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BCL-2 family isoforms in apoptosis and cancer

Chloe F. A. Warren[1,2], Michelle W. Wong-Brown[1,2] and Nikola A. Bowden[2,3]

Abstract

The BCl-2 family has long been identified for its role in apoptosis. Following the initial discovery of BCL-2 in the context of B-cell lymphoma in the 1980s, a number of homologous proteins have since been identified. The members of the Bcl-2 family are designated as such due to their BCL-2 homology (BH) domains and involvement in apoptosis regulation. The BH domains facilitate the family members' interactions with each other and can indicate pro- or anti-apoptotic function. Traditionally, these proteins are categorised into one of the three subfamilies; anti-apoptotic, BH3-only (pro-apoptotic), and pore-forming or 'executioner' (pro-apoptotic) proteins. Each of the BH3-only or anti-apoptotic proteins has a distinct pattern of activation, localisation and response to cell death or survival stimuli. All of these can vary across cell or stress types, or developmental stage, and this can cause the delineation of the roles of BCL-2 family members. Added to this complexity is the presence of relatively uncharacterised isoforms of many of the BCL-2 family members. There is a gap in our knowledge regarding the function of BCL-2 family isoforms. BH domain status is not always predictive or indicative of protein function, and several other important sequences, which can contribute to apoptotic activity have been identified. While therapeutic strategies targeting the BCL-2 family are constantly under development, it is imperative that we understand the molecules, which we are attempting to target. This review, discusses our current knowledge of anti-apoptotic BCL-2 family isoforms. With significant improvements in the potential for splicing therapies, it is important that we begin to understand the distinctions of the BCL-2 family, not limited to just the mechanisms of apoptosis control, but in their roles outside of apoptosis.

Facts

- BCL-2 family members play an integral role in apoptosis, but also contribute to many other cellular functions.
- Isoforms of almost all of the BCL-2 family members have been identified and some are well characterised.
- Therapeutics targeting BCL-2 show great promise for the treatment of cancer.

Open questions

- What is the functional role of uncharacterised BCL-2 family member isoforms in apoptosis and normal cellular functions, in particular the BCL-2 isoform BCL-2β?
- Is the presence and varied functional characteristics of BCL-2 family isoforms being considered in the development of therapeutics targeting BCL-2?
- Is there potential to target BCL-2 family member isoforms that are expressed higher in cancer?

Introduction

The BCl-2 family has long been identified for its role in apoptosis. Following the initial discovery of BCL-2 in the context of B-cell lymphoma in the 1980s, a number of homologous proteins have since been identified[1–3]. The members of the Bcl-2 family are designated as such due to

Correspondence: Nikola A. Bowden (Nikola.bowden@newcastle.edu.au)
[1]Faculty of Health and Medicine, School of Biomedical Sciences and Pharmacy, University of Newcastle, Newcastle, NSW, Australia
[2]Hunter Medical Research Institute, Newcastle, NSW, Australia
Full list of author information is available at the end of the article.
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their BCL-2 homology (BH) domains and involvement in apoptosis regulation. The BH domains facilitate the family members' interactions with each other, and can indicate pro- or anti-apoptotic function[4,5]. Traditionally, these proteins are categorised into one of three subfamilies; anti-apoptotic, BH3-only (pro-apoptotic), and pore-forming or 'executioner' (pro-apoptotic) proteins. Subfamily categorization has been traditionally based on BH and transmembrane domain and anti- or pro-apoptotic function status, as well as pore-forming ability (as shown in Table 1).

The role of the BCL-2 family in apoptotic regulation is typically described as the anti-apoptotic and pro-apoptotic BH3-only members existing in a state of competitive flux to influence the activation of the pore-forming executioners[6,7]. The ratio of pro- to anti-apoptotic subfamily members present in a cell can be altered by a number of signalling pathways, effectively relaying information on cellular stress, such as available nutrients, DNA damage, and protein processing[8]. Once the executioners are activated, the molecules come together to form pores in the outer mitochondrial membrane (MOM) and thus trigger mitochondrial outer membrane permeability (MOMP), and therefore apoptosis[9–11].

The BH domains are considered central to subfamily categorization as they facilitate the interaction of family members. BH3 was initially highlighted as an important domain as it was demonstrated to be vital for the interaction of the anti-apoptotic BCL-X_L and the executioner BAK, as well as for its apoptotic activity. The BH3 domain is vital for the correct folding of a hydrophobic pocket, within which BCL-2 members can interact[12,13]. Consequently, point mutations or deletions of the BH3 domain have been shown to significantly reduce the pro-apoptotic activity of a number of BH3-only proteins[14]. The BH4 domain is thought to be similarly significant for the anti-apoptotic subfamily; deletion of the BH4 domain can switch function to pro-apoptotic, while retention of the BH4 domain alone is sufficient to block changes in mitochondrial potential[14].

Beyond this understanding of a competitive flux, there are several hypotheses regarding how the BCL-2 family members interact, including direct and indirect interactions amongst family members (summarised in Supplementary Table 1). Each of the BH3-only or anti-apoptotic proteins have patterns of activation, localisation and response to specific death or survival stimuli. Binding selectivity between members of the different classes of BCL2 proteins also varies, for example, some BH3-only proteins bind non-specifically to several BCL2 prosurvival proteins while others tend to bind in a more specific manner. Similarly, BCL2 prosurvival family members can selectively bind to and limit activity of BAX or BAK. All of these interactions can vary across cell or stress types, or developmental stage, and this can cause the delineation of the roles of BCL-2 family members. Added to this complexity is the presence of relatively uncharacterised isoforms of many of the BCL-2 family members.

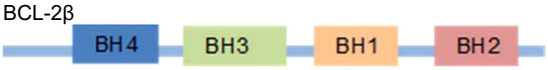
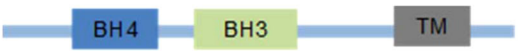
Table 1 BCL-2 subfamilies and members

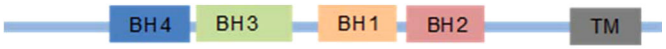
Subfamily	Activity	BH Domain Status	Members
Anti-apoptotic	Anti-apoptotic	Presence of BH4 domain	BCL-2 BCL-X_L BCL-W BCL-B (BCL2L10) MCL-1L
		Absence of BH4 domain	MCL-1 BFL-1/A1 BCL2L12[13]
Pore-forming executioners	Pro-apoptotic	Multi-domain	BAX BAK[104] BOK[105]
BH3-only	Pro-apoptotic	Activator–binds to pro-apoptotic and anti-apoptotic Bcl-2 multiregion proteins[13]	BIM BID Puma Mule[13,106]
		Sensitizer–displaces activator BH3-only proteins from anti-apoptotic proteins to promote apoptosis[13]	BAD Noxa BIK/BLK BMF HRK/DP5 Beclin-1
	Potential pro-apoptotic		BCL-Rambo (BCL2L13)[107] BCL-G (BCL2L14)[107] MCL-1S[108] MCL-1ES[108]

BCL-2 anti-apoptotic subfamily

This review focuses on the BCL-2 anti-apoptotic subfamily and known isoforms. Traditionally, members of this family are identified by their anti-apoptotic activity as well as the presence of BH4 and transmembrane domains for anchoring to cellular membranes[15]. Some members of this anti-apoptotic subfamily lack some of these physical features, have isoforms translated from the same gene which actually have pro-apoptotic activity, or can have their activity modulated by post-translational modification, as reviewed below and summarised in Table 2.

