Recapitulation of Embryological Programmes in Renal Fibrosis – The Importance of Epithelial Cell Plasticity and Developmental Genes

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Key Words

Epithelial-mesenchymal transition · Tubulointerstitial fibrosis · Transforming growth factor- β · Gremlin · Diabetic nephropathy · Bone morphogenic proteins

Abstract

Chronic fibrosis represents the final common pathway in progressive renal disease. Myofibroblasts deposit the constituents of renal scar, thus crippling renal function. It has recently emerged that an important source of these pivotal effector cells is the injured renal epithelium. This review concentrates on the process of epithelial-mesenchymal transition (EMT) and its regulation. The role of the developmental gene, gremlin, which is reactivated in adult renal disease, is the subject of particular focus. This member of the cysteine knot protein superfamily is critical to the process of nephrogenesis but quiescent in normal adult kidney. There is increasing evidence that gremlin expression reactivates in diabetic nephropathy, and in the diseased fibrotic kidney per se. Known to antagonize members of the bone morphogenic protein (BMP) family, gremlin may also act downstream of TGF- β in induction of EMT. An increased understanding of the extracellular modulation of EMT and, in particular, of the gremlin-BMP axis may result in strategies that can halt or reverse the devastating progression of chronic renal fibrosis.

Introduction

Chronic kidney disease (CKD) imposes an enormous burden on society. Progression of CKD is irreversible, culminating in end stage renal failure (ESRF). Effective strategies slow the rate of progression of CKD but therapies to halt or reverse the process are currently absent. ESRF is a devastating diagnosis, both in human and health economic terms, currently managed by dialysis or transplantation. These strategies of renal replacement therapy (RRT) account for a large proportion of any developed world health care budget, yet unfortunately, the number of ESRF patients on RRT is increasing at a significant rate [1]. This can largely be ascribed to a dramatic rise in both incidence and prevalence of diabetic nephropathy (DN) in both the developed and developing world. This trend is most pronounced in the United States and is illustrated by 2004 US Renal Data System data. In 1980, DN was listed as the primary aetiology underlying ESRF in 20% of the prevalent population; by 2004, it was the underlying diagnosis in 45% of the prevalent population. The growing prevalence reflects both increasing incidence and longer patient survival [2].

Chronic progressive fibrosis is the 'final common pathway' to ESRF through which a variety of progressive renal diseases, including DN, advance. Scarred kidneys classically display the triad of glomerulosclerosis, interstitial fibrosis and tubular atrophy. Rather than histopathologic

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changes at the level of the glomerulus, it is the development of tubulointerstitial fibrosis (TIF) in primary glomerular disease that most closely correlates with rate of progression of chronic renal dysfunction. Irrespective of primary aetiology, TIF, once established, appears to follow a uniform, predictable course [3].

Recently, the concept of phenotypic plasticity of the renal tubular epithelial cell (TEC) has been established. This unique ability allows the injured epithelial cell to convert to a fibroblastic phenotype, capable of migration into the tubulointerstitium and thus of contribution to fibrogenesis [4, 5]. Delineation of the molecular mechanisms of this 'reversed embryogenesis' [4], or epithelial-mesenchymal transition (EMT), creates exciting new possibilities for the discovery of both novel disease biomarkers and therapeutic targets. This review will address current concepts of the molecular mechanisms of EMT, with particular focus on the recapitulation of developmental genes such as gremlin in the adult disease state.

EMT

Characteristic features of renal fibrosis include an atrophied tubular architecture and an accumulating extracellular matrix (ECM) that relentlessly deposits and expands the interstitial space [6]. Interstitial myofibroblasts are the primary effector cells in this process and their accumulation has been demonstrated to predict disease progression in many forms of CKD, including DN [7, 8]. Originally postulated to be either locally activated renal fibroblasts that migrate into the tubulointerstitium or bone-marrow-derived mesenchymal precursor cells, seminal work from Strutz et al. [9] suggested that TECs can transition to fibroblastic phenotype via EMT.

