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BH3 mimetics to improve cancer therapy; mechanisms and examples

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Abstract

Tumor cell survival is highly dependent on the expression of certain pro-survival Bcl-2 family proteins. An attractive therapeutic approach is to inhibit these proteins using agents that mimic the Bcl-2 homology 3 (BH3) domains of the proapoptotic Bcl-2 family members, which neutralize these proteins by binding to their surface hydrophobic grooves. A number of BH3 mimetic peptides and small molecules have been described, a few of which have advanced into clinical trials. Recent studies have highlighted ABT-737, a bona fide BH3 mimetic and potent inhibitor of antiapoptotic Bcl-2 family members, as a promising anticancer agent. This review summarizes recent advances in understanding the mechanisms of action of BH3 domains and several classes of BH3 mimetics, as well as the prospects of using these agents to improve cancer therapy.

Keywords

apoptosis; cancer; drug resistance; BH3 mimetic; ABT-737

1. Introduction

The majority of anticancer agents used in the clinic were empirically discovered through large-scale testing of synthetic chemicals and natural products in cancer cell lines and tumor models. These agents lack specificity and are often ineffective against common epithelial tumors. Recent advances in cancer biology revealed alterations in several key pathways underlying tumorigenesis, and provided molecular targets for developing new therapies (Hanahan and Weinberg, 2000; Vogelstein and Kinzler, 2004). For example, imatinib (Gleevec), which targets the Bcr-Abl fusion protein, is a very effective treatment for a subset of chronic myeloid leukemia (Sawyers, 2002). It is hoped that these so called “targeted drugs” will improve specificity while reducing side-effects, and move oncology practice toward individualized treatment based on the genetic composition of the tumors (Anderson et al., 2006).

Apoptosis is an evolutionally conserved process that is required for development and tissue homeostasis. It also serves as a barrier to oncogenic transformation. Resistance to apoptotic cell death is a hallmark of cancer and contributes to chemoresistance (Hanahan and Weinberg, 2000; Johnstone et al., 2002). Several key pathways controlling apoptosis are commonly

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altered in cancer (Vogelstein and Kinzler, 2004). For instance, more than half of human tumors contain mutations in the *p53* tumor suppressor gene, virtually all of which abolish the ability of *p53* to trigger apoptosis (Vogelstein et al., 2000). Overexpression of certain antiapoptotic proteins, such as Bcl-2, Bcl-X_L, Akt, nuclear factor- κ B (NF- κ B), or inhibitor of apoptosis protein (IAP) family, are found in many types of human tumors (Reed, 2003). Defective apoptosis regulation drives neoplastic cells to gain additional tumorigenic features, including extended lifespan, further genetic mutations, growth under stress conditions, and tumor angiogenesis (Hanahan and Weinberg, 2000). Cancer cells become highly dependent on these genetic and epigenetic changes for survival, which seem to be ideal targets for development of novel anticancer drugs, as such drugs may selectively kill cancer cells while sparing normal cells whose survival does not rely on such changes (Demarchi and Brancolini, 2005).

The unfolding of the complex pathways involved in apoptosis signaling in the past decade has stimulated intensive efforts to restore apoptosis in cancer cells for therapeutic purposes (Mashima and Tsuruo, 2005; Yu, 2006; Mollinedo and Gajate, 2006). These efforts have led to several potential anticancer drugs, such as TNF-related apoptosis-inducing ligand (TRAIL), and inhibitors of the Bcl-2 protein family, IAPs and MDM2 (Reed and Pellecchia, 2005). One of the most promising approaches is to inhibit tumor cell survival using agents that mimic proapoptotic Bcl-2 homology 3 (BH3) domains, which play an essential role in apoptosis by neutralizing antiapoptotic Bcl-2 family proteins.

2. BH3 domains as critical inhibitors of the antiapoptotic Bcl-2 family members

Apoptosis in mammalian cells is regulated by two major pathways, one involving the mitochondria (intrinsic pathway) and the other involving the death receptors (extrinsic pathway). Apoptosis induced by anticancer agents is mainly regulated through the mitochondria by the Bcl-2 family of proteins, the evolutionarily conserved apoptotic regulators that integrate a variety of inter- and intracellular signals (Danial and Korsmeyer, 2004). The Bcl-2 family, including 17 or more members, all contain characteristic regions of homology termed as BH (Bcl-2 Homology) domains (Adams and Cory, 2007). Members of this family can be divided into three groups based on their structures and functions. The antiapoptotic (pro-survival) group, including Bcl-2, Bcl-X_L, Mcl-1, Bcl-w and A1, contain 4 BH domains. They protect cells from diverse cytotoxic conditions by inhibiting cell death. The second group, including Bax and Bak, are proapoptotic and contain multiple BH domains (Adams and Cory, 2007). The third group is also proapoptotic and termed “BH3-only proteins”. This group includes at least 8 proapoptotic members (Bad, Bid, Bik, Bim, Bmf, Hrk, Noxa, and PUMA) that display sequence homology with other Bcl-2 family members only within the amphipathic and α -helical BH3 segments (Fig. 1) (Huang and Strasser, 2000). The multiple BH3-only proteins are believed to fine tune apoptotic response in mammalian cells (Adams and Cory, 2007).

