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REVIEW

The BCL-2 protein family, BH3-mimetics and cancer therapy

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Escape from apoptosis is a key attribute of tumour cells and facilitates chemo-resistance. The 'BCL-2-regulated' or 'intrinsic' apoptotic pathway integrates stress and survival signalling to govern whether a cancer cell will live or die. Indeed, many pro-apoptotic members of the BCL-2 family have demonstrated tumour-suppression activity in mouse models of cancer and are lost or repressed in certain human cancers. Conversely, overexpression of pro-survival BCL-2 family members promotes tumorigenesis in humans and in mouse models. Many of the drugs currently used in the clinic mediate their therapeutic effects (at least in part) through the activation of the BCL-2-regulated apoptotic pathway. However, initiators of this apoptotic pathway, such as p53, are mutated, lost or silenced in many human cancers rendering them refractory to treatment. To counter such resistance mechanisms, a novel class of therapeutics, 'BH3-mimetics', has been developed. These drugs directly activate apoptosis by binding and inhibiting select antiapoptotic BCL-2 family members and thereby bypass the requirement for upstream initiators, such as p53. In this review, we discuss the role of the BCL-2 protein family in the development and treatment of cancer, with an emphasis on mechanistic studies using well-established mouse models of cancer, before describing the development and already recognised potential of the BH3-mimetic compounds.
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Facts

- Cancer development and progression are facilitated by enhanced cell survival signalling.
- Loss of initiators of apoptosis or overexpression of inhibitors of apoptosis are frequently observed in haematological and solid cancers.
- BH3-mimetic compounds offer a novel approach for treating chemo-resistant cancers by blocking select pro-survival BCL-2 family members.

Open Questions

- Do all cancers require high expression of pro-survival BCL-2 family members for their development and sustained growth?
- Can a therapeutic window be established for BH3-mimetic drugs?
- Will direct induction of apoptosis using BH3-mimetic compounds reduce the emergence of therapeutic resistance?
- What are the optimal drugs to partner BH3-mimetics for combination therapy of different cancers?

The complexity of multicellular animals is built upon a foundation of cell and tissue specification that facilitates coordination of intra-organismal processes and interaction with the surrounding environment. Cooperation between cells is essential, as are mechanisms to detect and remove 'rogue' cells that lose the ability to respond appropriately to developmental and homeostatic cues. Failure of these mechanisms can have dire consequences, such as the development of cancer or autoimmune disease.[1] A critical tumour-suppression mechanism is the cell's intrinsic ability to self-destruct through a process of programmed cell death known as apoptosis.[2] Indeed, evasion from apoptosis cooperates with oncogenic mutations that deregulate cell growth and cell

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Abbreviations: BCL-2, B cell lymphoma gene 2; BH, BCL-2 homology region; BAX, BCL-2-associated protein X; BAK, BCL-2-antagonist/killer; MCL-1, myeloid cell leukaemia gene 1; A1, BCL-2-related protein A1; BIM, BCL-2 interacting mediator of cell death; PUMA, p53 upregulated modulator of apoptosis; BID, BH3 interacting-domain death agonist; BAD, BCL-2-associated death promoter; BIK, BCL-2-interacting killer; HRK, Harakiri; BMF, BCL-2-modifying factor; tBID, truncated BID; MOMP, mitochondrial outer membrane permeabilisation; FOXO, forkhead box protein O; ER, endoplasmic reticulum; CHOP, C/EBP homologous protein; AKT, v-akt murine thymoma viral oncogene homolog; ASCIZ, ATM/ATR-substrate CHK2-interacting zinc finger protein; DLC1, dynein light chain 1; miRNA, microRNA; MYC, v-myc avian myelocytomatosis viral oncogene homolog; IgH, immunoglobulin heavy chain; IgL, immunoglobulin light chain; AML, acute myeloid leukaemia; CLL, chronic lymphocytic leukaemia; BOK, BCL-2 related ovarian killer; BL, Burkitt lymphoma; RNAi, RNA interference; HDACi, histone deacetylase inhibitors; MEK, MAPK/ERK kinase; EGFR, epidermal growth factor receptor; CML, chronic myeloid leukaemia; VEGFR, vascular endothelial growth factor receptor; BCR-ABL, break point cluster region – Abelson kinase fusion protein
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cycling in tumorigenesis. Evasion of apoptosis is therefore considered a requisite characteristic of tumour formation, one of the so-called 'Hallmarks of Cancer'.[3]

Apoptosis constitutes the ordered, genetically encoded process that removes not only damaged cells but also those that have become superfluous to the function of the organism.[4] Apoptosis enables cells to be eliminated with minimal disruption to surrounding cells and is thereby distinct from necrotic cell death, which is often unregulated and results in the release of cellular debris that can prompt tissue inflammation. It is important to note that some other forms of programmed cell death, known as pyroptosis,[5] and necroptosis (also called programmed necrosis),[4,6] have risen to prominence. However, the contributions of these forms of cell death to morphogenesis during animal development, adult tissue homeostasis as well as the genesis and treatment of cancer remain to be elucidated.

