Bid activation in kidney cells following ATP depletion in vitro and ischemia in vivo

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Am J Physiol Renal Physiol 286: F803–F809, 2004. First published December 16, 2003; 10.1152/ajprenal.00093.2003.—Bid is a proapoptotic Bcl-2 family protein, which on activation translocates to mitochondria and induces damage to the organelles. Activation of Bid depends on its proteolytic processing into truncated forms of tBid. Bid is highly expressed in the kidneys; however, little is known about its role in renal pathophysiology. In this study, we initially examined Bid activation in cultured rat kidney proximal tubular cells following ATP depletion. The cells were depleted of ATP by azide incubation in the absence of metabolic substrates and then returned to normal culture medium for recovery. Typical apoptosis developed during recovery of ATP-depleted cells. This was accompanied by Bid cleavage, releasing tBid of 15 and 13 kDa. Bid cleavage was abolished in cells overexpressing Bcl-2, an antiapoptotic gene. It was also suppressed by caspase inhibitors. Peptide inhibitors of caspase-9 were more effective in blocking Bid cleavage compared with inhibitors of caspase-8 and caspase-3. Provision of glucose, a glycolytic substrate, during azide incubation inhibited Bid cleavage as well, indicating that Bid cleavage was initiated by ATP depletion. Consistently, Bid cleavage was also induced following ATP depletion by hypoxia or mitochondrial uncoupling. Of significance, cleaved Bid translocated to mitochondria, suggesting a role for Bid in the development of mitochondrial defects in ATP-depleted cells. Finally, Bid cleavage was induced during renal ischemia-reperfusion in the rat. Together, these results provide the first evidence for Bid activation in kidney cells following ATP depletion in vitro and renal ischemia in vivo.

Apoptosis; caspase; acute renal failure; ischemia-reperfusion

ISCHEMIC INJURY TO the kidneys leads to acute renal failure, which is associated with high mortality (23). Although the development of ischemic acute renal failure involves multiple factors and may proceed in several phases, it is ultimately precipitated by sublethal and lethal damage to the tubular cells (13, 22, 38). Under these conditions, massive cell death in the necrotic form can be identified, and as a result, termed acute tubular necrosis. On the other hand, recent studies have suggested an apoptotic component in ischemic injury to the kidneys (5, 35). By morphology, apoptotic cells were identified in ischemia-reperfused kidneys (33). Biochemically, renal ischemia-reperfusion led to the expression and activation of caspases, a unique family of cysteine proteases responsible for disassembly of apoptotic cells (17). Endonuclease activation was also documented (4). In addition, regulation of apoptotic regulatory genes including Bcl-2 and Bax has been shown in ischemia-reperfused kidneys (3, 14). Finally, several pharmacological agents appeared to ameliorate renal tissue damage during ischemia-reperfusion, at least in part, by diminishing apoptosis (9, 18, 19). Despite these important observations, the molecular basis underlying apoptosis during renal ischemia-reperfusion remains largely unknown (5, 35).

Apoptosis is a highly regulated process that plays a central role in the maintenance of tissue homeostasis (31). Originally defined by cell morphology, apoptosis has now been characterized at the biochemical and molecular level. Two major pathways of apoptosis have been delineated (15). In the extrinsic pathway, death ligands bind to death receptors, leading to oligomerization of the receptor protein and subsequent formation of death-inducing signaling complexes and the activation of caspase-8 (2). In the intrinsic pathway, cellular stress leads to mitochondrial disruption, followed by the release of apoptogenic molecules such as cytochrome c (6). In the cytosol, cytochrome c associates with Apaf-1, resulting in the recruitment and activation of caspase-9. These two pathways, triggered differently, converge at the level of executioner caspases, promoting the development of stereotypical features of apoptotic morphology (15).

Important regulators of apoptosis include Bcl-2 family proteins (1, 16). Defined by the presence of Bcl-2 homology (BH) domains, these proteins can be proapoptotic or antiapoptotic. Specific function of individual members is determined by the presence and organization of the BH domains. For example, antiapoptotic members, such as Bcl-2 and Bcl-XL, contain four BH domains, whereas some proapoptotic molecules, such as Bax and Bak, contain three (BH1–3) and others only one, the BH3 domain (1, 16).

