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Phenotypic transitions and fibrosis in diabetic nephropathy

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The cause of renal fibrosis in diabetic nephropathy is widely believed to be phenotypic switching of fibroblasts to an activated state. However, emerging evidence suggests that diabetes also alters the phenotype of normal, non-fibroblast kidney cells, such as mesangial cells, tubular epithelial cells, and bone marrow-derived progenitors. Experiments have shown that cytokines, high glucose, and advanced glycation end products induce profibrotic changes in kidney cell phenotype by the processes of myofibroblast transdifferentiation and epithelial–mesenchymal transition. As a result, differentiated kidney cells become reprogrammed to secrete and accumulate extracellular matrix. This revised view implies that inhibiting phenotypic transitions in nonfibroblasts might limit fibrosis in diabetic nephropathy.

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In susceptible individuals, diabetes can cause progressive renal fibrosis, eventually reducing functioning renal mass. Fibrosis arises, in part, through 'activation' of renal fibroblasts to secrete and remodel the extracellular matrix. In nondiabetic kidney disease, studies strongly suggest that matrix-producing effector cells also arise from phenotypic transitions in mesangial cells and tubular epithelial cells. Profibrotic switches in the phenotype of mesangial and epithelial cells might also promote fibrosis in diabetic nephropathy.

Phenotypic changes of two types have been observed in experimental and clinical studies of diabetic nephropathy: myofibroblast transdifferentiation (MFT) and epithelial–mesenchymal transition (EMT). Yet, surprisingly little research has focused on MFT and EMT in progressive renal fibrosis in diabetes. The following review proposes a role for MFT and EMT in the pathogenesis of fibrosis in diabetic nephropathy and identifies crucial questions for future research.

PHENOTYPE SWITCHING AND FIBROSIS IN CHRONIC KIDNEY DISEASE

In patients with diabetic nephropathy, fibrosis develops in the glomerulus and tubulointerstitium (see Ziyadeh and Sharma[1]; Mason and Abdel Wahab[2]; Caramori and Mauer[3]; Wolf *et al.*[4]; Fogo and Kashgarian[5]; Eddy[6] for review), consistent with the development of fibrosis in most chronic kidney disease. Although renal fibrosis in diabetes is generally viewed as irreversible, some studies suggest that remission or regression is possible[6–11]. In diabetes, extracellular matrix accumulates in the glomerular mesangium, and in large cross-sectional studies, the increment in mesangial matrix correlates inversely with capillary filtration surface area and glomerular filtration rate.[3] Glomerular fibrosis occurs early in the progression from incipient to overt nephropathy.[2,3] In some patients, tubulointerstitial fibrosis also appears early in diabetic kidney injury, but it is more prominent later in the disease and closely correlates with the decline in renal function.[3,12–15] In patients with advanced fibrotic lesions, transglutaminases crosslink proteins, forming a matrix resistant to degradation by metalloproteinases. Profibrotic mediators, including transforming growth factor β (TGFβ), connective tissue growth factor, angiotensin II, endothelin-1, and fibroblast growth factor have been identified,[1,2,16–18]

most of which are also elevated in diabetic nephropathy.[2] Whether or not renal fibrosis is reversible, inhibition of these profibrotic mechanisms remains a promising therapeutic target in the diabetic kidney.[9,18]

A crucial advance in our understanding of renal fibrosis in nondiabetic kidney disease is that multiple cell types are responsible for accumulation and remodeling of extracellular matrix.[19–21] Although fibroblasts are critical effectors of renal fibrosis, changes in the phenotype of intrinsic, nonfibroblastic kidney cells play an important role (Figure 1). MFT and EMT result from reprogramming of cells by cytokines and growth factors, and MFT and EMT have been widely characterized in normal embryonic development and in diverse pathological processes.[20–28] In tissue injury, MFT and EMT produce effector cells that secrete matrix proteins, especially the fibrillar collagens and fibronectin, which serve as scaffolds for assembly and remodeling of the extracellular matrix into a fibrotic lesion.[29,30] Cells undergoing MFT and EMT also secrete protease inhibitors, cytokines, growth factors, and inflammatory mediators that amplify the fibrotic process by recruiting neighboring cells in a paracrine manner (Figure 2). As discussed below, effector cells in renal fibrosis can also arise from bone marrow-derived progenitors. Important questions to consider are: does MFT and EMT occur in diabetic nephropathy? If so, does MFT and EMT contribute to renal fibrosis?

