Renal repair and recovery

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Objective: **To review the cellular and molecular mechanisms of renal repair and recovery after acute kidney injury (AKI).**

Data Source: **The data were summarized from published research articles.**

Results: **In AKI, there is an acute inflammatory response, epithelial cell necrosis and apoptosis, and shedding of epithelial cells into the tubular lumen. Recent work demonstrates that repopulation of damaged renal tubules occurs primarily from proliferation of tubular epithelial cells and resident renal-specific stem cells, with some contribution of paracrine factors from bone marrow–derived mesenchymal stem cells. In addition, growth factors seem to play a critical role in the repair process in animal models of renal injury. However, attempts to use growth factors in the clinical setting to attenuate human AKI or accelerate renal repair have not yet been successful. The endothelium also plays a critical role in the pathogenesis of AKI. Lastly, in human studies, the effect of dialysis on renal recovery remains poorly understood.**

Conclusions: **Experimental animal models of AKI demonstrate that renal recovery and repair involves proliferation of tubular epithelial cells and stem cell populations and the coordinated contribution of multiple growth factors. Future efforts to improve recovery from AKI and improve patient outcomes may include novel therapies based on manipulation of populations of stem cells and augmenting repopulation of renal tubules. (Crit Care Med 2008; 36[Suppl.]:S187–S192)**

KEY WORDS: renal repair; recovery; acute renal failure; acute kidney injury; mesenchymal stem cell; growth factors; endothelial cells; dialysis

As reviewed by Thadhani et al. (1) and Waikar et al. (2), in the clinical setting, acute kidney injury (AKI) is often multifactorial. Distinct pathogeneses include sepsis, ischemia, and nephrotoxins. However, our understanding of the pathophysiology of these diseases relies heavily on animal models, in which a single pathogenic process usually predominates. These animal models have important strengths and limitations, in particular, uncertainty as to how well the models replicate human disease. Our understanding of repair and recovery is significantly predicated on these models. In this article, we will review common elements of the pathophysiology of different types of AKI. We will then focus on what is known about the cell types that may contribute to renal recovery and on the role of growth factors in renal repair and recovery. It is also increasingly clear from human studies and animal models that AKI can result in chronic kidney disease. We will briefly discuss the role of growth factors in this process. Lastly, we will summarize the effect of renal replacement therapy on recovery from AKI.

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Renal Architecture

The renal tubules are lined by epithelial cells that under normal conditions are highly polarized and are critical for the filtration and secretion functions of the kidney (described by Fish and Molitoris (3)). The apical side of these cells is characterized by membrane projections called microvilli that contain bundled F-actin filaments; these microvilli increase the surface area of the epithelial cell exposed to the tubular lumen. The actin cytoskeleton is critical to maintain the cell's shape and polarized functions; it is a dynamic structure characterized by a highly regulated, steady-state equilibrium between F-actin filaments and G-actin monomers. Cells are connected near the apical surface by a junctional complex that is made up of several protein complexes, including tight junctions and adherens junctions. The tight junction forms the border between the apical and basolateral surfaces of the cell that segregates proteins and phospholipids to the appropriate cell surface (gate function) and that blocks paracellular permeability (fence function). Proteins found as part of the tight junction include occludin, ZO-1, ZO-2, and cingulin.

The basolateral cell surface also contains a distinct subset of cell surface proteins. For example, the sodium- and potassium-activated adenosine triphosphatase is localized to the basolateral side of the cell and is critical for sodium resorption from the tubular lumen and transport to the interstitium. On the basolateral surface, transmembrane integrins bind to extracellular matrix proteins via Arg-Gly-Asp peptide sequences. Integrins form part of specialized protein complexes at extracellular matrix binding sites called *focal adhesion complexes* (4). The presence of integrins and focal adhesion complexes allow the renal tubular epithelial cell to be firmly adherent to the basement membrane. In addition, focal adhesion complexes signal to the actin cytoskeleton via focal adhesion kinase and other signaling molecules, and thus, changes in cell-matrix interactions can lead to actin

cytoskeleton rearrangement in renal tubular cells.

