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**TGF-β: the connecting link between nephropathy and fibrosis**

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**Abstract**

Renal fibrosis is the usual outcome of an excessive accumulation of extracellular matrix (ECM) that frequently occurs in membranous and diabetic nephropathy. The result of renal fibrosis would be end-stage renal failure, which requires costly dialysis or kidney transplantation. Renal fibrosis typically results from chronic inflammation via production of several molecules, such as growth factors, angiogenic factors, fibrogenic cytokines, and proteinase. All of these factors can stimulate excessive accumulation of ECM components through epithelial to mesenchymal transition (EMT), which results in renal fibrosis. Among these, transforming growth factor-beta (TGF-β) is proposed to be the major regulator in inducing EMT. Besides ECM protein synthesis, TGF-β is involved in hypertrophy, proliferation, and apoptosis in renal cells. In particular, TGF-β is likely to be most potent and ubiquitous profibrotic factor acting through several intracellular signaling pathways including protein kinases and transcription factors. Factors that regulate TGF-β expression in renal cell include hyperglycemia, angiotensin II, advance glycation end products, complement activation (C5b-9), and oxidative stress. Over the past several years, the common understanding of the pathogenic factors that lead to renal fibrosis in nephropathy has improved considerably. This review will discuss the recent findings on the mechanisms and role of TGF-β in membranous and diabetic nephropathy.

**Introduction**

Renal fibrosis can be defined as the accumulation of abnormal amounts of collagen, fibronectin, and other related fibrogenic molecules in the kidney and occurs in virtually every type of chronic kidney disease. The results of renal fibrosis would be end-stage renal failure, which requires costly dialysis or kidney transplantation. In normal physiological processes deposition of extracellular matrix (ECM) is required for normal wound healing, but the excessive deposition of fibrous material is the pathological feature of renal fibrosis. A tight balance between synthesis and breakdown of matrix proteins is therefore required to maintain normal function. Various resident and nonresident renal cells control this balance via the release of mediators which act to regulate collagen and matrix metalloproteinase (MMP) production. Loss of this regulation can ultimately lead to deposition of fibrotic material and scar formation. Recruitment of inflammatory cells, induction of reactive oxygen species (ROS), complement activation (C5b-9), release of transforming growth factor-beta (TGF-β), and other fibrogenic cytokines, hyperglycemia, and activation of MMPs, vasoactive molecules (e.g. angiotensin II (Ang-II) and endothelin-1), and ECM-cell interaction molecules (e.g. integrins, hyaluronic acid) are the various culprit factors involved in renal fibrosis. All factors are complimentary to each other and synergistically contribute to the development of renal fibrosis. Among these factors, TGF-β has been long considered as a key mediator in renal fibrosis and induces renal fibrosis largely by activating its downstream Smad and non-Smad signaling pathway. Indeed, the upregulations of TGF-β have been linked to diabetic nephropathy, membranous nephropathy and other chronic renal disease. Fundamental mechanism behind TGF-β induced renal fibrosis is epithelial to mesenchymal transition (EMT) that leads to morphological and phenotypic changes of tubular epithelial cells and it may also result in detachment of cells from the tubular basement membrane and migration into the interstitium. During the last 2 decades, the potential role of fibrosis in the progression of nephropathy has also been recognized. In this review, a few significant factors that emerge to be involved in driving this fibrosis process in membranous and diabetic nephropathy are discussed, with special importance on TGF-β. Our knowledge of the role of TGF-β in renal fibrosis is extensive; however, the majority of the research is dedicated to exploring the consequences of TGF-β signaling in renal fibrosis. The aim of the current review is to discuss the role of various factors that upregulate TGF-β and synergistically contribute to the development of renal fibrosis in membranous and diabetic nephropathy.
Epithelial to mesenchymal transition

