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Review Article

Smads as therapeutic targets for chronic kidney disease

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A B S T R A C T

Renal fibrosis is a hallmark of chronic kidney disease (CKD). It is generally thought that transforming growth factor-β1 (TGF-β1) is a key mediator of fibrosis and mediates renal scarring positively by Smad2 and Smad3, but negatively by Smad7. Our recent studies found that in CKD, TGF-β1 is not a sole molecule to activate Smads. Many mediators such as angiotensin II and advanced glycation end products can also activate Smads via both TGF-β-dependent and independent mechanisms. In addition, Smads can interact with other signaling pathways, such as the mitogen-activated protein kinase and nuclear factor-kappaB (NF-κB) pathways, to regulate renal inflammation and fibrosis. In CKD, Smad2 and Smad3 are highly activated, while Smad7 is reduced or lost. In the context of fibrosis, Smad3 is pathogenic and mediates renal fibrosis by upregulating miR-21 and miR-192, but down-regulating miR-29 and miR-200 families. By contrast, Smad2 and Smad7 are protective. Overexpression of Smad7 inhibits both Smad3-mediated renal fibrosis and NF-κB-driven renal inflammation. Interestingly, Smad4 has diverse roles in renal fibrosis and inflammation. The complexity and distinct roles of individual Smads in CKD suggest that treatment of CKD should aim to correct the imbalance of Smad signaling or target the Smad3-dependent genes related to fibrosis, rather than to block the general effect of TGF-β1. Thus, treatment of CKD by overexpression of Smad7 or targeting Smad3-dependent miRNAs such as downregulation of miR-21 or overexpression of miR-29 may represent novel therapeutic strategies for CKD.

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Introduction

Renal fibrosis is a major pathologic feature of chronic kidney disease (CKD) and is mediated by multiple mediators, including growth factors, cytokines, metabolic toxins, and stress molecules via multiple mechanisms and pathways. Among them, transforming growth factor-β1 (TGF-β1) is a key mediator and has been shown to play a pathogenic role in CKD [1–3].

TGF-β is a founding member of the TGF-β superfamily that includes activins, inhibins, growth and differentiation factors, and bone morphogenetic proteins. TGF-β1 is synthesized by all cell types within the kidney. TGF-β1 is secreted as latent precursors (latent TGF-β1) complexed with latent TGF-β binding proteins (LTBP) [4]. TGF-β1 becomes active when TGF-β1 is released from the latency-associated peptide (LAP) and dissociated from LTBP via proteolytic cleavage by plasmin, reactive oxygen species, thrombospondin-1, and acid [4]. Active TGF-β then binds its receptors and functions as autocrine and paracrine manners to exert its biologic and

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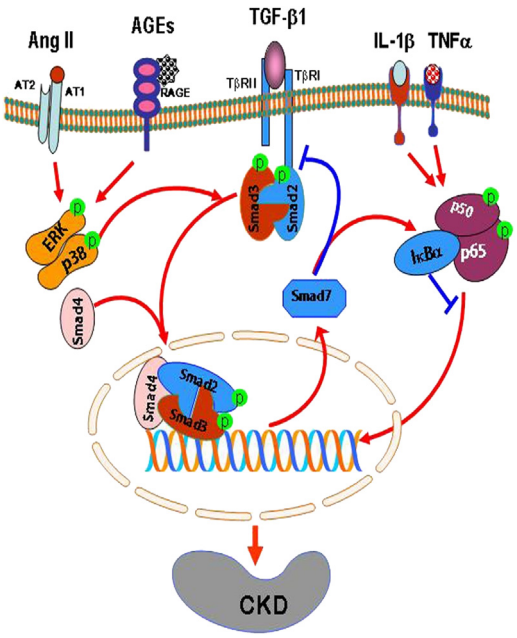

