Current Perspective

Regeneration of Injured Renal Tubules

Makoto Yoshida1,* and Shigeyoshi Honma1

1Department of Pharmacology, Faculty of Pharmacy, Takasaki University of Health and Welfare, Gunma 370-0033, Japan

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Abstract. Acute kidney injury (AKI), clinically defined by high serum creatinine and low urine flow, has many complicated pathophysiological features including tubular and glomerular injury. Although renal tubules are thought to be constituted by highly differentiated epithelial cells, it is possible to repair injured nephrons by the healing process. Several studies have revealed that AKI, especially AKI caused by ischemia/reperfusion injury or nephrotoxic medication, depends on a number of factors, including activation of transcriptional factors, endothelial injury of peritubular small vessels, immune responses, and inflammatory processes associated with necrosis and apoptosis of renal tubular epithelium. For regeneration of injured tubules, partly dedifferentiated progenitor-like cells fill the injured site and constitute the tubular structure and function, although the source of these cells is still under debate. It is essential to understand the molecular, cellular, and genetic mechanisms of AKI and tubular regeneration for the development of therapies to prevent and treat kidney injury.

Keywords: kidney, regeneration, tubule, acute kidney injury (AKI)

1. Introduction

There is increasing interest in the treatment of renal cell damage using artificial stem cells such as embryonic stem cells or inducible pluripotent stem cells. Although the kidney is a relatively complex organ consisting of various types of tissues, including tubules, vasculature, and interstitial cells, efforts to construct functional nephrons using premature cells are now partly successful. Recently, Song et al. (1) constructed a whole kidney using epithelial and endothelial cells on a rat cadaveric renal extracellular matrix scaffold. It is possible that the seeding of human umbilical venous endothelial cells and rat neonatal kidney cells on kidney scaffolds could be used to construct glomerular, tubular, and vascular architecture. An in vitro functional study (1) using rats revealed that the regenerated kidney filtered a standardized perfusate, reabsorbed glucose and sodium, and produced urine, although the rates were approximately 10% – 50% of those levels observed in cadaveric kidneys.

The mature kidney is thought to be an organ incapable of tubule generation because normal nephron development ends in the early stages of infancy as a result of the loss of progenitor mesenchyme (2). However, this does not rule out the possibility of a repair process for injured renal tubules with newly developed tubular epithelial cells. Damaged renal functions in acute kidney injury (AKI) can be restored by functional recovery of intact nephrons and intrinsic repair processes of injured structures. In this mini review, we summarize recent developments in acute renal tubular damage and recovery with accompanied processes.

2. Major contributors to AKI

AKI has a wide spectrum that includes several types of injury ranging from minor dysfunction to serious complications that require dialysis. Recently, a clinical definition and classification of AKI has been proposed by the Kidney Disease Improving Global Outcomes working group (3). In this guideline, AKI is defined as any of the following three criteria: 1) increase in serum creatinine by ≥ 0.3 mg/dl within 48 h, 2) increase in serum creatinine to ≥ 1.5 times baseline that is known or presumed to have occurred within the prior 7 days, or 3) decrease in urine volume to < 0.5 ml·kg\(^{-1}\)·h\(^{-1}\) for 6 h. Although
serum creatinine levels and urine volume are affected by some physiological and pathophysiological conditions other than those of the kidney, the above multiple diagnosis criteria of AKI can be helpful for prompt recognition and therapeutic intervention of renal failure.

The most common primary cause of AKI is ischemia and restoration of blood supply and re-oxygenation is known to be associated with exacerbated tissue injury, namely ischemia/reperfusion injury. Renal ischemia causes impairment of oxygen and nutrient delivery to cells of the kidney. Regions of the renal tubule most prone to ischemic injury are the S3 segments of the proximal tubule and the medullary thick ascending limb of the loop of Henle. Tissue hypoxia results in severe damage to the tubular epithelial cells, particularly in the proximal tubules of the outer medulla, which cannot convert from oxidative to glycolytic metabolism (4).

