Mechanisms controlling cell life and death decisions

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Programmed cell death or apoptosis is essential for both the development and maintenance of tissue homeostasis in multicellular organisms. The BCL-2 family members are critical regulators of the apoptotic program, whereas the caspase proteases are the major executioners of this program. 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 BID is one of our primary tools to study these mechanisms.

The role of mitochondrial Mtch2/Mimp in apoptosis and embryogenesis

In the extrinsic death pathway, apoptosis is initiated through activation of the TNF/Fas receptors. Activation of these receptors results in the cleavage of pro-apoptotic BID into truncated BID (tBID), which translocates to the mitochondria to induce oligomerization of pro-apoptotic BAX, followed by mitochondrial outer membrane permeabilization (MOMP). This event results in the release of apoptogenic factors such as cytochrome c. However, the mechanism by which tBID triggers MOMP is largely unknown. Mitochondria carrier homolog 2/Met-induced mitochondrial protein (Mtch2/Mimp) was identified in our lab as part of a complex with tBID in cells signaled to die by TNF (Grinberg et al., 2005; Gross, 2005; Schwarz et al., 2007) (see Figure). Mtch2/Mimp is a novel and previously uncharacterized 33-kDa protein, which is related to the family of mitochondrial carrier proteins. We have revealed that knocking out Mtch2/Mimp in mice results in embryonic lethality, and analysis of time pregnancies revealed that Mtch2/Mimp-/- embryos are not viable beyond E7.5. Histological analysis of the Mtch2/Mimp+/+ and the Mtch2/Mimp-/- embryos indicated that the null embryos are morphologically abnormal and do not seem to undergo complete gastrulation. Moreover, Mtch2/Mimp mRNA is highly expressed in the extraembryonic (ExEm) region of E7.5 wild type embryos, and this region is largely impaired in the Mtch2/Mimp-/- embryos. Thus, Mtch2/Mimp might play a critical role in the formation of the ExEm region. To study the connection between Mtch2/Mimp and apoptosis we generated Mtch2/Mimp-/- stable embryonic stem (ES) cell lines carrying either an empty vector or Mtch2/Mimp. Using these lines we demonstrated that the presence of Mtch2/Mimp sensitizes cells to tBID-induced MOMP. Thus, we discovered that Mtch2/Mimp is critical for normal embryonic development, and is an important positive regulator of tBID-induced apoptosis at the mitochondria. Future studies using a newly generated Mtch2/Mimp conditional knockout mouse will enable us to further elucidate the role of Mtch2/Mimp in apoptosis and embryogenesis.

Using blue-native gel electrophoresis (BN-PAGE) we have found that Mtch2/Mimp exists as part of a large mitochondrial complex that contains tBID, BAX and additional unidentified proteins. We have named this multi-protein complex the "Mtch2/Mimp-eosome". Two independent and complimentary approaches are being taken to identify the components comprising the Mtch2/Mimp-eosome: 1) Chemical cross-linking. We are using Mtch2/Mimp and several BCL-2 family members (e.g., BAX and BIM) as baits to identify interacting proteins comprising this complex; 2) A siRNA screen to identify resident mitochondrial proteins involved in the tBID-Mtch2/Mimp signaling pathway. For this screen we are using a SMART pool library designed against all the known mitochondrial proteins. This library includes siRNAs against 708 mitochondrial proteins, and was designed by Dharmacon according to our specifications. We are in the process of establishing a number of live imaging systems to follow MOMP.

