Biology of Renal Recovery: Molecules, Mechanisms, and Pathways

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Key Words

Acute kidney injury · Cell cycle · Fibrosis · Hypoxia-inducible factor-1 α · Inflammation · Mitochondria

Abstract

Acute kidney injury (AKI) contributes to progressive kidney disease. Although significant advances have been made in the understanding of mechanisms of AKI, less is known about the biological basis that links the initial injury to progressive interstitial fibrosis, tubular dysfunction, and capillary rarefaction. The round table discussion focused on mechanisms of renal recovery and fibrosis following AKI. The knowledge gained by understanding these pathways will serve to identify novel therapeutic targets in the future.

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Introduction

Acute kidney injury (AKI) results in temporary loss of kidney function. In some cases, this initial injury leads to progressive kidney disease and end-stage renal disease. However, the kidney also has a remarkable ability to undergo reparative processes leading to regeneration and recovery following AKI. Over the last 2 decades, there has been great progress to identify mechanisms that lead to

regenerative processes. This review will cover the mechanisms of recovery from AKI incorporating the discussion at the round table in San Diego, Calif., USA, at the CRRT (Conference on Continuous Renal Replacement Therapies) 2014 and current literature regarding the source of cells that contribute to the regenerative process, including stem cells, epithelial cells, pericytes, and bone marrow-derived cells. This review will also cover the maladaptive process of tubulointerstitial fibrosis, i.e. the deposition of extracellular matrix (ECM) components that leads to loss of kidney function.

Aging and Senescence

With age, the capacity for repair decreases, and chief among the biological processes that account for aging is somatic senescence. Senescence refers to an irreversible growth arrest with viable cells lacking mitogenic potential, so cells do not die but their ability to grow is impaired [1]. Telomeres are nucleoproteins consisting of repetitive DNA sequences and specific proteins that are located at

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the end of all eukaryotic chromosomes. Telomeres control chromosome stability, genetic integrity, and cell viability, and telomerase enzymes are thought to play a role in cell senescence [2]. Other factors such as oxidative stress, DNA damage, and mitochondrial injury contribute to non-telomerase-dependent cell senescence. These factors contribute to the increase in the incidence of AKI and decreased capacity for repair in the aging population [1, 3].

Cell Injury

In most animal models of ischemia-reperfusion injury, the S3 segment of the proximal tubule is most affected and tubule cells undergo apoptosis and necrosis [4]. Hypoxia is an early stimulus that inactivates prolyl hydroxylase thus inhibiting hypoxia-inducible factor (HIF)-1 α degradation. HIF-1 α then translocates to the nucleus and binds to HIF-1 β leading to transcription of HIF target genes resulting in angiogenesis, apoptosis, cell proliferation, cell survival, and glucose metabolism, factors that lead to both oxygen delivery and adaptation to oxygen deprivation [5]. Heme oxygenase-1 and galectin-1 are important HIF-1 α target genes. Heme oxygenase-1 is known to suppress inflammation and AKI [6, 7], whereas galectin-1 is thought to suppress inflammation and may be cardioprotective in myocardial infarction [8]. HIF prolyl hydroxylase inhibitors are currently under development and some are in use in clinical trials (ClinicalTrials.gov).

Mitochondria exist in dynamic equilibrium and are a major source of energy in proximal tubule cells; thus, they play a central role in cell survival, injury, and death [9]. They undergo constant fusion and fission to maintain the health of cells. Mitochondrial fusion is mediated by mitofusin (Mfn1 and Mfn2) and optic atrophy (OPA1), and fission is mediated by dynamin-related protein (Drp1) and mitochondrial fission (Fis1). Alterations in these proteins lead to impaired mitochondrial function and cell death through lower ATP levels, release of proapoptotic proteins, and increased oxidant levels. Following mitochondrial injury, mitochondria are sequestered by a process referred to as mitophagy. The engulfment of injured mitochondria may prevent cell death [9] and regulation of mitophagy may be a novel strategy to prevent cellular death following ischemia-reperfusion injury. Rapamycin attenuates cisplatin nephrotoxicity, in part, through enhanced mitophagy, whereas chloroquine blocks mitophagy and exacerbates AKI [10]. The synthesis of new mi-

tochondria, referred to as mitochondrial biogenesis, may be an important mechanism that assists in renal recovery. The peroxisome proliferator-activated receptor- γ coactivator-1 α (PGC-1 α), a transcriptional coactivator, binds to several transcription factors that regulate mitochondrial biogenesis. In sepsis, deficiency in proximal tubule-specific PGC-1 α leads to more sustained loss in glomerular filtration rate compared to wild-type controls [11], and the sirtuin (SIRT1) activator induces deacetylation of PGC-1 α , a marker of activation and enhanced recovery of mitochondrial biogenesis and renal recovery [12].

