Editorial Review

Inhibitors/antagonists of the TGF-β system in kidney fibrosis

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Abstract
Renal fibrosis is a major hallmark of chronic kidney disease, regardless of the initial causes, and prominent renal fibrosis predicts poor prognosis for renal insufficiency. Transforming growth factor (TGF)-β plays a pivotal role in the progression of renal fibrosis, and therapeutic interventions targeting TGF-β have been successful and well tolerated in animal models. However, these interventions might have adverse effects by inducing systemic inflammation due to the strong bifunctional role of TGF-β (pro-fibrotic and anti-inflammatory). This review of the current literature focuses on the inhibitors/antagonists of TGF-β, and discusses possible therapeutic approaches targeting them, describing the effectiveness of orally active bone morphogenetic protein 7 mimetics in reversing established fibrosis. It will conclude with a brief discussion of possible future directions for research.

Keywords: Bmp antagonist; Bmp7; CKD; fibrosis; TGF-β

Introduction
Renal fibrosis characterized by the proliferation of scar-producing myofibroblasts and the accumulation of extracellular matrix proteins is a major hallmark of chronic kidney disease (CKD), regardless of the initial causes [1, 2]. The degree of renal fibrosis is also considered a reliable predictor of renal prognosis. Over the last decades, the origins of scar-producing myofibroblasts have been the subject of intensive investigation. Although pathologists have traditionally held the view that scar-producing myofibroblasts originate from resident fibroblasts, other origins such as circulating fibrocytes, tubular epithelial-to-mesenchymal transition (EMT) and endothelial–mesenchymal transition have been proposed [3, 4]. More recent studies utilizing genetic fate mapping have supported the traditional view and demonstrated that the resident fibroblasts transform into scar-producing myofibroblasts and are the main contributors to fibrosis [5, 6].

Another subject that has been intensively investigated is the reversibility of renal fibrosis: whether scar-producing myofibroblasts can be reverted to the original cell state or controlled in ways that reduce the matrix production [7, 8]. Recent human studies testing the effectiveness of antihypertensive drugs support the idea that these agents can retard the progression of renal diseases to some extent. However, whether any therapeutic intervention can achieve the regression of renal fibrosis and restore renal function remains unclear.

This review focuses on the therapeutic approaches targeting the inhibitors/antagonists of transforming growth factor-β (TGF-β), a key mediator in the pathogenesis of renal fibrosis. In particular, it looks at a recent paper by Sugimoto et al. [9], which describes the effectiveness of orally active bone morphogenetic protein 7 (BMP7) mimetics in reversing established fibrosis.

Blocking TGF-β functions in renal diseases
TGF-β is an original member of the TGF-β superfamily comprising activins, inhibins, growth and differentiation factors (GDF) and BMPs. TGF-β has a broad spectrum of biological functions in a variety of cell types, and is recognized as a key mediator in the pathogenesis of renal fibrosis [1].

TGF-β binds to its receptor, TGF-β receptor IIβ, which activates the TGF-β receptor I (TβRI) kinase. Activated TβRI then phosphorylates receptor-regulated Smads (R-Smads), Smad2 and Smad3 (Figure 1). Phosphorylated Smad2 and Smad3 form an oligomeric complex with Smad4. The complex translocates into the nucleus and regulates the target gene transcription, including Smad7 [10], which is an inhibitory Smad that negatively regulates R-Smads activation [11].

It is widely accepted that TGF-β and its downstream Smad cascade is a key mediator in the pathogenesis of renal fibrosis both in experimental models and in human kidney diseases [12–14]. Up-regulation of TGF-β and its downstream Smad cascade is prevalent in many types of kidney diseases. Recently, it has been reported that injured tubule epithelial cells arrested at G2/M phase of the cell cycle produce high amounts of TGF-β [15].

