Epithelial-mesenchymal transition and the reversal of renal fibrosis

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Abstract

Epithelial-mesenchymal transition (EMT) is a process in which epithelial cells loose their epithelial characteristics and acquire phenotypic features characteristic for mesenchymal cells. EMTs occur in a number of processes including embryonic development, cancer progression and tissue fibrogenesis in adult organs. Much progress has been made in recent years to elucidate the mechanisms inducing EMT in these processes. Moreover, signal transduction cascades have been identified. Here,
current concepts of EMT in these distinct settings will be discussed focusing on the role of EMT in renal fibrogenesis. Moreover, in recent years it has become evident that fibrotic processes are potentially reversible even in humans. The central role of EMT in the reversibility of renal fibrosis is outlined.

Introduction

Chronic progressive renal disease is characterized by the triad of glomerulosclerosis, tubular atrophy and tubulointerstitial fibrosis [1]. It has only recently been widely accepted in the field of nephrology that involvement of the tubulointerstitium is at least as important as glomerular disease for the prognosis of any given renal disease. However, the fact that the tubulointerstitial space plays an essential role in the progression of renal disease is not surprising given the fact that tubules and interstitium occupy more than 90 per cent of kidney volume [2]. Historically, the importance of the tubulointerstitial area for renal function was noted as early as 1844 when Henle and Pfeufer in Switzerland described the case of a 25-year old girl with Bright’s disease who died of anasarca [3]. Similarly, Toynbee in England had come to concurring conclusions at the same time [4]. However, the interest in tubulointerstitial changes in glomerulopathies waned subsequently and these changes were finally considered trivial [5]. Spühler and Zollinger, studying patients with primary interstitial nephritis induced by consumption of non-steroidal anti-inflammatory drugs, are credited with the rediscovery of the significance of the tubulointerstitial space for renal function [6]. Again 5 years later, Hutt and coworkers rediscovered the significance of the tubulointerstitium in non-primary interstitial inflammation focusing on primary glomerular disease [7]. This group examined biopsy specimens from 15 patients with acute manifestations of glomerulonephritis and compared their histologic findings with the respective clinical outcome. Unlike their expectations, the glomerular changes did not correlate with renal function as evaluated by the creatinine clearance whereas changes in the tubulointerstitial space did. These findings were substantiated by studies from Risdon and coworkers as well as Schainuck and Striker [8-10]. Stimulated by these studies, German pathologist Adalbert Bohle set out to examine the significance of tubules and interstitium systematically focusing on a number of specific forms of glomerular disease. He was able to establish a robust correlation between the morphometrically determined interstitial volume and renal function (albeit measured solely by serum creatinine values) in studies on mesangioproliferative [11], membranous [12], focal-sclerosing [13] and membranoproliferative [14] glomerulopathies. All of these studies concurred that the amount of interstitial infiltration and the development of interstitial fibrosis were relatively accurate predictors of renal function 5 or more years
after the initial observation [15]. At the same time, the group established the significance of the tubulointerstitial space for secondary forms of glomerulopathies including diabetic nephropathy [16] and glomerular amyloidosis [17]. The findings by Bohles’ group were subsequently confirmed by a number of researchers including Jepsen and Mortensen [18], Katafuchi et al. [19], Abe et al. [20], Alexopoulos et al. [21], Lane et al. [22], and Ziyadeh et al. [23]. Furthermore, tubulointerstitial changes have been shown to be of functional importance for non-glomerular disease such as nephrosclerosis [24], chronic pyelonephritis [25], and polycystic kidney disease [26].

**Renal fibrogenesis**

The process and outcome of tissue fibrosis has been compared to regular wound healing. Accordingly, three phases of renal fibrogenesis can be distinguished: induction, inflammatory, and post-inflammatory phases [27]. Variability in these phases may account for heterogenous outcomes. Renal fibrogenesis differs from typical wound healing, however, in that true resolution is a rare event. Conversely, matrix synthesis in most cases continues resulting in destruction of normal organ architecture and subsequent loss of renal function. Matrix synthesis may even continue despite resolution of the primary inflammatory process [2]. The three phases are the induction phase, the inflammatory phase and the post-inflammatory phase [1]. Here we will focus on the first two phases since they are critical for the development of EMT.

