

Molecular Mechanism in Renal Fibrosis – A Review

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Abstract

Renal fibrosis is the hallmark of various chronic kidney diseases (CKD). Transforming growth factor beta (TGF- β) is recognized as a vital mediator in renal fibrosis as it induces production of extracellular matrix to cause renal scarring. In chronic kidney disease, fibroblast dysfunction causes renal fibrosis and renal anaemia. Renal fibrosis is mediated by the accumulation of myofibroblasts, whereas renal anaemia is mediated by the reduced production of fibroblast-derived erythropoietin, a hormone that stimulates erythropoietin. Despite their importance in chronic kidney disease, the origin and regulatory mechanism of fibroblasts remain unclear. The majority of erythropoietin-producing fibroblasts in the healthy kidney originate from myelin protein zero-Cre (P0-Cre) lineage-labeled extra renal cells, which enter the embryonic kidney at E13.5. In the diseased kidney, P0-Cre lineage-labeled fibroblasts, but not fibroblasts derived from injured tubular epithelial cells through epithelial-mesenchymal transition, transdifferentiated into myofibroblasts and predominantly contributed to fibrosis, with concomitant loss of erythropoietin production. Erythropoietin production in transdifferentiated myofibroblasts was restored by the administration of neuroprotective agents, such as dexamethasone and neurotrophins. Mature tubular epithelial cells in adult kidney can undergo epithelial-to-mesenchymal transition (EMT), a phenotypic conversion that is fundamentally linked to the pathogenesis of renal interstitial fibrosis. Emerging evidence indicates that a large proportion of interstitial fibroblasts are actually originated from tubular epithelial cells via EMT in diseased kidney. Tubular EMT is proposed as an orchestrated, highly regulated process that consists of four key steps: (1) loss of epithelial cell adhesion; (2) de novo α -smooth muscle actin expression and actin reorganization; (3) disruption of tubular basement membrane; and (4) enhanced cell migration and invasion. Of the many factors that regulate EMT in different ways, transforming growth factor- β 1 is the most potent inducer that is capable of initiating and completing the entire EMT course, whereas hepatocyte growth factor and bone morphogenetic protein-7 act as EMT inhibitors both in vitro and in vivo.... Multiple cellular and molecular events, such as tubular atrophy, microvascular rarefaction and tissue hypoxia, promote scar formation and ensure a vicious progression to end-stage kidney failure. This Review outlines our current understanding of the cellular and molecular mechanisms of renal fibrosis, which could offer novel insights into the development of new therapeutic strategies.

INTRODUCTION

Renal fibrosis, particularly tubulointerstitial fibrosis, is the common final outcome of almost all progressive chronic kidney diseases. Renal fibrosis is also a reliable predictor of prognosis and a major determinant of renal insufficiency. Irrespective of the initial causes, renal fibrogenesis is a dynamic and converging process that consists of four overlapping phases: priming, activation, execution and progression. No resolving inflammation after a sustained injury sets up the fibrogenic stage (priming) and triggers the activation and expansion of matrix-producing cells from multiple sources through diverse mechanisms, including activation of interstitial fibroblasts and pericytes, phenotypic conversion of tubular epithelial and endothelial cells and recruitment of circulating fibrocytes. Tubular EMT, by definition, is a process in which renal tubular cells lose their epithelial phenotype and acquire new characteristic features of mesenchyme. Obviously, this phenotypic conversion not only illustrates an incredible plasticity of the differentiated tubular epithelial cells after development; it is also fundamentally linked to generation of the matrix-producing fibroblasts under pathologic setting. It is of interest to point out that the majority of renal tubules in adult kidney except collecting duct are developmentally derived from the metanephric mesenchyme through mesenchymal to epithelial transdifferentiation (MET) (1). Elevated expression of genes encoding extracellular matrix proteins (ECM), and an excessive accumulation of ECM components within the

tubule interstitium and glomeruli are the major characteristic features of renal fibrosis [2, 3]. Furthermore, renal fibrosis may also be considered as an irreversible wound-healing process that occurs after the initial insults of various injuries [4]. There are many cell types participating in the pathogenesis of renal fibrosis, including fibroblasts, myofibroblasts, mesangial cells, tubular epithelial cells, pericytes, endothelial cells, vascular smooth muscle cells, podocytes, and infiltrated inflammatory cells, such as macrophages, fibrocytes, and lymphocytes [5]. Fibroblasts are interstitial mesenchymes that structurally support epithelia by producing ECM.

