BH3 domains: intracellular death-ligands critical for initiating apoptosis

Recent studies have clarified how the BH3 domain, a short peptide motif found in certain BCL-2 family proteins, triggers key mitochondrial events associated with apoptosis.

BCL-2 family proteins are integral components of a genetically defined, evolutionarily conserved apoptosis pathway (Adams and Cory, 1998). Defective control of this pathway, leading to the suppression of apoptosis, is a hallmark of cancer (Green and Evan, 2002) and is linked in some cases to the deregulation of BCL-2 family members. Members of this family are characterized by the presence of up to four distinct segments of amino acid homology, termed BCL-2 homology (BH) domains. The multi-BH domain family members operate to either suppress apoptosis (e.g., BCL-2, BCL-X_L) or promote apoptosis (e.g., BAX, BAK), whereas the BH3-only subfamily members identified to date (e.g., BAD, BID) function exclusively to promote cell death. As their name implies, BH3-only proteins share the BH3 domain but appear otherwise unrelated. Structure/function studies revealed that BH3 domains function as uniquely important "death domains" in these proteins, essential for both their proapoptotic activity and, not coincidently, for their ability to bind to multidomain BCL-2 family members (Bouillet and Strasser, 2002).

A convergence of genetic and biochemical studies has established that BH3-only proteins act at an upstream point in an apoptotic signal-transduction cascade that leads ultimately to cytochrome c release from mitochondria and activation of caspases. BH3-only proteins, of which there are at least eight in mammalian cells, serve to integrate diverse apoptotic stimuli into a common cell death pathway governed by BCL-2 and its multi-BH domain relatives (Bouillet and Strasser, 2002). A remarkable number of seemingly unrelated cell death stimuli—including ligation of Fas and related TNF family receptors, deprivation of growth factors, DNA damage, and loss of cell matrix attachment—have all been linked to the activation of specific BH3-only family members through a variety of transcriptional and posttranslational mechanisms. Nevertheless, a common theme has emerged from the analysis of multiple BH3-only proteins: the BH3 domain is "unleashed" in response to a cell death stimulus and initiates mitochondrial events associated with apoptosis by targeting mitochondrial-localized multidomain BCL-2 family members.

The mechanism by which BH3 domains deliver their deadly signal has been elucidated by the analysis of genetically modified mice, together with in vitro assays which probed the function of BCL-2 family members in isolated mitochondria. The two multidomain proapoptotic proteins, BAK and BAX, serve as essential effectors of the mitochondrial apoptotic pathway and are necessary for induction of cell death by BH3-only proteins (Wei et al., 2001). The BH3-only protein, BID, binds (via its BH3 domain) and activates both BAK and BAX, triggering the oligomerization of these proteins in mitochondrial membranes and the subsequent release of cytochrome c into the cytoplasm (Wei et al., 2000). BID also interacts with multiple antiapoptotic BCL-2 family members, including BCL-2, BCL-X_L, and Bfl-1/A1 (Wei et al., 2000; Werner et al., 2002). These antiapoptotic proteins, by contrast to the direct effector role ascribed to BAK and BAX, appear to function principally as regulators, sequestering BH3-only proteins and thus preventing activation of BAK and BAX (Cheng et al., 2001).

Certain BH3-only proteins display selective binding to specific BCL-2 family members. For example, BID binds to both pro- and antiapoptotic BCL-2-related proteins, whereas BAD binds only antiapoptotic proteins such as BCL-2 and BCL-X_L. In this issue of *Cancer Cell*, Letai et al. (2002) report that the binding selectivity and distinct functional activity of BH3-only proteins are recapitulated in the con-

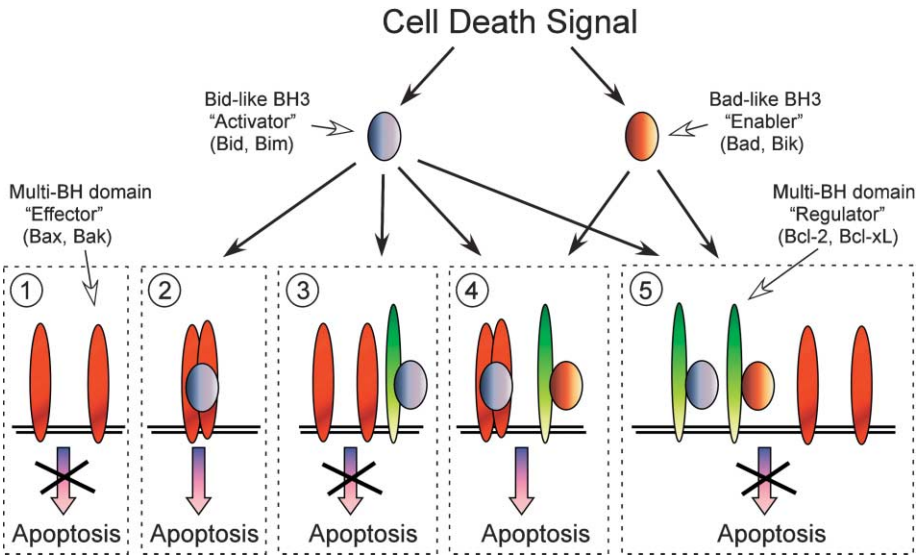

Figure 1. A model for the control of apoptosis by BH3 domain-only proteins

Cell death signals can engage two distinct classes of BH3-only proteins, BID-like "activators" and BAD-like "enablers." BID-like proteins directly bind and activate mitochondrial-localized BAK and BAX (1), triggering BAK/BAX oligomerization and apoptosis (2). Antiapoptotic BCL-2 proteins sequester BID-like proteins, preventing their interaction with BAK/BAX (3). BAD-like proteins sensitize or enable apoptosis by binding antiapoptotic BCL-2 family proteins, and thus preventing the sequestration of BID-like BH3 activators (4). Overexpression of BCL-2 or related proteins in cancer cells may sequester both classes of BH3-only proteins and thereby block cell death (5). (Figure after Letai et al., 2002.)