Table 2 Roles within the anti-apoptotic Bcl-2 subfamily[3,19,54,59,108–115]

Name of gene/ role of isoform/level of endogenous expression	Gene splice control mechanism / name of isoform / primary structure
BCL-2 Anti-apoptotic: highly expressed in B-cell lymphomas with t(14:18) translocation [3]	BCL-2α 
Role unknown: Low expression in healthy cells compared to BCL2α; increased ratio of expression in blood and bone marrow of CML patients [109]	BCL-2β 
BCL-X/BCL2L1 Anti-apoptotic: confers survival when overexpressed in cell lines deprived of growth factor [19]. Overexpression in tumours can increase risk of metastasis [110].	$BCL\text{-}X_L$ 
Pro-apoptotic: inhibits BCL-2 from enhancing survival, generally expressed in cells with high turnover rate [19], can sensitise cells to chemotherapy agents [54].	$BCL\text{-}X_S$ 
MCL-1 Anti-apoptotic: increases when cells are exposed to cell-survival inducing tumorigenic compounds, expressed in viable cells [108].	MCL-1L 
Pro-apoptotic [108]: Very low expression in oral cancer tissue compared to MCL-1	MCL-1S 
Pro-apoptotic [59]: Very low expression in oral cancer tissue compared to MCL-1 [111]	MCL-1ES 

BCL-W/BCL2L2 (BCL2-like 2) Anti-apoptotic: increased expression confers resistance to multiple cytotoxic insults [112] Unknown.	BCL-W (Isoform 1)  BCL-W (Isoform 2) 
BFL-1/A1 Anti-apoptotic: Confers survival advantage in monocytes [113], correlation between expression and stomach cancer development [114]. Anti-apoptotic: The protein is localised to the nucleus and has anti-apoptotic activity [115].	BFL-1/A1 (Isoform 1)  BFL-1/A1 (Isoform 2) 

BCL-X

The BCL-X or BCL2L1 (BCL2-like 1) gene has 44% homology to BCL-2. It has two well-known isoforms, BCL-X_L and BCL-X_S (Table 2), as well as a number of other characterised isoforms[16,17]. The two major isoforms arise from alternative splicing of BCL-X; splicing at the distal end of the 5′ splice site within the first coding exon for production of BCL-X_L and at the proximal end for BCL-X_S. Interestingly, the two isoforms have a different role in apoptosis. While BCL-X_L is anti-apoptotic, BCL-X_S is pro-apoptotic. Overexpression of BCL-X_L, but not BCL-X_S, confers survival in IL-3-dependent cells following IL-3 deprivation[18]. Transfection of these IL-3-dependent cells with BCL-X_S reinstates their sensitivity to IL-3 removal, regardless of levels of anti-apoptotic BCL-2[19]. This protective effect has been seen in several cell types, in response to chemotherapeutic drug treatment and growth factor removal[20].

The BCL-X_L protein is comprised of seven alpha-helices, where the two central hydrophobic helices (α5 and α6) are surrounded by five amphipathic helices (α3, α6, α1, α2, and α7). The BH1, BH2, and BH3 domains sit in close proximity, and form a hydrophobic cleft for binding other family members. The C-terminal transmembrane domain extends from the α7 helix. The N-terminal helix (α1) is essential for maintenance of structure stabilisation as it forms extensive interactions with the other helices. The BH3 domain is contained within the α2 helix, the BH1 domain across the α4 and α5, and BH2 across α6 and α7[21].

The protein structure of BCL-X_S has not been comprehensively described, but the loss of both BH1 and BH2 domains would significantly alter the hydrophobic binding cleft[22]. While BCL-X_L exerts its anti-apoptotic regulation by formation of heterodimers with both BAX and BAK, the pro-apoptotic function of BCL-X_S is derived from its capacity to disrupt the BAK/VDAC complex through its interaction with voltage-dependent anion channel (VDAC), thus freeing BAK for activation[23]. This highlights the difference in binding capacity between the two isoforms.

Since this discovery of the alternate functions of the two variants, the mechanisms of splicing control of the BCL-X gene have been a matter of some interest. It has been demonstrated that switching of splicing favour is induced by cellular stress, specifically DNA damage[24,25], protein synthesis stalling[26], and protein kinase C inhibition[27,28]. The induction of generic cellular stress via treatment with the drug ceramide as well as the combination of epigallocatechin-3-gallate (EGCG) and non-steroidal anti-

inflammatory (ibuprofen) have also been shown to shift splice favour[29,30].

Immunoprecipitation assays on known regulatory regions have identified proteins essential to BCL-X splice control, such as HNRNPK and PTBP1[31,32]. In addition, investigation of known pro-apoptotic transcription factor targets (SC35 via E2F1)[25], RNA binding proteins (SAM68, SAP155)[33,34], splice modulators (hnRNP F/H, SRp30c, RBm25, Akt SUMO, TCERG1)[35–39], and pathways demonstrated to shift Bcl-Xs splicing favour (SB1 and RBM25)[24,37], have identified a number of other spliceosomal and RNA binding proteins which are involved in BCL-X splicing regulation. As well as these proteins, a lncRNA, RNA INXS, transcribed from the opposite strand of BCL-X, and proto-oncogenes FBI-1 and FYN, are capable of modulating SAM68 activity in favour of BCL-X_S splicing[40,41].

BCL-X_L is also known to be involved in calcium signalling regulation via IP3R and VDAC1[42,43], can regulate Ca^{2+} homeostasis when localised at the endoplasmic reticulum[44] and reduce mitochondrial Ca^{2+} uptake[45]. The RNA-binding protein HuR, a translational repressor of BCL-X_L, can also affect maintenance of mitochondrial morphology, which regulates cellular apoptosis, through translational control of BCL-X_L expression[46]. BCL-X_L has been linked to non-apoptotic cell death by binding the tumour suppressor Beclin 1, subsequently inhibiting autophagy[47]. The overexpression of BCL-X_L has been shown to protect endothelial cells from TNF-mediated apoptosis and is involved in inflammatory response by inhibiting the activation of NF-κB and thus the upregulation of proinflammatory genes[48]. Interestingly, BCL-X_L has also been shown to have apoptosis-independent function in metastasis in pancreatic neuroendocrine tumour and breast cancer cell lines via nuclear promotion of epithelial–mesenchymal transition, migration, invasion and stemness[49] and in chemoresistance via RAS interaction and influence on EMT and regulation of cancer-initiating cell (CICs)[50].

Interestingly, despite the mass of research conducted on BCL-X_L and BCL-X splicing control, there is relatively little known about BCL-X_S. It is a BAK-dependent pro-apoptotic protein[23,51,52], but any roles outside of apoptosis regulation have not yet been identified. Induction of an increased ratio of BCL-X_S to BCL-X_L or overexpression of BCL-X_S in cancer cell lines have been shown to have a pro-apoptotic effect[20,53–55].

MCL-1

MCL-1 (myeloid leukemia sequence 1) was initially discovered due to its upregulation in the MC-1 hematopoietic cell line during the differentiation from monocyte to macrophage[56]. At the time of discovery, the MCL-1L transcript was the only known transcript, and it was rapidly designated as anti-apoptotic after overexpression was observed to protect cells from heat shock[57]. However, there are now three known isoforms of the gene; MCL-1L, MCl-1S and MCL-1ES (Table 2). Similar to the BCL-X isoforms, the three proteins have different roles in the regulation of apoptosis; MCL-1L is anti-apoptotic, while MCL-1S and MCL-1ES are both pro-apoptotic[58,59].

The C-terminal domain of MCL-1L is 350 amino acids long and has sequence homology with BCL-2. A central hydrophobic helix (α5) is surrounded by a set of amphipathic helices, which pack tightly against it (α1, α2, α3, α4, α5, α6, α7), where α3 and α4 are less densely packed, and BH1 is contained within α5 and α6. Helices α2, α3 and α4 form the characteristic hydrophobic binding groove and contain the BH3 domain, where α5 and α8 form the base of the groove[60]. MCL-1L also harbours a C-terminal transmembrane domain[58,60]. Unlike other members of the BCL-2 family, the MCL-1L N-terminus contains a PEST sequence that is associated with rapidly degrading proteins, as well as multiple sites for phosphorylation and caspase cleavage sequences[61]. These post-translational modifications can change protein stability and function, and consequently, MCL-1L has a high rate of turnover within the cell and its degradation can be modulated at several points along the N-terminus[58,62].

Alternatively, skipping of the second exon of the MCL-1 gene gives rise to the 271 amino acid MCL-1S (Table 2). This variant retains the BH3, BH4 and PEST domains, but not the BH1, BH2 and transmembrane domains. This gives rise to an isoform with features characteristic of a BH3-only protein, in which heterodimerization with anti-apoptotic MCL-1L can block its pro-apoptotic activity. In addition, MCL-1S is incapable of binding with BAX, BAK and BIM, whereas BCL-X_L interacts strongly with these family members[58]. Besides this initial study that described the key features of MCL-1S, there is relatively little known about the protein.

