Mimicking the BH3 domain to kill cancer cells

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Abstract

Cancer cells demonstrate deviant behavior that induces apoptotic signaling. In order to survive, cancer cells typically acquire changes enabling evasion of death signals. One way they do this is by increasing the expression of anti-apoptotic BCL-2 proteins. Anti-apoptotic BCL-2 family proteins antagonize death signaling by forming heterodimers with pro-death proteins. Heterodimer formation occurs via binding of the pro-apoptotic protein's BH3 domain into the hydrophobic cleft of anti-apoptotic proteins. The BH3 mimetics are small molecule antagonists of the anti-apoptotic BCL-2 members that function as competitive inhibitors by binding to the hydrophobic cleft. Under certain conditions, antagonism of anti-apoptotic BCL-2 family proteins can unleash pro-death molecules in cancer cells. Thus, the BH3 mimetics are a new class of cancer drugs that specifically target a mechanism of cancer cell survival, to selectively kill cancer cells.

Introduction

For decades, cytotoxic chemotherapy, along with surgery and radiation, has been one of the three important modalities used to treat cancer. As most chemotherapeutics were discovered by empirical screens, the molecular mechanisms of how they kill cells are poorly understood. An ideal chemotherapeutic drug would target only neoplastic cells, signaling their removal from the body without damaging adjacent cells. However, conventional chemotherapy is associated with many toxicities, largely due to the presence in normal cells of the targets of most conventional agents, ie DNA and microtubules. A truly cancer-selective therapy must target a molecule or property that is selectively present in cancer cells to avoid toxicity to normal cells.

In order for a cancerous cell to develop it must obtain the ability to surmount essential checkpoints that would normally send a deregulated cell to its demise. Cancer cells attain the capacity to evade the body's own immune response and to grow in a stressful environment, where both oxygen and nutrients are limited. In order to maintain a high proliferation rate, tumor cells generate their own growth signal, while, simultaneously becoming insensitive to growth inhibitory effects (Hanahan and Weinberg, 2000; Johnstone *et al.*, 2002). The cells obtain mutations in key cellular proteins that allow the developing cancer to grow, albeit breaking certain cellular rules, such as genomic instability, oncogene activation, loss of adhesion, along with breach of cell cycle checkpoints. Each of the breaches mentioned can cause activation of the mitochondrial (intrinsic) apoptotic pathway. The oncogene c-myc, has been shown to activate apoptosis (Evan *et al.*, 1992), similarly anoikis or loss of adhesion leads to the activation of pro-apoptotic factors (Puthalakath *et al.*, 1999; Puthalakath *et al.*, 2001). Cancer cells have a unique requirement to overcome the death signaling engendered by these behaviors. Targeting the mechanisms cancers use to escape apoptosis offers the possibility, therefore, of a wide therapeutic index.

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The BCL-2 family

The BCL-2 family of proteins governs whether a cell continues to live or instead commits to death via the mitochondrial apoptotic pathway. Interactions between pro- and anti-apoptotic BCL-2 family proteins control the decision making process at the mitochondrion, modulating the cells sensitivity to death (Kim *et al.*, 2006; Letai *et al.*, 2002; Wei *et al.*, 2000). BCL-2, BCL-X$_L$, MCL-1, BCL-W and BFL-1 are the anti-apoptotic members of the family (Boise *et al.*, 1993; Choi *et al.*, 1995; Cleary and Sklar, 1985; Gibson *et al.*, 1996; Kozopas *et al.*, 1993; Tsujimoto *et al.*, 1985). They share sequence homology in four α–helical BCL-2 homology domains (BH) BH1-4. While the multidomain pro-apoptotic proteins BAX, BAK and BOK, the promoters of apoptosis, share homology in the domains BH1 through BH3 (Chittenden *et al.*, 1995; Oltvai *et al.*, 1993). BOK expression is largely limited to reproductive tissues, and less is known about its function, though it is widely supposed that it behaves much like BAX and BAK (Hsu *et al.*, 1997). Lastly, the group sharing the least homology is the BH3-only pro-death group, which includes PUMA, NOXA, BIM, BID, HRK, BMF, BAD and BIK (Boyd *et al.*, 1995; Inohara *et al.*, 1997; Nakano and Vousden, 2001; O'Connor *et al.*, 1998; Oda *et al.*, 2000; Wang *et al.*, 1996; Yang *et al.*, 1995). They are so named because they demonstrate homology only in the BH3 domain. The BH3 domain, along with the presence of BAX and BAK, are absolutely essential for the death function of the BH3-only proteins (Cheng *et al.*, 2001; Wei *et al.*, 2001; Zha and Reed, 1997).