EMT is the process by which renal epithelial cells undergo a phenotypic conversion that give rise to a matrix producing myofibroblasts in response to various renal injuries (Figure 1). Myofibroblasts are believed to be the key effector cells in renal fibrosis responsible for the synthesis and deposition of ECM components. EMT transition is characterized by loss of cell-to-cell contact, breakdown of the basement membrane, loss of proteins such as E-cadherin, nephrin, podocin, and zonula occludens-1 (ZO-1), cytoskeletal reorganization, and transition to a spindle-shaped morphology concomitant with acquisition of new mesenchymal markers such as α-smooth muscle actin (α-SMA), vimentin, type I collagen, and fibronectin. EMT plays a central role in normal embryonic development and tissue repair, cancer progression, and chronic inflammation. Although it has been established that EMT could also contribute to renal, lung, and liver fibrosis in animal models, it is controversial whether the complete EMT process truly occurs in human diseases. Previous in vitro studies suggested that renal epithelial cells can undergo EMT, characterized by loss of epithelial markers and gaining of mesenchymal markers, under the treatment of various profibrotic cytokines, particularly TGF-β. Previous data have pointed out the significant role of EMT in renal fibrosis by examining the phenotypic conversion of renal tubular cells in animal models as well as in renal biopsy samples from patients with chronic kidney disease. EMT also occurs following fibrotic tissue repair after injury and contributes to organ fibrosis – a process known as fibrogenesis. In renal fibrosis, renal interstitial fibroblasts derive not only from mesenchymal stem cells in the bone marrow, but also from proximal tubular kidney epithelial cells that undergo EMT. Several reports specify that TGF-β is principally associated with this process. Moreover, transgenic mice with increased expression of TGF-β develop renal fibrosis. In addition, the clinical relevance of EMT has also been established in a study characterizing kidney biopsies: a significant relationship was established among epithelial cells containing EMT features, extent of interstitial fibrosis, and renal functional impairment. Moreover, the renal tubular expression of fibroblast markers such as α-SMA, vimentin, and fibroblast-specific protein 1 in fibrotic kidneys is well documented. Most of the in vitro studies clearly showed that renal tubular epithelial cells when exposed to profibrotic cytokines (especially TGF-β1) lose cell polarity, downregulate epithelial markers, acquire mesenchymal features, and express mesenchymal markers. This pattern of transformation was taken as a common evidence for EMT and was proposed to be valid as indirect evidence of EMT in vivo. However, the gaining of EMT markers in vivo is often the center of the argument and possibly characterizes a “partial EMT”, lacking the final transition to a “fully fibroblastic phenotype”. These studies do not add any significant evidence for a role of EMT in renal fibrosis, which clearly requires a “fully fibroblastic phenotype”. Moreover, the origin of myofibroblasts in kidney is of great interest because these cells are responsible for scar formation in fibrotic kidney disease. Previous fate-tracing experiments on kidney epithelial cells failed to identify a single fibroblast originating from the tubular epithelium, but confirm that interstitial pericytes are myofibroblast progenitors in fibrotic kidney disease. At present, the existence of EMT in renal fibrosis has been debated in several lineage-tracing studies, with conflicting findings. Types of differences may have arisen from varying experimental conditions such as different disease models, different strains of mice, and type of genetic alteration used. EMT or partial EMT is regulated by numerous growth factors, cytokines, hormones, and often combination of various conditions. One of the most important signaling pathway includes the TGF-β/Smad and other pathways may include integrin/integrin-linked kinase (ILK), and Wnt/β-catenin signaling. The TGF-β is a potential profibrotic factor in the kidney as it can initiate and maintain EMT. As mentioned above, factors
that play a critical role in regulating EMT individually or in combination, including IL-1β, Ang-II, MMP-2, plasmin, tissue-plasminogen activator, ROS, and advance glycation end products (AGEs). TGF-β is most potent profibrogenic cytokine that regulates cell differentiation, proliferation, ECM production, and apoptosis. Earlier, it was considered that TGF-β induced EMT in normal epithelial cells but in vitro data suggested that number of other different epithelial cells, including renal proximal tubular, alveolar epithelial, cardiac endothelial, and other cells undergo EMT-like changes. The molecular mechanism involving EMT is likely to be complex, a number of signaling networks and mediator have been identified in regulating this process.

Activation of latent TGF-β

TGF-β isoforms are synthesized as pro-proteins that contain pro-region and pro-region. Proteases like furin cleaved TGF-β into C-terminal mature peptides and N-terminal LAP (latency-associated peptide). Together with LAP and TGF-β form small latent complexes that are transported to extracellular matrix where can further covalently bind to latent TGF-β binding protein (LTBP) to form a large latent complexes (LLC). LLC accumulates in ECM as an inactive TGF-β with the support of transglutaminase-induced (TGase) crosslinks. Protease releases the soluble LCC from ECM and is activated by plasmin, MMPs, and thrombospondin (TSP) from LAP and LTBP. Once the active TGF-β is released from the ECM, it is capable of signaling.

