Gene Expression in Nephrotoxic and Ischemic Acute Renal Failure

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ABSTRACT

The commitment to DNA synthesis by the kidney to recovery from ischemic and nephrotoxic acute renal failure is accompanied by a pattern of gene expression that bears a striking resemblance to that exhibited by growth factor-stimulated cells in culture. Prominent among them is the expression of the immediate early genes that code for transcriptional factors that are rapidly and briefly expressed well before the onset of DNA synthesis. Other genes are activated that code for small secreted peptides. These proteins have cytokine-like activity that may be involved in the recruitment and activation of other cells that serve the regenerative response in some way. The expression of several additional genes, which are relatively kidney specific and developmentally regulated, are actually reduced during renal failure, suggesting that the commitment to DNA synthesis by the kidney may require dedifferentiation. Many different cell types participate in the increased DNA synthesis provoked by ischemic and nephrotoxic damage, including tubule cells removed from the site of greatest injury, as well as those outside of the tubule compartment, suggesting that paracrine, autocrine, and juxtacrine factors support the growth-promoting process. A prominent site of altered gene expression during acute renal failure is the thick ascending limb, which undergoes both positive and negative changes in expression and which seems to be a prominent site of reaction to nephrotoxic stimuli at the molecular level. Studying the interaction between the regulatory sequences of a select group of genes with their transactivating factors and the transduction pathways that activate them should identify the initial growth-promoting signal and the subsequent steps leading to renal regeneration. It is likely that a better understanding of these processes will lead to new strategies that can be applied to promote the full and more rapid recovery of the injured kidney.

Key Words: Cell cycle, immediate early genes, transacting factors

Recovery from ischemic and nephrotoxic acute renal failure requires the replacement of damaged tubule cells with new ones that restore the continuity of the renal epithelium (1). During this process, normally quiescent renal cells increase their nucleic acid synthesis and undergo mitosis. These phenomena occur in all forms of acute renal injury, including that caused by ischemia, gentamicin, and folic acid and cisplatin administration (2-6). There is also a temporal relationship between the enhanced renal DNA synthesis and reduced renal function (7), suggesting, perhaps, some causal relationship between the two. Little is known, however, about the control of renal regeneration after injury. This review serves to summarize what is known about the molecular response to renal injury.

RENALE REGENERATION AND THE CELL CYCLE

The general characteristics of the renal DNA synthetic response to injury are shown in Figure 1. Several hours after the release of a 50-min period of renal hilar clamping, renal DNA synthesis increases rapidly to a peak before falling toward normal values several days later. This burst in renal DNA synthesis is typical of all nephrotoxic insults, differing only in its rapidity and extent, depending perhaps on the severity of the damage to the kidney.

The renal DNA synthetic response to injury is reminiscent of the growth response of cells in culture as they enter the growth cycle (Figure 2). Nongrowing,
Figure 1. \(^{3}H\)thymidine SA in whole-kidney DNA in sham-operated animals \((N = 5)\) and animals 2 \((N = 3)\), 16 \((N = 1)\), 24 \((N = 2)\), and 96 h \((N = 2)\) after ischemia. * \(P < 0.001\).

Quiescent cells have low rates of DNA synthesis and are said to be in the G0 phase of the cycle. These cells can enter the cell cycle when stimulated to grow by the addition of a growth factor such as platelet-derived growth factor, epidermal growth factor (EGF), or insulin-like growth factor (IGF). After the binding of a growth factor to its receptor, growth-promoting signals are generated that commit the cell to a new phase of the cell cycle, termed G1. During the transition to the G1 phase, the cells do not change their appearance in any obvious way. Nonetheless, they express many genes that enable them to progress through the initial phase of the cell cycle. Prominent among them are the so-called immediate early genes (8), the expression of which begins almost immediately after stimulation by a growth factor, lasts only briefly, and does not depend on new protein synthesis (9). Although only a few of the nearly 100 genes that are known to participate in the early growth response have been fully characterized, some code for well-known DNA-binding transcription factors that presumably initiate a genetic cascade ending in cell division. Others code for small secreted proinflammatory polypeptides and proteases, the role of which in cell division is less clear. After a delay that varies among different cell types, the cells move into the next phase of the cycle, or G2, where new protein and RNA synthesis occurs and the cells increase in size. The newly synthesized proteins and mRNAs are crucial for progression through the remainder of the G1 phase, as well as through the S phase, when DNA synthesis occurs. Additional protein and RNA synthesis is required for the cells to complete the cycle and undergo mitosis.

