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Two roads to death – Bax targets mitochondria by distinct routes before or during apoptotic cell death

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Recent studies have revolutionized our understanding of how the crucial apoptosis effectors Bax and Bak target mitochondria to kill cells. We recently reported that an important determinant of the localization, oligomerization, and apoptotic function of Bax is an interaction with either mitochondrial voltage-dependent anion channel 2 (VDAC2) (in healthy cells) or Bak (in apoptotic cells).[1]

The Bcl-2 family proteins Bax and Bak1 (Bcl-2-antagonist/killer 1) are essential mediators of the intrinsic pathway of apoptosis as without them a cell is refractory to many apoptotic stimuli.[2] Despite this clear importance, key aspects of how Bax and Bak are regulated and how they self-associate to damage the mitochondrial outer membrane (MOM) to kill cells in response to toxic insult remain obscure. Bak is constitutively anchored in the MOM, whereas Bax is predominantly cytosolic, although a population of Bax can be detected at the mitochondria in most cells. Recent paradigm-shifting studies have challenged the long-held premise that Bax actively translocates to the MOM upon activation. Rather, Bax constitutively associates with mitochondria but is constantly trafficked to the cytosol. Upon apoptotic signaling this "retrotranslocation" is impaired leading to accumulation of Bax at mitochondria, a common hallmark of most apoptotic cells.[3,4] The mechanism governing Bax subcellular distribution is unclear and may be dependent on[4] or independent[3] of interaction with pro-survival Bcl-2 proteins. Understanding the regulatory mechanism is paramount as the levels of Bax and Bak at mitochondria are key determinants of the cellular response to chemotherapeutic agents.[3]

Our recent studies have provided insight into how Bax accumulates on mitochondria.[1] We show that, like Bak, the population of Bax that constitutively resides at mitochondria in numerous cell types is a component of a high-molecular weight complex involving voltage-dependent anion channel 2 (VDAC2).[1,5] The association with VDAC2 is important for the mitochondrial targeting of both Bax and Bak in healthy cells as their constitutive mitochondrial populations are reduced in cells deficient in VDAC2.[6] Following an apoptotic stimulus Bax can be recruited to the MOM by associating with mitochondrial Bak and thus can bypass the lack of VDAC2 to participate in mitochondrial membrane permeabilization. This bifurcated route to mitochondria (depicted in **Fig. 1**) is important for Bax apoptotic function, as Bax cannot efficiently mediate cell death in the absence of both VDAC2 and Bak.[1]

The VDACs have a checkered history in the regulation of apoptosis. Initially, they were thought to be constituents of the pore necessary for the efflux of apoptogenic factors including cytochrome *c*; however, the finding that cells lacking all 3 isoforms of VDAC can still undergo apoptosis refuted this. Subsequently, VDAC2 was implicated as a negative regulator of Bak.[7] Thus, Bak was proposed to be hyperactive in VDAC2-deficient cells, leading to the observed sensitization of these cells to apoptotic stimuli and the lethality of *Vdac2*$^{-/-}$ mice. Although we, and others, have confirmed the sensitization of *Vdac2*$^{-/-}$ fibroblasts,[1,8] we now show that Bak in these cells is not in fact hyperactive. Rather, mitochondria isolated from *Vdac2*$^{-/-}$ fibroblasts are actually more resistant to cytochrome *c* release as a result of reduced levels of mitochondrial Bak and Bax, indicating that in order for these cells to die Bak and Bax must be recruited from the cytosol.[1]

The reliance on VDAC2 and/or Bak for efficient Bax-mediated apoptosis may represent a new mechanism by which cells that are dependent on Bax to undergo cell death could evade apoptosis, potentially leading to tumor development. Furthermore, our study suggests that the Bax interaction with VDAC2 is a potential target to protect cells that are devoid of full-length Bak from cell death insults such as cerebellar granular neurons following ischemic stroke.

