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The Many Talents of TGF- β in the Kidney

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Abstract

Purpose of review: Pre-clinical data suggests that transforming growth factor- β (TGF- β) is arguably the most potent profibrotic growth factor in kidney injury. Despite this, recent clinical trials targeting TGF- β have been disappointing. These negative studies suggest that TGF- β signaling in the injured kidney might be more complicated than originally thought. This review examines recent studies that expand our understanding of how this pleiotropic growth factor affects renal injury.

Recent findings: There are recent studies showing new mechanisms whereby TGF- β can mediate injury (e.g. epigenetic effects, macrophage chemoattractant). However, more significant are the increasing reports on cross-talk between TGF- β signaling and other pathways relevant to renal injury such as Wnt/ β -catenin, YAP/TAZ, and klotho/FGF23. TGF- β clearly alters the response to injury, not just by direct transcriptional changes on target cells, but also through effects on other signaling pathways. In T cells and tubular epithelial cells, some of these TGF- β -mediated changes are potentially beneficial.

Summary: It is unlikely that inhibition of TGF- β per se will be a successful antifibrotic strategy, but a better understanding of TGF- β 's actions may reveal promising downstream targets or modulators of signaling to target therapeutically for chronic kidney disease.

Keywords

renal injury; fibrosis; growth factors

Introduction

Transforming growth factor- β (TGF- β) is a pleiotropic growth factor that plays important roles in organ development, immune responses, cancer biology, and the response to injury. In the kidney, TGF- β is considered a key mediator of fibrosis, the accumulation of extracellular matrix (ECM), in both the glomerulus (glomerulosclerosis) and the

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tubulointerstitial compartment (tubulointerstitial fibrosis) after injury. Tubulointerstitial fibrosis progression is a common pathologic feature of all renal injuries (e.g. hypertension, diabetes, glomerulonephritides) that lead to loss of renal function and eventual end-stage renal disease. Recent studies confirm the importance of tubulointerstitial fibrosis on renal biopsies as a prognostic indicator of renal survival even in glomerular injuries(1, 2). Given the rise in chronic kidney disease globally, TGF- β has received much attention as a potential anti-fibrotic target.

There are three mammalian isoforms of TGF- β ($-\beta 1$, $-\beta 2$, $-\beta 3$) that are part of the TGF- β superfamily which also include bone morphogenic proteins and activins. The TGF- β ligands are secreted as inactive proteins due to noncovalent binding to the latency-associated peptide (LAP), and this complex is tethered to the ECM by the latent TGF- β binding proteins(3). TGF- β is activated through proteolytic cleavage from the LAP by several proteases such as matrix metalloproteinases (MMPs) and plasmin(4). In addition, integrins containing αv can bind to RGD domains of the LAP and facilitate mechanical release and activation(5). Augmented mechanical tension as well as increased expression of integrins and MMPs are induced in the fibrotic kidney, leading to many potential activators of TGF- β in injury. Active TGF- β ligands all bind the TGF- β type II receptor (T β RII) which then heterodimerizes to the TGF- β type I receptor (T β RI)(6). The activated T β RI (also called ALK5) phosphorylates Smad2 and 3 which bind to the common Smad4 and accumulate in the nucleus to alter gene expression(6, 7). In addition to activating Smad-dependent (i.e. canonical) transcriptional responses, the activated receptor complex activates many other signaling proteins such as mitogen-activated protein kinase (MAPK), AKT, and Rho GTPases(8). The diversity of signaling pathways downstream of the activated T β RII/T β RI complex likely accounts for its pleiotropic effects that vary based upon target cell type and microenvironment.

There is abundant evidence that TGF- β promotes renal fibrosis from rodent models in which TGF- β activity has been systemically modulated. Transgenic mice with increased circulating TGF- β developed glomerulosclerosis and those with increased tubular production of TGF- β sustained tubulointerstitial fibrosis in the absence of any additional injury(9, 10). Conversely, systemically inhibiting TGF- β ameliorated tubulointerstitial fibrosis and glomerulosclerosis in the unilateral ureteral obstruction (UUO) and glomerulonephritis models, respectively(11, 12). Genetic inhibition of Smad3, but not Smad2, reduced ECM after injury by UUO, supporting a role for TGF- β /Smad3 signaling in renal fibrosis(13, 14). Most cells express the TGF- β receptor complex, so fully understanding how TGF- β signaling in diverse cell populations alters the response to injury has been challenging.

