Death by association: BH3 domain-only proteins and liver injury

E. S. Baskin-Bey and G. J. Gores
Mayo Clinic College of Medicine, Rochester, Minnesota

Baskin-Bey, E. S., and G. J. Gores. Death by association: BH3 domain-only proteins and liver injury. Am J Physiol Gastrointest Liver Physiol 289: G987–G990, 2005; doi: 10.1152/ajpgi.00371.2005.—Apoptosis, a prominent form of cell death, is a prime feature of many acute and chronic liver diseases. Apoptosis requires mitochondrial dysfunction, which is regulated by proteins of the Bcl-2 family. Whether or not a cell should live or die is controlled by the interaction of multidomain Bcl-2 proteins with proapoptotic BH3 domain-only proteins of this family. Current models suggest multidomain, antiapoptotic Bcl-2 proteins prevent mitochondrial dysfunction by sequestering and/or preventing activation of its proapoptotic relatives. BH3-only proteins initiate cell death by neutralizing and or ligating multidomain prosurvival Bcl-2 proteins. Thus BH3 domain-only proteins are paramount in the apoptotic process as exemplified by the role of the BH3 domain-only protein Bid in liver injury. In this concise review, we will focus on how these BH3 domain-only proteins are regulated in the cell, their association with the Bcl-2 family of proteins, and finally, current information regarding their involvement in liver apoptosis and injury.

apoptosis; mitochondria; death receptors

LIVER CELL DEATH IS A UNIVERSAL feature of most chronic and acute human hepatobiliary diseases. The consequences of sustained liver cell death include hepatic fibrosis, which can culminate in cirrhosis, portal hypertension, and organ failure. Because both sustained and massive acute cell death can be associated with considerable morbidity and loss of life, rational strategies to inhibit liver cell death are of broad scientific and clinical interest. Potential therapeutic strategies, however, must be predicated on a fundamental understanding of the cellular mechanisms mediating cell death. Although tremendous progress has been made in our understanding of cell death, there are considerable gaps in knowledge regarding the specific processes causing liver cell death.

Apoptosis, a prominent form of cell death, is a cardinal feature of many acute and chronic liver diseases including that occurring during viral hepatitis, cholestasis, mutations in α1-antitrypsin, copper overload states, unhealthy alcohol use, nonalcoholic fatty liver disease, and ischemia-reperfusion liver injury (9). In most liver diseases, apoptosis is associated with activation of death receptors (24). After ligation by their cognate ligands, death receptors aggregate resulting in the formation of cytosolic, multimeric protein complexes triggering death signals. In hepatocytes and other liver cell types, these signals converge on mitochondria resulting in mitochondrial dysfunction and cell death (4). Intracellular stress (reactive oxygen species, sustained increases in cytosolic calcium, endoplasmic reticulum stress, etc.) can also cause cell death independent of death receptors (12). However, these lethal intracellular death signals also converge on mitochondria. Thus mitochondrial dysfunction is a “checkpoint” for many forms of cellular injury.

Mitochondrial dysfunction in cell death involves permeabilization of the outer mitochondrial membrane releasing cytochrome c, endonuclease G, apoptosis-inducing factor, and high temperature requirement A2/Omi into the cytosol, which are all death effectors (4). Outer mitochondrial membrane permeabilization is regulated by members of the B cell lymphoma 2 (Bcl-2) family of proteins. This family can be further stratified based on the homology domains and function. All of the members of this family have protein domains homologous to Bcl-2, which have four such domains labeled Bcl-2 homology domains (BH1, -2, -3, and -4) (18). Antiapoptotic Bcl-2 multidomain proteins include Bcl-2, Bcl-xL (Bcl-extra long), A1, Bcl-w, and Boo (Bcl-2 homolog of ovary). Myeloid cell leukemia factor-1 (Mcl-1) is the only prosurvival Bcl-2 protein with 3 BH domains (BH1, -2, and -3) (18). The proapoptotic, multidomain (BH1, -2, and -3) members of this family include Bcl-2-associated X protein (Bax), Bcl-2 antagonist of cell death (Bak), Bcl-2-related ovarian killer (Bok), and Bcl-extra short (Bcl-xS); the latter has BH3 and BH4 domains only. The influence and function of these anti- and proapoptotic Bcl-2 proteins are regulated by the BH3 domain-only family members. In this respect, cell fate is at the mercy of the BH3 domain-only proteins. Indeed, this model is supported by model systems and genetic studies demonstrating that the BH3 domain-only protein EGL-1 is necessary for cell death in C. elegans.