The concept of epithelial cell plasticity is central to organogenesis and more specifically, to renal embryology. The mammalian kidney derives from two distinct origins. The collecting system stems from epithelial wolffian duct, but the majority of adult renal epithelia (that is, from glomerulus to the beginning of collecting tubule) originates from metanephric mesenchyme. This latter group derives via the process of mesenchymal to epithelial transition (MET). This essential capacity of epithelial and mesenchymal cells to interconvert during development ensures that cells can acquire migratory capacity and thus traverse the extracellular environment, ultimately settling in distinct areas to form organs [10]. Conversely, in adult tissue, mesenchymal and epithelial cells have traditionally been considered as terminally differentiated.

It is postulated that EMT occurs in differentiated adult renal epithelium when the tubular cell responds to injury by losing its epithelial phenotype and acquiring the characteristic features of mesenchyme. EMT may thus allow epithelial cells to escape from an apoptotic fate [11] and enable migration into the tubulointerstitium. An additional pool of matrix-producing fibroblasts is thus created. Although this cell population can effect repair of injured tissue, it is proposed that this situation is maladaptive and favours fibrogenesis [5]. Thus, EMT is a process in which the programmes of renal embryology are reversed, allowing cell dedifferentiation back to original embryological mesenchymal phenotype. It is important to note that evidence to date has only observed EMT occurring in those kidney cells with lineage originally of MET derivation [4, 5]. Almost all *in vitro* studies that document EMT have employed proximal tubular cells as a model system. However there is recent evidence that distal tubular cells also undergo EMT in response to injury [12] and that glomerular parietal epithelial cells undergo EMT in *in vivo* models of CKD [13]. This makes intuitive sense as glomerular epithelial cells and distal tubular cells derive from the same embryonic mesenchymal origin. Conversely, there is evidence that collecting duct epithelium does not undergo phenotypic change on exposure to the multifunctional growth factor, TGF- β 1, consistent with the fact that it is of differing embryological origin [14].

EMT is characterized by a number of defined cellular changes. Initially, a transition in morphology from the cobblestone-like cell sheet typical of an epithelial phenotype to the elongated, fusiform cell sheet characteristic of fibroblasts occurs. The epithelial cells lose polarity and cell-cell adhesion and gain mesenchymal properties including motility. Secondly, a loss of epithelial markers (e-cadherin, cytokeratin, ZO-1) is demonstrated. A corresponding increase in mesenchymal markers such as vimentin, α -SMA and/or fibroblast-specific protein-1 (FSP-1) also occurs [15].

EMT in Renal Fibrosis

Murine FSP-1, an S100-binding protein, was identified as a mesenchymal marker in a landmark study suggesting phenotypic conversion of the TEC [9]. Some TECs were noted to become FSP-1 positive in murine models of CKD [16, 17]. Thus a new hypothesis was generated; EMT serves as a potential source of fibroblasts in the fibrotic kidney. Okada et al. [15] evaluated the effects

of various cytokines on murine TECs in vitro, demonstrating that the combination of transforming growth factor- β 1 (TGF- β 1) and epidermal growth factor (EGF) was most efficacious in inducing EMT. Evidence of EMT was subsequently demonstrated in a number of other animal models of CKD [14, 18]. Significant evidence for EMT in human disease has been demonstrated; in 133 human renal biopsies, the number of tubular cells demonstrating evidence of EMT clearly correlated with both serum creatinine and the degree of TIF [19]. Tubular EMT is also relevant in experimental and human DN; there is evidence that this is mediated by an advanced glycation end-product (AGE)–receptor for advanced glycation end-product (RAGE) interaction and thus may play a critical role in DN pathogenesis [20].