Although the precise mechanisms of ischemic injury are unknown, activation of transcriptional factors, like hypoxia-inducible factor (HIF) and nuclear factor-κB (NF-κB), endothelial injury of peritubular small vessels, Toll-like receptor (TLR) signaling, immune responses, and inflammatory processes, are associated with necrosis and apoptosis of renal tubules (5 – 9).

Hypoxia is associated with alterations in transcriptional control of the expression of several genes. Well-known transcription factors relating to hypoxia are HIF and NF-κB; both of these are regulated by oxygen-sensing prolyl-4-hydroxylase domain–containing proteins (PHD) (5). Under normal oxygen conditions, PHD induces hydroxylation of specific prolyl residues in HIF using oxygen as a cofactor and then initiates a proteasomal degradation of HIF α subunit. Another oxygen-dependent enzyme, factor-inhibiting HIF, also reduces the transcriptional activity of HIF by the hydroxylation of asparagyl residues in HIF. In contrast, hypoxia is associated with the stabilization of the α-subunit of HIF, thereby allowing formation of an αβ heterodimer. Binding of the HIF heterodimer can either induce or repress the transcription of several target genes, including erythropoietin, vascular epidermal growth factor, glucose transporter-1, and NF-κB (10). Furthermore, hypoxia activates NF-κB by suppression of PHD-dependent hydroxylation of IκB kinase-β (11). The activation of NF-κB induces the transcription of cytokines and chemokines leading to inflammation. However, the role of the PHD-HIF pathway in AKI is a matter of debate because HIF has been implicated in mediating the cytoprotective effects of ischemic preconditioning. A recent report has revealed that pre-ischemic but not post-ischemic inhibition of PHD ameliorated ischemia/reperfusion injury, inflammation, and fibrosis, suggesting a relatively protective effect of HIF and HIF-activated gene products on AKI (12).

Endothelial cells of peritubular small vessels play critical roles in the pathophysiology of AKI (6). When the endothelium is injured by ischemia, small arterioles constrict more than normal and decrease the blood supply to renal tubular cells, along with concomitant increases in tissue levels of vasoconstrictors, such as endothelin-1, angiotensin II, thromboxane A₂, and prostaglandin H₂, and decreases in the activity of nitric oxide and other vasodilators (13). Furthermore, there are enhanced endothelium–leukocyte interactions due to increased expression of cell adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1), on damaged endothelial cells (14). Activated leukocytes generate pro-inflammatory and chemotactic cytokines and impair barrier function of injured endothelial cells, further enhancing immune and inflammatory processes.

Althought microorganism invasion is not included in the ischemia/reperfusion-induced immune response, pattern-recognition molecules, such as TLR, are involved in signaling events that recruit and activate immune cells and the complement system. Mice with a genetic deletion of Tlr4 are protected from kidney ischemia, and experiments using bone marrow chimeric mice suggest that kidney-intrinsic TLR4 signaling has a predominant role in mediating kidney injury (7). Signaling by TLR2, another TLR, has also been reported to contribute to AKI and inflammation during ischemia/reperfusion injury (8). TLR4 and TLR2 are activated by endogenous ligands, such as high-mobility group box 1, released from necrotic cells.

In addition to necrosis of renal tubular cells, apoptosis is involved in the processes of ischemia/reperfusion injury. Renal tubule injury is associated with several apoptotic indicators such as chromatin condensation, DNA fragmentation, caspase 3 activation, apoptosis-inducing factor or cytochrome c release, conformational Bax activation, and loss of intact Bcl-2. Pharmacological or bioengineered inhibition of some of these factors reduces apoptosis in proximal tubules of the cortex and outer medulla and improves renal function after ischemia, as indicated in a recent review (9).

The use of nephrotoxic medication is another common cause of kidney injury. Cisplatin, a well-characterized chemotherapy agent, induces acute and chronic renal tubular injury, which limits its efficacy as an anticancer treatment. Although DNA is the primary biological target of cisplatin, it has several other intracellular targets. Its nephrotoxicity is associated with activation of oxidative stress and mitogen-activated protein kinase (MAPK) families (15 – 17). Oxidative stress activates transcription of inflammatory mediators, including NF-κB and cyclooxygenase-2 (COX-2). Antioxidant
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treatment can ameliorate cisplatin-induced acute renal injury and fibrosis (15, 18). The anti-inflammatory and renal protective effects of COX-2 inhibitor on cisplatin-induced AKI are accompanied by inhibition of NADPH oxidase and MAPKs, suggesting that feed-forward deteriorating processes between oxidative stress and inflammation are involved in cisplatin nephrotoxicity (17).

