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Review Article

Role of the TGF-β/BMP-7/Smad pathways in renal diseases

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Abstract

TGF-β (transforming growth factor-β) and BMP-7 (bone morphogenetic protein-7), two key members in the TGF-β superfamily, play important but diverse roles in CKDs (chronic kidney diseases). Both TGF-β and BMP-7 share similar downstream Smad signalling pathways, but counter-regulate each other to maintain the balance of their biological activities. During renal injury in CKDs, this balance is significantly altered because TGF-β signalling is up-regulated by inducing TGF-β1 and activating Smad3, whereas BMP-7 and its downstream Smad1/5/8 are down-regulated. In the context of renal fibrosis, Smad3 is pathogenic, whereas Smad2 and Smad7 are renoprotective. However, this counter-balancing mechanism is also altered because TGF-β1 induces Smurf2, a ubiquitin E3-ligase, to target Smad7 as well as Smad2 for degradation. Thus overexpression of renal Smad7 restores the balance of TGF-β/Smad signalling and has therapeutic effect on CKDs. Recent studies also found that Smad3 mediated renal fibrosis by up-regulating *miR-21* (where *miR* represents microRNA) and *miR-192*, but down-regulating *miR-29* and *miR-200* families. Therefore restoring *miR-29*/*miR-200* or suppressing *miR-21*/*miR-192* is able to treat progressive renal fibrosis. Furthermore, activation of TGF-β/Smad signalling inhibits renal BMP-7 expression and BMP/Smad signalling. On the other hand, overexpression of renal BMP-7 is capable of inhibiting TGF-β/Smad3 signalling and protects the kidney from TGF-β-mediated renal injury. This counter-regulation not only expands our understanding of the causes of renal injury, but also suggests the therapeutic potential by targeting TGF-β/Smad signalling or restoring BMP-7 in CKDs. Taken together, the current understanding of the distinct roles and mechanisms of TGF-β and BMP-7 in CKDs implies that targeting the TGF-β/Smad pathway or restoring BMP-7 signalling may represent novel and effective therapies for CKDs.

Key words: bone morphogenetic protein-7 (BMP-7), inflammation, renal fibrosis, Smad, transforming growth factor-β (TGF-β)

INTRODUCTION

The TGF-β (transforming growth factor-β) superfamily contains highly pleiotropic molecules, such as TGF-β, BMPs (bone morphogenic proteins), activins, inhibins, GDFs (growth differentiation factors) and GDNFs (glial-derived neurotrophic factors) [1]. It is now well accepted that TGF-β acts as an essential mediator in kidney diseases given its multiple functions in renal fibrosis, inflammation, cell growth, apoptosis and differentiation, whereas BMP-7 exhibits its antifibrotic and anti-inflammatory properties [2–5].

TGF-β/BMP signalling is mediated by heterodimeric receptors with serine/threonine receptor kinases and cytoplasmic proteins called Smads [1]. TGF-β binds its TβRII (type II TGF-β receptor) to activate TβRI (type I TGF-β receptor) to convey intracellular signals through phosphorylation of R-Smads (receptor-regulated Smads), including Smad2 and Smad3. On the other hand, BMP-7 binds BMPRs (BMP receptors) to activate R-Smad1/5/8. Phosphorylated R-Smads will form heteromeric complexes with a common partner, Smad4 (co-Smad). The complex then translocates into the nucleus to induce the transcription of their target genes. However, the

Abbreviations used: BMP, bone morphogenic protein; BMPR, BMP receptor; CKD, chronic kidney disease; *Col1A2*, collagen type 1α2 gene; ECM, extracellular matrix; EMT, epithelial–mesenchymal transition; EndoMT, endothelial–mesenchymal transition; ERK, extracellular-signal-regulated kinase; FSP-1, fibroblast-specific protein-1; IL, interleukin; IκBα, inhibitory κBα; KO, knockout; MAPK, mitogen-activated protein kinase; MC, mesangial cell; MCP-1, monocyte chemoattractant protein-1; *miR*, microRNA; MMP, matrix metalloproteinase; NF-κB, nuclear factor κB; α-SMA, α-smooth muscle actin; TEC, tubular epithelial cell; TGF, transforming growth factor; LTBP, latent TGF-β-binding protein; TIMP, tissue inhibitor of metalloproteinase; TNF-α, tumour necrosis factor-α; TβRI etc., type I TGF-β receptor etc.; USAG-1, uterine sensitization associated gene-1; VEGF, vascular endothelial growth factor; ZEB, zinc finger E-box-binding homeobox.

