

ited not only p110 α but also its downstream effector, the protein kinase TOR. Broad inhibitory action was essential for shutting down the growth of glioma cells. However, in order to be effective, the promiscuity of the drug had to be targeted to PI3K and TOR. Then, and only then, did vice become a virtue, with PI-103 achieving the effects of combination therapy as a single agent (Figure 1).

Specificity of target combination

Given the complexity of cellular signaling and the ability of cells to compensate for loss of function, it comes as no surprise that more than one kinase needs to be inhibited to achieve a significant change in the cellular phenotype. The effects on multiple targets need to be complementary and then are synergistic. The action of the new PI3K inhibitor lends support to a model of PI3K signaling that includes as an essential feature a negative feedback loop originating from TOR and targeting an upstream component of the signaling chain (Hay, 2005; Wulschleger et al., 2006). An inhibitor directed to TOR alone weakens this negative feedback and results in activation of the PI3K signaling pathway. Only the dual PI3K-TOR inhibitor can prevent this compensatory effect (Figure 1) (Fan et al., 2006). Among the

compounds tested, the inhibitor PI-103 is also the most effective in reducing Akt phosphorylation. A particularly gratifying quality of the dual PI3K-TOR inhibitor PI-103 is its lack of toxicity. This fact allays fears that PI3K inhibitors may induce intolerable side effects on essential cellular activities such as insulin signaling. With the identification of p110 α and TOR as a critical target combination in glioma, the stage is set for rapid progress in the field of PI3K inhibitors.

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At the gates of death

Apoptosis that proceeds via the mitochondrial pathway involves mitochondrial outer membrane permeabilization (MOMP), responsible for the release of cytochrome c and other proteins of the mitochondrial intermembrane space. This essential step is controlled and mediated by proteins of the Bcl-2 family. The proapoptotic proteins Bax and Bak are required for MOMP, while the antiapoptotic Bcl-2 proteins, including Bcl-2, Bcl-xL, Mcl-1, and others, prevent MOMP. Different proapoptotic BH3-only proteins act to interfere with the function of the antiapoptotic Bcl-2 members and/or activate Bax and Bak. Here, we discuss an emerging view, proposed by Certo et al. in this issue of *Cancer Cell*, on how these interactions result in MOMP and apoptosis.

In his classic film *Rashomon*, Akira Kurosawa told the story of a samurai's death from four distinct points of view and presented his rain-soaked protagonists, and us, with the changing nature of truth. In this issue of *Cancer Cell*, Certo et al. (2006) provide a new perspective on how cells die and provide a possible resolution to a controversy that focuses on the heart of this process, at the gates of death. As in *Rashomon*, we can find four different and perhaps not completely incompatible

viewpoints on how an important form of cell death occurs.

Most physiological cell deaths in animals occur through apoptosis, and most apoptosis in mammals proceeds by the mitochondrial pathway, wherein mitochondrial outer membrane permeabilization (MOMP) allows the proteins of the intermembrane space to diffuse into the cytosol (Green, 2005). MOMP is most likely a result of formation of a proteolipid pore, although this has not been visual-

ized. Upon MOMP, holocytochrome c contacts APAF-1, inducing the latter to recruit and activate caspase-9. Caspase-9 in turn cleaves and thereby activates executioner caspases, which then orchestrate apoptosis. Even without downstream caspase activation, however, MOMP appears to be sufficient to commit most cells to die, and death can proceed following MOMP in a caspase-independent manner. Therefore, MOMP is a critical decision point at which cell life and death is determined.