TGF-β mediates progressive renal fibrosis by stimulating extracellular matrix production, while inhibiting its degradation. TGF-β is also considered to induce EMT of...
the injured tubule epithelial cells [16], whereas the in vivo relevance of EMT remains controversial. In diabetic nephropathy, TGF-β also mediates mesangial matrix accumulation [17, 18].

Several strategies to inhibit the TGF-β signaling pathway have been proposed: the neutralizing antibody, decorin, soluble receptors and small-molecule inhibitors for receptor serine/threonine kinases [13, 19] (Figure 1). The administration of neutralizing antibodies against TGF-β is the most intensively studied approach, and has been successful and well tolerated in several types of animal models. The administration of the anti-TGF-β antibody prevented [20, 21] and, in some cases, even reversed the early features of diabetic nephropathy [22]. The administration of anti-TGF-β antibody in combination with an angiotensin-converting enzyme (ACE) inhibitor or an angiotensin receptor blocker exerted additional beneficial effects in diabetic nephropathy [23].

These positive outcomes in animal studies encouraged researchers to perform clinical trials with anti-TGF-β antibodies and other TGF-β blockers in fibrotic diseases. TGF-β2 neutralizing antibody (lerdelimumab) effectively decreased the scarring after glaucoma surgery [24]. On the other hand, a TGF-β1 neutralizing antibody (CAT-192, metelimumab) could not provide any efficacy in patients with systemic sclerosis, and instead resulted in serious adverse events [25]. A pan-TGF-β neutralizing antibody (GC-1008) has been tested for its efficacy in patients with focal segmental glomerulosclerosis and certain cancers; however, these studies are still in their early phases. The blockers for intrinsic receptor serine/threonine kinases are currently being investigated in patients with certain cancers.

Pirfenidone [5-methyl-1-phenyl-2(1H)-pyridone] is an orally active small molecule which is known for its anti-fibrotic action [13, 26]. Pirfenidone inhibits TGF-β at multiple steps: it reduces TGF-β promoter activity, TGF-β protein secretion, TGF-β-induced Smad2 phosphorylation and generation of reactive oxygen species [27]. In vivo administration of pirfenidone attenuates interstitial fibrosis as well as mesangial matrix expansion in kidney-disease models. The beneficial effect of pirfenidone has also been proved in patients with focal segmental glomerulosclerosis [28]. A recently completed placebo-controlled randomized clinical trial further demonstrated the beneficial effect of pirfenidone on improving estimated glomerular filtration rate in overt diabetic nephropathy [29].

In addition to its pro-fibrotic function, TGF-β possesses another role of inhibiting inflammation. Systemic inhibition of TGF-β utilizing the above-mentioned strategies might augment inflammation, which can lead to severe adverse effects. One promising strategy to circumvent the toxicity is the local administration of TGF-β blockers, which has been tested in animal models.

**Therapeutic strategies targeting factors regulating TGF-β signaling**

Another approach to circumvent toxicity is to target the signaling pathway of TGF-β. BMP7 is a 35-kDa homodimeric secretory protein and a member of the TGF-β superfamily that antagonizes the pro-fibrotic function of TGF-β. BMP binds to serine/threonine kinase receptor Type I and II. In mice, three Type I receptors have been identified—BMPR1a/Alk3, BMPR1b/Alk6 and ActR1a/Alk2. Although these Type I receptors are highly homologous, their preference for the ligands, tissue distribution and the roles during embryogenesis and in adult tissues is diverse.

**Fig. 1.** TGF-β and BMP7 signaling. TGF-β-dependent signaling promotes fibrosis and inhibits inflammation, whereas BMP7-mediated signaling inhibits fibrosis, inflammation and cell death.
Type I receptor, which phosphorylates R-Smads (Figure 1). Subsequently, these R-Smads form an oligomeric complex with Smad4, and translocate to the nucleus, where they activate transcription of target genes. Smad1, Smad5 and Smad8 are R-Smads in BMP signaling pathways, whereas Smad2 and Smad3 are those in TGF-β signaling pathways, although the specificity of the activation of R-Smads by TGF-β superfamily members are not as strict as previously considered [30]. In addition to the Smad signaling pathway, other signaling pathways such as mitogen activated protein kinases are activated by BMPs in certain cell types [31, 32].