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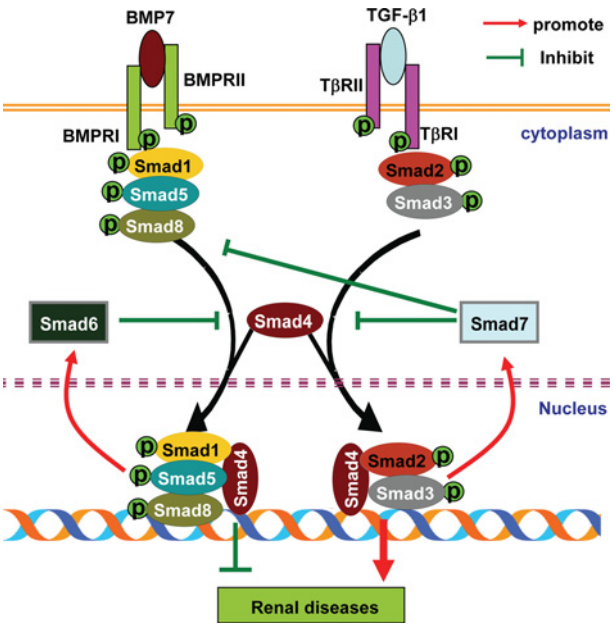

Figure 1 TGF-β1/BMP7/Smad signalling in kidney disease
In kidney diseases, TGF-β1 triggers Smad2/3, whereas BMP7 activates Smad1/5/8. Then, the phosphorylated Smad2/3 or Smad1/5/8 form complexes with Smad4 and shuttle into the nucleus to regulate gene transcription by binding to DNA sequences or cofactors. In addition, TGF-β1 and BMP7 also activate the inhibitory Smads (Smad6 and Smad7) to negatively regulate their signals. Generally, Smad6 has inhibitory effects mainly on the BMP7 signals and Smad7 acts as a general antagonist for the entire TGF-β family. BMPRI etc., BMPR type I etc.; p, phosphorylation.

Smad-mediated signals induced by TGF-β/BMP are closely controlled by negative-feedback mechanisms via their inhibitory Smads (I-Smads), termed Smad6 (primarily for BMP signalling) and Smad7 (primarily for TGF-β signalling) [6,7] (Figure 1). Thus the Smad4/Smad1/5/8 and Smad2/3/4 complexes regulate the transcription of two different sets of genes. Aside from the Smad-dependent pathways, both TGF-β and BMP-7 can also signal through the non-canonical pathways, such as p38 and ERK (extracellular-signal-regulated kinase) MAPK (mitogen-activated protein kinase), to exert their distinct biological activities [8–10].

The present review focuses on interpreting the functional role and regulatory mechanisms of TGF-β/Smads and BMP-7/Smads in the progression or regression of CKDs (chronic kidney diseases). The therapeutic potential of targeting the TGF-β/BMP-7/Smad signalling pathways is also discussed.

TGF-β AND BMP-7 IN RENAL FIBROSIS

Renal fibrosis, the common pathological feature of CKDs, is characterized by excessive accumulation of ECM (extracellular matrix). It is well accepted that progressive renal fibrosis leads to the end-stage renal disease [11–13]. TGF-β is a well-known mediator in renal fibrosis [2,14], whereas the antifibrotic effects of BMP-7 have drawn more attention [3,4,15].

Role of TGF-β in renal fibrosis

TGF-β1, the most abundant isoform of TGF-β family members, can be secreted by all types of renal cells and infiltrated inflammatory cells as the latent precursor, called latent TGF-β1, which binds to LTBP (latent TGF-β-binding protein). TGF-β1 is released from the LAP (latency-associated peptide) and LTBP when exposed to many factors, such as ROS (reactive oxygen species), plasmin and acid [16–19]. The mature TGF-β1 then binds to TβRII to recruit TβRI and initiates the downstream signals, including both Smad-dependent and Smad-independent pathways [8].

Emerging evidence has demonstrated a central role for TGF-β1 in renal fibrosis in both experimental and human kidney diseases. TGF-β1 is significantly up-regulated in the fibrotic kidney regardless of the initial causes of kidney diseases [2,18,20]. Overexpression of mature TGF-β1 in rodent liver is capable of promoting the progression of fibrosis in kidneys, revealing a functional importance of TGF-β1 in CKDs [21,22]. The pro-fibrotic effect of TGF-β1 is confirmed further by the findings that blockade of TGF-β1 with neutralizing TGF-β antibodies or antisense oligonucleotides significantly ameliorates renal fibrosis *in vivo* and *in vitro* [23]. Collectively, the underlying mechanisms by which TGF-β1 mediates fibrogenesis are summarized as follows: (i) TGF-β1 induces matrix production through Smad3-dependent or non-canonical mechanisms; (ii) TGF-β1 inhibits ECM degradation by suppressing MMPs (matrix metalloproteinases) and inducing the natural inhibitor of MMPs [TIMPs (tissue inhibitor of metalloproteinases)] [23]; and (iii) furthermore, TGF-β1 is believed to induce myofibroblast formation through tubular EMT (epithelial–mesenchymal transition) [24].