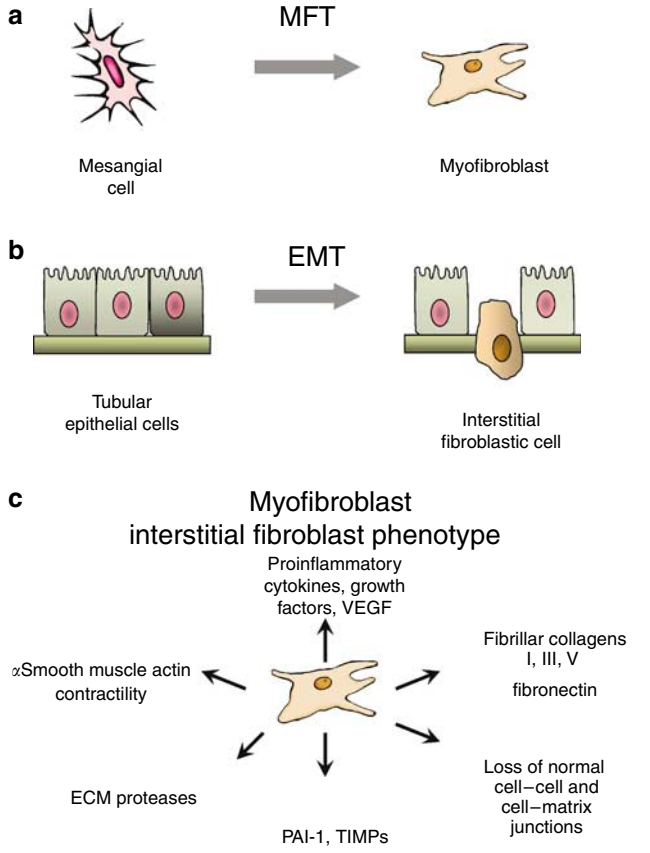

Figure 1 | MFT and EMT produce effector cells in renal fibrosis. **(a)** MFT alters the normal phenotype of glomerular mesangial cells to produce myofibroblasts that secrete excess extracellular matrix. Prolonged transdifferentiation of mesangial cells can lead to fibrosis. Fibroblasts or microvascular pericytes in the tubulointerstitium might also undergo MFT. It is unclear whether mesangial or fibroblast MFT is reversible. **(b)** EMT is another phenotypic transition that produces profibrotic effector cells. Tubular epithelial cells respond to stress signals by undergoing EMT and producing fibroblastic cells that migrate across the tubular basement membrane into the interstitium. In chronic kidney disease, effector cells deriving from EMT are postulated to cause tubulointerstitial fibrosis, which is closely associated with progression to end-stage kidney disease. **(c)** Examples of the phenotypic changes that result from MFT and EMT. Although MFT and EMT have been well studied in nondiabetic kidney disease, their role in diabetic nephropathy is being investigated.

PHENOTYPIC TRANSITIONS IN FIBROSIS: NOMENCLATURE

Before continuing, it is necessary to consider the definitions of MFT and EMT to avoid confusion. In this review, MFT and EMT are defined as processes that fundamentally alter the structure and function of mesenchymal, epithelial, or progenitor cells depending on whether the target cell is mesenchymal or epithelial. MFT and EMT are considered by some investigators to belong to the general class of phenotypic transitions known as metaplasias.[31–34] Changes in cell growth sometimes accompany MFT and EMT, but this

