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REVIEW

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The role of epithelial-to-mesenchymal transition in renal fibrosis

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Abstract Epithelial-to-mesenchymal transition (EMT) involving injured epithelial cells plays an important role in the progression of fibrosis in the kidney. Tubular epithelial cells can acquire a mesenchymal phenotype, and enhanced migratory capacity enabling them to transit from the renal tubular microenvironment into the interstitial space and escape potential apoptotic cell death. EMT is a major contributor to the pathogenesis of renal fibrosis, as it leads to a substantial increase in the number of myofibroblasts, leading to tubular atrophy. However, recent findings suggest that EMT involving tubular epithelial cell is a reversible process, potentially determined by the surviving cells to facilitate the repopulation of injured tubules with new functional epithelia. Major regulators of renal epithelial cell plasticity in the kidney are two multifunctional growth factors, bone morphogenic protein-7 (BMP-7) and transforming growth factor β1 (TGF-β1). While TGF-β1 is a well-established inducer of EMT involving renal tubular epithelial cells, BMP-7 reverses EMT by directly counteracting TGF-β-induced Smad-dependent cell signaling in renal tubular epithelial cells. Such antagonism results in the repair of injured kidneys, suggesting that modulation of epithelial cell plasticity has therapeutic advantages.

Keywords Epithelial-to-mesenchymal transition · Renal fibrosis · Bone morphogenic protein 7 · Transforming growth factor β

Abbreviations *ALK:* Activin-like kinase · *bFGF:* Basic fibroblast growth factor · *BMP:* Bone morphogenic protein · *ECM:* Extracellular matrix · *EGF:* Epithelial growth factor · *EMT:* Epithelial-to-mesenchymal transition · *FSP1:* Fibroblast specific protein 1 · *IL-1:* Interleukin 1 · *LAP:* Latency-associated polypeptide · *MET:* Mesenchymal-to-epithelial transition · *MMP:* Matrix metalloproteinase · *TBM:* Tubular basement membrane · *TGF:* Transforming growth factor

Introduction

Chronic progressive fibrosis of the kidney remains an unsolved challenge for nephrologists, as it still almost inevitably leads to end-stage renal failure, requiring replacement therapy [1, 2]. Scarring of the kidney, which is caused by a progressive fibrosis leading to impairment of kidney function, occurs due to a variety of primary insults, such as diabetes mellitus, hypertension, primary glomerulopathies, autoimmune diseases, toxic injury or congenital abnormalities [1, 3, 4]. Scarred kidneys are almost uniformly characterized by the triad of glomerulosclerosis, interstitial fibrosis and tubular atrophy, implicating common mechanisms which are independent of the underlying primary disease [1, 4]. While resident renal fibroblasts are traditionally considered to be the principal mediators of renal fibrosis, recent studies have highlighted the involvement of tubular epithelial cells, the epithelial parenchyme of the kidney, in progression of chronic renal disease [1, 3]. Tubular epithelial cells possess a unique plasticity, which enables them to convert between the epithelial and mesenchymal phenotypes [5, 6]. While specific therapeutic options to inhibit progression of chronic renal disease are still not available in the clinic, modulation of epithelial-to-mesenchymal transition (EMT) offers a novel therapeutic target to potentially inhibit renal fibrogenesis.

The epithelial-to-mesenchymal transition

EMT is necessary for embryonic development, tumor progression and organ fibrosis [7, 8]. During EMT, epithelial characteristics are lost and a mesenchymal phenotype is acquired [7]. The cell morphology changes from a cuboidal to a fibroblastic shape and intercellular epithelial adhesion molecules such as E-cadherin and zonula occludentes protein ZO-1 are replaced by mesenchymal cytoskeletal markers such as fibroblast-specific protein 1 (FSP1) and vimentin [7, 9, 10]. Instead of interacting with the extracellular matrix (ECM) at the basal cell surface, the transdifferentiated cells acquire the ability to invade the ECM [7, 11].