BMP7 is highly expressed in the kidney, and its genetic deletion in mice leads to severe impairment of kidney development resulting in perinatal death [33, 34]. Recently, several reports indicated that the administration of pharmacological doses of BMP7 inhibits and repairs acute and chronic renal injury in animal models (reviewed in ref [31, 32]). The first report was published in 1998, in which Vukicevic et al. [35] demonstrated the effect of recombinant BMP7 in the treatment of acute renal failure after bilateral renal artery occlusion. They showed that BMP7 preserved renal function and increased the survival rate. The effectiveness of BMP7 in diabetic nephropathy was also confirmed by the pharmacological method as well as by generating transgenic mice overexpressing BMP7 in podocytes and proximal tubules [36, 37]. In 2003, Zeisberg et al. [38] demonstrated that the administration of BMP7 to chronic kidney injury caused by nephrotoxic nephritis led to repair of severely damaged renal tubular epithelial cells, in association with reversal of chronic renal injury. The same group further demonstrated the effectiveness of BMP7 in animal models for Alport syndrome [39], lupus nephritis and diabetic nephropathy [40].

BMP7 exerts several functions in various types of kidney cells: it antagonizes TGF-β-dependent fibrosis [36], and reduces apoptosis of tubular epithelial cells and podocytes [41, 42]. In contrast to the anti-TGF-β strategies that might augment inflammation, BMP7 attenuates renal expression of inflammatory cytokine [43, 44] and reduces the infiltration of inflammatory cells. Collectively, BMP7 promotes various aspects of repair processes during kidney diseases.

The problem of systemic administration of BMP7 protein is the low availability in the kidney: only 0.5% of the administered BMP7 dose/g tissue is targeted for BMP7 receptors in the kidney, explaining the need for a huge amount of BMP7 for its renoprotective action [35], which might exert adverse effects in different tissues.

The recent study by Sugimoto et al. [9] suggested a new therapeutic strategy that may overcome the problem. Among BMP Type I receptors, Alk3 is predominantly expressed in tubular epithelial cells, whereas Alk6 is expressed in osteoblasts. The authors first demonstrated that Alk3 deletion in tubular epithelial cells led to the enhancement of TGF-β signaling, epithelial damage and fibrosis, indicating the essential role of Alk3 signaling in the renoprotective effect of BMP7 [9]. The authors next screened a library of small peptide agonists of BMP signaling, and identified THR-123 that acts specifically through Alk3. Unlike recombinant BMP7 protein, orally administered THR primarily localized to the kidney cortex, possibly due to its specific binding to Alk3. The administration of THR-123 suppressed inflammation and apoptosis, and reversed established fibrosis in several types of animal models. Furthermore, THR-123 in combination with ACE inhibitor showed additive therapeutic effects. THR-123’s property of being orally active promises potential utility in the clinical situation. Although the authors reported that THR-123 induced no osteogenic activity, Alk3 is widely expressed in various tissues, and a recent report demonstrated that BMP/Alk3 signaling might reduce serum iron and augment anemia of inflammation [45]. Further study is required to analyze the possible side effects of THR-123.

Another therapeutic approach might be to enhance the bioactivity of endogenous BMP7 [46]. Endogenous BMP7 is highly expressed in distal tubules, collecting ducts and podocytes [47]. Endogenous BMP signaling is also present in podocytes and collecting ducts in healthy kidneys, and additionally in proximal tubules during kidney injury [48], supporting the possibility that endogenous BMP7 also contributes to the maintenance of the kidney.