Pivotal work by Iwano et al. [21] allowed the quantitative, unambiguous identification of those renal fibroblasts in the interstitial pool that originally derived from tubular epithelium. These authors demonstrated that genetically tagged proximal TECs in transgenic mice exhibited a degenerate morphology, became disorganized and migrated into the interstitium. The ability to track the precise fate and movement of these cells enabled these authors to demonstrate that over one third of the fibroblast pool were of epithelial origin. These results established the very significant contribution of EMT to renal fibrosis [21]. Supportive evidence was provided by an in vivo model in which selective blockade of EMT was performed. Mice lacking tissue plasminogen activator (tPA) demonstrated blocked tubular EMT but no effect on local interstitial fibroblast activation. In the absence of EMT, progression of myofibroblast accumulation was blunted, and the kidneys were protected from the development of interstitial fibrosis after sustained ureteral obstruction. This study confirmed a definite role for EMT in the pathogenesis of renal interstitial fibrosis in the whole animal [22].

Mechanisms and Models of Tubular EMT

A cellular model of EMT has been constructed by Yang and Liu [23], primarily based on data from in vitro experiments. Four pivotal events occur during EMT in a highly regulated fashion: firstly, loss of epithelial adhesion; secondly, de novo expression of mesenchymal markers such as α -SMA, and cytoskeletal reorganization; thirdly, tubular basement membrane disruption and finally, acquisition of migratory and invasive capacity by the transformed cell.

There are several known inducers of EMT, including TGF- β 1 [24], connective-tissue growth factor (CTGF) [25], EGF [15], fibroblast growth factor-2 [26], AGEs [20], angiotensin II [27], interleukin-1 [28], matrix metalloproteinase-2 [29], and type I collagen [15]. Chief amongst this growing list of regulators is the profibrotic cytokine TGF- β 1, now well recognized as a major effector in the initiation and progression of renal disease. TGF- β 1 signalling pathways can initiate and drive to completion the entire EMT process in the renal tubular cell in vitro [24]. This growth factor may be the common downstream effector that mediates the actions of some or all of the other factors that induce EMT [4]. Multiple intracellular signalling pathways are involved in EMT. In brief, the Smad pathway, Rho A, mitogen-activated protein kinases, extracellular regulated protein kinases (ERK) and c-Jun N-terminal kinases are involved in TGF- β 1-induced EMT [30–34]. Of note, recent work has indicated that TGF- β 1 induces generation of cellular reactive oxygen species (ROS) and that these mediate EMT in renal tubular epithelial cells directly through activation of mitogen-activated protein kinases and indirectly through ERK-directed Smad-2 phosphorylation [35].

The increasing body of evidence that supports the concept of epithelial cell plasticity has enhanced understanding of the complex molecular pathways of EMT. One way in which this has been achieved is through large-scale transcriptomic analysis. Recent work from our group compared the transcriptomes of in vitro (tubular EMT) and in vivo (adriamycin nephropathy) models of tubular fibrosis; an example of one gene particularly identified was claudin-1, which appears to play an integral role in the structural integrity of the tubular epithelium [36].

Mediators of EMT – The Bone Morphogenic Proteins

Bone morphogenic proteins (BMPs) are secreted proteins forming a subgroup within the TGF- β superfamily of cysteine-knot cytokines. The group, containing over 30 members, was originally described for their ability to induce the formation of ectopic bone and cartilage in vivo. The BMPs, and particularly BMPs-2, -4 and -7, are known to play an important role in renal development [37–39].

The effects of BMP-7, like the other members of this family, antagonize TGF- β /Smad-dependent signalling in renal TECs. BMP-7 appears to have a unique role in nephrogenesis and, particularly, in the modulation of MET [40]. BMP-7 is a survival factor for the metanephric

mesenchyme and BMP-7 knockout mice exhibit severe renal dysgeneses and die shortly after birth from renal failure [37].

BMP-7 in the adult is primarily expressed in the kidney, and particularly localizes to TECs and glomerular epithelial cells. It appears integral to the maintenance of a differentiated renal epithelial cell phenotype [40]. The robust expression of BMP-7 in normal kidney is, however, dramatically reduced in the diseased, fibrotic kidney, and specifically in in vivo models of DN [41]. Thus, expression of BMP-7 opposes that of TGF- β 1, which increases in acute and chronic renal disease. It has now been demonstrated that exogenous BMP-7 attenuates progressive loss of renal function and renal fibrosis, reducing injury in in vivo models including obstructive nephropathy, tubulointerstitial nephritis, lupus nephritis and DN [42–44].