A third isoform, MCL-1ES, has also been identified. MCL-1ES occurs as a result of alternative splicing within the first exon at a non-canonical donor-acceptor site. The resultant protein is 197 amino acids long and lacks the PEST sequence and BH4 domain present in the other MCL-1 isoforms (Table 2). This isoform displays a pro-apoptotic function, with overexpression of this isoform resulting in decreased resting cell viability and mitochondrial integrity, all leading to cell death[59]. Interestingly, the effects are amplified when MCL-1ES is co-transfected with MCL-1L, an anti-apoptotic family member[59]. Further work has demonstrated that MCL-1ES localisation to the mitochondria and consequent pro-apoptotic activity is dependent on its heterodimerization with MCL-1L[59,63]. Interestingly, the effect of overexpression on apoptosis is BAX/BAK-dependent, and preliminary studies indicate that MCL-1ES can form the

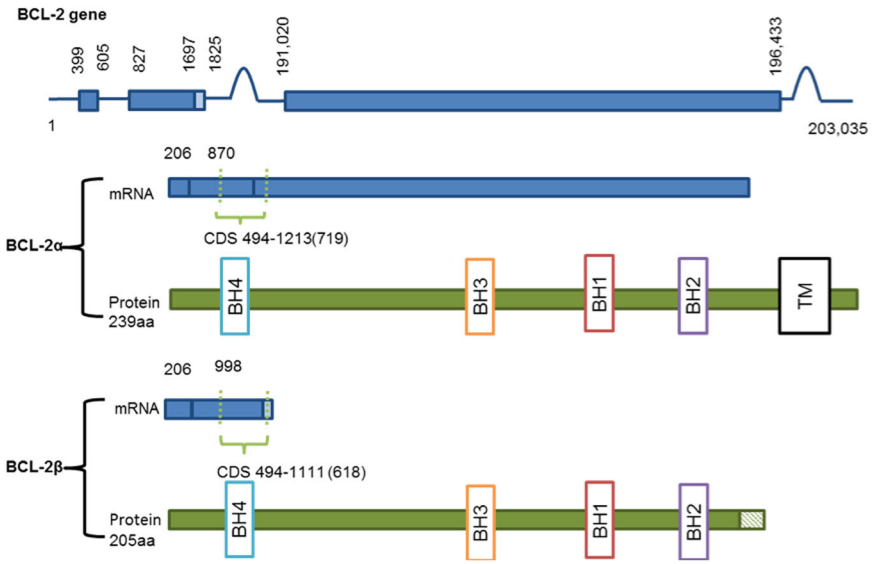


Fig. 1 Schematic diagram of BCL-2. Comprised of three exons, with the first two exons encoding the four BH domains and exon 3 encoding the transmembrane domain, with BCL-2β lacking the transmembrane domain

mitochondrial pores for the initiation of apoptosis by the release of cytochrome c and activation of MOMP. This activity is dependent on the BH3 domain of MCL-1ES[63].

Although studies have been performed on splicing control between MCL-1L and MCL-1S, the mechanisms of MCL-1ES splicing control are still unknown. Much of the work in delineating MCL-1 splicing regulators was performed in parallel with BCL-X investigations. For example, treatment of prostate cancer cell lines with EGCG/ibuprofen switched splicing favour to the pro-apoptotic variant for both MCL-1 and BCL-X in a protein phosphatase 1 (PP1)-dependent manner[30]. In addition, a study by Moore et al. (2010) identified that the splicing regulator ASF/SF2, and protein kinases PLK1 and WEE1, can shift splicing in favour of MCL-1S[64]. The same study identified SAP155 as a driver for transcription of pro-apoptotic splice variants for both MCL-1 and BCL-X, and this result has since been validated by other studies[64,65]. These data indicate that MCL-1 splicing regulation is associated with cell cycle control.

Of the three isoforms, only MCL-1L has been found to have roles outside of apoptosis. Like BCL-2 and BCL-X_L, MCL-1L can regulate autophagy, mitochondrial morphology, and calcium signalling via its interaction with IP3R[66], and is involved in cell cycle control[61] and lipid metabolism[67].

BCL-2

BCL-2 was the first member of the family to be identified, due to its role in B-cell lymphoma. A chromosomal translocation between chromosomes 14 and 18 in this disease, t(14:18), causes enhancement of BCL-2 transcription, which confers a survival advantage to the cancerous cells[1–3]. The BCL-2 gene is comprised of three exons; the first two exons encode the four BH domains, whereas the exon 3 encodes the transmembrane domain that anchors the protein to intracellular membranes[68,69] (Fig. 1). There are two isoforms of BCL-2; BCL-2α and BCL-2β. While BCL-2α is anti-apoptotic[3,70–72], BCL-2β is yet to be fully characterised. It lacks exon 3 and thus the transmembrane-anchoring domain, but otherwise shares the same BH domains and general structure of BCL-2α (Fig. 1). BCL-2β also has an isoform-specific 9-amino acid stretch at its C-terminal domain[73] (Table 2).

Roles of BCL-2α

The structure of the BCL-2α protein is similar to BCL-X_L, with two central hydrophobic helices (α1 and α2) surrounded by five α-helices, and a C-terminal transmembrane domain. Like BCL-X_L, this characteristic hydrophobic groove is comprised of helices 3, 4, 5 and 6. The structure of the BCL-2β protein is yet to be ascertained, but is known to lack the transmembrane domain, although the significance of this is unclear. While several studies have concluded that a C-truncated BCL-2α is incapable of localising to appropriate organelles, bind target proteins or regulate apoptosis[74–78], others have disputed the significance of a transmembrane domain[79–81]. However, it is important to note that all these studies have been performed on truncated BCL-2α but not on wildtype BCL-2β.

BCL-2α binds to BAX via its BH1 and BH2 domains, and this interaction is central to its role in apoptosis regulation, as demonstrated in cell lines in response to

Table 3 Alternative roles for BCL-2α, other than apoptosis

Cellular process	Description of feature	Role of Bcl-2
Autophagy	Autophagy is a survival mechanism resorted to during starvation, wherein intracellular contents can be recycled for nutritional value.	BCL-2α is capable of inhibiting autophagy via its interaction with Beclin-1, although only when localised at the ER membrane[116]
Apoptosis via p53	p53 is a major tumour suppressor.	BCL-2α can prevent p53 from up regulating pro-apoptotic genes. Interestingly, p53 can also negatively regulate the BCL-2 protein[88,117]
Transcription factor control	Transcription factors regulate gene expression.	BCL-2 can regulate the transcription factors NF-κB, AP1, CRE and NFAT by blocking them from entering the nucleus
Regulation of Ca^{2+} at the endoplasmic reticulum	The ER is the central storage centre for Ca^{2+}, a major cellular signalling molecule.	BCL-2 is capable of modulating the activity of IP3R (a Ca^{2+} channel)[42]
Nucleotide excision repair (NER)	NER repairs bulky, helix distorting DNA damage induced by UV irradiation.	Overexpression of BCL-2 attenuates cyclobutane pyrimidine dimer (CPD) removal and the stalling of DNA replication following exposure to UV light[82]
Base excision Repair (BER)	BER occurs throughout the cell cycle to repair non-helix distorting lesions, such as mismatched or damaged single bases.	Overexpression of BCL-2 downregulates BER via APE1 blockage[85,118,119]
Mismatch repair (MMR)	MMR repairs bases which have been mis-incorporated during DNA replication and recombination.	BCL-2 can inhibit MMR via its direct interaction with MSH2[86,120]
Double-strand break repair (DSBR) and non-homologous end joining (NHEJ)	NHEJ is a mechanism of DSBR that rejoins short DNA overhangs (microhomologies) on the ends of either strand of the broken DNA.	Cells with higher expression of BCL-2 had lower levels of end joining and vice versa. This was thought to be due to the ability of BCL-2 to interact with KU proteins, which form a molecular scaffold for the DSBR machinery[87,121]. BCL-2 can also regulate DSBR via its interaction with BRCA1[89]
DSBR and single-strand break repair (SSBR) via PARP1	PARP1 is involved in SSBR and DSBR.	BCL-2 can relocate to sites on the chromatin, where it can directly interact with and inhibit PARP1. This interaction can be disrupted by BH3-only BCL-2 family members (and BH3 mimetic drugs)[122]

cellular stress[5]. However, in several models of stress, such as gamma irradiation, IL-3 deprivation of dependent cells, glucocorticoid and cytotoxic drug treatment, and heat shock, it has been shown that, where BCL-2 is upregulated and capable of binding BAX, the capacity of cells to undergo apoptosis is reduced[3,70–72]. These investigations have emphasised the significance of the BH domains.