Figure 1. TGF-β/Smads and crosstalk pathways in renal fibrosis and inflammation. After binding to TβRII, TGF-β1 activates the TβRI-kinase, which phosphorylates Smad2 and Smad3. The phosphorylated Smad2 and Smad3 then bind to Smad4 and form the Smad complex, which translocates into the nucleus and regulates the target gene transcription, including Smad7. Smad7 is an inhibitory Smad that functions to block Smad2/3 activation by degrading the TβRI and Smads and to inhibit NF-κB-driven inflammatory response by inducing IκBα, an inhibitor of NF-κB. Note that Ang II and AGEs can activate Smads independent of TGF-β1 via the ERK/p38/MAPK crosstalk pathway. Red arrow lines (symbols) indicate positive regulation and blue lines (symbols) indicate negative regulation. AGE, advanced glycation end-products; Ang II, angiotensin II; ERK, extracellular-signal-regulated kinases; IkBa, IκBα; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-kappaB; TGF-β, transforming growth factor-β. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

pathologic activities via activation of the Smad-dependent and independent signaling pathways [5]. Of these pathways, Smad signaling is a major mechanism by which TGF-β1 mediates CKD [2,3,6].

As shown in Fig. 1, after binding of TGF-β1 to its receptor II (TβRII), TGF-β receptor type I (TβRI)-kinase becomes phosphorylated, resulting in the activation of Smad2 and Smad3, and two receptor-associated Smads (R-Smads) by phosphorylation. The phosphorylated Smad2 and Smad3 then bind to the common Smad4 (Co-Smad) to form the Smad complex, which translocates into the nucleus to regulate the target gene transcription, including Smad7. Smad7 is an inhibitory Smad (I-Smad) and specifically binds E3 ligases (Smurfs and Arkadia) to negatively regulate Smad2 and Smad3 activation and functions by targeting the TβRI and Smads for degradation via the ubiquitin proteasome degradation mechanisms [7,8].

In CKD, TGF-β/Smad signaling is deregulated, resulting in overactivation of Smad2 and Smad3 but a loss of Smad7. Thus, the imbalance of Smads may be a key mechanism leading to renal scar formation and end-stage kidney disease.

In this paper, the current understanding of the distinct role of individual Smads and the regulating mechanisms of Smads and Smad-crosstalk pathways in renal fibrosis and inflammation are introduced and the advances in treatment of CKD by targeting the Smad pathway are described.

Diverse roles of TGF-β1 in renal fibrosis and inflammation

It has been long recognized that TGF-β1 is a key mediator in CKD [1–3]. TGF-β1 mediates progressive renal fibrosis by stimulating extracellular matrix (ECM) production while inhibiting its degradation. In addition, TGF-β1 also induces the transformation of tubular epithelial cells or endothelial cells to myofibroblasts through epithelial-mesenchymal transition (EMT) or endothelial-mesenchymal transition (EndoMT) [9,10]. The functional role of TGF-β1 in EMT and renal fibrosis is demonstrated by the ability of blocking TGF-β1 with neutralizing TGF-β antibodies, decorin, and antisense oligonucleotides to prevent or ameliorates renal fibrosis *in vivo* and *in vitro* [3,11]. Direct evidence for a role of TGF-β1 in renal fibrosis comes from studies that mice overexpressing an active form of TGF-β1 in liver develop progressive liver and renal fibrosis [12,13].

Although the critical role of TGF-β1 in renal fibrosis has been well recognized, little attention has been paid to the role of TGF-β1 in renal inflammation. TGF-β1 is also known as an anti-inflammation cytokines [14]. Mice deficient for TGF-β1 results in the development of a lethal inflammation and death at 3 weeks of age [15]. Similarly, conditional deletion of TβRII or TGF-β1 gene from T cells develops autoimmune diseases [16,17]. By contrast, mice overexpressing human latent TGF-β1 are protected against progressive renal inflammation and fibrosis in obstructive and immunologically induced crescentic glomerulonephritis [18–20]. Thus, TGF-β1 also plays a vital role in anti-inflammation. Results from these studies implicate the complexity of TGF-β1 in the pathogenesis of kidney disease and the necessity to understand the diverse roles and mechanisms of active *versus* latent TGF-β1 under disease conditions.