Death signals emanating from diverse cellular locations caused by DNA damage, growth factor deprivation and oncogene activation are directed through the mitochondrial pathway by activation (transcriptional or post-translational modifications) of BH3-only proteins (Evan *et al.*, 1992; Nakano and Vousden, 2001; Oda *et al.*, 2000). In viable cells BAX and BAK exist as monomers, however, upon receiving death signals from the activated BH3-only proteins, they homooligomerize and insert into the mitochondrial membrane (Eskes *et al.*, 2000; Korsmeyer *et al.*, 2000; Wei *et al.*, 2001). Insertion of homooligomerized BAX and BAK causes outer mitochondrial membrane permeabilization (MOMP). Permeabilization is followed by release of cytochrome *c* and other pro-apoptotic factors from the mitochondria. Peremabilization of the mitochondrial outer membrane is the key step in commitment to death via the mitochondrial apoptotic pathway (Fig 1).

There is general agreement that BAX and BAK require activation prior to effecting permeabilization of the mitochondrion. While, some reports emphasize the importance of activator BH3 proteins in facilitating this transition (Kim *et al.*, 2006; Kuwana *et al.*, 2002; Letai *et al.*, 2002; Wei *et al.*, 2000) other reports stress the neutralization of the anti-apoptotic members (Willis *et al.*, 2005; Willis *et al.*, 2007). The former describes a select few of the BH3-only proteins termed activators (BIM, tBID and perhaps PUMA) that have the ability to bind directly to BAX and BAK causing their activation (Kim *et al.*, 2006; Kuwana *et al.*, 2005; Letai *et al.*, 2002). In the latter case, BH3-only proteins are sensitizers that cause death by binding the anti-apoptotic BCL-2 proteins causing release of proapoptotic proteins (Fig 2) (Willis *et al.*, 2005; Willis *et al.*, 2007).

Following its release, cytochrome *c* (Korsmeyer *et al.*, 2000) binds in a complex with APAF-1, caspase-9 and dATP/dADP to form the apoptosome (Zou *et al.*, 1999). From the apoptosome platform the downstream effectors of death, the caspases, are activated (Acehan *et al.*, 2002; Zou *et al.*, 1997; Zou *et al.*, 1999).

Apoptotic road blocks in cancer

Delineation of the mitochondrial apoptotic pathway leads to an important question, how does a cancer cell survive the apoptotic signaling events activated by its aberrant behavior?

One mechanism is to select for a block in apoptotic signaling at the mitochondria by increasing the expression of anti-apoptotic BCL-2 members. There are many mechanisms known by which BCL-2 is expressed at high levels in cancer cells. BCL-2 was originally cloned from the breakpoint of the chromosomal translocation t(14; 18) in patients with follicular lymphoma (Cleary and Sklar, 1985; Tsujimoto *et al.*, 1985). The translocation placed BCL-2 next to enhancer elements of the immunoglobulin promoter causing an increased expression of BCL-2. The BCL-2 translocation occurs in approximately 90% of follicular lymphomas and in about one third of diffuse large B cell lymphomas (Weiss *et al.*, 1987). However, BCL-2 overexpression has also been linked to gene amplification (Rao *et al.*, 1998), hypermethylation of the BCL-2 gene (Hanada *et al.*, 1993) or chromosomal deletions causing the loss of micro-RNAs involved in the silencing of BCL-2 (Cimmino *et al.*, 2005). A series of genetic studies in mice really emphasized the oncogenic potential of BCL-2 (Letai *et al.*, 2004; Strasser *et al.*, 1990; Vaux *et al.*, 1988). Work examining the combined action of c-myc and BCL-2 in mice showed that for neoplastic progression there is a requirement for the deregulated cellular proliferation to be coupled with a compensatory inhibition of apoptosis.