Bid is a unique BH3 domain-only proapoptotic protein (36). Unlike others, Bid activation depends on the proteolytic processing of intact Bid into truncated forms of tBid. tBid, generated thereby, translocates to mitochondria and leads to disruption of the organelles and the release of apoptogenic molecules such as cytochrome c (20, 24). Bid processing can be conducted by several proteases; however, caspase-8 has been shown to be the major protease responsible for Bid cleavage during death receptor-mediated apoptosis (41). Caspase-8-mediated Bid processing therefore bridges the extrinsic death receptor-mediated pathway of apoptosis to the intrinsic mitochondrial pathway (41). This provides a mechanism to amplify the execution signal and exacerbate the pace of cell demise (21, 43).

Bid is expressed in an abundant manner in the kidneys (36). However, no information has been available regarding a physiologically role of this important proapoptotic molecule in and ischemia-reperfusion led to the expression and activation of caspases, a unique family of cysteine proteases responsible for disassembly of apoptotic cells (17). Endonuclease activation was also documented (4). In addition, regulation of apoptotic regulatory genes including Bcl-2 and Bax has been shown in ischemia-reperfused kidneys (3, 14). Finally, several pharmacological agents appeared to ameliorate renal tissue damage during ischemia-reperfusion, at least in part, by diminishing apoptosis (9, 18, 19). Despite these important observations, the molecular basis underlying apoptosis during renal ischemia-reperfusion remains largely unknown (5, 35).

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renal cells. On the other hand, recent studies identified a central role for Bid in neuronal cell death during brain ischemia (30, 42). Under these considerations, we examined Bid activation in an in vitro model of renal cell ATP depletion and in an in vivo model of rat kidney ischemia-reperfusion. In the in vitro model, Bid was cleaved into truncated forms of tBid, following ATP depletion. tBid translocated to mitochondria. Bid activation was attenuated by Bcl-2 overexpression and was also suppressed by caspase inhibitors. In the in vivo model, Bid cleavage into tBid was demonstrated during renal ischemia as well as early reperfusion. Together, these results suggest the potential involvement of Bid in renal cell injury during ischemia-reperfusion.

MATERIALS AND METHODS

Materials. Rat kidney proximal tubular epithelial cells were from Dr. U. Hopfer at Case Western Reserve University, Cleveland, OH. The cells were maintained as described (39). Bcl-2-overexpressing cells were obtained by stable transfection with the Bcl-2 expression vector pBLCX-bcl-2 as described previously (32). The vector was a gift from Dr. J. Yuan at Harvard Medical School, Boston, MA. The rabbit polyclonal antibody against Bid was a gift from Dr. X. M. Yin at the University of Pittsburgh School of Medicine, Pittsburgh, PA. The rabbit polyclonal antibody specific to the active form of caspase-3 was a gift from Dr. A. Srivivasan at Idun Pharmaceuticals (La Jolla, CA). Reagents were also purchased from the following sources: cell-permeable peptide inhibitors of caspases (VAD, IETD, LEHD, DQMD) from Enzyme Systems Products (Dublin, CA); carbonyl cyanide-m-chlorophenyl hydrazone (CCCP) from Calbiochem-Novabiochem (San Diego, CA); all secondary antibodies from Jackson ImmunoResearch (West Grove, PA); and other reagents from Sigma (St. Louis, MO).

Renal ischemia. Male Sprague-Dawley rats of 250–275 g were purchased from Charles River. For experiments, the animals were anesthetized with one intraperitoneal injection of 50 mg/kg pentobarbital sodium and placed on a homeothermic table to maintain core body temperature of ~37°C. Flank incisions were made to expose both renal pedicles for clamping to induce ischemia. For ischemia-only group, kidneys were harvested at the end of 35 min of clamping. For reperfusion groups, clamps were released after 35 min of clamping and kidneys were collected after indicated periods of reperfusion. Color changes of the kidneys during the initiation of clamping and release of the clamps were monitored to indicate renal ischemia and reperfusion. Sham operation was conducted by an identical procedure except both renal pedicles for clamping to induce ischemia. For ischemia-reperfusion, clamps were released after 35 min of clamp-}