Initial studies investigating TGF- β and renal fibrosis focused on this growth factor's effects on fibroblasts. TGF- β promotes activation of fibroblasts to myofibroblasts with increased α -smooth muscle actin (α -SMA) expression, synthesis of ECM components, and fibroblast proliferation(15–17). Myofibroblasts, potent producers of ECM, are widely considered the main cell type responsible for fibrosis progression. There is also data that TGF- β sensitizes epithelial cells to apoptosis and tubular degeneration leading to tubular atrophy, a component of tubulointerstitial fibrosis(10, 11). Despite the detrimental effects of TGF- β in murine models of CKD, recent clinical trials did not show a benefit in blocking TGF- β for diabetic

nephropathy or focal segmental glomerulosclerosis(18, 19). There are many potential explanations for these negative studies, but it raises the possibility that TGF- β signaling in the injured kidney may not be completely understood. This review highlights some of the newer data involving TGF- β and the kidney including potentially beneficial effects of this Jekyll-and-Hyde protein (Table 1) and new interactions between TGF- β and other growth factors/signaling pathways (Figure 1). The newer data still point to an important profibrotic role for TGF- β and renal injury but paint a more nuanced and complicated picture of how this multi-faceted growth factor affects the response to injury.

TGF- β and Renal Inflammation

In homeostasis, TGF- β has well-defined anti-inflammatory effects, and mice lacking TGF- β 1 or T β RII die soon after birth with overwhelming inflammation(20, 21). The role of TGF- β on inflammation after renal injury is more complex. Macrophages play key roles in the response to acute kidney injury (AKI) with proinflammatory M1 (classically activated) macrophages infiltrating early in animal models of AKI. Reducing macrophage infiltration at this early stage has been shown to be beneficial(22). At later stages following AKI, a reparative M2 (alternatively activated) macrophage subtype predominates, and macrophage depletion can impair recovery and promote chronic kidney disease (CKD)(23). The role of macrophages in CKD is less clear, but macrophage infiltration on human CKD biopsies predicts worse renal outcomes(24). TGF- β signaling can induce a M2 macrophage polarization which may suppress inflammation but, if persistent, can also promote fibrosis through the production of pro-fibrotic growth factors such as TGF- β 1(25, 26). However, selective deletion of TGF- β 1 from macrophages did not reduce fibrosis after murine models of injury, suggesting that other macrophage-derived growth factors may be mediating fibrosis(27). A recent study deleted T β RII in macrophages and showed reduced tubulointerstitial fibrosis and macrophage infiltration following injury(28). TGF- β -infused Matrigel plugs in vivo acted as a powerful chemoattractant for wildtype but not T β RII^{-/-} macrophages(28). Furthermore, fewer labeled bone-marrow derived T β RII^{-/-} monocytes migrated to the injured kidney compared to WT monocytes after an ischemic AKI/CKD model(28). Interestingly, the conditional deletion of T β RII didn't alter the ratio of M1 and M2 macrophages. These studies indicate that TGF- β promotes fibrosis, in part, through its role as a macrophage chemoattractant, but macrophages likely promote fibrosis through production of cytokines other than TGF- β 1.

T cells are another inflammatory cell type with divergent effects depending upon the microenvironment. TGF- β can promote an anti-inflammatory, Foxp3+, regulatory T cell subset (Treg) which enhances repair after ischemic injury(29, 30). However, in the presence of IL-6, TGF- β induces T cell differentiation to a more inflammatory T17 subtype(31). Several studies describe both pro- and anti-inflammatory effects of TGF- β after renal injury depending upon the type of injury and method of altering TGF- β signaling(32–35). A recent study showed that exogenous TGF- β may reduce inflammation and fibrosis through effects on the Wnt/ β -catenin pathway. The transcription factor β -catenin, the canonical signaling protein of Wnt, usually binds to LEF/TCF transcription factors. The transcription factor FoxO is known to compete with LEF/TCF for binding to β -catenin(36), and TGF- β increased β -catenin/FoxO1 interactions(37). When coupled with an inhibitor of β -

catenin/TCF interactions (ICG-001), exogenous TGF- β 1 reduced kidney fibrosis through the induction of Tregs(37). This effect was abrogated by the depletion of Tregs in both the ischemia/reperfusion (I/R) and UUO models. This study exemplifies how TGF- β can mediate opposing effects on injury response based upon its interactions with other growth factor pathways and signaling proteins.