The balance between proapoptotic and antiapoptotic Bcl-2 members mediated through protein-protein interactions determines the fate of cells, to survive or to die (Danial and Korsmeyer, 2004). Structural studies revealed that the BH1, BH2 and BH3 domains in the antiapoptotic proteins fold into a globular domain containing a hydrophobic groove on its surface (Sattler et al., 1997). The α -helical BH3 domains of proapoptotic proteins bind to this hydrophobic groove and neutralize the antiapoptotic proteins (Petros et al., 2000). In healthy cells, basal levels of antiapoptotic proteins prevent Bax and Bak from being activated. Upon reception of apoptotic signals, BH3-only proteins are activated and competitively bind to the hydrophobic grooves of the antiapoptotic proteins through the BH3 domains (Fig. 2) (Cheng et al., 2001). This serves to displace Bax and Bak, and allows them to form multimers and permeabilize the mitochondrial outer membrane (Danial and Korsmeyer, 2004). Most if not all apoptotic signals transmitted

by BH3 domains converge through Bax and Bak (Zong et al., 2001). Once a cell becomes committed to apoptosis, a cascade of downstream events are triggered to execute cell death, including collapse of mitochondrial membrane potential, release of the apoptogenic mitochondrial proteins such as cytochrome *c*, SMAC/Diablo and AIF, and activation of caspases (Green and Kroemer, 2004; Wang, 2001).

BH3 domains have different capabilities of inducing apoptosis in different cell types, owing to their differential binding specificities for antiapoptotic proteins (Chen et al., 2005; Kuwana et al., 2005; Letai et al., 2002). The BH3 domains of PUMA and Bim can bind to all five antiapoptotic proteins (Fig. 2). In contrast, those of Bad and Bmf preferentially interact with Bcl-2, Bcl-X_L, Bcl-w, but not with Mcl-1 or A1. The BH3 domains of Bid, Bik and Hrk strongly bind to Bcl-X_L, Bcl-w, and A1, but only weakly to Bcl-2 and Mcl-1. The BH3 domain of Noxa binds to Mcl-1 and A1, but not to other antiapoptotic proteins (Chen et al., 2005; Kuwana et al., 2005). On the other hand, some evidence suggests that several BH3-only proteins such as Bid and Bim directly bind to and activate Bax and Bak (Cartron et al., 2004; Kuwana et al., 2005). An alternative hierarchical model among BH3-only proteins was thus proposed, in which Bid, Bim, and perhaps PUMA, are involved in directly activating Bax and Bak, while other BH3-only proteins function by engaging pro-survival proteins, thereby freeing up these proteins to interact with Bax and Bak (Certo *et al.*, 2006; Kim *et al.*, 2006). However, emerging evidence indicates that all BH3-only proteins indirectly activate Bax and Bak by binding to antiapoptotic proteins (Fig. 2), as apoptosis induced by many stimuli is intact in mice deficient in both Bid and Bim (Willis et al., 2007).

3. Rationale for targeting tumors with BH3 mimetics

Apoptosis deregulation in cancer cells appears to primarily affect the signaling pathways upstream of Bax/Bak and mitochondria, leaving the downstream core apoptotic machinery mostly intact (Danial and Korsmeyer, 2004; Reed, 2003). This presents a great opportunity for restoring apoptosis in cancer cells by manipulating the balance between the pro- and antiapoptotic Bcl-2 family members. In the last several years, a number of approaches have been used to identify Bcl-2 family inhibitors that mimic the actions of the proapoptotic BH3 domains (Cory and Adams, 2005; Fesik, 2005). Such approaches are appealing for several reasons. Theoretically, manipulating the pro-survival proteins using BH3 mimetics should be more effective than targeting the upstream signaling steps, which are often defective due to oncogenic alterations. Biologically, proapoptotic BH3 domains bind directly to the hydrophobic grooves of pro-survival proteins with high affinity, and are necessary and sufficient for initiation of apoptosis (Baell and Huang, 2002). Pharmacologically, the sizes of α -helical BH3 domains, 14–24 amino acids as defined by different groups, are relatively small and amenable for structure-based design to identify small-molecule mimics.

