Progression of chronic kidney disease: too much cellular talk causes damage

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Tubulointerstitial fibrosis, tubular atrophy, and peritubular capillary rarefaction are major hallmarks of chronic kidney disease. The tubulointerstitium consists of multiple cell components including tubular epithelial, mesenchymal (fibroblasts and pericytes), endothelial, and inflammatory cells. Crosstalk among these cell components is a key component in the pathogenesis of this complex disease. After severe or recurrent injury, the renal tubular epithelial cells undergo changes in structure and cell cycle that are accompanied by altered expression and production of cytokines. These cytokines contribute to the initiation of the fibrotic response by favoring activation of fibroblasts, recruitment of inflammatory cells, and loss of endothelial cells. This review focuses on how augmented growth factor and cytokine production induces epithelial crosstalk with cells in the interstitium to promote progressive tubulointerstitial fibrosis after renal injury.

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The hallmark of chronic kidney diseases (CKDs) is tubulointerstitial fibrosis (TIF), which has the histopathologic features of extracellular matrix (ECM) accumulation, tubular atrophy, inflammatory cell infiltration, and peritubular microvasculature loss. This pathology is the common endpoint of CKDs of multiple etiologies including glomerular insults, repeated acute kidney injury (AKI), and chronic tubulointerstitial injuries. The tubulointerstitium consists of multiple cell components including tubular epithelial, mesenchymal (fibroblasts and pericytes), endothelial, and inflammatory cells, all of which contribute to fibrosis progression. In addition, altered matrix metalloproteinase enzyme activity and disruptions in the tubular basement membrane can promote growth factor release and communication between the epithelial and interstitial compartments.[1] The interplay among these cells is highly complex and, although initially aimed at tubular repair and recovery after injury, may become unregulated and accelerate tubular atrophy and TIF progression.

Tubular epithelia, in particular the proximal tubules, are targeted by acute and chronic injuries. The injured epithelia dedifferentiate and proliferate, resulting in repair after AKI.[2] When epithelial injury occurs repetitively or persists over time, tubular apoptosis may occur and lead to progressive TIF. This was nicely demonstrated with the diphtheria toxin model in which mice with repeated tubule-specific injury leading to apoptosis developed interstitial fibrosis, tubular atrophy, and inflammation.[3] These dying epithelial cells may elicit proinflammatory cytokines and other growth factors that promote inflammation and fibrosis, but more research is required to elucidate the mechanisms whereby epithelial apoptosis leads to TIF.[4] Tubular apoptosis may be sufficient but not necessary to promote TIF as even sublethal epithelial injury alters the structure and function of these tubules in ways that can also lead to progressive renal dysfunction. More than a decade ago, it was postulated that the injured epithelia undergo epithelial to mesenchymal transformation and transform into interstitial mesenchymal cells that are responsible for ECM production and ultimately fibrosis.[5] Many factors including transforming growth factor-β (TGF-β), hypoxia-inducible factor-1α, and integrin-linked kinase have been implicated in epithelial to mesenchymal transformation *in vivo*,[6–8] and prevention of TGF-β–mediated signaling by bone morphogenetic protein-7 was proposed to

be beneficial in reversing TGF-β–mediated TIF.[6] Although the origin of fibroblasts remains controversial, a recent study showed that only 5% of fibroblasts originate from epithelial to mesenchymal transformation,[9] and lineage-tracing studies have shown that fibroblasts derive from resident mesenchymal cells and pericytes that are platelet-derived growth factor receptor-β positive.[10,11]

Regardless of whether epithelia undergo epithelial to mesenchymal transformation, the injured proximal tubule clearly dedifferentiates and undergoes cell-cycle changes. The dedifferentiated epithelial cells acquire a partial mesenchymal phenotype that is associated with increased production of profibrotic cytokines. Although injured epithelia are unlikely to be the main producers of ECM, they are important producers of growth factors that have paracrine effects on resident fibroblasts/pericytes. Consistent with this, deletion of Snail and Twist, transcription factors that promote dedifferentiation by repressing E-cadherin, reduced TIF after renal injury.[12,13] In addition, chronically injured epithelia become arrested in G2/M, and this cell cycle dysfunction is also associated with excessive production of profibrotic growth factors.[14] G2/M cell cycle arrest induced increased JNK (c-jun N-terminal kinase) activity, which augmented production of TGF-β and CTGF/CCN2 (connective tissue growth factor).[14] Thus, the chronically injured epithelial cells undergo changes in cell structure and cell cycle that are accompanied by increases in cytokine production (Table 1). This review focuses on how epithelial injury, through augmented growth factor production that induces either autocrine signaling or crosstalk with interstitial cells, promotes progressive TIF after renal injury.