Acute Kidney Injury: Mechanisms of Injury and Repair

Cellular damage in AKI involves three forms of injury: sublethal damage resulting in depolarization of cells (and therefore loss of normal cellular function) and cell death through apoptosis and necrosis (1, 5, 6). Although there has been controversy about which segment of the tubule is the most affected, it is clear that the proximal tubule, and in particular the S3 segment, undergoes significant morphologic changes and therefore has been the subject of much study (7).

Specific animal models and human disease vary with regard to the relative balance of these pathophysiological processes. In renal biopsies from patients with ischemic kidney injury (e.g., after cadaveric kidney transplantation), the majority of tubules may seem fairly normal, with limited overt cellular necrosis. However, in animal models of either toxin- or ischemia-mediated kidney injury, necrosis is typically widespread. These differences may be due to the fact that in animal models, severe ischemia is induced by exposure to high doses of a nephrotoxin or by cross-clamping of the renal artery. In animal models of septic AKI, cellular morphology often seems relatively normal (8). These differences highlight an important distinction between the clinical entity of AKI and animal models (9–11) and may partially explain why many of the successful interventions for AKI in animal models have not been efficacious in clinical trials.

Despite these differences, common injury processes underlie all forms of AKI. For recovery to occur, these must be repaired. Sublethal cellular injury has been described both in animal models and in human renal allografts. In animal models, short ischemic times lead to loss and fusion of the apical microvilli, whereas longer ischemic times result in shedding of microvilli into the tubular lumen, loss of integrity of the actin cytoskeleton, and ultimately cell death (12). Ischemia leads to dissolution of focal adhesion complexes with changes in the cell-matrix signaling that contribute to actin cytoskeleton remodeling (4). In addition, junctional complexes are disrupted after AKI, leading to increased paracellular permeability due to loss of the "gate" function and loss of cell polarity due to loss of the "fence" function. Consequently, the sodium- and potassium-activated adenosine triphosphatase that is typically localized exclusively on the basolateral surface can be found on the apical surface after ischemic injury. Mislocalization of these channels contributes to the inability of the proximal tubule to reabsorb sodium, as is commonly seen in AKI (13). Thus, the recovery process must result in reestablishment of polarity of sublethally injured cells and in the establishment of polarity in regenerated epithelial cells. Reestablishment of polarity seems to require signaling cues from adjacent cells and from the extracellular matrix.

With more severe injury, epithelial cells die through apoptosis and necrosis, resulting in loss of tubular integrity. Apoptosis is a form of programmed cell death that is characterized by cell shrinkage, nuclear condensation and fragmentation, and rapid clearance by phagocytosis. Most normal cells constitutively express the machinery necessary for apoptosis but are prevented from undergoing apoptosis by the presence of survival factors. Loss of these survival or growth factors leads to triggering of apoptosis via a "default" pathway (6). Depending on the nature and duration of injury (in particular with more severe or prolonged injury), cells may also undergo necrosis. Viable renal tubular epithelial cells may also be shed into the lumen of the tubule (14), which contributes to the loss of epithelial integrity. Thus, one of the first steps in renal recovery is the repopulation of the tubular compartment, followed by differentiation of cells to mature tubular epithelial cells. As we will explore in more detail, the nature of the cells that contribute to this repopulation process remains controversial. Three different types of cells play roles in this process in various model systems: renal tubular epithelial cells, renal-specific progenitor cells, and bone marrow–derived mesenchymal stem cells.

In addition, AKI is frequently characterized by damage to other cellular compartments, in particular the endothelial compartment. Microvascular injury plays a critical role in both septic and ischemic AKI (15, 16). Given the enormous oxygen tension gradient within the normal kidney, small changes in oxygen delivery may greatly exacerbate tissue hypoxia. Endothelial cell damage may lead to leukocyte activation and sludging within the capillaries and to release of inflammatory mediators. In ischemic AKI, neutrophils, monocytes, and T cells are recruited to the injured tissue and modulate injury (17–19).

