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Molecular Mechanisms of TGF-β Signaling in Renal Fibrosis

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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. The precise roles of individual Smads, receptors, and co-repressors have been recently characterized, and the results reveal the complexity of TGF-β signaling during CKD. Smad2 and Smad7 play protective roles; however, Smad3 plays a pathogenic role in CKD. Smad4 enhances Smad3-mediated renal fibrosis. Heat-shock protein also plays an essential role in TGF-β/Smad-mediated renal fibrosis. Recent findings demonstrate that microRNAs are critical downstream effectors of TGF-β/Smad3 signaling in renal fibrosis. Thus, targeting the downstream TGF-β/Smad3 signaling pathway by gene transfer of either Smad7- or Smad3-dependent microRNAs, and by applying Smad3 inhibitor and Smad7 agonist may offer a specific and effective therapeutic strategy for renal fibrosis in CKD.

Keywords Renal fibrosis · TGF-β signaling · Chronic kidney disease · Smads · MicroRNAs · Matrix pathobiology

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Introduction

One of the biggest problems in nephrology is the progression of chronic kidney diseases (CKD), because it leads to the development of end-stage renal disease (ESRD) that requires renal replacement therapies such as dialysis and transplantation [1]. Renal fibrosis, characterized by tubulointerstitial fibrosis and glomerulosclerosis, is regarded as a central event in the progression of CKD, although the etiology and causative mechanisms of CKD differ [1].

Elevated expression of genes encoding extracellular matrix proteins (ECM), and an excessive accumulation of ECM components within the tubulointerstitium 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 [1].

Transforming growth factor beta (TGF-β) has been recognized as an important mediator in the process of both inflammation and fibrosis in a variety of kidney diseases [1, 2, 5–7]. Detailed discussion of the pathogenesis of tubulointerstitial and glomerulosclerosis has been extensively reviewed [1, 8–11]. In this review article, we focus on the recent findings of TGF-β/Smad signaling in fibrosis.

TGF-β Signaling in Renal Fibrosis

Over the last two decades, TGF-β1 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 [2]. Dysregulation of TGF-β1 is believed to be one of the major pathogenic mechanisms in the progression of CKD because TGF-β1 can increase ECM production and alter the degradation of ECM components [7, 8, 12, 13]. Studies from both in human and experimental models of CKD confirm that TGF-β1 and its receptors are highly upregulated in the fibrotic kidney [2, 7]. Overexpression of active TGF-β1 also causes glomerular and interstitial fibrosis in transgenic mice [14]. Suppression of TGF-β activity attenuates renal fibrosis in experimental models of kidney diseases [15–17]. 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 [1, 2].

TGF-β employs its action by stimulating dimerization of type I and type II TGF-β receptors (TβRI and TβRII) and activates the downstream mediators called Smad2 and Smad3 by phosphorylation [18]. 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 [19]. 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 (Fig. 1).