Attaining a precise understanding of the mechanisms by which BMP-7 achieves its renoprotective function is thus of high priority. BMP-7 has been shown to reduce the release of proinflammatory cytokines [45]. In the renal mesangial cell, BMP-7 counters the proinflammatory effects of TGF- β 1 [46]. In TECs, an in vitro model demonstrated that BMP-7 reversed EMT by directly counteracting TGF- β 1-induced signalling [12]. Zeisberg et al. [47] recently reported that BMP-7 induced some phenotypic changes consistent with MET in adult fibroblasts (reacquisition of e-cadherin and reduced motility). In addition, they reported detection of a novel interstitial cell population co-expressing FSP-1 and e-cadherin in an in vivo mouse model of chronic fibrosis treated with recombinant BMP-7. Thus, the interesting possibility arises that adult fibroblasts may also retain plasticity and thus potentially contribute to healthy epithelial cells in repaired renal tubular structures.

The ability of BMP-7 to reduce inflammatory mediator production, coupled with the potential to reverse TIF, is clearly crucial for maintenance of the structural and functional integrity of the tubular epithelium. BMP-7 appears to counteract endogenous mediated TGF- β 1 action [48]. Antagonism of the effects of BMP-7 will clearly have deleterious effects, disrupting renal homeostasis and tipping the balance towards progressive inflammation and fibrosis.

Gremlin: A Model for the Reactivation of Developmental Genes in Diabetic Nephropathy and Tubulointerstitial Fibrosis

Gremlin is a highly conserved, secreted protein that fulfils a pivotal function in diverse processes of growth, differentiation and development, in many cases by antagonizing the activity of BMPs [49]. Gremlin is a cysteine knot protein that can heterodimerize with specific BMPs, thus preventing their interactions with TGF- β receptors. Other members of this cysteine knot superfamily include the tumour suppressor DAN and the head-inducing factor Cerberus [50]. Originally identified as a dorsalizing factor in *Xenopus laevis*, gremlin appears to favour antagonism of BMP-2, -4 and -7 [51, 52]. Gremlin is preferentially expressed in fibroblasts and appears to play a role in cell cycle arrest; overexpression in rat and mouse cell lines leads to growth inhibition and cell cycle arrest [53].

Gremlin is a key regulator of BMP activity in embryonic development and is known to be the principal BMP antagonist that modulates early limb outgrowth and patterning in the mouse embryo [54]. A primary role for gremlin in nephrogenesis is reinforced by the observation that ablation of gremlin expression causes death in homozygous mice within 48 h of birth due to complete renal agenesis [54, 55]. As previously mentioned, the targets of gremlin binding, BMP-2, -4 and -7, also have essential functions in renal development.

Recent work has established that gremlin mediates its action via induction of epithelial to mesenchymal feedback signalling. Metanephric renal (and limb bud) organogenesis occurs via BMP antagonism and thus gremlin is confirmed as the essential extracellular signal which initiates renal development [55].

In 1999, our group employed suppression subtractive hybridization to identify 15 genes differentially induced when human mesangial cells were treated with elevated ambient glucose [56]. This group includes two novel development genes. One of these was 'Induced in High Glucose' (IHG)-2, later identified via cloning in silico as the developmental gene, gremlin [57]. This key discovery generated the hypothesis that reactivation of developmental genes may occur in adult kidney disease, and specifically, that gremlin may play a role in the pathogenesis of DN. In vitro models of DN were utilized to validate the discovery of this pivotal gene. Elevated levels of gremlin were observed in extracts from model systems such as kidney mesangial cells treated with high glucose, or exposed to cyclical mechanical strain (a model of the glo-

merular hypertension of diabetes). TGF- β 1 was shown to induce gremlin expression in human mesangial cells in vitro, while the stimulatory effect of high glucose on gremlin expression was attenuated by the addition of anti-TGF- β 1 antibody [57].