Recovery from acute tubular injury is, as of yet, a poorly understood process but involves recruitment of inflammatory cells, which may be important sources of paracrine growth factors, and proliferation and differentiation of surviving cells to form polarized epithelial tubules (20). Growth factors may exert important anti-apoptotic and proliferative effects on damaged cells. Renal-specific or bone marrow–derived mesenchymal stem cells may accelerate the repopulation of tubules by direct proliferation or through paracrine effects. Thus, the recovery process may recapitulate many aspects of renal development (21); indeed, a number of proteins expressed in the developing kidney are re-expressed after acute renal injury.

Recovery from Acute Kidney Injury: What Is the Progenitor Cell for the Tubular Epithelium?

After ischemic injury, a number of different cell types have been implicated in the repair process. First, surviving renal tubular epithelial cells dedifferentiate (21). Existing renal tubular epithelial cells contribute to the recovery process through proliferation and migration to denuded areas of the tubule. After renal injury, proximal tubular cells express vimentin, an intermediate filament protein that is found in undifferentiated mesenchymal cells but not differentiated kidney cells; these cells also express proliferating cell nuclear antigen, a marker of mitogenic activity (22). In contrast, injured cells express neither vimentin nor proliferating cell nuclear antigen. In these studies, the majority of surviving cells expressed vimentin, suggesting that many cells dedifferentiated as part of the repair/recovery process. Thus, surviving renal tubular epithelial cells appear to play a critical role in the regeneration process.

Several different types of renal stem cells have been implicated in the recovery process, both organ-specific stem cells and mesenchymal stem cells in renal repair (reviewed by Cantley (23)). Renal-specific stem cells have been described by several independent groups. These cells can be identified using bromo-deoxyuridine, which incorporates into the DNA of

dividing cells. Techniques have been developed to label cells that are slowly going through the cell cycle, suggesting that these cells are slowly proliferating progenitor cells. These cells have been found in the renal tubules and the papilla (24, 25). Moreover, these renal progenitor cells have phenotypic plasticity and are capable of expressing epithelial markers *in vitro* when subjected to appropriate extracellular cues (24, 25). After ischemic renal injury, these cells proliferate and seem to contribute to repopulation of the tubule in animal models. However, study of the role of these cells in renal repair has been limited by the lack of markers that specifically identify this population. Furthermore, the potential use of these cells as a treatment strategy for AKI is unclear because these cells are challenging to isolate and maintain in culture *in vitro*.

Similarly, a number of studies have focused on the role of bone marrow–derived stem cells in renal recovery. Two different types of stem cells can be found in the bone marrow: hematopoietic stem cells and mesenchymal stem cells. Bone marrow–derived hematopoietic stem cells are identified by their lack of expression of lineage-specific markers (CD4, CD8, CD45R, CD11b, Gr-1, and Ter-119) and by their expression of CD117 (c-kit) and Sca-1 on the cell surface. Bone marrow–derived hematopoietic stem cells can differentiate into any mature blood cell type. In contrast, mesenchymal stem cells can be isolated from the bone marrow based on their ability to adhere to plastic (23, 26) and can differentiate into a variety of cell types.

Clinical transplantation studies originally suggested that recipient-derived cells can be identified in the parenchyma of the donor kidney after engraftment, suggesting that stem cells might play a role in tissue homeostasis (27, 28). Subsequent studies in animal models initially suggested that bone marrow–derived stem cells migrate to the kidney and accelerate the renal recovery process by repopulating the tubule after ischemia–reperfusion injury. However, the quantification of the contribution has varied in different experimental systems and is dependent on how the stem cells are labeled as "foreign" (that is, whether cells are labeled enzymatically by the presence of the β-galactosidase gene or by the presence of a Y chromosome in a XX recipient animal) (29, 30). In all these systems, the majority of cells that repopulate the tubules seem to be derived from either renal epithelial cells that have proliferated or the renal-specific stem cells, not from marrow-derived cells.