Like MCL-1L and BCL-X_L, BCL-2α is the most extensively studied isoform and is involved in autophagy via interaction with Beclin 1, as well as calcium signalling, and has roles outside of apoptosis regulation[82,83]. Interestingly, the interaction between BCL-2 and Beclin 1 occurs at the same site as BH3-only proteins and so competition for the site exists between these proteins[84]. It has also been implicated in DNA repair, including nucleotide excision repair, base excision repair, mismatch repair and double-strand break repair[82,85–87]. In addition, BCL-2α can regulate a number of major transcription factors, including p53[88], NF-κB, AP1, CRE and NFAT[77]. The different roles of BCL-2α are summarised in Table 3.

Current evidence for a role of BCL2β

Despite the accumulation of evidence for the many roles of BCL-2α, there has been very little investigation into the role of BCL-2β. Functional protein studies on BCL-2 primarily focuses on the wildtype BCL-2α. Where the BCL-2β isoform is addressed, a recombinant version of a C-terminal-truncated BCL-2α is used for characterisation studies and it has been assumed this structure shares the same function as BCL-2β, as both lack the transmembrane domain[73]. Characterisation studies on the BCL-2β isoform have so far been limited to cloned versions of the genes, which do not accurately reflect the naturally-occurring sequence[74,76,78,89].

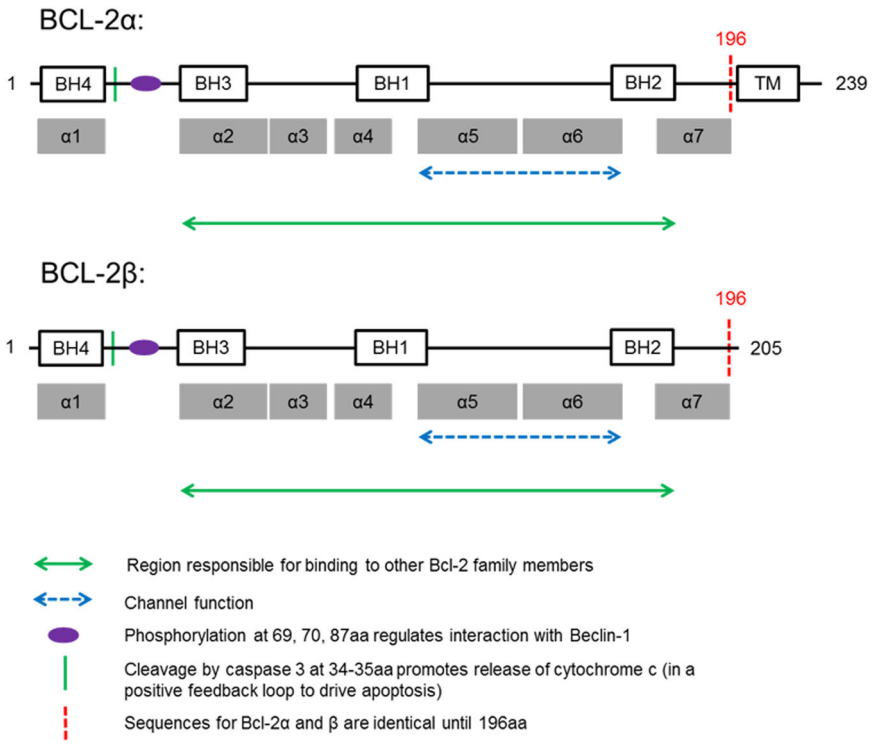


Fig. 2 Primary protein structures of BCL-2α and BCL-2β. This figure is based on experiments on BCL-2 and the highly homologous BCL-X_L. It illustrates the similarities between the isoforms. BH1, BH2, and BH3 are required for heterodimerisation with BCL-2 family members[5,21,123]. Channels are formed by α-helices 5 and 6[124]. Phosphorylation by MAPK8 (mitogen-activated kinase 8) at specific residues between BH4 and BH3 can modify binding to Beclin-1[125]. Caspase-3 cleavage at amino acids 34–35 can abrogate protein function[126]. The two proteins are identical up to amino acid 196, where they start to differ, with BCL-2β lacking a transmembrane domain and having a specific C-terminal 9-amino acid sequence[73]. This figure was adapted from Belka and Budach (2002)[127]

Studies that have assessed the significance of the transmembrane domain on the capacity for BCL-2 to regulate apoptosis and p53 and to interact with BAX and BRCA1 concluded that the domain was vital for the efficiency of BCL-2 in these roles[74,76,78,90]. In contrast, studies that have concluded that the transmembrane domain is not essential for function were focused on the separate steps of apoptosis activation and/or apoptosis across different cell types[79,80]. Despite these inconsistencies in the literature, it is important to note that C-terminal-truncated BCL-2α does not accurately represent BCL-2β, due to the isoform's specific 9-amino acid C-terminal sequence (see Fig. 2).

Targeting the BCL2 family for therapeutic purposes

As the BCL-X and BCL-2 families have essential roles in apoptotic regulation and were initially discovered in the cancer setting, they have garnered interest as therapeutic targets. Several studies have tried to regulate apoptosis using retroviral systems[91], alteration of localisation apparatus[92], activity-blocking antibodies[93], RNAi[94] and miRNAs[95]. However, the most successful method so far for targeting the BCL-2 family has been through BH3-mimetic molecules.

BH3-mimetics

Initial proof-of-concept studies that highlighted the potential of BH3-mimetics showed that small molecules which bound to the hydrophobic groove of BCL-X_L could block anti-apoptotic function[96]. Since then, many different BH3-mimetics have been developed, and these are summarized in Table 4.

Several studies have highlighted the significance of low MCL1 expression conferring sensitivity to BH3-mimetics in cell lines[97,98]. MCL-1L is one of the most potent of the BCL-2 family as it has a significantly high affinity with pro-apoptotic members. Several molecules with the potential for binding MCL-1L have been developed, A-1210477 was an early molecule proposed to act directly on MCL-1L to promote apoptosis in cell lines[99,100]. More recently, S63845 a small molecule that binds with high affinity to the BH3-binding groove of MCL1 has been shown to kill MCL1-dependent cancer cells, including multiple myeloma, leukaemia and lymphoma cells[101].

Manipulation of splicing

Splicing alters the function of BCL-2 members, therefore there is potential to target this therapeutically by manipulation of gene splicing to favour pro-apoptotic

Table 4 BH3-mimetics and their targets

Inhibitor	Detail for development	Molecular targets	Stage of clinical trials
ABT-737	Lead compound. Mimics BH3 domain of BAD[128]	BCL-2, BCL-X_L, BCL-W	–
ABT-263 (Navitoclax)	Based on ABT-737 but has longer half-life and is orally bioavailable[129]	BCL-2, BCL-X_L, BCL-W	Phase 1/2[130–133]
ABT-199 (Venetoclax)	Derivative of ABT-263[134]	BCL-2	Approved for use in chronic lymphocytic leukaemia (CLL) patients with 17p deletion[135]
S55746	Synthetic small molecule and orally available. BCL-2 selective, and no significant binding to BFL-1 and MCL-1 were observed[136]	BCL-2	Phase 1 (NCT02920697, NCT02920541, NCT02603445)
WEHI-539	Small molecule derived from hydrazinylbenzothiazole cores[137]	BCL-X_L	Preclinical[138]
A-1155463	Small molecule, more potent and chemically-stable than WEHI-539[139]	BCL-X_L	Preclinical[139]
A-1331852	Small molecule, orally bioavailable[139]	BCL-X_L	Preclinical[139, 140]
A-1210477	Derivative of indole-2-carboxylic acid core. Has high affinity to MCL-1, and synergizes with navitoclax to induce apoptosis in multiple cancer cell lines[99]	MCL-1	Preclinical[141, 142]
S63845	Synthetic small molecule inhibitor. Higher affinity for human MCL-1 compared to A1210477[101] Acts synergistically with ABT-199[143]	MCL-1	Phase 1 (NCT02979366)[144–150]

transcripts. Introduction of splicing-switching oligonucleotides that alter BCL-X splicing from BCL-X_L to the pro-apoptotic BCL-X_S in melanoma cell culture and tumour xenografts was shown to reduce tumour load[102]. Additionally, the transcription factor FBI-1 has been shown to have a role in alternative splicing by interacting with splicing factor SAM68, thus reducing binding of SAM68 to BCL-X and resulting in the preferential splicing of anti-apoptotic BCL-X_L[41]. The silencing of FBI-1 expression restores the ability of SAM68 to induce splicing of pro-apoptotic BCL-X_S[41].

