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Cheating Death: New Molecules Block BAX

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Abstract

BAX is a formidable BCL-2 family protein that executes cellular suicide in response to physiologic and pathologic stress. A new article in *Nature Chemical Biology* (Garner *et al.* <https://doi.org/10.1038/s41589-018-0223-0>) reports small molecules that inhibit the conformational activation of BAX, informing a pharmacologic approach to blocking unwanted cell death in human disease.

BCL-2 family proteins regulate the critical balance between cellular life and death during health and disease. This homeostatic Yin and Yang derives from protein interactions between two classes of BCL-2 proteins, those that kill ('proapoptotic') and those that protect ('antiapoptotic'). BAX is a cardinal death protein that lies dormant in the cytosol of essentially all cells until triggered by stress signals [1]. Select members of another class of BCL-2 proteins, called 'BH3-only' proteins, function as afferent stress sensors and can deploy their BCL-2 homology 3 (BH3) domain helix to directly bind to and activate BAX. In response to BH3-triggering, this 21 kDa globular protein composed of nine α -helices [2] undergoes a dramatic conformational reorganization, transforming from a cytosolic monomer into a mitochondrial outer membrane-embedded oligomer that effectively destroys the power plants of the cell [3]. Upon permeabilization by BAX, the mitochondria release a series of signaling proteins that irreversibly propel the apoptotic cascade. Whereas BAX-mediated apoptosis contributes to beneficial cellular pruning during development and organism homeostasis, conditions of premature or unwanted cell death such as in neurodegeneration, ischemic injury, bone marrow failure, infertility, and many other diseases could benefit from pharmacologic restraint of BAX.

Despite the central role of BAX as an arbiter of cell death, there are currently no drugs that directly modulate its apoptotic activity. Indeed, BAX has been a difficult protein to generate and store in the laboratory, explore by traditional small-molecule screening, and characterize its dynamic conformational states. Given the lethal consequences of renegade BAX activation, a series of natural mechanisms alternatively preserve its inactive state or trap its conformationally activated form, providing multiple levels of 'security'. The C-terminal α 9 helix of monomeric BAX itself serves as an auto-inhibitory feature both by plugging the C-terminal hydrophobic pocket and by sequestering its mitochondrial targeting functionality [2]. In addition, the identification of an inactive dimeric form of BAX in the cytosol

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demonstrated how interaction between the C-terminal surface of one monomer and the N-terminal face of another can likewise preserve the inactive state [4]. The canonical mode of inhibiting activated BAX involves trapping its conformationally exposed BH3 helix in a groove located on the surface of anti-apoptotic BCL-2 proteins [5], thereby preventing the propagation and oligomerization of BAX. A non-canonical mode of BAX inhibition preserves the inactive state through conformational restraint imposed by direct interaction with the BH4 domain of antiapoptotic proteins [6]. Viruses target BAX with their own battery of proteins, including antiapoptotic BCL-2 homologs, to enforce host cell survival during infection. Cytomegalovirus expresses a unique protein, called vMIA, which interacts with BAX at a distinct binding site to arrest conformational activation [7]. In each case the goal of these natural protein-interaction mechanisms is to stabilize the BAX hydrophobic core to maintain the inactive, globular state or to trap structural components essential to propagating BAX activation. Could small molecules do the same?

Gavathiotis and colleagues recently identified a pair of structurally similar, carbazole-based molecules that inhibit BAX by doing exactly that, namely stabilizing key structural features of the hydrophobic core [8]. The compounds, dubbed BAX activation inhibitors 1 and 2 (BAI1, BAI2), emerged from a screen of small molecules that could block BH3-triggered BAX poration of liposomes. Interestingly, these same molecules were first identified as inhibitors of the channel activity of BAX [9]. A rigorous multidisciplinary workflow incorporating biochemical, structural, and cellular analyses demonstrated that BAIs (i) bind to inactive BAX at a deep hydrophobic pocket, (ii) allow for interaction with triggering BH3 ligands but prevent the induced conformational activation, (iii) block the membrane translocation and oligomerization of BAX, and importantly, (iv) suppress apoptosis induced by pharmacologic stimuli in a dose-responsive and BAX-dependent fashion.

How do BAIs suppress BH3-triggered direct BAX activation? The answer derives from an understanding of the direct and allosteric effects of BH3 triggering (Figure 1A). Select BH3 domains, such as those found in BIM, BID, and PUMA, can engage BAX at a ‘trigger site’ formed by the confluence of α -helices 1 and 6 at the N-terminal surface of BAX [10]. This binding interaction displaces the α 1– α 2 loop that partially overlies the trigger site and represents the initiating conformational change of BAX activation. As a consequence of this loop opening, a series of conformational changes ensue, including exposure of the crucial BAX BH3 helix and allosteric release of the C-terminal α 9 helix implicated in mitochondrial translocation [11]. Importantly, these changes are transmitted through the hydrophobic core of the globular protein, and the key helical components separate from the central α 5– α 6 hairpin. Nuclear magnetic resonance (NMR) analyses revealed that BAIs bind to a deep hydrophobic pocket formed by the convergence of α -helices 3, 4, 5, and 6 (Figure 1B). Applying paramagnetic relaxation enhancement (PRE) NMR using a soluble probe and hydrogen–deuterium exchange mass spectrometry analysis, both of which measure BAX conformational changes reflected by access of the protein backbone to either the PRE probe or deuterium exchange, revealed protection of residues adjacent to the BAI-binding site but also within the hydrophobic core. Thus, by apparently strengthening the core hydrophobic interactions of BAX, BAIs prevent the conformational release of the BAX BH3 and C-terminal α 9 helices, and thus impose an allosteric constraint on BH3 triggering

(Figure 1C). Indeed, the capacity of BAIs to block translocation of BAX to the mitochondria keeps this latent lethal weapon of the apoptotic cascade away from its target organelle.

The discovery of a novel hydrophobic pocket amenable to small-molecule targeting provides a new opportunity to develop inhibitors of BAX and thus of BAX-mediated apoptosis. The potential therapeutic utility is vast, particularly considering the broad impact of preventing pathologic cell death. The study by Gavathiotis and colleagues demonstrates that BAX can indeed be restrained by small molecules, and provides a blueprint for the development of BAX-inhibitory drugs.

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Figure 1. BAX Activation Inhibitor (BAI) Molecules Restrain the BH3-Triggered Conformational Activation of Proapoptotic BAX.

(A) In response to cellular stress, select BH3-only proteins deploy their BH3-helices (light blue) to directly bind to a regulatory site formed by surface residues of BAX helices $\alpha 1$ and $\alpha 6$ (dark blue), thereby triggering the initial conformational change that involves displacement and ‘opening’ of the $\alpha 1$ - $\alpha 2$ loop (green). Allosteric mobilization of the BAX BH3 (cyan) and the C-terminal $\alpha 9$ (purple) helices ensues, leading to BAX translocation, self-association, and permeabilization of the mitochondrial outer membrane. (B) Computational docking based on the measured chemical-shift perturbations by nuclear magnetic resonance analysis of ^{15}N -labeled BAX upon BAI1 titration demonstrated that the small molecule (orange) engages select residues of α -helices 3, 4, 5, and 6 (red) that localize to a hydrophobic pocket. (C) The proposed allosteric mechanism of action of BAIs involves

reinforcing the hydrophobic interactions of the BAX core, which ‘locks down’ BAX by preventing the conformational release of the BAX BH3 and C-terminal $\alpha 9$ helices upon BH3 triggering.

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