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# Fibrotic changes mediating AKI to CKD transition

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## Abstract

End-stage renal disease (ESRD) is common, costly and 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 recent progress 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.

### Keywords

Acute renal failure; Fibrosis; Ischemia-reperfusion injury

## 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 recovery, many others, perhaps even the majority, develop future CKD and are at increased risk of developing end stage renal disease (ESRD). The pathophysiologic mechanisms that explain how an episode of AKI can lead to CKD and risk of ESRD are the subject 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 morphological 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* demonstrated using genetic lineage analysis that

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injured PT cells expressing kidney injury marker-1 (Kim1) undergo dedifferentiation and proliferation in order to repair injured tubules [4]. 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 expression of Vimentin and Snail2 [4,6]. In many cases, proximal tubule 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 remain 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 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, expression of Sox9 is low in uninjured kidneys, and shows a dramatic upregulation 24 hours 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* showed that Sox9+ cells contributed to repair of proximal, distal and loop of Henle segments after folic acid injury and IRI [8]. Using the Sox9 driven Cre-ER$^2$ model crossed with R26R-tdTomato, *Kumar et al* 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 [7]. 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, peritubular 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 TGFβ and CTGF [11]. As additional insults occur to the kidney, this population of Kim1+ dedifferetiated 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 1a–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 PDGFRβ, and are located adjacent to blood vessels, in a perivascular niche [15]. Evidence supports expression of both Ihh and Shh by tubular epithelium in kidney, with Shh expression seen in the collecting duct, 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 proximal tubule injury [16]. Kramman *et al* showed that loss of $Gli1^+$ cells lead to increased Kim1 expression in the PT cells 10 days after ablation, which normalized by day 21 [16]. This transient increase of Kim1 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 Kim1 expression, suggesting that loss of $Gli1^+$ pericyte function may lead to AKI-like physiology [15,16]. To address this question, *Kramann et al* showed that during acute injury, $Gli1^+$ pericytes detach from endothelial cells, expand into the cortex, and give rise to αSMA expressing myofibroblasts (online suppl. Fig S1b–c) [15]. These myofibroblasts secrete extracellular matrix (ECM) proteins, such as collagens and fibronectin, that 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 $Gli1^+$ perictyes 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 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* showed that Wnt4 ligand expression is restricted to the papillary collecting duct (CD) epithelial cells in adult kidney, and not expressed within the cortex or medullary CD [19]. Interestingly, during fibrosis, medullary myofibroblasts upregulate Wnt4 ligand [19]. In fact, exogenous Wnt4 induced myofibroblast differentiation *in vitro* implicating Wnt4 as a fibrotic-inducing ligand. However, 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].

Taking a different approach, *Maarouf et al* revealed that PT specific and inducible expression of Wnt1 was sufficient to induce interstitial fibrosis in the absence of any other injury [20]. Wnt1 ligand had previously been shown to be secreted by PT epithelial *in vitro*, and 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 activation of canonical Wnt signaling and myofibroblast proliferation [20]. Several other studies are in agreement with these results, for example 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 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 S1 b–c) [18].

Another development pathway reactivated in AKI-CKD transition is the notch signaling pathway (online suppl. Fig S1 b–c). Notch signaling regulates various cellular processes, depending on the cell type. In stem cells, notch is involved in differentiation, and is often over-expressed in many carcinomas [25, 26]. Notch plays a crucial role in both podocyte and proximal tubule development in the kidney, with many different notch ligands and receptors being expressed throughout nephrogenesis [27]. 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 proximal tubule 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* later showed that activated notch signaling was not unique to diabetic nephropathy, with notch-1 expression correlating with degree of tubulointerstitial fibrosis in a wide variety of kidney diseases [30]. In animal models, *Djudjaj et al* demonstrated that genetic deletion of notch-3 in UUO lead to a reduction in tubular injury, along with a decrease in collagen deposition [31]. Finally, *Bielesz et al* found that inhibiting notch, both genetically and pharmacologically using γ-secretase inhibitor, reduced tubulointerstitial fibrosis in UUO and folic acid models [32]. 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. Table S1 provides a summary of inhibitors of the Hh-Gli, Notch and Wnt pathways and progress in bringing them towards the clinic. Some of these agents are already in clinical trials for other indications. 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 suffering from kidney injury.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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