Plasticity resulting in cells shifting between epithelial and mesenchymal phenotypes is essential in embryonic development as it permits anchored epithelial cells to reorient in a developing organism [12]. In this regard, EMT is known to contribute to the formation of mesoderm during gastrulation and also to the formation of connective tissue from somitic epithelium [13, 14]. In adults, EMT is speculated to occur involving resident epithelia in response to injury, as an additional source of myofibroblasts/fibroblasts which are essential for repair of injured tissue [14, 15]. During kidney fibrosis however, enhanced conversion of renal tubular epithelial cells into myofibroblasts/fibroblasts is considered unfavorable, as it leads to disruption of polarized renal tubular epithelial layers and an increase in fibrotic scar formation [15].

The EMT in the kidney

In recent years, EMT associated with adult injured kidneys has become of increasing interest. Initial reports have demonstrated that FSP1 (S1004A), a member of the S100 family of calcium-binding proteins exclusively expressed in fibroblasts, can be detected in tubular cells of injured kidneys in both acute and chronic disease [16]. This finding has been confirmed by numerous independent studies, which have detected renal tubular epithelial cells in the process of EMT in different animal models of chronic renal disease and also in human kidney biopsies [15, 16, 17, 18, 19, 20, 21]. A reciprocal correlation of increasing numbers of tubular epithelial cells involved in EMT and a decline of excretory renal function suggests a pathogenic role for the EMT in the progression of chronic renal disease [5]. By utilizing a model of γGT-LacZ transgenic mice, which allows the indisputable identification of cells derived from proximal tubular epithelium in the kidney, Iwano et al. have recently demonstrated that more than one third of renal interstitial fibroblasts, the main mediators of renal interstitial fibrosis, are derived from renal tubular epithelium via EMT [15]. Further evidence for the importance of the EMT in the progression of chronic renal disease has been provided by the observation that reversal of EMT results in improvement of renal function and decreased mortality in a mouse model of crescentic glomerulonephritis [5].

A model for EMT in the kidney

Mechanistic insights into the regulation of EMT involving renal tubular epithelial cells are predominantly based on in vitro studies. The main inducers of EMT in renal tubular epithelial cells can be categorized into growth factors and enzymes, which facilitate disruption of the tubular basement membrane integrity [3, 10, 22, 23]. Transforming growth factor β1 (TGF-β1) has been identified as the main inducer of EMT in the kidney and in other organ systems [8, 24, 25, 26]. Growth factors with a capacity to induce EMT of tubular epithelial cells also include epithelial growth factor (EGF), basic fibroblast growth factor (bFGF) and Interleukin-1 (IL-1) [9, 22, 27]. In addition to stimulation by growth factors, disruption of the underlying tubular basement membrane (TBM) by MMP-2 or other MMPs, can induce EMT of tubular epithelial cells [10, 28]. The main inducers of EMT, TGF-β1 and disruption of the TBM, can also promote cell death via apoptosis (TGF-β1) or anoikis (disruption of the TBM), suggesting that EMT serves as a physiologic pathway that allows injured tubular epithelial cells to escape cell death [29, 30, 31]. Hence, EMT-derived fibroblasts can facilitate immediate repair of injury and potentially also serve as a pool of vital cells with capacity to repopulate the injured tubular epithelium [5, 15]. This concept is strengthened by the observation that constant exposure of tubular epithelial cells to TGF-β1 induces apoptosis in a substantial number of cells,