Reciprocally, cancer cells can evade death through mutations that inactivate the effector arm of apoptosis, such as, the mutations of BAX observed in some solid tumors and hematopoietic malignancies (Meijerink *et al.*, 1998; Meijerink *et al.*, 1995; Rampino *et al.*, 1997). An especially common defect of cancers is a mutation in the tumor suppressor gene p53 (Greenblatt *et al.*, 1994) which in turn has a negative effect on activation of apoptosis, as the BH3-only proteins NOXA and PUMA are transcriptional targets of p53 (Jeffers *et al.*, 2003; Nakano and Vousden, 2001; Oda *et al.*, 2000; Shibue *et al.*, 2003; Villunger *et al.*, 2003). However, there are also indirect mechanisms for thwarting p53 function including mutations in ATM, a kinase involved in the phosphorylation and activation of p53 (Banin *et al.*, 1998).

A recent paper segregated each of these blocks in apoptosis into three classes. In a Class A block, there is a loss of activator BH3-only proteins. A Class B block is caused by a failure to activate the effector arm of apoptosis through loss or inactivation of BAX or BAK. Finally, increased expression of an inhibitor protein such as BCL-2/MCL-1 causes a class C block (Deng *et al.*, 2007) (Fig 3). A novel technique called BH3 profiling can detect the class of apoptotic block a cancer cell has evolved in order to evade apoptosis. BH3 profiling exploits the binding pattern of sensitizer BH3 proteins to the anti-apoptotic proteins (Certo *et al.*, 2006; Letai *et al.*, 2002) (Fig 2). An example of how this works is if the cancer cell has selected for upregulation of MCL-1 to avoid apoptosis, the sensitizer BH3 peptide NOXA would cause release of cytochrome *c* from the isolated mitochondria, while the BAD peptide would be ineffective, diagnosing a MCL-1 dependent Class C block. BH3 profiling has been used to categorize diffuse large cell lymphoma cell lines into each of the three classes of apoptotic blocks and shown that membership in the class C block correlates with increased drug sensitivity (Del Gaizo Moore *et al.*, 2007; Deng *et al.*, 2007).

How does increased expression of an anti-apoptotic correlate with enhanced drug sensitivity? From results generated by overexpression studies in cell lines, it is easy to envision that increased expression of BCL-2 would provide additional opposition against chemotherapy (Martinou *et al.*, 1994; Miyashita and Reed, 1993; Takahashi *et al.*, 2003; Zhang and Insel, 2001). However, as mentioned previously, during oncogenesis the abnormal phenotype of oncogene activation and genomic instability acquired for deregulated growth initiates apoptotic signals, in the form of activated BH3-only proteins. A compensatory increased level of an anti-apoptotic protein is selected to buffer the death signals generated. Therefore, the anti-apoptotic proteins are filled with death initiator signals or are "primed" for death and the cancer cell is often dependent or addicted to the anti-

apoptotic protein (Certo *et al.*, 2006; Del Gaizo Moore *et al.*, 2008; Letai *et al.*, 2004). The relatively "full" state of these selected anti-apoptotic proteins does not afford for the binding of many subsequent pro-apoptotic proteins. This is in contrast to the case of forced overexpression in cell lines, in which most of the overexpressed anti-apoptotic proteins are "empty" and therefore competent to sequester subsequent pro-death signaling molecules.

