Defining the Acute Kidney Injury and Repair Transcriptome

Sanjeev Kumar, MD, MRCP, PhD, Jing Liu, PhD, and Andrew P. McMahon, PhD, FRS

Summary: The mammalian kidney has an intrinsic ability to repair after significant injury. However, this process is inefficient: patients are at high risk for the loss of kidney function in later life. No therapy exists to treat established acute kidney injury (AKI) per se: strategies to promote endogenous repair processes and retard associated fibrosis are a high priority. Whole-organ gene expression profiling has been used to identify repair responses initiated with AKI, and factors that may promote the transition from AKI to chronic kidney disease. Transcriptional profiling has shown molecular markers and potential regulatory pathways of renal repair. Activation of a few key developmental pathways has been reported during repair. Whether these are comparable networks with similar target genes with those in earlier nephrogenesis remains unclear. Altered microRNA profiles, persistent tubular injury responses, and distinct late inflammatory responses highlight continuing kidney pathology. Additional insights into injury and repair processes will be gained by study of the repair transcriptome and cell-specific translatome using high-resolution technologies such as RNA sequencing and translational profiling tailored to specific cellular compartments within the kidney. An enhanced understanding holds promise for both the identification of novel therapeutic targets and biomarker-based evaluation of the damage-repair process.

Semin Nephrol 34:404-417

Keywords: Acute kidney injury, repair, transcriptome, TRAP, miRNA, development, cancer

The clinical syndrome of acute kidney injury (AKI) is characterized by an abrupt (within 48 h) decrease in kidney function, frequently caused by ischemia reperfusion injury (IRI), sepsis, or nephrotoxic insult.[1–3] Despite advances in medical care, patients with AKI continue to have high morbidity and mortality; in-hospital mortality rates in critically ill patients with AKI approach 50% to 70%.[3,4] Furthermore, survivors also have a strikingly higher risk of developing chronic kidney disease (pooled adjusted hazard ratio, 8.8; 95% confidence interval, 3.1-25.5), and end-stage renal disease (pooled adjusted hazard ratio, 3.1; 95% confidence interval, 1.9-5.0) compared with non-AKI patient groups.[5]

The histologic features of human ischemic AKI include loss of the brush border typical of the proximal tubular epithelium, sloughing of tubular epithelial cells into the lumen resulting in focal loss of tubular epithelial cells, infiltration of inflammatory cells, and the appearance of Tamm-Horsfall protein-rich casts in the urine.[6] After AKI, a repair process restores renal tubular epithelium and kidney function. The cellular mechanisms of repair have been scrutinized intensively using mouse genetic approaches. Agreement is increasing that surviving cells within the renal tubular epithelium repair tubular damage in the mouse, and likely the human kidney (see article by Marcus Moeller in this issue). Whether repair is a general capacity shared by surviving cells, or a more specific function ascribed to a small subset of identifiable epithelial cells, has engendered considerable debate (see article by Marcus Moeller in this issue). It is clear that the reparative process is not as efficient or effective as desired: fibrosis is evident despite the reacquisition of biochemical parameters such as plasma creatinine removal, and progression to chronic kidney disease is a frequent long-term outcome.[5]

Fibrosis is associated with injury-invoked appearance of α-smooth muscle actin–positive myofibroblasts. In fibrosis, Yang et al[7] suggested G2/M-arrested proximal tubular cells activate c-jun NH_2-terminal kinase signaling, initiating production of profibrotic cytokines. In fibroblasts, hypermethylation of RAS protein activator like 1, an inhibitor of the Ras oncoprotein, leads to prolonged fibroblast activation and fibrogenesis.[8] Once triggered, myofibroblasts synthesize a distinct collagen I–rich extracellular matrix that may promote further fibrosis.

Initial suggestions that most fibrotic cells arise from an epithelial-to-mesenchymal conversion of renal tubule cells have been challenged; a revised view of an extratubular origin for myofibroblasts is supported by several fate-mapping studies. One view holds that

Department of Stem Cell Biology and Regenerative Medicine, Eli and Edythe Broad-California Institute of Regenerative Medicine (CIRM) Center for Regenerative Medicine and Stem Cell Research, The Keck School of Medicine of the University of Southern California, Los Angeles, CA.

Financial support: Supported by grants from the National Institute for Diabetes and Digestive and Kidney Diseases and CIRM (A.P. M.), and by the John McKay Fellowship from the University Kidney Research Organization (S.K.).

Conflict of interest statement: none.