The term 'apoptosis' was first coined by Kerr *et al.*[7] to describe a form of cell death distinguished from necrosis by a characteristic morphology. Apoptosis is associated with cell shrinkage and membrane blebbing to yield small vesicles, which are subsequently engulfed by neighbouring phagocytic cells.[8–10] In addition, molecular events, such as inter-nucleosomal DNA cleavage and translocation of phosphatidyl-serine to the outer leaflet of the plasma membrane, are indicative of apoptosis and frequently used experimentally as markers of apoptosis.

In this review, we summarise the literature describing the mechanisms by which apoptosis signalling is governed with particular focus on their importance in cancer development as demonstrated by observations from various experimental mouse models and also from studies of human cancer. We close with an analysis of the role of the BCL-2 family for mediating the activity of many commonly used anticancer therapeutics, including the promising new class of agents known as BH3-mimetics.

The BCL-2-Regulated Apoptotic Pathway

The BCL-2-regulated apoptotic pathway (also known as 'intrinsic', 'stress' or 'mitochondrial' pathway) is evolutionarily highly conserved, with homologues of critical genes found in animals as distantly related as worms and humans.[11–13] Indeed, many of the early insights into the roles of components of this cell death pathway were derived from studies using the model organism *Caenorhabditis elegans* (e.g., Vaux *et al.*).[12] Initiation of the BCL-2-regulated apoptotic pathway is controlled, as the name implies, by interactions between members of the BCL-2 protein family.[14–19] This family consists of three groups of structurally related proteins: the pro-survival BCL-2-like proteins, the multi-BH domain pro-apoptotic BAX/BAK proteins, and the pro-apoptotic BH3-only proteins.

The BCL-2 protein family. The pro-survival BCL-2 family proteins (BCL-2, BCL-XL, BCL-W, MCL-1, A1/BFL-1) share homology within four BCL-2 homology domains (BH1–4). These proteins form a characteristic helical bundle fold, which is critical for their ability to bind to the pro-apoptotic BCL-2 family members and thereby exert their antiapoptotic function.

The pro-apoptotic BAX/BAK subfamily members also contain four BH domains (BH1–4). In their inactive state, their structure is very similar to that of BCL-2 pro-survival proteins,[20] but BAX and BAK are able to undergo substantial conformational change during apoptosis.[21] BAX and BAK bind to and are inhibited by different BCL-2-like pro-survival proteins to different extents.[22,23] BOK shares significant homology across all four BH domains to BAX and BAK; however, its role in apoptosis remains unclear,[24] although it may cooperate with BAX in the attrition of primordial follicle oocytes during ageing.[25]

The pro-apoptotic BH3-only proteins (BIM, PUMA, BID, BAD, BIK, BMF, NOXA, HRK) share only the BH3 domain with each other and their more distant relatives.[19,26,27] These proteins are unstructured in isolation but assume an α-helical fold when bound to BCL-2 pro-survival family members.[28] The exception to this rule is BID, which is produced as an inactive globular protein that is converted into its active form, tBID (truncated BID), through caspase-8-mediated cleavage.[29,30] The BH3-only proteins are able to bind members of the BCL-2-like pro-survival subfamily and some of them can also bind to BAX and BAK, but there are substantial differences in their selectivity of interaction.[17–19,23,31–33]

