Regulatory mechanisms controlling the balance between cell life and death

Atan Gross

Yehudit Zaltsman

Michal Schwarz

Hagit Niv

Keren Yacobi

Iris Kamer

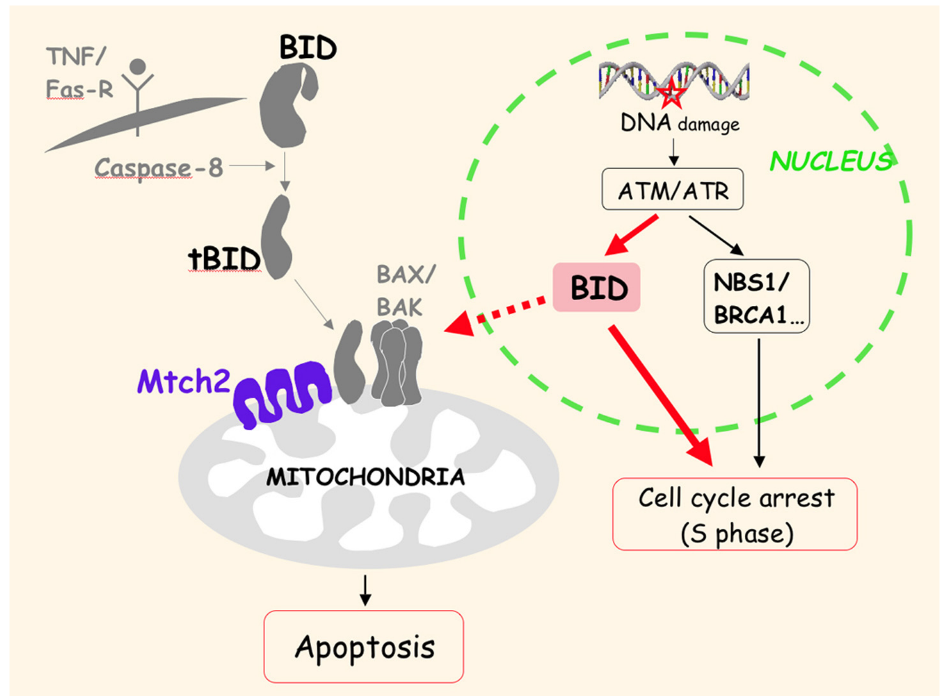
Galia Oberkovitz

Liat Shachnai

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 that act as sentinels of intercellular damage. In our laboratory, we are focused on elucidating the mechanisms that balance between cell life and death, and we are using BID as our primary tool to study these mechanisms. We are also using the rat ovary as a model system to study the balance between cell survival and apoptosis in a physiological context.

Regulation of Mitochondrial Function during Apoptosis

Activation of the TNF-R1 receptor results in caspase-8-mediated cleavage of *BID* into truncated *BID* (p15 t*BID*), which translocates to the mitochondria (see figure). At the mitochondria, t*BID* induces the activation of BAX and BAK, which are the executioners of the mitochondrial apoptotic program. To identify proteins that interact with t*BID* at the mitochondria in apoptotic cells we used cross-linkers. Using this approach, we have revealed that in TNFα-activated cells, t*BID* becomes part of a 45kD cross-linkable mitochondrial complex (Grinberg et al., 2002). We have later purified this complex and identified mitochondrial carrier homolog 2 (Mtch2) as the t*BID* partner in this complex (Grinberg et al., 2005, Gross, 2005). Mtch2 is a novel and previously uncharacterized 33kD protein, which is related to a family of carriers that catalyze the transport of metabolites across the inner mitochondrial membrane. We have also reported that Mtch2 resides in a large protein complex, and that TNFα leads to the recruitment of t*BID* and



Schematic model for the double agent role of BID in cell life and death. Left: In the TNF/Fas death-receptor pathway, BID is cleaved to generate tBID, which translocates to the mitochondria to interact with BAX/BAK and Mtch2, a novel tBID target that we have recently identified. Right: Following DNA damage, ATM and ATR are activated and can cause either cell cycle arrest or apoptosis. We can now add BID to the list of ATM targets, which is important for both cell cycle arrest at the S phase and apoptosis.