Figure 2. Simplified schematic shows TGF-β synthesis and activation. TGF-β isoforms are synthesized as pro-proteins that contain pre-region and pro-region. Proteases like furin cleaved TGF-β into C-terminal mature peptides and N-terminal LAP (latency-associated peptide). Together with LAP and TGF-β form small latent complexes that are transported to extracellular matrix where can further covalently bind to latent TGF-β binding protein (LTBP) to form a large latent complexes (LLC). LLC accumulates in ECM as an inactive TGF-β with the support of transglutaminase-induced (TGase) crosslinks. Protease releases the soluble LCC from ECM and is activated by plasmin, MMPs, and thrombospondin (TSP) from LAP and LTBP. Once the active TGF-β is released from the ECM, it is capable of signaling.
Thrombospondin-1 (TSP-1)\textsuperscript{42,43}, integrins, such as $\alpha V\beta 6$ or $\alpha V\beta 8$\textsuperscript{44}. Activated TGF-$\beta$ induces renal scarring largely by activating its downstream Smad and non-Smad signaling pathway.

**Smad-dependent signaling**

In most cells, three types of cell surface proteins mediate TGF-$\beta$ signaling: TGF-$\beta$ receptor I (T$\beta$RI), II (T$\beta$RII), and III (T$\beta$RIII)\textsuperscript{45}. TGF-$\beta$ exerts its cellular effects by binding to T$\beta$RI and T$\beta$RII serine/threonine kinase receptors\textsuperscript{46}, leading to binding of anchoring proteins SARA\textsuperscript{47} to Smad2/3 and facilitate their phosphorylation (Figure 3). Afterwards, phosphorylated complex of Smad2/3 forms a higher-order complex with Smad4 and accumulate in the nucleus. In nucleus, this active Smad2/3/4 complex regulates the transcription of $\alpha$-SMA, collagen I, PAI-1, and MMP-2\textsuperscript{48,49}. Experimental study revealed that Smad complex has a nuclear localization-like sequence for proper translocation to the nucleus and this sequence is recognized by importins\textsuperscript{50}. Moreover, this complex is retained in the nucleus where they affect transcription of target genes via direct DNA binding or by association with numerous DNA binding protein families which participate as Smad cofactors, such as AP1, CREB/ATF, HOX, RUNX, FOX, E2F, and zinc finger proteins\textsuperscript{51}. These co-factors vary in various cell types, in that way determining the cell type-dependent responses\textsuperscript{50}. In addition to Smad co-factors that positively regulate or enhance transcriptional outputs, Smad binding proteins were identified that attenuate TGF-$\beta$ signaling by interfering with Smad functions. These negatively acting Smads are required to prevent the inappropriate activation of TGF-$\beta$ signaling, or to turn off the pathway following normal activation. There are some inhibitory Smad binding proteins were identified such as Smad6 and Smad7 and these proteins provide another venue for regulatory inputs controlling the activity of Smads. Smad6/7 inhibits phosphotransferase reaction between SARA and Smad2/3 by blocking their interaction with T$\beta$RI and cover