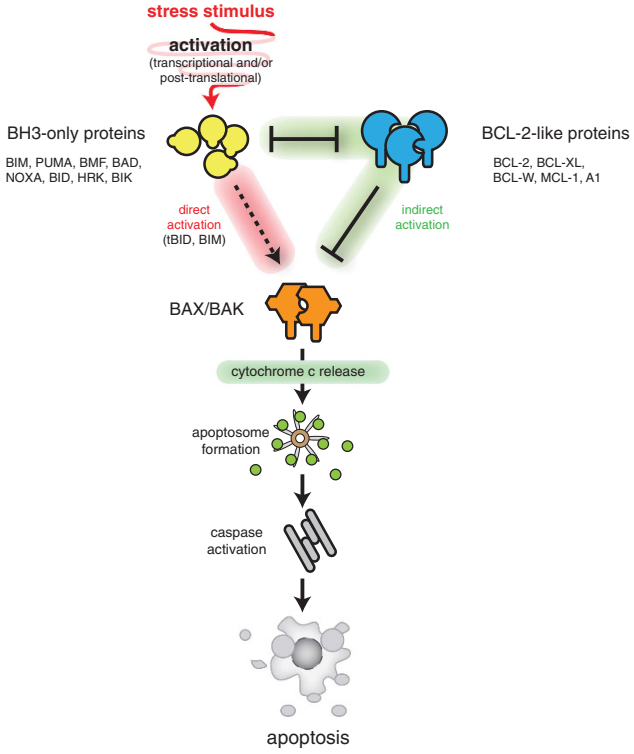

Figure 1 The BCL-2 family members interact to regulate initiation of apoptosis. In healthy cells, the BCL-2-like pro-survival proteins safeguard mitochondrial outer membrane integrity and cell survival by preventing the activation of BAX and BAK. Under conditions of stress, the BH3-only proteins are activated transcriptionally and/or posttranscriptionally to induce apoptosis by releasing BAX/BAK from inhibition by the BCL-2-like proteins or in the case of certain BH3-only proteins (notably BIM, tBID, PUMA) by activating BAX/BAK through direct binding. Once activated, BAX/BAK cause mitochondrial outer membrane permeabilisation (MOMP) with consequent release of apoptogenic molecules (e.g., cytochrome *c*, SMAC/DIABLO) that cause activation of the caspase cascade that culminates in cellular demolition

Activation of the BCL-2-regulated apoptotic pathway. The BCL-2 regulated apoptotic pathway is initiated through the transcriptional and/or posttranscriptional activation of the BH3-only proteins in response to various upstream signalling events. Some BH3-only proteins (notably BIM, tBID, PUMA) cause activation of BAX/BAK through direct binding, and all BH3-only proteins can activate BAX/BAK indirectly by binding to and inhibiting the pro-survival BCL-2-like proteins (Figure 1).[19,34–39] Activated BAX/BAK cause outer mitochondrial membrane permeabilisation (MOMP), which allows cytosolic release of apoptogenic factors (e.g., cytochrome *c*, Smac/DIABLO) that cause activation of the caspase cascade.[40] It remains unclear whether binding of BH3-only proteins to the pro-survival BCL-2-like proteins or their direct binding to BAX/BAK is more critical for the initiation of apoptosis.[37,38,41] It is, however, clear that some of the BH3-only proteins are more potent inducers of apoptosis (e.g., BIM, PUMA, tBID) than others (e.g., BAD, NOXA, BMF). Pertinently, the potent BH3-only proteins bind avidly to all pro-survival BCL-2 family members and can also engage BAX/BAK, whereas the less potent ones have more select binding specificities for the pro-survival BCL-2 family members and reportedly do not bind to BAX or BAK.[31,32]

Mechanisms of BH3-only protein activation. Because of their position at the apex of the BCL-2-regulated apoptotic pathway, the BH3-only proteins act as a fulcrum, determining whether the scales tip in favour of cell death or in favour of cell survival.

The mechanisms that lead to activation of the BH3-only proteins vary between members of this subfamily and also according to the apoptotic stimulus.[26,27,42] Transcriptional activation features prominently; however, emerging evidence also identifies posttranscriptional mechanisms, such as those involving microRNAs (miRNAs), as important in certain contexts.

For example, following DNA damage the tumour-suppressor p53 is posttranslationally activated and then transcriptionally upregulates PUMA and NOXA.[43–45] E2F1 is also able to induce PUMA and NOXA.[46] PUMA as well as BIM expression were reported to be induced by the transcription factor FOXO3a in response to cytokine withdrawal.[47–49] However, mutation of all known FOXO transcription factor-binding sites in the *Bim* gene had no impact on haematopoietic cell homeostasis and apoptosis,[50] indicating that this mode of induction may not be critical for BIM activation. In response to ER stress, BIM expression can be transcriptionally induced by CHOP.[51]

Various posttranslational processes were reported to regulate the stability and thereby control the activity of BH3-only proteins. BIM and BAD were reported to be negatively regulated by phosphorylation.[42,52] Phosphorylation of BAD by AKT was shown to cause its sequestration in the cytosol by 14-3-3 proteins, thereby restraining its pro-apoptotic activity.[53] Mice lacking BAD are largely normal, and their cells do not show marked resistance to the apoptotic stimuli tested.[54,55] The role of BAD in programmed and stress-induced cell death is therefore probably relatively subtle and ancillary to the action of more potent BH3-only proteins (e.g., BIM, PUMA). Phosphorylation of BIM by ERK was reported to be critical for the antiapoptotic activity of this kinase.[56–60] However, a recent study has shown that ERK-mediated direct phosphorylation of BIM does not have a major role in the control of the pro-apoptotic activity of this BH3-only protein within the whole animal.[61] Both BIM and BMF were shown to be sequestered by binding to elements of the cytoskeleton, thereby restraining their pro-apoptotic activity.[62,63] Interestingly, loss of the transcription factor ASCIZ, with consequent reduction in its target dynein light chain 1, which reportedly links BIM to the dynein motor complex,[62] causes abnormal death of B lymphoid cells, and this can be blocked by concomitant loss of BIM.[64] This suggests that this mode of BIM regulation has a critical role in normal physiology.