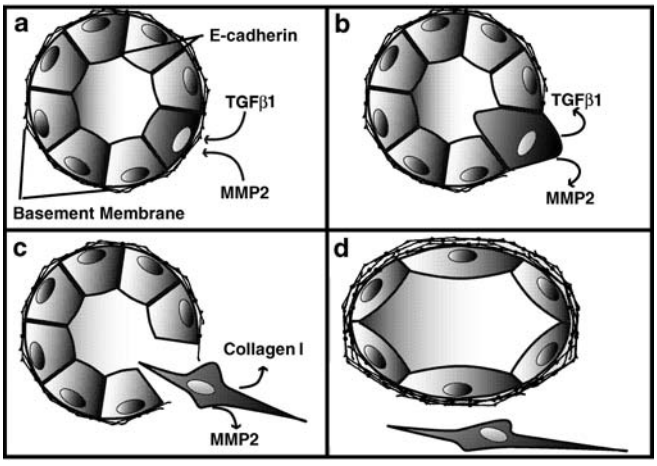

Fig. 1a–d A working model of EMT in chronic kidney disease. **a** In normal kidneys tubular epithelial cells are tightly connected with their neighboring cells via intercellular adhesion molecules such as E-cadherin. On their basal side they are tightly connected with the tubular basement membrane (TBM), while the apical side is facing the tubular lumen. In chronic renal disease, tubular epithelial cells become activated, when growth factors such as TGF-β1 or TBM-degrading proteases such as MMP-2 are released from infiltrating mononuclear cells and interstitial fibroblasts. **b** Activated tubular epithelial cells lose expression of E-cadherin and secrete TGF-β1 and MMP-2, further facilitating degradation of TBM and enhancing EMT in an autocrine manner. **c** Cells in EMT acquire mesenchymal migratory capacity and traverse across the disrupted TBM into the interstitial microenvironment. **d** EMT-derived fibroblasts within the interstitium contribute to progression of chronic renal disease by facilitating deposition of interstitial ECM. Located within the fibrotic interstitial microenvironment, they possess a typical mesenchymal cytoskeleton

while the remaining cells, which survive, undergo EMT [32, 33]. Based on these findings, a hypothetical model of EMT in chronic renal disease is proposed (Fig. 1). In the normal kidney, tubular epithelial cells are tightly connected with their neighboring cells via intercellular adhesion molecules such as E-cadherin [24]. On their basal side they interact with the TBM, while the apical side faces the tubular lumen [24]. In the initial phases of renal tubular injury, the epithelial cells initially respond to TGF-β1 and/or MMP-2 (which are potentially released by infiltrating mononuclear cells) by acquiring an "activated" state [6]. If the pathogenic insult persists, activated tubular epithelial cells can either die or undergo EMT [6]. The process of EMT, which includes various intermediate stages, is initiated by loss of E-cadherin expression and autocrine TGF-β1 and MMP-2 secretion, which further facilitate degradation of the TBM and enhance the EMT [6]. Cells derived via EMT then acquire a migratory capacity and traverse across the disrupted TBM into the interstitial microenvironment exhibiting features of a mesenchymal phenotype [19, 23]. EMT-derived fibroblasts within the interstitium contribute to progression of chronic renal disease by deposition of interstitial ECM [34]. EMT and apoptosis both contribute to loss of tubular epithelium, leading to tubular atrophy and disease progression.

The role of E-cadherin in EMT during renal fibrosis

While numerous distinct signaling pathways have been described as initiators of EMT in different settings, all of them culminate in the loss of E-cadherin [24, 35, 36]. E-cadherin is an epithelial cell specific intercellular adhesion molecule, which by itself can induce mesenchymal-to-epithelial transition (MET), when overexpressed in cells of mesenchymal lineage [37]. Several different studies have demonstrated that E-cadherin is an important determinant for maintenance of the epithelial phenotype [5, 38, 39]. Metastatic cancers are associated with loss of E-cadherin [40]. In many different carcinoma cell lines E-cadherin functions as a suppressor of invasiveness [41]. Several studies demonstrate that loss of cell-cell adhesion is due to decreased E-cadherin expression [42]. Identification of the transcriptional regulators of E-cadherin, such as Snail and SIP1, have further revealed the importance of E-cadherin expression for EMT and a possible role in the progression of cancer [43, 44, 45]. Furthermore, the conversion of an epithelial cell into a fibroblast, with its numerous intermediate stages, is reflected by a reciprocal expression of E-cadherin and FSP1. E-cadherin expression gradually decreases while FSP1 expression increases inversely [5, 46]. Such studies have led to the speculation that E-cadherin is a potential epithelial master gene [24, 47].

