Apoptosis: it’s BAK to VDAC

The voltage-dependent anion channel (VDAC) of the mitochondrial outer membrane (MOM; Mannella & Kinnally, 2008)—also known as mitochondrial porin—has long been implicated in regulating the mitochondrial response to certain cell death stimuli (Galluzzi & Kroemer, 2007). This includes a potential role in MOM permeabilization (MOMP) during apoptosis, which is a necessary step in the release of cytochrome c—a critical cofactor for downstream activation of caspases—from the organelle. Although controversial, it is possible that VDAC is a component of the permeability transition pore complex, and therefore its influence could also extend to the inner membrane, regulating membrane potential and ATP production. Among the strongest indications that VDAC has a role in the regulation of cell death are the reported interactions between VDAC and members of the BCL2 family of proteins. BCL2 proteins are important regulators of the mitochondrial pathway to apoptosis (Youle & Strasser, 2008). The multi-BH-domain-containing, pro-apoptotic members BAX and BAK are the essential gateways to MOMP, and it has been suggested that both physically interact with VDAC (Galluzzi & Kroemer, 2007). In particular, Cheng and colleagues (2003) concluded that a minor VDAC isoform—known as VDAC2—acts to physically restrain BAK in the MOM in non-stimulated viable cells. However, death stimuli, including that elicited by truncated BID (tBID)—an activated, pro-apoptotic, BH3-only BCL2 family protein—displaced inactive BAK from VDAC2, promoting BAK oligomerization. In view of this collective evidence, it was a surprise when gene deletion studies suggested that all three Vdac gene products are dispensable for mitochondrial-dependent cell death in mouse embryonic fibroblasts (MEFs; Baines et al. 2007). In addition to necrotic cell death driven by defects in the permeability transition pore—elicited, for example, by oxidative stress or calcium overload—a range of other stresses were also unaffected by the deletion of Vdac1–Vdac3.

A compelling study by the Hajnoczky group, published in this issue of EMBO reports, now reprises the role of VDAC2 in regulating BAK, but suggests a function for VDAC2 that is diametrically opposed to the antagonism of BAK by VDAC2 reported by Cheng and colleagues (2003): VDAC2 promotes tBID-induced apoptosis by recruiting newly synthesized BAK to mitochondria. What is going on? Can three such divergent conclusions—no role, antagonism, or displace inactive BAX from VDAC2, BAX and BAK at the mitochondrial outer membrane. (A) Upper panel: recruitment and activation of BAX at the MOM by tBID (shown in yellow, with its exposed BH3 domain in green). Lower panel: the activation of BAK by tBID probably parallels that of BAX, with the exception that inactive BAK is anchored at the MOM by its TM segment. (B) Interaction of nascent BAK with VDAC2 at a presumptive binding site within BAK (shown as a turquoise oval) results in BAK insertion into the MOM through its TM segment. Inactive BAK might then be available for interactions with binding partners, including VDAC2 and MCL1. In cells that express excess BCL2 or BCL-XL, but are primed to undergo apoptosis because tBID has been generated, these pro-survival proteins bind to tBID and activated monomeric BAK, preventing BAK oligomerization and MOM permeabilization. MCL, myeloid cell leukaemia; MOM, mitochondrial outer membrane; tBID, truncated BID; TM, transmembrane; VDAC, voltage-dependent anion channel.

Reconstitution experiments using purified proteins or peptides and liposomes of known lipid composition have suggested a core pathway to MOMP, defined exclusively by BCL2 family proteins (Kuwana et al. 2005; Lovell et al. 2008); however, non-BCL2 components are unquestionably layered on top of this pathway to support its execution and regulation in the complex milieu of the intact cell. The available evidence (Shore & Nguyen, 2008) supports an emerging model that focuses on BAX as the effector of MOMP, and tBID as the ‘activator’ BH3-only protein (Fig 1A, upper panel). The process starts with the generation of active tBID from inactive BID; tBID then rapidly migrates to the membrane where it probably functions as a receptor for inactive cytosolic BAX, stimulating the conformational transition of BAX to its active, monomeric, membrane-integrated form through the insertion of the transmembrane (TM) segment and integration of helices 5 and 6 into the bilayer, leaving the N terminus available for proteolysis or binding to an antibody. This pathway might be amplified by the ability of activated BAX, similarly to tBID, to recruit more molecules of inactive BAX from the cytosol. Activated BAX can undergo auto-oligomerization, yielding a MOMP-competent complex. In most respects, BAK probably follows a similar process...
(Ruffolo & Shore, 2003), with the exception that conformationally inactive BAK is anchored in the membrane through its TM segment (Fig 1A, lower panel), rather than being free in the cytosol. One important consequence of this distinction between BAK and BAX might be that membrane-anchored inactive BAK is available to interact with other proteins that are located in the same membrane.