The expression of the BH3-only proteins can be modulated posttranscriptionally through the activity of miRNA. These short (17–25 nucleotides) RNA species bind in a sequence-specific manner to several target mRNA transcripts and inhibit their translation either through translation inhibition or mRNA destabilisation. Although the change in mRNA transcript abundance for any single miRNA target is mostly relatively minor, individual miRNAs are able to exert marked effects by targeting multiple mRNA species-encoding proteins that act within the same signalling pathway.

With respect to the BH3-only proteins, several miRNA have been implicated in the regulation of BIM expression, including the miR-17∼92 cluster in mice[65,66] and in human cancer cell lines also (miR-32, miR-17-5p, miR-106-25).[67–69] PUMA was

Table 1 The role of the BCL-2 protein family members in tumour development

Gene	Role in tumorigenesis	Model	References
Bim (Bcl2l11, Bod)	Tumour suppressor	*Eμ-Myc*	89,90
Puma (Bbc3)	Tumour suppressor	*Eμ-Myc*	91
Puma (Bbc3)	Required for tumour initiation	Irradiation-induced lymphoma	111,112
Bax	Tumour suppressor	*Eμ-Myc*	92
Bmf	Tumour suppressor	Irradiation-induced lymphoma	110
Noxa (Pmaip1)	Tumour suppressor	Irradiation-induced lymphoma	111
Bad (Bbc2)	Conflicting reports	Irradiation-induced lymphoma	54,55
Bcl2	Oncogene	*Eμ-Myc*	86
Bclx (Bcl2l1)	Oncogene; required for lymphoma initiation	*Eμ-Myc*	87,90,93,94
Mcl1	Oncogene; required for sustained lymphoma growth	*Eμ-Myc*	88,97
Mcl1	Oncogene; required for sustained lymphoma growth	p53-deficient thymic lymphoma	108
Mcl1	Required for sustained lymphoma growth	*AML*	113,114

reported to be regulated by miR-483-3p, miR-221 and miR-222.[70,71]

As for the pro-survival BCL-2 family members, BCL-2 and MCL-1 appear to be the prominent targets of miRNA-mediated regulation. Both are targeted by miR-29 and miR-153,[72–74] and BCL-2 expression is also regulated by miR-15, miR-16,[75] miR-195[76] and the p53-inducible miR-34.[77] BCL-XL expression appears to be controlled by miR-491.[78]

Role of the BCL-2-Regulated Apoptotic Pathway in Mouse Models of Tumorigenesis

Experimental models have been utilised to delineate the roles of the various BCL-2 family proteins during tumorigenesis (Table 1). Because of the prominent expression of the pro-survival BCL-2 family members in the haematopoietic system,[79] a large proportion of these studies have focussed on the role these proteins have during leukaemia and lymphoma development.

***Eμ-Myc* lymphoma model.** MYC expression is thought to be deregulated in ~70% of human cancers.[80] In Burkitt lymphoma (BL), this is due to a chromosomal translocation that subjugates the *c-Myc* gene to the control of the immunoglobulin heavy (IgH) or light chain gene enhancers. The *Eμ-Myc* transgenic mice were generated to model this malignancy with expression of the *c-Myc* proto-oncogene driven by the IgH gene enhancer, *Eμ*.[81] Early in life, these mice contain abnormally increased numbers of large, cycling B-cell progenitors,[82] which comprise the nascent neoplastic cells.[83] Upon acquisition of oncogenic mutations that cooperate with MYC in neoplastic transformation, clonal malignant pre-B or sIg^+ B lymphomas emerge from the pool of preleukaemic B lymphoid cells.[83] On a C57BL/6 background, median (50%) survival is ~110 days, and all animals succumb to lymphoma within ~350–400 days. Tumour cells from lymphoma-bearing mice can be readily transplanted into syngeneic (immune-competent) recipients or adapted to grow indefinitely *in vitro* as cell lines.[81,83] These two features and the ability to identify and study a preneoplastic cell population (see above) made this the most widely used animal model of human cancer (>1400 publications).