Thus, direct contribution to the new tubular architecture and epithelial cell mass is not the predominant mechanism of mesenchymal stem cell benefit. Rather, mesenchymal stem cells may exert their beneficial effects via paracrine mechanisms (31, 32). Most recently, Bi et al. (33) have demonstrated that infusion of conditioned media from mesenchymal stem cells into animals that have received cisplatin to produce a toxic renal injury diminished tubular cell apoptosis and renal damage, again supporting a paracrine mechanism of action. Recent studies have also suggested that migration of mesenchymal stem cells requires the presence of CD44R on the cell surface and up-regulation of hyaluronan in the parenchyma of the injured kidney (34). Further understanding of the mechanism of action of mesenchymal stem cells is clearly required before these cells can be used therapeutically to accelerate renal recovery.

Recovery from Acute Injury: Role of Growth Factors

A number of growth factors enhance proliferation of tubular epithelial cells in animal models and in *in vitro* cell culture–based systems (11, 35). In animal models, administration of exogenous growth factors has been shown to accelerate renal recovery from injury. Although some of these polypeptide growth factors have failed to show any significant benefit in human clinical trials, these studies highlight important components of the process of renal recovery. Differences in treatment effect between animal models and clinical trials may be attributable to differences in the timing of growth factor administration relative to injury; in animal models, growth factors are typically administered at the time of injury or immediately after injury. In contrast, AKI is typically advanced when therapy is initiated in human studies, highlighting our need for new biomarkers that can be used to identify AKI earlier in the course of the disease (11).

Epidermal Growth Factor. Epidermal growth factor (EGF) is a ubiquitous polypeptide growth factor capable of stimulating proliferation of many types of epithelial cells. EGF activates cellular signaling by engaging the EGF receptor, a receptor tyrosine kinase that is found on the surface of adult kidney cells. Administration of EGF to animals with ischemic- or toxin-mediated AKI shortens recovery time (36), likely due to downstream activation of cell survival pathways. Both renal epithelial cells and progenitor cells have been shown to proliferate in response to EGF (25, 37). Mice with a targeted mutation in the EGF receptor have delayed recovery from nephrotoxic AKI (38), suggesting that this pathway may play an important role in renal repair. However, given the pleiotropic effects of EGF in multiple tissue types, therapeutic strategies based on this signaling pathway will require careful targeting to the tissue type of interest.

Insulin-like Growth Factor-1. Although insulin-like growth factor-1 (IGF-1) is minimally expressed in the adult human kidney, its receptor is abundantly expressed on proximal tubule cells. After renal injury, expression of IGF-1 is up-regulated in surviving proximal tubule cells. In addition, recruited inflammatory cells such as macrophages produce IGF-1 (39). Not only is IGF-1 mitogenic, it induces expression of EGF receptor (40) and may enhance proliferation of remaining tubular cells through the EGF/EGF-receptor signaling pathways. IGF-1 promotes renal blood flow and leads to an increase in glomerular filtration rate, likely via production of prostaglandins and nitric oxide (41); improved renal blood flow may enhance the recovery process. Finally, IGF-1 promotes anabolism and protein synthesis (42, 43), which might aid in the recovery from critical illness. Unfortunately, despite the promise of IGF-1 in animal models, clinical trials in humans failed to demonstrate a benefit of IGF-1 in AKI (44).

α-Melanocyte Stimulating Hormone. α-Melanocyte stimulating hormone (α-MSH) is an anti-inflammatory cytokine derived from pro-opiomelanocortin (reviewed by Kohda et al (45)). Receptors for α-MSH are found on macrophages, neutrophils, and renal tubules. Endogenous α-MSH production is up-regulated in inflammatory states, and α-MSH down-regulates leukocyte activation. Interestingly, α-MSH is protective even in intercellular adhesion molecule-1 (ICAM-1)–deficient animals, in which neutrophil recruitment is attenuated (46). α-MSH also seems to have direct effects on renal tubules, including down-regulation of inducible nitric oxide synthase, which may attenuate the extent of injury. However, the role of endogenous α-MSH

in renal repair and recovery remains unclear.

