

Fibrotic Changes Mediating Acute Kidney Injury to Chronic Kidney Disease Transition

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Abstract

End-stage renal disease (ESRD) is common, costly, and it results from progressive chronic kidney disease (CKD). ESRD claims many lives every year. It is increasingly recognized that episodes of acute kidney injury (AKI) predispose to the future development of CKD and ESRD. While our understanding of the pathophysiology of the AKI to CKD transition is improving, there are no validated therapeutic strategies to prevent this transition. In this review, we summarize the recent progress made in defining the cellular and molecular events underlying the AKI to CKD transition and highlight potential therapeutic targets and strategies to reduce the incidence of CKD following AKI.

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Introduction

Acute kidney injury (AKI) and chronic kidney disease (CKD) are both precursors to end-stage renal disease (ESRD), which led to over 46,000 deaths in 2013 [1]. While some patients with AKI make an apparent full re-

covery, many others, perhaps even the majority, develop CKD in the future and are at an increased risk of developing ESRD. The pathophysiologic mechanisms that explain how an episode of AKI can lead to CKD and risk of ESRD are subjects of intense investigation. Understanding the cellular and molecular mechanisms underlying progression from AKI to CKD is required in order to develop novel therapeutics and diagnostic tools to treat this “silent killer.”

Tubular Injury and Repair

AKI is characterized by a variety of cellular and structural injuries (online suppl. Fig. 1; for all online suppl. material, see www.karger.com/doi/10.1159/000474960) [2]. The proximal tubule (PT) is particularly susceptible to injury due to high metabolic activity. PT injury leads to dedifferentiation, characterized by distinct morpho-

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logical changes including loss of brush border, decreased cell size, fewer mitochondria, and elongated shape. Dedifferentiated cells also express injury markers and developmental genes, such as Pax2, while their proliferative rate increases [3]. Kusaba et al. [4] demonstrated using genetic lineage analysis that injured PT cells expressing kidney injury marker-1 (Kim-1) undergo dedifferentiation and proliferation in order to repair injured tubules. Kim-1 is a phosphatidylserine receptor that allows surviving epithelia to clear the tubule lumen of apoptotic fragments [5]. PT cells may also undergo a partial epithelial-to-mesenchymal transition (EMT) including enhanced migratory capacity during repair as demonstrated by the expression of Vimentin and Snail2 [4, 6]. In many cases, PT can be fully reconstituted without apparent long-term structural damage in a process of successful repair (online suppl. Fig. S1b). However, some patients will undergo maladaptive repair, in which most function is restored, but an underlying fraction of cells remains damaged. This failed repair contributes to ongoing fibrotic processes and progresses over time to CKD (online suppl. Fig. S1b, c).

The progression of PT cells through the various states of differentiation and repair is being defined at the molecular level. A characteristic indication of successful repair is the subsequent downregulation of Kim-1 during redifferentiation, along with other dedifferentiation markers, such as Pax2, and EMT markers Vimentin and Snail1 [6]. The Sry-box family transcription factor, Sox9, is also induced during injury [7]. Similar to Kim-1, the expression of Sox9 is low in uninjured kidneys, and shows a dramatic upregulation 24 h after ischemia reperfusion injury (IRI) in murine models [7]. These Sox9⁺ injured PT cells are actively proliferating, with half of Sox9⁺ cells co-expressing Kim-1 [7]. Indeed, Kang et al. [8] showed that Sox9⁺ cells contributed to the repair of proximal, distal, and loop of Henle segments after folic acid injury and IRI. Using the Sox9-driven Cre-ER² model crossed with R26R-tdTomato, Kumar et al. [7] showed that PT cells that initiated de novo expression of Sox9 after injury contributed the most to repair, further implicating dedifferentiation as the predominant mechanism of epithelial repair. Additionally, both PT-specific deletion of Sox9 in repair, and global deletion of Kim-1 during injury, exacerbated injury and delayed repair [7–9].