Agents mimicking the BH3 domains are likely to provide some degree of selectivity against cancer cells. These cells often express high levels of Bcl-2-like antiapoptotic proteins to evade the apoptotic fate imposed by unscheduled cell proliferation, activation of oncogenes, or DNA damage (Amundson et al., 2000; Kirkin et al., 2004). Cancer cells are often much more sensitive to inhibition of pro-survival proteins compared with their normal counter parts (Hanahan and Weinberg, 2000). Furthermore, differential binding specificities of BH3 domains suggest that it might be possible to design BH3 mimetics to target specific antiapoptotic proteins that are overexpressed in a particular type of cancer for improved specificity.

4. Peptide BH3 mimetics

In principle, peptides containing BH3 domain sequences should mimic the actions of BH3-only proteins and may be explored as pharmaceutical lead molecules (Shangary and Johnson, 2002). BH3 peptides longer than 14 amino acids can retain an α -helical structure and some

biological activities (Shangary et al., 2004). In cell-free assays, these peptides bind to hydrophobic grooves of antiapoptotic proteins, and disrupt complexes formed between proapoptotic and antiapoptotic Bcl-2 family proteins (Letai et al., 2002). Similar to BH3-only proteins, BH3 peptides induce oligomerization of Bax and Bak, permeabilization of mitochondrial membrane, and release of cytochrome *c* (Chen et al., 2005; Kuwana et al., 2005; Letai et al., 2002).

Despite these proof-of-principle studies, the use of BH3 peptides as therapeutic agents is limited by their unfavorable pharmacological properties, including poor cellular permeability, bioavailability, solubility, and metabolic stability *in vivo* (Denicourt and Dowdy, 2004). Several methods have been used to overcome these limitations. Intracellular uptake of BH3 peptides can be enhanced by tagging them with peptide transduction domains from *Drosophila* antennapedia protein or human immunodeficiency virus-1 TAT protein (Shangary and Johnson, 2003). Chemical modifications can also be used to optimize BH3 peptides. For example, a Bid BH3 peptide modified by a chemical staple to maintain its α -helical conformation has much improved pharmacological properties (Walensky et al., 2004). It is conformationally stable, resistant to degradation by proteases, and has an increased ability to penetrate cell membranes and affinity of binding to Bcl-2 family proteins. It can directly activate Bax, and induce apoptosis in leukemia cells *in vitro* and *in vivo* (Walensky et al., 2006). The α -helical conformation of a Bak BH3 peptide can be stabilized using unnatural amino-acid residues (Sadowsky et al., 2007; Sadowsky et al., 2005). These approaches may be generally applicable to different BH3 peptides, as well as other biologically active peptides, such as those used to inhibit p53-HDM2 interactions, for improving stability and delivery (Bernal et al., 2007).

5. Small-molecule Bcl-2 and Bcl-X_L inhibitors

Compared to BH3 peptides, small-molecule BH3 mimetics appear to hold greater promise for targeting the Bcl-2 protein family. Targeting protein-protein interactions using small molecules are in general very difficult. However, the deep hydrophobic groove on the surface of Bcl-X_L makes it feasible to develop highly specific inhibitors (Petros et al., 2000). This notion, coupled with the therapeutic relevance of Bcl-2 family proteins, has stimulated considerable interest in identifying small molecules capable of binding to Bcl-2 and/or Bcl-X_L, inhibiting their anti-apoptotic functions, and thereby promoting apoptosis (Reed and Pellecchia, 2005). A number of such compounds have been identified through a variety of methods, including computational modeling, structure-based design, and high-throughput screening of natural product and synthetic libraries (Table 1).

5.1 Investigational Bcl-2 and Bcl-X_L inhibitors

The first small-molecule Bcl-2 inhibitor reported is HA14-1, a synthetic chromene molecule identified by a computational modeling approach (Wang et al., 2000). At micromolar range, it disrupts Bax and Bcl-2 interactions and promotes mitochondrial dysfunction and cytochrome *c* release (An et al., 2004; Manero et al., 2006; Milanese et al., 2006). HA14-1 induces apoptosis in several tumor cell lines and enhances their apoptotic responses to γ -radiation and anticancer agents, such as TRAIL, the proteasome inhibitor bortezomib, flavopiridol, imatinib, and MAPK kinase inhibitors (Dai et al., 2004; Hao et al., 2004; Milella et al., 2002; Pei et al., 2003; Pei et al., 2004; Sinicrope et al., 2004; Tortora et al., 2007). HA14-1 also overcomes chemo- and radio-resistance caused by Bcl-2 overexpression (An et al., 2007; Manero et al., 2006; Oliver et al., 2007).