Differential roles of Smad in renal fibrosis have been clarified. In both patients and animal models with CKDs, Smad2/3 is highly activated in the fibrotic kidney [25–34]. Smad3 is confirmed to be pathogenic because deletion of Smad3 inhibits fibrosis in obstructive nephropathy [29], hypertensive nephropathy [35], diabetic nephropathy [26] and drug-toxicity-related nephropathy [32]. Furthermore, Smad3 can directly bind to the promoter region of collagens to induce their synthesis [36–38]. In addition, TGF-β also induces TIMP-1 through Smad3 to inhibit ECM degradation and overexpression of Smad3 inhibits MMP-1 activity in fibroblasts [39], suggesting a pathogenic role of Smad3 during renal fibrosis.

In contrast, Smad2 plays a protective role in renal fibrosis even though Smad2 and Smad3 have more than 90% structural similarity and both physically interact with each other in response to TGF-β1 [1]. Several studies demonstrate different functional roles of Smad2 and Smad3 in mediating the action of TGF-β1 [40–42]. These observations are supported further by our finding that conditional KO (knockout) of Smad2 from the kidney TECs (tubular epithelial cells) enhanced Smad3-dependent renal fibrosis *in vivo* and *in vitro,* including increasing phosphorylation of Smad3, its nuclear translocation, Smad3-responsive promoter activity and Smad3 binding to the *Col1A2* (collagen type 1α2 gene) promoter [43]. Taken together, Smad2 plays a protective

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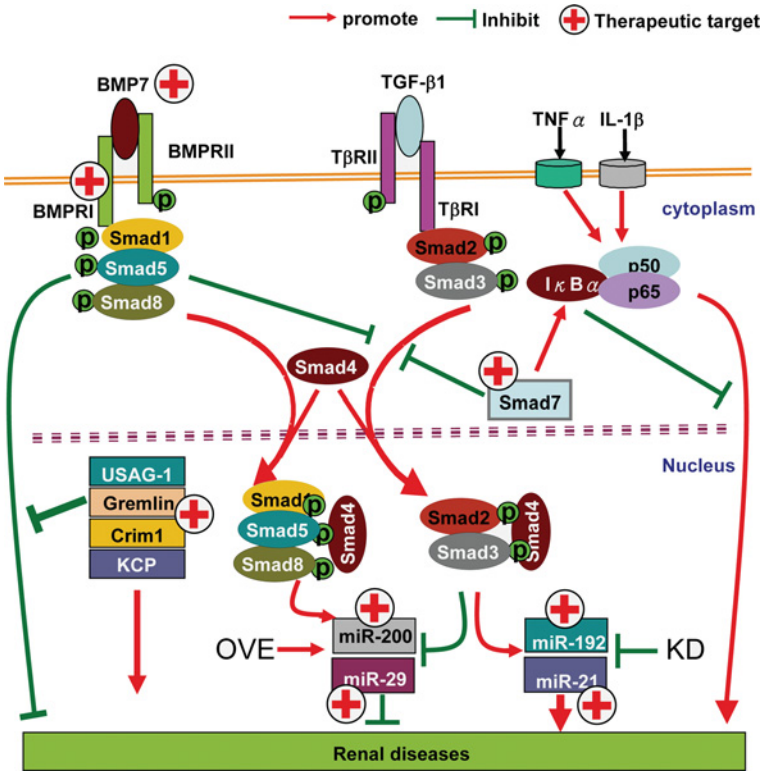

Figure 2 Therapeutic targets of TGF-β1/BMP7/Smad signalling for kidney disease
(i) Administration of BMP7 attenuates renal inflammation and fibrosis. (ii) Inhibition of BMP antagonists, such as USAG-1, Gremlin, Chordin-like1, Crim1 and Kielin/Chordin-like protein (KCP), protects against renal injury; (iii) THR-123, a small-peptide agonist of BMP signalling which functions through the Alk3 receptor to inhibit renal inflammation and fibrosis. (iv) Overexpression of Smad7 suppresses NF-κB-driven renal inflammation and TGF-β/Smad3-mediated fibrosis. (v) Alteration of TGF-β/BMP-7/Smad-regulated microRNAs, such as inhibition of *miR-21* and *miR-192*, while up-regulating *miR-29* and *miR-200*, suppresses renal fibrosis. BMPRI etc., BMPR type I etc.; KD, knockdown; OVE, overexpression; p, phosphorylation.

role in renal fibrosis by counter-regulating Smad3 signalling; however, the effect of deleting Smad2 on non-Smad-dependent pathways remains to be determined.