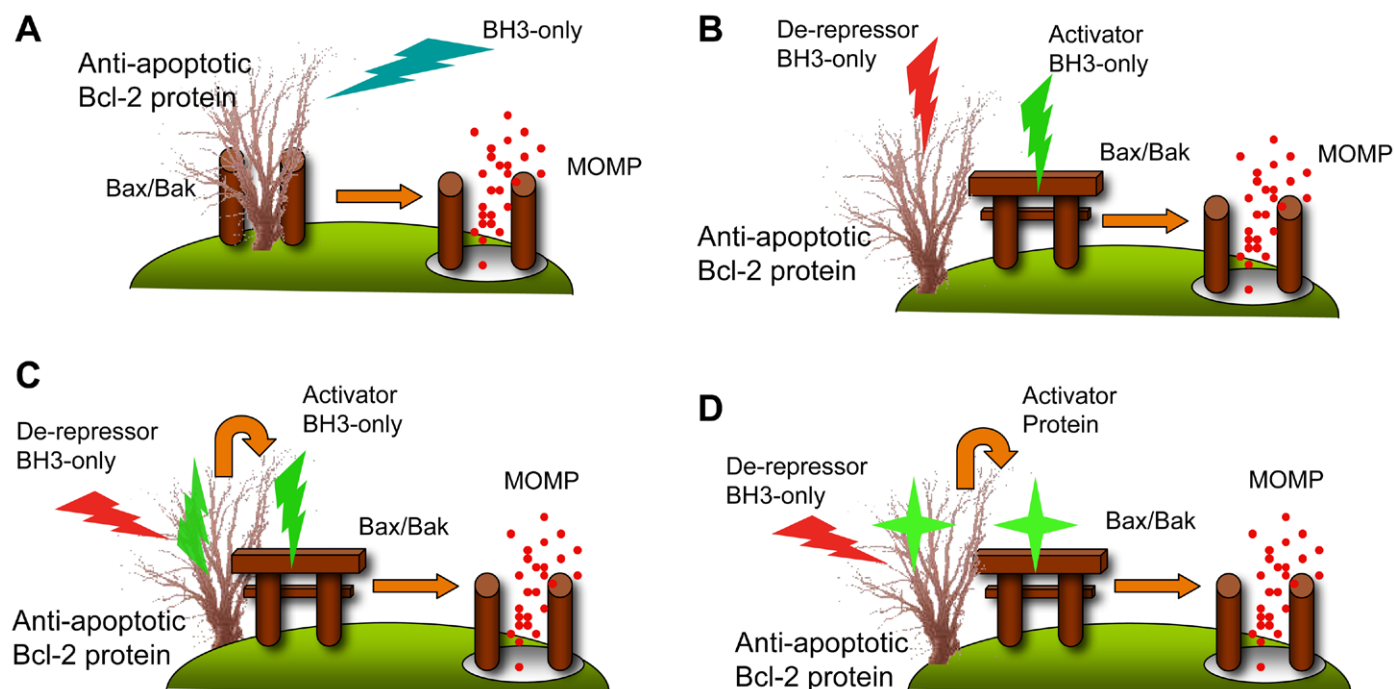


Figure 1. Four versions of the death of a cell

A: In this view, Bax and Bak (the gate) are constitutively active on the surface of the mitochondrial outer membrane and are held in check by the antiapoptotic Bcl-2 proteins (the tree). A lightning strike BH3-only protein neutralizes this inhibition, releasing Bax and Bak to permeabilize the mitochondria, and death proceeds.

B: In this view, Bax and Bak are inactive unless they interact with "direct activator" BH3-only proteins such as Bid and/or Bim (green lightning). Antiapoptotic Bcl-2 proteins can prevent this activation, and are in turn inhibited by other BH3-only proteins that act as "sensitizers" or "de-repressors" (purple lightning).

C: A third view, wherein only cells that are "primed for death" sequester direct activator BH3-only proteins on antiapoptotic Bcl-2 family members. Sensitizer/de-repressor BH3-only proteins displace these direct activators, which now activate Bax and Bak to cause MOMP and apoptosis.

D: Yet another view, with aspects of all of the others, where cells primed for death sequester not only Bid and/or Bim, but also other proteins capable of activating Bax and Bak. Alternatively, physicochemical conditions in cells may activate Bax and/or Bak, which are then sequestered by antiapoptotic Bcl-2 proteins. Sensitizer/de-repressor BH3-only proteins release the sequestered Bax and Bak, or the proteins that activate them, resulting in MOMP and apoptosis.

MOMP is controlled by the members of the Bcl-2 family of proteins, defined by the sharing of from one to four Bcl-2 homology (BH) domains. Two proapoptotic multidomain (sharing BH1, -2, and -3) proteins, Bax and Bak, appear to be essential for MOMP (Wei et al., 2001) and probably directly form the pore in the outer membrane (Kuwana et al., 2002). In the absence of Bax and Bak, MOMP does not occur, and such cells deprived of survival factors (a condition that normally promotes MOMP and apoptosis) instead undergo autophagy as a means of long-term survival (Lum et al., 2005). Antiapoptotic Bcl-2 proteins prevent MOMP and promote cell survival. A third set of Bcl-2 proteins share only the BH3 domain and promote apoptosis, but the precise manner in which they do this is the controversy that concerns us here.

The first version of how MOMP proceeds by the function of Bax and Bak proposes that these multidomain Bcl-2 family proteins are constitutively active and are held in check by the antiapoptotic Bcl-2 proteins (Chen et al., 2005) (Figure 1A). The BH3-only proteins neutralize the antiapoptotic proteins and release Bax and Bak, which cause MOMP, and death proceeds. However, because different antiapoptotic Bcl-2 proteins are neutralized by different BH3-only proteins, death results only when all of the functional antiapoptotic Bcl-2 proteins are effectively targeted. In this "death by default" model, the presence of the right combination of BH3-only proteins is necessary and sufficient for the decision to undergo apoptosis.