In vivo models provided evidence to support the initial hypothesis. Elevated levels of gremlin were observed in glomeruli micro-dissected from kidneys of the streptozotocin STZ-induced diabetic rat model [57]. Increased levels of gremlin expression correlated with a marked decrease in tubular BMP-7 expression early in experimental DN [41]. Thus, it is possible that gremlin acts downstream of TGF- β 1 to downregulate BMP-7. Supportive evidence for this was seen in the Goto-Kakizaki rat model of non-obese type II diabetic rats which, at 9–10 months, demonstrated some phenotypic evidence consistent with EMT; tubular e-cadherin was lost and α -SMA upregulated on immunocytochemistry of renal tissue. Quantitative PCR results included significant increases in gremlin, TGF- β 1 and CTGF mRNA in renal extracts from the rats at 9–10 months [58]. These findings coincided with histological changes consistent with moderate DN and TIF [58]. The 5/6 nephrectomy model of glomerular hypertension also demonstrated increased gremlin expression in kidney tissue, thus suggesting a more global role in renal fibrosis [59].

Recent work from our group has pointed to a role for gremlin in the pathogenesis of TIF. Preliminary work with TECs indicated that TGF- β 1 induced gremlin expression [59]. The first evidence of increased expression of gremlin in human CKD was then demonstrated. Diagnostic kidney biopsies were obtained from the European Renal Complementary DNA Bank, and gremlin expression and localization compared between normal control and tissues with primary aetiologies including DN, rapidly progressive glomerulonephritis, IgA nephropathy and minimal change disease. Gremlin expression was found to be undetectable in normal adult kidney; however, there was a significant increase in gremlin expression in the 10 DN biopsies studied. In situ hybridization demonstrated marked gremlin expression which correlated with more advanced disease. There was clear gremlin localization, primarily to the tubular compartment, and a positive correlation with fibrosis severity and elevations in serum creatinine. Importantly, gremlin expression was found to co-localize with TGF- β 1 expression, supporting the hypothesis that TGF- β 1 modulates gremlin expression in the tubulointerstitial compartment. Quantitative studies with real time PCR were performed; gremlin mRNA was found to be significantly elevated in

the DN biopsies compared to normal control (2.6 fold; $p < 0.01$). Again, a direct correlation was seen between serum creatinine and TIF score. A significant increase in gremlin expression was also seen in the rapidly progressive glomerulonephritis group [60].

It thus appears that gremlin is an embryologically expressed gene critical to the regulation of renal morphogenesis that becomes quiescent after birth. This developmental gene may be reactivated in the adult diseased kidney, with the re-emergence being linked to an attempted tissue repair that becomes maladaptive [50]. The high abundance of gremlin in the tubular compartment in advanced DN and its correlation with fibrosis severity and creatinine points to an important role for gremlin in the pathogenesis of TIF. Co-localization with TGF- β 1 points to this profibrotic cytokine having a role in the modulation of gremlin in the tubular compartment, as it does in the glomerular compartment. As a secreted modulator of TGF- β 1 signalling, gremlin may be an attractive option as a diagnostic marker and possibly as a target for antifibrotic therapy.

Table 1 summarizes the roles of BMP-7, gremlin and TGF- β 1 in renal embryology, normal adult kidney and diseased adult kidney. Figure 1 is a schematic outlining the proposed extracellular modulation of EMT by TGF- β , gremlin and BMP-7.

EMT and Potential Therapeutic Targets

The fate of the tubular epithelial cell that has undergone EMT in response to injury is fascinating. Potentially, the transformed cell may proliferate to expand the fibroblast population, undergo apoptosis, or revert back to epithelial type [4]. This last possibility offers exciting potential for therapeutic development – if on exposure to regenerative cues the cell is able to redifferentiate back to epithelial form, and then it would appear possible to reverse renal fibrosis.