There is a striking parallel between the changes in gene expression induced by ischemic renal damage and those induced by growth factor stimulation of quiescent cells in culture. c-Fos mRNA, the prototype of the nucleus-binding members of the immediate early genes, increases almost immediately after the release of renal hilar clamping (Figure 3). Its induction is short lived and precedes the peak of renal DNA synthesis. A similar increase is observed for the immediate early gene Egr-1 (6, 10). The c-Fos and Egr-1 proteins contain DNA-binding motifs that regulate the transcription of other genes, and it is highly likely that these genes regulate the expression of downstream target genes that serve the renal regenerative response.

Another group of immediate early genes, the members of the small cytokine-like genes, are also expressed during renal ischemic injury. The expression of KC, which codes for a small secreted glycoprotein with potent chemotactic (11) and mitogenic (12) properties, increases to a maximum 1 h after ischemia.
and returns to normal by 4 h in a pattern typical of the immediate early gene response. The expression of JgE, a potent monocyte chemoattractant (13), increases up to 48 h after the release of the hilar clamp. In contrast to its behavior in cells stimulated to grow in vitro, which is brief, the expression of JgE after ischemia is prolonged and lasts up to 1 wk (14).

Table 1 lists the genes of the cell cycle in various forms of nephrotoxic and ischemic damage that have been explored thus far. The increased expression of many members of the immediate early or competence gene families, including transcription factors, members of the small cytokine superfamily, structural genes, and intracellular second messengers, has been described. In addition, the expression of a newly cloned putative DNA-binding protein, Kid-1, is decreased after ischemic and folic acid–induced injury (15), demonstrating that both positive and negative regulation of gene expression occurs in the early molecular responses to renal injury. Many of the genes that are activated during the later stages of the G0/G1 transition, as well as during G, and S, are also expressed.

WHAT STIMULATES RENAL DNA SYNTHESIS?
The similarity between immediate early gene induction by growth factors and by renal injury has led