Deregulated MYC expression promotes neoplastic transformation by causing aberrant cell proliferation. However, under conditions of stress, such as limited supply of growth factors or nutrients, deregulated MYC expression also increases the predisposition of cells to undergo apoptosis.[84,85] Preleukaemic B lymphoid cells from *Eμ-Myc* mice are highly prone to undergo apoptosis,[85] and apoptosis constitutes a major mechanism to suppress/delay lymphoma development in *Eμ-Myc* mice. Accordingly, overexpression of pro-survival BCL-2 family members (e.g., BCL-2,[86] BCL-XL[87] or MCL-1[88]) greatly accelerate lymphoma development in *Eμ-Myc* mice. Loss of BIM,[89,90] PUMA[91] or BAX[92] (but curiously not loss of BAK) also accelerate MYC-induced lymphomagenesis, indicating that these pro-apoptotic proteins are major tumour suppressors in this context.

Studies using gene-targeted mice or pharmacological inhibitors revealed that endogenously controlled expression of BCL-XL,[90,93,94] but not BCL-2,[95] is essential for MYC-induced lymphoma development. This may be explained by the fact that BCL-XL, but not BCL-2, is expressed at readily detectable levels in pro-B/pre-B cells, the population of preleukaemic cells from which malignant lymphoma is thought to arise in *Eμ-Myc* mice. However, lymphomas initiated by combined overexpression of MYC and BCL-2 need high BCL-2 expression for their continued survival.[96]

Interestingly, although BCL-XL is critical for the development of pre-B/B lymphoma in *Eμ-Myc* mice, it is dispensable for the sustained survival and expansion of these tumours.[97] Instead, MCL-1 is essential, with loss of even a single allele of *Mcl-1* abrogating the *in vivo* growth of malignant *Eμ-Myc* lymphomas, unless they have acquired a mutation in the tumour-suppressor gene *p53*.[97]

p53-deficient mice, a model of Li–Fraumeni syndrome. Mutation or loss of *p53* is the most frequent mutation in human cancer and is frequently associated with poor prognosis and chemoresistance.[98,99] Furthermore, a rare genetic disorder, Li–Fraumeni syndrome results from the inheritance of a single mutated copy of the *p53* gene.[100,101] These patients are characterised by a high incidence of early onset of various cancers, particularly lymphoma, leukaemia and several forms of sarcoma, which develop following the somatic loss of the remaining wild-type *p53* allele in cancer-initiating cells.

The $p53^{+/-}$ heterozygous mice recapitulate the human condition[102,103] and mice completely deficient for p53 rapidly (within 150–280 days) develop thymic lymphoma with 100% penetrance on the C57BL/6 genetic background. These mice have been widely used to examine the importance of p53-mediated tumour suppression and the consequences of its loss during lymphomagenesis. As p53 induces apoptosis through the BH3-only proteins PUMA and (to a lesser extent) NOXA,[43–45] it was a great surprise that mice double deficient for both PUMA and NOXA displayed no propensity to tumour formation.[104] Even the combined loss of p53's ability to induce apoptosis, cell cycle arrest and senescence ($Puma^{-/-}Noxa^{-/-}p21^{-/-}$ mice) did not render mice tumour prone.[105–107] The precise mechanisms by which p53 suppresses tumour development therefore remain unclear.

A recent study has delineated the roles of the different pro-survival BCL-2 family members required for lymphoma development and expansion in p53-deficient mice.[108] MCL-1 was found to be critical for both the development and sustained growth of lymphoma initiated by p53 deficiency, whereas BCL-XL was dispensable. This study showed that even for p53-deficient tumours therapeutic targeting of MCL-1 may represent an effective treatment strategy.

γ-radiation-induced thymic lymphoma model. Thymic lymphoma can be induced in mice by repeated exposure to low doses of γ-radiation.[109] This is thought to facilitate neoplastic transformation through the sequential accumulation of oncogenic mutations in immature haematopoietic progenitors in the bone marrow, ultimately culminating in malignant lymphoma. Following exposure to 1.5 Gy γ-irradiation weekly for 4 weeks, mice typically succumb to thymic lymphoma around 150–200 days. In this experimental model, NOXA and BMF have been shown to suppress lymphoma

development,[110,111] while the role of BAD remains contentious with one report describing accelerated thymic lymphomagenesis in BAD-deficient mice while another publication reported no effect.[54,55] Possible explanations for the discrepancy might include differences in γ-radiation dosing and schedule used or differences in genetic background (C57BL/6[55] *versus* complicated mixed background[54]). Loss of BIM alone did not accelerate lymphoma development; however, mice deficient for both BAD and BIM showed accelerated tumour development.[55]

Remarkably, loss of PUMA completely abrogated γ-radiation-induced thymic lymphoma development.[111,112] This striking finding could be attributed to the profound resistance of PUMA-deficient white blood cells to DNA damage-induced apoptosis. The persistence of these cells obviated the need for mobilisation and burst of proliferation of haematopoietic stem/progenitor cells that would normally occur to repopulate the depleted haematopoietic system,[111,112] a process that appears to be required for lymphoma development in this model.[109] These observations suggest that accumulation of DNA lesions, some of them potentially oncogenic, is not sufficient to induce tumour formation unless it is also accompanied by a drive for proliferative expansion of the mutation bearing leukaemia/lymphoma-initiating cells.