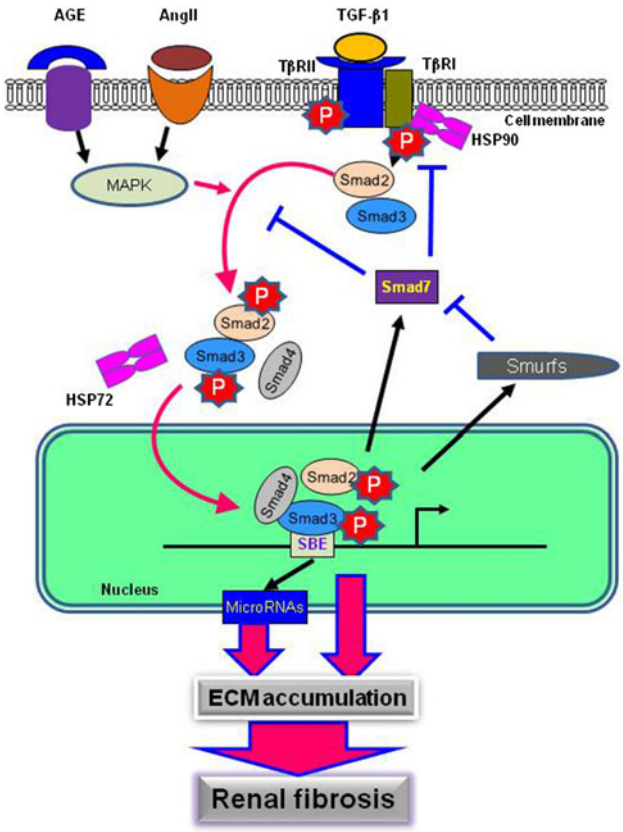

Fig. 1 TGF-β/Smads signaling and its cross-talk pathway in renal fibrosis. Once TGF-β1 binds to TβRII, TβRII activates TβRI kinase, which in turn phosphorylates Smad2 and Smad3. After forming a heterodimer with Smad4, phosphorylated Smads (Smad2 and Smad3) translocate into the nucleus to induce the transcription of target genes such as microRNAs and Smad7. In a normal wound-healing situation, Smad7, as an inhibitory Smad, inhibits Smad3 phosphorylation and causes TβRI to degrade. In the diseased condition, however, TGF-β induces Smurfs to remove Smad7 protein. Activation of TβRI and Smad3 is no longer restricted and, thus, TGF-β signaling and renal fibrosis is enhanced. In addition, AGE and AngII are able to directly activate Smad3 via the ERK/p38/mitogen-activated protein kinase (MAPK) cross-talk pathway

Precursor of TGF-β

TGF-β can be produced by both resident kidney cells and infiltrating leukocytes [5]. Three isoforms of TGF-β (TGF-β1, 2, and 3) are broadly expressed and they are secreted as latent precursors. These latent forms of TGF-β (latent TGF-β) form a complex with latent TGF-β binding proteins (LTBP-1, -3, and -4) [2, 12]. Among these isoforms, TGF-β1 is the vital mediator in fibrosis [7, 12, 13]. Plasmin, reactive oxygen species, thrombospondin-1, and acid promote proteolytic cleavage of active TGF-β1 from the latency-associated peptide (LAP) and LTBP [2, 12]. Although the latent TGF-β is generally believed to act as a precursor of active TGF-β, studies from our laboratory demonstrate that latent TGF-β actually plays a protective role in renal fibrosis [20–22]. By employing transgenic mice overexpressing latent TGF-β1 in the skin, renal fibrosis is dramatically reduced in obstructive and immunologically-induced crescentic glomerulonephritis, despite higher levels of circulating latent form of TGF-β1 [20–22]. Although the detailed mechanism is waiting to be explored, elevation of endogenous of Smad7 may be one of the mechanisms for latent TGF-β to protect kidney from 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 [12, 18]. Thus, the activated TβRI receptor activates Smad2 and Smad3 via phosphorylation of their C-terminal serines. In an experimental animal model, TβRI and intestinal wall collagen

deposition are elevated during intestinal fibrosis, which is inhibited by application of TβRI inhibitor, SD-208 [23]. In vitro studies, knockdown of TβRI expression by siRNA, and treatment with SD-208 inhibit gene expression of TβRI and collagen I in isolated myofibroblasts. These findings support the fact that TβRI also actively participates in fibrosis.

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 in renal injury [25].

However, specific deletion of *TβRII* in the collecting duct leads to an elevation of both TGF-β and fibrosis after UUO [26]. Results from in vitro studies suggest that inhibiting TβRII in collecting duct enhances renal fibrosis through paracrine TGF-β signaling between epithelial and interstitial cells [26]. These results contradict to the reports that deletion of *TβRII* in tubular epithelial cells suppresses renal fibrosis in UUO and AKI models [24, 25]. This discrepancy may probably relate to the different cell types in which *TβRII* was deleted. As TβRII may play a critical role in maintaining epithelial integrity [27], deletion of *TβRII* in epithelial cells diminishes their susceptibility to stretch-induced injury. Thus, the absence of TβRII is beneficial to renal fibrosis. Furthermore, the local environments of medulla versus cortex in the kidney may govern the outcomes of renal fibrosis in the UUO model because the major site of fibrosis is within renal cortex, not medulla. These results reveal the complexity of TGF-β signaling during CKD.

