CYCLIND dysregulation (an NF-κB target gene), correlate or cooperate with genetic activation of NF-κB.

By using NF-κB target gene expression profiles to identify MM patients likely to have *TRAF3* deletions among cohorts of patients treated with dexamethasone versus bortezomib, Keats et al. found that only 2/20 patients with *TRAF3* inactivation responded to dexamethasone, whereas 17/19 patients among the *TRAF3* inactivation group responded to bortezomib. These results suggest that constitutive activation of the NF-κB pathway through *TRAF3* inactivation is correlated with dexamethosone resistance and bortezomib sensitivity. Although the exact molecular basis for the differential sensitivity of individual MM patients to bortezomib versus dexamethasone is not clear, it appears that *TRAF3* status, which can be assessed fairly simply by conventional molecular techniques, should dictate treatment with proteasome inhibitors.

These two studies add to the growing body of evidence demonstrating that mutations in NF-κB pathway genes are rather common occurrences in a variety of malignancies (Courtois and Gilmore, 2006). Why certain NF-κB-activating gene mutations appear to occur only in some types of tumors is not clear. Nevertheless, the current studies take us one step closer to understanding the molecular underpinnings of MM and toward designing and designating more effective therapies for individuals with MM.

REFERENCES

Annunziata, C.M., Davis, R.E., Demchenko, Y., Bellamy, W., Gabrea, A., Zhan, F., Lenz, G., Hanamura, I., Wright, G., Xiao, W., et al. (2007). Cancer Cell, this issue.

Carrasco, D.R., Tonon, G., Huang, Y., Zhang, Y., Sinha, R., Feng, B., Stewart, J.P., Zhan, F., Khatry, D., Protopopova, M., et al. (2006) Cancer Cell, *9*, 313–325.

Courtois, G., and Gilmore, T.D. (2006). Oncogene *25*, 6831–6843.

Hideshima, T., Neri, P., Tassone, P., Yasui, H., Ishitsuka, K., Raje, N., Chauhan, D., Podar, K., Mitsiades, C., Dang, L., et al. (2006). Clin. Cancer Res. *12*, 5887–5894.

Jourdan, M., Moreaux, J., Vos, J.D., Hose, D., Mahtouk, K., Abouladze, M., Robert, N., Baudard, M., Reme, T., Romanelli, A., et al. (2007). Br. J. Haematol. *138*, 160–168.

Keats, J.J., Fonseca, R., Chesi, M., Schop, R., Baker, A., Chng, W.-J., Van Wier, S., Tiedemann, R., Shi, C.-X., Sebag, M., et al. (2007). Cancer Cell, this issue.

Mulligan, G.B., Mitsiadis, C., Bryant, B., Zhan, F., Chng, W.J., Roels, S., Koenig, E., Fergus, A., Huang, Y., Richardson, P., et al. (2007). Blood *109*, 3177–3188.

Richardson, P.G., Mitsiadis, C., Schlossman, R., Munsh, N., and Anderson, K. (2007). Oncologist *12*, 664–689.

Shaughnessy, J.D., Jr., Zhan, F., Burington, B.E., Huang, Y., Colla, S., Hanamura, I., Stewart, J.P., Kordsmeier, B., Randolph, C., Williams, D.R., et al. (2007). Blood *109*, 2276–2284.

Trecca, D., Guerrini, L., Fracchiolla, N.S., Pomati, M., Baldini, L., Maiolo, A.T., and Neri, A. (1997). Oncogene *14*, 791–799.

Xia, Z.-P., and Chen, Z.J. (2005). Sci. STKE *272*, pe7.

Life, Death, BH3 Profiles, and the Salmon Mousse

Douglas R. Green[1,*]
[1]Department of Immunology, St. Jude Children's Research Hospital, Memphis, TN 38105, USA
*Correspondence: douglas.green@stjude.org
DOI 10.1016/j.ccr.2007.07.011

New drugs that neutralize the antiapoptotic members of the Bcl-2 family hold promise for rational cancer therapies, both alone and in combination with other agents. An understanding of how and why such agents may trigger apoptosis on their own, and how resistance to these drugs can occur, depends on the complexity of the Bcl-2 family interactions that control mitochondrial outer membrane permeabilization (MOMP). By extracting mitochondria from tumor cells and exposing them to peptides corresponding to the regulatory BH3-only proteins, MOMP predicts not only which cells will undergo apoptosis in response to Bcl-2 antagonists, but also why other cells may be resistant.

