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Apoptosis in ischemic renal injury: Roles of GTP depletion and p53

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Apoptosis in ischemic renal injury: Roles of GTP depletion and p53.

Apoptosis is increasingly recognized as a major mode of cell demise after ischemic injury to the kidney. The mediators of apoptotic cell death are many and include changes in intracellular pH, calcium, free radicals, ceramide, and adenosine triphosphate (ATP) depletion. Recently, we identified guanosine triphosphate (GTP) depletion as an independent trigger for apoptotic death after chemical anoxia in vitro. We further demonstrated that GTP salvage with guanosine inhibits tubular cell apoptosis after ischemic injury in vivo. This inhibition of apoptosis was accompanied by a significant protective effect on renal function. We also showed that p53 is the mediator of apoptosis in the setting of GTP depletion and ischemic injury. Indeed, salvage of GTP with guanosine prevented the ischemia-induced increase in p53 protein. Further, pifithrin-alpha, a potent and specific inhibitor of p53, inhibited apoptosis and protected renal function with a profile similar to that seen with guanosine. Finally, the protective effects of pifithrin-alpha involved both down-regulation of the transcriptional activation of Bax and a direct inhibition of p53 translocation to mitochondria. We propose that GTP depletion and activation of p53 are major inducers of apoptotic cell death after ischemic renal injury. In this setting, guanosine and pifithrin-alpha are potent inhibitors of apoptosis and are thus potentially useful in preventing and ameliorating functional injury to the ischemic kidney.

Ischemic renal injury has been traditionally associated with tubular cell necrosis along with obstructive cast formation, disruption of architecture, and a significant inflammatory response. Early attempts at preventing and treating ischemic injury were primarily targeted at this "necrotic" phenotype and generally met with moderate success, at least in animal models of this disease.

Only recently did apoptosis emerge as a significant mode of cell death during ischemic renal injury [1, 2]. While the contribution of apoptotic cell death to functional deterioration of the organ is obvious in conditions like myocardial infarction and stroke, it is less clear how apoptotic dropout of tubular cells can impact glomerular filtration rate (GFR). Nevertheless, recent reports have demonstrated that interference with the apoptotic program does translate into a protective effect on renal function [3–5]. This opened new avenues for potential therapeutic modalities targeting various steps of the highly organized and fairly well-understood process of apoptotic cell demise.

THE MEDIATORS OF CELL DEATH DURING ISCHEMIC RENAL INJURY

The apoptotic program itself has been extensively characterized and is fairly well preserved among cell types, tissues, and species. However, the primary triggers of this program are many and do show organ, species, and insult specificities. In particular, ischemia/reperfusion (I/R) injury is accompanied by a myriad of changes in signaling molecules and metabolic effectors that can, independently or in concert, trigger cell death in various forms. These include changes in intracellular pH, calcium, ceramide, free radicals, hypoxia and adenosine triphosphate (ATP) depletion. While all of these factors are grossly deranged during abrupt necrotic cell death, they can also be specific effectors of apoptotic death under certain circumstances. The current literature suggests that the mode of cell death that results from ischemic injury depends in part on the severity of the primary alterations in these factors. For example, severe ATP depletion invariably causes necrosis that is likely related to cessation of all vital enzymatic functions and Na/K

ATPase shutdown. Conversely, milder ATP depletion, when sustained, can specifically trigger the apoptotic cascade. Thus, necrosis and apoptosis appear to be part of a spectrum of cells death profiles and they often coexist, reflecting the differential severity of the insult in various parts of the tissue.

GUANOSINE TRIPHOSPHATE (GTP) DEPLETION AS AN IMPORTANT POTENTIAL TRIGGER FOR APOPTOSIS

Guanine nucleotides are favorite targets to induce apoptosis in activated lymphocytes and malignant cells. Thus, the importance of targeting and inhibiting the GTP synthetic pathway is supported by abundant data in the immunobiology and cancer literature [6]. This "privileged" status of guanine nucleotides is based on the very small size of GTP pools compared to ATP. Indeed, in most cells, ATP and GTP are in equilibrium with a ratio of about 7 to 1. This makes GTP significantly more rate-limiting for nucleic acid synthesis under conditions of nucleotide depletion. For example, a 50% reduction in both ATP and GTP translates into a more significant depletion in the absolute amount of GTP as compared to ATP. In addition, the GTP/GDP ratio is an important regulator of the function of a variety of signaling GTPases. These molecules cycle between active GTP-bound and inactive GDP-bound states. This cycle is normally controlled by a myriad of regulating proteins such as GEFs, GAPs, and GDIs. However, the ambient GTP/GDP ratio has also been shown to affect the GTPase cycle and can thus impact the function of these proteins. GTPases such as members of the Ras and Rho families are important regulators of cell proliferation and apoptosis. Therefore, GTP depletion can modulate cell death through these signaling molecules beyond its effect on nucleic acid synthesis.

IS GTP DEPLETED DURING ISCHEMIC INJURY?