ATP depletion. Three approaches were used to deplete the cells of ATP. First and in the majority of the experiments, cells were subjected to 10 mM azide incubation in glucose-free Krebs-Ringer bicarbonate solution (composition in mM: 115 NaCl, 3.5 KCl, 25 NaHCO₃, 1 KH₂PO₄, 1.25 CaCl₂, and 1 MgSO₄; gassed with 5% CO₂). In the second approach, cells were incubated in glucose-free Krebs-Ringer bicarbonate solution with 15 μM CCCP, a mitochondrial uncoupler (10). In the third approach, cells were subjected to severe hypoxia in glucose-free Krebs-Ringer bicarbonate solution (12, 32). To remove residual oxygen and maximize the degree of hypoxia, EC Oxynre, a biocatalytic oxygen-reducing agent, was added at 1.2 U/ml in the incubation medium. After ATP depletion for indicated periods, groups of cells were returned to the full culture medium to simulate in vivo reperfusion. Morphological examination of apoptosis. Cells were stained for 2–5 min with 5 μg/ml of Hoechst 33342 in PBS at room temperature. Cell morphology was monitored by phase-contrast microscopy. Nuclear staining by Hoechst 33342 was examined by fluorescence microscopy. Typical apoptotic morphology included cellular shrinkage, nuclear condensation and fragmentation, and formation of apoptotic bodies.

Immunofluorescence of active caspase-3. Indirect immunofluorescence was performed as described previously with modifications (11, 32). Briefly, cells were grown on collagen-coated glass coverslips for experiments. After incubation, cells were fixed with 4% paraformaldehyde for 30 min at room temperature. The fixed cells were incubated for 1 h in a blocking buffer containing 2% normal goat serum. The cells were subsequently incubated for 1 h with the primary antibody, a rabbit polyclonal antibody specific for active caspase-3 (34). Finally, the cells were exposed to a Cy3-labeled goat anti-rabbit secondary antibody. Staining was examined by fluorescence microscopy.

Immunoblot analysis. Proteins were analyzed by immunoblotting using NuPAGE Gel Systems as described previously (11, 12, 32). Briefly, samples were resolved under reducing conditions on 10 or 12% Bis-Tris gels in MES running buffer and electroblotted onto polyvinylidene difluoride membranes. The blots were blocked with 5% fat-free milk and then exposed to the primary antibody overnight at 4°C. Finally, the blots were incubated with the horseradish peroxidase-conjugated secondary antibody, and antigens on the blots were revealed by reactions with chemiluminescent substrates (Pierce, Rockford, IL). The reactivity of the antibody was significantly lower for tBid than that for intact Bid. Thus the same blots were exposed separately to reveal Bid and tBid, with longer exposure for tBid.

Cellular fractionation. To analyze the intracellular distributions of Bid and tBid, cells were fractionated into cytosolic and membrane-bound organellar fractions enriched with mitochondria using low concentrations of digitonin. Digitonin at low concentrations selectively permeabilizes the plasma membranes, without solubilizing mitochondria. This method has been successfully used by us and others to study protein translocations during apoptosis (26, 29, 32, 37). Briefly, cells were exposed to 0.05% digitonin in isotonic sucrose buffer (in mM: 250 sucrose, 10 HEPES, 10 KCl, 1.5 MgCl₂, 1 EDTA, and 1 EGTA; pH 7.1) for 2 min at room temperature to collect the soluble part as the cytosolic fraction. The digitonin-insoluble part was further extracted with 2% SDS buffer to collect the membrane-bound organellar fraction. Because Bid and tBid redistribution mainly takes place between the cytosol and mitochondria (20, 24), immunoblot analysis of the organellar fraction is expected to reveal mitochondrial content of the molecules.

RESULTS

Apoptosis during recovery of ATP-depleted cells. An important determinant of ischemic cell injury is the depletion of cellular ATP (23). Thus to model renal ischemia-reperfusion, we subjected cultured rat kidney proximal tubular cells to azide treatment in the absence of metabolic substrates to induce ATP depletion. ATP-depleted cells were subsequently returned to full culture medium for recovery or “reperfusion.” Significant amounts of apoptosis developed within a few hours of recovery. As shown in Fig. 1A, many cells exhibited typical apoptotic morphology with a shrunk configuration and apoptotic bodies. Nuclear condensation and fragmentation were also evident (Fig. 1B). Of interest, caspase activation was demonstrated in the same population of cells. As shown by immunofluorescence, the active form of caspase-3 was revealed specifically in cells with typical apoptotic morphology (Fig. 1C). Vital dye exclusion assays suggested that necrotic cell death under the experimental conditions was very limited (not shown). In general, ~40% cells underwent apoptosis during 2 h of recovery period following 3 h of azide treatment. Without azide exposure, control cells had less than 5% apop-
During azide incubation alone (lanes 2–4). Of note, as indicated in MATERIALS AND METHODS, the antibody reacted at significantly higher affinity with full-length Bid of 22 kDa, compared with the truncated Bid of 15 and 13 kDa. Thus the blots were usually subjected to low exposure to reveal the 22-kDa Bid and high exposure to reveal the 15- and 13-kDa tBid, respectively. Similar two-step exposure was employed for experiments described in subsequent figures.