Another way to manipulate splicing is by targeting SAP155, a splicing factor which acts on MCL-1 and BCL-X. Inhibition of this protein by meaymycin B, and potent inhibitor of SAP155, has been used to switch splicing in favour of pro-apoptotic MCL-1s in cell culture[65]. Interestingly, the combination of meaymycin B with BH3-mimetic ABT-737 also induces apoptosis[65]. The activity of SAP155 has also been successfully downregulated using an anti-SAP155 antibody, which induced an increase in the pro-apoptotic BCL-X_S isoform compared to BCL-X_L, and this method can be used to prime the cell for response to apoptosis-inducing treatment[33].

Targeting splicing factors to favour the expression of pro-apoptosis isoforms is appealing but the non-specific nature of splicing factors will need to be addressed for this to be a superior target than BH3 mimetics. A more targeted approach to manipulating splicing is the use of specific antisense oligonucleotides. Antisense oligonucleotides designed to knock-down exon 2 in MCL-1 pre-mRNA can shift splicing pattern from MCL-1L to MCL-1S[103]. This increases the expression of pro-apoptotic MCL-1S and reduces the level of anti-apoptotic MCL-1L, and was shown to induce apoptosis in basal cell carcinoma and gastric adenocarcinoma cell lines[103]. Manipulation of splicing remains an area of interest that requires further development to be a targeted as a treatment with clinical potential.

Summary

The BCL-2 family is involved in the regulation of apoptosis and therefore plays a vital role in protecting against cancer. Targeting the apoptotic pathway directly is a valid option for improving or developing new chemotherapies, but it is imperative that we understand the molecules, which we are attempting to modify, manipulate or mimic. As demonstrated in this review, there are gaps in knowledge regarding isoforms of anti-apoptotic BCL-2 family isoforms. Further studies focusing on understanding the variety of splice variants and isoforms and their biological role in apoptosis is required for targets of this pathway to reach their full potential.

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Author details
[1]Faculty of Health and Medicine, School of Biomedical Sciences and Pharmacy, University of Newcastle, Newcastle, NSW, Australia. [2]Hunter Medical Research Institute, Newcastle, NSW, Australia. [3]Faculty of Health and Medicine, School of Medicine and Public Health, University of Newcastle, Newcastle, NSW, Australia

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References

1. Tsujimoto, Y. et al. Cloning of the chromosome breakpoint of neoplastic B cells with the t(14;18) chromosome translocation. *Science* **226**, 1097–1099 (1984).
2. Tsujimoto, Y. et al. Involvement of the bcl-2 gene in human follicular lymphoma. *Science* **228**, 1440–1443 (1985).
3. Tsujimoto, Y. Stress-resistance conferred by high level of bcl-2 alpha protein in human B lymphoblastoid cell. *Oncogene* **4**, 1331–1336 (1989).
4. Petros, A. M. et al. Solution structure of the antiapoptotic protein bcl-2. *Proc. Natl Acad. Sci. USA* **98**, 3012–3017 (2001).
5. Yin, X. M., Oltvai, Z. N. & Korsmeyer, S. J. BH1 and BH2 domains of Bcl-2 are required for inhibition of apoptosis and heterodimerization with Bax. *Nature* **369**, 321–323 (1994).
6. Certo, M. et al. Mitochondria primed by death signals determine cellular addiction to antiapoptotic BCL-2 family members. *Cancer Cell.* **9**, 351–365 (2006).
7. van Delft, M. F. & Huang, D. C. How the Bcl-2 family of proteins interact to regulate apoptosis. *Cell Res.* **16**, 203–213 (2006).
8. Hetz, C. & Glimcher, L. The daily job of night killers: alternative roles of the BCL-2 family in organelle physiology. *Trends Cell Biol.* **18**, 38–44 (2008).
9. Nechushtan, A. et al. Bax and Bak coalesce into novel mitochondria-associated clusters during apoptosis. *J. Cell Biol.* **153**, 1265–1276 (2001).
10. Chipuk, J. E. et al. Direct activation of Bax by p53 mediates mitochondrial membrane permeabilization and apoptosis. *Science* **303**, 1010–1014 (2004).
11. Martinou, J. C. & Green, D. R. Breaking the mitochondrial barrier. *Nat. Rev. Mol. Cell Biol.* **2**, 63–67 (2001).
12. Petros, A. M., Olejniczak, E. T. & Fesik, S. W. Structural biology of the Bcl-2 family of proteins. *Biochim. Biophys. Acta* **1644**, 83–94 (2004).
13. Shamas-Din, A. et al. BH3-only proteins: orchestrators of apoptosis. *Biochim. Biophys. Acta* **1813**, 508–520 (2011).
14. Hossini, A. M. & Eberle, J. Apoptosis induction by Bcl-2 proteins independent of the BH3 domain. *Biochem. Pharmacol.* **76**, 1612–1619 (2008).
15. Alberts, B., *Molecular Biology of the Cell: Reference Edition* (Taylor & Francis, New York, NY, USA 2008).
16. Hossini, A. M. et al. A novel Bcl-x splice product, Bcl-xAK, triggers apoptosis in human melanoma cells without BH3 domain. *Oncogene* **25**, 2160–2169 (2006).
17. Ban, J. et al. Identification of a human cDNA encoding a novel Bcl-x isoform. *Biochem. Biophys. Res. Commun.* **248**, 147–152 (1998).
18. Vaux, D. L., Cory, S. & Adams, J. M. Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. *Nature* **335**, 440–442 (1988).
19. Boise, L. H. et al. bcl-x, a bcl-2-related gene that functions as a dominant regulator of apoptotic cell death. *Cell* **74**, 597–608 (1993).
20. Minn, A. J., Boise, L. H. & Thompson, C. B. Bcl-x(S) anatagonizes the protective effects of Bcl-x(L). *J. Biol. Chem.* **271**, 6306–6312 (1996).
21. Muchmore, S. W. et al. X-ray and NMR structure of human Bcl-xL, an inhibitor of programmed cell death. *Nature* **381**, 335–341 (1996).
22. Chang, B. S. et al. The BH3 domain of Bcl-x(S) is required for inhibition of the antiapoptotic function of Bcl-x(L). *Mol. Cell. Biol.* **19**, 6673–6681 (1999).
23. Plotz, M. et al. Disruption of the VDAC2-Bak interaction by Bcl-x(S) mediates efficient induction of apoptosis in melanoma cells. *Cell Death Differ.* **19**, 1928–1938 (2012).
24. Shkreta, L. et al. The DNA damage response pathway regulates the alternative splicing of the apoptotic mediator Bcl-x. *J. Biol. Chem.* **286**, 331–340 (2011).
25. Merdzhanova, G. et al. E2F1 controls alternative splicing pattern of genes involved in apoptosis through upregulation of the splicing factor SC35. *Cell Death Differ.* **15**, 1815–1823 (2008).
26. Boon-Unge, K. et al. Emetine regulates the alternative splicing of Bcl-x through a protein phosphatase 1-dependent mechanism. *Chem. Biol.* **14**, 1386–1392 (2007).
27. Revil, T. et al. Protein kinase C-dependent control of Bcl-x alternative splicing. *Mol. Cell. Biol.* **27**, 8431–8441 (2007).
28. Li, C. Y. et al. Regulation of alternative splicing of Bcl-x by IL-6, GM-CSF and TPA. *Cell Res.* **14**, 473–479 (2004).
29. Massiello, A. et al. Identification of two RNA cis-elements that function to regulate the 5′ splice site selection of Bcl-x pre-mRNA in response to ceramide. *J. Biol. Chem.* **279**, 15799–15804 (2004).
30. Kim, M. H. Protein phosphatase 1 activation and alternative splicing of Bcl-X and Mcl-1 by EGCG + ibuprofen. *J. Cell. Biochem.* **104**, 1491–1499 (2008).
31. Bielli, P. et al. Regulation of BCL-X splicing reveals a role for the polypyrimidine tract binding protein (PTBP1/hnRNP I) in alternative 5′ splice site selection. *Nucleic Acids Res.* **42**, 12070–12081 (2014).
32. Revil, T. et al. Heterogeneous nuclear ribonucleoprotein K represses the production of pro-apoptotic Bcl-xS splice isoform. *J. Biol. Chem.* **284**, 21458–21467 (2009).
33. Massiello, A., Roesser, J. R. & Chalfant, C. E. SAP155 Binds to ceramide-responsive RNA cis-element 1 and regulates the alternative 5′ splice site selection of Bcl-x pre-mRNA. *FASEB J.* **20**, 1680–1682 (2006).
34. Paronetto, M. P. et al. The RNA-binding protein Sam68 modulates the alternative splicing of Bcl-x. *J. Cell Biol.* **176**, 929–939 (2007).
35. Garneau, D. et al. Heterogeneous nuclear ribonucleoprotein F/H proteins modulate the alternative splicing of the apoptotic mediator Bcl-x. *J. Biol. Chem.* **280**, 22641–22650 (2005).
36. Cloutier, P. et al. Antagonistic effects of the SRp30c protein and cryptic 5′ splice sites on the alternative splicing of the apoptotic regulator Bcl-x. *J. Biol. Chem.* **283**, 21315–21324 (2008).
37. Zhou, A. et al. Novel splicing factor RBM25 modulates Bcl-x pre-mRNA 5′ splice site selection. *Mol. Cell. Biol.* **28**, 5924–5936 (2008).
38. Risso, G. et al. Modification of Akt by SUMO conjugation regulates alternative splicing and cell cycle. *Cell Cycle* **12**, 3165–3174 (2013).
39. Montes, M. et al. TCERG1 regulates alternative splicing of the Bcl-x gene by modulating the rate of RNA polymerase II transcription. *Mol. Cell. Biol.* **32**, 751–762 (2012).
40. DeOcesano-Pereira, C. et al. Long non-coding RNA INXS is a critical mediator of BCL-XS induced apoptosis. *Nucleic Acids Res.* **42**, 8343–8355 (2014).
41. Bielli, P., et al., *The transcription factor FBI-1 inhibits SAM68-mediated BCL-X alternative splicing and apoptosis*. EMBO reports, 419–427 (2014).
42. White, C. et al. The endoplasmic reticulum gateway to apoptosis by Bcl-X(L) modulation of the InsP3R. *Nat. Cell Biol.* **7**, 1021–1028 (2005).
43. Huang, H. et al. An interaction between Bcl-xL and the voltage-dependent anion channel (VDAC) promotes mitochondrial Ca^{2+} uptake. *J. Biol. Chem.* **288**, 19870–19881 (2013).
44. Eno, C. O. et al. Distinct roles of mitochondria- and ER-localized Bcl-xL in apoptosis resistance and Ca^{2+} homeostasis. *Mol. Biol. Cell.* **23**, 2605–2618 (2012).
45. Rong, Y. & Distelhorst, C. W. Bcl-2 protein family members: versatile regulators of calcium signaling in cell survival and apoptosis. *Annu. Rev. Physiol.* **70**, 73–91 (2008).
46. Durie, D. et al. HuR controls mitochondrial morphology through the regulation of Bcl translation. *Translation (Austin).* **1**, e23980 (2013).