Address reprint requests to A. McMahon, Department of Stem Cell Biology and Regenerative Medicine, Eli and Edythe Broad-CIRM Center for Regenerative Medicine and Stem Cell Research, The Keck School of Medicine of the University of Southern California, 1425 San Pablo St, Los Angeles, CA 90089. E-mail: amcmahon@med.usc.edu

0270-9295/ - see front matter

http://dx.doi.org/10.1016/j.semnephrol.2014.06.007

perivascular fibroblasts (pericytes) are the chief culprit,[9] whereas another associates fibrosis resident non-pericyte intertubular fibroblasts and bone marrow–derived fibroblasts.[10] The origins of injury-associated myofibroblasts are discussed in article by Benjamin Humphreys.

Harnessing and enhancing the kidney's intrinsic mechanisms of repair, and developing approaches to suppress and reverse renal fibrosis, are major goals of renal regenerative medicine. These strategies are founded, and dependent, on our detailed knowledge of the molecular and cellular events at play. New approaches to interrogate underlying mechanisms have enhanced resolution at the molecular level by enabling systematic, relatively unbiased, quantitative measurement of transcriptional and translational events. Further, the move from whole-organ analysis to a breakdown of responses in specific cellular compartments is increasing cellular resolution. These advances will facilitate the identification of new targets augmenting renal repair processes and suppress renal scarring.

Here, we provide a brief overview of the cellular responses initiated by AKI, with a particular focus on the repair processes after ischemic AKI, review studies that have performed whole-kidney or cell-specific gene/transcript expression analysis temporally in the setting of murine and human AKI, and discuss the role of next-generation RNA-sequencing (RNA-seq) and translating ribosome affinity purification (TRAP) profiling in transcriptional and translational analyses, respectively, of the renal repair process.

BRIEF OVERVIEW OF CELLULAR RESPONSES AFTER ISCHEMIC AKI

Injury and Repair of Nephron

Renal Tubule Damage

The proximal tubule is divided into three molecularly, histologically, and topographically distinct segments: S1, S2, and S3.[11] The S3 segment, although highly developed in rodents, is not as pronounced in human beings. The epithelial cells in the straight S3 segment of the rodent proximal tubules located in the outer stripe of the outer medulla are exquisitely sensitive to ischemic insults. Histologically, the ischemic injury is readily discernible in this stripe in animal models of ischemic AKI induced by clamping of the renal pedicle. The S1 and S2 segments of the proximal tubule also respond to injury but the S3 segment shows the most marked cell loss after AKI in the mouse kidney.[12,13] Although the medullary thick ascending limb (TAL) of the loop of Henle also resides in the outer medullary region, the TAL is relatively resistant to IRI. However, an AKI-like phenotype can be induced experimentally by targeting apoptosis specifically within the TAL.[14] IRI regimens that effectively target the S3 segment of the proximal tubule (PT) have little effect on cells of the TAL. The differential sensitivities off adjacent tubular epithelial cell types may reflect a distinct ability of TAL cells to switch from oxidative to glycolytic metabolism,[15] to mount anti-apoptotic response (activating extracellular signal-related kinase and BCl-2 proteins),[16] and increased expression of insulin-like growth factor-1 (IGF-1) and hepatocyte growth factor (HGF).[17]

Both proximal and distal tubules undergo cell death in human AKI although biopsy specimens of renal allografts show significantly greater apoptosis in distal tubules whereas proximal tubular epithelial cells show more marked proliferation.[18] Focal areas of tubular epithelial cell loss in the TAL and proximal tubular S3 segment have been reported in patients with ischemic acute tubular necrosis.[19]

Ischemia-induced renal tubular adenosine triphosphate depletion is likely an initiating insult in rodent IRI-associated AKI. Critical alterations in tubular dynamics, metabolism, and structure ultimately lead to necrotic and/or apoptotic cell death. These include depletion of cellular energy stores, loss of basolateral distribution of Na^+K^+adenosine triphosphatase and β-integrins (loss of polarity), disruption of the actin cytoskeleton and adherent and tight-junctions (shedding of brush border and sloughing of cells), accumulation of intracellular calcium, accumulation of hypoxanthine, and generation of reactive oxygen species.[20]

Renal Tubule Repair

Damaged renal tubular epithelium may be repaired by surviving epithelial cells, other cell types resident within the kidney, or cells that move into the injured organ. Only direct experimental analysis can distinguish among these possibilities; consequently, the most robust conclusions are founded on fate-mapping strategies using mouse genetics. By using approaches that label renal tubule cells exclusively, Humphreys et al[21] argued that repair by surviving cells within the proximal tubule epithelium is a broad mechanism. Further analysis of clone size and differentiation markers suggests that repair in S1/S2 segments is not mediated by a rare stem cell but is general property of differentiated proximal tubule epithelial cells activated on injury.[22] A contrasting view argues for repair from a small subset of CD24+, CD133+ cells that reside within human renal tubules.[23,24]

Non-Nephron Components of Injury and Repair

Macrophage, Leukocytes, and Neutrophils

One of the earliest cellular responses to renal damage, seen within the first few hours after the triggering stimulus, is neutrophil and macrophage infiltration;