to have a mode of constraint that does not rely upon association via the Bax/Bak BH3 domain (see text).

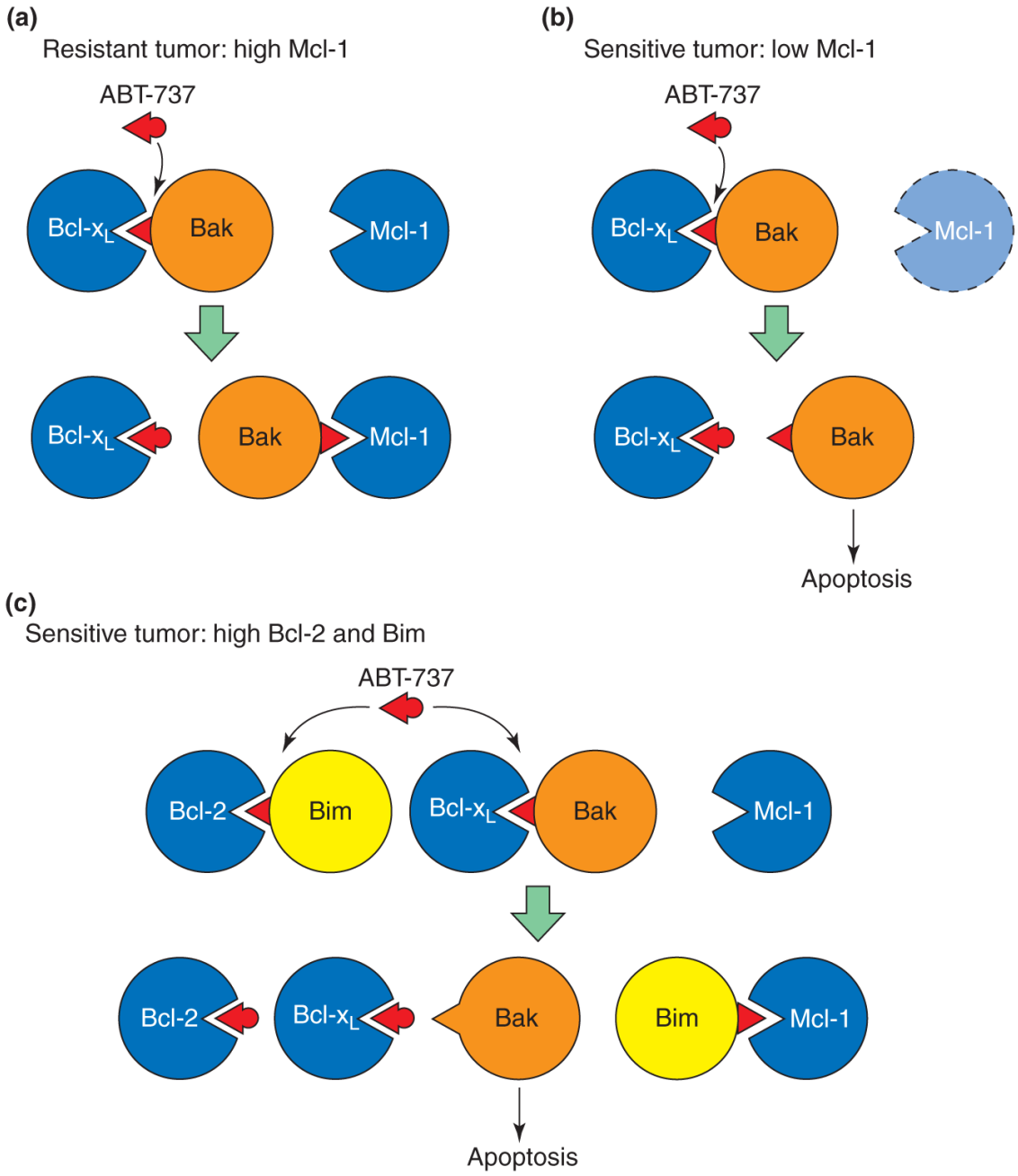

Figure 3.