Figure 3. TGF Smad signaling. At the cell membrane, the TGF-$\beta$ binds to the type II TGF-$\beta$ receptor kinase and recruits the type I receptor and induces transphosphorylation of the type I receptor. The activated type I TGF-$\beta$ receptor then phosphorylates receptor-associated Smad2 and Smad3. The adaptor protein Smad anchor for receptor activation (SARA) facilitates the phosphorylation of Smad2/3 near the cell surface. Phosphorylated Smad2/3 associate with Smad4 and translocate into the nucleus, where they activate transcription of TGF-$\beta$ target genes. This cascade then leads to mRNA and protein synthesis of various factors that support EMT. The inhibitory Smad binding proteins such as Smad6/7 inhibits TGF-$\beta$ signaling by preventing T$\beta$RII’s activation of Smad2/3. Smad ubiquitination regulatory factor-1 (Smurf1) and Smurf2 are an ubiquitin ligase that specifically targets Smad2/3 for degradation. Smurf 1/2 can also interact with inhibitory Smad6/7 and thereby targets the TGF beta receptor for degradation.
its access to Smad2/352,53. Further, it was reported that Smad-dependent transcriptional activity can also be inhibited via direct binding to either transcriptional co-repressors (SnoN, Ski, and TGFf) of TGF-β target genes or to intermediary proteins that recruit such repressors. For example, interaction between Smad complex and co-repressors prevent gene transcription through inhibition of Smads54–56. Furthermore, Smad7 binds to Smad ubiquitination regulatory factor-2 (Smurf2) to form an E3 ubiquitin ligase that targets the TGF-β receptor for degradation via proteasomal and lysosomal pathways and facilitates the inhibitory effect of Smad757. EMT also inhibited by hepatocyte growth factor (HGF), via upregulation of the Smad transcriptional corepressor SnoN that leads to formation of a transcriptionally inactive SnoN/Smad complex, thereby blocking the effects of TGF-β58. The majority of TGF-β targeted genes regulated in EMT rely on Smad3-dependent transcriptional regulation59. A requirement for Smad signaling in mediating EMT is clearly illustrated in vivo in Smad3 knockout mice after unilateral urethral obstruction. These Smad3 null mice are resistant to induction of EMT and show reduced extracellular accumulation after obstructive injury. Moreover, the cultures of renal tubular epithelial cells from Smad3 null animals show a block in EMT and a reduction in auto induction of TGF-β60. Similarly, upregulation of Smad3 together with constitutively active TpR1 synergistically enhanced the EMT response of non-transformed mammary epithelial cells51. Earlier studies in renal proximal tubule cells (PTCs) demonstrated Ang-II-induced tubular EMT was Smad3-dependent60. Together, these data suggest the central significance of Smad signaling in mediating EMT both in vitro and vivo. Furthermore, Smad2 and Smad3 have differential roles in EMT different cell types. For example, mice with double knockout of Smad2 and Smad3, it is observed that Smad3 but not Smad2 was required for a number of TGF-β-induced functions including induction of EMT61. However, in human proximal tubular epithelial cells, increased connective tissue growth factor (CTGF) and decreased E-cadherin were Smad3-dependent, whereas Smad2 negatively regulates the post-transcriptional modification of miR-2164. Recently, the emerging roles of long noncoding RNAs (IncRNAs) in Smad3-dependent renal inflammation and fibrosis have also been recognized65.

Smad-independent signaling

Role of TGF-β signaling in EMT is determined not only by Smad signaling pathway, but also by the ability of TGF-β to activate non-Smad signaling pathways (Figure 4). Non-Smad pathways of TGF-β can indirectly participate in EMT, apoptosis, and differentiation and matrix formation66. Non-Smad pathways of TGF-β stimulates parallel downstream signaling pathways involve (i) mitogen-activated protein kinases (MAPK) pathway such as extracellular signal-regulated kinase (ERK)1/2, Jun-N terminal kinase (JNK), and p3867,68, (ii) growth and survival kinases phosphatidylinositol-3-kinase (PI3K), Akt, and the small GTP-binding proteins (Ras, RhoA, Rac1, and Cdc42)69–71, (iii) Notch72, and (iv) Wnt/β-catenin pathway73,74. In most cases, activation of these cooperative pathways are best known for their roles in TGF-β-induced EMT.

TGF-β-induced MAPK pathway activation

The MAPK family operates three parallel signaling pathways: ERK, JNK, and p38 MAPK and expressed in whole kidney and detectable in various renal cell culture models75. These three parallel signaling pathways are activated by different receptors and are involved in numerous events related to renal fibrosis. In particular, the previous evidence has suggested that TGF-β activates both ERK and JNK pathways in mesangial cells76. The ERK activation is generally stimulated by various growth factors and activation of its cascade stimulates mitosis, cell differentiation, and in some cells, hypertrophy and growth arrest77. Thus, ERK activation is important for EMT, which is one of the major biological functions of TGF-β. Preincubation with an ERK inhibitor reduces TGF-β-stimulated Smad phosphorylation and collagen production, suggesting that ERK activity is essential for TGF-β signaling78. Similarly, JNK pathway also plays a pathogenic role in renal fibrosis and tubular apoptosis. Previous study demonstrated that JNK inhibitor significantly suppressed renal fibrosis and tubular apoptosis, suggesting a pathogenic role for JNK signaling in renal fibrosis and tubular apoptosis79. Signaling by JNK has been demonstrated to be involved in fibronectin synthesis induced by TGF-β80. Furthermore, TGF-β activates p38 MAPK pathway via MKK3 protein kinase, and stimulated mesangial cells from MKK3-deficient mice had a reduction in pro-alpha I collagen expression compared with wild-type mice81. In addition, TGF-β induced synthesis of ECM proteins like fibronectin via a p38-dependent mechanism82. Another pathway of p38 MAPK induced EMT involves direct phosphorylation of glycogen synthase kinase-3β (GSK-3β) leading to inhibition of its activity resulting in accumulation of β-catenin in nucleus83. However, the previous study suggested p38 MAPK activation is dependent on TGF-β through functional β1-integrin, and p38 MAPK activity is crucial but is not enough to induce EMT84. Taken together, these results suggest that activation of MAPK signaling contributes to and may even be required for TGF-β-induced EMT.

