Killing cancer cells by flipping the Bcl-2/Bax switch

Impairment of apoptosis, the physiologic cell death process, is central to cancer development and renders tumors refractory to cytotoxic therapy. Bcl-2, the oncoprotein activated in follicular lymphoma, inhibits the conserved cell death pathway triggered by diverse cytotoxic agents, as do several close relatives. A small-molecule antagonist of these proteins has now been designed by Oltersdorf et al. Strikingly, ABT-737 sensitizes many tumors to cytotoxic agents and is effective as a single agent against certain lymphomas and solid tumors, provoking stable regression in some tumor xenografts. Hence, this work validates Bcl-2-like proteins as important new targets in cancer therapy.

To eliminate superfluous or damaged cells, multicellular organisms have evolved an efficient cell suicide mechanism involving dedicated caspases that cleave multiple intracellular proteins. The major switch for their activation is controlled by opposing factions of the Bcl-2 family (Cory and Adams, 2002). Whereas Bcl-2 and its closest relatives (Bcl-x_L, Bcl-w, A1, and Mcl-1) promote cell survival, the structurally similar Bax and Bak instead promote cell death (Figure 1A). The life-death switch is flipped by distant cousins (e.g., Bim, Bad, Bid, Puma, and Noxa) that share only the signature pro-death BH3 (Bcl-2 homology region 3) domain. Once these "BH3-only" proteins are unleashed by diverse intracellular stress signals, their BH3 domain docks to an extended hydrophobic groove on the pro-life Bcl-2-like proteins (Liu et al., 2003; Sattler et al., 1997), thereby neutralizing them. By a poorly understood mechanism (see below), this leads to aggregation of Bax and Bak on the endoplasmic reticulum and mitochondrial membranes, promoting release of apoptogenic proteins such as cytochrome c to trigger caspase activation.

In many tumors, Bcl-2 or other pro-life relatives are often overexpressed, or signaling via BH3-only proteins is impaired, typically due to mutation of the p53 tumor suppressor, which would otherwise induce Puma and Noxa to trigger apoptosis (Villunger et al., 2003). Nevertheless, nearly all tumors retain the core apoptotic machinery. Therefore, small molecules that directly target the Bcl-2-like proteins by mimicking the BH3 domain (Figure 1A) should be highly effective anticancer drugs (Cory and Adams, 2002). One promising approach is to create stabilized forms of BH3 peptides (Walensky et al., 2004). An alternative route is to screen for small organic molecules that supplant BH3 function. Due to the challenge of targeting protein-protein interactions, however, only candidates with low affinity (μM versus the nM binding of BH3 peptides) have previously emerged, and the evidence that they act as BH3 mimetics remains poor.

Now, however, Oltersdorf and colleagues have developed a very promising small molecule BH3 mimetic by using a structure-based approach to target the groove on Bcl-x_L (Oltersdorf et al., 2005). An initial high-throughput NMR-based screen of a chemical library revealed two leads that bound its groove at different nearby sites, precisely where conserved BH3 residues dock (Figure 1B). Guided by the topography of the Bcl-x_L groove, these low affinity (mM) leads were then bridged to form a much higher affinity (36 nM) derivative. Further modifications minimized binding to human serum albumin and improved affinity for Bcl-x_L and Bcl-2. The final compound, ABT-737 (Figure 1C), binds Bcl-x_L, Bcl-2, and Bcl-w with high affinity (low or even sub nM), even in 10% human serum, but has far lower affinity for the more divergent Mcl-1 and A1.

Significantly, ABT-737 was cytotoxic to B lymphoid tumor cell lines and primary cells from human follicular lymphomas. This probably reflects its binding to Bcl-2-like proteins, because the control enantiomer, which binds very poorly, had ~30-fold lower activity. Notably, ABT-737 also kills cells from