A series of small molecules named BH3Is, including a thiazolidin derivative BH3I-1 and a benzene sulfonyl derivative BH3I-2, were identified by screening a chemical library using a BH3 peptide displacement assay (Degterev et al., 2001). BH3Is disrupt interactions between

Bcl-X_L and proapoptotic Bcl-2 family proteins at low micromolar range. BH3I-2', an analog of BH3I-2, inhibits mitochondrial respiration, damages inner mitochondrial membrane, and induces apoptosis via caspase-dependent and -independent mechanisms (Degtarev et al., 2001; Feng et al., 2003; Milanese et al., 2006). It also sensitizes TRAIL-induced apoptosis in leukemia and prostate cancer cells (Hao et al., 2004; Ray et al., 2005).

The mitochondrial electron transfer inhibitor Antimycin A and analogs were identified as Bcl-X_L inhibitors based on computational modeling (Tzung et al., 2001). They interact with the hydrophobic BH3 binding groove of Bcl-X_L, compete with BH3 peptides to bind to Bcl-2, and induce caspase-independent apoptosis in cells expressing high levels of Bcl-X_L (Tzung et al., 2001). Antimycin A analogs inhibit the effects of Bcl-X_L on energy metabolism (Schwartz et al., 2007), and exhibit antitumor activities in preclinical models (Wang et al., 2005).

Several additional small-molecule inhibitors of Bcl-2 and/or Bcl-X_L have been described, including certain theaflavins and epigallocatechins (EGCGs), which are abundant constituents in black and green tea (Leone et al., 2003), chelerythrine, which is a widely known protein kinase C inhibitor (Chan et al., 2003), terphenyl derivatives (Kutzki et al., 2002; Yin et al., 2005), and NSC365400 (compound 6) (Enyedy et al., 2001). Despite their abilities to bind to Bcl-2 and/or Bcl-X_L and induce apoptosis in cancer cell lines, these molecules by themselves are not considered good drug candidates, because their binding affinity for Bcl-2 and Bcl-X_L are in micromolar range (Zhai et al., 2006), which are much lower than those (normally in nanomolar range) generally associated with drug candidates for targeting protein-protein interactions. They also lack favorable pharmacological properties essential for clinical applications.

5.2. Bcl-2 and Bcl-X_L inhibitors tested in clinical trials

Among the small-molecule Bcl-2 and Bcl-X_L inhibitors identified, only two classes, including gossypol derivatives and GX015-070, have advanced into clinical trials. Gossypol is an orally-available compound found in cottonseeds originally used as an herbal medicine in China (Pellecchia and Reed, 2004). Gossypol has been known to have anti-tumor activities for a long time (Stein et al., 1992), but only recently was identified as a Bcl-2 inhibitor by structure-based methodology (Kitada et al., 2003). It binds to the BH3-binding grooves of Bcl-2, Bcl-X_L and Mcl-1, displacing BH3 peptides with a sub-micromolar inhibitory concentration of 50% (IC₅₀) (Kitada et al., 2003). Gossypol promotes an allosteric conformational change in Bcl-2 and loss of mitochondrial membrane potential in a Bax/Bak-independent fashion (Lei et al., 2006). Gossypol-induced apoptosis involves cytochrome *c* release from mitochondria and activation of several caspases (Mohammad *et al.*, 2005a; Zhang *et al.*, 2003). The anticancer activities of gossypol have been demonstrated in cells derived from head and neck squamous cell carcinoma (HNSCC), colon, prostate, and pancreatic cancers (Mohammad et al., 2005b; Oliver et al., 2004; Oliver et al., 2005; Wolter et al., 2006; Xu et al., 2005; Zhang et al., 2007b). Gossypol also sensitizes cancer cells to apoptosis induced by γ -irradiation and TRAIL (Bauer et al., 2005; Xu et al., 2005; Yeow et al., 2006), and improves the efficacy of cyclophosphamide-adriamycin-vincristine-prednisolone (CHOP) regimen in lymphoma cells (Mohammad et al., 2005a).

As a highly reactive compound, gossypol does not have desirable pharmacologic properties and exhibited some toxicity in clinical trials (Kitada et al., 2003; Van Poznak et al., 2001). Several attempts have been made to generate semi-synthetic analogs of gossypol with improved pharmacologic properties. Apogossypol was synthesized and characterized using a combination of approaches including molecular modeling, magnetic resonance (NMR)-based structural analysis, fluorescence polarization assays, and cell-based assays (Becattini et al., 2004). Apogossypol binds to and inhibits Bcl-2 and Bcl-X_L with high affinity and induces apoptosis in tumor cell lines in sub-micromolar range. TW-37 is another gossypol derivative

rationally designed based on computer modeling of the BH3 domain of Bim (Wang et al., 2006). It binds to Bcl-2, Bcl-X_L, and Mcl-1 with high affinity. Preclinical studies showed that TW-37 combined with MEK inhibitors potently inhibits the growth of melanoma cells (Verhaegen et al., 2006), and is effective against chemoresistant diffuse large cell lymphoma cells with little toxicity to normal peripheral blood lymphocytes (Mohammad et al., 2007).

