Molecular mechanism that regulate Bid-induced apoptosis

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Apoptosis is a highly regulated mechanism of cell death that is required for normal development and maintenance of tissue homeostasis. One of the key events in many types of apoptosis is the release of mitochondrial cytochrome c to the cytosol. Cytochrome c then triggers the formation of a complex containing procaspase-9 and APAF-1, which leads to activation of caspase-9. Caspase-9 is an initiator caspase that activates procaspases, resulting in a cascade of proteolytic events and apoptotic death. Diverse upstream death signals appear to be coupled to downstream events in mitochondria through the activation of members of a subgroup of the Bcl-2 family, BH-3 only proteins, which contain only one of the four domains that define Bcl-2 proteins, the BH-3 domain. Several BH-3 only proteins can be regulated by different posttranslational mechanisms. Signaling pathways activated by certain growth factors induce phospholylation of Bad, allowing 14-3-3 scaffold proteins to bind and sequester it from mitochondria. Bim is normally sequestered to the microtubular dynein motor complex. Certain apoptotic stimuli free Bim, allowing it to translocate to mitochondria. Bid, another BH-3 only protein, can be cleaved by caspase-8 after Fas/TNF-R1 engagement. The p15 form of truncated Bid (tBid) translocates to mitochondria and induces cytochrome c release, leading to the activation of downstream caspases and apoptosis.

In order to explore the apoptosis regulatory mechanism related to Bcl-2 family member proteins, we searched for proteins that interacted with Bid using the yeast two-hybrid system. One positive clone, termed cloneX1, that specifically interacted with tBid was isolated. CloneX1 cDNA encodes a 218 amino acid protein and contains a carboxyl-terminal hydrophobic tail. Transient transfection of clone X1 into Cos 7 cells followed by indirect immunofluorescence analysis revealed an intracellular membrane-staining pattern. Clone X1 shares no significant homology with the members of Bcl-2 family proteins. The interaction was independent of the BH3 domain of Bid, since it could also interact with BH3-mutated Bid (G94E) in which the amino acid essential for interaction among Bcl-2 family proteins was mutated. Northern blotting analyses showed the clone X1 mRNA was abundant in the brain and liver. Reverse transcriptase-polymerase chain reaction (RT-PCR) analyses showed a number of cell lines (Cos 7, HeLa, HEK293T, HepG2, Jurkat) also expressed clone X1 mRNA. Functionally, the transient overexpression of clone X1 in Cos 7 cells partially prevented Bid-induced cell death. These results suggest that clone X1 could function as a regulator of Bid-induced apoptotic pathway. Clone X1 may represent a new type of regulator of cell survival and apoptosis regulation.