### Table 1. The expression of cell cycle–related genes during renal regeneration

<table>
<thead>
<tr>
<th>Phase</th>
<th>Model</th>
<th>Direction</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genes of the G0/G1 Transition</td>
<td></td>
<td></td>
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<tr>
<td>Transcription Factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c-fos</td>
<td>Cisplatin</td>
<td>↑</td>
<td>Safirstein et al. (5)</td>
</tr>
<tr>
<td></td>
<td>Ischemia</td>
<td>↑</td>
<td>Safirstein et al. (6); Ouellette et al. (10); Rosenberg and Paller (26)</td>
</tr>
<tr>
<td></td>
<td>Folic acid</td>
<td>↑</td>
<td>Cowley et al. (27); Norman et al. (28)</td>
</tr>
<tr>
<td>c-myc</td>
<td>Folic acid</td>
<td>↑</td>
<td>Cowley et al. (29); Norman et al. (28); Asselin et al. (30)</td>
</tr>
<tr>
<td>Egr-1</td>
<td>Ischemia</td>
<td>↑</td>
<td>Ouellette et al. (10); Safirstein et al. (6)</td>
</tr>
<tr>
<td>NGF, B (glucocorticoid receptor like)</td>
<td>Folic acid</td>
<td>↑</td>
<td>Kujubu et al. (31)</td>
</tr>
<tr>
<td>Kid-1</td>
<td>Ischemia</td>
<td>↓</td>
<td>Witzgall et al. (15)</td>
</tr>
<tr>
<td></td>
<td>Folic acid</td>
<td>↓</td>
<td>Witzgall et al. (15)</td>
</tr>
<tr>
<td>Small cytokine superfamily</td>
<td>Ischemia</td>
<td>↑</td>
<td>Safirstein et al. (14)</td>
</tr>
<tr>
<td>KC</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>JE</td>
<td>Ischemia</td>
<td>↑</td>
<td>Safirstein et al. (14)</td>
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<tr>
<td>Structural genes</td>
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<tr>
<td>Actin</td>
<td>Ischemia</td>
<td>↑</td>
<td>Safirstein et al. (5); Rosenberg and Paller (26)</td>
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<tr>
<td></td>
<td>Folic acid</td>
<td>↑</td>
<td>Cowley et al. (27); Norman et al. (28)</td>
</tr>
<tr>
<td>Vimentin</td>
<td>Folic acid</td>
<td>↑</td>
<td>Norman et al. (28)</td>
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<tr>
<td>Signaling</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Ki-ras (G-protein)</td>
<td>Folic acid</td>
<td>↑</td>
<td>Cowley et al. (27)</td>
</tr>
<tr>
<td>Ha-ras</td>
<td>Folic acid</td>
<td>↑</td>
<td>Cowley et al. (27)</td>
</tr>
<tr>
<td>c-ros (tyrosine kinase)</td>
<td>Ischemia</td>
<td>↑</td>
<td>Safirstein (unpublished observation)</td>
</tr>
<tr>
<td>Proteases</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Tissue factor</td>
<td>Ischemia</td>
<td>↑</td>
<td>Safirstein (unpublished observation)</td>
</tr>
<tr>
<td>G &amp; S Phase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADP-ATP Translocase</td>
<td>Folic acid</td>
<td>↑</td>
<td>Norman et al. (28)</td>
</tr>
<tr>
<td>Histone H4</td>
<td>Folic acid</td>
<td>↑</td>
<td>Cowley et al. (27)</td>
</tr>
<tr>
<td>Histone H4ac</td>
<td>Ischemia</td>
<td>↑</td>
<td>Rosenberg and Paller (26)</td>
</tr>
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to the investigation of growth factors that might underlie the renal DNA synthetic response. EGF would seem to be a likely candidate because the EGF gene is highly expressed in the normal kidney (16) and EGF is mitogenic for the proximal tubule (17), the tubule segment that is most prominently damaged by nephrotoxic and ischemic damage. Although renal injury has profound effects on the expression of preproEGF, its expression actually falls and remains low for a prolonged period of time (Figure 4). Also, the total renal content of EGF falls (18), as does the renal excretion of EGF (5,6). A small, soluble, and active species of EGF, however, is retained by the kidney during ischemia (18), perhaps formed as a consequence of the posttranscriptional effects of ischemia on preproEGF processing (6). Further, the renal EGF receptor number increases (6,12). The significance of these changes in the renal EGF system is unknown at present, but it would appear that the increase in the EGF receptor is responsible for the beneficial effects of exogenously administered EGF and transforming growth factor-alpha in ischemic and perhaps other forms of renal injury (19,20).

The increased renal expression of other growth factors known to be mitogenic to proximal tubules in vitro, such as IGF-1 (21,22) or hepatocyte growth factor (23,24) has been demonstrated after ischemic and nephrotoxic injury. The increase occurs after the onset of the immediate early gene response, however, suggesting that these factors may be important to events beyond entry into the cell cycle rather than initiators of the cell cycle.

The expression of genes not clearly related to the cell cycle has also been demonstrated after the induction of acute renal failure (ARF) (Table 2). This list includes diminished expression of the Tamm-Horsfall (TH) gene, which, like preproEGF, is transcribed by the thick ascending limb. Also, renin mRNA decreases in ARF. The expression of genes responsive to injury in other systems, such as heat shock protein and clusterin, increases. Finally, the expression of the superoxide dismutase gene is decreased during ischemia.

WHAT CELLS EXPRESS THE IMMEDIATE EARLY GENES?