GX015-070 is an indole-derivative and a broad-spectrum inhibitor of pro-survival Bcl-2 family proteins developed by Gemin X Biotech (Reed and Pellecchia, 2005). It has been tested in clinical trials, alone or in combination with other anticancer drugs, for treating several hematological malignancies and non-small cell lung cancer (NSCLC) (Table 1) (www.clinicaltrials.gov). It activates the mitochondrial apoptotic pathway by displacing Bak from Mcl-1 and Bcl-X_L, upregulating Bim, and inducing Bax and Bak conformational changes, mitochondrial depolarization, and caspase activation (Perez-Galan et al., 2007; Trudel et al., 2007a). It synergizes with the proteasome inhibitor bortezomib to kill mantle cell lymphoma (MCL) cells, but shows no significant cytotoxicity to peripheral blood mononuclear cells (PBMC) from healthy donors (Perez-Galan et al., 2007). As a single agent or in combination with melphalan, dexamethasone, or bortezomib, GX015-070 is effective against cultured or patient-derived multiple myeloma cells (Trudel et al., 2007a). It also combined synergistically with cisplatin or the epidermal growth factor receptor (EGFR) inhibitor gefitinib against NSCLC cells (Li et al., 2007).

Although the above small molecules can inhibit Bcl-2 and/or Bcl-X_L and induce apoptosis in cancer cells, there is no evidence indicating that any of them function primarily as a BH3 mimetic. They often promote caspase-independent cell killing and do not require Bax/Bak for their cytotoxicity in both short-term and long-term assays (van Delft et al., 2006), suggesting that their killing is mediated by unidentified mechanisms that are distinct from the well-defined mitochondrial pathway.

6. ABT-737, a bona fide BH3 mimetic and a promising drug candidate

6.1 Development and characterization of ABT-737

The most potent and specific Bcl-2/Bcl-X_L inhibitor discovered to date is a synthetic small-molecule compound called ABT-737 (Table 1). It was developed by Abbott Laboratories using a combination of approaches, including NMR-based screening, parallel synthesis and structure-based design (Oltersdorf et al., 2005). The Bcl-X_L hydrophobic binding groove was first divided into two smaller half-sites, each of which was individually targeted by a small molecule. The two lead compounds were then chemically linked to form a fusion product, which was subjected to chemical modifications to further improve its affinity for Bcl-2 and Bcl-X_L, and decrease its binding to human serum albumin (Bruncko et al., 2007). The final molecule ABT-737 has extremely high affinity for Bcl-X_L, Bcl-2 and Bcl-w, with a dissociation constant (*K_i*) below 1 nM for each of them, although it binds poorly to Mcl-1 and A1 (Oltersdorf et al., 2005) (Table 1).

Mechanistic studies revealed that ABT-737 is similar to the BH3 domain of Bad (Oltersdorf et al., 2005). By itself it does not bind to Bax, but disrupts the complex of Bax and Bcl-2 and triggers conformational alteration of Bax. In cell-free assays, it does not directly induce cytochrome *c* release from purified mitochondria, but relieves the inhibitory effects of Bcl-2 and Bcl-X_L on cytochrome *c* release. In contrast, a stabilized BH3 peptide derived from Bid can directly induce cytochrome *c* release from purified mitochondria (Walensky et al., 2004). ABT-737 also displaces BH3-only proteins such as Bim from its binding partners. It does not directly induce apoptosis in most cell lines analyzed, but enhances the apoptotic effects of many death signals (Oltersdorf et al., 2005). It was recently shown to promote autophagy by relieving the inhibition of Bcl-2 and Bcl-X_L on Beclin-1 (Maiuri et al., 2007). Importantly, the

effects of ABT-737 are completely abrogated in Bax and Bak deficient cells (van Delft et al., 2006). This strict dependence on Bax and Bak distinguishes ABT-737 from other small-molecule Bcl-2/Bcl-X_L inhibitors (van Delft et al., 2006), and suggests it functions as an authentic BH3 mimetic.