TUBULAR INJURY

1. Proximal Injury

Light chain disease, particularly with κ light chains bearing the V κ I sub-group, or non-polar amino acids can be associated with an acquired Fanconi syndrome, blocking PTC transport of glucose, amino acids and phosphate. These light chains have greater resistance to proteolysis and appear on electron microscopy as intra-lysosomal crystalline deposits within PTCs [6]. More commonly light chains cause subtle changes in the PTCs, with increased lysosomes, focal swelling of mitochondria, apical cytoplasm blebbing and loss of brush border described on electron microscopy [7]. Increased FLCs in the tubular ultrafiltrate results in greater PTC endocytosis, which can lead to a series of pro-inflammatory changes within the PTC cell including activation of redox pathways, as

hydrogen peroxide is generated on endocytosis of FLCs, with activation of c-Src, a redox-sensitive tyrosine kinase [8], increased NF- κ B and phosphorylation of mitogen-activated protein kinases (MAPKs), resulting in the transcription of inflammatory and profibrotic cytokines (IL-6, IL-8, MCP-1, TNF- α and TGF- β), leading to inflammatory cell recruitment and tubulo-interstitial fibrosis as often seen on renal biopsy specimens. Activation of the redox-sensitive MAPK apoptosis signal-regulating kinase 1 (ASK1) by FLCs results in increased PTC apoptosis [9]. In addition, *in vitro*, FLCs can induce a phenotypic switch in PTCs to a fibroblastic phenotype.

2. Distal Tubule Injury

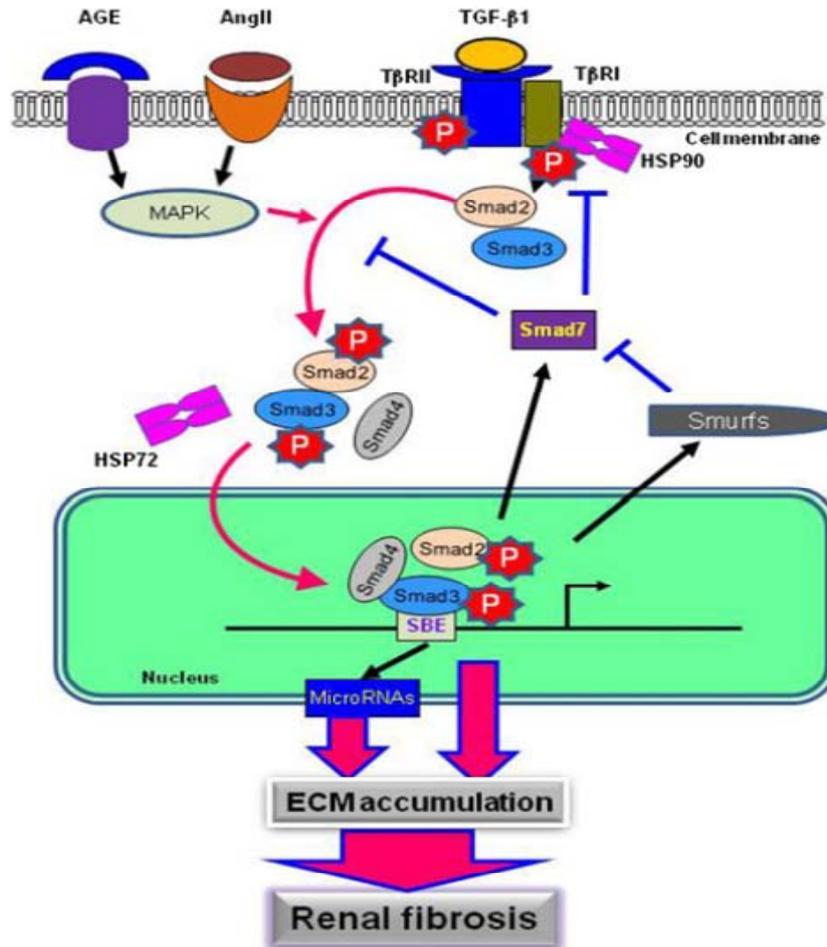
As PTC-mediated endocytosis of FLCs is saturable, in plasma cell dyscrasias large amounts of free monoclonal light chains can pass on into the distal tubule. Cast nephropathy, the classic histopathological appearance associated with myeloma, is caused by the precipitation of FLCs in the lumen of the distal nephron causing intra-tubular obstruction, which then subsequently leads to interstitial inflammation and fibrosis [10]. The risk of developing cast nephropathy generally increases with increasing urinary FLCs above 2 g/day, but not all FLCs are nephrotoxic. As FLCs are composed of different amino acids, they have different isoelectric points (pIs) in solution due to the differences in electrical charge of individual amino acids. As the pH of the solution approaches that of the protein pI, the net protein electrical charge approaches zero, and as such protein precipitation becomes more likely. In the healthy kidney, renal tubular fluid becomes more progressively acidic as it passes towards the distal tubule, and as such FLCs with a low pI are more likely to precipitate distally than proximally.