Acute myeloid leukaemia (AML) models. AML can be induced experimentally in mice by transducing haematopoietic stem/progenitor cells with expression constructs encoding protein products encoded by recurrent chromosomal translocations found in human AML (e.g., MLL-ENL, AML-ETO9a). Using this system, MCL-1, but not BCL-XL, BCL-2 or BCL-W, was shown to be critical for the sustained survival of AML cells *in vitro* and *in vivo*.[113,114]

Collectively, these results characterise the process of tumour formation as a sustained effort of nascent neoplastic cells to cope with stress conditions imposed by the oncogenic lesions, genomic instability and potentially cytotoxic signals from their environment. Mutations that sensitise cells to apoptosis, such as loss of pro-survival BCL-2 family members, act to suppress tumour development, whereas loss of pro-apoptotic BCL-2 family members expedite neoplastic transformation.

Role of the BCL-2-Regulated Apoptotic Pathway in Human Cancer

The BCL-2 family of proteins have also been shown to be critical for the development, progression and treatment responses of human cancers (Table 2). Indeed, the *BCL-2* gene itself was discovered following its identification as the oncogene activated by the t(14;18) chromosomal translocation in follicular lymphoma.[115] The seminal discovery by Vaux *et al*.[116] that enforced BCL-2 expression could protect cells from growth factor deprivation-induced death revealed for the first time that defects in apoptosis could cause cancer.

Studies using human cancer-derived cell lines and patient samples revealed abnormalities in the expression of several anti- as well as pro-apoptotic BCL-2 family members in a broad range of malignancies. In addition to the critical role of BCL-2 in follicular lymphoma, high levels of BCL-2 expression have also been observed in neuroblastoma and chronic lymphocytic leukaemia (CLL).[117–120] In at least some cases of CLL, this is likely due to loss of miR-15a and miR-16-1, which can repress BCL-2 expression.[75] Amplifications of the *Mcl-1* or *Bcl-x* gene loci have been identified as frequently occurring somatically acquired copy number aberrations in lung, breast[121,122] and giant-cell tumours of the bone.[123] RNAi-mediated knockdown of MCL-1 or BCL-XL induced killing of some cell lines derived from such cancers, suggesting that these pro-survival BCL-2-like proteins are essential for their sustained survival.[121] Furthermore, MCL-1 levels were found to be high in primary AML samples and antagonising MCL-1 activity (using inducible expression of BIM variants that only bind to and inhibit MCL-1) impaired the *in vitro* survival of primary human AML cells.[113,114] BFL-1, the human homologue of A1, was shown to be overexpressed and associated with chemo-resistance in various cancers, including B-CLL.[124–126]

Pro-apoptotic BCL-2 family members were also found to be deregulated in human cancers. The genomic regions harbouring *PUMA* and *BOK* commonly show somatically acquired loss of copy number in various cancer types.[121] Loss of BAX function appears likely to have a role in colon cancer development, with frame-shift mutations in the *BAX* gene detected in ~50% of colon cancers of the microsatellite mutator phenotype.[127] Combined loss of BAX and BAK was observed in a small number of AML samples from heavily pretreated patients; treatment may have selected for tumour

Table 2 Aberrations in BCL-2 protein family members in human cancer

Gene	Expression	Cancer	References
BIM (BCL2l11, BOD)	Genomic loss	Mantle cell lymphoma	128
BIM (BCL2l11, BOD)	Epigenetic silencing	Burkitt lymphoma	129
PUMA (BBC3)	Genomic loss	Various	121
PUMA (BBC3)	Epigenetic silencing	Burkitt lymphoma	130
BMF	Genomic loss	Advanced lung, breast cancer	131
BOK	Genomic loss	Various	121
BAX	Genomic loss	Colon cancer	127
BCL2	Overexpressed	Follicular lymphoma, neuroblastoma, CLL	115,117–120
BCL2L1 (BCLX)	Amplified	Lung cancer	121,122
MCL1 (BCL2L3)	Amplified	Lung cancer, breast cancer	121
MCL1 (BCL2L3)	Overexpressed	AML	114
BFL-1 (BCL2A1)	Overexpressed	B-CLL; various solid cancer	124

cells with loss of both of these multi-BH domain pro-apoptotic BCL-2 family members.[113] Homozygous loss of the *Bim* gene is seen in ~15–20% of mantle cell lymphomas.[128] Moreover, in BL, the *BIM* and *PUMA* genes were found to be silenced by epigenetic alterations, such as hyper-methylation.[129,130] Furthermore, the region harbouring the *BMF* gene is lost in late stage lung and breast cancer.[131]

These observations demonstrate that abnormalities in anti- as well as pro-apoptotic BCL-2 family members can contribute to the development of cancer in humans.

