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RESEARCH HIGHLIGHT

BAX and BAK become killers without a BH3 trigger

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Cell Research (2019) 29:967–968; https://doi.org/10.1038/s41422-019-0253-5

It is widely believed that triggering mitochondria-dependent apoptosis requires that certain pro-apoptotic BH3-only proteins (e.g., BID, BIM, etc.) of the BCL-2 family directly engage and activate the family's essential effector proteins BAX and BAK, whose oligomerization then permeabilizes the mitochondrial outer membrane (MOM), committing the cell to apoptosis. However, by reconstituting a cell line engineered to lack all eight of the accepted BH3-only proteins, Huang et al. obtained evidence that these apoptotic triggers primarily target the anti-apoptotic BCL-2 relatives BCL-XL and MCL-1, concomitant neutralization of which enabled membrane-mediated spontaneous activation of BAX/BAK.

Interactions between three factions of the BCL-2 protein family control mitochondria-dependent apoptosis.[1] When activated by stress, the eight accepted BH3-only members (BID, BIM, PUMA, BIK, BAD, BMF, NOXA, HRK), which share only the BH3 interaction domain with the family, use that domain to stably bind certain or all of their pro-survival relatives (BCL-2, BCL- XL, MCL-1, BFL-1, BCL-W), neutralizing their ability to sequester BAX and BAK, which then permeabilize the mitochondrial outer membrane (MOM), provoking the cell's proteolytic demolition.

How the BH3-only proteins provoke activation of BAX and BAK remains a long-standing puzzle. The Direct Activation Model (Fig. 1a) posits that certain BH3-only proteins (most notably BIM and BID) are 'activators' that can directly engage BAX and BAK transiently and convert them into a form that oligomerizes on the MOM, whereas the others (e.g., BAD) are 'sensitizers' that engage only pro-survival family members, lowering the apoptotic threshold.[2] An alternative Indirect Activation Model (Fig. 1b) suggests that the BH3-only proteins need to only bind most of the pro-survival proteins in the relevant cell to elicit apoptosis, and that BAX/BAK activation may be autonomous.[3,4] The current consensus,[5] retains aspects of both models, with the pro-survival proteins engaging all BH3-only proteins and any BAX or BAK commencing activation; however, BAX/BAK activation is still widely assumed to require a direct BH3 trigger, mainly because certain BH3-only proteins, or their BH3 peptides, can stimulate BAX to lyse artificial membranes (e.g., liposomes), mimicking mitochondrial permeabilization.

The conundrum has been that every BH3-only protein except BAD has shown some such 'activator' activity,[6] and it is likely that redundancy in their functions has limited the conclusions drawn by deleting only one or a few BH3-only proteins. To circumvent this problem, the Luo lab undertook the Herculean task, using CRISPR/Cas9 in a cell line, of deleting complete subsets of family members, or the entire BCL-2 family.[7,8] Their findings with these cell lines strongly support an indirect activation model (Fig. 1c).

Their previous knockout (KO) in cancer cell line HTC116 of all eight accepted BH3-only proteins (OctaKO cells) or all BCL-2 family members (BCL-2 allKO)[8] suggested that simply neutralizing both MCL-1 and BCL-XL might activate BAX/BAK. To test this hypothesis, Huang et al. deleted the gene encoding MCL-1 or BCL-XL from the OctaKO cells with CRISPR/Cas9 and reconstituted these cell lines with doxycycline (dox)-inducible genes for each individual BH3-only protein.[7] Consistent with known affinities of BH3 domains for pro-survival family members,[3] BAD3SA, a stabilized mutant of the BAD BH3-only protein, which specifically binds BCL-2, BCL-XL or BCL-W, killed OctaKO cells expressing BCL-XL but not those expressing MCL-1, whereas NOXA, which engages MCL-1 but not BCL-XL, killed those expressing MCL-1 but not BCL-XL. Thus, the apoptosis required only 'sensitizers' and not an 'activator.'

Since survival of OctaMCL-1KO cells required BCL-XL, Huang et al. tested whether the putative activators BIM and tBID would kill them faster than 'sensitizer' BAD3SA, as the Direct Activation Model would predict. However, BAD3SA, which cannot bind BAX or BAK, killed OctaMCL-1KO cells just as fast. To address whether activators use both an indirect and direct mechanisms, they replaced the BH3 domains of BIM and tBID with that of BAD3SA. These chimeras, which should lack all 'activator' activity, killed OctaMCL-1KO as rapidly as WT tBID or BIM, but not OctaBCL-XLKO cells. Thus, 'activator activity' for BAX/BAK, rather than an intrinsic property of certain BH3-only proteins, depends on the cell's pro-survival profile.

