

## EMT and TGF-beta in renal fibrosis

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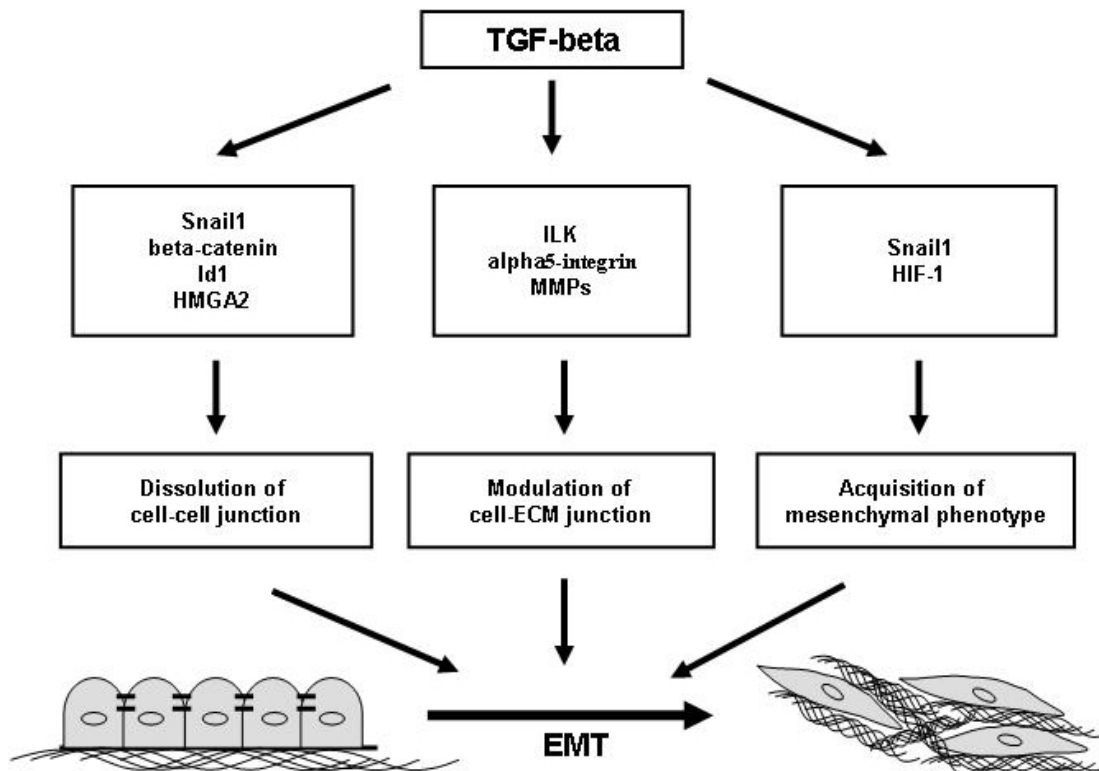
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## 1. ABSTRACT

Transforming growth factor-beta1 (TGF-beta1) is a member of TGF-beta superfamily and the principal mediator contributing to the development and progression of renal fibrosis in a variety of disease settings. A critical effect of TGF-beta1 is the induction of epithelial-mesenchymal transition (EMT), which likely explains the continuous replenishment of fibroblasts during the progression of tissue fibrosis. Since we first identified EMT as the origin of fibroblasts in renal fibrosis, the signaling underlying EMT has been intensively studied in the field of nephrology. During the past five years, detailed mechanisms by which TGF-beta1 induces EMT have been clarified, and novel therapeutic approaches targeting TGF-beta1-mediated EMT are now being proposed.

## 2. INTRODUCTION

Epithelial-mesenchymal transition (EMT) is an important physiological event that occurs frequently during embryonic development (1). However, numerous reports have shown that EMT can also occur during pathological states, such as tumor metastasis and tissue fibrosis (2-6). It is well established that tissue fibroblasts are the principal effector cells in the accumulation of extracellular matrix (ECM), but the origin of those fibroblasts was not known until recently (7). Since our report that some fraction of the tissue fibroblasts involved in renal fibrosis originates from EMT, many studies have confirmed that EMT plays a critical role in the development and progression of renal fibrosis (4).