Although the model of the cell cycle as applied to the kidney has yielded some new insights into the process of renal repair, the kidney’s cellular heterogeneity complicates matters. It is clear that many

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**TABLE 2. The expression of genes not related to the cell cycle**

<table>
<thead>
<tr>
<th>Genes Responsive to Cell Injury</th>
<th>Model</th>
<th>Direction</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heat shock protein</td>
<td>Ischemia</td>
<td>↑</td>
<td>Van Why et al. (32)</td>
</tr>
<tr>
<td>Clusterin</td>
<td>Glycerol, Folic acid</td>
<td>↑</td>
<td>Correa-Rotter et al. (33,34)</td>
</tr>
<tr>
<td>Superoxide dismutase</td>
<td>Ischemia</td>
<td>↓</td>
<td>Rosenberg and Paller (26)</td>
</tr>
<tr>
<td>Growth Factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatocyte growth factor</td>
<td>CCI</td>
<td>↑</td>
<td>Nagaiko et al. (24)</td>
</tr>
<tr>
<td>EGF</td>
<td>Ischemia</td>
<td>↑</td>
<td>Ishibashu et al. (25)</td>
</tr>
<tr>
<td>IGF-I</td>
<td>Ischemia</td>
<td>↓</td>
<td>Satirstein et al. (5)</td>
</tr>
<tr>
<td>Other</td>
<td>Ischemia</td>
<td>↑</td>
<td>Satirstein et al. (5)</td>
</tr>
<tr>
<td>Tamm-Horsfall</td>
<td>Ischemia</td>
<td>↓</td>
<td>Satirstein et al. (14)</td>
</tr>
<tr>
<td>Renin</td>
<td>Ischemia</td>
<td>↓</td>
<td>Rosenberg and Paller (26)</td>
</tr>
<tr>
<td>Endothelin</td>
<td>Ischemia</td>
<td>↑</td>
<td>Terado et al. (36)</td>
</tr>
<tr>
<td>Parathyroid hormone-related protein</td>
<td>Ischemia</td>
<td>↑</td>
<td>Soifer et al. (35)</td>
</tr>
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</table>
different cells participate in the renal DNA synthetic response to injury, including the cells relining the injured tubules, the mildly affected early proximal tubule cells, and the interstitial and perivascular cells (2–4, 7). However, not all cells participate in this response, most notably, the cells of the glomerulus, indicating that the growth-promoting signal is not delivered to all cells.

The response of the cells of the thick ascending limb is interesting in this regard. Although not prominently involved in the cellular necrosis provoked by either ischemic or nephrotoxic damage, it is clear that the thick ascending limb is a key element in the molecular response to injury. These cells undergo both positive and negative regulation of gene transcription during renal injury, gaining the ability to express the nuclear-binding protein early growth response gene 1 product (EGR1) (37) while losing the ability to make EGF and TH, which they express normally to a high degree. Whether the thick ascending limb responds to a self-generated signal or responds to one arising from outside of the cell membrane is unknown, but the close proximity of the thick ascending limb to the injured pars recta of the proximal tubule (Figure 5) and other cellular elements in the outer stripe of the outer medulla suggests that such communication is possible. A full understanding of the renal regenerative response will require identification of the cells that act as initiators and targets of the growth-promoting signals.

LIVER REGENERATION

The cells of the liver also regain the capacity to enter the cell cycle after either two-thirds hepatectomy or toxic injury (38), and what is known about regeneration in the liver is likely to yield some insight into the process in the kidney.

DNA synthesis is initiated 12 to 16 h after partial hepatectomy and continues until liver mass is restored to its initial size. Under these circumstances, nearly 95% of the remaining hepatocytes participate in this response. Many of the immediate early genes expressed by the kidney during the G0/G1 transition are also expressed by the regenerating liver, including c-Fos, c-Jun, egr-1, actin, JE, and KC (39). Not all of the immediate early genes respond to partial hepatectomy in the same way as that induced in serum-activated tissue culture cells. Most notable was the difference in the time course of the expression of the Jun and Fos family of transcription factors (39). Because these proteins are active only as dimers, which show specificity for DNA targets based on their composition, it is conceivable that differences in the mix of these dimers could shape the particular characteristics of the liver's regenerative response. It would be of interest to see if similar differences in the timing of the expression of Fos and Jun family members also occurs after nephrotoxic damage. Differences were also observed in the time course and intensity of the secreted immediate early proteins' response depending on which cell type was stimulated. As pointed out earlier, this is true for JE expression in the kidney, which is expressed for a much more prolonged period of time in the kidney than in the endothelial cells (13) or after aortic vessel injury (40). These differences may also shape the response of a given tissue to injury.