3. Repairing theories

Acute injury of renal tubular cells by anoxia or nephrotoxic medications results in necrosis, apoptosis, and detachment of cells from the tubular basement membrane. Migration of new cells into the injured region reconstitutes a functional tubular epithelium. However, the origin of the new epithelial cells is still under debate. Three possible sources of these cells are postulated: 1) renal stem or progenitor cells, 2) dedifferentiation of injured tubular cells, and 3) immigration of bone marrow-derived cells (Fig. 1).

Epithelial cells in the skin or gastrointestinal tract are known to turnover at a high rate, and their source is stem or progenitor cells at basal areas of tissues. While the magnitude of renal cell turnover is lower than these organs, it is possible that there is a similar repairing mechanism for tubular epithelium, particularly with acute injury. Cell division of the adult kidney has been suggested by pulse-chase labeling studies using a nucleotide analog in rodents. Daily administration of bromodeoxyuridine (BrdU) for 7 days incorporated it into DNA of cells (called label-retaining cells: LRC) in renal tubules of normal rat kidney (19). Acute ischemia/reperfusion injury increased the number of LRC that were mostly positive to proliferating cell nuclear antigen (PCNA), a cell division marker that specifically recognizes early G1 and S phases of the cell cycle. Isolated LRC have the potential to form tubule-like structures in collagen gel and to differentiate into several types of renal tubular cells when transplanted into rat metanephros isolated from embryos (20). Pathological studies of kidney samples from human AKI patients have also revealed elevated numbers of PCNA-positive cells in tubules (21).

It is possible that renal stem or progenitor cells outside the nephron migrate into damaged nephrons and then regenerate tubules. A pulse-chase study using BrdU in the healthy rat kidney showed that numerous LRCs were present in renal papilla (22). During the repair phase of transient renal ischemia, these cells entered the cell cycle and the BrdU signal quickly disappeared from the

Fig. 1. Proposed cell sources to repair damaged renal tubules in acute kidney injury (AKI). These include 1) proliferation and differentiation of stem or progenitor cells in tubules, 2) injured but surviving tubular epithelial cells that dedifferentiated to mesenchyme state by epithelial–mesenchymal transition (EMT), and 3) immigration and differentiation of stem cells in bone marrow or extra-tubular spaces. Delayed or improper treatment of AKI may result in chronic kidney disease (CKD) accompanied with intercellular fibrosis.
papilla, despite the absence of apoptosis in this part of the kidney. Some renal papillary cells, including LRC, migrated to the medulla and integrated into collecting ducts (23). However, another time-course study has failed to detect the migration of papillary LRC to injured tubules of the outer medulla or cortex following ischemia (24).

The existence of tubular stem cells in the kidney has also been suggested from gene expression and functional assays. One cell line dissected from a single nephron of an adult rat showed great potential to grow and expressed both mature and immature tubular cell markers related to kidney development (25). Another study reported mouse proximal tubule progenitor-like cells that expressed nuclear factor of activated T-cell cytoplasmic 1 (NFATc1), a transcription factor (26). NFATc1-labeled proximal tubular cells were relatively resistant to mercuric chloride–induced renal injury and repaired damaged proximal tubule segments.

In humans, cell surface antigens, such as CD24 and CD133, are used for isolating renal stem cells because they are known markers of other adult stem cells. Several reports have suggested that multipotent CD24−CD133+ tubular cells are present in the human kidney. A proximal tubular cell population with high aldehyde dehydrogenase activity has been shown to express CD24, CD133, and mesenchymal markers, like vimentin (27). These cells displayed sphere-like clusters of epithelial cells in culture and anchorage-independent growth features, two classic stem cell properties. A recent study has shown that a human CD24+CD133+CD106− tubular progenitor population displayed a committed phenotype toward the tubular lineage (28). When these human cells were injected into severe combined immune-deficient mice with AKI, regenerated tubular structures were observed in different portions of the nephron, and it also reduced the morphological and functional kidney damage.