**Figure 1.** TGF-beta1 as a master regulator of EMT

EMT is a multi-step process involving the dissolution of tight junctions and adherens junctions, modulation of cell-ECM junctions, reorganization of the actin cytoskeleton, induction of mesenchymal gene expression, and acquisition of cellular motility. Numerous reports have shown that transforming growth factor-beta1 (TGF-beta1) is involved with these steps, and TGF-beta1 is now thought to be the master regulator governing the induction of EMT (8, 9) (Figure 1). Because epithelial cells are tightly bound to one another through cell-cell and cell-ECM junctions, dissolution of these junctions is necessary before epithelium can undergo EMT. Once epithelium loses its cellular polarity through the loss of these junctions, it acquires mesenchymal phenotypes, moves to interstitial areas, and participates in the accumulation of ECM. The mechanisms underlying this process are quite complex, and much remains to be learned about the molecular events mediating it. Identification of the key molecules (e.g., TGF-beta1) involved in the EMT process is essential for the development of novel therapies aimed at inhibiting the progression of fibrosis. In this review, we discuss the recent advances in our understanding of the biology of TGF-beta1-induced EMT, focusing on its role in renal fibrosis.

### 3. DISSOLUTION OF CELL-CELL ADHESION

E-cadherin is a well-known prototypical adhesion molecule localized in adherens junctions, which are linked to the cortical actin cytoskeletons of cells. Repression of

E-cadherin is a hallmark of EMT and is considered to be an integral step in the EMT process. A variety of transcriptional factors, including Snail1, Slug (Snail2), SIP-1, ZEB-1, E12/E47 and Twist, are known to bind directly to E-boxes in the promoter regions of E-cadherin (8, 10, 11). In particular, studies have emphasized the key role played by the zinc-finger transcription factor Snail1 which TGF-beta1 has been shown to upregulate via both Smad-dependent and -independent signaling pathways (12-14). Madin-Darby canine kidney (MDCK) cells transfected with Snail1 show downregulated E-cadherin expression, increased expression of mesenchymal markers (vimentin and fibronectin), and transformation to a fibroblastoid phenotype (11). In transgenic mice, tamoxifen-induced Snail1 activation leads to development of renal fibrosis with the loss of epithelial features in renal tubules (15). Snail1 was also recently reported to be a critical regulator of fibroblast function *in vitro* and *in vivo*, and to continue to activate cellular motility and proliferation, even after terminal differentiation of mesenchymal cells (16).

Snail1 also binds directly to E-boxes in the promoter regions of two tight junction proteins, claudin and occludin, resulting in complete repression of their promoter activity. Snail1 is thus able to simultaneously repress expression of genes encoding both adherens and tight junctions (17). Claudin, occludin, two partitioning-defective proteins (PAR3 and PAR6) and zonula occludens (ZO)-1 are all membrane proteins localized at tight junctions, which are responsible for establishing and

maintaining epithelial cell polarity. Occludin binds to the TGF-beta type I receptor (TbetaRI) and promotes its recruitment to tight junctions. TGF-beta1-mediated induction of EMT is followed by the additional recruitment of TGF-beta type II receptor (TbetaRII) to the same junction complexes. In this way, occludin regulates TbetaRI localization for efficient TbetaRI-dependent dissolution of tight junctions during EMT (18). At the same time, Id1, an early TGF-beta1-inducible protein, suppresses expression of E-cadherin and ZO-1. Id1 also prevents HEB, a basic helix-loop-helix transcription factor, from binding to the E-box by sequestering it through formation of a heterodimeric HEB/Id1 complex, thereby blocking its transactivation of E-cadherin gene transcription (19).

Another molecule that has been shown to suppress E-cadherin gene transcription is T cell-specific transcription factor/lymphoid enhancer factor-1 (TCF/Lef1), which is associated with Wnt/beta-catenin signaling (13, 20). In renal proximal tubular cells, TGF-beta1-induced beta-catenin is required for synthesis of alpha-smooth muscle actin (alpha-SMA) as a marker of EMT (21). The Wnt signaling pathway is a necessary component that drives EMT in several embryonic processes (22). Wnt signaling promotes stabilization of cytoplasmic beta-catenin through phosphorylation of glycogen synthase kinase 3beta (GSK-3beta), which prevents beta-catenin's degradation (23). beta-Catenin is normally degraded via the ubiquitin proteasome pathway, which is mediated by a complex of proteins that includes adenomatous polyposis coli (APC), Axin and GSK-3beta (24). Dissociation of this complex as a result of GSK-3beta phosphorylation increases the stability of cytoplasmic beta-catenin and promotes its binding to TCF/Lef1. Subsequent nuclear translocation of the beta-catenin-TCF/Lef1 complex leads to a reduction in E-cadherin, thereby promoting EMT.

