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Smad7 as a therapeutic agent for chronic kidney diseases

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1. ABSTRACT

Increasing evidence shows that transforming growth factor-β TGF-β1 (TGF-β1) is upregulated and plays a diverse role in renal fibrosis by stimulating extracellular matrix (ECM) production, while inhibiting renal inflammation. Recent studies have identified that TGF-β1, once activated, signals through its downstream signaling pathway to exert its biological effects. It is now well accepted that TGF-β regulates fibrosis positively by receptor-associated Smads including Smad2 and Smad3, but negatively by an inhibitory Smad, called Smad7. We and other investigators have shown that gene transfer of Smad7 is able to inhibit renal fibrosis in a number of experimental models of chronic kidney diseases, including obstructive nephropathy, remnant kidney disease, and autoimmune crescentic glomerulonephritis. Blockade of Smad2/3 activation is a major mechanism by which overexpression of Smad7 inhibits renal scarring. Furthermore, our recent findings also demonstrate that Smad7 plays a critical role in anti-inflammation in chronic kidney diseases by blocking the NF.κB-dependent inflammatory pathway. Thus, Smad7 has a unique role in both anti-renal fibrosis and inflammation. These findings also indicate that targeting the TGF-β/Smad signaling pathway by overexpressing Smad7 may provide a novel, specific, and effective therapy for chronic kidney diseases.

2. INTRODUCTION

It is well characterized that renal inflammation and fibrosis are common features of chronic kidney diseases, including diabetic and hypertensive nephropathy. Although TGF-β has long been considered as a key mediator in renal fibrosis, little attention has been paid to the role of TGF-β in anti-renal inflammation. TGF-β1 is produced and secreted *in vivo* as a latent complex, consisting of mature dimeric TGF-β1, a latency-associated peptide (LAP), and a latent TGF-β binding protein (LTBP). LAP binds to the N-terminal of TGF-β, thereby preventing TGF-β from binding to its receptors, while LTBP-1 binds the LAP-TGF-β complex and prevents TGF-β from interacting with local matrix proteins. TGF-β must be liberated from LAP to become activated. The factors involved in the liberation of TGF-β from LAP include plasmin, thrombospondin-1, reactive oxygen species and acid (pH). Once being released, active TGF-β functions in both autocrine and paracrine manners to regulate cell proliferation, apoptosis, differentiation, chemotaxis, ECM production, cell migration, and inflammatory/immune responses.

It is now well established that after binding to its receptors, TGF-β acts by stimulating its downstream

mediators, Smad2 and Smad3, to mediate renal fibrosis (1-5). However, the signaling mechanisms of TGF-β in anti-renal inflammation remain largely obscure. Our recent studies find that TGF-β exerts its anti-renal inflammation by inducing renal Smad7, an inhibitor of Smad signaling, thereby blocking renal inflammation by inactivating NF.κB signaling (6). Therefore, although Smad7 is induced by TGF-β, unlike TGF-β that exhibits diverse roles in renal fibrosis and inflammation, Smad7 has an unique role in anti-renal fibrosis on the one hand and anti-renal inflammation on the other.

The major task of this chapter is to review the molecular basis and the role of TGF-β/Smad signaling in chronic kidney diseases, particularly focusing on the role and mechanisms of Smad7 as an agent of anti-renal fibrosis and inflammation. A therapeutic targeting on the TGF-β/Smad signaling pathway using ultrasound-microbubble-mediated inducible Smad7 gene transfer in renal inflammation and fibrosis will be emphasized.

3. TGF-β AND ITS SIGNALING PATHWAY IN CHRONIC KIDNEY DISEASES

3.1. TGF-β and Smad signaling in renal fibrosis

TGF-β has long been considered as a key mediator in the pathogenesis of renal fibrosis in both experimental and human kidney diseases (1-3, 7). TGF-β stimulates extracellular matrix (ECM) production by increasing the synthesis of ECM proteins on the one hand, while acting to inhibit their degradation on the other (7). In addition, TGF-β mediates renal fibrosis by inducing the transformation of tubular epithelial cells to myofibroblasts (EMT) (8). Gene delivery of TGF-β1 to the normal rat kidney causes renal fibrosis that can be prevented or ameliorated by blockade of TGF-β with a neutralizing TGF-β antibodies, decorin, and antisense strategies, demonstrating the important role of TGF-β in renal fibrosis (7). The most direct evidence for a role of TGF-β in renal fibrosis comes from the finding that mice overexpressing an active form of TGF-β1 in liver result in progressive liver and renal fibrosis (9, 10).