Although the fraction of PT that fail to repair after AKI is unknown, after moderate to severe AKI, a minority of PT cells and segments are characterized by ongoing injury and dedifferentiation. These cells fail to downregulate Kim-1, and chronic Kim-1 expression promotes proinflammatory cytokine secretion, peritu-

bular inflammation, and interstitial fibrosis [5, 10]. These cells will also undergo G2/M cell cycle arrest, leading to secretion of pro-fibrotic growth factors, such as TGF β and CTGF [11]. As additional insults occur to the kidney, this population of Kim-1 + dedifferentiated cells will increase, leading to CKD due to loss of PT integrity and function.

Tubulointerstitial Fibrosis and Signaling

While tubular epithelial cell damage is a core component in AKI to CKD transition, other growth factor and cytokine signaling pathways play important roles [12]. Aberrant paracrine signaling from the hedgehog (Hh) signaling pathway between the tubular cells and the interstitium contributes to tubulointerstitial fibrosis and capillary rarefaction, for example (online suppl. Fig. S1a, b). Hh ligands, including Sonic hedgehog (Shh) and Indian hedgehog (Ihh), signal by binding to their transmembrane receptor patched (Ptch1) [13], which activates intracellular transcription factors called Gli proteins [14]. These transcription factors include Gli1, Gli2, and Gli3. Gli1 expression marks a population of MSC-like pericytes in the outer medulla and inner cortex. These cells express PDGFR β and are located adjacent to blood vessels in a perivascular niche [15]. Evidence supports the expression of both Ihh and Shh by tubular epithelium in kidney, with Shh expression seen in the collecting duct (CD), and Ihh expressed in S3 segment of the PT [13]. While Ptch1 is expressed in many kidney cell types, enrichment of the receptor is seen in perivascular interstitial cells [13].

During homeostasis, Gli1⁺ pericytes stabilize vascular networks within the kidney through close contacts with microvascular endothelial cells (online suppl. Fig. S1a). Ablation of these pericytes is sufficient to drive capillary rarefaction in which the density of capillaries is significantly reduced. Most strikingly, ablation of Gli1⁺ cells also induces transient PT injury [16]. Kramann et al. [16] showed that loss of Gli1⁺ cells lead to increased Kim-1 expression in the PT cells 10 days after ablation, which normalized by day 21. This transient increase of Kim-1 in PT cells is similar to the type of injury witnessed in AKI (online suppl. Fig. S2). Additionally, both mild and severe IRI mouse models show increased capillary rarefaction and Kim-1 expression, suggesting that loss of Gli1⁺ pericyte function may lead to AKI-like physiology [15, 16]. To address this question, Kramann et al. [16] showed that during acute injury, Gli1⁺ pericytes detach from endothe-

lial cells, expand into the cortex, and give rise to α SMA expressing myofibroblasts (online suppl. Fig. S1b, c) [15]. These myofibroblasts secrete extracellular matrix proteins, such as collagens and fibronectin, which accumulate in the interstitium, leading to increased hypoxia, decreased renal function, and eventually CKD. These data suggest that Gli1^+ pericytes are one of the main progenitor cells contributing to the AKI-CKD transition. Indeed, ablation of these Gli1^+ pericytes after unilateral ureteral obstruction (UUO) significantly decreased interstitial fibrosis [15]. Collectively these observations suggest that the Hh pathway is a promising target for anti-fibrotic therapies. In support of this, Gli2 knockout in Gli1^+ pericytes ameliorated kidney fibrosis, as did treatment with the Gli inhibitor, GANT61 [16].