The induction phase is critical for the subsequent accumulation of matrix proteins. This phase is characterized mainly by the influx of infiltrating mononuclear cells. In almost all forms of primary or secondary glomerular disease, interstitial infiltrates have been described, including primary glomerulonephritis, systemic vasculitis, chronic renal ischemia and acute renal allograft rejection [28]. However, fibrosis may occur even without prior interstitial inflammation as demonstrated by Lavaud and colleagues in the Zucker obese rat [29], at least experimentally. Infiltrating mononuclear cells are composed of monocytes/macrophages and lymphocytes, particularly T-lymphocytes [30]. The degree of interstitial infiltration correlates with renal function in several forms of immune-mediated renal disease, including lupus nephritis and membranous glomerulonephritis [31]. Müller and colleagues were able to demonstrate that the number of tissue CD4+ cells in particular displays a close correlation with renal function [30]. Conversely, the association of CD14+ monocytes/macrophages with subsequent kidney damage has remained controversial [32]. Kondo et al. showed convincingly that mast cells are also recruited to the kidney, and that the number of infiltrating mast cells showed a good correlation with the degree of interstitial fibrosis [33]. The localization of inflammatory mononuclear cells is either within the interstitial
space or between tubular epithelial cells and the tubular basement membrane. This latter process has been termed tubulitis; however, its functional significance is still unclear [34].

The tubular epithelial cell has a central role in the formation of interstitial inflammation due to exposure to high-grade proteinuria which often accompanies primary glomerular disease. Filtered proteins may be directly tubulotoxic or chaperon’s cytokines emanating from upstream injured glomeruli and circulation. In fact, many clinical studies have shown that patients with a higher degree of proteinuria have a more rapid decline in renal function [35]. The central role of the tubular epithelial cell in secondary interstitial inflammation is outlined in figure 1.

Figure 1. Graphic illustration of the central role of the tubular epithelial cell in the formation of secondary interstitial inflammation. Tubular epithelial cells get stimulated mainly by proteinuria and inflammatory cytokines, and respond by synthesizing and secreting various chemokines (after [1]).

The endothelial cell is the second key cell in the mediation of interstitial infiltrates since all infiltrating cells need to migrate through this mechanical barrier. However, knowledge about changes in these cells during the early phase of fibrogenesis is surprisingly limited. The importance of this cell type was exemplified by a study by de Greef et al. in acute renal failure. This group observed that vascular endothelial cells of the vasa recta in the outer medulla displayed de novo expression of the co-stimulatory molecule B7-1 starting 2 hours after reperfusion. Blocking this molecule by neutralizing antibodies
caused complete abrogation of reperfusion injury [36]. It is currently unclear how these findings relate to renal fibrogenesis in chronic kidney disease. Clearly, further studies are needed to define the role of vascular endothelial cells in renal fibrogenesis.