The role of ATM mediated *BID* phosphorylation in the DNA damage response

In 2005, we have reported that BID is also localized to the nucleus and is required for cell cycle arrest at the S phase and inhibition of apoptosis following DNA damage. This surprising and new pro-survival function of BID is regulated by its phosphorylation on serines 61 and 78 by the ataxia-telangiectasia mutated (ATM) kinase, a key guardian of genome integrity (Kamer et al., 2005; Zinkel et al., 2005; Gross, 2006; Zinkel et al., 2006; Zinkel et al., 2007)(see Figure). We also found that BID's pro-survival activity involves its nucleo-cytoplasmic shuttling since DNA damage triggers the nuclear export of BID, and artificially trapping BID in the nucleus inhibits its ability to induce cell cycle arrest at the S phase (Oberkovitz et al., 2007). To assess the relevance of our findings to the *in vivo* setting, we generated a BID knock-in mouse, in which the endogenous BID gene has been replaced with a gene that drives the expression of a non-phosphorylatable BID protein ($BID^{S61A/S78A}$ or BID-AA). Using cells from the BID-AA mice we found that primary T and B cells demonstrate a defect in the intra S-phase DNA damage checkpoint, increased chromosomal damage, and increased apoptosis in response to DNA damage. Thus, BID's S phase arrest function seems to be critical for T and B cells to preserve genomic stability and to survive following.

To determine how BID is physically executing its function(s) in the DNA damage response, we took a biochemical approach to purify proteins that associate with BID using the formaldehyde cross-linker. We revealed that BID is found as part of a 50-kDa cross-linked complex in healthy cells and in cells treated with DNA damage, suggesting that BID may execute its

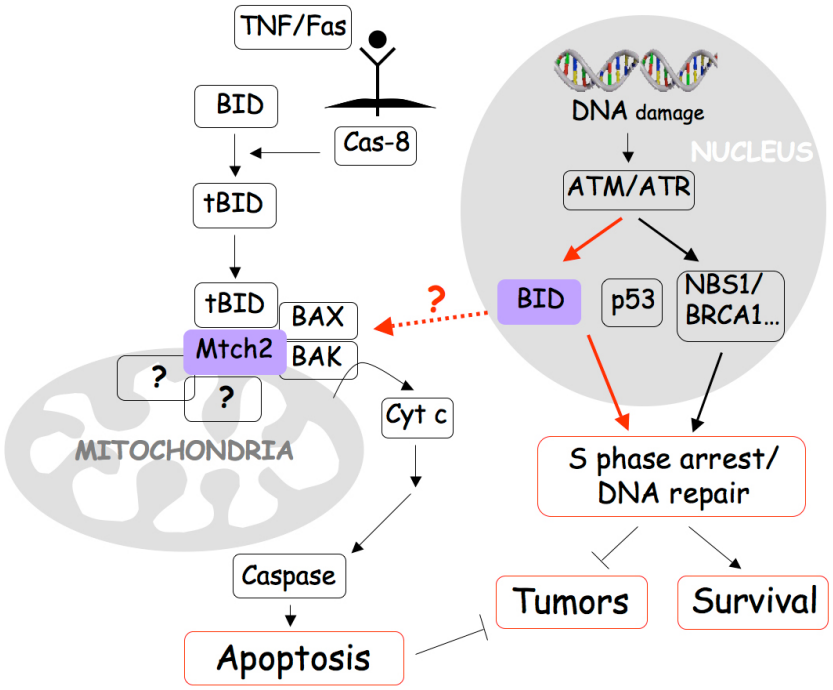

Fig.1 *BID as a double agent. Left: In the TNF/Fas death-receptor pathway, BID is cleaved to generate tBID, which translocates to the mitochondria to interact with the Mtch2/Mimp-eosome and BAX/BAK to induce MOMP. Right: Following DNA damage, ATM and ATR are activated and can lead to either survival or apoptosis. BID is an ATM target, which is important for cell cycle arrest, DNA repair and survival. It still remains unknown how BID connects between the DNA damage pathway in the nucleus and the apoptosis pathway at the mitochondria. Both pathways that involve BID are eventually critical for inhibition of tumorigenesis.*

function by a stable interaction with another protein. Finally, BID was recently shown to be phosphorylated on Ser61 in the Eμ-*myc* mouse model. Interestingly, we found that breeding BID-AA and wild-type mice with Eμ-*myc* mice resulted in accelerated onset of Myc-induced B cell lymphomas in the BID-AA background, suggesting that BID phosphorylation is playing a role in Myc-induced lymphomagenesis. These studies and the studies described above further strengthen the link between BID and the DNA damage response. We anticipate that if BID is indeed playing such a pivotal role in cell fate decisions in lymphoid tissues then our studies will have important implications for tumor development in the lymphoid lineage, as well as for genomic instability syndromes.

Selected publications

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