Design of BCL-2 targeting drugs

Once it was understood that cancer cells depended on BCL-2 or related anti-apoptotic proteins for survival - it became apparent that this could be of great practical significance, as a mechanism to specifically target cancer cells (Letai *et al.*, 2004). One of the first strategies employed to target the BCL-2 family, was the use of antisense oligonucleotides to knockdown the mRNA expression of BCL-2 (Cotter *et al.*, 1994). Oblimersen (Genasense) from Genta is an 18-mer phosphorothioated antisense oligonucleotide directed against the open reading frame of BCL-2 (Klasa *et al.*, 2002). The clinical effect of the drug has been modest in clinical trials. (O'Brien et al., 2005), and it is not clear how well the drug lowers BCL-2 protein levels in cells *in vivo*.

The next strategy that emerged was to antagonize the function of BCL-2 rather than reduce its levels. This was approached mechanistically following delineation of the crystal structure of BCL-X_L, which revealed that the BH1- BH3 domains formed a hydrophobic groove (Muchmore *et al.*, 1996), where the α-helix of the BH3-only proteins bind (Sattler *et al.*, 1997). The structural analysis of BCL-X_L bound to the BAK BH3 peptide (Sattler *et al.*, 1997) was a proof of concept experiment indicating that it could be possible to create small molecules that bound to the hydrophobic groove of BCL-X_L, inhibiting its anti-apoptotic function. Since this preliminary experiment, numerous small molecules with varying degree of specificity and effectiveness have been synthesized and the mechanism by which they induce apoptosis is depicted in (Fig 4).

A number of natural compounds that function as cell permeable, small molecule mimics of the BH3 domain were found using a library screening process including Tetrocarcin A, Antimycin and gossypol (Kitada *et al.*, 2003; Nakashima *et al.*, 2000; Tzung *et al.*, 2001). Gossypol is found in cottonseeds, it is a natural polyphenol capable of displacing BH3 peptides from BCL-X_L with a dissociation constant in the low micromolar range (Becattini *et al.*, 2004; Kitada *et al.*, 2003). An altered version of gossypol without the two reactive aldehyde groups has been synthesized. However, the new compound termed apogossypol has actually reduced affinity for BCL-X_L (Becattini *et al.*, 2004). TW-37 is another gossypol derivative that was designed using the structure of the BIM BH3 domain as a model; it binds with high affinity to BCL-2, BCL-X_L and MCL-1 (Verhaegen *et al.*, 2006). Two molecules were discovered from a chemical library screen, tested for the ability to disrupt the BCL-X_L/ BAK BH3 complex and they were named BH3I-1 and BH3I-2 (Degterev *et al.*, 2001). In contrast to random screening of compounds another approach to drug discovery is computer aided design of a specific ligand molecule based on the structure of the recipient receptor. This method enabled the discovery of the organic compound HA14-1 (Wang *et al.*, 2000). In general, most of these molecules display a biochemical affinity for certain anti-apoptotic BCL-2 proteins, linked to their ability to kill certain cells, and even in some cases improve survival of xenografts (Degterev *et al.*, 2001; Kitada *et al.*, 2003; Mohammad *et al.*, 2007). However, the mechanism of cell killing is often unclear, and it is not clear that the primary event is antagonism of anti-apoptotic BCL-2 proteins (van Delft *et al.*, 2006).

ABT-737

Of the compounds discovered to date, there is the most evidence to support killing via direct interaction with the BCL-2 family for the BH3 mimetic ABT-737 (Abbott Laboratories).

