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New Insights into Epithelial-Mesenchymal Transition in Kidney Fibrosis

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Abstract

Epithelial-mesenchymal transition (EMT), a process by which differentiated epithelial cells undergo a phenotypic conversion that gives rise to the matrix-producing fibroblasts and myofibroblasts, is increasingly recognized as an integral part of tissue fibrogenesis after injury. However, the degree to which this process contributes to kidney fibrosis remains a matter of intense debate and is likely to be context-dependent. EMT is often preceded by and closely associated with chronic interstitial inflammation and could be an adaptive response of epithelial cells to a hostile or changing microenvironment. In addition to tubular epithelial cells, recent studies indicate that endothelial cells and glomerular podocytes may also undergo transition after injury. Phenotypic alteration of podocytes sets them in motion to functional impairment, resulting in proteinuria and glomerulosclerosis. Several intracellular signal transduction pathways such as TGF*β*/Smad, integrin-linked kinase (ILK) and Wnt/*β*-catenin signaling are essential in controlling the process of EMT and presently are potential targets of antifibrotic therapy. This review highlights the current understanding of EMT and its underlying mechanisms to stimulate further discussion on its role, not only in the pathogenesis of renal interstitial fibrosis but also in the onset of podocyte dysfunction, proteinuria, and glomerulosclerosis.

Kidney fibrosis is an inevitable outcome of all kinds of progressive chronic kidney disease (CKD).[1] Despite a great deal of intense study, comprehensive understanding of the pathogenesis of renal scar formation after injury remains a daunting task that poses a major obstacle toward designing effective therapeutic strategies. In the past several years, epithelial-mesenchymal transition (EMT), a process by which fully differentiated epithelial cells undergo transition to a fibroblast phenotype, has emerged as an important pathway leading to generation of matrix-producing fibroblasts and myofibroblasts in diseased kidney.

The concept of EMT, originally formulated in embryonic development and tumor metastasis,[2,3] highlights the tremendous plasticity of differentiated epithelial cells and prompts an appreciation for the role of epithelia in the evolution of fibrotic lesions in adult kidney. We and others have written comprehensive reviews on EMT in kidney fibrosis approximately 6 years ago.[4,5] Since then, EMT has become arguably one of the most interesting topics in the field of renal fibrosis and has attracted a great deal of attention.[6–12]

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DISCLOSURES

None.

EMT IN KIDNEY FIBROSIS

Many studies from different laboratories illustrate that tubular epithelial cells *in vitro* undergo phenotypic conversion after incubation with fibrogenic TGF-β1 (TGFβ); the transition is characterized by loss of epithelial proteins such as E-cadherin, zonula occludens-1 (ZO-1) and cytokeratin, and acquisition of new mesenchymal markers including vimentin, α-smooth muscle actin (α-SMA), fibroblast-specific protein-1 (FSP1), interstitial matrix components type I collagen, and fibronectin.[4,8] These alterations in protein expression are usually accompanied by morphologic changes to a fibroblastoid appearance and an enhanced migratory capacity. Several years ago, we proposed that EMT is an orchestrated, highly regulated process that consists of four key steps: loss of epithelial cell adhesion, *de novo* α-SMA expression and actin reorganization, disruption of tubular basement membrane, and enhanced cell migration and invasion.[4,13]

Conclusive demonstration of EMT *in vivo* in the setting of kidney diseases appears very challenging. Nevertheless, Iwano and colleagues[14] provide the most convincing evidence for EMT *in vivo* as a source of interstitial, matrix-producing fibroblasts. Using genetically tagged proximal tubular epithelial cells, they show that up to 36% of all FSP1-positive fibroblasts within the interstitial space originate from renal proximal tubules after unilateral ureteral obstruction. This landmark study clearly illustrates the significant contribution of EMT to the pathogenesis of chronic kidney fibrosis in that model. Interestingly, recent studies using a similar cell lineage-tracing technique show that a substantial number of interstitial fibroblasts also come from capillary endothelia by endothelial-to-mesenchymal transition (EndoMT).[15,16] Because endothelial cells are a specialized type of epithelia, this EndoMT represents another form of EMT that occurs in the injured kidney. This finding illustrates that the originality and multiplicity of interstitial fibroblasts in diseased kidney are much more complex than one previously thought. Evidence for EMT *in vivo* is emerging in various other animal models of CKD, including obstructive nephropathy,[13,15] diabetic nephropathy,[17,18] remnant kidney after 5/6 nephrectomy,[19,20] experimental GN,[21,22] nephrotoxic serum nephritis,[23] and chronic allograft nephropathy.[24–27]