Immune Cells, Inflammation, and Regeneration

Following injury, processes are initiated that lead to the regeneration of the tubule epithelium or, under pathological circumstances, to fibrosis, in which normal parenchymal cells are replaced with connective tissue. Following initial injury, acute inflammatory infiltration mediates damage within the kidney. Dendritic cells are abundant in the kidney interstitial compartment and interact with exogenous molecules (pathogen-associated molecular patterns) released from pathogens, endogenous molecules (danger-associated molecular patterns) released from injured cells, and with resident or infiltrating immune cells, including lymphocytes, natural killer T cells, epithelial cells, and fibroblasts [13]. Once activated, dendritic cells induce injury either directly or through inflammatory signals. Major cell types that contribute to the initial inflammation after AKI are inflammatory monocytes, neutrophils, lymphocytes, and natural killer T cells [14–16].

Monocytes are thought to survey the status of endothelial cells in tissues such as the kidney, and when the endothelium is damaged, they recruit neutrophils to elicit inflammation [17]. Inflammatory monocytes then help clear away apoptotic neutrophils and endothelial cells after injury. Thus, monocytes play a prominent role in renal injury as well as repair.

Several cell types regulate regeneration after injury. Monocytes and macrophages orchestrate multiple phases of tissue repair. Their exact role is somewhat obscured by the heterogeneity within monocyte/macrophage populations, perhaps due to phenotypic changes in macrophages over time within damaged tissue or recruitment of unique populations from outside the injury [18]. Changes in the phenotype reflect their changing role throughout the repair process, first promoting initial inflammation, thus

increasing initial injury which exacerbates scar formation, but eventually also promoting clearance of excess scar tissue. Macrophages were initially seen as either one of two classic phenotypes: M1 or M2, inflammatory and anti-inflammatory, respectively. This dichotomy, however, does not seem to fully encapsulate the diverse phenotypes of macrophages during wound repair or fibrosis. M2 macrophages have been further subdivided into M2a, M2b, and M2c phenotypes based on their profiles of cytokine production and function [19]. Macrophages show plasticity in response to environmental signals and therefore their phenotypes may not be terminal.

Macrophages also play a crucial role in the regeneration of the kidney after injury and reduction of the scar area. Toll-like receptor ligands, such as lipopolysaccharides, and cytokines, such as interferon- γ , induce monocytes to differentiate into M1 macrophages. M1 macrophages promote a proinflammatory environment within the kidney through the production of tumor necrosis factor- α , which results in apoptosis of endothelial cells within the kidney. These macrophages can be identified by cell surface expression of CCR2. Blockade of CCR2 reduces initial renal inflammation and injury [20]. Clodronate liposomes specifically deplete M1 macrophages and decrease injury [21]. M1 macrophages also play a role in increasing fibrosis in mice [22]; however, it remains to be determined if they exacerbate the initial inflammatory injury or if they inhibit repair.

While typical inflammatory cytokines and pathogen- and danger-associated molecular pattern molecules lead to the classical activated macrophage phenotype, different combinations of cytokines and immune activators induce the alternative phenotypes of macrophages. M2b macrophages produce interleukin (IL)-10 and transforming growth factor- β that promote an anti-inflammatory environment. IL-10 and platelet-derived growth factor produced by M2b macrophages stimulate the differentiation of fibroblasts into myofibroblasts, which lay down ECM after tissue destruction. M2c macrophage phenotypes help turn the ECM over thus decreasing the fibrotic area. They may also serve to stimulate the proliferation of renal endothelial cells. This may be due to the ability of macrophages to produce cytokines that promote tubular regeneration [23].