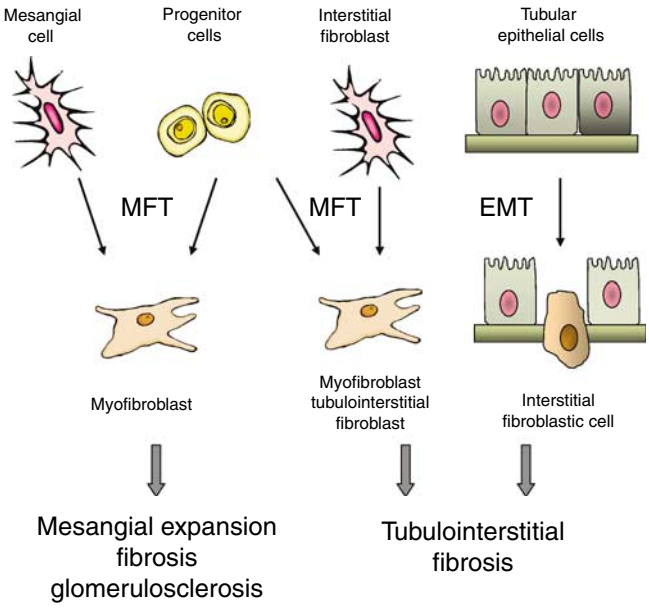

Figure 2 | In diabetic nephropathy, diverse cell types undergo MFT and EMT to produce profibrotic effectors. At least four distinct cell types undergo metaplastic changes in the diabetic kidney: (from left to right) mesangial cells, bone marrow-derived progenitors, interstitial fibroblasts, and tubular epithelial cells. Both MFT and EMT contribute to tubulointerstitial fibrosis. It is unclear whether microvascular pericytes of the peritubular capillaries can also be targeted by MFT. MFT results in myofibroblasts that contribute to fibrosis in the glomerulus and tubulointerstitium. In contrast, EMT of tubular epithelial cells produces fibroblastic cells that migrate into the interstitial space. In practice, the lack of stage-specific markers precludes a precise distinction between the phenotypes of myofibroblasts and fibroblastic cells that derive from the epithelium. There might, in fact, be significant overlap.

is not always the case.[33] In nature, metazoan development depends on MFT- and EMT-like changes in progenitor or stem cells, and on the reverse process of EMT the mesenchymal epithelial transition.[35] In adult cells with fixed physiological functions, MFT and EMT can have deleterious effects and often play a critical role in pathophysiology that involves altered cell growth or dysplasia.

Phenotypic changes in the form of MFT drive glomerulosclerosis by altering the phenotype of mesangial cells and possibly bone marrow-derived progenitors (Figure 1). MFT of fibroblasts, and perhaps also extraglomerular vascular pericytes, contributes to tubulointerstitial fibrosis. Mesangial cells are microvascular pericytes that provide structural support for glomerular capillaries and resist the high hydraulic pressure required to achieve ultrafiltration.[36–38] In patients with diabetes, mesangial cell hypertrophy, expanded mesangial matrix, thickening of the glomerular basement membrane, and obliteration of glomerular capillaries characterizes glomerulosclerosis. In response to injury, mesangial cells can transdifferentiate into myofibroblasts, a specialized population of mesenchymal cells that secrete extracellular matrix, particularly the fibril-forming collagens and fibronectin (Figure 2). Production of interstitial collagens by myofibroblasts contrasts with the lack of collagen I synthesis by mesangial cells in the normal, nondiabetic kidney.[2,39] Mesangial MFT, sometimes called 'activation', is postulated to participate in endothelial and podocyte injury and in secondary tubulointerstitial injury. MFT also precedes expansion of the mesangial matrix and glomerulosclerosis,[2,40,41] consistent with a pathogenic role. Whether the same mechanisms regulate MFT in mesangial cells and phenotypic activation of fibroblasts is unknown.

In contrast to MFT, tubular epithelial cells undergo a unique phenotypic change known as EMT (Figure 1), which drives tubulointerstitial fibrosis in response to growth factors and profibrotic cytokines such as TGFβ.[20,21,26,27,42] EMT of tubular epithelium challenges our long-held view that these cells are terminally differentiated and reflects the remarkable phenotypic plasticity of epithelial cells.

A function for EMT in the etiology of renal fibrosis was first suggested in rodent models of anti-tubular basement membrane disease[43] and reduced renal mass by 5/6 nephrectomy.[44] EMT has also been reported in obstructive nephropathy, anti-glomerular basement membrane disease, and nephrotoxic serum nephritis.[27] A hallmark of EMT is the loss of E-cadherin at adherens junctions, distortion of epithelial cell polarity, and disorganization of cell–cell adhesion junctions.[45] This loss of epithelial barrier function results in effector cells that migrate into the interstitial extracellular matrix (Figure 2). Tubular cells that undergo EMT also secrete matrix proteins and inflammatory mediators. EMT of tubular epithelium and MFT of intertubular pericytes might be especially important because clinical studies have associated epithelial injury and tubulointerstitial fibrosis with increased risk of progression in diabetic nephropathy.[12,13]

