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The role of BCL-2 family members and caspases in cell life and death

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The BCL-2 family members are major regulators of the apoptotic process, whereas caspases (cysteine proteases) are the major executioners. The cell death-regulating activity of the BCL-2 family members appears to depend on their ability to modulate mitochondrial function. BCL-2 family members are divided into pro- and anti-apoptotic molecules. In our laboratory, we are primarily focused on understanding the function of the pro-apoptotic members and of the caspases in mitochondrial-dependent and independent pathways. We are also interested in elucidating the non-apoptotic functions of these proteins. Our studies are divided into three major lines of research:

Defining the mitochondrial-dependent and independent functions of BID

BID, a pro-apoptotic BCL-2 family member, plays a critical role in the TNFα/Fas death receptor pathway in-vivo. Receptor activation leads to caspase cleavage of cytosolic BID, and the truncated product (tBID) translocates to the mitochondria to induce cytochrome c (Cyt c) release, which, in turn, activates a downstream caspase program (Fig. 1). Several studies have indicated that tBID functions by inducing the formation of BAX or BAK oligomers, which may form channels for the release of Cyt c. We have recently found that TNFα also triggers the oligomerization of tBID. Moreover, enforced dimerization of tBID in intact cells leads to Cyt c release without inducing the oligomerization of BAX or BAK. Thus, tBID may act by two alternative pathways to induce the mitochondrial apoptotic program. Our future goal is to determine the mechanism of action of the tBID oligomer and its physiological importance.

tBID is not the only active form of BID, since a non-cleavable BID mutant is fully capable of inducing apoptosis. Non-cleavable BID induces caspase cleavage in a Cyt c-dependent and independent manner. Interestingly, cytosolic BID is found as part of a high molecular weight protein complex, which may be related to its Cyt c-independent function. Our future goals are to define the exact cytosolic function of BID and identify the proteins that are involved in this mitochondrial-independent pathway.

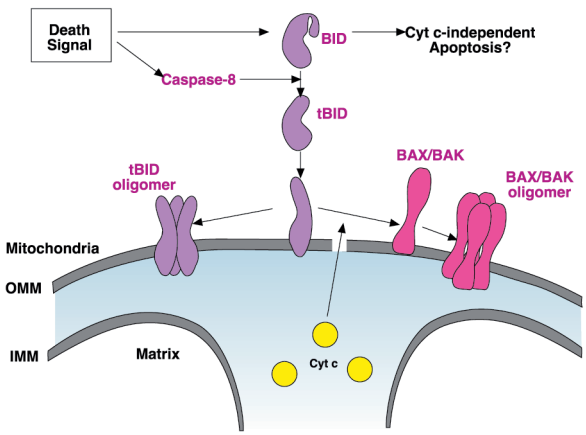

Fig. 1 *Proposed mechanisms for BID and tBID-induced apoptosis. BID is cleaved by caspase-8 to truncated tBID, which translocates to the outer membrane of mitochondria (OMM). At the OMM, tBID either homooligomerizes or induces the oligomerization of BAX/BAK, leading to Cyt c release. Alternatively, cytosolic uncleaved BID may activate a death pathway independent of Cyt c.*

Exploring the cellular functions of BAX using yeast as a model system

BCL-2 family members and caspases do not exist in yeast. Nevertheless, yeast has been demonstrated to be a relevant model system to explore the cellular functions of the BCL-2 family members. We are using the yeast *S. cerevisiae* to study the pro-apoptotic BAX molecule. Yeast are able to grow under both respiratory and fermentative conditions, making them a unique tool to assay the functional involvement of mitochondria in the effects induced by BAX. Using the tetracyclin expression system enables growth in both respiratory and fermentative conditions. Interestingly, wild type yeast cells are much more resistant to BAX when grown in ethanol (respiration conditions) as opposed to glucose (fermentation conditions). Moreover, in glucose, BAX was capable of killing non-respiring rho0 cells (cells lacking mitochondrial DNA). Thus, BAX can induce death independent of mitochondrial respiration. Identifying the cellular target(s) of BAX in yeast may shed new light on its mode of

action in mammalian cells. We have also found that BAX acts specifically to delay the exit from the G1 phase in yeast and has no effect on the progression through the S and G2/M phases. Our future goal is to define how these cell cycle aberrations are related to the cell death process and whether they are relevant to the process in mammalian cells.

Studying the role of caspases in the rat ovarian follicle

Apoptosis, or programmed cell death (PCD) plays a prominent role in the postnatal ovarian cycle and atretic degeneration due to PCD, rather then ovulation, is the ultimate fate of the vast majority of ovarian follicles in mammals. Using the well-characterized model of inducing atresia of preovulatory follicles in-vivo by hypophysectomy, we have found that DNA fragmentation has occurred but effector caspases (i.e., caspase-3, -6, -7) were not activated. To further analyze this phenomenon, we have used an in-vitro model of inducing atresia of preovulatory follicles by serum starvation. Using this model, we have found that DNA fragmentation has occurred but only very low levels of caspase-3 activation were detected. Moreover, inhibition of effector caspases using the broad caspase inhibitor, zVAD-fmk, only partially inhibited DNA fragmentation. In this model, addition of gonadotropins (i.e., luteinizing hormone (LH)) induces ovulation and inhibits atresia. As expected, addition of LH inhibited DNA fragmentation but surprisingly also induced high levels of caspase-3 activation. Our goal is to further define the role of effector caspases in the process of ovulation and determine whether caspases are essential for this process in-vivo. This project is performed in collaboration with Dr. Alex Tsafriri.

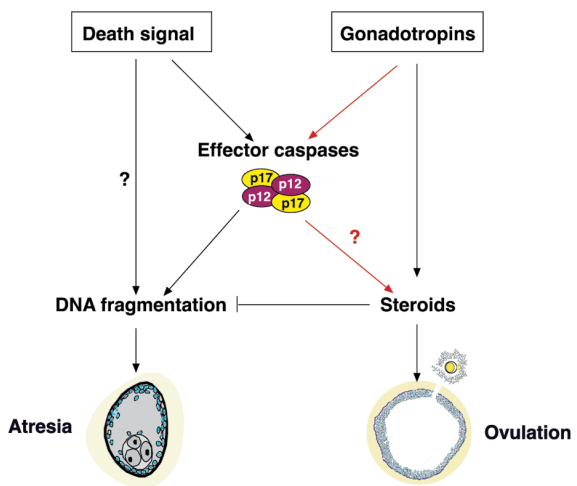

Fig. 2 *The possible duel role of caspases in the rat preovulatory follicle. A death signal leads to DNA fragmentation and atresia of preovulatory follicles. On the other hand, gonadotropins lead to steroid production and ovulation and in addition to the inhibition of atresia. Effector caspases seem to play a role in both pathways.*

Selected Publications

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