Once all prominent pro-survival proteins are neutralized, does BAX/BAK activation require a direct 'activator'? To address this issue, they generated '2 + 5' KO cells, which lack both BAX and BAK and the five pro-survival family members, and reconstituted them and the BCL-2 allKO cells with dox-inducible BAX. The similar rates of BAX activation in these two cell lines indicated that, in the absence of the pro-survival proteins, or upon their inactivation, BAX activation is autonomous and does not rely on endogenous BH3-only proteins. Thus, pro-survival family members may be the only physiologically relevant targets of BH3-only proteins, as indirect activation models suggest (Fig. 1b, c).

Recent pharmacological studies[9] reached similar conclusions. BH3 mimetic drugs targeting both BCL-XL (A-1331852) and MCL-1 (S63845) readily killed the OctaKO cells and parental HCT116 cells, arguing that the sole physiological function of BH3-only proteins is to neutralize these pro-survival proteins.

Collectively, these studies argue strongly that BH3-only proteins must primarily, and perhaps exclusively, target all pro-survival family members prominent in a cell. Indeed, they question whether direct engagement of BAX or BAK by BH3-only proteins has physiological relevance. There are, however, potential caveats. Firstly, additional BH3-only proteins may remain to be identified — if so, the OctaKO line could prove valuable for identifying

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Published online: 15 November 2019

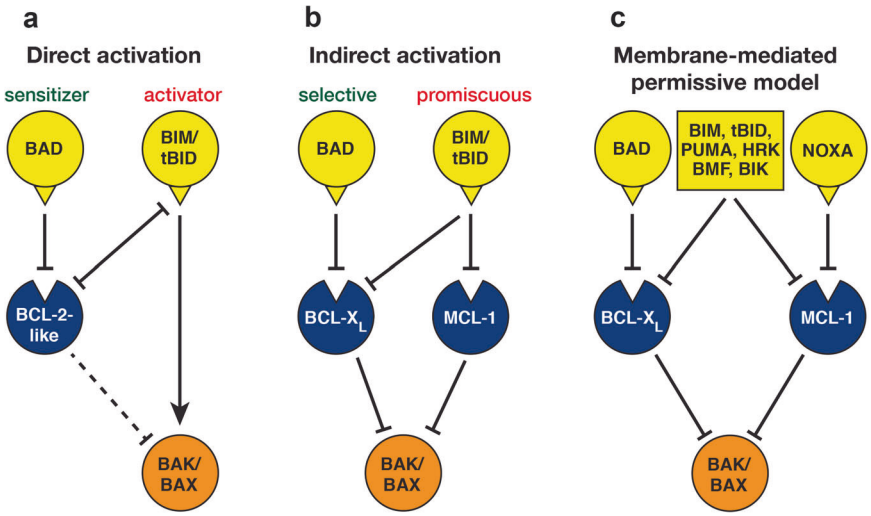

Fig. 1 Models for the activation of BAK and BAX to drive apoptosis. **a** The direct activation model posits that BH3-only proteins are either *sensitizers* (e.g., BAD), which can only engage and neutralize pro-survival family members, or *activators* (e.g., BIM and activated BID (tBID)), which can also bind BAX and BAK and trigger their rearrangement into lethal oligomeric forms that damage the MOM and seal commitment to apoptosis. **b** The indirect activation model suggests that BH3-only proteins can only engage pro-survival family members, but certain ones are more potent because they can bind and neutralize all pro-survival members (are *promiscuous*), whereas others (like BAD and NOXA) are less potent because they are *selective* for a subset of pro-survival members. **c** The membrane-mediated permissive Model is an indirect activation model that emphasizes BCL-XL and MCL-1 as key targets for inactivation by BH3-only proteins to free BAX and BAK for death duty. In this model, once freed from pro-survival restraint, activation of BAX and BAK is autonomous, probably requiring only association with the MOM for BAX

them. Secondly, the findings rely entirely on derivatives of the HTC116 cancer cell line, and its apoptotic responses may be perturbed by mutations or epigenetic changes acquired during its evolution, or its extensive gene editing.

Putting these caveats aside, the findings[7–9] suggest that a BH3-only protein may not be required or sufficient to drive BAX/BAK activation. Huang et al. suggest that membrane contact may suffice for BAX.[7] Its activation might also be controlled autonomously by whether its membrane anchor is released or sequestered in its surface groove; by post-translation modifications such as phosphorylation; by reduced retro-translocation of BAX to the cytosol, leading to MOM accumulation;[10] or by pro-survival engagement of BAX at a site independent of its BH3. Since BAK is bound to the MOM in healthy cells, its activation may differ from that of BAX.

ADDITIONAL INFORMATION

Competing interests: The author declares no competing interests.

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