IDENTIFYING PHENOTYPIC TRANSITIONS IN DIABETIC NEPHROPATHY: MARKERS OF MFT AND EMT

MFT and EMT alter expression of cytoskeletal proteins and reorganization of actin filaments, making it possible to use morphological and immunohistochemical markers to identify cells that have undergone MFT or EMT. For example, myofibroblasts have an elongated, spindle-shaped morphology with extended cell processes.[46] α-Smooth muscle actin (αSMA) – absent in normal mesangial, tubular epithelial, and fibroblastic cells – is prominent in myofibroblasts and is the most common marker used to localize MFT and EMT *in vivo*. Upregulation of αSMA facilitates myofibroblast contraction in wound healing and fibrosis. αSMA also functions as a mechanotransducer that responds to signals received at focal adhesions. Fibroblast-specific protein 1 (also known as S100A4 and MTS1) is another marker which has been used in clinical and experimental studies. It specifically localizes to fibroblasts,[27,43] but probably does not differentiate between 'activated' and resting fibroblasts. Glomerular mesangial cells do not express fibroblast-specific protein 1[43] Not surprisingly, upregulation of procollagen Iα2 marks activated fibroblasts and myofibroblasts and is also a useful marker to identify cells which have undergone MFT and EMT. However, a drawback to using procollagen Iα2 is that expression can be demonstrated in other cell types as well.[47]

Although these markers have been used successfully to identify metaplastic cells, the lack of specific myofibroblast markers hinders studies of MFT and EMT, particularly in animal models. At present there are no genetic markers of MFT or EMT. The development of selective, 'stage-specific' MFT and EMT markers is important, and noninvasive biomarkers are needed for clinical studies involving MFT and EMT.

PHENOTYPIC TRANSITIONS IN EXPERIMENTAL MODELS OF DIABETIC NEPHROPATHY

Analysis of MFT and EMT in diabetic animals demonstrates that prominent changes in renal cell phenotype occur in diabetes. For example, kidneys from the KKay mouse model of diabetic nephropathy have been shown to express MFT markers,[48] and in the obese Zucker *fa/fa* diabetic rat, MFT was observed in the tubulointerstitium but not in glomeruli.[49] In the Zucker rat, matrix deposition correlated with MFT of tubulointerstitial fibroblasts, implicating MFT in the development of tubulointerstitial fibrosis.[49] MFT has also been reported in glomeruli from rats made diabetic with streptozotocin (STZ).[50,51] In addition, STZ mice displayed evidence of MFT in tubulointerstitial fibroblasts. Typically, glomerular MFT markers temporally and spatially associate with accumulation of collagen type I, IV, and with a transcriptional effector of TGFβ signaling, SMAD1.[50,51]

In addition to MFT, several studies suggest that EMT of tubular epithelial cells occurs in diabetes. EMT markers in the kidney have been reported in Wistar Kyoto and Sprague–Dawley normotensive rats treated with STZ,[42,52] and EMT was attenuated by a crosslink breaker that neutralized the

effect of advanced glycation end products (AGEs) *in vivo*, which suggests a role for AGEs.[52] Consistent with this notion, the receptor for AGEs has been shown to induce EMT by a TGFβ-dependent pathway in cultured tubule epithelium.[52] Taken together, these results demonstrate that MFT and EMT occur in the diabetic kidney and that they can be studied experimentally in rodent models. Future studies of MFT and EMT in diabetes should reveal mechanisms of regulation and the functional role in renal fibrosis.

DO MFT AND EMT OCCUR IN PATIENTS WITH DIABETIC NEPHROPATHY?

Markers of MFT and EMT have been observed in the kidneys of patients with diabetic nephropathy. For instance, renal biopsies from healthy subjects did not reveal any αSMA-positive myofibroblasts,[53,54] demonstrating that MFT is rare in normal kidney. In contrast, biopsies performed on diabetic patients documented the presence of MFT markers in the tubulointerstitium[52–55] and in glomeruli associated with mesangial matrix expansion.[52,53,56]

EMT has also been identified in biopsies of patients with diabetic nephropathy[55] and strong correlations were observed between EMT and fractional area of the interstitium (an index of tubulointerstitial fibrosis) and collagen type III.[53,54] In fact, EMT, MFT, proteinuria, and the decline in renal function are tightly correlated, leading two groups to recommend αSMA immunostaining as a biomarker for renal fibrosis in patients with diabetes.[53,54] Taken together, these findings suggest that normal cells in the diabetic kidney are undergoing MFT and EMT, contrasting earlier theories that the cause of renal fibrosis is only because of phenotypic switching of fibroblasts.

PHENOTYPIC TRANSITIONS OF BONE MARROW-DERIVED PROGENITOR CELLS: A NOVEL PATHWAY TO RENAL FIBROSIS

Although the experimental and clinical studies discussed above suggest that MFT and EMT occur in diabetic nephropathy, it is sometimes difficult to precisely identify the cells that undergo MFT and EMT. Apparently, many derive from intrinsic mesangial cells, tubular epithelial cells, and perhaps pericytes. However, recent experiments suggest another possibility and are leading investigators to wonder if cells undergoing MFT and EMT might arise from bone marrow-derived progenitors (Figure 2).