PI3K/Akt pathway in TGF-β-mediated EMT

Similar to various other fibrotic factors that act through tyrosine kinase receptors, TGF-β has been shown to rapidly activate PI3K/Akt pathway71,85. Consistent with this observation, the regulatory subunit of PI3K was found to interact with the TpR1 and TpR1I receptors, and the PI3K activity increases upon TGF-β stimulation86. Activation of the PI3K/
Akt pathway by TGF-β plays a major role in tubular EMT\(^{87}\). Inhibitors of PI3K and Akt were found to inhibit TGF-β-induced loss of ZO-1 and E-cadherin and a gain in vimentin and α-SMA expression\(^{88}\). Further, PI3K induced EMT in epithelial cells is a Snail-dependent fashion, means Snail activation is required for TGF-β-induced EMT\(^{89}\). Moreover, activation of Snail in adult transgenic mice induced renal fibrosis and Snail was found to be overexpressed in human fibrotic kidney\(^{90}\).

**Rho-like GTPases in TGF-β-mediated EMT**

Rho-like GTPases, comprised of the Rho, Rac, and Cdc42 subfamilies of proteins, are key regulators of variety of crucial cellular activities including morphology, motility, proliferation, and gene regulation that accompany EMT. TGF-β has been found to induce activation of Rho, Rac, and Cdc42 in different cell systems; however, most studies have focused on the role of RhoA and its effector kinase ROCK in TGF-β-induced EMT\(^{91}\). TGF-β rapidly activates RhoA in epithelial cells, and that blocking RhoA or its downstream target p160 (ROCK), by the expression of dominant-negative mutants, inhibited TGF-β mediated EMT. These data suggest that TGF-β rapidly activates RhoA-dependent signaling pathways to induce stress fiber formation and mesenchymal characteristics\(^{70}\). The GTPase RhoA appears to play a role particularly in the regulation of cytoskeletal and adherent junction rearrangement\(^{84}\). In addition to its role in cytoskeletal rearrangement, RhoA has been shown to play role in TGF-β-mediated morphological change and activation of the α-SMA promoter during EMT\(^{92}\). Previous studies identified a new pathway whereby TGF-β controls EMT through recruitment of the TGF-β receptor to tight junctions where it interacts with the polarity protein Par6 (partitioning-defective 6)\(^{93}\). TGF-β downregulates RhoA protein levels by phosphorylation of Par6 that triggers binding of the E3 ligase SmurF1 to the complex followed by ubiquitination and degradation of RhoA. Subsequently, this leads to the dissolution of tight junctions, rearrangement of actin cytoskeleton and EMT\(^{93}\).

**Notch signaling pathway in TGF-β-mediated EMT**

The Notch signaling pathway consists of several receptors, their ligands, negative and positive regulators, and
transcription factors. The TGF-β signaling can induce expression of Notch ligands, such as Jagged-1 (JAG1), which activate Notch signaling, leading to EMT and epithelial cell cycle arrest in cell models in vitro. Previous study demonstrated that functional inactivation of γ-secretase, an activator of Notch receptor, completely prevents EMT induced by TGF-β, indicating a role for Notch signaling components downstream of TGF-β. Furthermore, the metalloprotease and γ-secretase proteolytic cleaved the Notch receptor that leads to release of the Notch intracellular domain from the plasma membrane and its subsequent translocation into the nucleus. Translocation of Notch intracellular domain mediates transcription of target genes, including basic helix-loop-helix transcription factors of the hairy and enhancer of split (Hes) family and the Hes-related repressor protein (HEY) families. Follow-up studies demonstrate expression of the hairy/enhancer-of-split-related transcriptional repressor HEY1, and JAG1, was induced by TGF-β at the onset of EMT in epithelial cells from mammary gland, kidney tubules, and epidermis. TGF-β-induced EMT was blocked by RNA silencing of HEY1 or JAG1, and by chemical inactivation of Notch.