Infiltration of inflammatory cells results in the activation and proliferation of resident fibroblasts. Activation of resident interstitial fibroblasts is characterized by the de-novo expression of $\alpha$-smooth muscle actin whose expression is normally restricted to vascular smooth muscle cells; hence, the name myofibroblast for these cells. These resident fibroblasts become activated by stimulation with cytokines including transforming growth factor (TGF)-ß1 [37], platelet-derived growth factor (PDGF) [38], and fibroblast growth factor (FGF)-2 [39] among others. While a number of studies in human glomerular disease described a good correlation between the number of $\alpha$-smooth muscle positive interstitial cells and renal function, these studies probably do not sample the complete fibroblast participation [40]. Fibroblast activation is uneven and the pool of fibroblasts engaged in fibrogenesis are heterogenous in their biochemical synthesis [41,42]. Immunohistochemical profiles in normal human kidneys suggest that a subpopulation of interstitial fibroblasts may express constitutively $\alpha$-smooth muscle actin as well as the PDGF receptor $\beta$-chain [43]. It is currently unclear if the increase in number of $\alpha$-smooth muscle actin positive cells is the result of residual proliferating smooth muscle cells, proliferation of the few fibroblasts constitutively expressing the protein, or by de novo expression of formerly non-expressing cells. Furthermore, for some fibroblasts activation is not associated with de novo expression of $\alpha$-smooth muscle actin [42]. Furthermore, the degree of change in phenotype in the transition from fibroblast to myofibroblast is often underappreciated since expression of $\alpha$-smooth muscle actin is not the only biochemical change to occur [44]. For example, Rodemann and Muller demonstrated that fibroblasts from fibrotic kidneys expressed a number of proteins not detectable in their normal kidney derived counterparts [45]. Recently, Ru and colleagues noted even among so-called myofibroblasts a higher degree of variability and complexity due to different levels of $\alpha$-smooth muscle actin expression [46]. Very recently, Faulkner et al. demonstrated very elegantly that in the model of accelerated angiotensin II- induced renal fibrosis early expansion of $\alpha$-smooth muscle actin-positive cells was the key step for the progression of the disease [47]. Collectively, these studies support the notion that fibroblasts from kidneys with interstitial fibrosis differ from fibroblasts in normal kidneys.

However, activated resident interstitial fibroblasts are not the only cells participating in extracellular matrix production. A number of studies have confirmed the existence of bone marrow-derived cells within the interstitium
and the mesenchymal-epithelial transition of bone marrow cells to tubular epithelium [49]. Preliminary evidence using genetically tagged fibroblast and tubular epithelial cells indicates that fibroblasts derived from bone marrow comprise about 12% of the resident interstitial population in normal murine kidneys [50]. In that study, this percentage did not change when an experimental model of progressive renal disease was induced. Conversely, up to 36 per cent of additional extracellular matrix producing cells derived from tubular epithelial cells by a process entitled epithelial-mesenchymal transition (EMT), the main topic of this review.

**Epithelial-mesenchymal transition in renal fibrogenesis**

In the literature, the terms ‘epithelial-mesenchymal transformation, transition’ or transdifferentiation’ are often used synonymously. However, the term “transformation” classically describes the oncogenic conversion of epithelia. The term “transdifferentiation” classically refers to differentiated cells changing into other differentiated cells [51]. For example, transdifferentiation has been observed in retinal pigmented cells that become lens epithelia [52]. Recently, the term epithelial-mesenchymal “transition” has been used preferentially to describe the conversion of terminally differentiated epithelia into cells with a mesenchymal phenotype [50]. EMT is a variant of transdifferentiation and a well recognized mechanism for dispersing cells in vertebrate embryos [53], forming fibroblasts in injured tissues [50, 54], or initiating metastases in epithelial cancer [55, 56]. Such EMT should be considered a distinct phenomenon as opposed to the transient, spontaneously reversible “scattering” of epithelial cells, which can be observed *in vitro*. The conversion of epithelial to mesenchymal cells was first described only in 1982 in a seminal study by Greenburg and Hay [57].