Smad pathways in chronic kidney disease

In CKD, Smad2 and Smad3 are highly activated and TGF-β is not a sole molecule to activate Smads [3,21]. As shown in Fig. 1, many mediators including advanced glycation end-products (AGE) and angiotensin II (Ang II) can activate Smad2 and Smad3 and mediate renal fibrosis including connecting tissue growth factor (CTGF) expression via both TGF-β-dependent and independent mechanisms [22–25]. The later involves the mitogen-activated protein kinase (MAPK)-Smad crosstalk pathway (Fig. 1). This is supported by the findings that deletion of TGF-β1 or TGF-β receptor II is unable to prevent AGE-induced Smad2 and Smad3 activation and fibrosis [22,23]. By contrast, blockade of the engagement of AGE to its receptor (RAGE) with the soluble RAGE or ERK/p38 MAP kinases with the specific inhibitors or dominant negative adenovirus can prevent AGE-induced Smad2/3 activation and renal fibrosis [22,23], identifying the RAGE-ERK/p38 MAPK-Smad2/3 crosstalk pathway in the development of diabetic complications. Similarly, under the hypertensive

conditions, Ang II can activate Smad2/3 to stimulate ECM production and EMT via the AT1-ERK/p38 MAPK-Smad2/3 crosstalk pathway in addition to the TGF-β-dependent mechanism [24–26]. The important role for the MAPK-Smad crosstalk pathway in AGE and Ang II-mediated renal fibrosis is further demonstrated by the ability of Ang II and AGE to induce Smad3-mediated fibrosis, including CTGF expression and EMT in kidney cells lacking TGF-β1 gene or TβRII, but not in those with a blockade of ERK/p38 MAP kinases [23,25,26]. Therefore, in CKD, many mediators like AGE and Ang II can bind to their own receptor and then activate the Smad pathway via the TGF-β-independent mechanism through the ERK/p38 MAPK pathway in addition to the TGF-β-dependent mechanism (Fig. 1). All of these studies reveal the complexity of the activation of Smads under disease conditions. These results may also implicate that targeting the TGF-β signaling at the receptor levels may not be an optimal therapeutic approach due to the existing of the intracellular crosstalk pathways.

Distinct roles of Smads in chronic kidney disease

Pathogenic role of Smad3 in renal fibrosis

Smads have distinct roles in renal fibrosis and inflammation. In the context of renal fibrosis, Smad2 and Smad3 are strongly activated in both experimental and human kidney diseases, including diabetic nephropathy [21–23,27–29], obstructive kidney diseases [30–33], remnant kidney disease [26,34], drug-associated nephropathy [35], and immunologically mediated glomerulonephritis [20,36]. Many fibrogenic genes, such as ColIa1, ColIa2, ColIIIa1, ColVa2, ColVIa1, and ColVIa3 and the tissue inhibitor of MMP-1 (TIMP-1), are the downstream targets of TGF-β/Smad3 signaling [37] suggesting that Smad3 may be a critical mediator of TGF-β/Smad signaling in fibrosis. An essential role for Smad3 in collagen matrix synthesis is confirmed by the findings that deletion of Smad3 from mice suppresses fibrosis in a number of rodent models, including diabetic nephropathy [27] obstructive nephropathy [30], and drug toxicity-related nephropathy [35]. Furthermore, the use of a Smad3 inhibitor to block TGF-β1-induced endothelial-myofibroblast transition *in vitro* and renal fibrosis in a type 1 diabetic kidney disease demonstrates a therapeutic potential for kidney disease by targeting Smad3 signaling [38].