It is now clear that after binding to its receptors, TGF-β signals through its signaling pathway, Smads, to mediate renal fibrosis (1-3). Indeed, many fibrogenic genes including COL1A1, COL1A2, COL3A1, COL5A1, COL6A1, COL6A3, COL7A1 and tissue inhibitor of MMP-1 (TIPM-1) have been shown to be Smad3-dependent (11). Recent findings that mice null for Smad3 are protected against renal fibrosis (12), radiation-induced skin fibrosis (13), and bleomycine-induced pulmonary fibrosis (14) demonstrate a critical role for Smad3 in fibrogenesis. In the context of renal fibrosis, Smad signaling is strongly activated in experimental and human kidney diseases (15-19), including diabetic nephropathy (15), obstructive kidney disease (16, 17), 5/6 nephrectomy (18), and hypertensive nephropathy (19).

In addition to TGF-β, many mediators in chronic kidney diseases are also able to activate Smad2/3 independently from TGF-β. In the diabetic conditions, we find that advanced glycation end products (AGEs), an essential mediator in diabetic complications, are able to activate Smad 2/3 directly and independently from TGF-β (15). This is evident by the findings that AGEs can induce a rapid activation of Smad2/3 in tubular epithelial cells, mesangial cells, and vascular smooth cells. This phenomenon occurs in the absence of TGF-β and its receptors and is mediated by the receptor for AGEs (RAGE) via the ERK/p38 MAP kinase-dependent crosstalk pathway (15). Similarly, in the hypertensive conditions, we and other investigators have also demonstrated that angiotensin II is able to activate the Smad signaling pathway to stimulate ECM production via the AT1 receptor-mediated, ERK/p38 MAP kinase-Smad cross talk pathway (19, 20). These interesting findings implicate that Smads may act as signal integrators, forming the crosstalk pathways among the fibrogenic molecules to mediate tissue scarring. Collectively, these new data also imply that targeting Smad signaling, instead of anti-TGF-β therapy, may represent a better therapeutic strategy for diabetic and hypertensive complications.

3.2. TGF-β and Smad Signaling in renal inflammation

As described above, while TGF-β and Smad signaling in renal fibrosis has been firmly established, little attention has been paid to the role of TGF-β in the disease conditions with renal inflammation. First, it is known that TGF-β exhibits its anti-inflammation properties by inhibiting macrophage activation, cytokine (IL-1 and TNFα) production, adhesion molecules (ICAM-1, ELAM-1) and chemokines (MCP-1, IL-8) expression (21, 22). TGF-β also exhibits chemotatic effect on neutrophils and macrophages (21, 22). Furthermore, a critical role for TGF-β in anti-inflammation is generated from the results of TGF-β gene knockout mice. Indeed, mice deficient in TGF-β1 develop lethal multiorgan inflammation at 3 weeks of age (23). In contrast, administration of TGF-β attenuates autoimmune diseases, including collagen-induced arthritis (24), allergic encephalomyelitis (25), and experimental colitis (26). The findings that T cells engineered to produce latent TGF-β1 reverse allergen-induced airway hyperactivity and inflammation and down regulate Th1-mediated autoimmune and Th2-mediated allergic inflammatory processes further demonstrate the importance of TGF-β as a useful therapeutic agent in inflammation and immune diseases (27-29). On the other hand, mice that are conditionally deleted for the TGF-β receptor II or TGF-β gene in T cells develop autoimmune diseases (30, 31). Results from all these studies suggest a critical role for TGF-β1 in anti-inflammation and immune-mediated diseases.