Department of Biological Regulation

972 8 934 3656

972 8 934 4116

atan.gross@weizmann.ac.il

www.weizmann.ac.il

BAX to this complex. More recently we found that knocking down the expression of Mtch2 sensitizes cells to TNFα and Fas-induced apoptosis and sensitizes mitochondria to the toxic effects of t*BID*. Thus, inhibiting the function of Mtch2 might be an important step in activating the mitochondrial apoptotic program in the TNFα/Fas pathway. Our present/future studies are focused on elucidating the importance and function of Mtch2 in healthy cells and in cells signaled to die via the TNF-R1/Fas death–receptor pathway using Mtch2 knock out cells and mice, and by identifying and later characterizing the components of the Mtch2-resident complex.

The Balance between Cell Cycle Arrest and Apoptosis in response to DNA Damage

In a second line of research in the lab, we are studying the involvement of *BID* in the response of cells to DNA damage. We have initially found that a caspase-8 non-cleavable *BID* mutant sensitizes *BID*-/- mouse embryonic fibroblasts (MEFs) to DNA damage-induced apoptosis (Sarig et al., 2003). These results suggested that full-length *BID* is involved in the response of cells to DNA damage. In search of the mechanism by which *BID* is activated in this pathway, we found that DNA damage leads to the phosphorylation of full-length *BID* by the ataxia-telangiectasia mutated (ATM) kinase, and that this phosphorylation is important for cell cycle arrest at the S phase and for inhibition of apoptosis (Kamer et al., 2005, Gross, 2006)(see figure). Thus, our studies demonstrated for the first time that *BID*, a member from the core apoptotic regulatory machinery (BCL-2 family) receives direct imputes from a key regulator of the cell cycle arrest/DNA repair machinery (ATM), and therefore is an excellent candidate to coordinate/balance between genotoxic stress responses and apoptotic cell death. Our more recent results demonstrate that whole-animal irradiation induces the prominent phosphorylation of *BID* in lymphoid organs. Thus, *BID*'s "balancing act" between cell survival and cell death might be most relevant to the homeostasis of lymphoid tissues, and therefore might have important implications for suppression of tumor development in these tissues. Our present/future studies are focused on further understanding this role of *BID* by determining the cellular location of phosphorylated *BID*, and by attempting to purify proteins that associate with the phosphorylated form. To determine the importance of *BID* phosphorylation *in vivo*, we have recently generated a *BID* knock-in mouse, in which the endogenous *BID* gene has been replaced with a gene that drives the expression of a *BID* protein carrying mutations in the ATM phosphorylation sites.

The Balance between Cell Survival and Apoptosis in the Rat Ovary

The ovary is an excellent experimental paradigm to explore the cellular functions of apoptotic players in a physiologically relevant process. In the ovary, atresia is a well-documented process, in which most of the growing ovarian follicles are eliminated by apoptosis. We have initially found that luteinizing hormone (LH) enhances caspase activity and apoptosis in the theca-interstitial cells of rat preovulatory follicles in culture (Yacobi et al., 2004). In the past two years we have continued to use cultured follicles to examine whether LH-induced caspase activation is related to the ability of LH to induce steroid production. In these studies, we used two inhibitors of steroid production: aminoglutethimide, an inhibitor of P450scc located at the mitochondria, and epostane, an inhibitor of 3βHSD located at the endoplasmic reticulum. We found that treatment with aminoglutethimide, but not with epostane, significantly reduced LH-induced caspase activation and apoptosis in theca-interstitial cells (Yacobi et al., submitted). Thus, inhibition of the early stages of steroidogenesis at the mitochondria inhibits LH-induced caspase activation. To examine whether caspase activity, in turn, might affect steroid production, we used two broad caspase inhibitors and found that inhibition of caspases attenuated LH-induced progesterone production. Thus, a functional link exists between steroid production and caspase activation in cultured follicles. A similar functional link might also exist *in vivo* since we found that the human chorionic gonadotropin (hCG)-induced increase in progesterone production in rats was accompanied by an increase in caspase activity. Thus, our studies reveal a novel linkage between two seemingly distinct processes in which LH-induced caspase activation is coupled to steroid production at the mitochondria. Our present/future studies are focused on defining the mechanistic details of the steroid-caspase link, and to determine the functional importance of this link *in vivo*. This project is performed in collaboration with Dr. Alex Tsafriri.

Selected publications

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