6.2 Anticancer effects of ABT-737

As a single agent, ABT-737 is most efficacious against small-cell lung carcinoma (SCLC) and several lymphoid malignancies including follicular lymphoma, diffuse large B cell lymphoma, chronic lymphocytic leukemia, acute lymphocytic leukemia, and acute myeloid leukemia (Del Gaizo Moore *et al.*, 2007; Deng *et al.*, 2007; Konopleva *et al.*, 2006; Oltersdorf *et al.*, 2005). It effectively kills tumor cells or cell lines from these diseases in the nanomolar range. ABT-737 alone also kills multiple myeloma cells in the micromolar range (Chauhan *et al.*, 2007; Kline *et al.*, 2007; Trudel *et al.*, 2007b). Apoptosis induced by ABT-737 is associated with dissociation of proapoptotic and antiapoptotic Bcl-2 family members, conformational change of Bax, cytochrome *c* release from the mitochondria, and activation of caspases (Chauhan *et al.*, 2007; Kojima *et al.*, 2006; Konopleva *et al.*, 2006; Oltersdorf *et al.*, 2005; van Delft *et al.*, 2006). ABT-737-induced apoptosis is caspase-dependent (van Delft *et al.*, 2006).

For most tumor cell lines, especially those derived from solid tumors, ABT-737 has weak single-agent cell killing activity (Oltersdorf *et al.*, 2005; van Delft *et al.*, 2006). However, it exhibited striking synergy when combined with a variety of anticancer agents. ABT-737 markedly enhanced the response of tumor cells to γ -irradiation and chemotherapeutic drugs with different modes of action, including the genotoxic agents etoposide, doxorubicin, cisplatin and melphalan, the antimetabolite drug arabinoside (Ara-C), the microtubule targeting agents paclitaxel and vincristine, the glucocorticoid dexamethasone, the anti-inflammatory agent thalidomide, and the proteasome inhibitor bortezomib (Chauhan *et al.*, 2007; Oltersdorf *et al.*, 2005; Trudel *et al.*, 2007b; van Delft *et al.*, 2006). Established combination treatment regimens, such as vincristine/dexamethasone/L-asparaginase (VXL) treatment for acute myeloid leukemia, also benefited from ABT-737 (Kang *et al.*, 2007). ABT-737 overcomes resistance to Bcr-Abl inhibitors imatinib and INNO-406 in leukemia cells with Bcr/Abl translocation (Kuroda *et al.*, 2007; Kuroda *et al.*, 2006). It also enhances the anticancer effects of several investigational agents, such as cyclin-dependent kinase (CDK) inhibitor roscovitine and MDM2 inhibitor Nutlin-3a (Chauhan *et al.*, 2007; Chen *et al.*, 2007). Furthermore, ABT-737 amplifies the activity of anticancer immune cells against melanoma cells (Lickliter *et al.*, 2007). ABT-737 treatment in general is well tolerated by normal hematopoietic cells and bone marrow cells (Konopleva *et al.*, 2006; Oltersdorf *et al.*, 2005). Thus, combination therapy with ABT-737 may lower the doses of conventional anticancer agents required for clinical responses to reduce collateral damage to normal cells, or ensure more stable remissions without lowering doses.

The anti-tumor activities of ABT-737 have been characterized in several animal models. ABT-737 caused complete regression of established SCLC xenograft tumors and produced cures in a high percentage of the treated mice. Tumor regression was attributed to caspase activation and apoptosis induction (Oltersdorf *et al.*, 2005). ABT-737 suppressed tumor growth in a xenograft mouse myeloma model (Trudel *et al.*, 2007b). It also suppressed aggressive leukemia driven by Raf-transformed myeloid cells, reduced tumor burden by about 50%, and significantly extended survival of the treated mice (Konopleva *et al.*, 2006). In E μ -myc/*bcl-2* bitransgenic mouse model, ABT-737 therapy prolonged the survival of recipient mice transplanted with the control or Bcl-2-transduced tumors by up to 30 days (van Delft *et al.*, 2006). In these studies, ABT-737 was well tolerated and did not produce significant weight loss (< 5%), but caused reduction in platelet and lymphocyte counts (Oltersdorf *et al.*, 2005).

6.3 Overexpression of Mcl-1 as a mechanism of ABT-737 resistance

ABT-737 efficiently binds to Bcl-2, Bcl-X_L and Bcl-w, but not to Mcl-1 and A1. While A1 is not expressed in most tumors, Mcl-1 is ubiquitously expressed in a variety of tumors, which might explain why most tumors are insensitive to ABT-737 (van Delft et al., 2006). Several groups reported that Mcl-1 expression plays a major role in causing ABT-737 resistance. Leukemia and SCLC cells expressing relatively higher levels of Bcl-2, Bcl-X_L and Noxa, and lower levels of Mcl-1 were found to be sensitive to ABT-737 (Del Gaizo Moore et al., 2007; Tahir et al., 2007). Conversely, those expressing high levels of Mcl-1 are resistant to ABT-737 (Konopleva et al., 2006; van Delft et al., 2006). Down-regulation of Mcl-1 by siRNA or Noxa-induced protein degradation overcomes ABT-737 resistance (Chen et al., 2007; Konopleva et al., 2006; van Delft et al., 2006). Unbiased genomic analysis and siRNA library screening also identified Mcl-1 and Noxa as modulators of ABT-737 sensitivity (Lin et al., 2007; Olejniczak et al., 2007). Furthermore, combinations of ABT-737 with agents that decrease Mcl-1 expression, such as CDK inhibitor roscovitine and protein synthesis inhibitor cycloheximide, markedly boosted the effects of ABT-737 on human leukemia and SCLC cell lines (Chen et al., 2007). These studies not only validated the specificity and molecular mechanisms of ABT-737, but also provided a rationale for targeting Mcl-1 for improving its therapeutic effects.