47. Lessene, G., Czabotar, P. E. & Colman, P. M. BCL-2 family antagonists for cancer therapy. *Nat. Rev. Drug. Discov.* **7**, 989–1000 (2008).
48. Badrichani, A. Z. et al. Bcl-2 and Bcl-XL serve an anti-inflammatory function in endothelial cells through inhibition of NF-kappaB. *J. Clin. Invest.* **103**, 543–553 (1999).
49. Choi, S. et al. Bcl-xL promotes metastasis independent of its anti-apoptotic activity. *Nat. Commun.* **7**, 10384 (2016).
50. Carne Trecesson, S. et al. BCL-XL directly modulates RAS signalling to favour cancer cell stemness. *Nat. Commun.* **8**, 1123 (2017).
51. Wang, C. & Youle, R. J. Predominant requirement of Bax for apoptosis in HCT116 cells is determined by Mcl-1's inhibitory effect on Bak. *Oncogene* **31**, 3177–3189 (2012).
52. Lindenboim, L. et al. Bak but not Bax is essential for Bcl-xS-induced apoptosis. *Cell Death Differ.* **12**, 713–723 (2005).
53. Taylor, J. K. et al. Induction of endogenous Bcl-xS through the control of Bcl-x pre-mRNA splicing by antisense oligonucleotides. *Nat. Biotechnol.* **17**, 1097–1100 (1999).
54. Sumantran, V. N. et al. Overexpression of Bcl-xS sensitizes MCF-7 cells to chemotherapy-induced apoptosis. *Cancer Res.* **55**, 2507–2510 (1995).
55. Hossini, A. M. et al. Conditional expression of exogenous Bcl-X(S) triggers apoptosis in human melanoma cells in vitro and delays growth of melanoma xenografts. *FEBS Lett.* **553**, 250–256 (2003).
56. Kozopas, K. M. et al. MCL1, a gene expressed in programmed myeloid cell differentiation, has sequence similarity to BCL2. *Proc. Natl Acad. Sci. USA* **90**, 3516–3520 (1993).
57. Reynolds, J. E. et al. Mcl-1, a member of the Bcl-2 family, delays apoptosis induced by c-Myc overexpression in Chinese hamster ovary cells. *Cancer Res.* **54**, 6348–6352 (1994).
58. Bae, J. et al. MCL-1S, a splicing variant of the antiapoptotic BCL-2 family member MCL-1, encodes a proapoptotic protein possessing only the BH3 domain. *J. Biol. Chem.* **275**, 25255–25261 (2000).
59. Kim, J. H., et al., MCL-1ES, a novel variant of MCL-1, associates with MCL-1L and induces mitochondrial cell death. *FEBS letters.* 2758–2764 (2009).
60. Day, C. L. et al. Solution structure of prosurvival Mcl-1 and characterization of its binding by proapoptotic BH3-only ligands. *J. Biol. Chem.* **280**, 4738–4744 (2005).
61. Thomas, L. W., Lam, C. & Edwards, S. W. Mcl-1; the molecular regulation of protein function. *FEBS Lett.* **584**, 2981–2989 (2010).
62. Morciano, G. et al. Mcl-1 involvement in mitochondrial dynamics is associated with apoptotic cell death. *Mol. Biol. Cell.* **27**, 20–34 (2016).
63. Kim, J. H. & Bae, J. MCL-1ES induces MCL-1L-dependent BAX- and BAK-independent mitochondrial apoptosis. *PLoS One* **8**, e79626 (2013).
64. Moore, M. J. et al. An alternative splicing network links cell-cycle control to apoptosis. *Cell* **142**, 625–636 (2010).
65. Gao, Y. & Koide, K. Chemical perturbation of Mcl-1 pre-mRNA splicing to induce apoptosis in cancer cells. *Acs. Chem. Biol.* **8**, 895–900 (2013).
66. Eckenrode, E. F. et al. Apoptosis protection by Mcl-1 and Bcl-2 modulation of inositol 1,4,5-trisphosphate receptor-dependent Ca^{2+} signaling. *J. Biol. Chem.* **285**, 13678–13684 (2010).
67. Escudero, S. et al. Dynamic regulation of long-chain fatty acid oxidation by a noncanonical interaction between the MCL-1 BH3 Helix and VLCAD. *Mol. Cell* **69**, 729–743 e7 (2018).
68. Seto, M. et al. Alternative promoters and exons, somatic mutation and deregulation of the Bcl-2-Ig fusion gene in lymphoma. *EMBO J.* **7**, 123–131 (1988).
69. Chen-Levy, Z., Nourse, J. & Cleary, M. L. The bcl-2 candidate proto-oncogene product is a 24-kilodalton integral-membrane protein highly expressed in lymphoid cell lines and lymphomas carrying the t(14;18) translocation. *Mol. Cell. Biol.* **9**, 701–710 (1989).
70. Bissonnette, R. P. et al. Apoptotic cell death induced by c-myc is inhibited by bcl-2. *Nature* **359**, 552–554 (1992).
71. Miyashita, T. & Reed, J. C. bcl-2 gene transfer increases relative resistance of S49.1 and WEHI7.2 lymphoid cells to cell death and DNA fragmentation induced by glucocorticoids and multiple chemotherapeutic drugs. *Cancer Res.* **52**, 5407–5411 (1992).
72. Katsumata, M. et al. Differential effects of Bcl-2 on T and B cells in transgenic mice. *Proc. Natl. Acad. Sci. USA* **89**, 11376–11380 (1992).
73. Tsujimoto, Y. & Croce, C. M. Analysis of the structure, transcripts, and protein products of bcl-2, the gene involved in human follicular lymphoma. *Proc. Natl Acad. Sci. USA* **83**, 5214–5218 (1986).