Clinical studies utilizing human kidney biopsies also suggest that EMT likely plays a role in the pathogenesis of human CKD. Tubular expression of mesenchymal markers such as vimentin and FSP1 is found in various progressive kidney diseases including diabetic nephropathy,[28,29] lupus nephritis,[30] pauci-immune crescentic GN,[31] IgA nephropathy,[32] and chronic allograft nephropathy.[26,33,34] Furthermore, the expression of these transitional proteins in tubular epithelial cells often is well correlated with declining renal function.[28,34]

The extent to which EMT contributes to renal fibrosis *in vivo* remains a matter of intense debate and is likely to be context-dependent.[35–37] There are numerous reasons why EMT is often underestimated in injured kidney, as discussed previously.[4] Although loss of epithelial markers such as E-cadherin, ZO-1, and cytokeratin is a universal feature of EMT, fibroblastic conversion has been more difficult to define because of a lack of specificity of many available phenotypic markers.[38] Commonly used mesenchymal markers include vimentin, α-SMA, FSP1, desmin, collagen I, fibronectin, N-cadherin, the transcription factor Snail, and matrix metalloproteinases 2 and 9 (MMP-2 and MMP-9, respectively). However,

most of these markers are not absolutely specific for fibroblasts because they are also present in other cells such as inflammatory cells and endothelial cells. Furthermore, tubular epithelial cells and endothelial cells after injury *in vivo* may not undergo a complete EMT in many circumstances; rather, they undergo a partial EMT, also known as pre-EMT or *in situ* EMT, in which these cells only change one or two phenotypic markers but not actually leave their local microenvironment.[34] The discussion surrounding the relative role, even the very existence, of EMT in renal fibrogenesis is likely to continue, particularly in human CKD, because it is impossible to obtain conclusive evidence by utilizing the cell lineage-tracing technique in humans for the obvious reasons.

EMT AND PERITUBULAR MICROENVIRONMENT

Renal fibrosis is generally considered the result of a failed tissue injury/repair response and the entire process can be arbitrarily divided into several phases.[1,39,40] Tubular epithelial cells initially produce various chemokines and cytokines in response to various environmental stresses, including high ambient glucose, protein overload, hypoxia, and increased reactive oxygen species, as well as other injurious stimuli such as persistent infection, auto-immune reactions, and chemical insults. The chemokine gradients built up around tubular and capillary compartments attract and direct the influx of inflammatory cells, including monocytes/macrophages and lymphocytes (particularly T cells), to the tubulointerstitial space. Infiltrating cells in turn activate and produce a mixture of soluble factors, including proinflammatory, profibrotic cytokines and MMPs.[1,40] This cytokine milieu creates a hostile microenvironment for tubular epithelial and endothelial cells, rendering them adaptable to changing cell phenotype for the sake of escaping apoptosis. Not surprisingly, many EMT regulatory genes such as Snail also play an important role in modulating cell survival.[41,42] In that regard, EMT could be viewed as an adaptive response of epithelial cells after chronic stress/injury.

Many factors and extracellular cues in the tubular and capillary microenvironment clearly play a critical role in regulating EMT in different phases of renal fibrogenesis.[4] The list of these EMT regulatory factors is constantly growing (Table 1, A and B) and includes various cytokines, growth factors, and proteases as well as other environmental cues. In the early inflammatory phase of renal fibrosis, cytokines produced by infiltrating cells play a decisive role in initiating EMT. This notion is substantiated by a study in which oncostatin M from conditioned media of activated peripheral blood mononuclear cells promotes tubular EMT *in vitro*.[43,44] Likewise, proinflammatory cytokine IL-1 is an important regulator of EMT.[45] Of interest, some proinflammatory cytokines such as a mixture of IL-1, TNFα, and IFN-γ profoundly potentiate tubular EMT triggered by TGFβ by inducing TGFβ receptor expression, although they have little effect by themselves.[46] TNFα-dependent NF-κB activation also stabilizes the transcription factor Snail by blocking its ubiquitination, providing another molecular linkage between inflammation and EMT.[47] The significance of renal inflammation in initiating and promoting EMT is also manifested by many observations that renal fibrosis is almost always preceded by and closely associated with chronic interstitial inflammation.[48–51]