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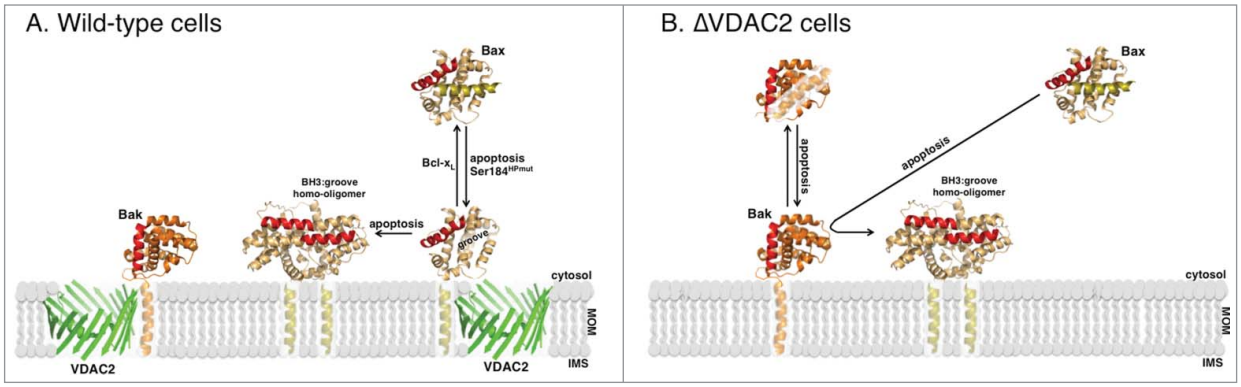

Figure 1. Bax mitochondrial localization and apoptotic function is governed by interactions with VDAC2 and Bak. (**A**) In a healthy wild-type cell, Bax (PDB 1F11) is in equilibrium between the mitochondrial outer membrane (MOM) and cytosol. This equilibrium is governed by interactions with voltage-dependent anion channel 2 (VDAC2, PDB, 4BUM) and other factors including Bcl-x_L.[3] The mitochondrial populations of both Bax and Bak (PDB 2IMS) form a high-molecular weight complex involving VDAC2. The interaction with VDAC2 involves the C-terminal transmembrane anchor of Bax (yellow) and Bak (orange). Hydrophobic mutation (HPmut) of the Bax tail residue Ser184 encourages interaction with mitochondrial VDAC2. Following an apoptotic stimulus, mitochondrial Bax is activated and dissociates from VDAC2 to form BH (Bcl-2 homology domain)3:groove homo-dimers (PDB 4BDU) and subsequent higher order oligomeric pores, the structure of which is currently unknown. (**B**) In VDAC2-deficient cells (ΔVDAC2), both Bax and Bak are redistributed to the cytosol. Following an apoptotic stimulus, Bax and Bak need to translocate to the MOM for cytochrome c release and cell death to occur. During apoptosis, Bax can be recruited to the MOM via interaction with mitochondrial Bak, thus allowing Bax to participate in MOM damage and leading to the release of apoptogenic factors from the intermembrane space (IMS). In the combined absence of both VDAC2 and Bak, Bax cannot efficiently mediate cell death.

The focus now is to understand the molecular mechanism involved in the Bax–VDAC2 interaction. Our studies indicate that the C-terminal transmembrane anchor of Bax is important for its interaction with VDAC2, consistent with VDAC2 being a β-barrel membrane-integrated pore. Mutation of the Bax C-terminal residue Ser184 to a hydrophobic residue is sufficient to promote the association of Bax with the MOM.[9] However, our observation that this was not the case in mitochondria from *Vdac2*$^{-/-}$ MEFs suggests that the mutation actually improves the stability of the association with VDAC2 rather than promoting a hydrophobic interaction with mitochondrial lipids. This is consistent with the reduced rate of dissociation of a Bax Ser184 mutant from the MOM compared with wild-type Bax.[3] Supporting a role for the C-terminus, mutation of hydrophobic residues in the C-terminal transmembrane anchor of each protein disrupted the association of mitochondrial Bax and Bak with the large VDAC2 complex. This is consistent with our previous finding that substitution of the Bak C-terminus with that of the mitochondrial fission regulator Fis1 allows Bak to target the MOM and mediate cell death, but disrupts its association with VDAC2.[10] Thus, a major determinant of mitochondrial Bax accumulation is the stability of its interaction with VDAC2. The constitutive mitochondrial localization of Bak may be explained by the stability of the interaction between its transmembrane anchor and VDAC2 compared with that of the Bax transmembrane anchor. Although the C-terminal transmembrane anchors are clearly important, whether other regions of Bax and Bak are also involved in the interaction with VDAC2 is of interest, particularly if such interaction involves cytosolic regions of Bax or Bak that are more likely to be targetable.

Although our study has provided insight into the regulation of Bax and Bak, key questions remain. For example, is the interaction with VDAC2 direct or do other proteins participate? Can proteins other than Bak and VDAC2, such as VDAC1 or Bcl-x_L, facilitate Bax mitochondrial targeting and thus apoptotic activity? Why, when Bax and Bak targeting to the MOM is impaired, are VDAC2 cells sensitized to apoptotic stimuli? These are important questions if the undoubted potential of Bak and Bax as therapeutic targets is to be exploited.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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