Bone marrow-derived progenitors in nonrenal models of organ fibrosis

To define the role of progenitor cells in MFT and EMT, irradiated mice are given bone marrow grafts from sex-mismatched controls or from mice expressing marker genes such as luciferase or green fluorescent protein. The extent to which donor cell engraftment contributes to MFT and EMT is analyzed by measuring the proportion of cells positive for the Y chromosome, using antibodies against luciferase or assessing fluorescence. Using these techniques in conjunction with markers of MFT or EMT, bone marrow-derived progenitor cells were identified as a source of myofibroblasts in peripheral tissues.

Typically, in response to injury, bone marrow-derived myofibroblasts have been shown to expand and contribute to fibrosis in the lung,[57,58] liver,[59] skin,[58] and peritoneum.[60] In a murine model of cirrhosis, ~70% of liver myofibroblasts derived from bone marrow and most of these myofibroblasts actively secreted collagen type I.[59]

In another study of patients with liver fibrosis of diverse etiologies, a significant proportion of myofibroblasts was shown to derive from bone marrow progenitors.[61] These patients had bone marrow transplants from opposite sex donors, so the derivation of myofibroblasts was classified by the presence of a Y chromosome in cells that were positive for myofibroblast markers. Although it is possible to use this approach to identify cells derived from bone marrow progenitors, it is difficult to assign an origin to the remainder of myofibroblasts that did not come from bone marrow progenitors.

Although the studies cited above suggest that myofibroblasts can derive from bone marrow progenitors, their contribution to fibrosis is debated. For instance, in a mouse model of bleomycin-induced lung fibrosis, bone marrow cells contribute little to the accumulation of fibroblasts and most of the collagen type I-producing cells are not from bone marrow.[62] In a model of cardiac fibrosis, bone marrow-derived progenitors do not produce myofibroblasts.[63] Using a sensitive green fluorescent protein under transcriptional control of the αSMA promoter, another study in mice has failed to detect significant numbers of αSMA-positive myofibroblasts that derive from bone marrow.[64] It is difficult to reconcile these disparate findings, but the results support the contention that fibroblasts are phenotypically heterogeneous and that the response of these fibroblasts depends on injury- and tissue-specific cues.[65]

Bone marrow-derived progenitors and renal fibrosis

In the kidney, the ability of bone marrow progenitors to produce effector cells appears to depend upon both nephron localization and the specific injury producing fibrosis. Previous studies have shown that cells from the metanephric mesenchyme and the bone marrow can transdifferentiate into renal myofibroblasts.[66] In mice, a population of mesangial cells arise from bone marrow, possibly from hematopoietic stems cells of a granulocyte/macrophage origin.[64,67–71] These bone marrow-derived mesangial progenitors can repopulate glomeruli after kidney injury[68,71,72] and a glomerulosclerosis phenotype can be conferred by bone marrow transplantation.[67] A role for bone marrow progenitors in tubulointerstitial remodeling remains uncertain.[72] In a model of renal fibrosis induced by ureteral obstruction and characterized by tubulointerstitial fibrosis, bone marrow progenitors produce a population of interstitial myofibroblasts,[26,73] but collagen type I transcription in these cells is not observed.[73] The possibility that collagen I synthesis is upregulated by post-transcriptional mechanisms was not investigated. In obstruc-

tion, the *Col1α2* promoter is most active in tubular cells,[47,73] suggesting that tubular epithelium, not bone marrow-derived progenitors, secrete collagen I. Similarly, in a renal model of endothelial injury, bone marrow progenitors do not contribute significantly to interstitial fibrosis.[74] Collectively, these results suggest that bone marrow-derived progenitors give rise to matrix-producing mesangial cells, but the contribution to tubulointerstitial fibrosis is less certain and might depend on the type of injury.

Diabetic nephropathy and bone marrow-derived progenitors

The contribution of bone marrow-derived progenitors to MFT and EMT in diabetes or more specifically in diabetic nephropathy is a question that researchers are investigating.