A fundamental question remained to be answered is that while mice overexpressing the bioactive form of hepatic TGF-β1 develop progressive renal injury associated with highly increased levels of circulating active TGF-β1 (10,11), T cells engineered to produce latent TGF-β1 prevents immune and inflammatory response (27-29). This significant finding suggests a unique counter-regulating role between active and latent TGF-β1 in the pathogenesis

of immune and inflammatory diseases and the unexpected complexity between latent and active TGF-β1 in renal pathology. We recently sought to test the hypothesis whether an increase in circulating latent form of TGF-β1 may have a protective role in renal inflammation. This hypothesis was tested in a non-immune-mediated progressive model of obstructive kidney disease and an immunologically-induced anti- glomerular basement membrane (GBM) crescentic glomerulonephritis, a most severe form of kidney disease that mimics rapidly progressive glomerulonephritis in human. In contrast to a previous report that transgenic mice that overexpress an active form of TGF-β1 in the liver develop progressive renal injury (10,11), mice that overexpress latent TGF-β1 in the skin exhibit a 8-10-folds increase in circulating levels of total TGF-β1 in plasma and a 2-3 folds increase in TGF-β1 within the kidney. Of them, over 90% are latent TGF-β1 (6, 32). However, all mice have a normal renal histology and function and, importantly, are protected against progressive renal injury in obstructive kidney disease and anti-GBM crescentic glomerulonephritis, including macrophage and T cell infiltration, Th1-mediated crescentic glomerulonephritis, prevention of renal functional impairments, and the development of renal fibrosis (6, 32). This novel observation suggests that, unlike active TGF-β1, latent TGF-β1 may have a unique role in anti-renal fibrosis and anti-renal inflammation.

4. SMAD 7 AS A THERAPEUTIC AGENT FOR CHRONIC KIDNEY DISEASES

4.1. Smad7 as an inhibitory Smad that negatively regulates TGF-β/Smad signaling

As described above, the TGF-β/Smad signaling pathway can be activated by TGF-β and many other profibrogenic factors including AGEs and angiotensin II via TGF-β-dependent and independent mechanisms. Thus, Smad signaling may act as a central pathway leading to fibrosis regardless of the initial pathogenic causes in disease conditions. Smad7, an inhibitory Smad, is induced by TGF-β but acts in a negative feedback loop to inhibit TGF-β activity by preventing the phosphorylation of Smad2/3 (1-5). Smad7 also inhibits TGF-β signaling by targeting TGF-β receptors for proteasomal degradation via recruitment of the ubiquitin ligases Smurf1 and Smurf2 (33,34). Smad7 is also induced by the STAT1 (signal transducer and activator of T cells 1) pathway following activation with interferon-γ (IFN-γ) or interleukin-7 (IL-7), or by activated NF.κB following stimulation of cells with TNF-α (35-37).

Increasing evidence has also shown that the ubiquitin-proteasome pathway can influence TGF-βsignaling through Smad7-ubiquitin degradation pathways (33, 34). It has been reported that both Smurf1 and Smurf2 (Smad ubiquitination regulatory factors) as well as arkadia are E3 ubiquitin ligases for Smad7 (33, 34, 38). Reduction of Smad7 protein resulting from enhanced ubiquitin-dependent degradation has been shown to play a pathogenic role in progression of tubulointerstitial fibrosis (39, 40). Smad7 also acts as an adaptor protein that recruits ubiquitin ligases such as Smurf2 to the TGF-β receptor complex to promote its degradation through proteasomal pathways (33, 34). Thus, in addition to function as an inhibitor of Smads, Smad7 also regulate TGF-β signaling via the ubiquitin-dependent mechanism.