TGF- β and Epithelial Cells

Increased TGF- β levels impair the epithelial response to injury by sensitizing renal tubules and podocytes to apoptosis and enhancing de-differentiation(11, 38). Epithelial de-differentiation may compromise tubule transport/function and can lead to increased production of profibrotic and pro-inflammatory cytokines(39, 40). More recent studies have expanded upon the cellular effects TGF- β exerts on epithelia. Similar to the protective TGF- β / β -catenin interactions noted above with T cells, our group reported that selective deletion of proximal tubular T β RII worsened tubular apoptosis and fibrosis in murine CKD models, in part, through reduced β -catenin activity(41). Excessive TGF- β signaling is clearly deleterious to injured tubules, but TGF- β mediates many diverse cellular responses and its inhibition may cause loss of some protective effects. Others have also reported potentially beneficial effects of TGF- β through its effects on autophagy(42). Our group found that proximal tubule cells lacking T β RII had increased G2/M arrest, associated with a profibrotic phenotype(43), after aristolochic acid exposure in vitro(41). Interestingly, treatment of wild type cells with TGF- β 1 (10ng/mL but not 1ng/mL) also induced G2/M arrest (unpublished data). TGF- β signaling can affect many different stages of the cell cycle (e.g. p15, p21), and the effect likely varies depending upon the dose and target cell type. One limitation is the ability to measure active TGF- β in vivo to know what doses in vitro correlate with renal injury. These divergent, dose-dependent effects of TGF- β have also been found in studies looking at TGF- β -dependent matrix production and inflammation(44, 45). How epithelial TGF- β signaling alters the cell cycle in renal injury and how these cell cycle changes affect tubular atrophy and tubulointerstitial fibrosis requires further investigation.

In addition to the β -catenin pathway, TGF- β signaling also affects expression of klotho, an essential subunit of the fibroblast growth factor-23 (FGF-23) receptor(46). Klotho is produced in renal tubule cells and is an important suppressor of aging, but its expression is reduced after renal injury and in human CKD(47–49). Recent data suggests that epithelial TGF- β signaling suppresses klotho expression through methylation. One group has shown that TGF- β mediates this response by induction of a methyltransferase (G9a) that targets histone H3 at a locus known to regulate klotho gene expression in cancer cells(50). Another group has shown that TGF- β alters methylation of the klotho promoter indirectly through miRNAs that inhibit methyltransferases(51). These recent studies suggest that TGF- β -dependent suppression of klotho may be another mechanism by which excessive TGF- β is deleterious to injured tubules.

TGF- β and Mesenchymal Cells

As mentioned earlier, TGF- β stimulates fibrosis by inducing fibroblasts to transform into myofibroblasts, increase ECM production, and augment proliferation, migration, and

adhesion. These TGF- β -dependent effects also occur with pericytes, a recently recognized potent source of myofibroblasts after injury(52, 53). More recent data add to our understanding of TGF- β and mesenchymal cells primarily by defining new modulators of TGF- β signaling and new interactions with other important signaling pathways. TGF- β -dependent epigenetic changes in epithelial cells were mentioned above, and many studies have identified a similar mechanism whereby TGF- β 1 alters fibroblast protein expression. TGF- β 1 induced methylation of lysine 4 of histone H3 (H3K4) by upregulation of SET-domain containing lysine methyltransferase 7/9 (SET7/9) in both renal fibroblasts and epithelial cells(54). Treating obstructed mice with siRNA to SET7/9 reduced renal fibrosis. Moreover, an inhibitor of SET7/9, sinefungin, inhibited H3K4 methylation and reduced TGF- β 1-dependent α -SMA expression in both renal epithelial cells and fibroblasts(54). TGF- β 1 upregulated SET7/9 in a Smad3-dependent manner, and TGF- β 1 increased mono-methylated H3K4 levels in the promoters of Col1a1, CTGF, PAI-1. The same group also reported an increase in SET7/9 expression in peritoneal fluid from peritoneal dialysis patients (compared to control human peritoneal mesothelial cells), and sinefungin reduced peritoneal fibrosis in murine models(55). These data suggest that altered methylation is another mechanism by which TGF- β 1 stimulates profibrotic gene expression in mesenchymal cells.