GSK-3beta phosphorylation has also been linked to the stable function of Snail1, which, like beta-catenin, is degraded via the ubiquitin pathway (25). Signaling pathways linked to phosphoinositide-3-kinase (PI3K) can also induce phosphorylation of GSK-3beta via downstream effectors such as integrin-linked kinase (ILK) and Akt (26). In addition, Snail1 and Slug promote formation of beta-catenin-TCF-4 transcription complexes that bind to the TGF-beta3 promoter to increase the gene's transcription. The resultant increase in TGF-beta3 signaling increases TCF/Lef1 gene expression, resulting in formation of beta-catenin-TCF/Lef1 complexes and thus initiation of EMT. Both TGF-beta1- and TGF-beta2-induced EMT appear to be TGF-beta3-dependent, establishing essential roles for multiple TGF-beta isoforms (27).

PAR3 and PAR6, two regulators of the apical-basal polarity of epithelial cells, were also reported to be key players during EMT. For instance, phosphorylation of PAR6 is required for TGF-beta1-dependent EMT in mammary gland epithelial cells. Ligand-activated TbetaRII phosphorylates PAR6, which in turn activates the E3 ubiquitin ligase SMURF1. SMURF1 then induces proteasomal degradation of RhoA, loss of the actomyosin

ring and breakdown of apico-basal polarity (28). TGF-beta1 induces transcriptional downregulation of PAR3, resulting in the cytoplasmic localization of aPKC and PAR6, downregulation of E-cadherin and loss of tight and adherens junctions. Conversely, forced expression of PAR3 leads to a marked inhibition of TGF-beta1-induced E-cadherin suppression (29). Snail1 inhibits Crumbs-3 expression, leading to the relocalization of PALS1 and PATJ, two components of the Crumbs-3 complex, as well as the relocalization of the PAR complex, which is followed by loss of apical-basal polarity. Both the PAR and Crumbs polarity complexes are displaced from tight junctions in MDCK cells undergoing Snail1-induced EMT (30). At the same time, Twist, another Snail family transcription factor, and FTS-1-binding proteins engage their respective promoters. The net result is the emergence of the EMT proteome and repression of epithelial proteins. Loss of E-cadherins and cytokeratins, rearrangement of actin stress fibers and expression of fibroblast specific protein 1 (FSP1), vimentin, interstitial collagens and, occasionally, alpha-SMA, mark the morphological transition of epithelial cells into fibroblasts (31).

#### 4. MODULATION OF CELL-ECM ADHESION

Renal fibrosis is characterized by excess accumulation of ECM in the interstitium. Tubular epithelial cells (TECs) are surrounded by ECM, so that their biological interaction likely plays a key role in the progression of renal fibrosis. Integrins are heterodimeric proteins comprised of alpha and beta subunits and serve as receptors for ECM proteins. In primary murine TECs, TGF-beta1 upregulates expression of alpha5-integrin, while knocking down alpha5-integrin attenuates TGF-beta1-mediated induction of mesenchymal markers like alphaSMA, suggesting integrin signaling is involved in the induction of the mesenchymal phenotype (32). The adaptor molecule disabled-2 (Dab2) is a positive mediator of TGF-beta1 signaling that acts by bridging the heteromeric TGF-beta1 receptor complex to Smad proteins. TGF-beta1 was recently reported to induce the transient accumulation of Dab2 at the membrane and to increase Dab2 binding to beta1 integrin, which occurs concomitantly with the promotion of EMT. Downregulation of Dab2 inhibits activation of integrin, as indicated by the reduction of TGF-beta1-induced phosphorylation of focal adhesion kinase and cellular adherence, leading to the inhibition of EMT (33).