Smad4 is a common Smad for TGF-β/BMP signalling and a key molecule for shuttling Smad2/3 and Smad1/5/8 into the nucleus [1]. Although the early embryonic lethality of Smad4 KO [44] hinders the study of functional role of Smad4 in the kidney disease, a recent study [45] with the generation of conditional Smad4-KO mouse has shed some light. The disruption of the *Smad4* gene in kidney tubules decreased ECM synthesis *in vivo* in obstructed kidneys and *in vitro* in TGF-β1-treated kidney interstitial fibroblasts [45]. Similarly, deletion of Smad4 from MCs (mesangial cells) also inhibits ECM deposition induced by TGF-β1 [46]. These studies demonstrate further that disruption of the *Smad4* gene attenuates renal fibrosis by decreasing Smad3-responsive promoter activity and inhibiting Smad3 binding to the *Col1A2* promoter, instead of impairing Smad3 phosphorylation and phospho-Smad3 nuclear translocation [45]. Taken together, these results indicate that Smad4 plays an essential role in regulating the ability of Smad3 to initiate the transcription of its targeted genes, instead of the nuclear shuttling of Smad3.

Under normal conditions, Smad7, an inhibitory Smad, is induced by TGF-β1 to block the overactivation of TGF-β signals via inhibition of TβRI and Smad2/3 [47–50]. In CKDs, TGF-β1 induces Smad7 transcription, but promotes Smad7 protein degradation via the Smad3-dependent Smurfs/arkadia-mediated ubiquitin–proteasome degradation pathway [47,48,51]. For example, Smurf2, one of the HECT (homologous with E6-APC terminus) family of E3 ubiquitin ligases for Smad7, can directly bind and promote Smad7 degradation via ubiquitination to enhance TGF-β/Smad-induced renal fibrosis [52]. The importance of Smad7 in renal disease is supported further by *in vivo* findings that disruption of the *Smad7* gene in mice leads to more severe renal fibrosis in both obstructive and diabetic nephropathy [27,53]. More importantly, gene therapy with overexpression of Smad7 significantly attenuates TGF-β/Smad signalling and renal fibrosis in different CKD models [27,31,33,54,55], suggesting the therapeutic potential of Smad7 in CKDs.

The emerging role of microRNAs (*miR*s) in TGF-β-mediated renal fibrosis has been reported recently [56,57]. TGF-β1 up-regulates *miR-21*, *miR-93*, *miR-192*, *miR-216a* and *miR-377* but down-regulates *miR-29* and *miR-200* families [56,57]. Our

laboratory has demonstrated further that the expression of *miR-21*, *miR-29* and *miR-192* are Smad3-dependent [58–60]. More importantly, microRNA therapy by knocking down *miR-21* and *miR-192* or overexpressing *miR-29* effectively inhibits expression of fibrotic markers both *in vitro* and *in vivo*, implying that TGF-β/Smad3 may exert its pro-fibrotic effects through these microRNAs. On the basis of these reports, microRNAs are the key downstream mediators of TGF-β1 and BMP-7 signalling during renal fibrosis or EMT. Targeting these microRNAs related to TGF-β and BMP-7 signalling pathways may represent a novel and specific antifibrosis therapy for kidney diseases.

Role of BMP-7 in renal fibrosis

As a morphogenetic protein, BMP-7 is essential for renal development because BMP-7-KO mice die after birth due to renal failure [61,62]. In contrast with TGF-β1, BMP-7 is highly expressed in the medullary tubules, glomerular parietal epithelial cells, podocytes and renal artery adventitial cells in the normal adult kidney [3,63–66]. Under disease conditions, expression of renal BMP-7 is significantly down-regulated, as detected in diabetic nephropathy [9] and ischaemic acute kidney injury [63,64]. Neutralization of the endogenous BMP-7 by antibodies increases the expression of fibronectin and collagen III in TECs, demonstrating a protective role of BMP-7 in fibrosis [9]. In addition, administration of rhBMP-7 (recombinant human BMP-7) or overexpressing BMP-7 is able to suppress renal fibrosis during diabetic nephropathy [67–69]. This therapeutic effect of BMP-7 is also confirmed by the findings that treatment of BMP-7 effectively prevents renal fibrosis in animal models of obstructive kidney disease [70], nephrotoxic serum nephritis [71], drug-toxicity-related nephropathy [72], Alport's syndrome and lupus-nephritis-like glomerulonephritis [73], suggesting BMP-7 may be an ideal therapeutic agent for treating kidney diseases [73,74].