4.2. Smad7 inhibits renal fibrosis in intro and *in vivo*

4.2.1. Overexpression of Smad7 inhibits renal fibrosis *in vitro*

Owing to the fact that Smad7 can act as a negative regulator of Smad signaling, overexpression of Smad7 is capable of inhibiting Smad2 and Smad3 activation, thereby terminating Smad signaling and blocking Smad-mediated collagen matrix production. *In vitro*, overexpression of Smad7 blocks fibrogenic effects of TGF-β, AGEs, angiotensin II, and high glucose on renal tubular epithelial cells, mesangial cells, and smooth muscle cells (15, 41-42). It has been previously shown that epithelial-mesenchymal transition (EMT) is a mechanism of tubulointerstitial fibrosis in both experimental and human kidney diseases and TGF-β is a major mediator in the process of EMT (8). We find that TGF-β-induced EMT and collagen matrix production are associated with over-activation of Smad signaling (41). This suggests that restored the balance of Smad signaling by overexpressing Smad7 may be able to inhibit TGF-β-induced EMT and fibrosis. To test this hypothesis, we established a doxycycline-regulated inducible Smad7 expressing tubular epithelial cell line. Levels of Smad7 transgene expression is tightly regulated by addition of doxycycline in a dose-dependent manner. Indeed, overexpression of Smad7 is capable of inhibiting TGF-β-induced activation of Smad signaling, thereby blocking TGF-β-induced EMT and fibrosis (41). Similarly, overexpression of Smad7 is also able to block TGF-β and high glucose-induced ECM production in mesangial cells and tubular epithelial cells (42, 43). Most recently, we report find that angiotensin II and AGEs can induce a rapid activation of Smad2/3 via the ERK/p38 MAP kinase-Smad crosstalk pathway (15, 19). Overexpression of Smad7 contains capacity to inhibit activation of Smad2/3 and renal fibrosis in response to AGEs (15). The ability of overexpression of Smad 7 to inhibit Smad2/3-mediated fibrosis in response to multiple fibrogenic factors provides strong evidence that Smad7 may be a therapeutic agent for inhibition of renal fibrosis and implies that specific targeting this pathway by overexpression of Smad7 may represent a novel therapeutic strategy for chronic kidney diseases.

4.2.2. Ultrasound-microbubble-mediated inducible Smad7 gene transfer

We then test whether overexpression of renal Smad7 is able to suppress renal fibrosis in rat model of obstructive kidney disease. It is believable that the targeted gene therapy will ultimately have a major impact on clinical treatment of diseases in the future. Although biologic gene delivery using viral vectors or non-biologic approaches, including gene gun, electroporation, liposome, and naked DNA injection, have been developed, the clinical use of gene therapy today remains problematic due to the disadvantages in the aspects of sufficiency and safety of gene delivery. In addition, controlling the transgene

expression at a therapeutic level without causing any side effects is another important challenge.

To overcome these disadvantages and problems described above, we have recently developed a novel, safe, effective, and controllable gene therapy to specifically inhibit TGF-β/Smad-mediated renal fibrosis using an ultrasound-microbubble-mediated inducible Smad7. This technique is safe since ultrasound itself is harmless to the body and is widely used clinically for many purposes such as physical therapy, diagnosis, guidance for deep organ biopsy, and local drug and genetic material delivery (44). Ultrasound contrast agents such as Optison is also safe and widely used clinically (44, 45). For example, Optison is in liquid form at room temperature, but it becomes gas-filled microbubbles with an average diameter of 3 μm at body temperature. Microbubbles can act as an agent to carry drugs or genetic materials for site-specific treatment and gene therapy (44, 45). Gene-bearing microbubbles can be injected intravenously or locally and then ultrasound energy is applied to the target region. As the microbubbles enter the region of insonation, they cavitate, locally releasing DNA. Cavitation also likely causes a local shockwave that increases cell permeability and thus improves cellular uptake of DNA (44, 45). The use of this technique substantially increases the gene transfection rate and transgene expression by up to a 1,000-fold compared to the naked DNA strategy and results in Smad7 transgene expression in more than 90% of kidney cells (16). Moreover, controlling the transgene expression at the desired therapeutic levels, while minimizing the side-effect, is also fundamental when designing the gene therapy. To avoid the undesirable side effects caused by overexpression of Smad7, including severe inflammation resulting from over-suppression of TGF-β signaling and massive apoptosis due to over-inhibition of the NF.κB signaling pathway, a doxycycline-regulated Smad7 gene is transfected into the kidney and Smad7 transgene expression is induced at a therapeutic level by controlling the doses of doxycycline in the drinking water (200 μg/ml in the drinking water). At this dose of doxycycline, Smad7 expression is induced at the therapeutic levels without detectable side-effects (16).