A recent study reported that the number of bone marrow-derived myofibroblast progenitors is 1.6-fold higher in patients with type 1 diabetes compared with nondiabetic controls,[75] suggesting that diabetes might induce a profibrotic state. The mechanism by which type 1 diabetes increases the number of bone marrow progenitor cells is uncertain but might be related to a relative deficiency in bone morphogenetic protein (BMP) 6, which inhibits transcription of genes that initiate the MFT response.[75]

In *db/db* mice, a genetic model of type 2 diabetes in which the mice lack the leptin receptor, bone marrow-derived progenitors can transfer the diabetic glomerular phenotype (i.e., mesangial expansion and glomerulosclerosis) to nondiabetic normoglycemic mice.[76] Studies in experimental models of diabetes seem warranted to define the role of progenitor cells in MFT, EMT, and renal fibrosis in diabetes (Figure 2). These studies seem especially relevant given that some have proposed the use of stem or progenitor cells to treat glomerular fibrosis *in vivo*.[77]

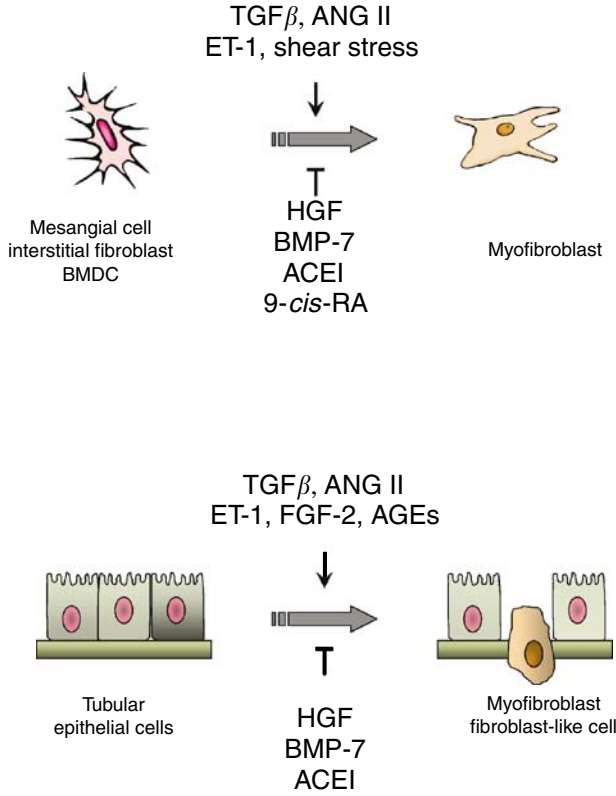

Figure 3 | Working model for regulation of MFT and EMT in the diabetic kidney. Induction of MFT and EMT requires a shift in the balance between positive and negative regulators. Numerous paracrine or autocrine factors stimulate MFT and EMT including TGFβ, endothelin-1 (ET-1), angiotensin II (ANG II), and perhaps high glucose (HG), fibroblast growth factor 2 (FGF2), AGEs, and BMDC, bone marrow-derived cells. Repression of MFT and EMT involves HGF, BMP-7, angiotensin-converting enzyme inhibitors (ACEI), and retinoic acid (RA).

MFT, EMT, AND THE PATHOGENESIS OF FIBROSIS IN DIABETIC NEPHROPATHY

Two lines of evidence suggest that MFT and EMT functionally contribute to fibrosis in diabetic nephropathy:

1. The same extracellular cues that induce MFT and EMT also increase fibrosis.
2. Inhibitors of MFT and EMT attenuate or block fibrosis and progressive kidney disease in animal models of diabetes (Figure 3).

Molecular mediators of MFT and EMT in diabetic nephropathy

The cytokine TGFβ, a critical profibrotic mediator,[2,4,16,18] is elevated in diabetes and strongly induces MFT and EMT (Figure 3). TGFβ induces αSMA and collagen type I in human mesangial cells[78] and also increases mesangial cell hypertrophy *in vitro* and *in vivo*. In tubular epithelial cells, TGFβ induces αSMA and represses E-cadherin,[79] demonstrating that TGFβ is a potent stimulus for EMT. The importance of TGFβ has also been demonstrated in mice overexpressing TGFβ in the kidney. Transgenic expression of TGFβ in the kidney causes robust fibrosis in the glomerulus and tubulointerstitium.

High glucose is another potential stimulus of MFT and EMT, but surprisingly, the extent to which hyperglycemia directly stimulates phenotypic transitions in kidney cells is unclear. Hyperglycemia induces synthesis of numerous matrix proteins by mesangial cells including collagen I, III, IV fibronectin, and laminin (reviewed by Mason and Abdel Wahab[2]). High extracellular glucose also promotes collagen accumulation in proximal tubule epithelial cells[80,81] and elevates AGEs. *In vivo*, these AGEs promote crosslinking of extracellular matrix proteins and slow degradation by endogenous proteases.[82,83] *In vitro*, AGEs stimulate EMT, which is attenuated by a crosslink breaker that neutralizes the effect of AGEs, suggesting a causative role.[42,52] Similarly, in cultured tubule cells, the receptor for AGEs induces EMT by a TGFβ-dependent pathway,[52] suggesting that AGEs may require TGFβ. Induction of EMT by AGEs also requires connective tissue growth factor.[84] Obviously, hyperglycemia is a huge factor in diabetes, but high glucose *per se* has not been established as a stimulus of the full complement of

metaplastic phenotypes (i.e., αSMA induction and E-cadherin repression).