Investigation of the regenerative response in the liver has also focused on what is responsible for initiating the growth response. The evidence so far suggests that multiple factors interact in initiating and supporting liver regeneration, including circulating hormones, neural and liver-derived growth factors, α-adrenergic agonists, and hepatocyte growth factor (39). During CCl4-induced liver injury, the Kupffer cell seems to be the source of the hepatocyte growth factor (41), and because this is a nonparenchymal cell, this has important implications for the process in the kidney, because there is a prominent nonparenchymal cell response to nephrotoxic injury as well (42).

Interestingly, and different from the situation in renal regeneration, the number and activity of the EGF receptor are actually reduced during hepatic regeneration (43) so that the increase seen after nephrotoxic injury is tissue specific. Of interest is the observation that a portion of the EGF-receptor complex is localized to the nucleus in regenerating hepatocytes, suggesting perhaps a nuclear site of

Figure 5. Thin section (5 μm) of the outer stripe of the outer medulla from rat kidney 24 h after a 50-min period of bilateral hilar clamping. Thick ascending limbs are labeled with a fluorescein isothiocyanate-labeled anti-TH antibody. Note intact thick ascending limbs surrounded by islands of necrotic tubules. TH staining is markedly reduced from normal intensity and consistent with reduced TH mRNA levels at this time (5).
action of the activated receptor in regenerating cells 

Carbon tetrachloride liver injury is followed by an even more impressive regenerative response than that provoked by partial hepatectomy (45); different from the situation of partial hepatectomy, in which the differentiatated function of the liver is well preserved, there appears to be a switch to a more dedifferentiated state of gene expression after such injury. Albumin gene expression falls and $\alpha$-fetoprotein gene expression increases, reiterating the pattern of gene expression in the fetus. The expression of other liver-specific genes, such as ligandin and cytochrome P-

that stem cells may emerge during regeneration after massive hepatic necrosis (46), whereas no such emergence of a stem cell can be demonstrated after partial hepatectomy (39). These differences in the molecular response to partial hepatectomy and hepatotoxic injury reveal the diversity of the regenerative response of the liver. To date, no systematic search of a renal stem cell has been undertaken.

**IMPLICATIONS FROM THE OBSERVED CHANGES IN GENE EXPRESSION**

Several themes emerge from the study of the molecular response to renal injury. The experimental paradigm of the progression through the cell cycle seems a good one, and applying it to renal regeneration has already identified several new target areas for future research. It has also led to the trial of several growth factors that show promise in ameliorating the course of renal failure (19,20,47–49). Several of the changes in gene expression suggest the possible participation of the inflammatory cascade in the regenerative or physiologic responses of the kidney to renal cell injury. The only well-characterized function of JE and KC is a chemotactic one, and their expression may help to recruit and activate leukocytes or activate endothelial cells in regions where these factors are expressed. This, in turn, may release factors that also affect vascular and epithelial cell function (50). Leukocyte depletion during ischemia (51) limits damage much like the protective effect observed after myocardial ischemia (52), suggesting a role for leukocyte activation in the process of renal damage. Thus, although probably serving some regenerative role, the robust and early expression of these genes may also have deleterious effects on the kidney. It may be that limiting or modifying the inflammatory cascade might lead to even better preservation of renal function after ischemia than observed with generalized leukocyte depletion.