The activity of endogenous BMP is precisely regulated by BMP antagonists. BMP antagonists function through direct association with BMP, thus prohibiting BMP from binding their receptors (Figure 1). The interplay between BMP and its antagonists fine tunes the level of available BMP and governs developmental and cellular processes. The presence of endogenous inhibitors in the kidney might explain the need for a large amount of BMP7 for its renoprotection, in addition to the low availability of exogenously administered BMP7 in the kidney.

Increasing evidence indicates the important roles of BMP antagonists in developing and diseased kidneys [31, 32]. Among the many BMP antagonists, the uterine sensitization-associated gene-1 (USAG-1) and gremlin are the most intensively studied (Table 1). USAG-1 (also known as Sostdc1, wise and ectodin) is a 28-kDa secretory protein, which acts as a BMP antagonist [49]. In adult tissues, the expression of USAG-1 is by far the most abundant in the kidney, and at the same time, USAG-1 is the most abundant BMP antagonist in adult kidneys. The localization of USAG-1 in the kidney is confined to distal tubules and overlaps with that of BMP-7 in distal convoluted tubules [47].

USAG-1 null mice exhibited prolonged survival, preserved renal function, reduced renal fibrosis in animal models of acute and chronic kidney injuries [50, 51]. Renal BMP signaling was significantly enhanced in USAG-1 null mice during renal injury, whereas the administration of neutralizing antibody against BMP7 abolished renoprotection in USAG-1 null mice, indicating that USAG-1 plays a critical role in the modulation of renoprotective action of BMP7 [50]. In addition to its function as a BMP antagonist, USAG-1 also modulates Wnt signaling [52], another molecule which promotes kidney fibrosis.

Another BMP antagonist, Gremlin, is a 28-kDa secretory protein, whose expression is not detected in adult healthy kidney but is found increased in kidney-disease models. While gremlin-null mice are neonatally lethal because of the lack of kidneys and septation defects
in the lung, allelic deletion of gremlin attenuates the progression of diabetic nephropathy [53]. Furthermore, in vivo delivery of gremlin siRNA plasmid reversed the early features of diabetic nephropathy [54], indicating the possible therapeutic applications targeting gremlin.

**Remaining questions for future research**

The above-mentioned studies indicate that impressive advancements have been taking place in the development of therapeutic strategies targeting fibrosis in advanced CKD in the last few decades, and orally active BMP7 mimetics, THR123 [9] is one of the most promising strategies.

Several questions remain to be clarified, however, before these therapeutic strategies can be applied in clinical practice. One of the most important questions is how far fibrosis and renal dysfunction are reversible in human diseases. If there is a ‘point of no return’, or a window of opportunity for the therapeutic approaches, we need methods to determine whether an intervention may be too late to improve patient outcomes. It is therefore important to define the ‘point of no return’ in the course of kidney disease, and search for the biomarkers indicating whether the given patient has reached this point.