Recently, several studies have demonstrated that the 90-kDa heat-shock protein, HSP90, interacts with both TβRI and TβRII to inhibit the Smad7-dependent ubiquitination of TβRI/TβRII [28, 29•, 30•, 31•]. Inhibition of HSP90 activity reduces TGF-β signaling and renal fibrosis by elevating Smad7/Smurf2-dependent ubiquitination of TβRI/TβRII [28, 29•, 30•, 31•]. Thus, one of the possible strategies to termination of TGF-β signal transduction is to induce ubiquitination-dependent degradation of TGF-β receptors.

Smads

Proteins of the Smad family (such as Smad2 and Smad3) are the first identified substrates of the TβRI kinase, and they play a central role in the transduction of receptor signals to specific target genes in the nucleus [12]. Smad2 and Smad3 are well-documented downstream mediators of TGF-β-induced fibrosis, as activation of Smad2 and Smad3 are found to be promoted in both experimental and human kidney diseases, such as obstructive kidney diseases [21, 32–35], remnant kidney disease [36, 37], hypertensive nephropathy [38], diabetic nephropathy [39–44], and chronic aristolochic acid nephropathy [45]. Owing to insertion in the DNA binding domain in Smad2, only Smad3 can directly bind to the specific DNA sequences, called Smad binding element (SBE), in the promoters of TGF-β target genes [46, 47]. During fibrosis, TGF-β, via Smad3 activation, induces the transcription of several genes important for ECM synthesis, such as collagen (ColIa2), fibronectin, connective tissue growth factor (CTGF), tissue inhibitor of MMP-1 (TIMP-1) Smad7, and plasminogen activator inhibitor-1 (PAI-1) [42, 48–51], revealing a pathologic role for Smad3. Ectopic expression of Smad3 stimulates TGF-β-induced fibronectin expression [52], and deletion of *Smad3* in mice suppresses in mouse models of diabetic nephropathy [39, 41], obstructive nephropathy [32], and chronic aristolochic acid nephropathy [45], confirming the critical role of TGF-β/Smad3 signaling during fibrosis. In addition, treatment of heat-shock protein 72 (HSP72) is capable of inhibiting tubulointerstitial fibrosis in a rat model of UUO, which is associated with its ability to suppress TGF-β1-induced phosphorylation and nuclear translocation of Smad3, but not Smad2 [53, 54].

Smad2, on the other hand, is not believed to bind DNA directly, but rather requires a nuclear DNA binding protein of the family Fast (Fast-1) to bind DNA, in association with Smad4, to activate transcription in response to TGF-β [55]. Mice homozygous for a deletion in *Smad2* die during embryogenesis, also hindering the investigation of the role of Smad2 in fibrosis [56]. However, specific deletion of *Smad2* gene in the kidney tubular epithelial cells demonstrates the protective role of Smad2 in fibrosis in a mouse model of UUO, because renal fibrosis is significantly enhanced when *Smad2* gene is deleted from kidney tubules [57•]. In vitro studies also show that knockdown of Smad2 expression in tubular epithelial cells enhances expression of collagen I, collagen III, and TIMP-1, but suppresses expression of the matrix-degrading enzyme MMP-2 after TGF-β1 treatment. More interestingly, *Smad2* deletion enhances TGF-β/Smad3 signaling in fibrosis because deletion of *Smad2* elevates Smad3 phosphorylation, nuclear translocation, promoter activity, binding of Smad3 to a collagen promoter (Col1a2), and autoinduction of

TGF-β1 [57•]. In contrast, restoring Smad2 expression suppresses TGF-β1-induced Smad3 phosphorylation and collagen I matrix expression [57•]. In conclusion, Smad3 signaling promotes renal fibrosis while Smad2 protects against TGF-β-mediated fibrosis by counteracting TGF-β/Smad3 signaling.

Smad4 is known as the common Smad in TGF-β/Smad signaling that associates with receptor-regulated Smad, such as Smad2 and Smad3. Owing to the lethality of *Smad4* knockout (KO) mice [58], its functional role in TGF-β1-driven renal fibrosis remains under-explored. However, a recent study shows that specific deletion of *Smad4* in kidney tubular epithelial cells suppresses renal fibrosis in a mouse model of UUO. In vitro studies confirm that deletion of *Smad4* in fibroblasts reduces TGF-β1-induced collagen I expression [59•]. Unexpectedly, deletion of *Smad4* does not inhibit the Smad3 phosphorylation and nuclear translocation but reduces the Smad3-mediated promoter activities and the binding of Smad3 to the ColIa2 promoter [59•].