Protective role of Smad2 in renal fibrosis

By contrast to Smad3, our recent study demonstrated that Smad2 is renoprotective in fibrosis [39]. In conditional Smad2 KO mice in which Smad2 is specifically deleted from tubular epithelial cells by crossing the Smad2 floxed mouse to the kidney specific promoter (Cadherin 16)-driven Cre transgenic mouse [39]. Unexpectedly, we found that deletion of Smad2 from the kidney significantly enhances renal fibrosis in a mouse model of obstructive nephropathy. This may promote Smad3 signaling, including Smad3 phosphorylation, nuclear translocation, promoter activities, and the binding of Smad3 to the collagen I promoter (COL1A2) [39]. Thus, although it is commonly believed that Smad2 and Smad3 bind together physically and work in a nonredundant manner in embryonic development, Smad2 may function to competitively inhibit Smad3 activation and the subsequent binding to its target genes under pathophysiologic conditions. Thus, the loss of Smad2 enhances Smad3-mediated collagen matrix expression in response to TGF-β1 and other fibrotic mediators, including Ang II, AGE, and nephrotoxin [23–26,35].

Differential role of Smad2 and Smad3 in EMT

Smad 2 and Smad3 also play a distinct role in EMT. Many studies have demonstrated that Smad3 plays a critical role in the EMT process in the kidney under various disease conditions [26,30,35]. Our recent study found that disruption of Smad3 – but not Smad2 – attenuates Ang II-induced EMT as identified by the loss of an epithelial marker E-cadherin and the gain of mesenchymal phenotype α-SMA [26]. This may be mediated by CTGF because the knockdown of Smad3 – but not Smad2 – blocks AGE and Ang II-induced CTGF expression and renal fibrosis [23,26]. The ability of blocking Smad3 to inhibit EMT and EndoMT in a variety of animal models confirms a critical role for Smad3 in the pathogenesis of CKD [10,38,40,41].

Diverse role of Smad2 and Smad3 in angiogenesis

Smad2 and Smad3 also have a distinct role in angiogenesis. Indeed, impaired angiogenesis leads to the progressive renal fibrosis [42,43], whereas excessive angiogenesis has been associated with the early development of diabetic nephropathy [44]. It is now understood that TGF-β acts by stimulating Smad2 and Smad3 to control the process of angiogenesis by diversely regulating expression of vascular endothelial growth factor (VEGF), a proangiogenic factor, and thrombospondin-1 (TSP-1), an antiangiogenic factor [45] Indeed, deletion of Smad3 – but not Smad2 – is capable of blocking TGF-β1-induced VEGF expression. Whereas, Smad2 – but not Smad3 – is critical for TSP-1 expression [45]. These findings demonstrate a critical role for Smad3 in angiogenesis while Smad2 is an antiangiogenic factor in response to TGF-β1. Furthermore, disruption of Smad2 – but not Smad3 – also inhibits TGF-β1-induced expression of VEGF-A antagonist, a soluble VEGF-A receptor sFlt-1, providing another evidence for a role of Smad2 in antiangiogenesis [45]. It should be pointed out that the differential regulating roles of Smad2 and Smad3 in angiogenesis under disease conditions are complicated. For example, in the early development of diabetic nephropathy, it is likely that the early activation of TGF-β/Smad3 may induce VEGF to cause glomerular hyperfiltration, resulting in the development of albuminuria. However, in the advanced diabetic nephropathy, it is also highly possible that the prolonged activation of Smad3 may stimulate renal fibrosis, which – together with Smad2 – may promote ischemic renal injury by impairing the angiogenesis. Nevertheless, impaired angiogenesis has

been known as a mechanism leading to progressive renal fibrosis in CKD [42,43].

Taken together, it can be concluded that Smad3, but not Smad2, mediates renal fibrosis. This new finding suggests that treatment of renal fibrosis should be specific by targeting on Smad3, rather than Smad2. However, because all of the results described above are from studies *in vitro* and in a mouse model of obstructive nephropathy, it is possible that Smad2 and Smad3 may function differently in other disease conditions and in human CKD.