It should be pointed out that, unlike viral-based gene transfer, this ultrasound-microbubble method mediates a gene transfer temporally, peaking at day 3, declining thereafter to less than 50% over two weeks, and reaching the basal level in 4 weeks. This observation is also noticed in peritoneal tissues (16). It should be also noted that an extensive and uncontrollable overexpression of Smad7 also causes massive cell death through apoptosis (16), which is consistent with previous reports that Smad7 is an inducer of cell apoptosis (46). This may be associated with the inhibition of a survival factor NF.κB and the activation of the JNK and p38 MAP kinase pathway (37, 46-49). The interaction between of Smad7 and these mediators (NF.κF, JNK, and p38) in apoptosis is cell type-specific since Smad7 induces apoptosis in mesangial cells by activating the capspase-3 pathway (49), while in podocytes Smad7 causes apoptosis is NF.κB-dependent, but p38 and caspase-3-independent (37). Thus, it is critical to control the level of Smad7 transgene expression within the diseased organs/tissues and to maintain a physiological balance within the TGF-β/Smad7, NF-κB, p38, and JNK signaling crosstalk pathways when attempting to target Smad signaling with Smad7. This implies that balancing the signaling pathways, rather than terminating or dominating the signals, is important when designing the therapeutic strategies to target the signaling pathway (s).

4.2.3. Overexpression of Smad7 inhibits renal fibrosis in rat models of kidney diseases

To explore the therapeutic potential of Smad7 in renal fibrosis, gene transfer of Smad7 using adenovirus or an ultrasound-mediated technique is used and has been shown able to substantially inhibit Smad2 and Smad3 activation and renal fibrosis in a rat model of obstructive nephropathy (16, 17). Interestingly, in contrast to the observation *in vitro* that Smad7 is induced by TGF-β, renal Smad7 is reduced in the diseased kidney associated with obstructive nephropathy, despite upregulation of renal TGF-β and over-activation of Smad2/3 (16, 40). Thus, the restoration of renal Smad7 results in substantial inhibition of Smad2/3 activation, thereby blocking renal fibrosis including α-SMA, collagen I and III mRNA expression and ECM accumulation (16). The critical role of Smad7 in inhibition of renal fibrosis is further supported by the finding that that an accelerated degradation of Smad7 by the ubiquitin-proteasome pathway enhances renal fibrosis in obstructive kidney disease (39, 40).

Overexpression of renal Smad7 is also able to inhibit progressive renal injury in a rat remnant kidney disease in which renal scarring is associated with local production of angiotensin II and hypertension (18). In this disease model, renal Smad7 is also reduced in association with upregulation of renal TGF-β and a marked activation of renal Smad2/3 (18). Ultrasound-microbubble-mediated inducible Smad7 gene transfer results in a higher renal Smad7, thereby inhibiting activation of Smad signaling and renal fibrosis (18). Progressive renal injury including an increase in proteinuria and serum creatinine as well as a fall of glomerular filtration rate is prevented, although levels of high blood pressure are not altered (18).

In addition to inhibition of renal fibrosis, gene therapy with Smad7 also blocks vascular Smad signaling and sclerosis that is associated with hypertension in a rat remnant kidney disease (18). This is consistent with a known role for Smad3 in vascular sclerosis in response to angiotensin II (19). Thus, treatment of Smad7 restores the balance of Smad signaling by inhibiting overactivation of Smad3 in response to high levels of TGF-β1, angiotensin II, and high blood pressure itself, resulting in inhibition of renal fibrosis. Taken together, results from these studies support the central mechanism of Smad signaling in fibrosis and demonstrate that specific blockade of Smad signaling by overexpressing Smad7 may represent a great therapeutic potential for chronic diseases associated with fibrosis.