Irrespective of the membrane-integrated active conformation adopted by monomeric BAX or BAK in the membrane in response to tBID, the TM segment seems to have an essential role, as its deletion typically renders physiologically relevant amounts of ectopic BAX and BAK ineffective in inducing cell death. The TM domain is important for membrane targeting to the resident locations of BAX and BAK, the mitochondria and endoplasmic reticulum. TM segments that confer targeting to the cognate membrane through the signal recognition machinery of the membrane are referred to as ‘signal-anchors’, whereas TM segments—usually located at either end of a protein—that mediate membrane insertion independently of the membrane signal recognition complex are called ‘tail-anchors’. In the case of BAX, the contribution of the membrane signal recognition machinery to mitochondrial protein targeting remains controversial (Ott et al., 2009). However, this was specifically addressed for BAX using protein knockdowns, which showed that BAX targets mitochondria independently of mitochondrial protein import receptors (Setoguchi et al., 2006). Knockdown of VDAC2, but not of VDAC1, compromised the mitochondrial localization of a construct tagged with three copies of the FLAG epitope at its N terminus and delivered into semi-permeabilized HeLa cells. Conversely, the overexpression of haemagglutinin (HA)-VDAC2—but not of HA-VDAC1—enhanced mitochondrial localization. However, coupling the BAK TM segment to the C terminus of green fluorescent protein (GFP) resulted in efficient targeting of GFP to mitochondria by a VDAC2-independent mechanism, whereas coupling GFP to full-length BAK retained the VDAC2 dependence for mitochondrial targeting of the fusion protein. Collectively, the results suggest that VDAC2 might interact with a remote site on BAK and unmask the targeting of nascent BAK to mitochondria, why do Vdac2−/− cells resist tBID-mediated apoptosis? BAX would be expected to compensate for the loss of BAK at mitochondria, as cytosolic BAX can be activated by tBid. By using a combination of approaches and cell types with targeted deletions of Vdac2, Bax, and BAK, the results suggest that the BAK–VDAC2–tBID axis is more efficient at initiating apoptosis than the BAX–tBID pathway (Roy et al., 2009). Nevertheless, the compounding feature of compensatory changes in cells harbouring targeted deletions of specific genes could be a source of variability between different studies.

In conclusion, an important consequence of VDAC2-mediated targeting of nascent BAK to mitochondria is the spatial separation of mitochondrial BAK and cytosolic BAX, thus opening the possibility for differential regulation of these otherwise redundant effectors of MOMP (Fig 1A,B). In addition to VDAC2, BAX has been reported to interact constitutively with MCL1 in the MOM of viable cells (Cuconati et al., 2003; Nijhawan et al., 2003). These and potentially other interactions are presumably in equilibrium and can shift to an interaction with tBid depending on the relative protein abundance and interaction affinities (Fig 1B). It is likely that mitochondrial BAK follows the same sequence of activation by tBID and antagonism by pro-survival BCL2 members, as described for BAX. Similarly to BAX, tBID induces a conformational activation of monomeric BAK (Ruffolo & Shore, 2003). BCL2 (and presumably BCL-XL), which can sequester tBID, also selectively interacts with this tBID-activated BAK conformer, preventing its auto-oligomerization and MOMP (Ruffolo & Shore, 2003). Owing to the spatial separation of BAK and BAX in viable cells, however, there are probably different mechanisms to prevent BAX or BAK from initiating MOMP.

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Gordon C. Shore is at the Department of Biochemistry and
Goodman Cancer Center, McGill University, McIntyre Medical
Building, Room 906B, 3,655 Promenade Sir William Osler,
Montreal, Quebec H3G 1Y6, Canada.
Tel: +1 514 398 7282; Fax: +1 514 398 7384;
E-mail: gordon.shore@mcgill.ca

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