ABT-737 was ineffective at activating apoptosis in cells doubly deficient of BAX and BAK, demonstrating that the mechanism of action is solely through the BCL-2 family (van Delft *et al.*, 2006). ABT-737 was discovered by Abott using a strategy of combining screening using nuclear magnetic resonance, structure based design and combinatory chemical synthesis. The strategy was named "SAR by NMR" (Oltersdorf *et al.*, 2005). The lead compound ABT-737 mimicked the BH3 domain of BAD and bound selectively to BCL-2, BCL-X_L and BCL-W. Of importance the binding efficiency for ABT-737 to the subset of the anti-apoptotic members was is in the nanomolar range, hence making *in vivo* therapeutically significant concentrations attainable. The crystal structure of ABT-737 bound to BCL-X_L shows the chloro-biphenyl and thio-phenyl moieties bind to the p2 and p4 pockets of the hydrophobic groove of BCL-X_L (Lee *et al.*, 2007). ABT-737, similar to the BAD peptide, binds with poor affinity to MCL-1 and BFL-1 with a dissociation constant in the micro-molar range (Fig 2) (Oltersdorf *et al.*, 2005).

Obatoclax is a synthetic derivative of prodiginines (GX015-070) from Gemin X Biotech (Nguyen *et al.*, 2007; Reed, 2003; Trudel *et al.*, 2007a). However, the affinity for binding to the BCL-2 family is inferior to ABT-737, again with dissociation constants in hundreds of nanomolar range. TW-37 has also been shown to bind to BCL-2, BCL-X_L and MCL-1 so it could potentially be a pan-BCL-2 mimetic (Verhaegen *et al.*, 2006). Similarly, to many other putative BH3 mimetics, obatoclax is not entirely dependent on BAX and BAK for its method of apoptosis induction (Konopleva *et al.*, 2008). A compound that binds with high affinity to all anti-apoptotic proteins is almost certainly going to be more toxic to cancer cells. Furthermore, such a drug will not stimulate selection for expression of alternative antiapoptotic BCL-2 family proteins, as none would escape inhibition. However, the concern is that there would be greater toxicity to normal cells, so that it is not clear that an improved therapeutic index would be obtained by a pan-BCL-2 family inhibitor. Our observation that cancer cells are far more likely to be "primed" than normal cells lends some hope to such an approach, but true confirmation would require extensive *in vivo* testing.

Clinical use of BH3 mimetics

Upfront therapy

Of the small molecule inhibitors of BCL-2 described thus far, four are at present in clinical trials – Genasense, TW-37, obatoclax, and ABT-263. In *in vitro* experiments, ABT-737 has mono-therapy toxicity to leukemia, lymphoma, and at higher concentrations is also able to induce apoptosis in multiple myeloma, glioma and small cell lung cancer cell lines (Chauhan *et al.*, 2007; Oltersdorf *et al.*, 2005; Tagscherer *et al.*, 2008; Trudel *et al.*, 2007b). Numerous studies including work carried out by our laboratory have demonstrated that primary cells from patients with chronic lymphocytic leukemia (Del Gaizo Moore *et al.*, 2007), acute myeloid leukemia (Konopleva *et al.*, 2006), acute lymphocytic leukemia (Del Gaizo Moore *et al.*, 2008), along with B-cell neoplasms like follicular lymphoma and marginal zone lymphoma (Vogler *et al.*, 2008) are extremely sensitive to ABT-737, with widespread apoptosis when treated with concentrations in the nanomolar range. Similarly, obatoclax the pan-BCL-2 inhibitor shows single agent efficiency in the killing of multiple myeloma (Trudel *et al.*, 2007a), AML cell lines and primary samples. However, it also causes cell cycle arrest independent of apoptosis (Konopleva *et al.*, 2008; Trudel *et al.*, 2007a). Although, TW-37 has been shown to bind BCL-2, BCL-X_L and MCL-1, the mechanism by which it induces apoptosis has not yet been elucidated. It causes a reduction in proliferation in pancreatic and B-cell lymphomas along with reducing tumor size in xenograft models, however, whether this is BCL-2 dependent needs to be clarified (Mohammad *et al.*, 2007; Wang *et al.*, 2008).