Regarding renal fibrogenesis, EMT was first demonstrated by our group in a murine model of anti-tubular basement membrane disease by cloning of a specific fibroblast marker, named fibroblast specific protein-1 (FSP-1), a member of the S100 family [54]. Its expression is constitutive in tissue fibroblasts under physiologic conditions [54], and its promoter contains a *cis*-acting element (FTS-1) highly specific for fibroblasts [58]. This specificity was demonstrated in an elegant experiment by placing the herpes virus thymidine kinase under the control of the FSP-1 promoter in transgenic mice, and specific killing of fibroblasts and preventing interstitial fibrosis when rescued by the addition of nucleoside analogs [59]. Analyzing two mouse models of chronic progressive renal disease, FSP-1 expression was robustly upregulated in the tubulointerstitium. The initial staining pattern was in close similarity to the distribution of collagen-producing cells in a rabbit model of
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anti-GBM disease with a perivascular accentuation [60]. In addition, however, *de novo* expression of this fibroblast-specific protein could be detected in tubular epithelial cells in both models of chronic progressive renal disease; suggesting a possible transition of these cells to a mesenchymal phenotype [61]. This phenomenon had been described in a number of organs including thyroid and mammary gland as well as retina (reviewed in [53]). As EMT is a dynamic process which involves the gradual loss of epithelial cell markers paired with a gain of mesenchymal markers, it is associated with various intermediate cell stages co-expressing epithelial cell markers as well as mesenchymal markers. In order to distinguish stress-fiber expressing epithelial cells from cells which are actively undergoing EMT, the loss of epithelial adhesion molecules such as E-cadherin and ZO-1 paired with increased expression of mesenchymal markers such as vimentin, \(\alpha\)-smooth muscle actin and FSP-1 and migratory activity are used as EMT criteria; although absolute criteria have not yet been defined. Very recently, EMT has been described in fibrotic processes in organs other than the kidney including lung [62, 63] and liver (Zeisberg, unpublished observations).

The occurrence of EMT in the kidney is not surprising. Since tubular epithelial cells (with the exception of collecting duct cells) are derivatives of the metanephric mesenchyme, they seem to be particularly well suited to undergo phenotypic changes towards a mesenchymal phenotype. This process can thus be categorized as recapitulation of the renal developmental programs [64]. EMT is an essential development feature in metazoans. Such EMT of primitive epithelial cells enables them to migrate and settle at distant sites of the embryo, where they might participate in the formation of epithelia via a mesenchymal-epithelial transition (MET) [65]. In mammals, EMT first occurs at the blastula stage during the formation of the parietal endoderm which later contributes to extraembryonic tissues. In the setting of the primitive streak, EMT takes place in the so-called marginal zone, where the primitive visceral endoderm is in contact with the trophoectoderm [55]. The newly formed mesodermal mesenchymal cells appear at the primitive streak and migrate to form the embryonic and extraembryonic mesodermal derivates. Thus, the kidney has evolved as a model system to study embryonic and adult plasticity between epithelia and mesenchyme [66]. Kidney development depends on a program of reciprocal inductive interactions between two mesodermal derivatives, the ureteric bud and the metanephric mesenchyme [67]. In mice, at approximately 10.5 days postcoitum, the ureteric bud contacts the metanephric mesenchyme, inducing these cells to condense and aggregate [67]. Subsequently, this induced population undergoes mesenchymal to epithelial transition (MET) to form a comma shaped node, which elongates to form an S-shaped tubule [68]. Further morphogenesis and differentiation of this S-shaped tubule results in the formation of the glomerulus and the distal and proximal
tubule elements of the mature nephron [68]. Because EMT of tubular epithelial cells in the adult kidney occurs in cells which originally stem from mesenchymal cells via MET, it has been proposed that EMT of adult cell is a rudiment of an embryonic cell plasticity, which could be potentially regulated by developmental morphogens [64]. In this regard, it is important to note that EMT has been described only in tubular epithelial cells originating from MET derivation. Collecting duct cells, which are of different embryologic origin, may be resistant to the development of EMT [69].

Other examples of EMT during development are the neural crest cells which give rise to cells of various lineages such as bone, muscle and the peripheral nervous system; the EMT of the somite walls giving rise to the sclerotome during somitogenesis; and EMT of the oral epithelium during palate formation. A specialized form of EMT, an endothelial-mesenchymal transition (EndMT) occurs during heart development [70]. This process occurs in the outflow tract and atrioventricular canal of the embryonic heart in a spatiotemporally restricted manner. The mesenchymal cells which form the atrioventricular cushion, the primordia of the valves and septa of the adult heart, are derived from the endocardium via an endothelial-mesenchymal-transition (EndMT) [70].