A significant role for modulation of EMT by exogenous agents has been described. Angiotensin II potentiates EMT and its inhibition leads to reduced renal fibrosis [27]. ROCK, a downstream effector kinase of RhoA that plays an important role in cytoskeletal disassembly in EMT, can be inhibited by Y-27632, a ROCK antagonist. This agent attenuates fibrosis in a murine model of obstructive nephropathy [67]. Experiments using ROS suggest that antioxidants and mitogen-activated protein kinase inhibitors may have a role in antagonism of EMT [35]. It has been demonstrated that the AGE-RAGE path-

Table 1. Summary of current data for gremlin, BMP-7 and TGF- β in renal development and disease

	Gremlin	BMP-7	TGF- β
General	<ul style="list-style-type: none"> – member of cysteine knot superfamily secreted peptide – heterodimerizes with BMPs; prevents interaction with serine/threonine kinase receptors – roles in growth, differentiation and development 	<ul style="list-style-type: none"> – member of TGF-β superfamily – secreted peptide – activates serine/threonine kinase receptors – diverse roles in growth and development 	<ul style="list-style-type: none"> – member of TGF-β superfamily – secreted peptide, 3 isoforms – ligand-induced activation of heteromeric transmembrane serine/threonine receptor kinases – multifunctional; regulates cell proliferation, differentiation, apoptosis, immune response and ECM remodelling
Renal organogenesis	<ul style="list-style-type: none"> – principle BMP antagonist modulating renal development [46] – in knockout mice, complete renal agenesis [46, 47] 	<ul style="list-style-type: none"> – mediates branching morphogenesis & modulates MET [33] – in knockout mice, severe renal dysgenesis [30] 	<ul style="list-style-type: none"> – inhibits branching morphogenesis in kidney [6] – loss of TGF-β2 leads to renal agenesis in female mice [61]
Diabetic nephropathy			
i) in vitro	<ul style="list-style-type: none"> – induced in mesangial cells high glucose [48] – TGF-β 1 [49] – cyclical mech. strain [49] 	<ul style="list-style-type: none"> – nil to date 	<ul style="list-style-type: none"> – high ambient glucose upregulates expression and bioactivity of TGFβ in mesangial, tubular and almost all other renal cell types [62–64]
ii) in vivo	<ul style="list-style-type: none"> – elevated levels renal tissue STZ rat model [34, 49] – GK rat model [50] 	<ul style="list-style-type: none"> – dramatic reduction in renal expression in STZ rat model [34] – exogenous recombinant BMP 7 attenuates progression DN [36] 	<ul style="list-style-type: none"> – overexpression of TGF-β in both glomerular and tubulointerstitial compartments of diabetic animals [65, 66]
iii) human	<ul style="list-style-type: none"> – Elevated levels in human DN biopsies [52] 	<ul style="list-style-type: none"> – nil to date 	<ul style="list-style-type: none"> – correlates with disease progression [67]
Tubulointerstitial fibrosis			
i) in vitro	<ul style="list-style-type: none"> – TGF-β1 induces gremlin expression in tubular cells [51] 	<ul style="list-style-type: none"> – counteracts TGF-β in renal mesangial and tubular cells [12, 39] – reduces release proinflammatory cytokines at level tubular cell [38] – induces some changes consistent with MET in renal fibroblast [40] 	<ul style="list-style-type: none"> – primary inducer of EMT; can initiate entire course in isolation [23] – secreted by the tubular cell as undergoes EMT; autocrine loop [6]
ii) in vivo	<ul style="list-style-type: none"> – nil to date 	<ul style="list-style-type: none"> – attenuates progressive fibrosis in multiple rodent models [35, 37] 	<ul style="list-style-type: none"> – increased expression associated with progression of renal fibrosis [68]
iii) human	<ul style="list-style-type: none"> – Colocalizes with TGF-β in tubulointerstitial compartment in DN [52] – increased expression in RPGN [52] 	<ul style="list-style-type: none"> – nil to date 	<ul style="list-style-type: none"> – localizes to tubulointerstitial compartment and correlates with fibrosis severity [52]
Summary	<ul style="list-style-type: none"> – induced by TGF-β1 – antagonises BMPs – pivotal role in modulating renal organo-genesis – not expressed in normal kidney – recapitulated in adult renal disease, with highest expression in tubular compartment 	<ul style="list-style-type: none"> – expression in normal & diseased kidney inversely proportional to TGF-β – pivotal role in renal organogenesis – highly expressed in normal adult kidney – lost in fibrosis – exogenous administration ameliorates fibrosis – key role in maintenance normal tubule homeostasis 	<ul style="list-style-type: none"> – multifunctional cytokine with pivotal role in EMT and renal fibrosis – induces gremlin – inhibits BMP 7 in vitro – increasing expression in acute and chronic renal disease, and specifically in DN – pleiotropic effects mean not an attractive therapeutic target
RPGN = Rapidly progressive glomerulonephritis.			