74. Peng, J. et al. The Bax BH3 peptide H2-H3 promotes apoptosis by inhibiting Bcl-2's pore-forming and anti-Bax activities in the membrane. *Sheng Wu Yi Xue Gong. Cheng Xue Za Zhi* **26**, 829–835 (2009).
75. Alnemri, E. et al. Overexpressed full-length human BCL2 extends the survival of baculovirus-infected Sf9 insect cells. *Proc. Natl Acad. Sci. USA* **16**, 7295–7299 (1992).
76. Froesch, B. A. et al. Inhibition of p53 transcriptional activity by Bcl-2 requires its membrane-anchoring domain. *J. Biol. Chem.* **274**, 6469–6475 (1999).
77. Massaad, C. A., Portier, B. P. & Taglialatela, G. Inhibition of transcription factor activity by nuclear compartment-associated Bcl-2. *J. Biol. Chem.* **279**, 54470–54478 (2004).
78. Tanaka, S., Saito, K. & Reed, J. C. Structure-function analysis of the Bcl-2 oncoprotein. Addition of a heterologous transmembrane domain to portions of the Bcl-2 beta protein restores function as a regulator of cell survival. *J. Biol. Chem.* **268**, 10920–10926 (1993).
79. Borner, C. et al. The protein bcl-2 alpha does not require membrane attachment, but two conserved domains to suppress apoptosis. *J. Cell Biol.* **126**, 1059–1068 (1994).
80. Kawatani, M. et al. Transmembrane domain of Bcl-2 is required for inhibition of ceramide synthesis, but not cytochrome c release in the pathway of inostamycin-induced apoptosis. *Exp. Cell Res.* **286**, 57–66 (2003).
81. Dremina, E. et al. Anti-apoptotic protein Bcl-2 interacts with and destabilizes the sarcoplasmic/endoplasmic reticulum Ca^{2+} -ATPase (SERCA). *Biochem. J.* **383**(Pt 2), 361–370 (2004).
82. Liu, Y., Naumovski, L. & Hanawalt, P. Nucleotide excision repair capacity is attenuated in human promyelocytic HL60 cells that overexpress BCL2. *Cancer Res.* **57**, 1650–1653 (1997).
83. Braun, F. et al. Protect and serve: Bcl-2 proteins as guardians and rulers of cancer cell survival. *Cell Cycle* **12**, 2937–2947 (2013).
84. Decuypere, J. P., Parys, J. B. & Bultynck, G. Regulation of the autophagic bcl-2/beclin 1 interaction. *Cells* **1**, 284–312 (2012).
85. Kuo, M. L. et al. Suppression of apoptosis by Bcl-2 to enhance benzene metabolites-induced oxidative DNA damage and mutagenesis: a possible mechanism of carcinogenesis. *Mol. Pharmacol.* **55**, 894–901 (1999).
86. Hou, Y. et al. Bcl2 impedes DNA mismatch repair by directly regulating the hMSH2-hMSH6 heterodimeric complex. *J. Biol. Chem.* **282**, 9279–9287 (2007).
87. Wang, Q. et al. Bcl2 negatively regulates DNA double-strand-break repair through a nonhomologous end-joining pathway. *Mol. Cell* **29**, 488–498 (2008).
88. Beham, A. et al. Bcl-2 inhibits p53 nuclear import following DNA damage. *Oncogene* **15**, 2767–2772 (1997).
89. Laulier, C. et al. Bcl-2 inhibits nuclear homologous recombination by localizing BRCA1 to the endomembranes. *Cancer Res.* **71**, 3590–3602 (2011).
90. Laulier, C. & Lopez, B. S. *The secret life of Bcl-2: apoptosis-independent inhibition of DNA repair by Bcl-2 family members. Mutat. Res.* **751**, 247–257 (2012).
91. Kagawa, S. et al. A binary adenoviral vector system for expressing high levels of the proapoptotic gene bax. *Gene Ther.* **7**, 75–79 (2000).
92. Bartholomeusz, G. A. et al. Activation of a novel Bcr/Abl destruction pathway by WP1130 induces apoptosis of chronic myelogenous leukemia cells. *Blood* **109**, 3470–3478 (2007).
93. Piche, A. et al. Modulation of Bcl-2 protein levels by an intracellular anti-Bcl-2 single-chain antibody increases drug-induced cytotoxicity in the breast cancer cell line MCF-7. *Cancer Res.* **58**, 2134–2140 (1998).
94. Agarwala, S. S. et al. LDH correlation with survival in advanced melanoma from two large, randomised trials (Oblimersen GM301 and EORTC 18951). *Eur. J. Cancer* **45**, 1807–1814 (2009).
95. Schultz, J. et al. MicroRNA let-7b targets important cell cycle molecules in malignant melanoma cells and interferes with anchorage-independent growth. *Cell Res.* **18**, 549–557 (2008).
96. Wang, J. L. et al. Structure-based discovery of an organic compound that binds Bcl-2 protein and induces apoptosis of tumor cells. *Proc. Natl Acad. Sci. USA* **97**, 7124–7129 (2000).
97. Yamaguchi, R. & Perkins, G. Mcl-1 levels need not be lowered for cells to be sensitized for ABT-263/737-induced apoptosis. *Cell Death Dis.* **2**, e227 (2011).
98. Suryani, S. et al. Cell and molecular determinants of in vivo efficacy of the BH3 mimetic ABT-263 against pediatric acute lymphoblastic leukemia xenografts. *Clin. Cancer Res.* **20**, 4520–4531 (2014).
99. Leverson, J. D. et al. Potent and selective small-molecule MCL-1 inhibitors demonstrate on-target cancer cell killing activity as single agents and in combination with ABT-263 (navitoclax). *Cell Death Dis.* **6**, e1590 (2015).