**Bid cleavage during apoptosis triggered by azide.** To determine whether Bid was activated during apoptosis of renal cells, we first analyzed Bid cleavage by immunoblotting, because Bid activation depends on its proteolytic processing into truncated forms of tBid. For this purpose, cells were incubated with azide in glucose-free Krebs buffer for 0–3 h alone, or followed by recovery in full culture medium. Whole cell lysates were collected in Laemmli buffer containing 2% SDS and analyzed by immunoblotting using a polyclonal antibody against Bid. The results are shown in Fig. 2. In control cells, intact Bid with an apparent size of 22 kDa was detected (lane 1). Bid was not processed during azide treatment (lanes 2–4). In sharp contrast, upon reperfusion or recovery, Bid was cleaved into truncated forms of tBid, which showed apparent sizes of 15 and 13 kDa (lanes 5–7). As a result, intact Bid decreased in the reperfused cells, accompanied by the appearance of 15 and 13 kDa tBid. Of note, reactivity of the antibody was significantly lower for tBid than that for intact Bid. Thus the same blots were exposed separately to reveal Bid and tBid, with longer exposure for tBid. Nevertheless, decreases in intact Bid and concomitant production of tBid indicated Bid processing during recovery of ATP-depleted cells.

**Bid cleavage following hypoxia or CCCP treatment.** To substantiate our observation that Bid was proteolytically processed during recovery of ATP-depleted cells, we tested other models of ATP depletion, namely, hypoxia and CCCP treatment. Severe hypoxia or anoxia leads to the arrest of cellular respiration and ATP production by oxidative phosphorylation. CCCP, on the other hand, dissipates mitochondrial membrane potential and as a result uncouples respiration from ATP generation in the organelles. As expected, in the absence of glycolytic substrates, both severe hypoxia and CCCP led to rapid declines of cellular ATP (data not shown). As for the azide-treated group, after 5 h of hypoxia or 2 h of CCCP incubation, cells were returned to the full culture medium for recovery. The results are shown in Fig. 3. Clearly, Bid cleavage was detected during recovery of the ATP-depleted cells, irrespective of the approaches for ATP depletion. Again, intact Bid decreased, which was accompanied by the production of tBid (lanes 2–4).

**Inhibition of Bid cleavage by caspase inhibitors and glucose.** To gain insights into the mechanisms underlying Bid cleavage following ATP depletion, we initially tested the effects of VAD, a broad-spectrum peptide inhibitor of caspases (11). The same experiment also tested the effects of glucose, the substrate of glycolysis, and N-acetylcysteine (NAC), an antioxidant. As shown in Fig. 4A, inclusion of 50 μM VAD or 5.5 mM glucose during azide incubation suppressed Bid cleavage, whereas 10 mM NAC was not effective. Because glucose facilitated glycolysis and prevented ATP depletion during azide treatment (data not shown), the results provide further support for a role of ATP depletion in triggering Bid cleavage.
The results also suggest that Bid cleavage might be mediated by caspases and can be dissociated from oxidative stress or the production of free radicals. To further determine the role of specific caspases in Bid cleavage, we examined the effects of peptide inhibitors with specificity toward caspase-8, caspase-9, or caspase-3 (Fig. 4B). All three inhibitors showed inhibitory effects on Bid cleavage (compare lanes 3–11 with lane 1). However, the caspase-9 inhibitor LEHD appeared to be most effective. At 20–50 μM, LEHD almost completely blocked Bid cleavage (lanes 6 and 7), whereas Bid cleavage was still evident in the presence of 50 μM IETD and DQMD (lanes 3 and 9), inhibitors of caspase-8 and -3, respectively. LEHD was also more effective in suppressing apoptosis. In the presence of 50 μM LEHD, apoptosis was reduced to 10–20% from ~40%. The results suggest an important role for a caspase-9-initiated proteolytic cascade in Bid activation and apoptosis in this experimental model.