Erythropoietin. Erythropoietin (EPO) has been shown to accelerate recovery from ischemic AKI (reviewed by Sharples et al (47)). It has been proposed that this is in part due to improved endothelial cell survival and function, resulting in improved oxygen delivery to the renal tubular epithelium. In addition, EPO administration before ischemia increases the number of cells expressing hypoxia-inducible factor-1α, which is known to be induced by ischemia and to be important in up-regulating genes, including EPO itself, that are involved in renal-cell survival and repair (48, 49). EPO also promotes proliferation of tubular epithelial cells in a cisplatin model of AKI. However, no studies have been done to examine whether EPO therapy can attenuate human injury; furthermore, the role of endogenous EPO in renal repair remains unclear.

Hepatocyte Growth Factor. As the name implies, hepatocyte growth factor (HGF) was initially identified as a mitogenic factor for hepatocytes. HGF was subsequently shown to have additional properties, including mitogenic, morphogenic, motogenic, and differentiating effects in kidney-derived cell lines and in the kidney itself (50, 51). HGF binds to c-met, a receptor tyrosine kinase, leading to the induction of a number of signaling pathways, including mitogen-activated protein kinase, PI3K, Src, and Grb2/Sos/Ras. Renal injury leads to up-regulation of both HGF and c-met within the kidney.

In animal models of toxic and ischemic AKI, HGF therapy at the time of injury markedly accelerated functional and histologic recovery (52, 53). Not only does HGF have important effects on cellular proliferation, it may prevent tubule damage by promoting adhesion of tubular cells to the basement membrane. This may in turn prevent cellular sloughing and loss (54) and activate anti-apoptotic signaling pathways (including PI3K and Bcl-xl) (55). In the late phases of recovery, HGF has antifibrotic effects that may have an important role in the prevention of long-term fibrosis and scarring (51).

Bone Morphogenetic Protein-7. Bone morphogenetic protein (BMP-7), also known as osteogenic protein-1, is a member of the transforming growth factor-β superfamily that has been shown to be essential for skeletal, kidney, and ocular development (56). BMP-7 expression persists in the adult kidney, in particular in the collecting tubule, the glomeruli, and the renal arteries. Although the role of endogenous BMP-7 expression in the maintenance of normal renal architecture remains unclear, a number of studies have examined the effects of BMP-7 both on acute and chronic kidney injury. Renal ischemia leads to decreased levels of BMP-7 messenger RNA in the rat kidney, likely due to local tissue damage (57). Administration of exogenous BMP-7 at the time of ischemic injury leads to attenuation of the severity of the injury, both histologically and biochemically (58). In the same study, BMP-7 administration also reduced levels of ICAM-1 expression in the damaged kidney, which may limit inflammatory cell–mediated injury. In cell culture models, BMP-7 seems to down-regulate proinflammatory cytokines, including interleukin-6, interleukin-8, monocyte chemotactic protein-1, and endothelin-2, a potent vasoconstrictor (59). Like HGF, BMP-7 seems to have important antifibrotic effects in the recovering kidney (60).

Transforming Growth Factor-β. Transforming growth factor-β (TGF-β) is a profibrotic growth factor that is a critical mediator of the epithelial-to-mesenchymal cell transition (60). Although TGF-β receptor expression is up-regulated after injury, TGF-β does not seem to play a critical role in early renal recovery because immediate treatment with TGF-β neutralizing antibodies does not slow renal recovery (61). However, treatment with neutralizing antibodies results in reduced capillary dropout and interstitial inflammation, suggesting that TGF-β may play a critical role in the long-term effects of AKI. More recent studies suggest that capillary dropout and increased interstitial cellularity are associated with salt-sensitive hypertension and increased albumin excretion, which may be responsible for the long-term effects of AKI (62). Indeed, TGF-β seems to be an important mediator of renal fibrosis in many different contexts.

Recovery from Acute Injury: Role of Adjacent Cells and Extracellular Matrix

Cellular contact with adjacent cells and with the extracellular matrix protects cells from apoptosis. After injury, major structural components of the basement membrane, including laminin and fibronectin III, and the cell surface receptors for these basement membrane proteins (e.g., integrins) are up-regulated (63, 64). Integrins are also mislocalized after renal injury; it has been suggested that this may allow epithelial cells to migrate as part of the recovery process (21). Normal cell–cell contact via cadherins also provides an anti-apoptotic signal in proximal tubular cells (65). Components of the extracellular matrix, notably hyaluronan, may be up-regulated after renal injury and seem to be critical for the recruitment of extrarenal cell types that contribute to the recovery process, including mesenchymal stem cells.