The major driving force behind EMT during the fibrogenic phase of renal fibrosis appears to be various profibrotic growth factors, including TGFβ,[6,13] basic fibroblast growth factor,[52] and connective tissue growth factor,[17,53] as well as angiotensin II,[54] the principal component of the renin-angiotensin system. Produced by stressed/injured tubular epithelial cells, infiltrating inflammatory cells, and/or residential activated fibroblasts, these factors establish a fibrogenic niche in the interstitial space that drives tubular epithelial and endothelial cells to transition. Among them, TGFβ is the most widely studied and likely the most potent EMT inducer.[6,38] As a sole factor, TGFβ can initiate and complete the entire course of EMT processes.[13] The essential role of TGFβ in EMT is also consistent with the observation that its expression is universally upregulated in every kind of CKD in experimental models and in clinical settings.[55]

The fibrogenic phase is often followed by tissue repair and remodeling.[40,56] In this stage, many matrix-degrading proteases are activated, secreted into the tubulointerstitial space, and participate in extracellular matrix remodeling. Surprisingly, these proteases also play a crucial role in promoting EMT. It has been reported that MMP-2 is necessary and sufficient for inducing tubular EMT *in vitro*, and overexpression of MMP-2 in transgenic mice promotes renal fibrosis.[57,58] MMPs also induce the proteolytic shedding of E-cadherin, which causes the nuclear translocation of β-catenin and the induction of Snail2 (Slug), leading to EMT in tubular epithelial cells.[59] Intriguingly, plasmin is demonstrated to bind to the tubular cell membrane receptor, protease-activated receptor-1, and initiates a cascade of signal transduction events leading to induction of EMT.[60] We have also reported that tissue-type plasminogen activator facilitates tubular EMT by inducing MMP-9 expression and promoting the destruction of tubular basement membrane integrity.[61,62]

Sustained injury eventually causes peritubular endothelial dysfunction and/or EndoMT, leading to hypoxia. In the advanced stage of CKD, renal parenchymal hypoxia, generation of reactive oxygen species, advanced glycation end products, and advanced oxidation protein products are predominant pathologic features.[27,63–67] In an elegant study by Higgins and colleagues,[68] the condition of hypoxia, through hypoxia-inducible factor-1 (HIF-1), is a powerful cue for inducing tubular EMT *in vivo* and *in vitro*. Stable expression of HIF-1α in tubular epithelial cells through ablation of von Hippel-Lindau tumor suppressor, a ubiquitin ligase responsible for HIF-1α degradation, consistently promotes interstitial fibrosis.[69] Recent studies further demonstrate that HIF-1α directly induces Twist, a key EMT regulatory transcription factor in kidney tubular cells subjected to hypoxia.[70] Likewise, advanced glycation end products are shown to induce tubular EMT in a TGFβ-dependent and -independent manner.[71,72] Although not tested, it would not be surprising if advanced oxidation protein products also induce EMT in diseased kidney.

Extensive studies also identify a diverse array of factors that negatively regulate EMT (Table 1). Hepatocyte growth factor and bone morphogenic protein-7 directly target TGFβ/Smad signaling and prevent, and even reverse in some cases, EMT and renal fibrosis.[23,73,74] The antifibrotic effects of several therapeutic agents such as vitamin D analogues, renin-angiotensin system inhibitors, statin, and rapamycin are, at least to some extent, attributable to their action in suppressing EMT.[75–79]

PODOCYTE EMT AND PROTEINURIA

In addition to tubular EMT and EndoMT as discussed above, recent findings indicate that glomerular podocytes also undergo phenotypic conversion, characterized by loss of podocyte-specific markers and gain of transitional features, a process reminiscent of EMT.[80] This implies that EMT in kidney diseases goes beyond the tubulointerstitial compartment. It is attractive to speculate that the transition of podocytes after injury may play a critical role in causing podocyte dysfunction, which ultimately leads to a defective glomerular filtration, proteinuria, and glomerulosclerosis.