Furthermore, BMP-7 can antagonize TGF-β1-induced suppression of matrix degradation, such as the production of PAI-1 (plasminogen activator inhibitor-1) [75]. It is also reported that BMP-7 blocks the activation of both Smad3-dependent and Smad-independent pathway, such as p38, ERK and MAPKs, via the activation of Smad1 and Smad5 [9,76,77]. Moreover, BMP-7 can also prevent Smad3 from binding to DNA sequences [78]. This inhibition by BMP-7 can then reduce TGF-β-triggered fibrotic gene expression [71]. In addition, blockade of macrophages infiltrating the kidney could be another mechanism by which BMP-7 inhibits renal fibrosis [79,80].

REGULATORY ROLE OF TGF-β AND BMP-7 IN EMT

Controversy about EMT

α-SMA (α-smooth muscle actin)-positive myofibroblasts are the principal effector cells in the fibrotic response and are believed to cause excessive deposition of interstitial ECM under pathological conditions. However, the origin of these α-SMA-positive myofibroblasts in the fibrotic kidneys remains controversial [81]. Since Strutz and co-workers [82] first detected the expression of FSP-1 (fibroblast-specific protein-1; a specific marker for fibroblast) in TECs under pathological conditions, the concept of EMT has drawn a great attention. By using a lineage tracing technique, increasing evidence shows the major source of myofibroblasts in the injured kidney is derived from resident fibroblasts, pericytes or other cell types, instead of TECs via EMT. This controversy may be attributed to multiple reasons, such as the selection of EMT markers, different tracing techniques, variations among animal models or different stages of renal diseases when observed [83].

TGF-β1 promotes, but BMP-7 inhibits, EMT *in vitro*

Although a considerable number of factors, such as IL (interleukin)-1β [84], CTGF (connective tissue growth factor) [85], AngII (angiotensin II) [86] and AGEs (advanced glycation end-products) [87], are able to induce EMT, TGF-β1 is well accepted as a key factor in mediating EMT [88,89]. *In vitro* studies consistently demonstrate that the presence of TGF-β1 leads the fully differentiated kidney TECs to lose their epithelial property, such as a reduction of E-cadherin, ZO-1 (zonula occludens-1) and cytokeratin, and to gain a mesenchymal property, including the phenotypic changes with *de novo* expression of α-SMA and FSP-1 and the capacity to migrate [90].

Previous studies have also indicated that the Smad3-dependent and Smad-independent mechanisms play essential roles in TGF-β1-induced EMT. Blockade of Smad3 activation by overexpressing Smad7 inhibits EMT. Moreover, knockdown of Smad3 in proximal TECs prevents TGF-β1-induced down-regulation of E-cadherin and suppresses TGF-β1-stimulated α-SMA expression [41], confirming the central role for TGF-β/Smad3 signalling in the process of EMT [91]. In addition, TGF-β1 can also induce EMT via Smad-independent mechanisms by activating MAPK [92], Akt/PKB (protein kinase B) [93] and RhoA pathways [94].

Consistent with the protective role of BMP-7 in renal fibrosis, treatment with BMP-7 is able to inhibit TGF-β1-triggered EMT *in vitro* in mouse TECs [71]. It is generally believed that BMP-7 impairs the TGF-β1-mediated suppression of E-cadherin expression by inducing Id2 [95]. However, a study has shown that BMP-7 itself reduced the E-cadherin expression in a Smad1/5-dependent mechanism in human TECs, but prevented TGF-β1-induced α-SMA expression [96]. It has also been reported that TGF-β/Smad signalling mediates EMT by down-regulating *miR-200a* and *miR-141*, thereby suppressing the E-cadherin transcriptional repressors, including ZEB (zinc finger E-box-binding homeobox) 1 and ZEB2 [97]. In contrast, treatment with BMP-7 blocks EMT significantly by inducing *miR-200* expression [98], providing an additional mechanism as to how BMP-7 regulates EMT [98].

Controversy of EMT *in vivo*

Although the phenomenon of EMT is well observed *in vitro*, whether EMT exists *in vivo* is still under debate. Until now, animal studies have proven the existence of EMT in different renal disease models, including diabetic nephropathy [85,99,100], obstructive nephropathy [29,101], a remnant kidney model [24,102,103] and experimental glomerulonephritis [104].

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However, limited evidence for EMT in patients with CKD has been reported [105]. This largely hampers the translational research of EMT clinically.

Unlike TGF-β, BMP-7 acts as a negative regulator of EMT because administration of BMP-7 inhibits TGF-β1-triggered EMT in a number of mouse models of kidney diseases [71,106,107], demonstrating that BMP-7 could be a key molecule to maintain the phenotype of renal TECs.

In contrast with the view of EMT in renal fibrosis, studies using cell-tracing techniques have failed to detect the EMT origin of myofibroblasts in obstructive nephropathy and found that pericytes may be a major source of interstitial myofibroblasts in the fibrotic kidney [108–111]. Thus, although EMT *in vitro* has been widely accepted, whether EMT occurs *in vivo* during renal fibrosis remains unclear.