Orchestrating the process of regeneration after AKI is an assortment of soluble molecules such as cytokines, growth factors, and peptide molecules such as endothelin. Administration of recombinant epidermal growth factor to rats after bilateral ischemia-reperfusion injury increased proliferation of tubules [24]. Similarly, studies

have shown that growth factors such as platelet-derived growth factor, insulin-like growth factor-1, and hepatocyte growth factor are induced within the kidney after injury. Treatment with these molecules increased the proliferation and regeneration of tubule cells after AKI [25]. IL-10 has been shown to be beneficial in decreasing the initial inflammatory damage after both ischemic and cisplatin-induced renal injury [26]. Transforming growth factor- β potentially inhibits the inflammatory response to AKI and stimulates the regrowth of tubules after injury [27]. Endothelin (ET)-1 acts through ETA or ETB receptors. Postischemic blockade of ETA (unlike blockade of ETB) decreased tubule/microvascular injury and preserved renal mass. These results suggested that ET-1 acting on ETA may block AKI progression to chronic kidney disease [28].

Regeneration versus Fibrosis

Following AKI, regenerating epithelial cells are likely derived from endogenous surviving epithelial cells or intrarenal stem cells. Increasingly, it is thought that engraftment of bone marrow-derived cells contributes to a small degree in regenerating kidneys following AKI, suggesting that bone marrow-derived cells do not directly repopulate tubules following injury. On the other hand, mesenchymal stem cells home to the site of injury and appear to exert indirect effects to repair injured epithelium through paracrine mechanisms, cell-to-cell communication through soluble factors, or microvesicles carrying genetic information [29–31]. Humphreys et al. [32] demonstrated that fully differentiated epithelial cells surviving injury have the capacity to dedifferentiate, proliferate, and differentiate into epithelial cells. In addition, recent studies have identified a population of vimentin-, CD24- and CD33-positive progenitor tubular cells that are involved in proximal tubule regeneration following AKI [33].

An alternative outcome to kidney regeneration is the maladaptive fibrotic response. Typically, myofibroblasts aid in wound contraction and help maintain tissue integrity after damage by depositing ECM components, such as collagen and fibronectin. However, after severe AKI, this process of matrix deposition is excessive, leading to loss of kidney function and eventually end-stage renal disease. The potential cellular origins of myofibroblasts, which have been the focus of much debate, include dedifferentiation of endothelial or epithelial cells (endothelial or epithelial to mesenchymal transition), infiltration of

bone marrow-derived populations, or differentiation of local interstitial cells into myofibroblasts. Major purported sources of myofibroblasts are perivascular fibroblasts and pericytes. These cells have a myriad of functions within the kidney, such as regulating medullary blood flow. In mice that express green fluorescent protein under the control of collagen-1 reporter, Lin et al. [34] demonstrated that both pericytes and perivascular fibroblasts express collagen-1 before injury and after unilateral ureteral obstruction. These cells undergo differentiation into myofibroblasts, detected by their upregulation of α -smooth muscle actin (α -SMA). However, bone marrow-derived cells have also been proposed as a source of collagen-producing cells within the kidney and contributed approximately 30% of the α -SMA-positive cells within the injured kidney. More comprehensive work using α -SMA reporter mice has suggested that many of the theorized cellular precursors contribute in varying extents to the population of myofibroblasts within the kidney [35].

Recent studies demonstrate the relationship between cell cycle and fibrosis [36]. Contralateral nephrectomy after unilateral ischemia in mice leads to G2/M arrest in proximal tubule cells resulting in abnormal production of profibrogenic factors. These results suggest that cell cycle arrest may contribute to the pathogenesis of chronic pro-

gressive kidney disease and that strategies to maintain proper cell cycle function in epithelial cells following injury may prevent fibrosis and chronic kidney disease following AKI.

Conclusion

The round table discussion at the CRRT 2014 provided a wealth of information summarizing the current knowledge in the field of AKI and spanning mechanisms of injury and renal recovery. The biological basis of renal recovery following AKI will likely lead to novel therapeutics designed to target these pathophysiological mechanisms. Combining novel therapeutic and diagnostic strategies – including novel damage biomarkers and physiological biomarkers such as real-time glomerular filtration measurement, renal blood flow, and other imaging modalities – will lead to advances in the care of patients with AKI [37–39].

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