Regarding EMT during fibrogenesis in adult kidneys, de novo expression of FSP-1 is not the only change observed in tubular epithelial cells undergoing EMT. Ng and coworkers described the de-novo expression of the myofibroblast marker α-smooth muscle actin in tubular epithelial cells starting at day 21 after 5/6 nephrectomy [71]. Tubular epithelial cells lost their apical-basal polarity and seemed to migrate into the interstitium as demonstrated by electron microscopy. Of particular note are the findings in that study that complete loss of epithelial characteristics occurred exclusively in areas of complete tubular disruption.

What causes the plasticity of EMT?. Okada et al. examined the effects of various cytokines on the expression of FSP-1 in tubular epithelial cells in vitro and found the most robust induction by the combination of TGF-β and epidermal growth factor (EGF) [72]. Fan and coworkers described a dose-dependent effect of TGF-β1 on the transition of a rat tubular epithelial cell line indicated by an increase in the number of α-smooth muscle actin positive cells and downregulation of the epithelial adhesion molecule E-cadherin [73]. Our group analyzed the effects of FGF-2 on four aspects of EMT: expression of epithelial and/or mesenchymal cell markers, migratory capacity, secretion of metalloproteinases (MMPs) and matrix synthesis in two murine epithelial cell lines. FGF-2 had similar effects as EGF but its main effect was potentiation of EMT induction by TGF-β1 [74]. Additional cytokines that are known to be capable of inducing EMT are interleukin-1 [75] and tumor necrosis factor-alpha [76], although the latter has not been proven in the kidney. In our study
only TGF-β1 was able to induce matrix synthesis, whereas all tested cytokines induced the expression of mesenchymal markers, cell motility and MMP-2 and -9 secretions. Stimulation by cytokines is probably not sufficient to induce complete epithelial-mesenchymal transition; thus, additional stimuli are necessary. A number of studies have demonstrated fairly convincingly that the extracellular matrix influences the tubular epithelial cell phenotype [77]. Disrupting the tubular epithelial membrane, for example, by inhibiting type IV collagen assembly with soluble α1NC1-domains resulted in EMT conversion to a mesenchymal phenotype [78]. Conversely, the tubular basement membrane did have a stabilizing function on the epithelial phenotype. These in vitro studies confirmed the aforementioned in vivo study by Ng et al. that complete transition to a mesenchymal cell can only be observed when the tubular basement membrane is disrupted [71]. This concept was further corroborated by a seminal study by Yang et al., using the unilateral ureteral obstruction (UUO) model of progressive fibrosis in mice deficient for PAI-1 (plasminogen activator inhibitor) [79]. Surprisingly, the PAI-1 deficient mice, unlike their normal counterparts, did not develop progressive renal failure. In contrast to the control mice, the activity of MMP-9 was robustly reduced in PAI-1 deficient mice. MMP-9 is one of the key enzymes involved in the degradation of type IV collagen, the main component of the tubular basement membrane, and its expression may be controlled by PAI-1. Reduced activity of MMP-9 may result in less degradation of type IV collagen and thus the tubular basement membrane. True to this concept, the integrity of the tubular basement membrane was largely preserved in the PAI-1 knock-out mice but not in control mice. Furthermore, control mice displayed robust staining for α-smooth muscle actin in tubular epithelial cells indicating EMT, whereas the PAI-1 deficient mice did not [79].

Based on these findings, we propose the following model of EMT during kidney fibrosis: In the normal kidney, tubular epithelial cells are tightly connected with their neighboring cells via intercellular adhesion molecules such as E-cadherin. On their basal side, they interact with the tubular basement membrane while the apical side faces the tubular lumen [53]. 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 [80]. If the pathogenic insult persists, activated tubular epithelial cells can either die or undergo EMT [64]. 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 tubular basement membrane and enhance EMT. Cells derived via EMT then acquire a migratory capacity and traverse across the disrupted tubular basement membrane into the interstitial microenvironment, exhibiting features of a mesenchymal phenotype [78, 81].
EMT-derived fibroblasts within the interstitium contribute to progression of chronic renal disease by deposition of interstitial ECM. EMT and apoptosis both contribute to loss of tubular epithelium, leading to tubular atrophy and disease progression.