A second version of the story holds that Bax and Bak are not constitutively active but are activated by a subset of

the BH3-only proteins (Letai et al., 2002; Kuwana et al., 2005) (Figure 1B). Two such "direct activators" have been identified, Bid and Bim, and either of these can trigger MOMP through activation of Bax and Bak. However, because antiapoptotic proteins can sequester these direct activator BH3-only proteins, neutralization of the antiapoptotic proteins may be necessary for apoptosis to occur. Other BH3-only proteins can perform this function, acting as "sensitizers" or "de-repressors" by neutralizing those antiapoptotic Bcl-2 proteins they bind (Letai et al., 2002; Kuwana et al., 2005). Thus, BH3-only proteins not only differ in which other Bcl-2 proteins they interact with (different antiapoptotic Bcl-2 proteins as well as Bax and Bak), but also perform different functions. Without activation of Bax and/or Bak, the sensitizer/de-repressor BH3-only proteins do not trigger MOMP

and thus do not cause apoptosis. This is a "life by default" scenario.

Now, Letai and colleagues (Certo et al., 2006) offer a third view that may, at first blush, help to reconcile this controversy. In agreement with other groups (Chen et al., 2005; Kuwana et al., 2005), they found that BH3-only proteins show diverse abilities to neutralize different antiapoptotic Bcl-2 family members. In this view (Figure 1C), however, neutralization of antiapoptotic Bcl-2 proteins leads to death only in cells that have been stressed to engage the direct activators of Bax and Bak but are kept alive by one or more of the antiapoptotic Bcl-2 family members. In such cells the mitochondria are poised to undergo MOMP upon exposure to sensitizer/de-repressor BH3-only proteins that neutralize the antiapoptotic proteins to which the cells have become "addicted." Similarly, the Bcl-2/Bcl-xL inhibitor ABT-737 (Oltsdorf et al., 2005) triggered apoptosis in growth factor-deprived cells sustained by Bcl-2, but not in the same cells maintained in their exogenous survival factor. Thus, BH3-only proteins that neutralize antiapoptotic Bcl-2 family members will cause apoptosis only in some cells that are poised to die, and not in others that do not require these antiapoptotic proteins to survive (as Bax and Bak are not activated).

This modified view of Bcl-2 function has direct implications for understanding apoptosis regulation in oncogenesis and the use of Bcl-2 inhibitors in treating cancer. Oncogenes such as c-Myc, which drive cells into cycle, also engage Bax/Bak-dependent apoptosis (Dansen et al., 2006). Antiapoptotic Bcl-2 family members can prevent such cell death and thereby promote oncogenic transformation. In such cases, Bcl-2 antagonists should trigger MOMP and apoptosis, without causing death in normal cells that are not so lethally poised. If so, then use of such agents holds promise for effective anticancer therapy, without the cost of widespread apoptosis of primary tissues.

As attractive as this idea may be, a simple prediction is likely to undermine it. Of all BH3-only proteins examined, only Bid and Bim have the capacity to act as direct activators of Bax and Bak, to poise cells for MOMP and death upon antagonism of the antiapoptotic Bcl-2 proteins. This predicts that, in the absence of Bid and Bim, apoptosis will be as profoundly impaired as is seen in cells deficient in Bax and Bak. Early indications are that this is not the case (T. Kaufman, D. Huang, and A. Strasser, personal communication).

Are there additional ways in which Bax and Bak can be activated? Perhaps. A slight change in perspective brings in a fourth version of the story at the gates of death. While Bid and Bim are currently the only BH3-only proteins that are implicated in the activation of Bax and Bak, might there not be other ways these proteins can become activated? Recently, cytosolic p53 has been shown to have this potential, and like Bid and Bim, to be sequestered by antiapoptotic Bcl-2 proteins until released by a sensitizer/de-repressor BH3-only protein, Puma (Chipuk et al., 2005). Other non-Bcl-2 proteins may similarly have this activity. Or perhaps Bax and Bak can sometimes be directly activated by conditions in the cell without a requirement for other proteins. Detergents have this effect experimentally, and mild heat may similarly directly activate Bax and Bak. If such activation is held in check by antiapoptotic Bcl-2 family proteins, then the basic concepts proposed by Letai et al. may be general. Some cells live under conditions where neutralization of antiapoptotic mechanisms causes death ("death by default"), while others live under conditions where such neutralization is not lethal ("life by default").

Four slightly different perspectives, each with different consequences, each building on the story that precedes it. And each affecting our understanding of apoptosis and how it may apply to the

treatment of cancers. Are any of these stories more than a flawed approximation of reality? We sit and wait out the rain, and puzzle over the truth at the gates of death. (Fade to black.)

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