As any poet or philosopher can tell us, it is in the contemplation of death that we gain insight into life. Indeed, it was in their seminal *The Meaning of Life* that Monty Python had a dinner guest challenge the figure of Death to explain how their entire party had somehow all died at the same time, to which Death ominously replied, "The salmon mousse." In much the same way, if perhaps less ethereally, the study of the principles of cell death

has long held the promise to preserve life, by, among other things, providing new insights for the treatment of cancer. In this issue of *Cancer Cell*, Deng and colleagues (2007) probe the mechanisms of sensitivity and resistance of tumor cells to the Bcl-2 antagonist ABT-737 and, in so doing, offer compelling evidence for an emerging view of the functions of the Bcl-2 family proteins in the control of apoptosis. This is a view that may, ultimately, give us the equivalent of a "salmon mousse" for cancer.

Our story concerns the intrinsic, or mitochondrial pathway of apoptosis, in which mitochondrial outer membrane permeabilization (MOMP) results in diffusion of proteins from the intermembrane space to the cytosol. Holocytochrome *c* thus gains access to APAF-1, leading to caspase activation and death. Even without caspase activation, MOMP generally results in cell death. The decision, MOMP or no MOMP, translating into death or survival of the cell, is made by the interactions of the Bcl-2 family proteins, both pro- and antiapoptotic. Of these, Bax and Bak are the proapoptotic "effectors," which are likely to be directly responsible for the permeabilization of the outer membrane. These are antagonized by the actions of the antiapoptotic Bcl-2 proteins, including Bcl-2, Bcl-xL, Mcl-1, and Bfl/A1. A third class of Bcl-2 proteins, the BH3-only proteins, appear to make the "thumbs up or thumbs down" decision by regulating the other family members. Recently, several drugs that act as BH3 mimetics have been identified, including ABT-737, which antagonizes the functions of Bcl-2, Bcl-xL, and Bcl-w and has shown potent single-agent proapoptotic activity in some experimental tumors, but not others (Oltersdorf et al., 2005). Importantly, ABT-737 is generally inactive in triggering apoptosis in normal cells, with an interesting exception being platelets.

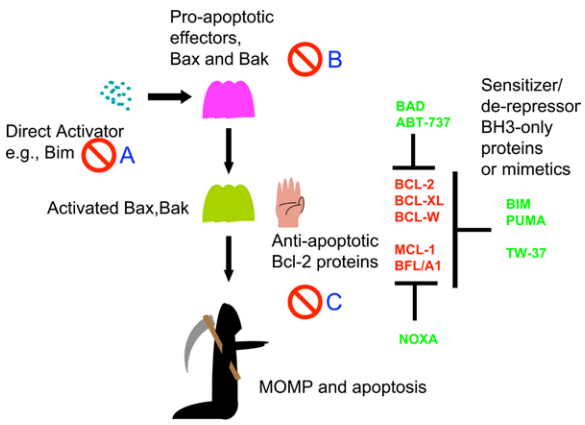

Figure 1. BH3 Profiling

Different BH3-only proteins or their corresponding BH3 peptides effectively neutralize different antiapoptotic Bcl-2 family members, and this may or may not lead to MOMP, depending on the status of the proapoptotic effectors Bax and Bak (the pink "salmon mousse"). These effectors do not promote death on their own, but do so if exposed to direct activator proteins or conditions (the green ptomaine), which include the BH3-only protein BIM but also other proteins as well. Activated Bax and Bak do not trigger MOMP; however, antiapoptotic proteins block MOMP (the hostess preventing the guests from eating), unless these inhibitors are effectively neutralized. Such neutralization can occur by binding to BH3-only proteins or BH3-mimetic drugs. MOMP, when it occurs, leads to cell death (the Grim Reaper). The pattern of MOMP occurrence in response to different BH3-only proteins predicts whether or not the drug ABT-737 will cause apoptosis and defines three classes of resistance to this drug, A, B, and C.