The Role of the BCL-2-Regulated Apoptotic Pathway in Cancer Therapy

Because of their role as mediators of apoptosis triggered by diverse cell stresses, the BCL-2 protein family members are key determinants of the response of tumour cells to a broad range of commonly used anticancer therapeutics (Figure 2). Accordingly, direct activation of the BCL-2-regulated apoptotic pathway using small-molecule mimetics of the pro-apoptotic BH3-only proteins is being developed as a novel strategy for cancer therapy.

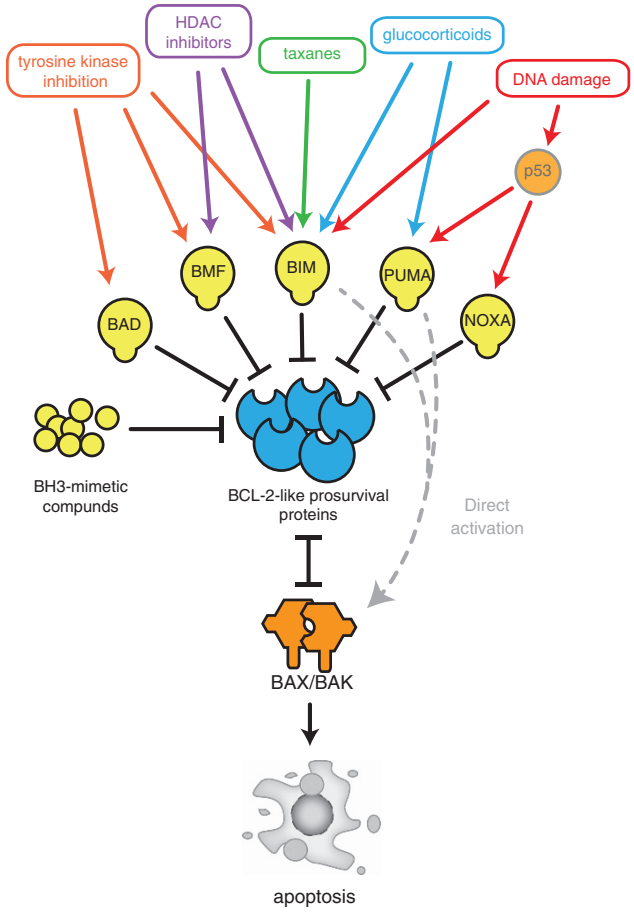

Figure 2 Many anticancer agents mediate tumour cell killing though activation of the BCL-2-regulated apoptotic pathway. BH3-only proteins are activated transcriptionally and/or posttranscriptionally in a cytotoxic stimulus-specific manner by many anticancer agents, often with 2–3 members cooperating to induce apoptosis. BH3-mimetic compounds bind directly to and block the BCL-2 pro-survival proteins and thereby elicit apoptosis even in cells lacking upstream activators of BH3-only proteins, such as the tumour-suppressor p53, which is critical for transcriptional induction of *Puma* and *Noxa*

The role of the BCL-2-regulated apoptotic pathway in the response to anticancer therapeutics was first demonstrated when it was found that non-transformed lymphoid cells from *Bcl-2* transgenic mice[132] and BCL-2-overexpressing lymphoma cells[133] were profoundly resistant to γ-irradiation, DNA damage-inducing chemotherapeutic drugs (e.g., etoposide) and glucocorticoids (e.g., dexamethasone). Similar protection from chemotherapeutic drug-induced apoptosis can be afforded by overexpression of any of the other pro-survival BCL-2 family members.[134–137]

Studies using gene-targeted mice or RNAi-mediated gene knockdown in cell lines revealed which pro-apoptotic BCL-2 family members are critical for cell killing by which anticancer agent. Consistent with the notion that BAX and BAK have essential overlapping functions in the BCL-2-regulated apoptotic pathway, cells from $Bax^{-/-}Bak^{-/-}$ mice are markedly resistant to diverse anticancer agents.[138–140] Notably, different anticancer agents require different BH3-only proteins for cell killing. PUMA is critical for therapeutic responses to γ-irradiation as well as to DNA-damaging drugs, with contributions from NOXA and also BIM (which does not appear to be a direct transcriptional target of p53) in at least certain non-transformed and malignant cell types.[141–145] PUMA and BIM together account for most of the pro-apoptotic activity of glucocorticoids.[141,143,146–149] BIM is also critical for taxane-induced cell killing.[150,151] Furthermore, BMF as well as BIM are critical for the killing of non-transformed lymphoid cells as well as certain lymphoma cells by inhibitors of histone deacetylases.[110,135] BIM (with BAD and BMF also contributing) is critical for the killing of tumour cells that are dependent on oncogenic kinases by therapeutic agents that block their activity, such as inhibitors of MEK (acting downstream of mutant B-RAF in melanoma or colon carcinoma),[152] EGFR (lung cancer),[153–155] BCR-ABL (CML)[156,157] and VEGFR signalling (tumour angiogenesis).[158] Notably, a gene polymorphism that impairs the expression of BIM was found to explain the *de novo* resistance of BCR-ABL-driven CML to Gleevec and mutant EGFR-driven lung cancer to Iressa/Tarceva in East Asian populations.[159]