6.4 Considerations of clinical applications of ABT-737

ABT-737 is likely to be most efficacious as a single agent for targeting tumors in which Mcl-1 expression is low, absent, or inactivated, such as follicular lymphoma, chronic lymphocytic leukemia, and SCLC. For tumors in which Mcl-1 is the predominant survival protein, ABT-737 is unlikely to be effective as a single agent, but may serve to enhance therapies that downregulate Mcl-1 (Dai and Grant, 2007; van Delft et al., 2006). Combination therapies using genotoxic agents and ABT-737 could be particularly effective, as many genotoxic drugs induce Mcl-1 degradation (Nijhawan et al., 2003), which should greatly potentiate the effects of ABT-737. Since Mcl-1 level is often maintained by cytokine signaling (Yang et al., 1995), it is likely that antagonists of certain growth factors may sensitize tumor cells to ABT-737. Furthermore, the rapid turnover of *Mcl-1* mRNA and protein provide the rationale for combining ABT-737 with inhibitors of transcription or translation (Chen et al., 2007), such as CDK inhibitors and multikinase inhibitors. Mcl-1 degradation is regulated by the ubiquitin E3 ligase Mule (Zhong et al., 2005), which may be manipulated to enhance the therapeutic effects of ABT-737.

Although ABT737 seems to be well tolerated in animals, its ability to inhibit several pro-survival proteins in normal cells might still be a concern for causing adverse effects. For example, ABT-737 causes dose-dependent acute thrombocytopenia by reducing the number of circulating platelets (Zhang et al., 2007a), whose turnover is regulated by apoptosis (Mason et al., 2007). Platelets are particularly sensitive to ABT-737, perhaps because of Bak-dependent apoptosis normally constrained by Bcl-X_L in these cells (Mason et al., 2007). How to avoid unwanted apoptosis in normal cells will be a major issue confronted in future clinical applications of ABT-737 and related compounds. Clinical trials for testing the efficacy and side effects of these compounds have been initiated (Adams and Cory, 2007), and the results are eagerly awaited.

7. Conclusions and future perspective

Studies of BH3 mimetics, in particular ABT-737, provide strong proof-of-principle that it is feasible to target pro-survival Bcl-2 family members in tumors by inhibiting protein-protein interactions. Several issues will be encountered as the BH3 mimetics move forward into various stages of preclinical development and clinical testing, including compound stability and

formulation, pharmacokinetics and metabolism, toxicity, and off-target effects. A key issue is their selectivity in normal versus cancer cells. It might be feasible to develop agents targeting individual pro-survival Bcl-2 family members based on their similar yet distinct hydrophobic BH3 binding grooves (Lee et al., 2007). The hope is then to be able to choose a particular combination based on the expression patterns of different pro-survival proteins in the tumors, and the sensitivity of normal cells to such a combination. A better understanding of BH3 domain biology in apoptosis regulation will be crucial. Furthermore, the status of Bcl-2 family members may serve as a valuable prognostic marker for predicting responses to ABT-737, or for selecting the right patient population and drug combination for clinical trials. For example, a recent study showed that the response to ABT-737 in lymphoma cells can be predicted by classifying the abnormalities of Bcl-2 family members using a strategy called “BH3 profiling” (Deng *et al.*, 2007).

Unlike agents that target specific alterations in a small fraction of tumors, such as Bcr/Abl translocation and EGFR activation, BH3 mimetics may be applicable to a wide range of malignancies, as apoptosis deregulation is common in most, if not all tumors. The already impressive success of ABT-737 illustrates that the combination of scientific disciplines, including molecular biology, protein structural analysis, computer modeling and synthetic chemistry, can have a major impact on the pace of developing novel experimental therapeutics. Although time will tell if there will be a major impact of BH3 mimetics in the clinic, the excitement generated from this approach will certainly drive the momentum of targeted cancer therapies. Combination of these therapies with the development and application of new biomarkers might take cancer treatment to a more rational and personalized level sooner than we had imagined.