Podocytes are specialized visceral epithelial cells that reside on the glomerular basement membrane (GBM) outside of the glomerular capillaries.[81,82] Similar to the cells in most parts of the nephron, podocytes are developmentally derived from the metanephric mesenchyme through mesenchymal to epithelial transdifferentiation. In this context, it seems not completely surprising that podocytes also undergo EMT, a process of reverse embryogenesis, under pathologic conditions. Recent studies demonstrate that podocytes in culture, upon incubation with TGFβ, reduce the slit diaphragm-associated proteins P-cadherin, ZO-1, and nephrin, changes consistent with loss of the epithelial feature.[80] Meanwhile, these cells begin to express the intermediate filament protein desmin, secret MMP-9, produce the interstitial matrix components fibronectin and collagen I, and upregulate the transcription factor Snail.[80] As a result, these alterations in cell phenotype eventually impair podocytes' filtration barrier function, as demonstrated by a paracellular albumin flux assay.[80]

Podocyte EMT also occurs in proteinuric kidney diseases.[80,83] In human biopsy samples of diabetic nephropathy and focal and segmental glomerulosclerosis, loss of nephrin and ZO-1 expression in glomerular podocytes is a common feature, whereas these cells express mesenchymal markers such as desmin, FSP1, MMP-9, and key EMT regulators Snail and integrin-linked kinase (ILK).[80,83,84] In a rat model of puromycin aminonucleoside nephropathy, several mesenchymal intermediate filament proteins such as desmin, vimentin, and nestin are upregulated predominantly in injured podocytes.[85] In an innovative cell lineage tracing study,[86] genetically tagged podocytes completely lost their molecular signatures such as Wilms tumor protein 1, synaptopodin, nephrin, and podocin, and presumably migrate to and repopulate in glomerular crescents during the early phases of cellular crescent formation in anti-GBM GN. These studies provide compelling evidence for profound phenotypic changes of podocytes *in vivo* under pathologic conditions. Of particular interest, several key EMT regulatory intracellular signal transduction pathways, including Wnt/β-catenin signaling,[87] ILK,[88] Snail,[80,89] and Jaggad/Notch signaling,[90,91] are often activated specifically in glomerular podocytes in various proteinuric kidney diseases, suggesting an active EMT program at work in podocytes after various insults.

The hypothesis of podocyte EMT offers a novel explanation for how injury causes podocyte dysfunction and a defective glomerular filtration barrier. We envision that EMT is an integral part of the spectrum of podocyte responses after injury. As illustrated in Figure 1, in response to injurious stimuli, podocytes undergo a range of adaptive changes, including hypertrophy, dedifferentiation, detachment, and apoptosis, depending on the severity and

duration of the injury.[80,92] The initial response may be cell hypertrophy, an adaptive change in cell size in an attempt to compensate for any lost function.[93] However, if the injury is progressive, podocytes will undergo EMT to escape from apoptosis, which results in the loss of highly specialized podocyte features and acquisition of new mesenchymal markers. This leads to an impaired glomerular filtration barrier, thereby ensuring the onset of proteinuria. More severe and/or longer injury induces podocyte detachment from GBM and/or apoptosis, resulting in podocyte loss, which certainly exacerbates proteinuria and leads to glomerulosclerosis. The involvement and relative contribution of these podocyte responses may not only depend on the severity and duration of a particular injury, but also vary in different disease models. For instance, podocyte hypertrophy may be a predominant feature in aging nephropathy, whereas cell loss could be a major finding in nephrotoxin-induced proteinuric glomerular diseases. We suspect that for many common glomerular diseases such as diabetic nephropathy, EMT could be a primary pathway leading to podocyte dysfunction and detachment, proteinuria, and glomerulosclerosis.