Inhibition of Bid cleavage by Bcl-2 expression. To further examine apoptosis-associated Bid cleavage, we compared wild-type proximal tubular cells with the cells stably transfected with Bcl-2, an anti-apoptotic gene. These two types of

cells were subjected to 3 h of azide treatment in glucose-free buffer alone or followed by 2 h of recovery in full culture medium. Consistent with previous observations, in wild-type cells, apoptosis developed within a couple of hours of recovery. The development of apoptosis was completely blocked in Bcl-2-transfected cells (data not shown). Of significance, Bid cleavage was also prevented by Bcl-2 overexpression (Fig. 5: lanes 3 and 7), suggesting a close relationship between the development of apoptosis and Bid processing.

Translocation of tBid to mitochondria. The action site for Bid within the cells is the mitochondria. tBid, generated via Bid processing, translocates to mitochondria to induce pathological alterations in the organelles, releasing apoptogenic factors including cytochrome c (20, 24). To determine whether the cleaved Bid in our experimental model was indeed active, we analyzed the distribution within the cells. To this end, cells were fractionated into cytosolic and membrane-bound organelar fractions enriched with mitochondria. The fractions were subjected to immunoblot analysis of Bid/tBid. The results are shown in Fig. 6. Consistent with previous observations, only intact Bid was detected in control cells, with the majority of Bid shown in the cytosol (lanes 1 and 3). In reperfused cells following azide treatment, intact Bid as well as tBid of 15 and 13 kDa was detected (lanes 2 and 4). Of significance, while the majority of intact Bid existed in the cytosol (lane 2), tBid moved to the mitochondrial fraction (lane 4). The translocation was partial for the 15-kDa tBid and virtually complete for the 13-kDa tBid. The results suggest that Bid after cleavage into tBid became active and may be involved in subsequent mitochondrial disruption.

Bid cleavage during renal ischemia-reperfusion. To extend the in vitro observations to in vivo systems, we analyzed Bid cleavage in a rat model of renal ischemia-reperfusion. The results are shown in Fig. 7. In sham-operated controls, intact Bid of 22 kDa was detected (lane 1). Ischemia of 35 min induced Bid cleavage, releasing tBid of 15 kDa but not the 13-kDa form. Reperfusion of the kidneys for 8 h led to further Bid cleavage. As a result, intact Bid was reduced significantly, accompanied by the production of both 15-
that Bid was processed into active forms during renal ischemia. The in vitro results were supported by the in vivo observation during Bid processing translocated to mitochondria, suggesting suppressed by caspase inhibitors. Importantly, tBid released apoptosis. Expression of Bcl-2, an antiapoptotic gene, inhibited depleted cells. This was accompanied by the development of processed into tBid during recovery following azide treatment (lanes 2 and 3). The results further suggest that Bid processing might be a result of caspase activation during the development of apoptosis.

DISCUSSION

This study examined Bid activation in an in vitro model of ATP depletion and in an in vivo model of renal ischemia-reperfusion. In the in vitro model, Bid was proteolytically processed into tBid during recovery or reperfusion of ATP-depleted cells. This was accompanied by the development of apoptosis. Expression of Bcl-2, an antiapoptotic gene, inhibited apoptosis as well as Bid processing. Bid processing was also suppressed by caspase inhibitors. Importantly, tBid released during Bid processing translocated to mitochondria, suggesting a role for Bid in the development of mitochondrial pathology. The in vitro results were supported by the in vivo observation that Bid was processed into active forms during renal ischemia and early reperfusion. Together, it is suggested that Bid might be involved in renal cell injury during ischemia-reperfusion.