A third factor that might induce MFT and EMT in the kidney is mechanical stress (Figure 3). Hemodynamic forces (i.e., transmural pressure and shear stress) cause mechanical stress that promotes glomerular injury early in the natural history of diabetes. However, whether or not hemodynamic stress leads to MFT in the diabetic kidney is unknown. What is known is that sheer stress and other mechanical forces promote MFT *in vitro*,[46,85] providing a rationale for future study. Mechanically stimulated MFT in mesangial cells might explain, in part, why systemic hypertension and glomerular hyperfiltration amplify kidney injury in diabetes. Indeed, mechanotransduction is an attractive candidate for linking hemodynamic stress to MFT of kidney cells.

Anti-metaplastic regulators block fibrosis in diabetic nephropathy

A second reason to implicate MFT and EMT in diabetic nephropathy is that negative regulators block renal fibrosis in diabetes. For example, hepatocyte growth factor (HGF, also known as scatter factor) is a potent anti-fibrotic hormone that blocks both MFT (in mesangial cells and interstitial fibroblasts) and EMT (of tubular epithelial cells) *in vitro* and *in vivo*.[50,86–88] That HGF inhibits EMT makes sense because HGF directs tubulogenesis in embryonic development by the opposite mechanism that initiates EMT: the mesenchymal–epithelial transition. In mice treated with STZ, renal delivery of an exogenous HGF gene reduced mesangial expansion, glomerular fibronectin, and collagen type I.[50,88]

HGF also:

- Reverses glomerulosclerosis in diabetic rats.[87]
- Reduces albuminuria and renal fibrosis. Inhibits MFT of interstitial fibroblasts in the diabetic mouse and rat kidney, thereby attenuating tubulointerstitial fibrosis.[50,87]
- Lowers renal tissue and urinary TGFβ, which might be a mechanism by which HGF blocks fibrosis in STZ diabetic rodent kidneys.
- Blocks fibrosis and improves renal function in *db/db* mice,[89] although a direct effect on the phenotypic transitions has not been tested extensively.

Additionally, HGF mediates the anti-fibrotic action of peroxisome proliferator-activated receptor-γ, a nuclear hormone receptor that regulates transcription. Natural and synthetic peroxisome proliferator-activated receptor-γ agonists increase HGF, which in turn blocks αSMA and matrix accumulation in mesangial cells and renal interstitial fibroblasts exposed to TGFβ.[90] Peroxisome proliferator-activated receptor-γ also activates transcription from the HGF promoter.

HGF mediates the anti-fibrotic action of another nuclear hormone receptor, the retinoic acid-receptor superfamily. Mesangial cells exposed to retinoic acid fail to develop into myofibroblasts when exposed to TGFβ.[91] Another ligand that binds to nuclear hormone receptors 1,25-dihydroxyvitamin D also represses renal fibrosis by an HGF-based mechanism.[92] This anti-fibrotic effect of HGF is also consistent with a role for MFT and EMT in the pathogenesis of renal fibrosis in diabetes. These results highlight the anti-fibrotic potential of retinoic acid, 1,25-dihydroxyvitamin D and peroxisome proliferator-activated receptor-γ agonists such as the thiazolidinediones and provide solid rationale for pursuing a therapeutic application for HGF.

However, the potential therapeutic role of HGF has been questioned in studies showing that chronically elevated HGF contributes to a decline in growth factor receptor, increases microalbuminuria, and promotes progression of diabetic nephropathy in *db/db* mice.[93] In addition, mice overexpressing HGF developed tubular hyperplasia, glomerulosclerosis, and a phenotype resembling polycystic kidney disease.[94] It is possible that these discrepant effects of HGF are explained by differences in the genetic background of model systems, so further studies on new models are necessary to determine the therapeutic potential of HGF in diabetic nephropathy. Moreover, HGF and its receptor c-Met promote cancer cell migration and expression of angiogenic factors,[95] so the proto-oncogenic action of HGF and c-Met might limit clinical utility.