TGF-beta1 induces Smad-dependent expression of ILK in TECs. ILK is an intracellular serine/threonine protein kinase that interacts with the cytoplasmic domains of beta-integrins and numerous other cytoskeleton-associated proteins. ILK is involved in the regulation of such integrin-mediated processes as cell adhesion, changes in cell shape and deposition of ECM. Forced expression of ILK in human proximal TECs suppresses E-cadherin and induces fibronectin and MMP-2, while expression of a dominant-negative ILK mutant abrogates TGF-beta1-initiated EMT, suggesting ILK plays a critical role in EMT (34). ILK also interacts with PINCH-1 (particularly interesting new cysteine-histidine rich protein-1), which

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appears to be essential for its activity (35). However, selective ablation of ILK gene in TECs *in vivo* has not yet been done, and the function of ILK *in vivo* remains to be determined.

Dissolution of integrin-mediated cell-ECM adhesion by MMP2, MMP9 or MMP14 is also associated with the induction of renal tubular EMT. Disruption of the tubular basement membrane (TBM) is a complementary step in the initiation of EMT. As collagen type IV, the main constituent of the TBM, is a specific substrate of MMP-2 and MMP-9, it would seem likely these two enzymes are also involved in initiating EMT. Consistent with that idea, in the remnant kidney model, TGF-beta1 stimulates the synthesis of both MMP-2 and its activator protease, MMP-14, in TECs, after which these two enzymes colocalize at sites of basal lamina disruption (36).

### 5. EMT AND TGF-BETA1 SIGNALING

Because the majority of TGF-beta1 target genes are controlled via Smad-dependent pathways, Smads are thought to be essential for TGF-beta1-induced EMT. The binding of TGF-beta1 to TbetaRII causes the receptor to form a heterodimer with TbetaRI and to then phosphorylate TbetaRI. Phosphorylated TbetaRI then selectively recruits and phosphorylates R-Smad proteins (Smad2/3), which, when released from the receptor complex, oligomerize with Smad4. The resultant Smad2/3-Smad4 complex enters the nucleus to promote transcription of target genes (8, 9). TGF-beta1 induces Smad2/3 phosphorylation in a TEC line. Although both Smad2 and Smad3 are phosphorylated and activated by TbetaRI, they have strikingly different effects on gene transcription. Complexes of phosphorylated Smad2 and Smad4 have been shown to upregulate expression of TCF/Lef1, which can then associate with beta-catenin or reassociate with Smads to promote transcription of EMT target genes (37). Notably, however, selective ablation of Smad3 signaling inhibits renal fibrogenesis *in vivo* and blocks EMT *in vitro*, indicating that Smad2 signaling is dispensable for EMT and the progression of renal fibrosis (38). Moreover, deletion of Smad2 induces EMT in hepatocytes and keratinocytes, suggesting Smad2 is a negative regulator of Smad3 and acts to maintain the epithelial cell phenotype (39, 40). On the other hand, Snail1 expression is ablated in Smad3-null cells, suggesting Snail1-induced EMT is also dependent on Smad3 signaling (38). High mobility group A2 (HMGA2) binds directly to the Snail1 promoter and acts as a transcriptional regulator of Snail1 expression (41). HMGA2 is induced via the Smad pathway during EMT and recruits other transcriptional regulators, including Slug, Twist and Id2, all of which reportedly repress E-cadherin (42). The rapid induction of Id1 in human TECs after TGF-beta1 treatment is also dependent on Smad signaling (19).

Smad7 is an inhibitory Smad protein, overexpression of which markedly suppresses TGF-beta1-induced Smads2/3 activation, thereby preventing EMT and collagen synthesis (43, 44). Conversely, selective ablation of Smad7 accelerates the progression of renal fibrosis in a

UUO model (45). Arcadia is an E3 ubiquitin ligase required for TGF-beta1 signaling during EMT. It stimulates EMT through degradation of Smad7, which suggests Smad signaling regulates EMT, both positively and negatively (46).

Gremlin, a bone morphogenic protein 7 (BMP-7) antagonist, is a downstream mediator of TGF-beta1, and has been shown to be upregulated in transdifferentiated renal proximal tubular cells and in human diabetic nephropathy, particularly in regions of tubulointerstitial fibrosis. Gremlin colocalizes with TGF-beta1, which is consistent with its importance as the effector of TGF-beta1-mediated EMT in renal fibrosis associated with human diabetic nephropathy (47, 48).