Table 1 | List of cytokines and growth factors produced by epithelial cells described in this review and some of their effects in the progression of chronic kidney disease

Factor	Effect	References
TGF-β	Profibrotic Tubular cell dedifferentiation Fibroblast to myofibroblast differentiation	23–25, 30
PDGF	Profibrotic Fibroblast proliferation Fibroblast to myofibroblast differentiation	35, 37
Hh	Profibrotic Recruitment of pericytes Fibroblast proliferation	40, 41, 44
CTGF	Profibrotic Fibroblast proliferation	31
Wnt	Profibrotic Fibroblast to myofibroblast differentiation	50, 51
MCP-1	Mobilization of macrophages	75–77
RANTES	Mobilization of macrophages	76, 78
CSF-1	Macrophage recruitment and adhesion Polarization into an M2 phenotype	80, 81
CX3CL1	Macrophage recruitment and adhesion Survival of profibrotic macrophages	82, 83
Thrombospondin-1	Activation of TGF-β Antiangiogenesis	93
VEGF	Endothelial cell proliferation and survival Macrophage recruitment	95–97
TIMP	Fibroblast proliferation Fibroblast to myofibroblast differentiation	55, 56
Lcn2	Fibroblast proliferation of ECM Epithelial production of ECM	53, 57

CSF-1, colony-stimulating factor-1; CTGF, connective tissue growth factor; CX3CL1, fractalkine; ECM, extracellular matrix; Hh, hedgehog; Lcn2, lipocalin; MCP-1, monocyte chemoattractant protein-1; PDGF, platelet-derived growth factor; RANTES, regulated on activation, T cell expressed, and secreted; TGF-β, transforming growth factor-β; TIMP, tissue inhibitor of matrix metalloproteinase 1; VEGF, vascular endothelial growth factor.

Epithelial/epithelial and epithelial/fibroblast crosstalk

Injured epithelia are potent producers of growth factors and cytokines such as TGF-β, platelet-derived growth factor (PDGF), hedgehog, and Wnt ligands. These growth factors may initially promote regeneration of the injured epithelia but, in persistent injury, have paracrine effects on surrounding cells such as fibroblasts, causing them to transform into myofibroblasts. Activated fibroblasts have increased stress fibers, and they proliferate and produce ECM components such as collagens leading to progressive TIF. Activated fibroblasts are difficult to study, in part because they were initially defined by ultrastructural features on electron microscopy, and there are no markers that identify these cells specifically.[15] The expression of α-smooth muscle actin is commonly used as an indicator of myofibroblasts, but we and others have found that α-smooth muscle actin inconsistently marks collagen type I–producing fibroblasts after injury.[16,17] Given the heterogeneity of fibroblasts isolated from injured kidneys, it is likely that many different subsets of fibroblasts exist with different expression profiles and functions.

There is strong evidence of epithelial/fibroblast crosstalk through growth factors *in vitro*, but defining it *in vivo* is difficult due to the various other cells (e.g., inflammatory cells, endothelial cells) that also produce profibrotic growth factors. In support of epithelial/fibroblast crosstalk, a rat model of tubular injury found that activated fibroblasts surrounded only those proximal tubules with evidence of injury.[18] Epithelial/fibroblast crosstalk is important in both renal development and tumorigenesis and is mediated by many of the same growth factors up-regulated after renal injury.[19–21] These growth factors include members of the TGF-β superfamily, Notch, Wnt, and Hedgehog pathways, and the importance of developmental signaling pathways in renal fibrosis was recently reviewed.[22] These pleiotropic growth factors most likely mediate all TIF regardless of etiology, but we currently have little information on the role of specific growth factors in different disease processes. One of the major reasons is that most studies investigating TIF progression utilize the unilateral ureteral obstruction model (UUO). Although this is considered the standard model of TIF, it has many limitations as it induces extreme injury in a short period of time, resulting in destruction of the kidney without producing functional data such as an altered glomerular filtration rate.