Wnt/β-catenin pathway in TGF-β-mediated EMT

The Wnt/β-catenin pathway is another major signaling pathway involved in EMT. Wnts compose a family of signaling proteins that play an essential role in kidney development, but their expression in adult kidney is thought to be silenced. Although relatively silent in normal adult kidney, Wnt/β-catenin signaling is re-activated after renal injury in a wide variety of animal models and in human kidney disorders. The protein β-catenin is the central regulator of Wnt/β-catenin signaling in EMT. Previous data suggested that cell contact integrity and β-catenin signaling regulate α-SMA expression during TGF-β induced EMT. The β-catenin pathway may offer new therapeutic targets to lessen progressive organ fibrosis.

Integrin-linked kinase in TGF-β-mediated EMT

Previous studies indicated that ILK is a key intracellular mediator of tubular EMT induced by TGF-β. This result is based on several observations that ILK expression is upregulated in a wide variety of chronic kidney diseases in both experimental and clinical levels. ILK is an intracellular serine/threonine protein kinase that interacts with the cytoplasmic domains of the β-integrins and may play an important role in EMT. Furthermore, there are two different mechanisms by which ILK induced EMT: (i) it acts as a protein kinase and (ii) as a scaffolding protein. As a protein kinase, ILK abolished TGF-β1-induced phosphorylation of Akt and GSK-3β and largely restored the expression of E-cadherin and ZO-1. Inhibition of ILK also blocked TGF-β1-mediated induction of fibronectin, Snail1, plasminogen activator inhibitor 1, and MMP2. As a scaffolding protein, ILK directly interacts with integrins and many other intracellular proteins, such as nephrin, α-parvin, and PINCH. Interaction with nephrin in normal glomerular podocytes provides molecular bridge that connects the cell-matrix integrin signaling with the cell-cell slit diaphragm signaling. Furthermore, ectopic expression of ILK suppresses E-cadherin, induces production of the ECM protein fibronectin, and enhances cell migration. ILK regulates E-cadherin at the transcriptional level, and repression is likely mediated via the transcriptional repressor Snai1 in some cell type. As discuss earlier, mechanism by which ILK induce EMT is mediated mainly by its protein kinase activity, because a kinase-dead mutant and small molecule inhibitor of ILK blocks TGF-β-mediated EMT.