To understand how this works, and to further delineate mechanisms of resistance to ABT-737, Deng et al. (2007) examined a number of ABT-737-sensitive or -resistant diffuse large B cell lymphoma lines (DLBCL) by their method of "BH3 profiling" (Certo et al., 2006). They isolated mitochondria from the different lines and exposed them to a panel of peptides corresponding to the BH3 regions of several BH3-only proteins and examined MOMP. In so doing, they identified three classes of resistance to ABT-737 and provided explanations for how they work.

This approach depends on an understanding of the functions of the different BH3-only proteins (Figure 1). There is general agreement that these proteins act to neutralize the antiapoptotic Bcl-2 proteins, and they show distinct preferences in this function (Certo et al., 2006; Chen et al., 2005; Kuwana et al., 2005), a function termed "sensitizer" or "derepressor" activity. The BH3-only protein BAD, for example, neutralizes Bcl-2, Bcl-xL, and Bcl-2, but neither Mcl-1 nor Bfl/A1, while another BH3-only protein, NOXA, neutralizes Mcl-1 (and to some extent Bfl/A1) but none of the others. BIM, PUMA, and probably BMF effectively bind and neutralize all of the different antiapoptotic Bcl-2 proteins. One prevalent view is that this neutralization of the antiapoptotic Bcl-2 proteins is not only necessary but also *sufficient* to cause MOMP via Bax and Bak (Willis et al., 2007).

Letai et al. (2002) and Kuwana et al. (2005) proposed an additional activity of some BH3-only proteins that is also necessary for MOMP: the activation of Bax and/or Bak. BH3-only proteins that have this "direct activator" function include BIM and BID. However, a recent study has shown that Bcl-2-inhibitable apoptosis proceeds in BID-BIM double knockout cells (Willis et al., 2007), leading to two possible conclusions; either the "direct activator" concept is wrong, or other mechanisms exist to activate Bax and Bak. I favor the latter and have proposed that such alternative mechanisms exist (Green, 2006). Cells in which direct activators of Bax and/or Bak are present are therefore "primed for death" such that disruption of the antiapoptotic Bcl-2 functions results in death (Certo et al., 2006).

It is in this context that Deng et al. (2007) characterize the DLBCL lines in their study and describe three classes of resistance to ABT-737. In the first (class A), Bax and Bak are functional, but no signal is present to activate these effectors, and therefore no death occurs when antiapoptotic Bcl-2 proteins are neutralized. Mitochondria from such

cells undergo MOMP in response to the direct activator BH3 sequences from BID or BIM, but not in response to the other BH3s. In this setting, neutralizing the antiapoptotic proteins with ABT-737 does not induce MOMP or death, because Bax or Bak is not engaged. In support of this idea, Deng et al. (2007) show that a cell line that is normally sensitive to apoptosis induction by ABT-737 is rendered resistant by knocking down one of the direct activator BH3-only proteins, BIM.

In their second class of resistance (class B), low or absent Bax and Bak appear to be responsible, and mitochondria from these cells do not undergo MOMP in response to any of the BH3 peptides. In such cells, neutralization of antiapoptotic proteins would not be expected to cause death unless some way to induce expression of Bax or Bak could be managed.

The third resistance class (class C) is one that they (Del Gaizo Moore et al., 2007) and others (Konopleva et al., 2006) had previously described for other types of lymphoma. In these cells, direct activators and Bax and/or Bak are present, and the cells are "primed for death," but the antiapoptotic Bcl-2 proteins sustaining survival are not affected by the drug. In AML and CLL, the presence of Mcl-1 correlated with such resistance, and mitochondria from such cells underwent MOMP in response to NOXA BH3. In the current study, one such resistant line was found to overexpress Bfl/A1.

Is this emerging, complex view important? The simpler view, discussed above, that neutralization of all available antiapoptotic Bcl-2 family proteins is both necessary and sufficient for apoptosis does not account for the data presented by Deng et al. (2007). For example, while both BIM and PUMA interact with all of the antiapoptotic Bcl-2 proteins with equivalent affinity (e.g., Chen et al., 2005), BIM but not PUMA was observed to induce MOMP in mitochondria from class A cells. One recent report (Kim et al., 2006) suggested that PUMA, like BIM and BID, is a direct activator BH3; however, this appears to be incompatible with the above results as well, at least under these experimental conditions.