Recent studies have also shed light on TGF- β interactions with the FGF23/klotho pathway in fibroblasts isolated from UUO-injured kidneys (UUOF). FGF23 stimulation of UUOF led to TGF- β -dependent JNK signaling(56). FGF23 signaled through the FGFR4 receptor and induced increased intracellular calcium via the channel TRPC6. This led to augmented reactive oxygen species (ROS) and TGF- β -dependent JNK activation(56). Smad-dependent TGF- β effects downstream of FGF23 have also been shown(57). The effects in both studies were present in UUOF but not normal (i.e. uninjured) fibroblasts, underscoring the limitations with using NRK-49F cells. These studies were performed in vitro and need to be assessed in the injured kidney, but they expand the known interactions between TGF- β and FGF/klotho pathways.

The Hippo pathway transcriptional co-activators YAP and TAZ also have important cross-talk with TGF- β signaling. Nuclear YAP and TAZ interact with Smads2/3 and promote Smad activity through nuclear retention, but cytosolic YAP and TAZ inhibit Smad activation. The localization of YAP/TAZ is altered by substrate stiffness and cell density, leading to increased TGF- β signaling with reduced cell density or increased matrix stiffness(58). Conversely, it was recently shown that TGF- β signaling can increase TAZ expression in both epithelial and mesenchymal cells from the kidney by two independent groups(59, 60). TGF- β increased TAZ transcription through p38-dependent signaling but did not alter TAZ nuclear localization(59). By increasing the total amount of TAZ (but not YAP), TGF- β signaling could augment TAZ signaling in the presence of an activator. In the context of renal injury, it is likely that TGF- β and YAP/TAZ act in a bi-directional, synergistic manner to promote fibrosis.

TGF- β and Vasculature

The renal microvasculature, including peritubular capillary endothelial cells, pericytes, and surrounding lymphatics, is emerging as an important component of the renal response to injury. The importance of TGF- β signaling on pericytes is well-documented and discussed under Mesenchymal Cells. A decrease in capillary density was initially noted one month after rats underwent the I/R ischemic injury, and these vascular defects persisted for 10 months after the acute injury concurrent with tubulointerstitial fibrosis(61). These results imply that the vascular defects from severe AKI play a pathophysiologic role in the subsequent development of fibrosis and CKD. The role of TGF- β signaling in the injured vasculature is complicated as TGF- β can promote expression of both the pro-angiogenic factor VEGF-A and the anti-angiogenic factor thrombospondin-1(62). More recent data suggest that excessive TGF- β signaling is deleterious to the injured endothelium. Heterozygous deletion of T β RII in the endothelium (T β RII^{flox/+};Tie2-Cre) was protective against fibrosis in both the folic acid nephropathy and UUO models(63). In the endothelial cells specifically, TGF- β can also activate the ALK1 receptor (rather than ALK5/ T β RI) which leads to Smads1/5 activation and pro-angiogenic signaling(63). Heterozygous knockout of T β RII in endothelial cells resulted in more proangiogenic Smad1/5 and less fibrotic Smad2/3 activation(63).

The lymphatic vessels, lined by lymphatic endothelial cells (LECs), undergo new growth, or lymphangiogenesis, during injury, but its role in renal injury and fibrosis remains unclear. The lymphatics drain interstitial fluid and return it to the intravascular space and are also involved in the trafficking of inflammatory cells(64). Lymphangiogenesis occurs in murine models of renal and peritoneal fibrosis, and the increase in lymphatic vessels in human diabetic nephropathy biopsies correlated with both the extent of inflammation and fibrosis(65). There are conflicting reports about whether TGF- β signaling stimulates or suppresses lymphangiogenesis, but most agree that the growth factor VEGF-C promotes growth of new lymphatic vessels(66). There are many potential explanations for the diverse effects of TGF- β on lymphatics, but most of the inhibitory studies examined the direct role of TGF- β on LECs in vitro(67, 68). By contrast, others found that TGF- β stimulated tubule epithelial cells and macrophages to produce VEGF-C, leading indirectly to lymphangiogenesis(69). More recently, a group found that connective tissue growth factor (CTGF) may be a downstream mediator of TGF- β -dependent lymphangiogenesis in renal injury(70). Lymphangiogenesis is likely an important modulator of the inflammatory response, and more studies are necessary to determine how TGF- β -dependent changes in lymph vessel growth and function impact the progression of renal fibrosis.

Conclusion

TGF- β affects many diverse cells' responses to renal injury (Table 1). Although excessive TGF- β signaling is clearly profibrotic, this growth factor mediates some protective effects on inflammation and epithelial cell function depending upon the microenvironment. Recent data also point to the importance of cross-talk between TGF- β and other signaling pathways in the development of renal fibrosis.