way is critical for the pathogenesis of diabetic complications. It now appears that AGEs play an important role in the development of renal fibrosis in a non-diabetic context. AGEs have been shown to be capable of EMT induc-

tion in the absence of exogenous TGF- β 1 [20]. Blockade of the AGE-RAGE interaction and RAGE-activated ERK-1/2 signalling resulted in a complete inhibition of tubular EMT; thus, blockade of these pathways point to

Fig. 1. Overview of the epithelial-mesenchymal transition as modulated by TGF- β , BMP-7 and gremlin. Free TGF- β and BMP-7 are bidirectionally regulated in receptor binding by positive or negative proteins in the extracellular space [61]. CTGF binds TGF- β 1 and enhances TGF- β binding to its extracellular receptor [62]. It is hypothesized that gremlin may also enhance TGF- β receptor binding. In addition, we have demonstrated that levels of gremlin mRNA are upregulated in an in vitro model of TGF- β -induced EMT, thus suggesting that gremlin may act downstream of TGF- β [59, 63]. Gremlin (like the other BMP antagonists noggin, follistatin and chordin) heterodimerizes with BMPs in the extracellular space and prevents them binding to BMP receptors [52]. CTGF has been shown to bind directly to BMP-2 and -4 and to inhibit interaction with the receptor [62]; CTGF is proposed to also have an inhibitory effect on BMP-7. Kielin/chordin-like protein (KCP)-1 is newly documented as a novel enhancer of BMP-7 binding [64]. Activation of the BMP-7 receptor induces phosphorylation of Smad-1, -5 and -8, which opposes epithelial-mesenchymal transition (EMT) in the nucleus [5].

Activation of the TGF- β receptor induces phosphorylation of Smad-2 and -3 and its nuclear import of Smad-4, resulting in transcriptional activation driving EMT [5]. Downstream kinases such as integrin-linked kinase (ILK) and PKB/Akt phosphorylate targets that mediate EMT progression [65]. Activation of the TGF- β receptor also results in activation of a number of other intracellular signalling mechanisms that promote EMT. These include: activation of Rho A which induces ROCK [31]; activation of ROS which induce ERK-directed activation of the Smad pathway [35], and the recently demonstrated regulation of the polarity protein Par-6. The phosphorylation of Par-6 results in Smurf-1 interaction, which targets RhoA for degradation, resulting in loss of tight junctions and ultimately EMT [66].

Thus TGF- β 1 and gremlin promote EMT, and BMP-7 inhibits EMT. The transitioning epithelia change into myofibroblasts by rearrangement of their actin cytoskeleton and expression of new proteins such as interstitial collagens, matrix metalloproteinases-2 and -9, and FSP-1 [4, 5]. Pharmacologic strategies that act to inhibit gremlin, a BMP antagonist, may thus inhibit EMT.

new therapeutic strategies [68]. Administration of an AGE cross-link breaker (ALT-711) reduced evidence of EMT in diabetic rats in association with reduced tubular AGE and TGF- β expression [20]. Strutz et al. [69] have identified several myofibroblast inhibitors, including the

methyl-xanthines, pentoxifylline and pentifylline, which robustly prevented fibroblast formation and proliferation per se as well as ECM formation [69].