Uromodulin (Tamm–Horsfall glycoprotein) is an 80 kDa glycosylated protein with a pI of 3.5, which is secreted by the thick ascending limb of the loop of Henle, and can self-aggregate into a gel, particularly with increasing electrolyte concentrations, and can also bind to and co-precipitate many low-molecular weight proteins. Such FLCs can bind via one of their CDR (CD3) to a specific nine amino acid sequence in the uromodulin protein structure, termed the light chain binding domain, and co-precipitate according to pI and local pH. However, light chain binding to uromodulin is variable with different binding affinities for different light chains, with the λ V family demonstrating the lowest binding affinity [11], as the secondary structure and key amino acid residues in the CDR3 region of FLCs are key important determinants of the molecular interaction with uromodulin. Such cast formation is a complex process depending not only on FLC pI and tubule fluid pH, but also upon the concentration and ionic composition of the tubule fluid, tubular flow rate, the concentration of uromodulin and FLCs, and the strength of their binding interaction [12]. It must be remembered that FLCs can precipitate without uromodulin. Thus, cast nephropathy can be acutely precipitated by dehydration, hypercalcaemia, and following the administration of frusemide, iodinated contrast media and non-steroidal anti-inflammatory drugs [13].

Intraluminal deposition of free light chains leads to increased tubular pressure, which leads to decreased glomerular filtration, directly in the glomerulus linked to the tubule, but also reduced glomerular filtration rates in other glomeruli due to activation of local neurohumoral activation. Persistent obstruction initially leads to local atrophy of the nephron segment proximal to the obstruction, and finally the whole nephron. Increased pressure within the tubule is transmitted into the renal interstitium causing a local inflammatory reaction, with an inflammatory cell infiltration. Uromodulin has been shown to directly activate macrophages and recruit polymorphonuclear leucocytes [14], via Toll-like receptor-4 and NF- κ B upregulation [15]. Typically cast nephropathy is observed to have an interstitial inflammatory cell component and intra-tubular giant cell formation with macrophages around deposited casts. Increasing intraluminal pressure leads to local tubular cell apoptosis and necrosis with consequent local tubular rupture and passage of tubular fluid and contents into the renal interstitium, increasing interstitial pressure, interstitial inflammatory reaction, resulting in increased interstitial fibrosis and reducing glomerular filtration rate in neighbouring nephrons.