Distinct role of Smad4 in renal fibrosis and inflammation

Although Smad4 is a common Smad in the signal transduction pathway of the TGF-β family, a recent study [46] found that Smad4 also plays a diverse role in renal fibrosis and inflammation. This is demonstrated by the finding that conditional deletion of Smad4 from the kidney enhances renal inflammation, including CD45+ leukocyte and F4/80+ macrophage infiltration and upregulation of proinflammatory cytokines, chemokine, and an adhesion molecule in a mouse model of UUO nephropathy [46]. By contrast, deletion of Smad4 inhibits progressive renal fibrosis. Although the exact mode of Smad4 in kidney disease remains unclear, the diverse roles for Smad4 in renal fibrosis and inflammation suggest that Smad4 may not be a therapeutic target for CKD.

Protective role of Smad7 in renal fibrosis and inflammation

Smad7 is an inhibitory Smad and has a protective role in renal fibrosis and inflammation. Smad7 inhibits renal fibrosis by negatively regulating Smad2 and Smad3 activation via its negative feedback mechanism. Smad7 is induced by TGF-β1 via the Smad3-dependent mechanism [47–49]. By contrast, Smad7 inhibits Smad2 and Smad3 activation by causing the degradation of TβRI and Smads via the ubiquitin–proteasome degradation mechanism [7,8,49]. In CKD, TGF-β1 and Ang II are able to induce Smad7 and E3 ligases (Smurfs and arkadia) mRNA expression; however, upregulation of the Smurfs and arkadia degrades Smad7 protein via a post-transcriptional modification mechanism [7,8,26,49]. Indeed, Smurf1 and Smurf2 as well as arkadia are E3 ubiquitin ligases for Smad7 [7,8,50] and physically interact with Smad7 [50,51]. Smad7 acts as an adaptor protein to recruit Smurf2 and arkadia to the TβRI to promote its degradation, including Smad7 itself [7,8,50,52]. Once Smad7 is degraded, activation of Smad2/3 and renal fibrosis is enhanced. This is clearly demonstrated by the recent finding that upregulation of renal Smurf2 causes an ubiquitin-dependent degradation of renal Smad7, resulting in enhanced TGF-β/Smad signaling and progressive renal fibrosis [51]. Similarly, upregulation of renal Smurf2 also causes a degradation of Smad transcriptional corepressor SnoN, leading to progressive renal fibrosis and EMT in a mouse model of obstructive kidney disease [53]. Thus, degradation of Smad7 from the diseased kidney promotes further Smad-dependent renal fibrosis. This is further supported by the findings that Smad7 KO mice develop more severe renal fibrosis in obstructive nephropathy and diabetic kidney disease [29,33].

Smad7 exerts its inhibitory role in renal inflammation by inducing IκBα expression, an inhibitor of nuclear factor-kappaB (NF-κB), thereby preventing NF-κB from activation (Fig. 1). In CKD, loss of renal Smad7 promotes renal inflammation, which is associated with activation of the NF-κB-dependent inflammatory pathway as evidenced in crescentic glomerulonephritis, diabetic nephropathy, and obstructive nephropathy [18,20,29,33,36]. In contrast, overexpression of Smad7 substantially inhibits DNA binding activity, nuclear translocation, transcriptional activity of NF-κB/p65, as well as NF-κB-dependent inflammatory responses induced by IL-1β and tumor necrosis factor alpha (TNF-α), which implicates a functional link between the Smad7 and NF-κB [18]. Indeed, Smad7 is able to induce IκBα expression, suggesting that TGF-β1 may act by stimulating Smad7 to induce IκBα expression, thereby inhibiting NF-κB activation and NF-κB-driven renal inflammation [18]. The protective role of Smad7 in both renal fibrosis and inflammation strongly indicates that Smad7 may be a therapeutic agent for CKD.