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** This study shows that TGF-b, in addition to an inhibitor of b-catenin/TCF interactions, can be protective in renal injury. The proposed mechanism is re-directed b-catenin signaling away from TCF and through the FoxO1 transcription factor. The combination of exogenous TGF-b plus enhanced b-catenin/FoxO1 signaling led to increased anti-inflammatory Treg differentiation.
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was abrogated with CTGF knockdown. A direct interaction between CTGF and VEGF-C was shown with surface plasmon resonance analysis.

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Key Points:

- TGF- β is a pleiotropic growth factor that mediates different cellular responses depending upon the target cell type and microenvironment.
- Many of TGF- β 's effects are likely due to interactions with other growth factor signaling pathways (Figure 1).
- Some TGF- β signaling in T cells and tubular epithelia may exert protective effects on injury through β -catenin signaling.

Figure 1. TGF- β interactions with other signaling pathways in renal injury.

Many effects of TGF- β on renal injury depend upon interactions with other growth factor pathways. This signaling cross-talk as well as the target cell types in which these interactions have been described are shown. TGF- β had protective effects on T cells and tubule epithelial cells through modulation of β -catenin signaling(37, 41). TGF- β increases expression of the Hippo transcription factor TAZ(59, 60). The protective klotho, a receptor for FGF23, is suppressed by TGF- β -dependent methylation in tubule epithelial cells(50, 51), and some profibrotic effects of FGF23 in fibroblasts are mediated by downstream TGF- β signaling(56, 57). TGF- β stimulated tubular epithelial cells and macrophages to produce VEGF-C, a potent inducer of lymphangiogenesis, and some of these effects may be mediated by CTGF(69, 70).

TGF- β , transforming growth factor- β ; FGF23, fibroblast growth factor 23; VEGF-C, vascular endothelial growth factor-C; CTGF, connective tissue growth factor.

Table 1.

TGF- β -dependent effects in renal injury

TGF- β -related Effect	Injury/Model	Effect on Renal Injury	Ref.
TGF- β acts as chemoattractant for macrophages	Deleted T β RII in macrophages (CD11b-Cre and LysM-Cre) I/R model of AKI and AKI/CKD and HB-EGF transgenic models	Reduced inflammation and fibrosis	28
TGF- β promotes regulatory T cells (Treg) differentiation	Exogenous TGF- β plus inhibitor of β -catenin/TCF I/R and UUO models	Reduced fibrosis	37
Genetic inhibition of TGF- β signaling in tubules increased apoptosis and G2 arrest after injury	T β RII deleted in proximal tubules (γ GT-Cre) Aristolochic acid injury and Uninephrectomy with angiotensin II	Fibrosis increased and renal function impaired	43
TGF- β inhibits klotho through methylation	Pharmacologic and genetic inhibition of TGF- β -dependent methyltransferase UUO model	Reduced fibrosis	50, 51
TGF- β promotes profibrotic gene transcription through SET7/9-dependent methylation of H3K4	siRNA to SET7/9 or sinefungin (SET7/9 inhibitor) UUO model and peritoneal fibrosis murine models	Reduced fibrosis and α -SMA expression	54,55
FGF23 stimulated TGF- β and JNK	Fibroblasts isolated from UUO kidneys	Increased myofibroblast differentiation and ECM production	56
TGF- β stimulates TAZ expression	C3H/10T1/2 cells (mouse mesenchymal cells) and LLC-PK1 (porcine tubule epithelial) or HK-2 cells*	*Epithelial de-differentiation, vimentin expression, cell cycle arrest	59, 60*
TGF- β signaling suppresses angiogenesis	Heterozygous T β RII deletion in endothelial cells (T β RM ^{fllox/+} /Tie2-Cre) Folic acid nephropathy and UUO	Protected against fibrosis	63
TGF- β stimulates epithelial cells and macrophages to produce VEGF-C	Induction of VEGF-C suppressed by T β RI/ALK5 inhibitor	Reduced lymphangiogenesis	69
CTGF as mediator of TGF- β -dependent lymphangiogenesis	CTGF knockout mice UUO model	Reduced VEGF-C and lymphangiogenesis	70

This table lists recently described TGF- β -dependent effects, the model in which these effects were observed, their impact on renal injury, and the corresponding reference(s).