text of isolated, synthetic BH3 peptides. Integrating these results with previous studies, Letai et al. propose a revised "rheostat" model (Figure 1) that represents a significant makeover from earlier versions that focused principally on the interaction of BCL-2 and BAX. Although it is likely to be vastly oversimplified, the model explains how the interplay amongst BH3-only proteins and multidomain BCL-2 family members regulate cell death (Letai et al., 2002). BAK and BAX function as mitochondrial-localized receptors for the "activator" or agonist class of BH3 peptide ligands, typified by the BID BH3 domain. Upon binding, these BID-like BH3 ligands trigger the oligomerization and activation of BAK and BAX receptors via an unknown mechanism. This key proapoptotic signaling event can be blocked by sequestration of BID-like BH3 ligands by BCL-2/BCL-X_L and related antiapoptotic multidomain proteins, which function essentially as decoy receptors in this model. A distinct functional class of BH3 ligands, typified by BAD and BIK BH3 peptides, do not bind and activate BAK/BAX receptors directly, but instead promote apoptosis indirectly by binding and neutralizing BCL-2/BCL-X_L decoy receptors. Presumably, this increases the effective activity of BID-like BH3 ligands by inhibiting their sequestration by BCL-2/BCL-X_L.

Two distinct functional classes of BH3 domains have, therefore, been defined by these studies. Using the terminology of Letai et al., BID-like BH3 domains "activate" apoptosis, through direct targeting of BAK/BAX, whereas BAD-like BH3 domains "sensitize" or enable apoptosis, by neutralizing BCL-2 and related antiapoptotic proteins. The BH3-only protein BIM appears to provide a second example of a BID-like BH3 domain, based on the ability of an isolated BIM BH3 peptide to activate BAX (and to a lesser degree BAK) and trigger cytochrome c release (Letai et al., 2002). This was unexpected since full-length BIM (BIML) does not show appreciable affinity for BAK or BAX and does not induce cytochrome c release in in vitro assays (Terradillos et al., 2002). Apparently, the presentation of the BIM BH3 domain is somehow masked in the context of BIML, since naturally occurring truncated isoforms of BIM, BIMS, and BIMAD bind to BAX and trigger its activated conformation (Marani et al., 2002). The selectivities of other known BH3-only proteins remain to be carefully examined in light of these findings. It will also be interesting to determine whether the principle of distinct BH3 domain activities applies to nonmammalian systems, including the *C. elegans* BH3-only protein EGL-1, and in *Drosophila* cell death pathways, where BH3 proteins have not yet been identified. Moreover, the ability of BAD-like BH3 domains to bind exclusively to antiapoptotic BCL-2 family members raises the possibility that BH3-only proteins with the inverse selectivity might exist: BID-like BH3 activators that bind exclusively to BAK/BAX. Such proteins would be predicted to be especially powerful inducers of apoptosis, immune from sequestration by BCL-2. BH3-only "activator" proteins in this class, if they exist, would not have been identified in BCL-2-interaction screening approaches used to identify most of the known BH3-only family members.

Understanding the functional intricacies of BH3 domains may ultimately pay off in efforts to develop novel anticancer agents that engage the apoptosis pathway selectively in tumor cells. In principle, cytotoxic insults that induce apoptosis through activation of BID-like BH3-only proteins are inherently different from stimuli that promote apoptosis through the activation of BAD-like BH3-only proteins. The latter, for example, may be insufficient to trigger cell death unless there are adequate levels of activated BID-like BH3 molecules provided by a separate apoptotic stimulus. Letai et al. provide initial experimental support for this scenario by examining the proapoptotic effects of cell-permeable BH3 peptides in a tumor cell line. A BID-BH3 peptide proved to be an effective inducer of apoptosis, and importantly, this activity depended on an intact BH3 domain and the function of endogenous BAK/BAX. By contrast, a BAD-BH3 peptide was not toxic to cells at the concentrations tested, but significantly enhanced the ability of the BID-BH3 peptide to induce apoptosis at otherwise sublethal concentrations. From the perspective of cancer drug development, agents that mimic the activity of a BAD-like BH3 domain might have a useful therapeutic window, sensitizing tumor cells to intrinsic or extrinsic apoptotic stimuli (e.g., activated oncogenes and chemotherapeutic drugs, respectively). In a much broader view, suppression of apoptosis has been implicated as a critical, if not essential, event for the development of cancer (Green and Evan, 2002). Unfortunately, the mechanisms that frequently block cell death in tumor cells, particularly in solid cancers, remain poorly defined. It should now be possible to exploit model BAD-like and BID-like BH3 peptides as research tools to help address the long-standing, important question of the precise contribution of BCL-2 family proteins to the survival of human tumor cells.

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