There are eight members of the BH3 domain-only protein family. These proteins include Hrk (hara-kiri), BH3 interacting domain death agonist (Bid), Bcl-2 interacting mediator of cell death (Bim), Bcl-2 modifying factor (Bmf), p53-53-kDa promoter-upregulated modulator of apoptosis (Puma), Noxa (named for “damage”), Bcl-2 antagonist of cell death (Bad), and Bcl-2 interacting killer (Bik) (18). BH3 domain-only proteins can neutralize or derepress antiapoptotic Bcl-2 proteins allowing proapoptotic proteins such as Bax/Bak-like proteins to induce apoptosis (22). In addition, a restricted
subset of these proteins may directly bind and activate Bax/ Bak-like proteins. Thus the regulation of expression and function of BH3 domain-only proteins is critical in mediating cell death. These proteins appear to be key executioners in the cell death program, and, therefore, understanding their expression and function is paramount if we are to develop cytoprotective strategies.

THE RELEASE OF KILLERS

Because multiple stimuli trigger apoptosis, it is very likely that each stimulus uniquely uses a subset of BH3 domain-only proteins. Also, the multiplicity of the multidomain Bcl-2 pro-

teins makes it likely that specific BH3 domain-only protein-multidomain Bcl-2 protein interactions are also important in regulating cell death.

Cytoskeleton abnormalities appear to promote apoptosis via Bmf and Bim. Bmf associates with the actin cytoskeleton of hematopoietic cells. Disruption of this cytoskeleton causes release of actin bound Bmf triggering apoptosis. This mechanism of cell death has been implicated in anoikis (cell death induced by release from cellular and structural attachments). Bim is associated with the microtubule network, and lethal disturbances in microtubule function result in the discharge of Bim. DNA damage (e.g., radiation) via p53 activation induces transcription of Noxa and Puma. Bad is normally phosphorylated and, as such, is sequestered in the cytoplasm by the scaffold protein 14-3-3. Growth factor deprivation has been reported to result in Bad dephosphorylation and cell death (18). Bid is uniquely regulated in apoptotic cascades. Bid is cleaved by death receptor-activated caspase 8 and/or 10 generating truncated Bid (tBid). This latter form of Bid functions as an operational BH3 domain-only protein causing cell death. Bik exists as a phosphoprotein. Mutation of the phosphorylation sites reduces its apoptotic activity without significantly affecting its ability to heterodimerize with Bcl-2. Regulation of Hrk remains poorly defined. In addition to the above, it is likely that all these proteins can be partly regulated by transcription, phosphorylation, and ubiquitination pathways. The role of micro-RNA in their regulation is also unknown but surely will prove to be important.

GAGGED AND BOUND

Considerable information has recently become available as to how these BH3 domain-only proteins mediate cell death. We will focus on Bcl-xL and Mcl-1’s control of Bak as a model system, which we believe to be the most developed (Fig. 1). Most BH3 domain-only proteins were discovered in protein interaction screens using Bcl-2-like proteins as bait (18). Many

Table 1. Selective binding of BH3 domain-only proteins to prosurvival Bcl-2 proteins

<table>
<thead>
<tr>
<th>Prosurvival Bcl-2 Proteins</th>
<th>A1</th>
<th>Bcl-2</th>
<th>Bcl-W</th>
<th>Bcl-xL</th>
<th>Mcl-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bad</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
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<tr>
<td>Bid</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
<td>Bik</td>
<td>X</td>
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<td>Bim</td>
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<tr>
<td>Bmf</td>
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<td>Hrk</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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</tr>
<tr>
<td>Noxa</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Puma</td>
<td>X</td>
<td>X</td>
<td>X</td>
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</tr>
</tbody>
</table>

Table 2. Expression of BH3 domain-only proteins in nonmalignant hepatocytes

<table>
<thead>
<tr>
<th>Known</th>
<th>Unknown</th>
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<tbody>
<tr>
<td>BID (Refs. 1, 8, 10, and 19)</td>
<td>BMF</td>
</tr>
<tr>
<td>NOXA (Ref. 5)</td>
<td>HRK</td>
</tr>
<tr>
<td>BAD (Ref. 13)</td>
<td>BIK</td>
</tr>
<tr>
<td>PUMA (Ref. 16)</td>
<td></td>
</tr>
<tr>
<td>BIM (Ref. 20)</td>
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</tbody>
</table>
BH3 domain-only proteins can bind with high affinity to Bcl-2-like proteins and trigger apoptosis. The BH3 domain of BH3 domain-only proteins binds to a clef lined with hydrophobic side chains formed predominantly by the BH1 domain (α-helix 5) of the prosurvival protein (14, 15). BH3 binds as an amphipathic α-helix (consisting of 9–16 amino acids) with its hydrophobic side contacting the Bcl-2 protein (15). Killing induced by each BH3 domain-only protein appears to reflect their ability to gain and bind tightly to prosurvival Bcl-2-like molecules present in the cell (3). The neutralization of these “protector proteins” permits spontaneous activation of Bax/Bak-like proteins instigating cell death (Fig. 1, model 1). However, Bid and Bim can also directly bind Bax/Bak-like proteins. In this model, other BH3 domain-only proteins are necessary to derepress the antiapoptotic proteins, but Bim and tBid are also required to ligate and activate Bax and/or Bak to induce cell death (Fig. 1, model 2).