Other possible cell sources to repair damaged tubules are intact or less-injured tubular epithelial cells around the detached cells. Because renal tubular epithelial cells are highly differentiated and quiescent, it is hard to expect that they will divide by themselves in that phenotype. Following injured cell detachment, the exposed basal membrane is progressively covered by cells with mesenchymal features, including a flattened appearance, lost polarity, and the expression of proteins that are characteristic of motile mesenchyme. Tubular epithelial cells that survive might dedifferentiate to the mesenchyme state and re-enter the cell cycle, and then deviated mesenchymal cells repair tubular damage. This epithelial–mesenchymal transition (EMT) is well investigated in oncology and developmental biology, and accumulated evidence suggest its participation in kidney fibrosis in chronic kidney disease (29). Using genetic fate–mapping techniques, Humphreys et al. have shown that regeneration of outer medulla nephrons after ischemia/reperfusion injury is predominantly accomplished by surviving, less-injured tubular epithelial cells (24). In this experiment, the Six2 reporter marked renal epithelium cells that have arose from the cap mesenchyme during nephrogenesis. Despite extensive cell proliferation, no dilution of cell-fate marker was observed after repair, arguing against the presence of a small population of unlabeled non-tubular cells with tubular regenerative capacity. In a subsequent two-step nucleotide analog pulse study, the researchers showed that the outer medulla tubule proliferation after ischemic injury occurs by self-duplication of epithelial cells through EMT, but not by either proliferation of intra-tubular or papillary LRC (30).

Another possible theory for the repair of injured tubules is intervention with bone marrow–derived stem cells. Transplanted kidneys from female donors to male patients involved renal tubules consisting of some extra-renal cells with Y chromosome. The percentage of Y chromosome–positive renal tubule cells ranged from 0.6% – 6.8% (31) and about 1% (32). Several studies with transplantation of whole bone marrow from male to female mice (31, 33) or from β-gal transgenic Rosa mice to wild-type recipients (33) have indicated that some tubular cells were derived from bone marrow stem cells during kidney regeneration. Although another group of researchers has been unable to reproduce these findings, they suggest a paracrine effect of renal interstitial cells derived from bone marrow stem cells that prevents apoptosis and enhances proliferation of injured epithelial cells (34).

Physiological and pathophysiological implications of each source of stem or progenitor cells for the repair of injured renal tubules are still unknown. Conflicting results may arise because of different timings for detection, sensitivity and selectivity of used cell markers relating to levels of differentiation, and severity of tubular damage. Even if it is a rare event, we cannot exclude its participation in the regeneration of tubules after injury. Although further precise studies are needed, it is possible to progress to intrinsic mechanisms for repairing injured tubules by stimulating each source of progenitor cell. Recent studies have revealed the molecular basis of recovery from AKI accompanied by a complex pattern of gene expression that induces several molecular regulators, including growth factors, adhesion molecules, and cell cycle regulators (35). Much more understanding of the pathophysiological roles of these factors will contribute to unraveling the novel pathways to repair tubular cells with AKI.
4. Conclusions

Accumulated tubular injury and delay or inhibition of tissue repair appears to lead to the progression of kidney damage. Prolonged stimulation of the immune system, inflammation, and EMT develops fibrosis of interstitial areas, induces chronic kidney disease, and further leads to end-stage renal disease that requires dialysis or renal transplantation (13). To prevent patients from succumbing to chronic kidney disease, it is important to regenerate tubular epithelium and regain its functions at the earliest stage possible. The existence of many factors affecting tubular injury and recovery indicate several possible ways to intervene in its processes. It is essential to understand further molecular, cellular, and genetic mechanisms of AKI and tubular regeneration for therapies to prevent and treat kidney injury.

References

30. Humphreys BD, Czerniak S, DiRocco DP, Hasnain W, Cheema R, Bonventre JV. Repair of injured proximal tubule does not