At this stage, the notion of podocyte EMT remains controversial because these cells possess sophisticated foot processes *in vivo* and already express a low level of vimentin at baseline.[94] Unlike tubular EMT, in which the transformed cells invade into the interstitial space and become matrix-producing cells, podocytes become motile after EMT, resulting in detachment from the GBM and leading to washout in urine or inclusion in glomerular crescent formation.[83,86] Despite these differences, tubular and podocyte EMT likely share similar hallmarks and could operate through communal mechanisms.

INTRACELLULAR SIGNALING AND MECHANISM OF EMT

The mechanism governing EMT has been studied in great detail in recent years. Because EMT can be induced by a wide variety of stimuli (Table 1), it is not difficult to imagine that a diverse array of intracellular signal pathways and mediators is potentially involved in regulating this process.[6,11] Depending on the specific pathophysiologic circumstances, these different signaling networks and mediators likely cooperate to induce a set of phenotypic changes that are consistent with EMT. In the setting of CKD, it is conceivable that three major signaling pathways (*i.e.*, TGFβ/Smad, integrin/ILK, and Wnt/β-catenin signaling) are essential for conferring tubular and podocyte EMT, although little is known about EndoMT. These pathways are intricately connected and integrated at different levels, and together they control a host of transcription regulators and signaling mediators that are imperative for EMT (Figure 2).

TGFβ Signaling

Mounting evidence establishes a crucial role for TGFβ signaling in mediating EMT.[6,38] TGFβ is the prototypic inducer of tubular and podocyte EMT,[13,80] whereas the effects of other mediators are often context-dependent, variable, and incomplete. Given the universal upregulation of its expression in the fibrotic kidney, TGFβ-induced EMT is particularly relevant to the pathogenesis of kidney fibrosis. Smad proteins mainly mediate the signals of TGFβ. Upon stimulation by TGFβ, transmembrane type II TGFβ receptor forms tight complexes with the type I receptor, leading to phosphorylation and activation of Smad2 and Smad3. Phosphorylated Smads then heteroligomerize with the common partner Smad4 and

translocate into the nucleus, where they control the transcription of TGFβ-responsive genes through interaction with specific *cis*-acting elements in the regulatory regions.[95,96] Of interest, various EMT related genes are the targets of TGFβ/Smad signaling, such as connective tissue growth factor, ILK, PINCH-1, β1-integrin, Wnt, Snail, Id1, α-SMA, collagen IA2, and MMP-2.[97–100]

The necessity of Smad signaling in EMT is clearly illustrated *in vivo* in Smad3 knockout mice after obstructive injury. Mice lacking Smad3 are protected from renal interstitial fibrosis and show reduced EMT and collagen accumulation after unilateral ureteral obstruction.[101] Consistent with this, primary tubular epithelial cells from the Smad3 null mice are resistant to induction of EMT and key EMT regulatory genes.[101,102] Targeting Smad signaling by inhibitory Smad7 also blocks tubular EMT and reduces renal fibrotic lesions.[103,104] Of note, Smad signaling in diseased kidney appears drastically hyperactive, not only because of TGFβ upregulation but also the dysregulation of Smad co-repressors and their regulators.[105–108] Blockade of Smad signaling is also mechanistically linked to the inhibition of EMT by hepatocyte growth factor and bone morphogenic protein-7.[23,109,110]

Smad-independent signaling of TGFβ apparently also plays a role in regulating EMT. Non-Smad pathways of TGFβ signaling involved in EMT include RhoA, p38 mitogen-activated protein kinase (MAPK), and phopshati-dylinositol-3-kinase/Akt. In most circumstances, activation of these non-Smad pathways provides the context for induction and specification of EMT and is necessary for some aspects of EMT. For instance, the small GTPase, RhoA, is important for morphologic changes, activation of α-SMA promoter, and cytoskeletal rearrangements during TGFβ-induced EMT.[111,112] TGFβ also activates p38 MAPK. However, studies show that TGFβ-mediated p38 MAPK activation is dependent on functional β1-integrin, and p38 MAPK activity is required but is not sufficient to induce EMT.[113] Recent studies identify a novel pathway in which p38 MAPK can inactivate glycogen synthase kinase-3β (GSK-3β) by direct phosphorylation at its C-terminus, leading to an accumulation of β-catenin (Figure 2).[114] Evidence indicates that the phopshatidylinositol-3-kinase/Akt pathway is also implicated in tubular EMT.[65,115] Although the underlying mechanism remains elusive, Akt-mediated cell survival and β-catenin accumulation through inhibition of GSK-3β could play an important role.