Anticancer drug-induced killing of tumour cells requires activation of BH3-only proteins by upstream signalling mediators, such as p53 or the glucocorticoid receptor. These upstream signal activators are frequently mutated, lost or silenced (e.g., due to epigenetic modifications) during tumour development or subsequently during emergence of therapy-resistant cancer cells.[14,27,160] To bypass such resistance mechanisms, a new class of therapeutics, known as 'BH3-mimetics', that directly activate apoptosis have been developed (Figure 2). BH3-mimetics bind and inhibit the pro-survival BCL-2 family members and thereby activate apoptosis in cancer cells.[16] ABT-737 and its clinical analogue ABT-263 (navitoclax) exemplify this new therapeutic class, with the latter compound currently in phase 2 clinical trials.[161,162] Both compounds bind to BCL-2, BCL-XL and BCL-W (but not to MCL-1 or A1) displacing the endogenous BH3-only proteins, which can then bind to MCL-1, A1 and some of them also to BAX/BAK. This causes killing of tumour cells via a BAX/BAK-dependent mechanism.[162–164]

However, as ABT-737 and ABT-263 bind only a subset of the BCL-2-like pro-survival proteins, overexpression of MCL1 or A1/BFL-1, both of which are not inhibited by these compounds, has the potential to confer resistance to therapy.[164] Some cancers (such as CLL) with very high expression of BCL-2 (and possibly also cancers expressing high levels of BCL-XL and/or BCL-W) respond robustly to the existing BH3-mimetics when used as single agents. However, in order to maximise treatment efficacy, in many cancers these BH3-mimetics are probably best employed in combination with drugs known to activate BH3-only proteins, such as BIM or PUMA, that can potently neutralise MCL-1 and/or A1.

Indeed BH3-mimetics have been found to potently synergise *in vitro* with various chemotherapeutic drugs in the killing of CLL[165] and many other cancer cells, including mouse xenograft models of human breast cancer.[152,153,157,166,167] Such combinatorial therapeutic strategies would effectively neutralise all pro-survival BCL-2 family proteins and thereby efficiently activate apoptosis in malignant cells.

BH3-mimetics also affect non-transformed cells; for example, ABT-737 and ABT-263 cause thrombocytopaenia because platelets rely on BCL-XL for their survival.[168,169] This problem can be circumvented in the context of BCL-2-dependent tumours by using ABT-199/venetoclax, a BH3-mimetic that only inhibits BCL-2 and is showing great promise for the treatment of CLL.[170] Moreover, to prevent unacceptable collateral damage to normal tissues, BH3-mimetics may best be used in combination therapies with drugs that only affect cancer cells, such as inhibitors of oncogenic kinases (e.g., Gleevec to inhibit BCR-ABL in CML, Vemurafenib to inhibit mutant BRAF in melanoma), rather than using them with cytotoxic drugs that cause DNA damage in both malignant as well as non-transformed cells.[160]

Concluding Remarks

Changes in expression and activity of members of the BCL-2 family (and their upstream regulators) can exert profound effects of cell survival and this is of particular relevance to the development of cancer and its clinical treatment. Mechanisms delineated using mouse models mimicking human malignancies have greatly advanced our understanding of how apoptosis suppresses tumour formation. Importantly, these findings have been mirrored by insights from clinical studies and this knowledge can now be harnessed to develop improved treatment strategies for patients. So far, the most exciting outcome from these advances has been the development of the BH3-mimetic drugs that directly activate apoptosis in cancer cells by binding and inhibiting select pro-survival BCL-2 family members. The first of such compounds, ABT-263/navitoclax and ABT-199/venetoclax, are currently generating much excitement as they progress through clinical trials and will hopefully prove efficacious for treatment of a wide variety of both haematological and solid cancers.

Conflict of Interest

ARD Delbridge and A Strasser are employed by The Walter and Eliza Hall Institute. The Walter and Eliza Hall Institute receives milestone payments from Genentech and AbbVie for the development of BH3-mimetics for cancer therapy.

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