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BID – a multi-faceted regulator of cell-death

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Programmed cell death or apoptosis is essential for both the development and maintenance of tissue homeostasis in multicellular organisms. Caspases, a family of cysteine proteases, are the major executioners of the apoptotic process, whereas the BCL-2 family members are critical regulators of this process. Members of the BCL-2 family include both anti- and pro-apoptotic proteins. The BH3-only proteins (e.g., BID) are an important subset of the pro-apoptotic proteins. In our laboratory, we are primarily focused on understanding the function of BID in cell-death processes. These studies are divided into two major lines of research: the mechanisms of action of BID at the mitochondria and the involvement of BID in the response of cells to DNA damage. In another line of research we are using the rat ovary as a model system to study life and cell-death processes in a physiological context. Currently, we are studying the involvement of caspase-3 in these processes.

Mitochondria in apoptosis: Exploring the role of a novel mitochondrial protein in tBID-induced apoptosis

BID 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 (see figure). It is commonly believed that the major apoptotic function of tBID is to induce permeabilization of the outer mitochondrial membrane (OMM), leading to Cyt c release from the inter membrane space (IMS). However, recently, new data have demonstrated that tBID, which resides in the OMM, also induces reorganization of the inner mitochondrial membrane (IMM), leading to rapid and complete release of Cyt c and likely other constituents of the IMS involved in modulating the apoptotic response. The mechanism by which tBID induces reorganization of the IMM and the importance of this process to apoptosis *in-vivo* is poorly understood. In our studies, we demonstrate that in apoptotic cells tBID interacts with a novel, uncharacterized 33kD mitochondrial protein (p33), which is related to a family of IMM channels/carriers. p33 is an excellent candidate for connecting tBID to the IMM since it is exposed on the surface of

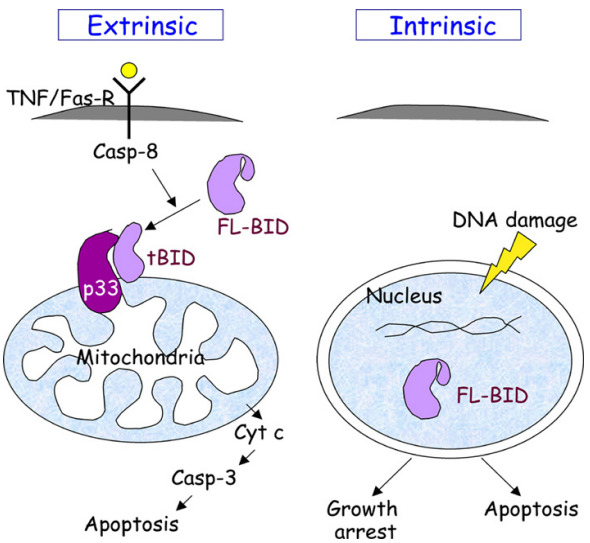

Fig. 1 *BID plays a role in both the TNF/Fas and DNA damage pathways. Left: In the TNF/Fas (extrinsic) pathway, cytosolic FL-BID is cleaved by caspase-8 to generate tBID, which translocates to the mitochondria to permeabilize the OMM via BAX/BAK. p33 is a novel mitochondrial protein, which we propose to be involved in tBID-induced remodeling of mitochondrial structure. Right: In the DNA damage (intrinsic) pathway, DNA damaging reagents can lead to either growth arrest or apoptosis. We propose that nuclear FL-BID is involved in the decision of whether cells with damaged DNA will survive or die.*

mitochondria and is predicted to reside in the IMM. Thus, p33 might be the mediator of tBID-induced remodeling of mitochondrial structure leading to apoptosis (see figure).

DNA damage and apoptosis: Is full-length BID involved in sensing DNA damage?

The BH3-only proteins are sensors of intracellular damage. These proteins are held in check by diverse mechanisms and seemingly at cellular locations in which they can sense/communicate a specific damage. Currently, it is unknown whether BID is involved in sensing a certain type of intracellular damage. In our studies, we demonstrate that full-length (FL) BID is partially localized to the nucleus in mouse embryonic fibroblasts (MEFs) and that certain types of DNA damage induce its rapid phosphorylation. In addition, we show that FL-BID is a potent inducer of apoptosis, which is required for DNA damage-induced apoptosis in MEFs. Thus, FL-BID might be involved in both sensing/processing DNA damage and in executing apoptosis in this pathway (see figure).

Apoptosis versus ovulation in the rat ovary: Is caspase-3 playing a role in both processes?

Atresia is a well-documented process, in which ovarian follicles are eliminated by apoptosis. Greater than 99% of ovarian follicles undergo atresia during reproductive life and less than 1% reach ovulation and differentiate into corpora lutea. Follicular atresia is primarily achieved by cell death of somatic granulosa cells (GC), whereas the peripheral theca cells (TC) do not seem to be involved in this process. The caspase-3 gene knockout has defined cell lineage specificity for apoptosis signaling in the ovary, however the exact role of caspase-3 during atresia is largely unknown. The experimental paradigm of atresia versus ovulation of rat ovarian follicles gives us the opportunity to analyze the cellular functions of caspase-3 during a physiologically relevant process.

We have used cultured rat preovulatory follicles to examine the regulation of caspase-3 in follicles undergoing apoptosis. Culturing follicles in the presence or absence of serum resulted in the induction of apoptosis of GC, which was accompanied by caspase-3 activation. Surprisingly, the addition of luteinizing hormone (LH), which induces ovulation and inhibits apoptosis, further increased caspase-3 activity. Immunohistochemistry studies of the LH treated follicles indicated that the active caspase-3 was predominantly localized to the peripheral TC. Moreover, the elevation in caspase-3 activity in TC was accompanied by an increase in apoptosis. Finally, we demonstrated that in freshly isolated preovulatory follicles and in antral follicles in intact ovaries, the expression level of pro-caspase-3 is significantly higher in TC than in GC. Thus, caspase-3 is mainly localized to TC and LH regulates its apoptotic activity in cultured follicles. The fact that TC are not involved in atresia *in-vivo*, suggests that caspase-3 might play a non-apoptotic role during ovulation. This project is performed in collaboration with Dr. Alex Tsafriri.

Selected Publications

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Acknowledgements:

Atan Gross is the incumbent of the Armour family career chair of cancer research. Our work is supported by the Israel Science Foundation, the Israel Cancer Research Foundation, Woman Health Research Center, and the Y. Leon Benoziyo Institute for Molecular Medicine.