KILLERS CHOOSE THEIR PREY

BH3 domain-only proteins exhibit distinct preferences of association to prosurvival Bcl-2-like proteins (Table 1). Bim and Puma target all prosurvival Bcl-2-like proteins equally. Bad and Bmf prefer Bcl-2, Bcl-xL, and Bcl-w. Bik and Hrk favor Bcl-xL, Bcl-w, and A1. Noxa associates with Mcl-1 and A1. Bid fancies Bcl-xL, Bcl-w, and A1 over Bcl-2 and Mcl-1 (3). Only Noxa, Puma, and Bim are capable of neutralizing Mcl-1. In contrast, multiple BH3 domain-only proteins may defuse Bcl-xL. Counteraction of both Bcl-xL and Mcl-1 by BH3 domain-only proteins is ultimately necessary for Bak activation and propagation of cell death. The lesson of this example is that several BH3 domain-only proteins likely act in concert to induce cell death.

TO LIVER OR NOT TO LIVER

The Bcl-2 family has a strong role in regulating death receptor-mediated apoptosis in hepatocytes (Table 2). Activation of the intrinsic, mitochondria-mediated, or extrinsic death receptor-mediated pathways occurs in the liver; however, the death receptor-mediated pathway appears to be more common, because the liver is remarkably sensitive to death receptor signaling (24). Hepatocyte apoptosis initiated by death receptors is regulated by Bid (1). Bid resides in the cytoplasm of the cell in an inactive full-length form. After death receptor (Fas, TNF-α, and TRAIL) activation, procaspase 8 and 10 are recruited to a multimeric receptor-based protein complex (2). Caspase 8 and 10 cleave Bid to generate tBid, which is then myristylated (25). tBid then translocates to the mitochondria, binds cardiolipin on the membrane surface, and induces mitochondrial dysfunction (7). tBid is thought to promote Bax and Bak activation, which is obligatory for mitochondrial cytochrome c release (11, 21). Indeed, Bid knockout hepatocytes are resistant to Fas (23) and TNF-α-induced cell death (8). Targeted reduction in Bid expression by antisense technology diminishes cholestatic liver injury following bile duct ligation in the mouse (10), c-Jun NH2-terminal kinase (JNK) inhibition blocks formation of tBid and hepatocellular apoptosis of hepatocytes after ischemia-reperfusion injury (19). We have also recently observed downregulation of Bax and Bak and upregulation of Mcl-1 in mice after treatment with a constitutive androstane receptor (CAR) agonist. This led to marked reduction in death receptor-mediated liver cell injury (unpublished data). The effect of CAR activation on expression of all BH3 domain-only proteins is unknown, but it is a subject of interest.

How does the current knowledge of tBid and liver cell death fit into the working models of BH3 domain-only protein regulation of cell death? In the model of BH3 domain-only protein derepression of antiapoptotic proteins (Fig. 1, model 1), tBid can neutralize Bcl-xL but not Mcl-1. Given that enhanced Mcl-1 protects against Fas-mediated hepatocyte apoptosis (17), other BH3 domain-only proteins, such as Noxa, Puma, and/or Bim, must also be involved in Fas-induced hepatocyte apoptosis. Appropriate knockout mice are available to test this hypothesis. This concept, therefore, can and should be rigorously tested.

What about the model of BH3 domain proteins directly ligating Bax and Bak? In this model (Fig. 1, model 2), tBid is capable of directly activating Bax/Bak, which explains, in part, the observations thatBid knockout mice are less susceptible to death receptor-mediated hepatocyte injury. However, this model also requires that Bak be liberated from Bcl-xL and Mcl-1. Liberation of Bak from these proteins cannot be accomplished solely by tBid because it does not bind Mcl-1. Therefore, this reasoning also predicts that additional BH3 domain-only proteins must also be involved in death receptor-mediated apoptosis. These concepts also help explain why multiple BH3 domain-only proteins are expressed by the hepatocytes (Table 2).

Other than Bid, not much information is available regarding the role of BH3-domain proteins in liver injury, although several are expressed (Table 2). Inactivation of Bid through phosphorylation by Akt reduces ischemic injury during rat liver graft preservation (13). TNF-α-induced apoptosis is repressed by increased expression of c-Jun in primary hepatocytes, which antagonizes p53-dependent expression of NOXA (5). PUMA expression is elevated in primary hepatocytes with loss of c-Jun or CREB function in bile acid (deoxycholic acid)-induced apoptosis (16). Prolonged insulin treatment downregulated Bim and caspase 3 activation in neonatal hepatocytes (20). However, all of these models require clarification as to the exact role of different BH3 domain-only proteins.

In summary, knowledge of the Bcl-2 subfamily BH3 domain-only proteins and their role in programmed cell death is critical for understanding cell injury in human liver diseases. These eight proteins have been proven to be intricately involved in regulation of apoptosis, but their roles in liver injury are largely unexplained. Further insight into the modulation of these killer proteins can prove useful for the development of therapeutic modalities.

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