ATP depletion is the primary result of inhibition of the respiratory chain during states of hypoxia, chemical anoxia, and ischemia. However, the ATP and GTP pools are tightly linked at various biochemical steps. The enzyme nucleotide diphosphate kinase catalyzes the reaction ATP + guanosine diphosphate (GDP) = adenosine diphosphate (ADP) + GTP. This keeps most ATP and GTP pools under strict equilibrium. Furthermore, ATP is a cofactor for the enzyme GTP synthetase while GTP is a cofactor for both adenosine monophosphate (AMP) deaminase and adenylosuccinate synthetase. Finally, GDP is an important regulator of the citric acid cycle. These biochemical facts predict that ATP and GTP pools should vary in parallel under most physiologic and pathophysiologic conditions. Indeed, we recently demonstrated that both chemical anoxia in vitro and renal is-

chemia in vivo are states of combined ATP and GTP depletion [7]. This suggested that GTP depletion could be an important and independent modulator of cell death phenotype under these conditions.

MODELS OF SELECTIVE NUCLEOTIDE DEPLETION POINT TO GTP DEPLETION AS THE PRIMARY INDUCER OF APOPTOSIS

Using potent and specific inhibitors of GTP and ATP synthesis like mycophenolic acid and alanosine, along with specific substrate-controlled media, we were able to develop models of sustained and selective ATP or GTP depletion in cell culture. These models linked the apoptotic phenotype specifically to the depletion of GTP. Furthermore, these models showed that ATP depletion, if unaccompanied by GTP depletion, results primarily in necrosis [7]. These models suggested that during states of generalized nucleotide depletion as occurs with ischemic renal injury, the reduction in GTP could be the primary culprit in triggering apoptosis. Therefore, we hypothesized that manipulating GTP pools during or after an ischemic insult might alter the apoptotic response observed under these conditions.

GTP SALVAGE WITH GUANOSINE AFTER ISCHEMIC INJURY INHIBITS TUBULAR CELL APOPTOSIS AND PROTECTS FUNCTION

In a model of bilateral renal artery clamp in the mouse and rat, we recently showed that intraperitoneal administration of guanosine normalized the GTP pools as early as 1 hour into the reperfusion phase [8]. ATP pools were not significantly increased. This selective and early repletion of GTP was accompanied by a major reduction in the number of apoptotic cells in all medullary tubular segments observed at 24 hours after reperfusion. More important, the repletion of GTP and inhibition of apoptosis did not alter the overall amount of tubular necrosis. Nevertheless, there was a dramatic improvement in renal function that was sustained even when examined at 72 hours after the insult. These results underscored the importance of GTP as a modulator of apoptosis during ischemic injury. They further pointed to apoptosis of tubular cells as a significant factor in the functional outcome of ischemic renal injury, independent of necrotic cell death. Other effects of guanosine unrelated to GTP repletion could not be excluded in these studies.

## p53 AS THE MEDIATOR OF GTP DEPLETION-INDUCED APOPTOSIS AFTER ISCHEMIC RENAL INJURY

p53 is the master regulator of cell cycle and apoptosis. It functions to induce cell cycle arrest or apoptosis

in response to DNA damage. It is activated by a variety of stimuli, including irradiation, hypoxia, and nucleotide depletion. It is the most frequently mutated gene in cancer and loss of p53 function correlates with the resistance of neoplasms to chemotherapy and radiation-induced apoptosis. A role for p53 in ischemia-induced apoptosis is well accepted in strokes but not in cardiac or renal ischemia [9]. A recent report suggested that down-regulation of inosine-5′-monophosphate dehydrogenase (IMPDH), the rate-limiting enzyme in GTP synthesis, is a requirement for the biologic activity of p53 [10]. Therefore, we hypothesized that GTP depletion-induced apoptosis during ischemic renal injury might be mediated by p53 [11].

We first examined the effect of ischemia on p53 and documented a significant increase in the protein levels, primarily in the renal medulla. We also showed that guanosine, which potently inhibits ischemia-induced tubular apoptosis, prevents this increase in p53. This suggested that p53 is the mediator of GTP depletion-induced apoptosis. Furthermore, pifithrin-alpha, a potent and specific inhibitor of p53, reproduced the protective profile seen with guanosine. That is, it inhibited apoptosis and protected function without significantly altering the necrotic damage postischemia. However, and unlike guanosine, pifithrin-alpha was protective when given up to 14 hours after the ischemic insult. This correlated well with the time course of p53 increase that occurs after ischemia.