Regulation of microRNAs by TGF-β/Smads during renal fibrosis

As shown in Fig. 2, recent studies showed that TGF-β regulates specific microRNAs to mediate renal fibrosis

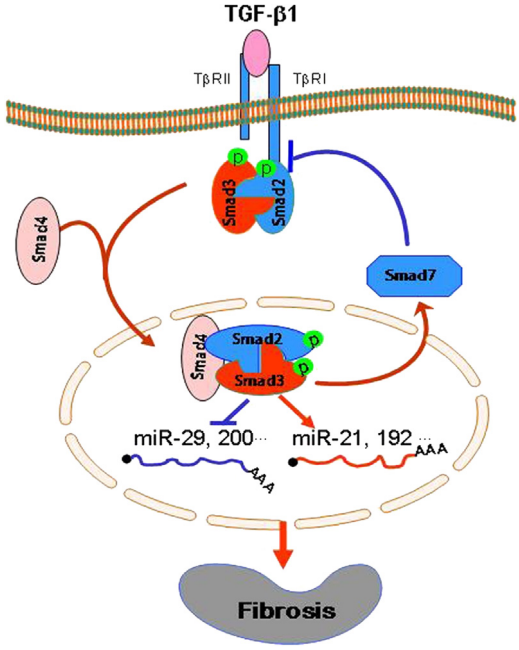

Figure 2. Smad3-dependent miRNAs in renal fibrosis. TGF-β1 acts by stimulating Smad3 to positively regulate miR-21 and miR-192, but negatively regulate the miR-29 or miR-200 families, to mediate renal fibrosis. Thus, overexpression of miR-29 or miR-200 or knockdown of miR-21 and miR-192 may represent novel and specific therapeutic strategies for renal fibrosis. Red arrow lines (symbols) indicate positive regulation and blue lines (symbols) indicate negative regulation. TGF-β, transforming growth factor-β. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

[21,54]. TGF-β1 upregulates miR-21, miR-93, miR-192, miR-216a, miR-377, but downregulates the miR-29 and miR-200 families [21,54]. We have recently identified that, among these TGF-β-dependent miRNAs, miR-21, miR-192, and the miR-29 family expression during renal fibrosis is tightly regulated by a TGF-β/Smad3 – but not Smad2 – dependent mechanism because the deletion of Smad3 – not Smad2 – inhibits the expression of miR-21 and miR-192, but enhances the miR-29 family expression in response to TGF-β1 [55–57]. Evidence supporting the interaction of Smad3 with miR-21, miR-192, and miR-29 also came from the findings that there are conserved Smad3-binding sites in the promoter region of all three miRNAs and that Smad3 can interact with their individual promoter region as detected by the ChiP assay [55–57]. The regulating role of TGF-β/Smad3 in miRNA expression and renal fibrosis is also detected in an experimental mouse model of obstructive nephropathy by miRNA microarray and real-time polymerase chain reaction (PCR). Mice null for Smad3 are protected against renal fibrosis along with the inhibition of miR-21 and miR-192 expression [55,56]. By contrast, severe renal fibrosis in the obstructive nephropathy is associated with a loss of miR-29, which is prevented in Smad3 knockout mice [57]. Of interest, expression of miRNAs can also interact with Smad3 to influence the Smad3 activity and functions. It is reported that overexpression of miR-200a could decrease Smad3 activity and attenuate TGF-β1-induced fibrosis [58].

Smad7 also plays a role in regulating Smad-dependent miRNA expression in response to TGF-β1. For example, in *in vitro*, overexpression of Smad7 in tubular epithelial cells abolished TGF-β1-induced miR-192 expression [55]. In *in vivo*, deletion of Smad7 enhanced Smad3 signaling, thereby promoting miR-192 expression and fibrosis in obstructive kidney disease. By contrast, overexpression of Smad7 blocks TGF-β/Smad signaling and thus inhibits miR-192 expression and renal fibrosis in the rat 5/6 nephrectomy model [55]. Again, results from the miRNA studies support the pathogenic role for Smad3 – but not Smad2 – and the protective role of Smad7 in renal fibrosis.