Endogenous antagonists of EMT should have limited toxicity and thus possess attractive therapeutic potential.

Exciting recent work has identified two endogenous antagonists of EMT both in vivo and in vitro; BMP-7 and hepatocyte growth factor (HGF) [12, 14]. Both of these factors, which appear to be inherently renoprotective, play a substantial role in embryonic nephrogenesis and in maintenance of adult kidney tubular epithelial cell homeostasis [4].

As detailed earlier in this review, recombinant human BMP-7 has resulted in significant renal recovery (in terms of both function and histology) in different rodent models of chronic renal injury in mice and rats [12, 42–44]. Importantly, in both the in vivo models of DN and unilateral urethral obstruction, the recovery of renal function seen with BMP-7 was greater than the benefit obtained from ACE inhibition [43, 44].

Important recent work by Lin et al. [64] has demonstrated that the novel protein kielin/chordin-like protein (KCP), acts as a BMP enhancer. This action is likely to attenuate renal fibrosis as KCP-null mice are more susceptible to the development of renal fibrosis in models of acute tubular necrosis and unilateral urethral obstruction. KCP is expressed in embryonic tissue but not in the normal adult. It appears to be reactivated in response to stress and to be involved in the regulation of renal fibrosis, acting as a potentiator of BMP signalling in a paracrine-like manner.

Liu et al. [70] have established that in a rat remnant kidney model, antagonism of HGF signalling with neutralizing antibody led to marked induction of renal tubular expression of the mesenchymal marker, α -SMA. In vitro studies subsequently demonstrated that HGF blocks TGF- β 1-induced EMT, reversing the phenotypic conversion [14]. Further work in a unilateral urethral obstruction animal model illustrated that administration of HGF protein or gene blocks EMT and attenuates TIF [27].

Recent data on gremlin highlights that there may also be a potential therapeutic role for the modulation of this BMP antagonist and this offers an exciting avenue for future research.

Conclusions

Tubular fibrosis, the ‘final common pathway’ in many forms of progressive glomerular disease, closely correlates with the inexorable decline towards ESRF and requirement for RRT. EMT is now established as an important pathway that generates myofibroblasts, the pivotal effector cells in the diseased tubulointerstitium. This epithelial cell plasticity, so important in normal organogenesis,

is likely to represent one possible response of the tubular epithelial cell confronted by sustained injury [5]. The transformed TEC has the potential to support tissue repair but unfortunately, the recapitulation of this developmental programme in the setting of the adult disease state appears to be maladaptive and the balance is tipped towards fibrogenesis. However, the unique ability of the cell to transition from epithelial to mesenchymal phenotype highlights an exciting area for potential therapeutic manipulation. As increasing evidence points to the cells ability to redifferentiate back to epithelial type with the appropriate regenerative cue, our need to precisely understand the complex mechanisms regulating EMT intensifies. One way in which this can be achieved is through large scale transcriptomic analysis. Recent work from our group compared the transcriptomes of in vitro (tubular EMT) and in vivo (adriamycin nephropathy) models of tubular fibrosis; an example of one gene particularly identified was claudin-1, which appears to play an integral role in the structural integrity of the tubular epithelium [36]. The discovery of gremlin as a potential player in the pathogenesis of DN was made through earlier techniques used to analyze the diseased renal transcriptome. Gremlin, a developmental gene pivotal in nephrogenesis, is quiescent in normal adult kidney and then reactivated in chronic kidney disease. First identified in in vitro models of DN, there is now increasing evidence that gremlin is reactivated in and modulates TIF per se, possibly downstream of TGF- β . Gremlin is a BMP antagonist and thus characterization of its interactions with these molecules in the tubulointerstitial compartment is of great significance. An increased understanding of BMP-7 and other endogenous antagonists of EMT has resulted in in vivo models of CKD that demonstrate renal recovery when these agents are administered. Future work delineating the BMP-gremlin axis in modulation of epithelial cell plasticity may result in pharmacologic strategies which achieve reversal of human chronic renal fibrosis.

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