MicroRNA-155 is regulated via the TGF-beta1/Smads pathway and contributes to epithelial cell plasticity by targeting RhoA. TGF-beta1 induces microRNA-155 expression and promoter activity through Smad4. Knocking down microRNA-155 suppresses TGF-beta1-induced EMT and tight junction dissolution, while ectopic expression of microRNA-155 reduces levels of RhoA protein and disrupts tight junction formation (49). Conversely, the microRNA-200 family acts to prevent TGF-beta1-induced EMT (50). These associations between microRNAs and EMT have been investigated mainly in the context of cancer biology and not in renal fibrosis. Whether or not microRNA expression is associated with the EMT observed in renal fibrosis has not yet been determined.

Treatment of rat kidney epithelial cells (NRK52) with TGF-beta1 leads to activation of PI3K and Akt, as evidenced by increased phosphorylation of Ser473 of Akt and GSK-3beta, and by the observation that TGF-beta1-induced EMT phenotypes are blocked by inhibitors of PI3K and Akt (51). Thus TGF-beta1 also signals EMT in a Smad-independent manner. Indeed, in several systems TGF-beta1 signaling through Ras GTPase is required for EMT. What's more, TGF-beta1 receptors can signal through PAR6-SMURF1 to mediate ubiquitination of RhoA, an inhibitor of TGF-beta1-dependent EMT. In MDCKII cells, TGF-beta1 promotes EMT via the Smad-independent Ras-Raf-MEK-ERK-AP-1 signaling pathway, which upregulates Snail1 expression. Subsequent suppression of E-cadherin correlates with upregulation of mesenchymal markers (i.e., vimentin and fibronectin) and a definitive change in cellular morphology, but does not correlate with Smad signaling.

### 6. EMT IN THE GLOMERULUS

TGF-beta1 is also known to be an important mediator of glomerular damage, acting through the following mechanism. TGF-beta1 initially induces EMT in glomerular epithelial cells (podocytes). Exposing immortalized mouse podocytes to TGF-beta1 suppresses expression of P-cadherin, ZO-1 and nephrin, while inducing expression of desmin, fibronectin and collagen type I. Ectopic expression of Snail1 also suppresses P-cadherin and nephrin in podocytes, which, given that TGF-

beta1 induces Snail1 (see above), suggests TGF-beta1 has the capacity to mediate EMT in podocytes. Consistent with these *in vitro* data, loss of epithelial markers (nephrin and ZO-1) by podocytes and gain of mesenchymal markers (desmin, FSP1 and MMP9) are also observed in human diabetic nephropathy (52). EMT can lead to the detachment of podocytes from the glomerular basement membrane, which in turn leads to glomerular sclerosis. TGF-beta1 is also associated with the subsequent formation of cellular crescents, within which expression of ILK is strongly induced. ILK-expressing cells within cellular crescents are also positive for protein gene product 9.5 (parietal epithelial cell marker), alphaSMA and TGF-beta1, suggesting TGF-beta1-mediated upregulation of ILK expression contributes to the induction of EMT in parietal epithelial cells, which in turn appears to contribute to the further formation of cellular crescents and global glomerular damage (53). Finally, TGFbeta1 induces mesangial accumulation of ECM by accelerating mesangial cell fibrogenesis (54).

The progression of kidney disease is more closely associated with tubulointerstitial fibrosis than with glomerular injury (55). The link between glomerular disease and tubulointerstitial fibrosis likely involves an interstitial microvascular circulation that is compromised by upstream glomerular disease. The obliteration of postglomerular capillaries as a result of glomerular sclerosis or severe glomerular injury due to crescent formation impairs peritubular perfusion because the glomerular efferent arterioles branch into the peritubular capillary networks surrounding the tubular segments. The resultant peritubular capillary loss or low blood flow reduces the oxygen supply to the interstitium, leading to chronic interstitial and tubular cell hypoxia, which can initiate and sustain interstitial scarring and tubular atrophy. We recently showed that stable expression of hypoxia inducible factor-1 (HIF-1) by TECs in a hypoxic state promotes renal fibrosis and that HIF-1 is essential for induction of EMT in TECs (56-58). HIF-1 induces activation of cellular motility through upregulation of lysyl oxidase genes (56). Moreover, recent studies indicate that TGF-beta1 increases the expression of the regulatory HIF-1alpha subunit and the binding of HIF-1 to DNA. TGF-beta1 stimulates HIF-1 accumulation and activity by increasing the stability of the HIF-1alpha subunit (59). In addition, hypoxia and TGF-beta1 act synergistically to enhance production of certain types of collagen in fibroblasts (60). Although the molecular basis of the functional interaction is not well understood, such crosstalk between HIF-1 and TGF-beta1 may play a key role in the progression of renal fibrosis.