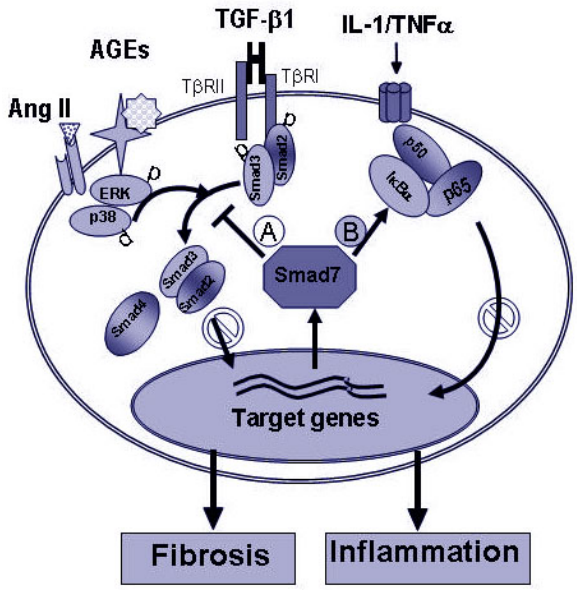

Figure 1. Mechanisms of Smad7 in anti-fibrosis and inflammation. (A) Overexpression of Smad7 blocks TGF-β, as well as AGEs and angiotensin II (Ang II),-induced Smad2/3 activation (phosphorylation), thereby preventing their nuclear translocation and renal fibrosis. (B) Overexpression of Smad7 induces IκBα expression, thereby preventing NF.κB from activation and inflammatory response.

4.3. Smad7 inhibits renal inflammation *in vitro* and *in vivo*

Since targeted deletion of TGF-β and its signaling pathways results in massive inflammation (21-23, 30, 31), it raises the fundamental question whether blockade of TGF-β/Smad signaling with Smad7 enhances renal inflammation. This has been tested *in vitro* in a stable Smad7 expressing tubular epithelial cell line (NRK52E) that Smad7 transgene expression is induced by addition of doxycycline. Overexpression of tubular Smad7 inhibits IL-1 and TNFα induced renal inflammation as determined by suppressing IL-1, TNFα, and ICAM-1 expression (6), in addition to inhibit renal fibrosis in response to TGF-β, AGEs, and high glucose (15, 41, 42). Thus, Smad7 has also anti-renal inflammation properties, in addition to function as an anti-renal fibrosis agent.

The functional role of Smad7 in suppression of renal inflammation is further evident in a mouse model of anti-GBM glomerulonephritis (32). Mice that transgenically express Smad7 on T cells are protected against crescentic glomerulonephritis (50). Our most recent study also found that renal fibrosis and inflammation in obstructive nephropathy are significantly enhanced in mice lacking Smad7 (unpublished data). All these findings strongly support the anti-inflammatory role of Smad7 in kidney disease.

The therapeutic effects of Smad7 on renal inflammation have been examined in a rat model of remnant kidney disease and autoimmune crescentic glomerulonephritis (51, 52). Overexpression of Smad7 not only inhibits renal fibrosis, but also blocks renal inflammation, including upregulation of renal IL-1, TNFα, ICAM-1, and iNOS (51, 52). In addition, overexpression of Smad7 also suppresses accumulation of macrophages and T cells in both glomeruli and tubulointerstitium, and prevents progressive renal functional injury (51, 52). All these studies demonstrate that Smad7 has therapeutic effect on renal inflammation.