The signal transduction mechanisms of EMT have been elucidated in recent years indicating that activation of integrin-linked kinase [82] and, alternatively, of the RhoA/ROCK [83] pathways are important, eventually resulting in loss of the epithelial-epithelial adhesion molecule E-cadherin. E-cadherin is an important maintainer of the epithelial phenotype which has been speculated to represent the epithelial master gene [64] (see below). In addition, Schramek and coworkers proposed that activation of the MAP kinase/Erk kinase pathway may be involved in the regulation of EMT [84]. Figure 2 illustrates the putative mechanisms involved in EMT.

**Figure 2.** Simplified scheme of EMT in renal fibrogenesis. Stimulation of tubular epithelial cells (TEC) with cytokines such as EGF, FGF-2 and TGF-β1 leads to a (partial) loss of epithelial phenotype and *de novo* expression of mesenchymal proteins (transitional cell). If there is complete disruption of the tubular basement membrane, complete transition to an activated fibroblast may ensue with synthesis of extracellular matrix proteins. Modified after [1].

**EMT during cancer progression and metastasis**

EMT is not only a key feature in embryonic development and renal fibrogenesis. In fact, EMT of cancer cells is increasingly being recognized as an important determinant of tumor progression [55, 85]. As of now, EMT in...
the setting of cancer lacks a clear definition. It needs to be noted that carcinomas (tumors derived from epithelia) and sarcomas (tumors of mesenchymal origin) are defined as different entities, which are not thought to interconvert (with the rare exception of sarcomatoid carcinomas). However, cancer cells acquire various intermediate stages of EMT which are reflected by the classical tumor-grading method. Thus, it is our current concept that, while genetic alterations and epigenetic changes account for the malignant transformation of cells (resulting into carcinomas in situ), EMT occurs in cancer cells in response to factors from the microenvironment, mediating their invasion across basement membrane barriers. In this regard, a recent study by Gupta and co-workers using a system of a defined malignant transformation in melanocytes (by transfection with a plasmid encoding the simian virus 40 early region, the catalytic subunit of the telomerase holoenzyme and the ras oncogene) has shown that only additional stimuli such as hepatocyte growth factor-induced MAPK signaling determined tumor invasion and metastasis [86]. Several recent studies have also demonstrated that inhibition of EMT in tumoral processes may decrease tumor invasion and metastasis; whereas an increase in EMT may accelerate tumor progression [56,87].

Due to the enormous diversity of cancer cell phenotypes, EMT involving cancer cells is a highly heterogenous phenomenon. However, EMT of cancer cells is commonly associated with decreased expression of E-cadherin, and mutations in this gene increase the metastatic potential of cancer cells [88, 89]. E-cadherin expression varies in different human tumors and there is an inverse relationship between levels of E-cadherin expression and the malignant grade or overall survival [89]. In normal epithelium, the cytoplasmic tail of E-cadherin associates with β-catenin, and decreased E-cadherin expression or loss-of-function mutations within the E-cadherin gene releases β-catenin [90]. Free β-catenin then interacts with the transcription factors lymphoid enhancer factor/T cell factor (LEF/TCF), and the resulting β-catenin/LEF/TCF complex binds specific responsive sequences that are directly involved in the induction of EMT [90]. The transcription factors Snail and Sip1 directly act as repressors of E-cadherin expression, and several studies have demonstrated their involvement in cancer cell EMT [91,92]. Increased ubiquination, which targets proteins for degradation, is another means of regulating E-cadherin levels. In addition to mutations within the E-cadherin gene, various exogenous stimuli control EMT of cancer cells [55]. Major exogenous regulators of EMT include members of the TGF-β superfamily, growth factors that bind to tyrosine kinase receptors such as EGF and FGF-2, MMPs and altered ECM-cell interactions. Downstream from tyrosine kinase receptors, a complex network of signaling mediates induction of EMT. Again, TGF-β is the most prominent of the factors within the tumor microenvironment that are known to induce EMT of cancer cells. Whereas TGF-β induces apoptosis and cell cycle arrest in normal
epithelia, in carcinoma cells it induces EMT and proliferation. Insights into the underlying mechanisms for such a dual role of TGF-β are emerging. For example, Ras-transformed EpH4 mouse mammary epithelial cells undergo EMT when exposed to TGF-β. Such Ras-mediated EMT is associated with either activation of the ERK/MAPK or the phosphatidylinositol 3-kinase (PI3K)-Akt/PKB signal transduction pathway [93]. Interestingly, as was shown in the same study, PI3K may inhibit induction of apoptosis by TGF-β in these cells [93]. Also, cells are protected from TGF-β-induced apoptosis when the Ras-mediated PI3K/Akt pathway is active. When injected into mice, Ras-transformed EpH4 cells progressively acquire a mesenchymal phenotype in association with autocrine production of TGF-β. Recent evidence implies that Smad proteins are also important regulators of TGF-β mediated-EMT [94].