ILK Signaling

ILK is an intracellular serine/threonine protein kinase that interacts with the cytoplasmic domains of the β-integrins and mediates the integrin signaling in diverse types of cells. ILK elicits its biologic activities through two principal properties: as a scaffolding protein and as a protein kinase.[116,117] As a scaffolding protein, ILK interacts with integrins and numerous intracellular proteins, such as α-parvin and PINCH.[116,118] We recently discovered that ILK also interacts with nephrin in normal glomerular podocytes, thereby building a molecular bridge that connects the cell-matrix integrin signaling with the cell-cell slit diaphragm signaling.[119] Not surprisingly, conditional knockout of ILK in a podocyte-specific manner results in massive proteinuria, glomerulosclerosis, and premature death in mice.[119,120] As a protein kinase, the catalytic activity of ILK renders it to directly phosphorylate several physiologically important downstream effector kinases including Akt and GSK-3β, leading

to the stabilization of β-catenin (Figure 2).[117] This in turn controls the expression of an array of genes that are required for the EMT program.

The involvement of ILK in tubular EMT has been established by several lines of evidence.[97] Intriguingly, many components of ILK signaling, including ILK, PINCH-1, and β1-integrin are induced simultaneously by TGFβ in a Smad-dependent manner.[97,98] ILK expression is also upregulated in a wide variety of CKDs in experimental and clinical settings.[84,97,121,122] Furthermore, ILK is independently identified as a key mediator of podocyte dysfunction and proteinuria in many forms of proteinuric kidney diseases[84] in which podocyte EMT is a predominant pathologic feature.[80] The action of ILK in regulating EMT is mediated primarily by its protein kinase activity, because a kinase-dead mutant and small molecule inhibitor of ILK blocks TGFβ-mediated EMT *in vitro*, prevents podocyte dysfunction and albuminuria after adriamycin administration, and inhibits renal interstitial fibrosis in obstructive nephropathy.[88,97,123] In this context, it is plausible that hyperactive ILK, a downstream signaling of TGFβ, plays a crucial role in mediating tubular and podocyte EMT and targeting its signaling could be a rational strategy for the treatment of fibrotic kidney disorders.

Wnt/β-Catenin Signaling

The role of Wnt/β-catenin signaling in regulating EMT during organ development and tumor metastasis is well established.[3,124] However, its implication in tubular and podocyte EMT in the setting of CKD has remained uncertain until recently.[87,125–127] Wnt proteins belong to a highly conserved family of secreted growth factors that play an essential role in organogenesis, tissue homeostasis, and tumor formation.[128,129] Wnt proteins transmit their signal across the plasma membrane through interacting with the Frizzled receptors and co-receptors LDL receptor-related protein-5/6. Upon binding to their receptors, Wnt proteins induce a series of downstream signaling events involving Disheveled, axin, adenomatosis polyposis coli, casein kinase-1, and GSK-3β, resulting in dephosphorylation of β-catenin. This leads to stabilization of β-catenin by escaping from ubiquitin-mediated degradation, allowing it to accumulate in the cytoplasm and to translocate into the nuclei, where it binds to T cell factor/lymphoid enhancer-binding factor-1 (LEF1) to stimulate the transcription of Wnt target genes.[129–131] In addition to this canonical pathway, Wnt proteins may also exert their activities through numerous β-catenin-independent, noncanonical intracellular signaling routes.