5. MECHANISMS OF SMAD7 ANTI-RENAL FIBROSIS AND INFLAMMATION

5.1. Signaling mechanism of Smad7 in anti-renal fibrosis

As shown in Figure 1, it is well accepted that TGF-β induces renal fibrosis by activating its downstream mediators, Smad2/3. In addition, AGE and angiotensin II, two critical mediators in diabetic and hypertensive kidney diseases, are also able to activate the Smad2/3 via the ERK/p38 MAP kinase-Smad crosstalk pathway (15, 19). In contrast, overexpression of Smad7 can counter-regulate Smad-mediated renal fibrosis in response to TGF-β, angiotensin II, AGE, and high glucose (15, 18, 41, 42). It is now clear that Smad7 negatively regulates TGF/Smad signaling via two possible mechanisms: (i) Smad7 binds to TGF-β receptor-1 thereby preventing recruitment and phosphorylation of Smad2 and Smad3 (53), and (ii) Smad7 acts as an adaptor protein that recruits ubiquitin ligases such as Smurf2 to the TGF-β receptor complex to promote its degradation through proteasomal-ubiquitin degradation pathways (33, 34, 40). *In vitro* studies using various types of cells consistently show that TGF-β stimulates Smad7 expression to exert its negative feedback mechanism (54). However, in chronic kidney diseases, the expression of renal Smad7 is reduced, despite upregulation of renal TGF-β1 and high levels of activated Smad2/3 (16, 18, 40). Although the mechanisms underlying the discrepancy of renal Smad7 expression between the *in vivo* and *in vitro* studies are unclear, it may be associated with the occurrence of ubiquitin-degradation of Smad7 at the same time when Smad7 binds ubiquitin ligases to cause TGF-β receptor degradation. Indeed, although upregulation of renal Smad7 mRNA is observed in obstructive kidney disease, an increase in the E3 ubiquitin ligase Arkadia within the diseased kidney may contribute to the decreased renal Smad7 at the protein level via the ubiquitin-degradation mechanism (40). A similar mechanism has also been reported in a rat model of obstructive kidney disease in which progressive renal fibrosis is associated ubiquitin-degradation of Smad transcriptional co-repressor Ski and SnoN (55, 56). Thus, reduction of renal Smad7 results in over-activation of TGF-β signaling and progressive renal fibrosis as shown in a rat anti-Thy1 model (57), mouse obstructive kidney disease (16,39, 40), remnant kidney disease (18), and autoimmune crescentic kidney disease (52). In contrast, overexpression of renal Smad7 inhibits Smad2/3 activation and renal fibrosis in response to TGF-β1, AGEs, high glucose in tubular epithelial cells and mesangial cells *in vitro* (15, 41, 42) and in a number of

disease models of progressive renal fibrosis (1). All these studies suggest that Smad signaling may be a key and common pathway leading to fibrosis regardless of the initial disease causes, while Smad7 is a common inhibitor of Smad-mediated renal fibrosis in chronic kidney diseases and it may indeed have prominent therapeutic potential for chronic kidney diseases.

While treatment of Smad7 blocks renal fibrosis, a significant role for endogenous Smad7 in anti-renal fibrosis is further demonstrated in our recent studies in obstructive and immunologically-induced crescentic glomerulonephritis (6, 32). In these studies, mice overexpressing latent TGF-β1 in the skin show a 8-10-fold increase in latent TGF-β1 with a marked upregulation of renal Smad7 without causing activation of Smad2/3 and renal fibrosis (6, 32). This novel observation is contradictive to the previous report that mice with overexpression of active TGF-β1 in the liver result in a 10-fold increase in bioactive TGF-β1 in circulation and develop progressive liver and renal fibrosis (9, 10). Although it is well known that Smad7 is induced by TGF-β, mechanisms by which latent TGF-β1 upregulates renal Smad7 without causing overactivation of Smad signaling remain largely unclear. It is possible that a persistent higher level of circulating latent TGF-β1 in mice that overexpress a latent form of TGF-β1 on skin may allow to maintain a low physiological level of active TGF-β1. This may favor to continuously activate and maintain the physiological level of TGF-β/Smad signaling within the kidney which increases renal Smad7 while balancing Smad2/3 actions without causing renal fibrosis. However, this could be lost when Smad2/3 became overactivated in response to higher levels of active TGF-β1 with progressive renal fibrosis (16, 18, 40). Indeed, renal Smad7, which normally is high, is significantly reduced in the fibrotic kidney associated with over activation of Smad2/3 (16, 18, 40), suggesting that TGF-β-induced Smad7 is regulated in a complex manner under disease conditions. In addition, it is possible that higher levels of latent TGF-β1 may be able to prevent renal Smad7 from ubiquitin-dependent degradation since post-modifications of Smad7 such as acetylation in response to TGF-β is able to protect Smad7 itself from Smurf-mediated ubiquitination and degradation (58, 59). Nevertheless, these observations suggest that overexpression of renal Smad7 in response to higher levels of latent TGF-β1 may be a critical mechanism of anti-renal fibrosis and indicate that Smad7 may be a therapeutic agent for renal fibrosis