In the normal situation, Smad7, an inhibitory Smad, acts as a safeguard mechanism to prevent prolonged activation of TGF-β signaling. TGF-β rapidly induces Smad7 expression because Smad3 binds to a SBE in the *Smad7* promoter to promote *Smad7* transcription [19, 51, 60, 61]. Smad7 then induces degradation of TβRI and Smads via the ubiquitin proteasome mechanism [62, 63]. During CKD, *Smad7* mRNA transcription is enhanced but Smad7 protein is reduced regardless of the upregulation of renal TGF-β1 and high levels of activated Smad2/3 [64]. It is because TGF-β upregulates the expression of E3 ubiquitin ligases, termed Smurf1, Smurf2, and arkadia, and these E3 ubiquitin ligases then physically interacts with Smad7 [36, 62–66]. After recruitment of the Smad7–E3 ubiquitin ligases complex to the activated TGF-β receptors, E3 ubiquitin ligases induce their degradation (including Smad7 itself) through proteasomal–ubiquitin degradation pathways [36, 62–66]. Thus, this aberrant expression of Smad7 results in enhancing further activation of TGF-β/Smad signaling and progressive renal fibrosis as evidenced in various rodent models of kidney diseases [35, 36, 64, 67•, 68]. This is further confirmed in *Smad7* KO mice in UUO, diabetic, and hypertensive models in which more severe renal fibrosis is developed [35, 43, 69].

Smad Co-repressors

As TGF-β receptors and Smads play a critical role in TGF-β signaling during renal fibrosis, Smad signaling is also controlled by a family of proteins known as Smad co-repressors which comprise the SnoN (Ski-related novel gene, non Alu-containing), Ski (Sloan-Kettering Institute proto-oncogene), and TGIF (TGF-β-interacting factor) [70]. After induction by TGF-β, SnoN and Ski bind to Smads and inhibit their transcriptional activity by disrupting functional Smad complexes or interfering with the nuclear translocation of Smad complexes [70, 71]. Thus, these Smad co-repressors effectively antagonize TGF-β/Smad-dependent gene transcription in order to protect the tissue from undesirable TGF-β responses. Studies in fibrotic kidneys in animal models of obstructive and diabetic nephropathy reveal that these co-repressors are diminished during renal fibrosis [68, 72, 73•, 74], suggesting a diminished negative controlling mechanism for TGF-β/Smad signaling.

In addition to targeting Smad7, Smurf2 can also target these Smad co-repressors (Ski, SnoN, and TGIF) for degradation in kidney cells which leads to progressive renal fibrosis and epithelial-to-mesenchymal transition (EMT) in a mouse model of obstructive kidney disease [67•, 68]. Studies in kidney biopsies from patients with various nephropathies reveal that Smurf2 is induced specifically in renal tubules [67•], suggesting further activation of TGF-β signaling and progressive renal fibrosis. Thus, the close association between Smurf2 expression and enhanced degradation of Smad co-repressors reveals that ubiquitin-mediated degradation of these Smad co-repressors may contribute to the development and progression of renal fibrotic diseases in animal models or humans.

Crosstalk Between TGF-β/Smad Signaling and Other Signaling Pathways

Besides TGF-β, ECM accumulation has been attributed to different factors involved in renal fibrosis, such as angiotensin II (AngII), advanced glycation end-products (AGEs), and high glucose, because these factors are also capable of activating Smad signaling via MAPK crosstalk mechanism to induce renal fibrosis [6].

In various pathological settings and cell types, AngII is able to regulate TGF-β expression and Smad activation, and, in turn, the endogenous production of TGF-β also promotes AngII responses [36, 75–77]. Inhibition of AngII signaling, however, suppresses TGF-β secretion and fibrosis [75, 77]. On the other hand, application of a TGF-β neutralizing antibody or truncated TβRII attenuates AngII-induced ECM synthesis [75, 77]. Similarly, high glucose condition or AGE regulates TGF-β expression and Smad activation. Treatment with a TGF-β neutralizing antibody or truncated TβRII can also suppress ECM expression and CTGF expression [40]. These results suggest a positive feedback mechanism in fibrosis in response to AngII, AGE, high glucose, and TGF-β1