Smad7 as a therapeutic agent in CKD

Because of a diverse role for TGF-β1 in renal inflammation and fibrosis, the blockade of the general effect of TGF-β may not be an ideal therapeutic approach for CKD. Thus, we proposed to target the downstream TGF-β signaling pathway by exploring the therapeutic potential of Smad7 [59]. As presented in Fig. 1, overexpression of Smad7 not only inhibits activation of Smad2/3, but also blocks NF-κB signaling by inducing IκBα. In CKD, renal Smad7 is degraded along with overactivation of Smad3 and NF-κB signaling, which leads to progressive renal fibrosis and inflammation [3,59]. These findings suggest that overexpression of Smad7 may be able to rebalance the Smad and NF-κB pathways and therefore inhibits renal fibrosis and inflammation. Results from *in vitro* studies support this hypothesis. Overexpression of Smad7 is able to block TGF-β1, Ang II, and AGE-induced activation of Smad2/3 and collagen matrix expression, including EMT [22–26,60]. In addition, we also found that overexpression of Smad7 is capable of inhibiting IL-1β-induced NF-κB activation and inflammatory responses *in vitro* [18]. Findings from *in vitro* studies provide strong rationale for developing the therapeutic approach to prevent and treatment CKD *in vivo*. To explore this therapeutic potential, four studies have been performed. Using a virus-based gene transfer technique, Smad7 is introduced into the kidney followed by ligation of the left ureter to produce obstructive nephropathy. Results show that overexpression of renal Smad7 can inhibit tubulointerstitial fibrosis [32]. Using an ultrasound-microbubble-mediated gene transfer technique, we deliver a doxycycline-regulated Smad7 gene into more than 90% the entire kidney cells, thereby inhibiting progressive tubulointerstitial fibrosis after the left ureteral ligation [31]. Using this gene therapy technique, we are also able to transfer the Smad7 gene into the kidney, resulting in attenuating renal fibrosis and inflammation in a rat model of remnant kidney disease [34,61], an immunologically induced crescentic glomerulonephritis in mice [36,62], and diabetic nephropathy in rats [29]. All of these studies clearly demonstrated that ultrasound-mediated gene transfer of Smad7 can effectively restore renal Smad7 to the normal levels under tight control of doses of doxycycline in the drinking water. As the resultant of overexpression of Smad7 in the diseased kidney, the balance of intrarenal Smad and NF-κB signaling pathways is achieved. Therefore, Smad3-mediated renal fibrosis and NF-κB-driven renal inflammation are inhibited and renal functional impairment is prevented. All of these studies demonstrate that Smad7 not only plays a negatively regulating role in renal fibrosis and inflammation, but it also acts as a therapeutic agent and has therapeutic effect on renal fibrosis and inflammation [59].

However, it should be noted that because of the counter-regulating role of Smad7 in a survival factor NF-κB [63], uncontrollable overexpression of Smad7 could be potentially harmful because higher levels of Smad7 can also induce cell death through apoptosis *in vivo* and *in vitro* [31,64–66]. Thus, it is critical to control the levels of Smad7 to maintain a physiologic balance between the TGF-β/Smad and NF-κB signaling pathways when attempting to target TGF-β signaling with the overexpression of Smad7. This could be controlled using the doxycycline-regulated Smad7 expressing system in which levels of Smad7 within the cells or tissues is tightly controlled by doses of doxycycline as reported in our extensive studies *in vivo* and *in vitro* [59].