PIFITHRIN-ALPHA DOWN-REGULATES THE TRANSCRIPTIONAL ACTIVATION OF BAX AND INHIBITS THE TRANSLOCATION OF p53 TO MITOCHONDRIA

p53 induces apoptosis primarily by activating proapoptotic proteins like Bax and Noxa that trigger programmed cell death via the intrinsic pathway. Indeed, the antiapoptotic effects of pifithrin-alpha after ischemia could be in part ascribed to a significant reduction in Bax protein. However, Bax was highly expressed in control tissues and its increase after ischemia was modest. We, therefore, examined alternative mechanisms by which the inhibition of p53 prevents apoptosis. One such mechanism was the recent demonstration of hypoxia-induced apoptosis by translocation of p53 to mitochondria in cell culture [12]. This translocation was sufficient to induce apoptosis and was transcription-independent. Using immunofluorescence labeling, we showed a significant p53 signal in ischemic kidneys that strongly localized to mitochondria. This mitochondrial p53 signal was inhibited by pifithrin-alpha, suggesting that migration of p53 to mitochondria is involved in the ischemic apoptotic response.
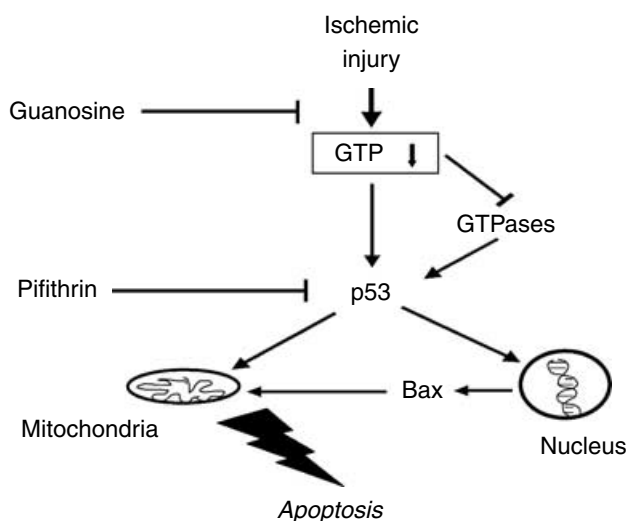
Fig. 1. Guanosine triphosphate (GTP) depletion and p53 in ischemia-induced apoptosis. This model proposes that GTP depletion and the activation of p53 are sequential steps underlying the apoptotic phenotype seen after renal ischemia. Guanosine and pifithrin-alpha restore GTP and inhibit p53, respectively, thus preventing apoptosis.

CONCLUSION

Our studies suggest that GTP depletion and the activation of p53 are sequential steps in ischemia-induced apoptosis. Inhibiting this pathway with guanosine or pifithrin-alpha significantly reduces tubular cell apoptosis and prevents the deterioration in renal function (Fig. 1). These studies also underscore the importance of apoptotic cell death in impacting renal function after ischemia. Apoptosis-based therapies should therefore be a focus of future investigation aimed at reducing the mortality and morbidity of ischemic renal injury.

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Cell cycle regulation: Repair and regeneration in acute renal failure

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Cell cyle regulation: Repair and regeneration in acute renal failure. Research into mechanisms of acute renal failure has begun to reveal molecular targets for possible therapeutic intervention. Much useful knowledge into the causes and prevention of this syndrome has been gained by the study of animal models. Most recently, investigation of the effects on acute renal failure of selected gene knock-outs in mice has contributed to our recognition of many previously unappreciated molecular pathways. Particularly, experiments have revealed the protective nature of two highly induced genes whose functions are to inhibit and control the cell cycle after acute renal failure. By use of these models we have started to understand the role of increased cell cycle activity after renal stress, and the role of proteins induced by these stresses that limit this proliferation.

The consequences of nephrotoxic renal injury include segment-specific changes in cell viability and reduced renal function. In experimental models necrosis of the S3 segment of the proximal tubule predominates and apoptosis occurs in a minority of cells, especially those of the distal nephron. Functionally, severe vasoconstriction, principally applied to the afferent arteriole, reduced glomerular filtration rate (GFR), and loss of autoregulatory responses characterize the renal microvascular response to injury. The kidney is also unable to generate maximum urinary concentration or to reclaim filtered sodium fully. Reversal of these changes coincides with the reestablishment of the normal renal epithelial barrier with new cells that reline the denuded tubules.

The process of regeneration and recovery begins shortly after injury, in which necrotic cells are accompanied by replicating cells lining the injured proximal tubule. The commitment to DNA synthesis is rapid and temporally coincides with the emergence of the morphologic and functional derangements. Data to be presented will support the hypothesis that renal injury and recovery are part of the same responses and that these processes depend on proper coordination of the cell cycle machinery. It will also be shown that the engagement of the cell cycle not only underlies recovery but is an important determinant of whether cells survive the injury itself.

CELL CYCLE PROGRESSION AND ITS REGULATION

Studies with eukaryotic models have elucidated that orderly progression through the cell cycle is regulated by the sequential synthesis, activation, compartmentalization, and degradation of proteins controlling both entry and exit from each phase of the cycle: G_1 (gap-1), S (DNA synthesis), G_2 (gap-2), and M (mitosis) (Fig. 1). One of the major controls on cell cycle progression is the regulation of phosphorylation of different substrates by interacting proteins consisting of a cyclin and a cyclin-dependent kinase (cdk). Cyclins, the regulatory subunit of the heterodimer, were originally found by nature of their cyclic oscillations during the sea urchin cell cycle [1]. The first