ROLE OF TGF-β AND BMP-7 IN RENAL INFLAMMATION

Although the role of TGF-β1 in renal fibrosis is well recognized, less attention has been paid to functions of TGF-β1 in renal inflammation. Previous studies have shown that deletion of TGF-β1 in mice leads to a lethal inflammatory response [112], whereas overexpression of latent TGF-β1 protected against progressive renal inflammation in several disease models [28,34]. Similarly, administration of TGF-β1 is capable of inhibiting pro-inflammatory cytokine expression and macrophage infiltration in glomerular diseases [113], but impaired TGF-β1 signalling by deletion of TβRII in TECs enhances renal inflammation by up-regulating TNF-α (tumour necrosis factor-α) and IL-1β, and promoting hyperactivation of NF-κB signalling [114]. These findings indicate the anti-inflammatory property of TGF-β1 in kidney diseases.

In addition to its anti-inflammatory effect, TGF-β1 can also induce MCP-1 (monocyte chemoattractant protein-1) expression to recruit inflammatory cells into the injured kidney via a Smad3-dependent mechanism [115,116]. It is reported that TGF-β1 stimulates expression of COX-2 (cyclo-oxygenase-2) and PGE_2 (prostaglandin E_2) to induce renal inflammation by Smad-independent mechanisms in human MCs [117]. Thus TGF-β1 regulates renal inflammation via complicated downstream mechanisms.

Smad7 is a key anti-inflammatory molecule in TGF-β1 signalling [118]. This is supported by the finding that deletion of the *Smad7* gene in mice largely enhances renal inflammation in obstructive [53] and diabetic [27] nephropathy. In contrast, overexpression of Smad7 effectively attenuates inflammatory cell infiltration and suppresses the production of inflammatory cytokines in the injured kidneys in different rodent models of kidney disease [27,33,55,119,120]. The possible mechanism may be largely attributed to the ability of Smad7 to induce expression of IκBα (inhibitory κBα), an inhibitor of NF-κB (nuclear factor κB), thereby inhibiting NF-κB-dependent inflammatory responses induced by IL-1β and TNF-α [118,121].

Smad4 also plays an anti-inflammatory role in kidney disease [45]. Conditional KO of *Smad4* from kidney TECs significantly enhances the recruitment of leucocytes and macrophages, and the expression of pro-inflammatory cytokines (TNF-α and IL-1β), chemokines (MCP-1) and adherent molecules [ICAM-1 (intercellular adhesion molecule-1)] in a mouse model of obstructive nephropathy [45]. Moreover, disruption of Smad4 function also inhibits Smad7 expression in obstructive nephropathy and impairs TGF-β1-induced Smad7 promoter activity *in vitro* [45]. This inhibition relieves the NF-κB from the suppression by IκBα and subsequently enhances NF-κB-driven renal inflammation [45].

Increasing evidence shows that BMP-7 plays a protective role on renal inflammation. Administration of BMP-7 reduces the severity of ischaemic renal injury and obstructive nephropathy by inhibiting the infiltration and activity of neutrophils, monocytes and macrophages [63,70]. Interestingly, treatment of BMP-7 is able to prevent the attachment of monocytes to tubular cells, to block macrophage infiltration and the release of TGF-β1 from diseased kidneys [79,80]. Results from *in vitro* studies on proximal TECs also reveal the anti-inflammatory effect of BMP-7 on the expression of pro-inflammatory cytokines (IL-6 and IL-1β) and chemokines (MCP-1 and IL-8) [122]. In addition, BMP-7 can block both constitutive and IL-1β-stimulated MCP-1 expression in human MCs by impairing JNK (c-Jun N-terminal kinase) signalling and AP-1 (activator protein-1) activity [123]. Thus BMP-7 is anti-inflammatory agent in kidney diseases.

REGULATION OF TGF-β AND BMP-7 IN RENAL CELL APOPTOSIS AND PROLIFERATION

It is well accepted that TGF-β1 is a pro-apoptotic molecule and capable of inducing apoptosis in several renal cell types, including TECs, podocytes and endothelial cells [2,124,125]. As tubular apoptosis is a critical event in acute kidney injury, treatment with an anti-TGF-β-neutralizing antibody can attenuate tubular apoptosis in the obstructed kidney [126]. Tubular apoptosis induced by TGF-β1 is Smad3-dependent because overexpression of Smad3 enhances TGF-β1-mediated apoptosis by up-regulating several pro-apoptotic genes, such as death-associated protein kinases and Bad [127–129]. On the other hand, TGF-β1 also induces podocyte apoptosis by activating the p38 MAPK and caspase 3 [130], indicating the pathogenic importance for TGF-β1 in kidney disease. Thus TGF-β/Smad signalling is one of key mechanisms for apoptosis under disease conditions.