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Bad	103	N	L	W	A	A	Q	R	Y	G	R	E	L	R	R	M	S	D	E	F	V	D	S	F	K	K	G	128
Bid	79	Q	E	D	I	I	R	N	I	A	R	H	L	A	Q	V	G	D	S	M	D	R	S	I	P	P	G	104
Bik	50	C	M	E	G	S	D	A	L	A	L	R	L	A	C	I	G	D	E	M	D	V	S	L	R	A	P	75
Bim	141	D	M	R	P	E	I	W	I	A	Q	E	L	R	R	I	G	D	E	F	N	A	Y	Y	A	R	R	166
Bmf	126	Q	H	Q	A	E	V	Q	I	A	R	K	L	Q	C	I	A	D	Q	F	H	R	L	H	V	Q	Q	151
Hrk	26	R	S	S	A	A	Q	L	T	A	A	R	L	K	A	L	G	D	E	L	H	Q	R	T	M	W	R	51
Noxa	18	P	A	E	L	E	V	E	C	A	T	Q	L	R	R	F	G	D	K	L	N	F	R	Q	K	L	L	43
PUMA	130	E	E	Q	W	A	R	E	I	G	A	Q	L	R	R	M	A	D	D	L	N	A	Q	Y	E	R	R	155
Bax	52	Q	D	A	S	T	K	K	L	S	E	C	L	K	R	I	G	D	E	L	D	S	N	M	E	L	Q	77
Bak	67	P	S	S	T	M	G	Q	V	G	R	Q	L	A	I	I	G	D	D	I	N	R	R	Y	D	S	E	92

Fig. 1.

Alignment of the BH3 segments of the proapoptotic Bcl-2 family members. The most conserved residues are shaded in dark gray, while the less conserved ones are shaded in light gray.

Fig. 2. A model of apoptosis mediated by Bcl-2 family members. The BH3-only proteins selectively bind to the hydrophobic grooves of the pro-survival Bcl-2 family members through their BH3 domains, which relieves their inhibition on Bax and Bak and triggers downstream apoptotic events.

Table 1

Small-molecule BH3 mimetics

Class	Developers	Targets (affinity)	Targeted diseases	Stage	References
HA14-1 and analogues	Raylight Chemokine Pharmaceuticals	Bcl-2 (μM)	Leukemia, myeloma, glioblastoma, prostate cancer, glioma, colon cancer, and prostate cancer	Preclinical	(An et al., 2007; Hao et al., 2004; Manero et al., 2006; Milanesi et al., 2006; Milella et al., 2002; Oliver et al., 2007; Pei et al., 2004; Sinicrope et al., 2004; Wang et al., 2000a)
BH3-Is and analogs	Harvard University	Bcl-X _L (μM)	Leukemia, prostate cancer, and non-small cell lung cancer	Preclinical	(Degterev et al., 2001; Feng et al., 2003; Milanesi et al., 2006; Ray et al., 2005)
Antimycin A and analogs	University of Washington	Bcl-2 (μM) Bcl-X _L (μM)	Leukemia and cervical cancer	Preclinical	(Campas et al., 2006; Schwartz et al., 2007; Tzung et al., 2001; Wang et al., 2005)
Gossypol and analogs (Apogossypol and TW-37)	University of Michigan/Ascenta Therapeutics/The Burnham Institute	Bcl-2 (sub-μM) Bcl-X _L (sub-μM) Mcl-1 (sub-μM)	Chronic lymphocytic leukemia, non-Hodgkin's lymphoma, glioblastoma, head and neck cancer, prostate cancer, and small-cell lung cancer	Phase I/II clinical trials	(Becattini et al., 2004; Gilbert et al., 1995; Mohammad et al., 2007; Mohammad et al., 2005a; Mohammad et al., 2005b; Oliver et al., 2004; Van Poznak et al., 2001; Verhaegen et al., 2006; Wang et al., 2006; Wang et al., 2000b; Zhai et al., 2006; www.clinicaltrials.gov)
GX015-070	Germin X Biotech	Bcl-2 Bcl-X _L (sub-μM) Mcl-1 (sub-μM)	Leukemia, lymphoma, multiple myeloma, and non-small cell lung cancer	Phase I/II clinical trials	(Li et al., 2007; Perez-Galan et al., 2007; Trudel et al., 2007a; www.clinicaltrials.gov)
ABT-737	Abbott Laboratories	Bcl-2 (nM) Bcl-X _L (nM) Bcl-w (nM)	Leukemia, lymphoma, myeloma, small-cell lung cancer, cervical cancer, and breast cancer	Preclinical	(Del Gaizo Moore et al., 2007; Deng et al., 2007; Kang et al., 2007; Kohl et al., 2007; Kojima et al., 2006; Konopleva et al., 2006; Kuroda et al., 2006; Oltschendorf et al., 2005; Tahir et al., 2007; Trudel et al., 2007b; van Delft et al., 2006)