**Reversibility of fibrosis**

One of the central questions in the field of renal fibrogenesis is the question of potential reversibility of fibrotic lesions. The disbalance between matrix synthesis and matrix degradation must be corrected towards matrix degradation. Until very recently, fibrotic lesions were thought to be irreversible. However, in the early nineties, Ma and colleagues demonstrated the potential reversibility of focal segmental glomerulosclerosis in an animal study. In that study, the angiotensin receptor blocker losartan was given to 18 months old Sprague Dawley rats resulting in regression of the spontaneous (albeit relatively mild) glomerulosclerosis [95]. Similarly, Boffa et al. recently studied the model of NO-deficient hypertensive rats and described very similar reductions of mesangial matrix expansion under losartan therapy over a period of 4 weeks [96]. Adamczak and coworkers performed one of the best studies to date to examine the potential reversibility of fibrosis in the 5/6th nephrectomy model in the rat [97]. This group waited for 8 weeks after nephrectomy for glomerulosclerosis and interstitial fibrosis to develop before starting treatment with either an angiotensin-converting enzyme (ACE) inhibitor at very high dosage (enalapril, at 48 mg/kg) or placebo. The results of that study were that progression was stopped in the inhibitor-treated group, and a robust regression of fibrotic changes regarding glomerulosclerosis (reduction of the mean glomerular sclerosis index from 1.22 to 0.77 after therapy) as well as tubulointerstitial fibrosis (reduction of the mean index from 0.99 to 0.63) could be observed. Similarly, accelerated regression of fibrotic lesions was noted under ACE inhibition in a reversible UUO model [98].

Our group has recently studied the role of bone morphogenetic protein (BMP)-7 in the reversibility of renal fibrosis. This morphogen is a 35 kD homodimer [99]. As implicated by the name, BMP-7 was isolated as a factor stimulating bone formation. However, BMP-7 is not exclusively important for bone metabolism. Generation of BMP-7-deficient mice demonstrated changes
not only of bone formation but also of lens development and, surprisingly, severe impairment of renal formation [100,101]. Renal impairment was so severe that the mice died only few days after birth due to renal failure. Morphologically, kidneys were hypoplastic and displayed robustly dilated collecting ducts separated by stromal cells and extracellular matrix indicating the absolute requirement of the morphogen for normal renal development [101]. BMP-7 expression begins at day 11.5 of mice development in the ureteric bud as well as the condensing mesenchyme, and acts as an important survival factor for these cells [102]. It is of particular interest that BMP-7 expression, unlike other morphogens, does persist post-natally, particularly in medullary collecting ducts and the distal tubule [103]. Although the exact function of postnatal BMP-7 expression is not entirely clear, it may function as a cellular differentiation factor [104]. Its effects are mediated via Alk 3 and Alk 6 receptors. Therapeutically, BMP-7 was first tested in acute renal failure. Vukicevic and coworkers were able to demonstrate that administration of BMP-7 [formerly known as osteogenic protein (OP)-1] reduced the severity of acute renal failure [105]. Regarding chronic renal failure, Hruska and coworkers administered BMP-7 to animals undergoing the UUO model, demonstrating that it could delay the loss of renal function by 5 days [106]. Interestingly, in that study BMP-7 was more effective than the ACE inhibitor enalapril. Similarly, our group demonstrated a positive effect on renal fibrogenesis in mouse models of lupus nephritis and Alport’s syndrome [107]. Moreover, when BMP-7 application was delayed in the model of nephrotoxic serum nephritis -in order to resemble the clinical situation more closely-, damage progression was stopped and a regression of fibrotic changes and tubular atrophy was observed in the treated animals [94]. Zeisberg et al. demonstrated that BMP-7 counteracted the effects of TGF-ß1 in a dose dependent manner, particularly those on EMT in tubular epithelial cells. In vivo, while placebo-treated mice displayed a robust positivity for FSP-1 and a simultaneous downregulation of E-cadherin in tubular epithelial cells, indicating EMT, BMP-7-treated animals did not; corroborating again the importance of EMT for the progression of renal disease. Similar results were recently obtained by Wang et al. in a model of diabetic nephropathy [104]. Thus, most studies concur on the robust anti-fibrotic effects of BMP-7. Conversely, BMP-7 treatment of rats undergoing protein overload proteinuria resulted in only modest effects on the disease course, possible due to the fact that tubular atrophy and EMT are not prominent features in this model [103]. At the present stage, BMP-7 represents one of the most promising strategies to prevent progression and possibly even revert fibrosis.