Several changes in gene expression suggest that the kidney, like the injured liver, may recapitulate a developmental program and dedifferentiate, at least partially, in order to commit to DNA synthesis, a view proposed by Toback (1) and reiterated by Bacallao and Fine (53). The reduced expression of preproEGF, TH, and kid-1 during ARF, each of which is not expressed significantly before birth, is consistent with that hypothesis. Also, many observations suggest that EGF gene expression in the kidney is associated with differentiation of function, rather than proliferation. Thus, EGF is transcribed to a high degree by the mature kidney only and mRNA for preproEGF is absent in the fetal kidney (54). EGF, when administered to normal rats, does not provoke renal DNA synthesis (20), and the increase seen during ARF may be dependent on the increase in EGF receptor number. EGF augments vasopressin and the Ca-mediated release of prostaglandin E2 from rat glomerular mesangial cells (55) and inhibits vasopressin and cAMP-induced water flow in rabbit cortical collecting ducts (56) by a cyclooxygenase-independent pathway. EGF excretion is also low in human renal diseases such as diabetic nephropathy, in which it is an early indicator of deteriorating kidney function (57); polycystic kidney disease (58); and renal transplantation (59), in which restoration of normal renal function is positively correlated with increasing EGF excretion. Thus, the repression of preproEGF gene expression during renal injury may be a necessary component of the regenerative response. Study of the renal regulation of EGF in experimental ARF should yield important information about the cause of reduced EGF expression in a wide variety of renal diseases.

**FUTURE RESEARCH DIRECTIONS**

ARF continues to be a major cause of hospital morbidity, and its high mortality rate has changed little in the postdialysis era (60). Ultimately, the goal of current research in renal regeneration is to maximize the renal regenerative program. The current emphasis on administering growth factors systemically is potentially hazardous because EGF and IGF have such pleiotropic effects. For example, EGF is a potent renovascular constrictor (61) and induces bone resorption in vivo (62), whereas IGF-I causes hypoglycemia (63). Obviously, any of these effects may limit their clinical usefulness. A more reasonable strategy would be to modify the regenerative response in as kidney specific a manner as possible. To achieve this goal, it will be necessary to identify what initiates renal regeneration, how the growth-promoting signal is transduced to the nucleus, and what the nuclear targets are that participate in regeneration.

A hypothetical scheme of the growth pathway is given in Figure 6. The binding of a growth factor to its receptor begins a phosphorylation cascade, which in the example given of a tyrosine kinase receptor,
begins with the phosphorylation of the receptor itself (64). Other growth factor receptors may initiate signaling without the necessity for autophosphorylation. A target of the phosphorylation cascade is a preformed transcription factor, which, when activated, moves to the nucleus and binds to DNA elements that regulate the rate of transcription of other genes. Because many of the genes that are activated during the immediate early gene response are transcriptional activators themselves, they transduce the growth factor signal to other genes, the transcriptional regulation of which is necessary to move the cell through the remainder of the cycle. These downstream targets include proteins that affect the transport of nutrients and enzymes necessary for DNA synthesis, as well as down-regulation of genes more closely linked to differentiated function than to commitment to the cell cycle. Also shown is a proposed scheme whereby new growth factor receptors would be necessary to complete the cell cycle. Thus, by studying the specific promoter and enhancer elements of the genes of the Go/G1 transition and the transactivating factors that bind to them, it may be possible to trace the growth-promoting signal back to its origin in the signal pathway. The immediate early genes would seem to be ideal candidates for such studies because much is known about their transactivation during cell growth (65). A similar approach may be used to trace the later downstream targets by studying a select group of genes not clearly related to the cell cycle but the expression of which changes during the regenerative response. PreproEGF, TH, or kid-1 would seem to be ideal for such studies because of their high level of expression by the kidney, their uniform down-regulation during renal injury, and their developmental pattern of expression. Because of the cellular heterogeneity of the kidney and the already demonstrated paracrine effects of nephrotoxic damage, the cells expressing these changes and how that expression is integrated into the regenerative response, will almost certainly be important. The goal for the future will be to manipulate the regenerative process, up-regulating beneficial limbs of the pathway while down-regulating potentially deleterious ones, so as to achieve full and rapid recovery of the kidney after injury.

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