5.2. Signaling mechanisms of TGF-β in anti-renal inflammation

While it is clear that TGF-β signals through Smad2/3 to mediate fibrosis, the signaling mechanisms by which TGF-β exerts its anti-inflammatory activity remain largely unclear. Beyond the anti-fibrotic effect, Smad7 is capable of suppressing renal inflammation. Overexpression of Smad7 in T cells has been demonstrated to prevent glomerulonephritis in an anti-GBM mouse model (50). Gene transfer of renal Smad7 also inhibits renal inflammation in remnant kidney disease and autoimmune crescentic glomerulonephritis (51, 52). Most recently, upregulation of endogenous renal Smad7 is also a central mechanism by which mice that transgenically express a latent form of TGF-β1 are protected against renal inflammation and renal fibrosis in both obstructive and anti-GBM glomerulonephritis (6, 32). Indeed, Smad7 gene therapy results in a substantial inhibition of renal inflammation including suppression of inflammatory cytokines (IL-1, TNFα), adhesion molecules (ICAM-1and VCAM-1), macrophage and T cell accumulation, and cell proliferation (51, 52).

Figure 1 illustrates the potential mechanism of in anti-inflammation by Smad7. The inhibitory effect of Smad7 on renal inflammation is associated with blockade of NF.κB activation (51, 52). It is well-known that NF.κB is a crucial transcriptional factor that regulates inflammatory responses in the kidney diseases. NF.κB is composed of p50 and p65 subunits, of which p65 is a potent transcriptional activator. A marked activation of p65 is closely correlated with the severity of renal inflammation in both obstructive, anti-GBM and autoimmune crescentic glomerulonephritis (6, 32, 52). It is also noted that the mouse Smad7 promoter contains a putative NF.κB regulatory site, implying a functional link between the NF.κB and Smad7 (60). Thus, overexpression of Smad7 substantially suppresses NF.κB activation as demonstrated by inhibition of NF.κB/p65 nuclear translocation, NF.κB transcriptional activity (reporter assay), NF.κB DNA binding activity, and NF.κB-dependent inflammatory responses induced by IL-1β and TNFα (6). NF.κB activation is regulated by its inhibitor, IκBα. Normally, IκBα binds to NF.κB p50/p65 subunits and thus prevents their activation from nuclear translocation. Once IκBα is phosphorylated or degraded, p50/p65 subunits become activated and translocate into the nucleus to stimulate the target genes. TGF-β may act by stimulating Smad7 to induce IκBα expression because Smad7 can upregulate expression of IκBα directly (61). Our recent finding further supports the notion that upregulation of renal Smad7 in latent TGF-β1 transgenic mice is associated with an increase in renal IκBα and prevention of IκBα phosphorylation/degradation (6). This is further support by *in vitro* finding that induced renal Smad7 is capable of upregulating IκBα, thereby blocking IL-1β and TNFα-induced NF.κB activation and inflammatory response. Therefore, an inhibition of NF.κB activation and NF.κB-driven inflammation by induction of IκBα expression while preventing its phosphorylation/degradation by Smad7 may be a key signaling pathway whereby TGF-β1 exerts its anti-inflammatory properties.

6. SUMMARY AND PERSPECTIVES

The discovery of the TGF-β signaling pathway via Smads has distinctly improved our understanding of the molecular mechanisms of renal fibrosis and inflammation in chronic kidney diseases. Understanding the specific role of individual Smads in the pathogenesis of renal fibrosis and inflammation is the first step towards designing

treatment for chronic kidney diseases. In the context of fibrosis, the imbalance of Smad signaling caused by increased Smad2/3 activation while decreased renal Smad7 may represent a common mechanism or pathway in the development of tissue scarring. This imbalance may also have impact on the development of renal inflammation due to overactivation of the NF.κB signaling. Thus, the restoration of renal Smad7 expression can balance this pathway by inactivating Smad2/3, thereby inhibiting renal fibrosis. TGF-β can also induce Smad7 to exert its anti-inflammatory activities by inducing IκBα, thereby blocking NF.κB activation and renal inflammation. The finding that mice overexpressing latent TGF-β1 are protected against renal fibrosis and inflammation by upregulation of renal Smad7 also implicates the therapeutic potential for chronic kidney disease by using latent TGF-β1. However, cautions should be taken when attempting to treat diseases with latent TGF-β1 since systemic effects of TGF-β1 are complicated, particularly under disease conditions. Therefore, local gene transfer of Smad7 to block the Smad2/3-dependent fibrosis pathway and the NF.κB-dependent renal inflammation may represent a better therapeutic strategy for chronic kidney diseases clinically.

7. ACKNOWLEDGEMENTS

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