In order to enhance its clinical potential ABT-737 has been modified to make the drug orally available. ABT-263 binds to serum proteins resulting in a longer oral half life. The tumoricidal action of ABT-263 was demonstrated in xenograft models of small cell lung cancer and acute lymphoblastic leukemia cancer cells, where ABT-263 caused complete regression of the tumors (Shoemaker *et al.*, 2008; Tse *et al.*, 2008). The drug is now in clinical trials for chronic lymphocytic leukemia, lymphoma and small cell lung cancer (Wilson *et. al.,* 2008). Notably, ABT-263 binds to BCL-2 with affinity <100 picomolar, making it several logs more potent than the other small molecules detailed above.

Intrinsic and acquired resistance therapy

The BH3 mimetics are often not very effective as single killing agents against some of the epithelial cancers, such as, the pancreatic, ovarian and breast cancers (Huang and Sinicrope, 2008; Kutuk and Letai, 2008; Witham *et al.*, 2007). However, the other niche for these drugs is in combination therapy, where the BCL-2 antagonist serves to inhibit BCL-2 mediated resistance, enabling killing by conventional chemotherapy. Numerous examples exist in the literature of an enhanced apoptotic response when the BH3 mimetics are combined with traditional therapies to treat various cancers such as melanoma (Verhaegen *et al.*, 2006), pancreatic (Huang and Sinicrope, 2008), glioma (Tagscherer *et al.*, 2008), breast (Kutuk and Letai, 2008), multiple myeloma (Trudel *et al.*, 2007b), and B-cell malignant models (Mohammad *et al.*, 2007; Tse *et al.*, 2008). Thus, it appears that the conventional therapies provide the additional priming event required to enable the Bcl-2 antagonists to kill the above examples of intrinsically resistant cancers. Resistance to conventional chemotherapy may also develop over time (acquired resistance). Upon initial treatment there is a dramatic response, which tapers over time due to the acquisition of resistance through various mechanisms, including alteration of the expression of Bcl-2 family members. A recent report, demonstrated the use of the BH3 mimetic ABT-737 in the treatment of acquired resistance in cancer cells, it was effective at resensitizing drug resistant breast cancer cell lines to apoptosis via paclitaxel. This study highlighted an important factor, during the acquisition of resistance against the chemotherapeutic drug, albeit *in vitro*, the breast cancer cell lines selected for increasing the expression of BCL-2 family members to gain resistance. ABT-737 in combination with other chemotherapeutics was efficient in overcoming this altered BCL-2 balance to reinstate killing (Kutuk and Letai, 2008), emphasizing the potential effectiveness of the use of BH3 mimetics in combination therapy.

Why are some cells resistant and some cells sensitive to ABT-737? Two conditions must be met for ABT-737 to kill cells. Firstly, BCL-2 (or BCL-X_L and BCL-W) need to be primed with death initiating signals. These signals may be BH3 activators such as BIM, or perhaps activated monomeric BAX or BAK. These pro-death proteins are then released upon ABT-737 binding to the hydrophobic groove of BCL-2, enabling activation of apoptosis (Fig 4) (Del Gaizo Moore *et al.*, 2007). Secondly, the displaced death initiating signal (eg BIM or BAX)) needs to exceed the quantity of empty MCL-1 and BFL-1, which are not targeted by ABT-737. Excess empty MCL-1 or BFL-1 would otherwise sequester displaced pro-death proteins so that apoptosis would not be activated. High levels of MCL-1 or BFL-1 have been shown to correlate with resistance to the ABT-737 (Konopleva *et al.*, 2006; Lin *et al.*, 2007; Tagscherer *et al.*, 2008; van Delft *et al.*, 2006). The resistance caused by MCL-1 can be overcome by inactivation via knockdown (Lin *et al.*, 2007) or through addition of agents that decrease MCL-1, such as seliciclib (Chauhan *et al.*, 2007; Chen *et al.*, 2007; Nguyen *et al.*, 2007). However, it is worthy of note that cells which have either lost or mutated BAX or BAK that fall into a class B block of apoptosis are also resistant to BH3 mimetics. Similarly, cells with reduced levels of BH3-only activator proteins (Class A) will also be unable to respond BH3 mimetics (Deng *et al.*, 2007). Thus, resistance to the BH3 mimetics is not solely dependent on the expression levels of MCL-1/BFl-1. Utilization of