In addition to its pro-apoptotic function, TGF-β1 diversely regulates cell proliferation in cell-type-dependent manner. In TECs, TGF-β1 induces a cell-cycle arrest at G_1-phase through decreasing the expression of c-myc and increasing the expression of $p15^{INK4b}$ and $p21^{CIP/WAF1}$, and thus delays the injured tubule from recovery [131,132]. However, it is notable that TGF-β1 fails to increase the proliferation of cultured podocytes *in vitro*, indicating that other pathways may be involved in the regulation of podocyte proliferation [133]. Moreover, TGF-β1 substantially induces the proliferation of renal fibroblasts during renal fibrosis, which is mainly mediated by basic fibroblast growth factor (FGF-2) [134]. Additionally, the effect of TGF-β1 on MCs

is dose-dependent. Low concentrations of TGF-β1 (<100 pg/ml) promote, but high concentrations (>250 pg/ml) inhibit, the proliferation of MCs [135,136].

In contrast with TGF-β1, BMP-7 protects TECs from apoptosis in kidney diseases [63,70,106]. Treatment of BMP-7 significantly reduces the number of apoptotic epithelial cells in the obstructed kidney [74]. A previous study has shown that addition of BMP-7 blocked aristolochic acid-induced injury in TECs by inhibiting cell apoptosis, deactivating caspase 3 activity and reducing levels of LDH (lactate dehydrogenase) [72]. Moreover, results from *in vitro* studies indicate that BMP-7 is able to block TGF-β1- or high-glucose-induced apoptosis in podocytes and renal injury by activating Smad5 [137–139]. BMP-7 also plays an opposite role of TGF-β1 on cell proliferation. In human TECs, addition of BMP-7 substantially induces cell proliferation [98]. This observation is confirmed further by *in vivo* findings that transgenic BMP-7 increases the number of PCNA (proliferating cell nuclear antigen)-positive podocytes in STZ (streptozotocin)-induced diabetic nephropathy [68]. Conversely, BMP-7 blocks aldosterone-induced MC proliferation by inactivation of the MAPK pathway [140]. Interestingly, BMP-7 induces cell proliferation or apoptosis in mouse inner medullary collecting duct (mIMCD-3) cells in a dose-dependent manner. Low doses of BMP-7 (0.25 nM) promote cell proliferation but inhibit cell apoptosis. In contrast, a high dose of BMP-7 (10 nM) suppresses cell proliferation but increases apoptotic response via a Smad1-dependent mechanism [141].

TGF-β AND BMP-7 IN RENAL MICROVASCULATURE

Loss of the glomerular and peritubular microvasculature is not only the outcome but also a driving force for progressive renal disease [142]. TGF-β1 is a key mediator in renal microvasculature because it regulates several endothelial functions, such as apoptosis, proliferation and migration [143]. It has been reported that, in addition to the direct pro-apoptotic effect of TGF-β1 on endothelial cells [144,145], TGF-β1 can also stimulate podocytes and TECs to produce a pro-angiogenic factor called VEGF (vascular endothelial growth factor), which, in turn, protects endothelial cells from apoptosis [142,146,147]. Under disease conditions, TGF-β1 induces EndoMT (endothelial – mesenchymal transition) to promote renal fibrogenesis [148,149]. This pathological process is Smad3-dependent because addition of SIS3, a specific inhibitor for Smad3, blocks TGF-β1-induced EndoMT and attenuates diabetic glomerulosclerosis [148]. Furthermore, TGF-β1 is able to diversely regulate the pro- and anti-angiogenic gene expression through its downstream mediators Smad2 and Smad3. Notably, deletion of Smad2 from mouse embryonic fibroblasts reduces the expression TSP-1 and the VEGF-A antagonist, whereas deletion of Smad3 inhibits TGF-β1-induced VEGF mRNA and protein expression [150].

BMP-7 is also a critical molecule in inducing angiogenesis [151–153]. Treatment of BMP-7 stimulates proliferation of HPAECs (human pulmonary artery endothelial cells). In contrast, administration of BMP-2 or BMP-7 is able to block TNF-α- or serum-deprivation-induced apoptosis in endothelial cells [154]. In addition, BMP-7 protects endothelial cells from apoptosis by inducing VEGF expression through two mechanisms: First, as podocytes and TECs are the key cell types of VEGF expression in the kidney, BMP-7 expression in these cells preserves higher levels of VEGF, thereby protecting endothelial cells from apoptosis via a paracrine-dependent mechanism [155]. Secondly, *in vitro* BMP-7 can directly activate the VEGF promoter to induce angiogenesis, which can be blocked by addition of Noggin, a BMP inhibitor [156].