AngII, AGE, high glucose, and TGF-β also share some intracellular mechanisms involved in fibrosis, including production of growth factors, activation of protein kinases, and the Smad pathway. In the context of renal fibrosis, Smad3, without TGF-β activation, can interact with

mitogen-activated protein kinase (MAPK) during AGE- or AngII-mediated fibrotic response. Under the diabetic conditions, AGE is capable of activating Smad 2/3 both in vivo and in vitro via the ERK/p38 MAP kinase-dependent mechanism [40, 77]. Similarly, under the hypertensive conditions, Smad3 signaling is activated by AngII via the AT1 receptor-mediated, ERK/p38 MAP kinase–Smad crosstalk pathway, in addition to the TGF-β-dependent mechanism [38, 77, 78]. Furthermore, both AGEs and AngII are able to activate Smad2/3 to stimulate CTGF expression in kidney cells lacking the *TGF-β1* gene or functional TβRII, but not in those with blockade of MAPK signaling by specific inhibitors, or dominant negative adenovirus to ERK1/2 and p38 [40, 77]. The identification of TGF-β-independent Smad pathways has advanced our understanding of complicated Smad signaling under disease conditions.

TGF-β-Dependent microRNAs in Renal Fibrosis

Aberrant microRNA expression is observed in the mouse models of renal fibrosis, demonstrating their critical roles in TGF-β-induced fibrosis. TGF-β1 is able to upregulate miR-21, miR-192, miR-491-5p, miR-382, miR-377, and miR-433 but downregulates the miR-29 and miR-200 families [6, 79•, 80]. These TGF-β-regulated microRNAs have been shown to modulate renal fibrosis.

Both miR-192 and miR-21 play a pathological role in kidney fibrosis through a feed-forward loop to amplify TGF-β signaling and promote fibrosis [81, 82, 83•, 84]. In addition, miR-377 induces fibronectin in MCs [85], while miR-491-5p induces Par-3 degradation in TECs [86]. MiR-382 participates in TGF-β1-induced loss of E-cadherin in human renal epithelial cells [87]. Conversely, members in miR-29 and miR-200 families play a protective role in renal fibrosis by inhibiting the deposition of ECM and preventing EMT, respectively. During renal injury, reduction of miR-29a and miR-29b enhances collagen expression [88•, 89•] and downregulation of miR-200a expression promotes TGF-β-dependent EMT with elevation of TGF-β2 and matrix proteins [90]. These findings suggest that microRNAs play a vital role in TGF-β induced renal fibrosis.

The study of the exact mechanism as to how TGF-β signaling regulates microRNA expression is still ongoing. TGF-β signaling is able to promote the processing of primary transcripts of some microRNAs, such as miR-21, into active form by the Drosha complex [91]. Our laboratory has also demonstrated that the expression of miR-21, miR-192, and the miR-29 family during renal diseases is TGF-β1/Smad3-dependent [81, 82, 89•]. Smad3 regulates the expression of these microRNAs by physically interacting with SBE in their promoters [81, 82, 89•]. Binding of Smad3 on SBE in the promoters may either elevate transcription and post-transcriptional processing of microRNAs, such as miR-21 and miR-192, or suppress the expression, such as miR-29b [81, 82, 89•]. In addition, regulation of TGF-β/Smad3-mediated microRNAs via maintaining renal miR-29b but suppressing miR-192 and miR-21 is another mechanism by which Smad7 protects kidneys from fibrosis under CKD conditions [6, 82].

More importantly, microRNAs can also regulate the TGF-β/Smad3 signaling. Reduction of Smad7 has been shown to be one of mechanisms by which miR-21 induces renal or pulmonary fibrosis [92•, 93]. Furthermore, overexpression of miR-200a also inhibits both Smad3 activity and TGF-β1-induced fibrosis [90]. In addition, both TGF-β1 and TGF-β2 reduce miR-200a expression in renal cells [90]. This reduction is also associated with an elevation of TGF-β2 expression, since TGF-β2 is one of the target genes for miR-200a, revealing a possible feedback between TGF-β2 and miR-200a. MiR-433 forms a positive feedback loop to amplify TGF-β signaling by suppressing Azin1-related polyamine synthesis [80]. All these results suggest the essential roles of microRNAs in TGF-β-induced renal fibrosis, and the complexity between TGF-β/Smads signaling and microRNAs under pathophysiological conditions.