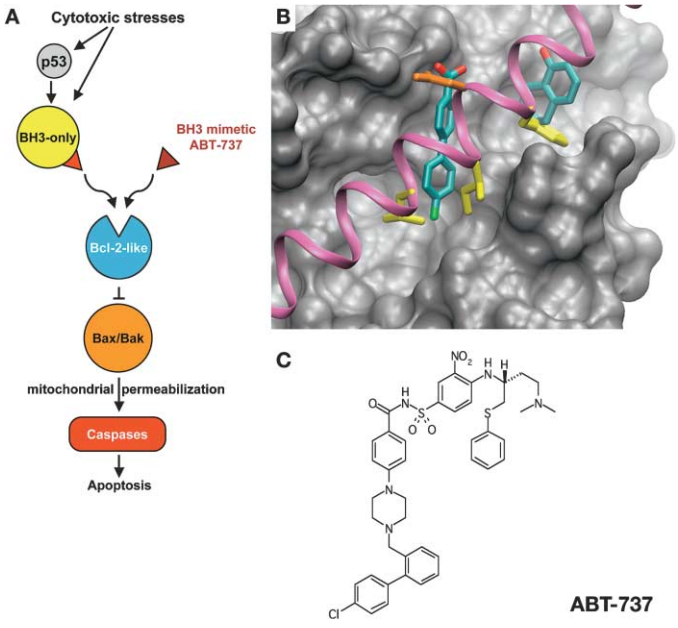

Figure 1. Targeting Bcl-2-like proteins to kill cancer cells

A: A BH3 mimetic such as ABT-737 engages the groove of a Bcl-2-like protein, freeing Bax or Bak to trigger membrane permeabilization and caspase activation. The inactivation of p53 function in most tumors precludes induction of its targets, such as the genes encoding the BH3-only proteins Noxa and Puma, which mediate its proapoptotic function. Even tumors with the p53 pathway inactive should be vulnerable to the appropriate BH3 mimetic.

B: Small precursors of ABT-737 bind like a BH3 domain to the groove of Bcl-x_L. The two lead compounds that led to ABT-737 (carbon backbone, blue; oxygen, red; fluorine, green) are superimposed with the Bim BH3 α-helix (mauve) on the surface of Bcl-x_L (gray). Four conserved BH3 amino acid residues critical for binding to Bcl-x_L are shown: leucine-94 (yellow), isoleucine-97 (yellow), aspartate-99 (orange), and phenylalanine-101 (yellow). The drug leads mimic the BH3 domain at the three hydrophobic (yellow) residues, binding to hydrophobic pockets on Bcl-x_L, and have an acidic moiety (red) that, like the aspartate, pairs with an essential arginine (Arg-139) in Bcl-x_L. The figure was adapted by Drs. Brian Smith and Peter Colman from the structures of Oltersdorf et al. (2005) and Liu et al. (2003) with permission.

C: Structure of ABT-737 (Oltersdorf et al., 2005).

most (13 of 15) chronic lymphocytic leukemias and most cell lines from small cell lung cancers (SCLC).

The drug also yielded very impressive results in vivo. Mice tolerated daily injections for three weeks with no adverse signs except a decline in platelets and lymphocytes. When *scid* mice implanted with a human follicular lymphoma cell line were treated with ABT-737, morbidity was delayed. Most strikingly, two different SCLC xenografts completely regressed in 77% of treated mice and did not grow back when treatment was stopped (monitoring continued for 58 to 107 days). Caspase activity assays suggested that the tumors regressed due to apoptosis.

Many cell lines derived from solid tumors resisted ABT-737 therapy. Nevertheless, ABT-737 markedly enhanced (2–20 fold) their response to radiation and a range of chemotherapeutic drugs with different modes of action (etoposide, doxorubicin, cisplatin, paclitaxel). Thus, combination therapy with ABT-737 may render such agents more effective at lower doses, reducing collateral damage to normal cells, or ensure more stable remissions with conventional doses.

In binding profile, ABT-737 resembles the BH3-only protein Bad. Whereas certain potent BH3-only proteins, notably Bid and Bim, have a BH3 domain that can provoke Bax/Bak-dependent permeabilization of mitochondria and synthetic liposomes, Bad and several others are relatively ineffectual by themselves, but enhance the potency of the first group (Kuwana et al., 2005; Letai et al., 2002). This has led to the hypothesis that Bid and Bim directly activate Bax and Bak, while the other, "sensitizing" BH3 proteins collude by engaging pro-survival proteins, freeing Bid and Bim to bind Bax and Bak (Letai et al., 2002). Evidence for direct binding of Bax and Bak by BH3-only proteins, however, remains scant.

An alternative view, derived from recent evidence that certain BH3-only proteins target specific subsets of the pro-survival proteins (Chen et al., 2005), is that efficient killing requires neutralization of multiple Bcl-2-like proteins, because more than one pro-survival protein restrains Bak and presumably also Bax (Willis et al., 2005). On this view, the potency of BH3-only proteins, such as Bim, reflects their ability to bind avidly to each pro-survival protein, whereas the less potent ones, like Bad, bind only a subset. Pertinently, killing by Bad, which binds tightly only to Bcl-2, Bcl-x_L, and Bcl-w, is enhanced by Noxa, which binds Mcl-1 and A1 (Chen et al., 2005), and Bad plus Noxa unleashes Bak (Willis et al., 2005).