POSSIBLE THERAPEUTIC STRATEGIES BY TARGETING THE TGF-β OR BMP PATHWAYS IN KIDNEY DISEASES

Therapeutic strategies by targeting TGF-β signalling

General blockade of TGF-β signalling

Considering the critical role of TGF-β1 signalling in the pathogenesis of renal inflammation and fibrosis, targeting TGF-β1 signalling may represent a novel therapeutic strategy for kidney diseases. Many studies show that blockade of the upstream of TGF-β signalling by neutralizing antibodies (anti-TGF-β2 IgG_4), antisense TGF-β oligodeoxynucleotides, soluble human TβRII (sTβRII.Fc) and specific inhibitors to TβR kinases (including GW788388 and IN-1130) is able to attenuate renal fibrosis in a number of experimental kidney disease models [157]. However, these strategies may also increase the risk of renal inflammation due to blocking the general anti-inflammatory property of TGF-β1.

Specific inhibition of TGF-β/Smad3 signalling

To overcome the potential side effect of the general blockade of TGF-β signalling, therapies by targeting the downstream mediators of TGF-β signalling, especially Smad3, may provide more specific and effective therapies for kidney disease. Treatment of diabetic kidney fibrosis using a Smad3 inhibitor (SIS3) reveals the therapeutic potential for renal fibrosis by specifically targeting downstream TGF-β signalling [148]. Furthermore, we and other investigators have also found that targeting the Smad3-dependent microRNAs by overexpression of *miR-29* and *miR-200* or down-regulating *miR-21* and *miR-192* may offer much more specific therapies for chronic kidney disease [57–60].

Smad7 therapy

Emerging evidence also demonstrates that overexpression of Smad7 is capable of attenuating TGF-β/Smad-mediated renal fibrosis and NF-κB-driven renal inflammation in different CKD models [157,158]. The ability of Smad7 to block the downstream mediators of both TGF-β/Smad and NF-κB pathways in renal fibrosis and inflammation suggests that Smad7 may be a powerful therapeutic agent for both acute kidney diseases and CKDs [158].

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Therapeutic strategies by targeting BMP-7 signalling

BMP-7 therapy

As described above, administration of BMP-7 is capable attenuating renal fibrosis and EMT, and effectively inhibiting renal inflammation in several animal models of kidney disease [67–73]. These studies demonstrate that BMP-7 exhibits therapeutic efficacy for renal inflammation, fibrosis and apoptosis. Thus it is highly possible that BMP-7 may be a therapeutic agent for kidney disease.

Specific Alk3 agonist

Considering the widespread expression of BMPRs outside of the kidney, it is possible that administration of pharmaceutical doses of BMP-7 may cause undesirable side effects. Thus development of a kidney-specific BMP-7 receptor agonist may offer a better therapy for kidney disease. It is well accepted that BMP-7 binds three type I receptors (Alk2, Alk3 and Alk6) to exert distinct biological functions. Among these, Alk3 is predominantly expressed in kidney TECs, whereas Alk6 mainly mediates BMP7-induced bone formation [159,160]. Therefore THR-123, a small-peptide agonist of BMP signalling which functions specifically through the Alk3 receptor, has been constructed and proved effective in the treatment of renal fibrosis without affecting osteogenic activity [161], indicating that Alk3 agonists may provide a more specific and promising therapy for CKDs.

Blockade of BMP antagonists

The activity of BMP is regulated not only by the expression of BMP itself, but also by a group of BMP antagonists. There are three subfamilies of BMP antagonists based on the size of cystine-knot, including the DAN family, Chordin and Noggin, and twisted gastrulation [162]. In the kidney, several BMP antagonists, such as Gremlin, Chordin-like1, Crim1, KCP (Kielin/Chordin-like protein) and USAG-1 (uterine sensitization associated gene-1), have been shown to be critical in the pathophysiological process of kidney diseases by modulating the activities of BMPs [163]. This is supported by the evidence from USAG-1-KO mice which are resistant to acute and chronic renal injury due to up-regulation of BMP-7 [164]. Thus targeting BMP antagonists may represent another therapeutic approach in treating renal diseases.

CONCLUSIONS

Recent research into the functional role of TGF-β and BMP-7 signalling pathways improves our knowledge on the molecular mechanisms of renal fibrosis and inflammation in CKDs. An understanding of the specific role of the individual signalling pathways at ligands–receptors, antagonists, intracellular signalling cross-talk pathways and transcriptional/post-transcriptional levels, including Smads-dependent microRNAs, in the pathogenesis of renal fibrosis and inflammation should enable us to develop specific and effective therapeutics for CKDs.

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