Multiple distinct genes in mammals encode Wnt proteins, creating a complex network of signaling systems. Interestingly, the vast majority of 19 mouse Wnt genes are induced concurrently in the fibrotic kidney after obstructive injury.[127] Induction of Wnts leads to the stabilization of β-catenin, resulting in its localization in the cytoplasm and nuclei of tubular epithelial cells, indicating a prevailed Wnt/β-catenin signaling in that model. Inhibition of Wnt signaling by Dickkopf-1, an endogenous Wnt antagonist that specifically inhibits the canonical Wnt/β-catenin signal pathway by binding to the LDL receptor-related protein-5/6 component of the receptor complex,[132] blocks the expression of Wnt target genes such as Twist, LEF1, c-myc, and fibronectin and ameliorates renal fibrosis after obstructive injury *in vivo*.[127] Likewise, activation of Wnt/β-catenin signaling is apparently involved in mediating

podocyte EMT by inducing Snail and suppression of nephrin, and mice with conditional ablation of β-catenin in glomerular podocytes are protected from proteinuria and podocyte dysfunction after administration of adriamycin.[87,89]

TGFβ, ILK, and Wnt signals are interconnected and converge at the activation of β-catenin (Figure 2), which leads to the activation of EMT transcriptional programs. In this context, it is of interest to note that many β-catenin target genes (*e.g.*, Snail, Twist, LEF1, and Jaggad1) are key EMT regulatory transcription factors and mediators. For instance, Snail is a zinc-finger protein that acts as transcription repressor by recognizing E-box elements in its target gene promoters.[133] Overexpression of Snail suppresses E-cadherin in tubular epithelial cells and inhibits nephrin and P-cadherin in podocytes.[75,80,89] *In vivo*, Snail activation is sufficient to induce EMT and kidney fibrosis in adult transgenic mice.[134] Twist is a basic helix-loop-helix transcription factor that is implicated in tubular EMT and kidney fibrosis.[70,135] Ectopic expression of Twist not only suppresses E-cadherin but induces mesenchymal markers such as fibronectin, vimentin, α-SMA, and N-cadherin.[136] Therefore, to some extent, β-catenin could function as a master switch that can integrate signal inputs from multiple pathways and control the EMT-related transcriptome.

CONCLUSIONS AND PERSPECTIVE

EMT has become one of the most fascinating topics in the studies of embryonic development, tumor metastasis, and organ fibrosis in recent years. The idea that epithelial cells after stress/injury can undergo conversion to give rise to fibroblasts, and thereby contribute to the pathogenesis of kidney fibrosis, is quite attractive and is receiving increasing attention. Undoubtedly, EMT prompts one to appreciate the role of epithelia and endothelia in the evolution of kidney fibrosis, thereby representing a paradigm shift in the field. A growing list of the extracellular factors and intracellular mediators that control EMT has been identified and could be exploited in developing future antifibrotic therapeutics.

Despite these progresses, many open questions remain. One fundamental issue is to what extent EMT contributes to renal fibrosis *in vivo*. A definitive answer has to rely on the cell lineage tracing technique *in vivo* by using a genetic model.[14,86] Because many cell types other than epithelial and endothelial cells (including interstitial fibroblasts, circulating fibrocytes, and vascular pericytes) also participate in matrix production in the fibrotic kidney,[14,15,37,137,138] dissection of the relative contribution of EMT to kidney fibrosis remains extremely difficult, if possible at all. Another challenge is to completely elucidate the key molecular mechanism controlling EMT. Given the diversity of known EMT regulatory factors, the underlying signal pathways could be immensely complex, with almost immeasurable cross-talks and feedbacks. What one needs may be to identify a converging “master switch” that integrates various signal inputs and controls the EMT transcriptional program. Finally, perhaps the most difficult challenge ahead is to develop a plan to translate many experimental innovations into clinically effective regimes. Several strategies targeting key EMT signaling appear to work in animal models.[5,110,123] It is hoped that well designed clinical trials will be carried out in the years to come.