Bid is a proapoptotic member of the Bcl-2 family proteins, which has only one BH domain (BH3) (41). Different from other BH3-only members, Bid exhibits several unique functional and structural features. Prominent among them is that Bid activation depends on proteolytic processing into active forms of tBid. Several types of proteases were shown to be able to process Bid. In death receptor-mediated apoptosis, caspase-8 is the major protease responsible for Bid cleavage (20, 24). During myocardial ischemia-reperfusion, Bid processing was shown to be mediated by calpain (7). Our previous study demonstrated the activation of several types of caspases during ATP depletion induced by severe hypoxia (11). Consistently, the current study showed that azide-induced apoptosis was suppressed by the general caspase inhibitor VAD and specific peptide inhibitors of caspase-9, -8, and -3. Our subsequent analyses showed that the caspase-9 inhibitor was most effective in blocking Bid processing (Fig. 4B). These results suggest an important role for a caspase-9-initiated proteolytic cascade in Bid processing and the development of apoptosis in this experimental model. Whether caspase-9 per se or downstream caspase proteolyses Bid remains to be determined.

It is interesting that, although Bid activation in vitro took place only during recovery or reperfusion of ATP-depleted cells (Fig. 2), in vivo Bid processing started from ischemia and extended to early reperfusion (Fig. 7). This observation suggests that the mechanisms responsible for Bid activation in vivo could be far more complex and may involve different proteases at different stages. Such a scenario is consistent with the fact that cell injury during renal ischemia-reperfusion is a multifactorial process, which may proceed as distinguished episodes (27). During ischemia, increases in intracellular Ca^{2+} may activate calpain. Meanwhile, mitochondrial damage may also have been initiated, releasing cytochrome c and leading to caspase activation. On activation, calpain and caspases may activate Bid separately or in a collaborative manner. On the other hand, after reperfusion, the death receptor-mediated extrinsic pathway of apoptosis might be important. The activation of the Fas system has been documented during renal ischemia-reperfusion in a model of unilateral occlusion (28). Presum-
ably, Fas activation leads to the activation of caspase-8, which subsequently proteolyses Bid into the active forms of tBid. Of note, Bid activation as indicated by the production of tBid was maximal at early reperfusion and decreased significantly after 24 hr of reperfusion (Fig. 7). It would be interesting to study the activation dynamics of calpain, caspase, Fas, and Bid systematically in the same animal model.

An important question is: what does Bid do during renal ischemia-reperfusion? Apparently, without experiments in Bid-deficient cells or Bid-null mice, we can only speculate at this moment. Based on our in vitro results showing tBid translocation to mitochondria, it is suggested that Bid/tBid might promote cell death at the mitochondrial level. Damage to mitochondria, outer membranes in particular, leads to the release of apoptogenic factors including cytochrome c, Smac, Omi, AIF, and endonuclease G (25). Cytochrome c, after being released into the cytosol, associates with Apaf-1, forming apoptosome to activate caspases (6). Smac and Omi can sequester IAP, a family of caspase inhibitory proteins, to liberate the caspases for full activation (21). On the other hand, AIF and endonuclease G released from mitochondria might promote cell death in a caspase-independent manner by facilitating nuclear fragmentation (15). Although it is unclear how Bid/tBid induces pathological alterations in the mitochondria, recent studies suggested an activation cascade for Bcl-2 family proteins during apoptosis (8). In this model, BH3 domain-only proteins such as Bid move to mitochondria and activate multi-BH domain proapoptotic proteins such as Bax and Bak, leading to their oligomerization and formation of pathological pores. Antiapoptotic proteins including Bcl-2 and Bcl-XL are positioned to block apoptosis at two separate steps, through the inhibition of Bax/Bak and BH3-only proteins including Bid. Consistent with this scenario, our previous study demonstrated Bax oligomerization in the outer membrane of mitochondria during ATP depletion of rat proximal tubular cells (26). More recently, we showed that transfection of Bid into HeLa cells led to the activation of Bax and Bak, permeabilizing mitochondria to release cytochrome c. Cotransfection of Bcl-2 prevented the insertion of tBid into mitochondrial membranes and as a result, it suppressed Bax/Bak activation, cytochrome c release, and cell death (40). Further investigations using Bid-deficient cells or animals should provide insights into the role of Bid in renal cell injury initiated by ATP depletion in vitro and ischemia in vivo.

In conclusion, this study provided the first evidence for Bid activation in kidney cells following ATP depletion in vitro and renal ischemia-reperfusion in vivo. Considering the important roles played by Bid in both extrinsic and intrinsic pathways of apoptosis, the activation of this molecule suggests its involvement in renal cell injury by ischemia-reperfusion. Genetic and pharmacological approaches targeting Bid or tBid may therefore provide new strategies to ameliorate tissue damage during ischemic acute renal failure.

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REFERENCES