Canonical Wnt signaling has been implicated in the AKI-CKD transition as well (online suppl. Fig. S1b, c) [17, 18]. Wnt ligands are present in the early mouse embryo, and play a critical role in nephrogenesis. Once nephrogenesis is complete, Wnt ligand expression decreases, and canonical Wnt signaling is repressed. However, during injury, both the epithelial and interstitial cells demonstrate activated canonical Wnt signaling [17]. DiRocco et al. [19] showed that Wnt4 ligand expression is restricted to the papillary CD epithelial cells in adult kidney, and not expressed within the cortex or medullary CD. Interestingly, during fibrosis, medullary myofibroblasts upregulate the Wnt4 ligand [19]. In fact, exogenous Wnt4 induced myofibroblast differentiation *in vitro* implicating Wnt4 as a fibrotic-inducing ligand. However, the knockout of Wnt4 before injury did not ameliorate fibrosis. This is consistent with the fact that the kidney expresses many different Wnt ligands, which may compensate for the absence of a single one. On the other hand, constitutive activation of the canonical Wnt pathway (through β -catenin stabilization) in kidney interstitial pericytes was sufficient to drive myofibroblast formation in uninjured kidneys [19].

Using a different approach, Maarouf et al. [20] revealed that PT-specific and inducible expression of Wnt1 was sufficient to induce interstitial fibrosis in the absence of any other injury. Wnt1 ligand had previously been shown to be secreted by PT epithelial *in vitro* and it is increased in injured kidneys [20]. A remarkable observation from this experiment was the complete absence of leukocyte infiltration or inflammatory cytokine expression in this model. Wnt1 induced interstitial fibrosis through the activation of canonical Wnt signaling and myofibroblast proliferation [20]. Several other studies are in agreement with these results, for example, the use of

antagonists of the Wnt pathway such as Klotho [21], DKK1 [22], sFRP4 [23], and the chemical inhibitor ICG-001 [18]. By contrast, several other studies have demonstrated a reparative effect of Wnt, specifically on the tubular epithelial cells. Complete ablation of β -catenin in tubule cells exacerbated AKI by increasing p53 and Bax expression [24]. This dual role of canonical Wnt signaling poses a dilemma. In AKI, Wnt signaling is necessary to force the proliferation and repair of tubule epithelial cells, while chronic overexpression of Wnt induces interstitial fibrosis and myofibroblast proliferation, potentially leading to AKI-CKD transition (online suppl. Fig. S1b, c) [18].

Another development pathway reactivated in AKI-CKD transition is the notch signaling pathway (online suppl. Fig. S1b, c). Notch signaling regulates various cellular processes depending on the cell type. In stem cells, notch is involved in differentiation, and is often overexpressed in many carcinomas [25, 26]. Notch plays a crucial role in both podocyte and PT development in the kidney, with many different notch ligands and receptors being expressed throughout nephrogenesis [27]. The expression of notch diminishes after development, with very little signaling in the mature kidney [27]. However, during AKI, the notch pathway is reactivated in tubular epithelial cells [28]. After IRI, an upregulation of notch2, delta-1, and Hes-1 was seen in the PT epithelial cells [28]. Additionally, jagged-1 and Hes-1 were found to be upregulated in the tubule epithelial of diabetic nephropathy patients [29]. Mariana et al. [30] later showed that activated notch signaling was not unique to diabetic nephropathy, with notch-1 expression correlating with the degree of tubulointerstitial fibrosis in a wide variety of kidney diseases. In animal models, Djurdjaj et al. [31] demonstrated that genetic deletion of notch-3 in UUO leads to a reduction in tubular injury, along with a decrease in collagen deposition. Finally, Bielez et al. [32] found that inhibiting notch, both genetically and pharmacologically using γ -secretase inhibitor, reduced tubulointerstitial fibrosis in UUO and folic acid models. This data indicates that notch signaling is also an important target for therapeutic intervention (online suppl. Fig. S1c).

Conclusion and Future Therapeutics

Despite the cellular complexity of AKI and CKD, progress has been made in defining critical pathways that represent attractive therapeutic targets. While many drug

candidates for the pathways listed above are in clinical trials for other indications, such as various cancers, few are being tested in kidney disease. While clinical trials in AKI and CKD pose a unique set of challenges, this growing list of candidate therapeutics to prevent the AKI to CKD transition suggests that a therapeutic breakthrough is possible in the next decade. Deciphering the balance between reparative vs. fibrotic signaling after acute injury should lead to novel therapeutics for patients experiencing kidney injury.

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Disclosure Statement

The authors has no conflict of interest to declare.