Perhaps more importantly, the emerging view laid out by Deng et al. (2007) is compatible with the idea that a therapeutic window may exist for BH3-mimetic drugs, such as ABT-737, to preferentially kill tumors rather than normal tissues. In any simpler view, no such window should logically exist, since tumors should be expected to have equal or higher levels of antiapoptotic proteins as compared to normal tissues, and thus be more resistant than normal cells to their neutralization. However if tumors are preferentially "primed for death" by direct activators of Bax and/or Bak, then they may have increased sensitivity to such neutralizers, unless they belong to one of the classes of resistance described by Deng et al. (2007). Such priming may occur, in part, because oncogenes that drive proliferation can also engage direct activators of Bax and Bak. For example, c-Myc drives the function of BIM (Egle et al., 2004).

In keeping with these notions, one recent study explores another drug, TW-37, which neutralizes Bcl-2 and Bcl-xL with affinities similar to those of ABT-737, but also effectively blocks Mcl-1 (with similar affinity to the other antiapoptotic proteins) (Verhaegen et al., 2006). A therapeutic window was found for melanoma versus primary cells in mice, a finding that is difficult to reconcile with simple "neutralization models" of Bcl-2 family function. However, if melanoma, like the lymphomas studied by Letai and colleagues, are "primed for death" by the engagement of direct activators of Bax and Bak, then the specificity of this drug for transformed cells can make sense. We shall see if the patterns of resistance defined by Deng et al. (2007) apply similarly to this agent. Ultimately, the functional mechanisms controlling the Bcl-2 family and MOMP may turn out to be very much a matter of life and death.

Fully effective tumor therapy requires that, like the guests at Monty Python's ill-fated dinner party, every targeted cell dies. Combinations of therapies to engage the mechanisms of apoptosis, for example, by conventional radiation or chcmotherapies, while inhibiting antiapoptotic mechanisms using BH3 mimetics, hold promise for new avenues of cancer therapy. But it is easy to kill tumor cells—the trick is leaving the healthy tissues intact. The BH3 profiling approach that Letai and colleagues have introduced represents a step toward teasing out such conditions.

REFERENCES

Certo, M., Del Gaizo Moore, V., Nishino, M., Wei, G., Korsmeyer, S., Armstrong, S.A., and Letai, A. (2006). Cancer Cell *9*, 351–365.

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. (2005). Mol. Cell *17*, 393–403.

Del Gaizo Moore, V., Brown, J.R., Certo, M., Love, T.M., Novina, C.D., and Letai, A. (2007). J. Clin. Invest. *117*, 112–121.

Deng, J., Carlson, N., Takeyama, K., Dal Cin, P., Shipp, M., and Letai, A. (2007). Cancer Cell, this issue.

Egle, A., Harris, A.W., Bouillet, P., and Cory, S. (2004). Proc. Natl. Acad. Sci. USA *101*, 6164–6169.

Green, D.R. (2006). Cancer Cell *9*, 328–330.

Kim, H., Rafiuddin-Shah, M., Tu, H.C., Jeffers, J.R., Zambetti, G.P., Hsieh, J.J., and Cheng, E.H. (2006). Nat. Cell Biol. *8*, 1348–1358.

Konopleva, M., Contractor, R., Tsao, T., Samudio, I., Ruvolo, P.P., Kitada, S., Deng, X., Zhai, D., Shi, Y.X., Sneed, T., et al. (2006). Cancer Cell *10*, 375–388.

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.C., Walensky, L.D., Sorcinelli, M.D., Weiler, S., and Korsmeyer, S.J. (2002). Cancer Cell *2*, 183–192.

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.

Verhaegen, M., Bauer, J.A., Martin de la Vega, C., Wang, G., Wolter, K.G., Brenner, J.C., Nikolovska-Coleska, Z., Bengtson, A., Nair, R., Elder, J.T., et al. (2006). Cancer Res. *66*, 11348–11359.

Willis, S.N., Fletcher, J.I., Kaufmann, T., van Delft, M.F., Chen, L., Czabotar, P.E., Ierino, H., Lee, E.F., Fairlie, W.D., Bouillet, P., et al. (2007). Science *315*, 856–859.