Plasticity of renal tubular epithelium

EMT in adult kidneys is associated with reiteration of renal developmental programs [48]. While most parenchymal epithelia are derived via branching from a primary epithelial sheet, the renal epithelium has two distinct embryological origins [49]. Most of the epithelium, which constitutes the nephron (from glomerulus to connecting tubule) is derived from the mesenchymal blastema, whereas the segment starting at the collecting tubule stems from the epithelial Wolffian duct [50]. Conversion of the metanephric mesenchyme into epithelium via MET is the central mechanism during kidney development [49, 51]. Formation of the epithelial nephron is initiated when the ureteric bud epithelium invades the mesenchymal blastema [50]. Condensing mesenchyme then adheres and forms an epithelial cyst, associated with expression of basement membrane proteins and formation of a lumen [52]. The growth factor BMP-7 plays an important role in regulation of nephrogenesis associated with MET, while TGF-β1 has been demonstrated to inhibit branching morphogenesis in the developing kidney [53, 54]. Interestingly, EMT in the injured adult kidney occurs in cells which are originally of MET-derived lineage [48]. In adult tubular epithelial cells which undergo EMT basal BMP-7 expression is substantially decreased, whereas expression of TGF-β1, the main inducer of EMT, is enhanced [5]. It was recently demonstrated that administration of BMP-7 can reverse TGF-β1-induced EMT in adult tubular epithelial cells,

suggesting that these cells maintained their capacity for transition back to renal tubular epithelial cells [5]. In summary, these findings suggest that the tubular epithelium, due to its unique mesenchymal origin, is plastic enough to acquire mesenchymal or epithelial phenotypes in disease and during repair. TGF-β1 and BMP-7, both members of the TGF-β superfamily, are increasingly being recognized as mutual antagonists in the kidney [5].

Bone morphogenic protein-7

Bone morphogenic proteins (BMPs) are a major subgroup of the TGF-β superfamily [55]. BMPs share high homology with activins and TGF-β proteins at the carboxy terminal domain [53]. Traditionally, BMPs are classified into three groups [55]. The first group contains BMP-2 and BMP-4, the second contains BMP-5, BMP-6 and BMP-7, and the third contains BMP-3 and BMP-8 [55]. BMPs in general control morphogenic pathways at different stages of development [56]. The cellular responses to BMP are mediated by type I and type II cell surface transmembrane serine/threonine kinase receptors [57, 58, 59].

BMP-7, also sometimes referred to as osteogenic protein-1 (OP-1), was originally identified as a potent osteogenic factor purified from bone [60]. Different studies have demonstrated a role for BMP-7 during mammalian kidney development [61, 62]. BMP-7 binds to the ALK3 and ALK6 type I serine/threonine kinase receptors [59, 63]. BMP-7 and its receptors are expressed in regions associated with mesenchymal-epithelial tissue interactions [64]. Homozygous BMP-7-deficient mice have dysplastic kidneys and die shortly after birth from renal failure [61, 62]. In these mutants, formation of S-shaped tubules is initiated, but is arrested at embryonic ~day 11.5 [61, 62]. BMP-7 regulates branching morphogenesis in the developing kidney, which is associated with MET, and functions as a survival factor for renal epithelium during kidney development [65, 66].

In the adult kidney, BMP-7 is increasingly being recognized for its potential to maintain tubular homeostasis in acute and chronic renal injury [67, 68]. Acute and chronic tubular injury is associated with decreased tubular BMP-7 expression, and administration of exogenous BMP-7 mediated repair of tubular injury and the return of renal function in different models of kidney injury [5, 69, 70, 71, 72, 73]. Tubular injury in chronic renal disease is associated with increased TGF-β1 expression in the kidney [74]. This reciprocal relationship between BMP-7 and TGF-β1 suggests that these two growth factors can function as physiological antagonists in the adult kidney [73]. Renal tubular epithelial cells respond to exposure to TGF-β1 by either undergoing apoptosis or EMT (Fig. 2), which eventually leads to tubular atrophy [6, 9]. While an anti-apoptotic effect of BMP-7 on tubular epithelial cells has not been demonstrated yet, BMP-7 is sufficient to reverse TGF-β1-induced EMT (Fig. 2) [5]. BMP-7 mediated reversal of EMT is independent of persisting TGF-β1 levels, suggesting that these two molecules directly counteract each other's signaling pathways within the cell [5].