Thus, a plausible hypothesis for why certain tumor cells are resistant to ABT-737 is that they express high levels of Mcl-1 or A1, which ABT-737 cannot neutralize. If so, these tumors may be vulnerable to a novel BH3 mimetic that binds a different subset of Bcl-2-like proteins. Alternatively, small molecules that downregulate or inhibit Mcl-1 or A1 may synergize with ABT-737.

ABT-737 provides strong proof of principle that targeting the Bcl-2 family will have benefit in cancer therapy, and clinical trials will be eagerly awaited. As the Bcl-2-guarded gateway to apoptosis appears to be the Achilles' heel of many tumors, BH3 mimetics with different target specificity seem destined to become valued weapons in the oncologist's armamentarium. More generally, the striking success of ABT-737 further boosts the case (Li et al., 2004; Vassilev et al., 2004) that small molecules can effectively inhibit protein-protein interactions, greatly expanding the universe of potential drug targets.

Acknowledgments

We thank our colleagues D.H.S. Huang, P. Colman, and B. Smith for valued discussions, and S.W. Fesik for early release of PDB files.

Suzanne Cory[1,2,*] and Jerry M. Adams[1,*]

[1]The Walter and Eliza Hall Institute of Medical Research
1G Royal Parade, Parkville, Victoria 3050 Australia
[2]Department of Medical Biology
The University of Melbourne
Parkville, Victoria 3010, Australia
*E-mail: cory@wehi.edu.au; adams@wehi.edu.au

Selected reading

Chen, L., Willis, S.N., Wei, A., Smith, B.J., Fletcher, J.I., Hinds, M.G., Colman, P.M., Day, C.L., Adams, J.M., and Huang, D.C.S. (2005). Mol. Cell *17*, 393–403.

Cory, S., and Adams, J.M. (2002). Nat. Rev. Cancer *2*, 647–656.

Kuwana, T., Bouchier-Hayes, L., Chipuk, J.E., Bonzon, C., Sullivan, B.A., Green, D.R., and Newmeyer, D.D. (2005). Mol. Cell *17*, 525–535.

Letai, A., Bassik, M., Walensky, L., Sorcinelli, M., Weiler, S., and Korsmeyer, S. (2002). Cancer Cell *2*, 183–192.

Li, L., Thomas, R.M., Suzuki, H., De Brabander, J.K., Wang, X., and Harran, P.G. (2004). Science *305*, 1471–1474.

Liu, X., Dai, S., Zhu, Y., Marrack, P., and Kappler, J.W. (2003). Immunity *19*, 341–352.

Oltersdorf, T., Elmore, S.W., Shoemaker, A.R., Armstrong, R.C., Augeri, D.J., Belli, B.A., Bruncko, M., Deckwerth, T.L., Dinges, J., Hajduk, P.J., et al. (2005). Nature *435*, 677–681.

Sattler, M., Liang, H., Nettesheim, D., Meadows, R.P., Harlan, J.E., Eberstadt, M., Yoon, H.S., Shuker, S.B., Chang, B.S., Minn, A.J., et al. (1997). Science *275*, 983–986.

Vassilev, L.T., Vu, B.T., Graves, B., Carvajal, D., Podlaski, F., Filipovic, Z., Kong, N., Kammlott, U., Lukacs, C., Klein, C., et al. (2004). Science *303*, 844–848.

Villunger, A., Michalak, E.M., Coultas, L., Müllauer, F., Böck, G., Ausserlechner, M.J., Adams, J.M., and Strasser, A. (2003). Science *302*, 1036–1038.

Walensky, L.D., Kung, A.L., Escher, I., Malia, T.J., Barbuto, S., Wright, R.D., Wagner, G., Verdine, G.L., and Korsmeyer, S.J. (2004). Science *305*, 1466–1470.

Willis, S.N., Chen, L., Dewson, G., Wei, A., Naik, E., Fletcher, J.I., Adams, J.M., and Huang, D.C. (2005). Genes Dev. *19*, 1294–1305.

DOI: 10.1016/j.ccr.2005.06.012