What is the current situation in humans regarding reversibility of fibrotic lesions?. Fioretto and coworkers showed fairly convincingly that regression of sclerotic changes appears to be possible [108]. In this study, eight type 1 diabetic
patients who underwent pancreas transplantation and who had some degree of renal involvement were followed up for a period of 10 years. Renal biopsies were taken at 0, 5, and 10 years after pancreas transplantation. Whereas there was a tendency for deterioration of sclerotic changes after the 5 years period, a definite reduction of mesangial matrix scores was noted 10 years after the normalization of glycemia. Although this study was performed in a small number of patients and the tubulointerstitial space was not evaluated, it demonstrates two things: First, sclerotic changes are reversible in humans; and second, regression of sclerotic changes requires a long time period in diabetics. The latter may be related to glycosilation of extracellular matrix proteins.

Reversibility of fibrotic lesions has been shown to occur in additional organs, particularly the liver. In experimental models of reversible cholestasis, regression of extracellular matrix depositions has been described in a number of studies. Moreover, similarly to Fioretto’s observation in the kidney, reversibility of fibrotic changes has been documented in the liver in clinical studies. Hammel et al. examined liver biopsies from 11 patients who had developed cholestasis with secondary liver fibrosis due to chronic pancreatitis [109]. At the time of the initial operation which abolished the cause of cholestasis, as well as at a mean period of 30 months later, open liver biopsies were performed. These did demonstrate a robust regression of periportal fibrotic changes. Moreover, reversibility of hepatic fibrosis has been described in patients with hepatitis B and C once the inducing cause had been resolved successfully [110]. In addition, there is at least one report of potential regression of sclerotic changes in the heart [111]. In summary, a large number of experimental and clinical studies demonstrate that the former paradigm of irreversibility of fibrotic lesions is not true, and that reversibility of fibrosis is possible. However, a number of questions remain unanswered. For example, the majority of the studies described here have not been performed in severe fibrotic conditions. Thus, it needs to be examined if there is a “no-return point” in this setting—as it seems to be the case-, and if so, how it can be defined. Furthermore, it is currently unknown if fibrosis can be reversible even if the inducing cause is not successfully treated. Finally, experimental studies have usually been conducted over relatively short time periods, and there is an urgent need for long time studies in both experimental as well as clinical settings.

In summary, this review illustrates the significance of renal fibrosis for the progression of renal disease and the role of EMT this process. In addition, participation of EMT in embrogenesis and tumor metastasis are described. Finally, the reversibility of fibrotic conditions and the contribution of EMT is being described.
References
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