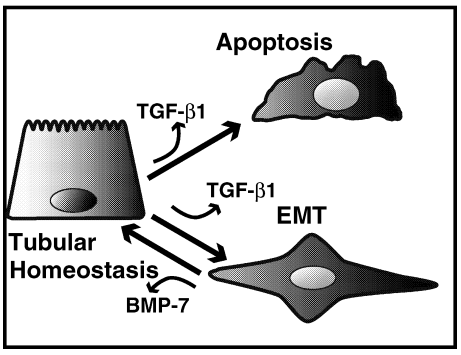

Fig. 2 Regulation of tubular homeostasis by TGF-β1 and BMP-7. When exposed to TGF-β1, tubular epithelial cells either respond by undergoing apoptosis or survive by undergoing EMT. BMP-7 re-induces the acquisition of an epithelial phenotype in EMT-derived fibroblasts

Counteraction of Smad-dependent TGF-β1 signaling by BMP-7 in renal tubular epithelial cells

All members of the TGF-β superfamily signal through heteromeric complexes of transmembrane type I and type II serine/threonine kinase receptors [75, 76]. Within this complex, the type II receptor kinase activates the type I receptor kinase, which subsequently phosphorylates Smad proteins, which function as signal transducers [35, 57, 58, 76]. Smads are subdivided into three classes, the receptor-regulated Smads (Smad1, -2, -3, -5 and -8), the common Smads (Smad4) and the inhibitory Smads (Smad6 and -7) [57]. Members of the TGF-β superfamily activate distinct downstream pathways involving serine/threonine kinase receptors and Smads [35, 58, 76, 77]. Each serine/threonine kinase receptor phosphorylates specific R-Smads [35, 76, 77, 78]. BMP-7 binds to ALK3 and ALK6 type I receptors, which function via Smad1, Smad5 and Smad8, whereas TGF-β1 binds to and ALK5 type I receptors, which activate Smad2 and Smad3 [59, 63] (Fig. 3). The phosphorylated Smad2/3 (TGF-β1 Smads) or Smad1/5/8 (BMP-7 Smads) form a hetero-complex with Smad4 (common Smad), which then shuttles into the nucleus and regulates the transcription of target genes in association with several co-transcriptional regulators (Fig. 3) [59, 63, 79]. While TGF-β1 directly inhibits E-cadherin expression and induces EMT in a Smad3-dependent manner, BMP-7 enhances E-cadherin expression via Smad5 and restores the epithelial phenotype [5, 80]. Such Smad-dependent action by BMP-7 in renal tubular epithelial cells is sufficient to reverse EMT in a mouse model of crescentic glomerulonephritis, resulting into repair of tubular atrophy and improvement of excretory renal function [5]. Direct counteraction between TGF-β1 and BMP-7 activation is unique, as it does not

Table 1 TGF-β1 and BMP7 actions in the kidney

TGF-β1	BMP-7
TGF-β1 inhibits branching morphogenesis in kidney development	BMP-7 is a mediator of branching morphogenesis associated with the mesenchymal-to-epithelial transition in kidney development
Progression of chronic renal disease is associated with increased TGF-β1 expression	Progression of chronic renal disease is associated with decreased BMP-7 expression
TGF-β1 induces epithelial-to-mesenchymal transition in chronic renal disease	BMP-7 reverses epithelial-to-mesenchymal transition in chronic renal disease
TGF-β1 activates interstitial fibroblasts in chronic renal disease	BMP-7 ameliorates fibroblast activation