Therapeutic Potential by Targeting the TGF-β Signaling Pathway

As TGF-β/Smad signaling plays an important role in ECM regulation, targeting TGF-β signaling is an attractive therapeutic approach for controlling fibrosis. However, the complexity of the TGF-β signaling pathway makes selection of appropriate therapeutic targets difficult. Especially, targeting a single element in TGF-β signaling pathway may not be effective.

During the past decades, numerous strategies have been developed to directly block TGF-β effects, including the use of TGF-β neutralizing antibodies [15, 16, 94, 95], antisense TGF-β oligodeoxynucleotides or soluble human TGF-β type II receptor [96], and specific inhibitors of TGF-β or its receptor kinases [17, 97, 98] (Table 1). However, intensive investigations by employing these strategies, including some human studies, did not produce any effective results which make them unavailable for the clinical use. The failure may be attributed to the fact that blockade of TGF-β signaling at the upstream levels may impair the potent anti-inflammation property of TGF-β and subsequently promote renal inflammation. On the other hand, existing intracellular Smad crosstalk pathways may hinder the strategies which target TGF-β and TGF-β receptor kinases.

As Smad3 and TGF-β-regulated microRNAs play a vital role of in TGF-β-induced fibrosis, we thus proposed to specifically target the downstream of TGF-β pathway by

Table 1 Potential therapeutic strategies for suppressing renal fibrosis

Level	Approach	Model	References
Suppression of TGF-β1 and its receptors	Antisense TGF-β ODN, neutralizing antibodies, TβR kinase inhibitors, HSP90 inhibitors, and soluble TβRII	DN, UUO	[15–17, 31•, 94–98]
Blockade of Smad signaling	Smad3 inhibitor, Smad7 agonist, and overexpression of Smad7 and HSP72	DN, UUO, RK	[3, 37, 43, 53, 64, 99•, 100•]
Suppression of downstream effectors	Anti-192, anti-miR-21, anti-mR-433, and overexpression of miR-29b and miR-200	DN, UUO	[79•, 80, 81, 89•, 92•]

UUO unilateral ureteral obstruction; *RK* remnant kidney; *DN* diabetic nephropathy; *ODN* oligodeoxynucleotide

exploring the therapeutic potential of Smad7 and TGF-β/Smad-dependent microRNAs. In the last decade, an ultrasound-microbubble-mediated gene transfer system has been developed in our laboratory to target specific genes or micrRNA which can avoid the potential side-effects of virus-based gene therapy. Our laboratory and others have demonstrated that restoration of renal Smad7 expression is capable of suppressing renal fibrosis in numerous rodent models, including diabetes, obstructive nephropathy, remnant kidney, and autoimmune disease [3, 37, 43, 64]. Furthermore, gene transfer of Smad7 is capable of protecting kidneys from fibrosis by regulating TGF-β/Smad3-mediated expression of miR-21, miR-192, and miR-29b in diseased kidneys [79, 82]. Most recently, by using the same gene transfer technique, we have demonstrated that overexpression of miR-29b, or knockdown of miR-21 and miR-433 in a mouse model of obstructive and diabetic kidney diseases, are capable of preventing or blocking renal fibrosis [79, 80, 81, 89•, 92•].

Recent studies have demonstrated that a specific inhibitor of Smad3 (SIS3), HSP72, and inhibitors of HSP90 are able to reduce renal fibrosis [31, 53, 99•]. On the other hand, a traditional Chinese herb, Asiatic acid, acts as an agonist of Smad7 to inhibit liver fibrosis by upregulating Smad7 expression [100•]. These novel agents may offer an alternative treatment for renal fibrosis. Thus, application of microRNAs, Smad7, HSP72, Smad3 inhibitor, HSP90 inhibitors, and Smad7 agonist in the treatment of kidney disease offers a novel, effective, and safe therapeutic approach to combat CKD.

Conclusions

Recent findings have been the major advances in our understanding about the role of TGF-β/Smad signaling in renal fibrosis. In general, Smad3 plays a pathogenic role in renal fibrosis and regulates its downstream specific microRNAs 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 renoprotective 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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Compliance with Ethics Guidelines

Conflict of Interest Arthur C.K. Chung and Hui Y. Lan declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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