Renal fibrosis in diabetes is also inhibited by BMP-7, another ligand that blocks EMT *in vitro* and *in vivo*.[28] In published studies, BMP-7 slowed progression of nephropathy in STZ rats[96] and *db/db* mice[97] and preserved glomerular filtration rate, glomerular size, and permeability better than or equal to Enalapril. Interestingly, the effects of BMP-7 occur without lowering blood pressure.[96] BMP-7 also inhibits collagen accumulation, making it a potent anti-fibrotic agent in this model. The extent to which the anti-fibrotic effect of BMP-7 reflects its ability to block EMT has not been directly tested. Like HGF, BMP-7 inhibits signals evoked by the TGFβ receptor in mesangial cells.[98,99] The *in vivo* relevance of BMP-7's antifibrotic action is supported by experiments in mice transgenic for noggin, an endogenous inhibitor of BMPs. Transgenic noggin mice develop massive expansion of the mesangial matrix and accumulation of fibronectin.[100]

Another link between BMPs and fibrosis in diabetic nephropathy is gremlin, the endogenous inhibitor of BMPs. Gremlin limits BMP availability, thereby reducing the threshold for induction of MFT and EMT by TGFβ, and it is markedly elevated in humans with diabetic nephropathy. Hyperglycemia and TGFβ induce the gremlin gene in mesangial cells[101] and the spatiotemporal pattern of gremlin expression is consistent with a role in tubulointerstitial fibrosis.[102]

CONCLUSIONS AND FUTURE DIRECTIONS

Our assumptions about the role of MFT and EMT derive primarily from experimental models of renal disease in rodents. Given the potential differences between humans and rodents, results derived in rats and mice must be interpreted with caution. It is unclear to what extent these results can be

extrapolated to humans with diabetic nephropathy. However, the role of MFT and EMT in human development is quite similar to that in rodents, supporting the notion that these phenotypic transitions are relevant to renal fibrosis in humans.

A model of fibrosis in diabetic nephropathy that encompasses MFT and EMT provides a conceptual framework for interpreting new findings and developing therapeutic approaches. For instance, an effective therapeutic approach will probably need to block the multiple phenotypic transitions that occur in diabetic nephropathy. In addition, a considerable body of work has delineated the regulation of MFT and EMT in development, which should yield productive clues for anti-fibrotic therapies.

Finally, there is understandable enthusiasm regarding remission or regression of renal fibrosis. Therefore, it will be important to determine whether these altered cells can revert to their normal phenotype or whether the cells must die out or be replaced. In development, EMT is reversible, but in fibrosis it is not clear whether reprogramming of the target cell is permanent.

Below are some pressing questions for research in this area:

1. What is the origin of fibrosis effector cells in diabetic nephropathy? Do they derive from progenitors, circulating fibrocytes, or only from intrinsic renal cells? It will be especially important to determine the contribution, if any, of hematopoietic stem cells to the emergence of myofibroblasts and fibroblastic cells in the diabetic kidney. If progenitors contribute to fibrosis, the phenotype of any progenitors used for renal replacement therapy would have to be tightly regulated.
2. Do infiltrating inflammatory cells induce MFT or EMT of normal kidney cells? Although some studies have demonstrated macrophages in the diabetic kidney, scant attention has been focused on the contribution of lymphocytes to induction of MFT, EMT, and the progression of fibrosis. Macrophages have been shown to induce fibrosis in the liver and a similar effect might be operative in the diabetic kidney.
3. What technical advances are necessary to study the functional role of MFT and EMT in renal fibrosis in diabetes? Noninvasive markers of renal fibrosis are urgently needed for animal model studies and for determining the natural history of fibrosis in patients with diabetic nephropathy. Currently, measurements of renal fibrosis in patients require renal biopsy, precluding large population-based studies. The development of anti-fibrotic strategies will rely on noninvasive markers for surrogate end points. Possible markers include serum and urine proteins (e.g., cytokines, collagen peptide fragments) and imaging. For example, TGFβ and connective tissue growth factor in urine tightly associates with albuminuria and renal injury in patients with type 1 and 2 diabetes.[103,104]
4. Finally, what are the cellular and molecular mechanisms that initiate and inhibit MFT and EMT in diabetic nephropathy? Although much is known about the regulation of MFT and EMT in nondiabetic kidney disease, the extent to which similar mechanisms operate in diabetic nephropathy is not completely understood. New therapeutic approaches might evolve from a better understanding of the mechanisms that control these profibrotic phenotypic transitions in diabetes.

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