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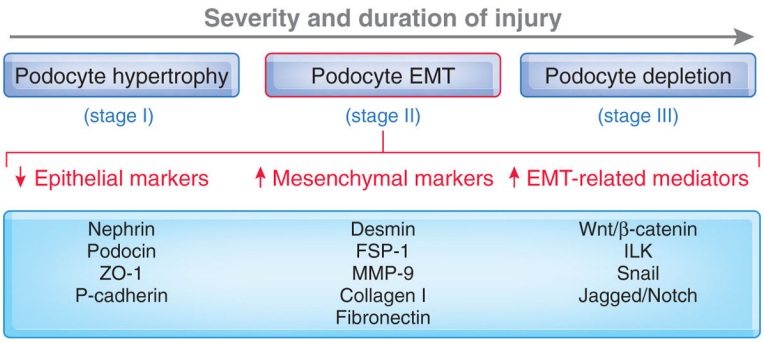

Figure 1.

Schematic presentation of the spectrum of podocyte responses after injury. Depending on the severity and duration of the injury, podocytes may respond to injurious stimuli in different ways, including hypertrophy, dedifferentiation and mesenchymal transition (EMT), detachment and apoptosis (depletion). EMT could be a primary pathway leading to podocyte dysfunction, proteinuria, and glomerulosclerosis in many common forms of proteinuric kidney diseases.

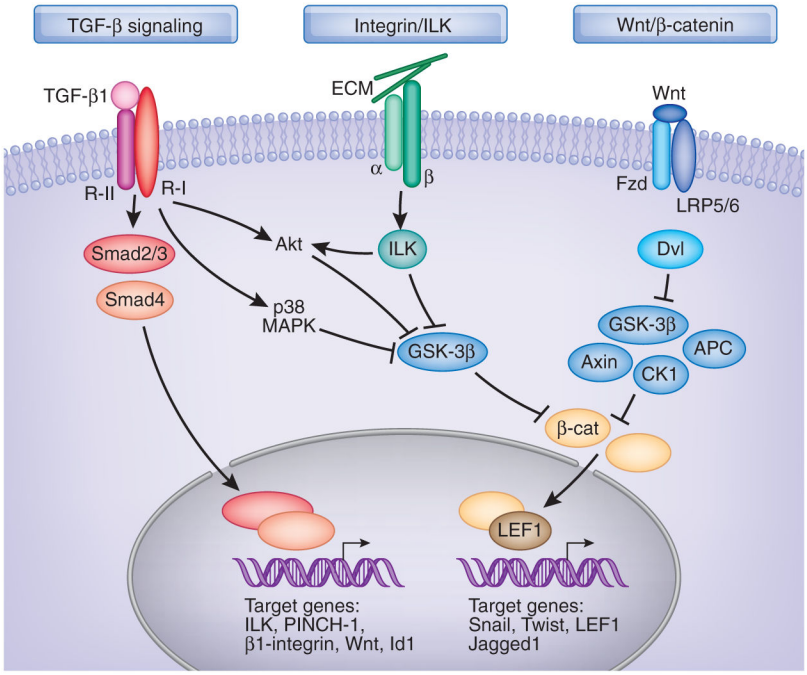

Figure 2.

Simplified schematic shows major intracellular signaling networks and mediators involved in the regulation of EMT in the fibrotic kidney. Although EMT can be induced by a wide variety of stimuli and potentially involves a diverse array of intracellular mediators, three major signaling pathways (*i.e.*, TGF*β*/Smad, integrin/ILK, and Wnt/*β*-catenin signaling) are essential for conferring tubular and podocyte EMT. These pathways are intricately connected and integrated at different levels. See text for details.

Table 1

Table 1A. Factors in the peritubular microenvironment that induce or promote EMT

Factors	References
Cytokines	
IL-1	45
oncostatin M	43, 44
Growth factors	
TGFβ1	6, 13, 38
FGF-2	52
connective tissue growth factor	17
Component of renin-angiotensin system	
angiotensin II	54
Proteases	
MMP-2	57
tissue-type plasminogen activator	61
plasmin	60
Environmental stresses	
hypoxia/reactive oxygen species	27, 66, 68
advanced glycation end products	17, 72

Table 1B. Factors in the peritubular microenvironment that suppress EMT

Factors	References
Growth factors	
hepatocyte growth factor	74
bone morphogenic protein-7	23
Nuclear receptor activator	
Vitamin D	75
Renin-angiotensin system inhibitors	
angiotensin II receptor blocker	76
Other	
statin	13, 77
rapamycin	78, 79