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### References


### Table 1. BMP modulating factors and their functions

<table>
<thead>
<tr>
<th>Common name</th>
<th>Gene name</th>
<th>Function</th>
<th>Renal expression</th>
<th>Phenotype of deficient mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAN</td>
<td>Nbl1</td>
<td>BMP antagonist</td>
<td>unknown</td>
<td>No apparent developmental phenotype [55], reduced inflammatory pain [56], Enlarged kidney and altered ureteric tree pattern [57]</td>
</tr>
<tr>
<td>Cerberus</td>
<td>Cer1</td>
<td>BMP antagonist</td>
<td>Expressed in developing kidney [57]</td>
<td>Defects of the L/R axis [58], Unknown</td>
</tr>
<tr>
<td>Coco</td>
<td>Dand5</td>
<td>BMP antagonist</td>
<td>Unknown</td>
<td>Post-natally lethal, and display kidney agenesis, lung defect, and limb deformities [61]</td>
</tr>
<tr>
<td>PRDC</td>
<td>Grem2</td>
<td>BMP antagonist</td>
<td>Absent in adult kidney, but emerges in interstitium and glomeruli of diseased kidney [59, 60]</td>
<td>Allletic deletion attenuates diabetic kidney disease [53]</td>
</tr>
<tr>
<td>Gremlin</td>
<td>Grem1</td>
<td>BMP antagonist</td>
<td>Unknown</td>
<td>Extra teeth and fused teeth [62], Resistant to kidney injuries [50, 51], High bone mass [63]</td>
</tr>
<tr>
<td>USAG-1</td>
<td>Sostde1</td>
<td>BMP antagonist, Wnt modulator</td>
<td>Expressed in distal tubules in adult kidney [47]</td>
<td>Lethal. Multiple abnormalities in ear, pharyngeal and cardiovascular system [64]</td>
</tr>
<tr>
<td>Sclerostin</td>
<td>Sost</td>
<td>BMP antagonist, Wnt modulator</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Chordin</td>
<td>Chrd</td>
<td>BMP antagonist in the presence of Twisted gastrulation</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>Chordin-like1</td>
<td>Chrdl1</td>
<td>BMP antagonist in the presence of Twisted gastrulation</td>
<td>Expressed in proximal tubules in adult kidney [65]</td>
<td>Failure of neural tube closure, limb deformities, loss of caudal vertebrae [67, 68], Display mild vertebral abnormalities, osteoporosis, and atrophin thymus [69, 70]</td>
</tr>
<tr>
<td>Noggin</td>
<td>Nog</td>
<td>BMP antagonist</td>
<td>Absent in adult kidney, but emerges in injured proximal tubules [66]</td>
<td>Post-natally lethal, and display skeletal defects and hypoplastic kidneys [72], Sensitive to kidney fibrosis [73]</td>
</tr>
<tr>
<td>Twisted</td>
<td>Twsg1</td>
<td>BMP antagonist</td>
<td>Expressed in tubules [65]</td>
<td>Failure of neural tube closure, limb deformities, loss of caudal vertebrae [67, 68], Display mild vertebral abnormalities, osteoporosis, and atrophin thymus [69, 70]</td>
</tr>
<tr>
<td>gastrulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crossveinless2</td>
<td>Bmpcr</td>
<td>BMP agonist</td>
<td>Expressed in developing kidney [71]</td>
<td>Sensitive to kidney fibrosis [73]</td>
</tr>
<tr>
<td>KCP</td>
<td>KCP</td>
<td>BMP agonist</td>
<td>Absent in adult kidney, but emerges in injured kidneys [73]</td>
<td></td>
</tr>
</tbody>
</table>
The primary or hereditary forms of distal renal tubular acidosis (dRTA) have received increased attention because of advances in the understanding of the molecular mechanism, whereby mutations in the main proteins involved in acid–base transport result in impaired acid excretion. Dysfunction of intercalated cells in the collecting tubules accounts for all the known genetic causes of dRTA. These cells secrete protons into the tubular lumen through H⁺-ATPases functionally coupled to the basolateral anion exchanger 1 (AE1). The substrate for both transporters is provided by the catalytic activity of the cytosolic carbonic anhydrase II (CA II), an enzyme which is also present in the proximal tubular cells and osteoclasts. Mutations in ATP6V1B1, encoding the B-subtype unit of the apical H⁺ ATPase, and ATP6V0A4, encoding the a-subtype unit, lead to the loss of function of the apical H⁺ ATPase and are usually responsible for patients with autosomal recessive dRTA often associated with early or late sensorineural deafness. Mutations in the gene encoding the cytosolic CA II are associated with the autosomal recessive syndrome of osteopetrosis, mixed distal and proximal RTA and cerebral calcification. Mutations in the AE1, the gene that encodes the Cl⁻/HCO₃⁻ exchanger, usually present as dominant RTA, but a recessive pattern has been recently described. Several studies have shown trafficking defects in the mutant protein rather than the lack of function as the major mechanism underlying the pathogenesis of dRTA from AE1 mutations.