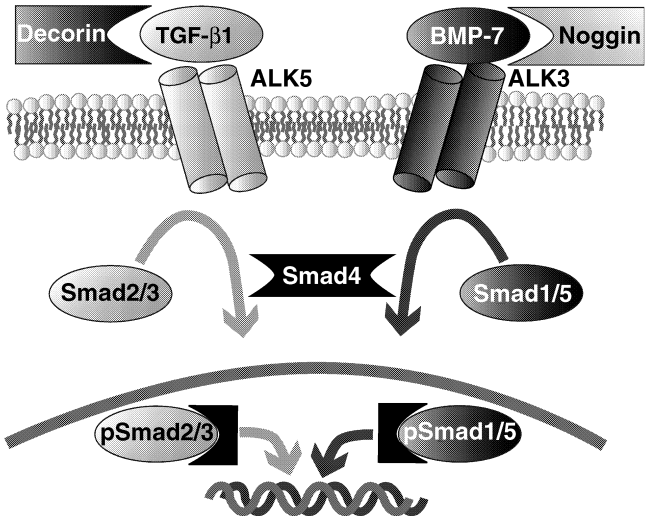

Fig. 3 BMP-7 counteracts TGF-β1 in a Smad-dependent manner. The schematic displays a simplified model of TGF-β1 and BMP-7 mediated signaling. TGF-β1 mediates its action via binding to its type I (ALK5) and type II receptors. The activated receptor phosphorylates Smad2 and/or Smad3, which then form heterocomplexes with Smad4. The heterocomplexes translocate into the nucleus and facilitate transcription of target genes. BMP-7 signaling is initiated by binding to type IIb receptors and type I receptors (ALK3, ALK6), which use Smad1, Smad5 and Smad8. After phosphorylation, Smad1/5/8 form heterocomplexes with Smad4 and translocate to the nucleus. Smad5-mediated BMP-7 signaling directly counteracts Smad3-dependent TGF-β1 induced EMT in tubular and mammary epithelial cells

involve extracellular trap-proteins, such as decorin, noggin or nodal [81, 82, 83]. Previously, physiologic negative regulators of TGF-β1 signaling with therapeutic utility have been identified. Soluble proteins such as TGF-β1-binding receptor-traps act by preventing access the receptor. Additionally, the proteoglycan decorin, the circulating protein α2-macroglobulin and the pro-region of the TGF-β1-precursor latency-associated polypeptide (LAP) have been identified as physiological regulators of TGF-β1-action [59]. Decorin has been used with limited toxicity in animal studies to prevent progression of chronic nephropathies [81, 84]. However, inhibition of TGF-β1 activity by decorin ameliorates progression of TGF-β1 induced chronic renal fibrosis, but reversal of disease pathogenesis associated with EMT was not demonstrated, as in the case of BMP-7. While decorin is an 'extracellular' competitor of TGF-β1 binding to its receptor its mechanism of action is different in comparison to that of BMP-7 action.

Conclusion

While TGF-β1 has long been identified as the major mediator of renal fibrosis, recent studies have provided increasing evidence that BMP-7 functions as its physiological antagonist in the kidney (Table 1) [5, 85]. BMP-7 is essential for MET-dependent nephrogenesis and branching morphogenesis, while TGF-β1 induces apoptosis of the metanephric mesenchyme and inhibits branching of the ureteric bud [86]. In the adult kidney, increased expression of TGF-β1 is associated with progression of chronic renal disease, while the expression of BMP-7 in the kidney is significantly decreased in injured kidneys [5, 74]. TGF-β1 is the main inducer of EMT in adult tubular epithelial cells, while BMP-7 reverses TGF-β1-induced EMT and restores tubular cell homeostasis [5]. TGF-β1 is a multi-functional growth factor and receptors for BMP-7 are widely distributed throughout the body. Thus, it is important to pursue studies to identify this antagonistic action on a given cell.

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