### 7. NEW THERAPEUTIC STRATEGIES AIMED AT INHIBITING TGF-BETA1-INDUCED EMT

Although EMT is an important element of the pathophysiology underlying the progression of renal fibrosis, there are at present no therapies aimed at preventing EMT as a means of treating chronic kidney disease in humans. Hepatocyte growth factor (HGF) and BMP-7 are well-known EMT antagonists. HGF binds to its

c-Met tyrosine kinase receptor and engages STAT3 during the formation of epithelial tubules. It also upregulates the expression of the Smad transcriptional co-repressor SnoN in tubular epithelial cells and negatively regulates EMT by interfering with Smad2/3 signaling. These inhibitory effects of HGF on EMT retard renal fibrogenesis in mice (61, 62).

In several models of kidney injury, administration of BMP-7 attenuates renal fibrogenesis while restoring the structure of tubular epithelial units (63). BMP-7 induces mesenchymal-epithelial transition (MET) by utilizing another set of Smads (Smad1/5), and reverses the EMT phenotype driven by TGF-beta1 (64). Although the precise mechanism and signaling pathways via which MET occurs remain unknown, a full understanding of this reciprocal phenomenon could form the basis for potentially highly effective novel therapeutic strategies. In a recent report, however, attenuation of TGF-beta1-induced EMT and corresponding induction of MET by BMP-7 could not be confirmed in human proximal TECs (65).

Treatment with BMP-2 also reverses the TGF-beta1-induced increase in fibronectin concomitantly with a significant downregulation of TbetaRI. BMP-2 shortens the half-life of TbetaRI through an effect on the ubiquitin proteasome degradation pathway, and reverses the TGF-beta1-induced increase in pSmad2/3, as well as the TGF-beta1-induced downregulation of inhibitory Smad7 (66). Interestingly, latent TGF-beta1 seems to protect against renal inflammation in a model of ureteral obstruction. In transgenic mice, overexpression of latent TGF-beta1 in keratinocytes reduced proteinuria by 50%, suppressed the formation of glomerular crescents by 70%, thereby preserving renal function (67). Progressive renal fibrosis was also prevented in these mice, and the protective effects were associated with elevated levels of latent, but not active, TGF-beta1 in plasma and renal tissue (68).

C-peptide reportedly interrupts TGF-beta1 signaling pathways and blocks development of EMT in HK2 human kidney proximal tubular cells. EMT-associated morphological alteration of proximal tubular cells, including increased vimentin expression, reduced E-cadherin expression, and cytoskeletal rearrangements can be prevented by treatment with C-peptide. C-peptide also blocks TGF-beta1-induced upregulation of expression of both TbetaRI and TbetaRII, and attenuates TGF-beta1-mediated Smad phosphorylation and Smad transcriptional activity. Thus C-peptide almost completely reverses the morphological changes induced by TGF-beta1 in proximal tubular cells, which suggests that it could serve as a renoprotective agent in diabetic nephropathy (69). If the results of clinical trials are promising, BMP-2, BMP-7, HGF, latent TGF-beta1 or C-peptide could become an important new adjuvant in the pharmacological armamentarium used to treat fibrogenesis.

GW 788388 is a new TbetaRI inhibitor that blocks TGF-beta1-induced Smad activation and target gene expression while reducing EMT and fibrosis. For instance, GW788388 given orally for 5 weeks reduced renal fibrosis

as well as the expression of key mediators of ECM in the kidneys of db/db mice (70). In addition, inhibition of histone deacetylase 6 (HDAC6) attenuated TGF-beta1-induced EMT, in part because reducing HDAC6 expression impairs the activation of Smad3 – e.g., the HDAC inhibitor trichostatin A prevented TGF-beta1-induced EMT in human TECs (71). At present, several clinical trials are investigating the effects of anti-TGF-beta1 antibodies in diabetic nephropathy. It is hoped that anti-TGF-beta1 therapy will become an important new tool to add to ACEI/ARB therapy for the treatment of human kidney diseases (72).