the BH3 profiling tool facilitates identification of the apoptotic block employed by cancer cells or the block acquired upon resistance to chemotherapy. Therefore, this tool would aid in the recognition of cancers that are likely to be susceptible to either pan- or selective BH3 mimetics, enabling a personalized approach to treatment (Del Gaizo Moore *et al.*, 2007; Del Gaizo Moore *et al.*, 2008; Deng *et al.*, 2007).

Conclusion

Huge inroads have been made in delineating the apoptotic pathway over the last two decades, in particular the mechanism by which the BCL-2 family functions through selective protein-protein interactions to control mitochondrial apoptosis. Simultaneously, an understanding of how cancer cells evade death at the molecular level has been achieved with the BCL-2 family playing a starring role in death avoidance. The stage is set for the next generation of therapeutics, the small-molecule inhibitors of anti-apoptotic BCL-2 proteins, to be tested and fully exploited clinically, either as single agents or in combination therapy.

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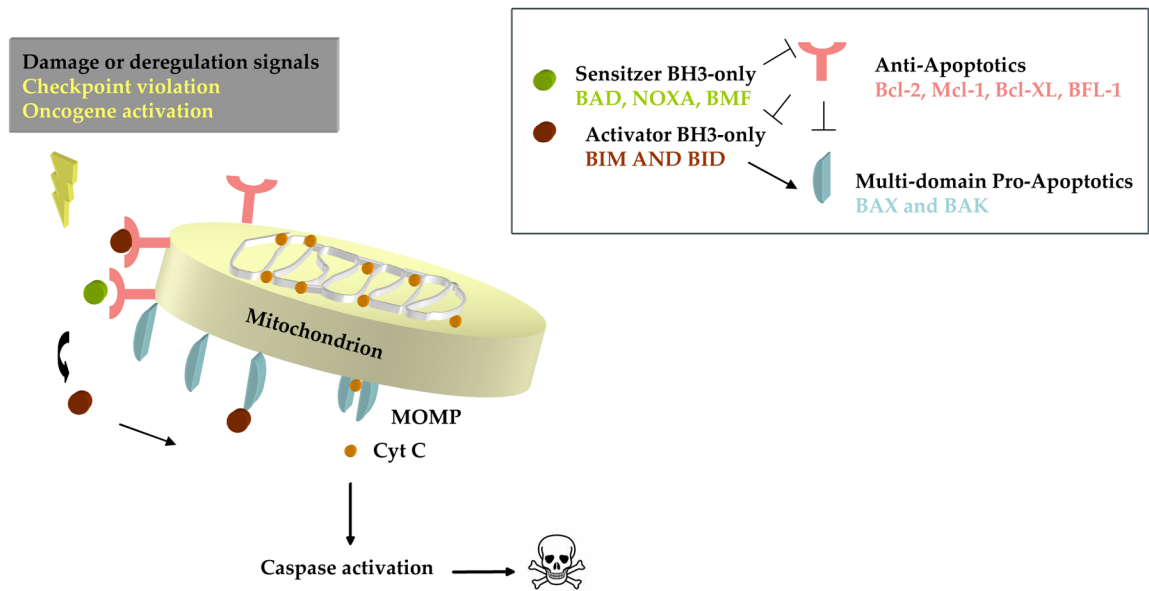
Fig 1. The mitochondrial apoptotic pathway
Cellular stress activates both sensitizer and activator BH3-only proteins. The sensitizer BH3-only proteins inhibit the anti-apoptotic BCL-2 family, while the direct activators cause the activation leading to oligomerization and insertion of BAX and BAK into the mitochondrial membrane. Mitochondrial outer membrane permeabilization (MOMP) causes the release of Cytochrome c, which forms a complex with caspase-9, APAF-1 and dATP/dADP triggering downstream apoptotic events.