TGF-B SIGNALING IN RENAL FIBROSIS

Over the last two decades, TGF- β 1 has been recognized as a vital mediator in the genesis of CKD and as pivotal in inducing production of ECM proteins which result in structural and functional changes in the kidney culminating in adverse results. Dysregulation of TGF- β 1 is believed to be one of the major pathogenic mechanisms in the progression of CKD because TGF- β 1 can increase ECM production and alter the degradation of ECM components. Studies from both in human and experimental models of CKD confirm that TGF- β 1 and its receptors are highly upregulated in the fibrotic kidney. Overexpression of active TGF- β 1 also causes glomerular and interstitial fibrosis in transgenic mice [16]. Suppression of TGF- β activity attenuates renal fibrosis in experimental models of kidney diseases [17-19]. *In vitro* studies confirm that TGF- β stimulates ECM synthesis, the myofibroblast differentiation, or transition of mesangial cells, interstitial fibroblasts, and tubular epithelial cells to become matrix-producing myofibroblasts. TGF- β employs its action by stimulating dimerization of type I and type II TGF- β receptors (TbRI and TbRII) and activates the downstream mediators called Smad2 and Smad3 by phosphorylation [20]. Phosphorylated Smad2 and Smad3 complex with Smad4 in the cytosol then translocate into the nucleus to regulate target gene expression. Alternatively, phosphorylated Smad2 and Smad3 can also form a stable association with Smad7, an inhibitory Smad, to interfere with their heteromerization with Smad4 [21]. The role of TGF- β /Smad signaling in renal fibrosis, however, is likely more complex than the canonical TGF- β /Smad pathway described above. Here, we will discuss the current findings in each major component of this complex signaling pathway during fibrosis.



TGF-β Receptors

Receptor phosphorylation not only activates TGF-β-induced signaling but also provides the basis for the roles of TβRII and TβRI kinases as signal transducers. It is well documented that, after TGF-β binding to TβRII, this constitutively phosphorylated TβRII binds to TβRI and activates TβRI via phosphorylation [22, 23]. Thus, the activated TβRI receptor activates Smad2 and Smad3 via phosphorylation of their C-terminal serine. TβRII also plays an important role in regulating renal fibrosis, because conditionally deleting TβRII in tubular epithelial cells of a mouse model of unilateral ureteral obstruction (UUO) suppresses tubulointerstitial fibrosis in the UUO kidneys [24]. It is interesting that this suppression is only associated with the inhibition of TGF-β/Smad3 signaling but not with the ERK/p38 MAP kinase pathway. These results are further confirmed by in vitro disruption of TβRII from kidney fibroblasts or tubular epithelial cells. Deletion of TβRII can successfully reduce TGF-β1-induced Smad signaling and fibrosis. Thus, impaired TGF-β/Smad3, but not the non-canonical TGF-β signaling pathway, is the key mechanism by which disruption of TβRII protects against renal fibrosis. Recent studies in acute kidney injury by selectively deleting TβRII in the proximal tubules of mice support the protective role of TβRII renal injury.

CONCLUSION

In general, Smad3 plays a pathogenic role in renal fibrosis and regulates its downstream specific micro RNAs such as miR-21, miR-192, and miR-29 which are involved in renal fibrosis. Smad4 plays a role in promoting Smad3-mediated renal fibrosis. In contrast, Smad2 plays a protective role in renal fibrosis to inhibit Smad3 phosphorylation and nuclear translocation while Smad7 is renal protective to prevent TGF-β/Smad signaling in renal fibrosis. However, under diseased condition, TGF-β promotes proteasomal-ubiquitin degradation of the Smad7 protein, resulting in an imbalance within the Smad signaling to induce renal fibrosis. MicroRNAs act as important downstream effectors of TGF-β/Smad signaling during fibrosis. Taken together, the advances of our understanding of the specific role of individual components of TGF-β/Smad signaling during renal fibrosis should provide us with valuable opportunities to search for new targets for drug intervention to combat renal fibrosis.

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