TGF-β and diabetic nephropathy

Diabetic nephropathy remains the most common cause of end stage renal failure as the incidence of diabetes rises rapidly worldwide. Nearly one-third of the patients with diabetes develop nephropathy. It is characterized by expansion of ECM, which eventually leads to renal fibrosis. Earlier data suggested the role of TGF-β as the key fibrotic factor has been well characterized using in vitro and in vivo experimental models as well as in patients with diabetic nephropathy. A significant increase in tubular and glomerular TGF-β expression in early and late stages of the disease in both type 1 and type 2 patients were observed. It has been revealed that the expression is strongly associated with the degree of glycaemic control in these patients. Accumulation of ECM components such as collagen has been demonstrated in diabetic glomeruli and mesangial cell incubated in high glucose. Previous studies suggest that high glucose not only activate ROS but also release Ang-II in mesangial cells linked to increased glomerular fibrosis. Ang-II has been implicated in the development of renal fibrosis but the defined mechanism of this effect remains unclear. Ang-II plays a significant role in activation of TGF-β via generation of ROS through the activation of NADPH oxidases in glomerular endothelia cell (GEC). Ang-II induced oxidative stress may activate latent TGF-β and subsequently, the TGF-β signaling system in GEC. Previous study reported that Ang-II and TGF-β are potent in vitro and in vivo agonists in increasing PAI-1 synthesis and may thus contribute to renal fibrosis by inhibiting degradation of ECM. Evidence suggests that in the classical renin-angiotensin system, renin not only plays a role in conversion of Ang-I to Ang-II but also unregulates TGF-β through receptor-mediated mechanism independent of Ang-II generation. Renin also increases level of TGF-β and stimulates an increase in PAI-1 and collagen. Experimental studies also imply that hyperglycaemia accelerates the process of AGEs formation, which has been suggested to be implicated in pathogenesis of diabetic nephropathy. AGEs are involved in the induction of glomerular ECM accumulation by upregulating the expression of type IV collagen and TGF-β in diabetic mice. Moreover, AGEs induce oxidative stress by generating excessive amount of ROS through activation of NADPH oxidase. High glucose level increased expression of αv β3 integrins and upregulates matrix-related function in cultured human glomerular epithelial cells and could contribute to matrix accumulation. Integrins, such as αvβ6 or αvβ8 can activate the TGF-β complex leading to activation of TGF-β either by tracional force (αvβ6) or proteolytic activity (αvβ8) by membrane type-1 MMP (MT1-MMP). The miRs are small noncoding
endogenous RNA strands that regulate gene expression in diabetic nephropathy. Recently, in vitro and in vivo diabetic nephropathy model have revealed that TGF-β1 also positively or negatively regulates the expression of several miRs that, in turn, amplify TGF-β1-signaling to further promote renal fibrosis. The expression of miR-192 was downregulated by TGF-β1 in vitro and its loss in vivo correlates with fibrosis in human diabetic nephropathy perhaps by enhancing TGF-β-mediated downregulation of E-cadherin that leads to EMT. The specific reduction of renal miR-192 decreases renal fibrosis and improves proteinuria, lending support for the possibility of an anti-miRNA-based translational approach to the treatment of diabetic nephropathy. Similarly, miR-377 was also associated to renal fibrosis in diabetic nephropathy. Its expression was upregulated in diabetic nephropathy, which led to increased fibronectin protein production. The expression of miR-29 was downregulated by high glucose or TGF-β1 in PTCs which led to increased collagen deposition in PTCs.

**TGF-β and membranous nephropathy**

Membranous nephropathy is associated with progressive injury of the kidney characterized by interstitial inflammation, fibrosis, and glomerular hypertrophy. The disease is the most frequent cause of nephrotic syndrome in adults worldwide. Complement membrane attack complex (C5b-9) plays an important role in the development of GEC injury and proteinuria in passive Heymann nephritis, an experimental model of human membranous nephropathy. TGF-β, α1(IV) collagen, α4(IV) collagen, S-laminin, and fibronectin were upregulated in patients with membranous nephropathy. The precise mechanisms of GEC injury and renal fibrosis have not been fully established. Previous studies have demonstrated that complement induces production of ROS via NADPH oxidase, MMP 9 via activation of transcription factor nuclear factor k B (NF-kB), and MMP 2 via arachidonic acid pathway result in renal fibrosis. ROS directly change the conformation of LAP through oxidation or indirectly activates proteolytic enzymes causing release of TGF-β. MPPs have also been concern in the cleavage of LAP and the release of mature TGF-β at the cell surface. Sublytic concentration of C5b-9 activates TSP-1 and releases active TGF-β in rat glomerular mesangial cells (GMCs). It is a 450-kDa trimeric ECM glycoprotein found GMCs. TSP-1 is a major physiological regulator of TGF-β1 activation suggesting that it may exert an important role in TGF-β driving the accumulation of ECM. Previous clinical report suggested that TSP-1 and TGF-β expression was increased in patients with focal segmental glomerulosclerosis and diabetic nephropathy. In various animal models, TSP-1 is co-localized with TGF-β that leads to renal fibrosis.

**Conclusions**

TGF-β plays important roles in the developments of renal fibrosis and contribute to fibrotic scar formation as seen in membranous and diabetic nephropathy. Our knowledge of the role of TGF-β in the renal fibrosis is extensive; however, the majority of this research is dedicated to exploring the consequences of TGF-β signaling in renal fibrosis. The studies reviewed herein show that the role for various extracellular factors and intracellular mediators that upregulates TGF-β and synergistically contribute to renal fibrosis via EMT. The molecular mechanism involving EMT is likely to be complex, a number of signaling networks and mediator have been identified in regulating this process. There is a rising list of the factors that control EMT has been identified and could be exploited in developing future antifibrotic therapeutics. Together, this review provides new mechanistic insights into the TGF-β upregulation by various factors in membranous and diabetic nephropathy. Better understanding of activation of TGF-β signaling and its role in EMT may provide novel tools for the prevention of renal fibrosis.

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**Declaration of interest**

The authors declare no conflict of interest.

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