Fig 2. The binding affinity of BH3-only peptides to the anti-apoptotic Bcl-2 family members
BH3-only peptides (Top) demonstrate a distinct binding pattern to the anti-apoptotic Bcl-2 family members (Left). Red depicts high affinity binding, orange depicts low- affinity binding and green depicts non-detectable binding. The BH3-only proteins are subdivided into activators and sensitizers. Modified with permission from (Deng et al., 2007).
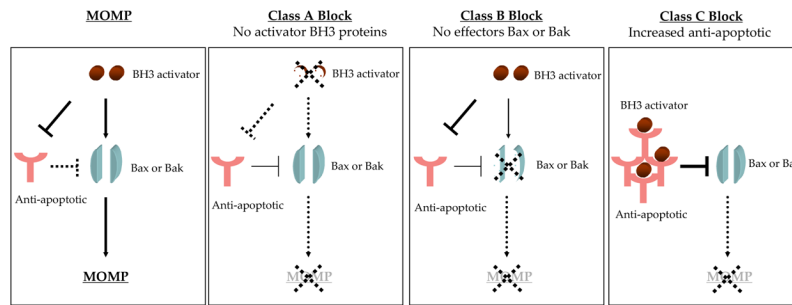
Fig 3. The apoptotic blocks utilized by cancer cells
In order to evade apoptosis cancer cells select for blocks in apoptotic signaling. These blocks have been categorized based on which group of the BCL-2 family is altered. A class A block is caused by a loss of BH3-only proteins, a Class B block is due to loss or inactivation of BAX and BAK and a class C block is caused by enhanced expression of anti-apoptotic BCL-2 proteins. Following activation and insertion of BAX and BAK into the mitochondrial memberane, the outer mitochondrial membrane is permeabilized (MOMP) enabling release of cytochrome *c* and activation of apoptosis.
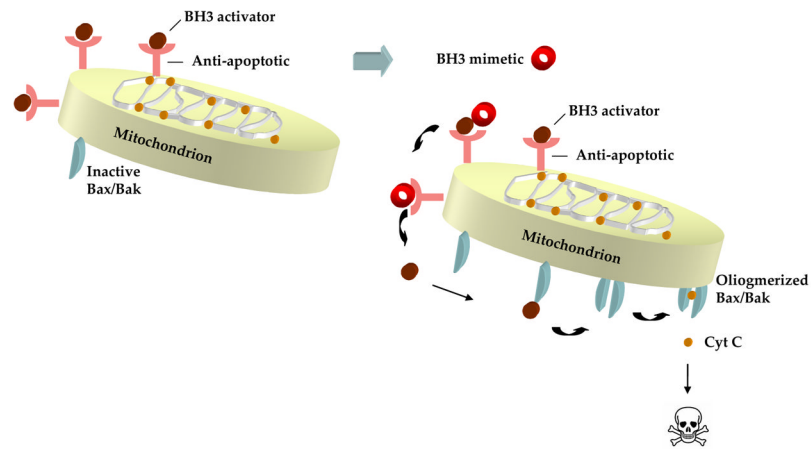
Fig 4. Model of BH3 mimetic- induced apoptosis
Anti-apoptotic Bcl-2 family members buffer the death signals activated by the deregulated growth of cancer cells. BH3 mimetics induce apoptosis by binding to the hydrophobic groove of the anti-apoptotic BCL-2 members displacing bound activator BH3-only proteins, enabling activation of BAX and BAK and the downstream apoptotic events.