Heat shock proteins (HSPs) are the main effectors of cellular repair, and the association between EMT and HSP expression in renal fibrosis has been analyzed. HSP47, a collagen-specific molecular chaperone, is a surrogate marker of collagen synthesis and assists in the assembly of procollagen. HSP47 colocalizes with FSP1 in the renal fibrosis observed in human IgA nephropathy, suggesting it is involved in the accumulation of collagen (73). Consistent with that idea, *in vivo* administration of HSP47 siRNA in the UUO model diminished the interstitial fibrosis (74). In addition, although TGF-beta1 upregulates the levels of total and phosphorylated HSP27, HSP27 protects E-cadherin expression and blocks EMT by downregulating Snail expression (75). HSP27 may be induced in renal fibrosis through a negative feedback loop affecting EMT. In TECs, TGF-beta1-induced EMT is also inhibited by selective expression of HSP72. Moreover, in the UUO model, oral administration of geranylgeranylacetone, a selective inducer of HSP72, significantly diminished the progression of renal fibrosis (76).

Early treatment of anemia using recombinant human erythropoietin (rhEPO) has also proven effective for the treatment of chronic renal failure. EPO is thought to exert several pleiotropic effects. rhEPO treatment reduced levels of TGF-beta1, alphaSMA and fibronectin expression, and inhibited the progression of renal fibrosis in the UUO model. The increased alphaSMA and vimentin expression and decreased E-cadherin expression caused by TGF-beta1 in MDCK cells was attenuated by co-administration of rhEPO, indicating the renoprotective effects of rhEPO may be mediated by inhibition of TGF-beta1-induced EMT (77).

Several reports have shown that vitamin D analogues are renoprotective in experimental animal models of chronic kidney diseases. A clinical trial also demonstrated the antiproteinuric effects of oral paricalcitol, a synthetic vitamin D analogue, in chronic kidney disease. Paricalcitol is able to target the EMT process through preservation of E-cadherin and inhibition of EMT markers, and *in vivo* paricalcitol suppresses renal expression of both TbetRI and Snail (78). Finally, rapamycin and mycophenolate mofetil (MMF) have a greater inhibitory effect on EMT *in vitro* than older immunosuppressives and may result in less fibrosis and a better long-term allograft survival (79). Thus, exploration of the drugs available for the treatment of chronic kidney disease is yet another treatment option.

## 8. CONCLUSIONS

More than 40% of all deaths in developed countries are attributable to chronic fibrosis, including renal fibrosis, liver cirrhosis, pulmonary fibrosis and cardiovascular fibrosis. Consequently, the search for antifibrotic drugs is a challenging and highly important project. TGF-beta1 is the pivotal factor controlling the progression of tissue fibrosis, but data on the clinical application of anti-TGF-beta1 therapy remains limited. Because tissue fibrosis is a kind of physiological adaptation to prevent expansion of areas of inflammation, thereby protecting organs from functional deterioration, controlling fibrosis in way that is beneficial to the body is not simple. However, recent advances in EMT biology should provide a variety of tools for establishing novel therapeutic approaches to the treatment of tissue fibrosis. For instance, it is possible that the combination of EMT inhibitors with conventional RAAS inhibition could improve renal survival in chronic kidney disease. And a method for inducing MET and the recovery from progressive fibrosis would be of incalculable clinical value and a landmark advance in the medical sciences.

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**Abbreviations:** BMP: bone morphogenic protein, EMT: epithelial-mesenchymal transition, EPO: erythropoietin, FSP: fibroblast specific protein, GSK: glycogen synthase kinase, HGF: hepatocyte growth factor, ILK: integrin-linked kinase, MET: mesenchymal-epithelial transition, MMP: metalloproteinase, PI3K: phosphoinositide-3-kinase, SMA: smooth muscle actin, TEC: tubular epithelial cell, TGF: transforming growth factor

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