Smad3-dependent miRNAs as therapeutic targets for renal fibrosis

Although TGF-β/Smad3 has been considered as a major pathway for fibrogenesis, the diverse roles of this pathway in fibrosis and immunity have hampered the development of anti-TGF-β treatment in general. Indeed, mice lacking either TGF-β_1 or Smad3 have impaired immunity that

results in fatal inflammation in multiple organs or the development of autoimmune disease [15,67]. Lessons learned from the Smad3 knockout mice strongly indicate that the development of therapeutic interventions for renal fibrosis involving this pathway should aim to specifically target the TGF-β/Smad3-dependent genes that are directly involved in fibrogenesis rather than block the general effect of the TGF-β/Smad3 signaling pathway.

The recent advances in miRNA research suggest that targeting miRNAs may be an alterative strategy for treatment of CKD. Indeed, several miRNAs, including miR-192, miR-21, and miR-29, are specifically regulated by Smad3 and mediate renal fibrosis in a Smad3-dependent manner [54–58]. Thus, in the context of renal fibrosis, we proposed a novel therapeutic strategy by specifically targeting the TGF-β/Smad3-dependent microRNAs that directly regulate the fibrosis genes (Fig. 2). The functional role of TGF-β/Smad3-dependent miRNAs in renal fibrosis is demonstrated *in vitro* that overexpression of miR-21 and miR-192 enhances – but knockdown of miR-21 or miR-192 inhibits – the collagen matrix expression in response to TGF-β1 [55,56]. By contrast, knockdown of miR-29 enhances fibrosis, whereas overexpression of miR-29 blocks collagen I expression in response to TGF-β1 [57]. Similarly, overexpression of miR-200a also downregulates TGF-β1-induced EMT and extracellular matrix expression in renal tubular epithelial cells [58].

More excitingly, we recently demonstrated that the ultrasound-microbubble-mediated gene transfer technique can also be used to deliver the miRNAs into the kidney and have proved that the ultrasound-mediated miRNA therapy is a novel and effective therapeutic approach for kidney disease. Indeed, ultrasound-mediated overexpression of miR-29b or knockdown of miR-21 before or after the established mouse model of obstructive nephropathy is capable of preventing or halting the progression of renal fibrosis [56,57]. These findings suggest that specific targeting the Smad3-dependent microRNAs related to fibrogenesis such as miR-21 and miR-29 may represent a novel and specific anti-fibrosis therapy for renal fibrosis. However, it should be aware that the regulating network of microRNAs is complicated. Different genes can be regulated by a single miRNA, and a single miRNA can regulate many different genes. It is also possible that miRNAs can regulate each other in the pathophysiologic processes via the co-regulatory mechanism. Thus, the complexity within the regulatory networking of TGF-β/Smads and miRNAs under pathologic conditions should be considered when developing miRNA as a potential therapeutic target for renal fibrosis.

Concluding remarks

The current understanding of the molecular mechanisms of TGF-β/Smads in renal fibrosis and inflammation in CKD has enabled us to develop specific therapeutic strategies for CKD. In general, Smad3 is a downstream key mediator of TGF-β/Smad signaling and plays a pathogenic role in renal fibrosis by upregulating miR-21 and miR-192, but downregulating miR-29 and miR-200 families to mediate renal fibrosis (Fig. 2). By contrast, Smad2 is renoprotective and suppresses renal fibrosis by competitively inhibiting Smad3 signaling. Smad4 is the common Smad and plays a diverse role in promoting Smad3-mediated renal fibrosis but suppresses NF-κB-driven renal inflammation by transcriptionally stimulating Smad7 expression. Most importantly, Smad7 is an inhibitor of both Smad3-mediated renal fibrosis and NF-κB-driven renal inflammation. In CKD, TGF-β/Smad signaling is imbalanced with highly activation of Smad2 and Smad3 but at a loss of Smad7. Therefore, therapies aimed to restore the balance of both Smad and NF-κB signaling pathways by overexpressing Smad7 or target Smad3-dependent miRNAs related fibrosis such as overexpression of miR-29 while inhibiting miR-21 may represent novel and effective therapeutic strategies for CKD.

Conflict of interest

The author has declared no conflict of interest to this submission.

Acknowledgments

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