Models for resistance/sensitivity of tumor cells to a selective BH3 mimetic like ABT-737 (red arrowhead) that binds tightly to Bcl-x_L, Bcl-2 and Bcl-w but not to Mcl-1 or A1. (**A**) High Mcl-1 (dark blue) renders the cell refractory, because Mcl-1 can capture the Bak released from Bcl-x_L by the drug. (B) In cells with little Mcl-1 (light blue) (or A1), the freed Bak can initiate apoptosis. (C) The high Bcl-2 (or Bcl-x_L) found in many tumors can sequester a high level of Bim [44] and prevent Bim from inducing apoptosis. Paradoxically, this can actually enhance *sensitivity* to ABT-737, because the Bim released by ABT-737 from Bcl-2 (or Bcl-x_L) can inactivate the ABT-737-insensitive Mcl-1 (or A1), freeing Bak to drive apoptosis.

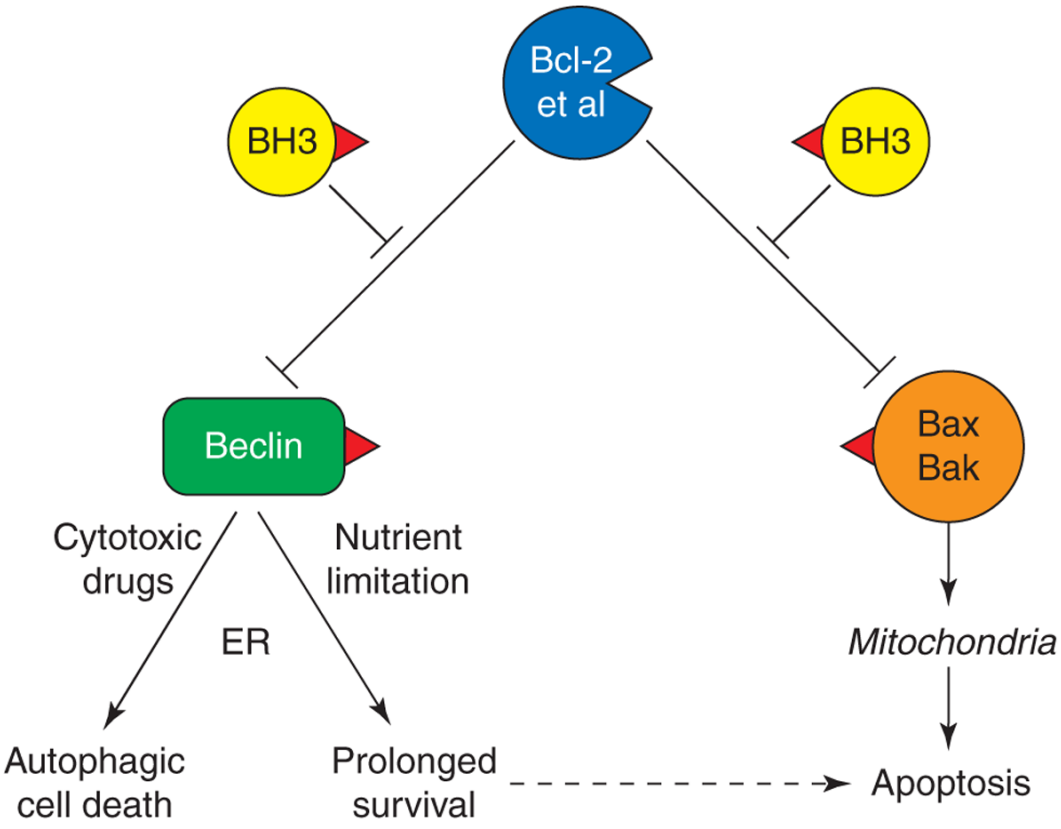

Figure 4.

Potential coupling of apoptosis and autophagy via Beclin 1 and Bcl-2 homologs. The model assumes that, in a favourable environment, pro-survival proteins such as Bcl-2 and Bcl-x_L prevent Beclin 1 from inducing autophagy by sequestering it, seemingly on the ER [52**, 53]. If so, the engagement of the pro-survival proteins by BH3-only proteins (or BH3 mimics) should trigger autophagy as well as apoptosis. In settings such as nutrient limitation or cytokine deprivation, autophagy temporarily prolongs survival by ensuring adequate ATP levels [49*, 52**], but in response to cytotoxic drugs, including some used in chemotherapy, it appears instead to promote autophagic cell death [55**]. How this dichotomy is controlled is not known.