Recovery from Acute Injury: Role of Endothelium

At present, specific mechanisms of endothelial repair and recovery remain unclear. However, it is clear that endothelial injury plays a critical role in the pathogenesis of AKI (15). Furthermore, the endothelium regulates leukocyte recruitment to areas of injury. Endothelial adhesion molecules, including E-selectin, P-selectin, and ICAM-1, become up-regulated after injury and recruit leukocytes to the site (21). This results in migration of leukocytes from the vessel lumen to the surrounding tissue and in T-helper cell stimulation. Multiple strategies to block ICAM-1, including anti–ICAM-1 antibodies and antisense oligonucleotides directed against ICAM-1, and studies in ICAM-1 deficient mice have all shown that ICAM-1 blockade is protective in the setting of AKI. Interestingly, a recent study has demonstrated that statins ameliorate ischemic renal damage. In this study, inflammatory cell infiltration, up-regulation of ICAM-1, and increased inducible nitric oxide synthase production were all attenuated by statin administration (66). Thus, renal injury clearly results in damage to the endothelial compartment, which in turn promotes tubular injury; the mechanisms of cellular repair require further study.

Strategies to Accelerate Renal Recovery: Human Studies

A number of clinical treatments have been proposed to enhance renal recovery from acute injury (11). These include strategies to increase urine flow and decrease tubular obstruction, such as the use of osmotic and loop diuretics, strategies to increase renal blood flow, such as the use of low-dose dopamine and atrial natriuretic peptide, and strategies to pro-

mote renal recovery using growth factors, including IGF-1. Unfortunately, none of these strategies has shown clinical benefit in human trials.

Thus, dialysis is the primary supportive therapy administered to patients with severe AKI. The effect of renal replacement therapy on renal recovery has been recently reviewed (67). In brief, the effect of several aspects of the dialysis prescription, including the timing of initiation of dialysis and dialysis dose, remain controversial or unknown. Both the timing and dose of dialysis may affect levels of inflammation and therefore affect renal recovery. A number of studies have focused on the effect of dialyzer membranes on mortality and renal recovery because the original cellulose-containing membranes activate complement and coagulation factors. These cellulose-containing membranes are frequently referred to as *bioincompatible* membranes. Newer synthetic membranes (polyacrylonitrile, polymethyl methylacrylate, polysulfone) and cellulose membranes containing synthetic side groups are more "biocompatible" and therefore cause less complement activation. Although these membranes initially were shown to have a positive effect on renal recovery and mortality, subsequent studies did not support these results. Several meta-analyses have been published, with varying conclusions; thus, the effect of biocompatible membranes on renal recovery seems to be limited, at best (68).

Lastly, the modality of dialysis may affect renal recovery. Continuous renal replacement therapy has a number of features that may improve renal recovery compared with intermittent hemodialysis (69), including 1) superior control of uremia, 2) prevention of intradialytic hypotension and thereby reduced risk of extending ischemic renal damage, 3) enhanced clearance of inflammatory mediators, and 4) better ability to provide nutritional support. The effect of modality on renal recovery has varied in studies to date; furthermore, it does not seem that modality improves overall survival (67). Thus, at present, these benefits for renal recovery must be considered largely theoretical.

CONCLUSIONS

AKI is a complex process, involving sublethal cell injury and apoptosis and necrosis of tubular cells. Recovery of the tubular epithelium requires proliferation and repolarization of remaining tubule cells. In addition, changes to the renal microvasculature contribute to tubular injury. Renal tubular epithelial cells and renal-specific and mesenchymal stem cells seem to contribute to the recovery process, although it seems that the mesenchymal stem cells have a primarily paracrine effect. A number of peptide growth factors have been studied in animal models and shown to play a role in the recovery process. However, to date, new therapies for human disease based on this work have been unsuccessful. Adjacent cells and the endothelium play a critical role in the recovery process. Lastly, additional studies are needed to understand the potential effect of renal replacement therapy on recovery from AKI.

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