100. Besbes, S. & Billard, C. First MCL-1-selective BH3 mimetics as potential therapeutics for targeted treatment of cancer. *Cell Death Dis.* **6**, e1810 (2015).
101. Kotschy, A. et al. The MCL1 inhibitor S63845 is tolerable and effective in diverse cancer models. *Nature* **538**, 477–482 (2016).
102. Bauman, J. et al. Anti-tumor activity of splice-switching oligonucleotides. *Nucleic Acids Res.* **38**, 8348–8356 (2010).
103. Shieh, J. J. et al. Modification of alternative splicing of Mcl-1 pre-mRNA using antisense morpholino oligonucleotides induces apoptosis in basal cell carcinoma cells. *J. Invest. Dermatol.* **129**, 2497–2506 (2009).
104. Oltvai, Z. N., Milliman, C. L. & Korsmeyer, S. J. Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. *Cell* **74**, 609–619 (1993).
105. Schendel, S. L. et al. Channel formation by antiapoptotic protein Bcl-2. *Proc. Natl Acad. Sci. USA* **94**, 5113–5118 (1997).
106. Wei, Y. et al. JNK1-mediated phosphorylation of Bcl-2 regulates starvation-induced autophagy. *Mol. Cell* **30**, 678–688 (2008).
107. Kirsch, D. G. et al. *Caspase-3-dependent cleavage of Bcl-2 promotes release of cytochrome c. J. Biol. Chem.* **274**, 21155–21161 (1999).
108. Belka, C. & Budach, W. Anti-apoptotic Bcl-2 proteins: structure, function and relevance for radiation biology. *Int. J. Radiat. Biol.* **78**, 643–658 (2002).
109. Reed, J. C. Proapoptotic multidomain Bcl-2/Bax-family proteins: mechanisms, physiological roles, and therapeutic opportunities. *Cell Death Differ.* **13**, 1378–1386 (2006).
110. Llambi, F. et al. BOK Is a Non-canonical BCL-2 Family Effector of Apoptosis Regulated by ER-Associated Degradation. *Cell* **165**, 421–433 (2016).
111. Czabotar, P. E. et al. Structural insights into the degradation of Mcl-1 induced by BH3 domains. *Proc. Natl. Acad. Sci. USA* **104**, 6217–6222 (2007).
112. Westphal, D. et al. Molecular biology of Bax and Bak activation and action. *Biochim. Biophys. Acta* **1813**, 521–531 (2011).
113. Bingle, C. D. et al. Exon Skipping in Mcl-1 Results in a Bcl-2 Homology Domain 3 Only Gene Product That Promotes Cell Death. *J. Biol. Chem.* **275**, 22136–22146 (2000).
114. Guillem, V., et al. BCL2 gene polymorphisms and splicing variants in chronic myeloid leukemia. *Leuk Res*. 1278–1284 (2015).
115. España, L. et al. Overexpression of Bcl-xL in Human Breast Cancer Cells Enhances Organ-Selective Lymph Node Metastasis. *Breast Cancer Res. Treat.* **87**, 33–44 (2004).
116. Palve, V. et al. Overexpression of Mcl-1L splice variant is associated with poor prognosis and chemoresistance in oral cancers. *PLoS One* **9**, e111927 (2014).
117. Gibson, L. et al. bcl-w, a novel member of the bcl-2 family, promotes cell survival. *Oncogene* **13**, 665–675 (1996).
118. Noble, K. E. et al. Monocytes stimulate expression of the Bcl-2 family member, A1, in endothelial cells and confer protection against apoptosis. *J. Immunol.* **162**, 1376–1383 (1999).
119. Choi, S. S. et al. A novel Bcl-2 related gene, Bfl-1, is overexpressed in stomach cancer and preferentially expressed in bone marrow. *Oncogene* **11**, 1693–1698 (1995).
120. Ko, J. K. et al. Bfl-1S, a novel alternative splice variant of Bfl-1, localizes in the nucleus via its C-terminus and prevents cell death. *Oncogene* **22**, 2457–2465 (2003).
121. Pattingre, S. et al. Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. *Cell* **122**, 927–939 (2005).
122. Bharatham, N., Chi, S. W. & Yoon, H. S. Molecular basis of Bcl-X(L)-p53 interaction: insights from molecular dynamics simulations. *PLoS One* **6**, e26014 (2011).
123. Jin, Z. et al. Bcl2 suppresses DNA repair by enhancing c-Myc transcriptional activity. *J. Biol. Chem.* **281**, 14446–14456 (2006).
124. Zhao, J. et al. Bcl2 inhibits abasic site repair by down-regulating APE1 endonuclease activity. *J. Biol. Chem.* **283**, 9925–9932 (2008).
125. Youn, C. K. et al. Bcl-2 expression suppresses mismatch repair activity through inhibition of E2F transcriptional activity. *Nat. Cell Biol.* **7**, 137–147 (2005).
126. Kumar, T. S. et al. Anti-apoptotic protein BCL2 down-regulates DNA end joining in cancer cells. *J. Biol. Chem.* **285**, 32657–32670 (2010).
127. Dutta, C. et al. BCL2 suppresses PARP1 function and nonapoptotic cell death. *Cancer Res.* **72**, 4193–4203 (2012).
128. Oltersdorf, T. et al. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. *Nature* **435**, 677–681 (2005).
129. Tse, C. et al. ABT-263: a potent and orally bioavailable Bcl-2 family inhibitor. *Cancer Res.* **68**, 3421–3428 (2008).
130. Rudin, C. M. et al. Phase II study of single-agent navitoclax (ABT-263) and biomarker correlates in patients with relapsed small cell lung cancer. *Clin. Cancer Res.* **18**, 3163–3169 (2012).
131. Gandhi, L. et al. Phase I study of Navitoclax (ABT-263), a novel Bcl-2 family inhibitor, in patients with small-cell lung cancer and other solid tumors. *J. Clin. Oncol.* **29**, 909–916 (2011).
132. Wilson, W. H. et al. Navitoclax, a targeted high-affinity inhibitor of BCL-2, in lymphoid malignancies: a phase 1 dose-escalation study of safety, pharmacokinetics, pharmacodynamics, and antitumour activity. *Lancet Oncol.* **11**, 1149–1159 (2010).
133. Roberts, A. W. et al. Substantial susceptibility of chronic lymphocytic leukemia to BCL2 inhibition: results of a phase I study of navitoclax in patients with relapsed or refractory disease. *J. Clin. Oncol.* **30**, 488–496 (2012).
134. Souers, A. J. et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. *Nat. Med.* **19**, 202–208 (2013).
135. Roberts, A. W. et al. Venetoclax in patients with previously treated chronic lymphocytic leukemia. *Clin. Cancer Res.* **23**, 4527–4533 (2017).
136. Casara, P. et al. S55746 is a novel orally active BCL-2 selective and potent inhibitor that impairs hematological tumor growth. *Oncotarget* **9**, 20075–20088 (2018).
137. Lessene, G. et al. Structure-guided design of a selective BCL-X(L) inhibitor. *Nat. Chem. Biol.* **9**, 390–397 (2013).
138. Merino, D. et al. Synergistic action of the MCL-1 inhibitor S63845 with current therapies in preclinical models of triple-negative and HER2-amplified breast cancer. *Sci Transl Med.* **9**, eaam7049 (2017).
139. Leverson, J. D. et al. Exploiting selective BCL-2 family inhibitors to dissect cell survival dependencies and define improved strategies for cancer therapy. *Sci. Transl. Med* **7**, 279ra40 (2015).
140. Punnoose, E. A. et al. Expression Profile of BCL-2, BCL-XL, and MCL-1 Predicts Pharmacological Response to the BCL-2 Selective Antagonist Venetoclax in Multiple Myeloma Models. *Mol. Cancer Ther.* **15**, 1132–1144 (2016).
141. Leverson, J. D. et al. Found in translation: how preclinical research is guiding the clinical development of the BCL2-selective inhibitor venetoclax. *Cancer Discov.* **7**, 1376–1393 (2017).
142. Phillips, D. C. et al. Loss in MCL-1 function sensitizes non-Hodgkin's lymphoma cell lines to the BCL-2-selective inhibitor venetoclax (ABT-199). *BloodCancer J.* **5**, e368 (2015).
143. Li, Z., S. He, S. & Look, A.T. The MCL1-specific inhibitor S63845 acts synergistically with venetoclax/ABT-199 to induce apoptosis in T-cell acute lymphoblastic leukemia cells. *Leukemia.* **33**, 262–266 (2019).
144. Kuwana, T. et al. Bid, Bax, and lipids cooperate to form supramolecular openings in the outer mitochondrial membrane. *Cell* **111**, 331–342 (2002).
145. Vela, L. et al. Direct interaction of Bax and Bak proteins with Bcl-2 homology domain 3 (BH3)-only proteins in living cells revealed by fluorescence complementation. *J. Biol. Chem.* **288**, 4935–4946 (2013).
146. Willis, S. N. et al. Apoptosis initiated when BH3 ligands engage multiple Bcl-2 homologs, not Bax or Bak. *Science* **315**, 856–859 (2007).
147. Llambi, F. et al. A unified model of mammalian BCL-2 protein family interactions at the mitochondria. *Mol. Cell* **44**, 517–531 (2011).
148. Letai, A. et al. Distinct BH3 domains either sensitize or activate mitochondrial apoptosis, serving as prototype cancer therapeutics. *Cancer Cell.* **2**, 183–192 (2002).
149. Leber, B., Lin, J. & Andrews, D. W. Embedded together: the life and death consequences of interaction of the Bcl-2 family with membranes. *Apoptosis* **12**, 897–911 (2007).
150. Kale, J., Osterlund, E. J. & Andrews, D. W. BCL-2 family proteins: changing partners in the dance towards death. *Cell Death Differ.* **25**, 65–80 (2018).