

## Renal Fibrosis

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### Summary

The kidney has unique attributes that are related to its complex structure and that affect the nature of fibrogenesis in this organ. It is divided into functional units, called nephrons, that have both a filtering and a reabsorbing component. Sclerosis may initiate in the sites of either of these components but ultimately involves both. The epidemiology and clinical manifestations of renal fibrosis suggest complex genetic and environmental influences on the development of fibrosis. Further, the different structures in the kidney manifest different mechanisms of fibrogenesis. These are determined by a combination of differences in the biology of the affected cells and the physical effects of nephron failure. Although therapy for renal fibrosis remains somewhat problematic, new insights into the mechanisms of the underlying diseases offer the promise of improved approaches to treatment.

**Key Words:** Kidney; fibrosis; TGF- $\beta$ ; glomerulosclerosis; nephron; basement membrane.

### 1. Introduction

The kidney serves a variety of functions essential to our physiology. The characteristic renal function that, when lost, requires replacement with dialysis or transplantation involves the cleansing of water-soluble impurities from the blood through the production of urine, and of maintenance of proper fluid and electrolyte homeostasis. In addition, the kidney regulates acid–base balance in tandem with the lung, controls bone metabolism through its roles in phosphate excretion and the metabolic activation of vitamin D, stimulates bone marrow function through the production of erythropoietin, and serves as a site for gluconeogenesis. Fibrosis of the kidney, whether it is a primary pathological process or the ultimate manifestation of organ involution and loss, can affect all of these functions, and thus remains an important clinical problem in both developed countries and the developing nations. In the United States in 2001, more than 400,000 patients were receiving treatment for chronic kidney failure, and the cost of treating this problem was approx \$22.8 billion (*1*).

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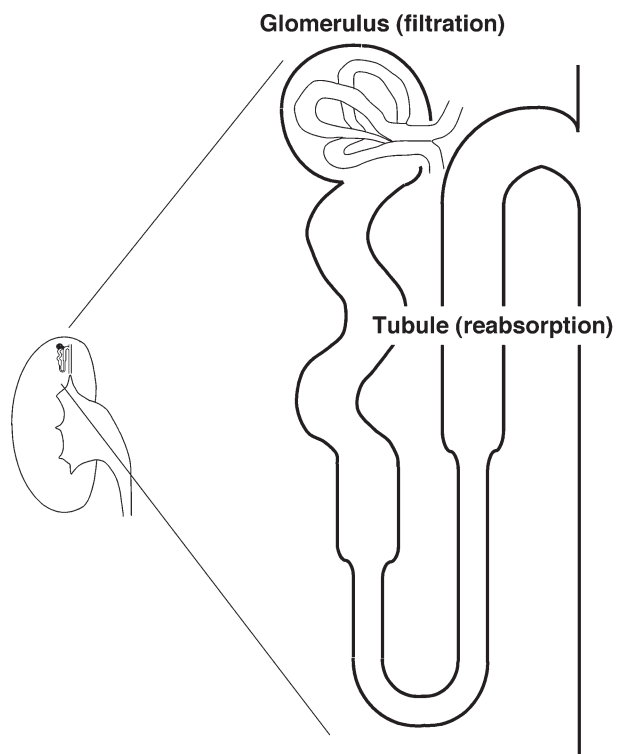


Fig. 1. Schematic depiction of the nephron. Water and small molecular solute pass from the glomerular capillaries into the urinary space, whereas most of the protein normally is restricted from passing through the filter. Ninety-nine percent of the filtrate is reabsorbed in the renal tubule. Reproduced from **ref. 37**, with permission.

Understanding renal fibrosis requires some sense of the functional anatomy of the kidney. It is a complex organ with highly specialized structures, each of which undergoes fibrosis in a unique way. The functional anatomy of the kidney is depicted schematically in **Fig. 1**. The major unit of the kidney is the *nephron*, which in turn is comprised of the filtering apparatus, the *glomerulus*, and an apparatus that regulates fluid and electrolyte homeostasis, the *tubule*. Although the glomerulus occupies only 3 to 5% of the renal mass, it is the initial site at which many acute and chronic inflammatory processes involve the kidney and has attracted a large share of the mechanistic analysis of renal fibrogenesis. The tubulointerstitium, however, is the site of significant inflammation and plays a role in the progression of chronic kidney disease. Fibrogenesis at both of these locations will be considered here.

The glomerulus is comprised of a ball (glomus) of capillaries surrounded by a cup of epithelial cells that serve as the beginning of the tubule. The approx 1,000,000 glomeruli in the kidneys filter the entire body water about five times per day. Ninety-nine percent of this filtrate is reabsorbed by the tubule emanating from each glomerulus, permitting precise regulation of fluid and electrolyte homeostasis. A fine balance between glomerular and tubular function prevents dehydration from occurring. This is effected through regulation of perfusion, hydrostatic pressure and filtration surface area in the glomerulus. A diagram of a cross-section of a capillary tuft within the glomerulus is shown in **Fig. 2A**. Several capillaries are draped over a stalk of *mesangial cells*, which have similarities to both macrophages and vascular smooth muscle cells. In this view, blood flow runs through the capillaries in a direction perpendicular to the plane of the page. The capillaries are comprised of an inner lining of fenestrated endothelial cells that permit the passage of solvent (water) and solute (electrolytes and other small molecules). An outer support structure is comprised of visceral epithelial cells that spread interdigitating process across the external surface of the capillary. The appearance of these interdigitating structures has led to their being called foot processes, and the unique cells from which they arise are termed *podocytes*. Because both the endothelial cells and the podocytes lay down a basement membrane, the result is a glomerular basement membrane (GBM) that is trilaminar, with a relatively electron-dense lamina densa between the lamina rara interna and lamina rara externa (**Fig. 2B**). Water and solute are extruded from the capillary space into the urinary space. Because of striking electrostatic properties (negative charge) as well as the role of the capillary membranes in filtering the plasma water, immune complexes often are trapped in the GBM or form *in situ* from circulating antibodies and glomerular antigens. This process activates the complement cascade or other inflammatory pathways, leading to the development of *glomerulonephritis*. If these events are sufficiently chronic to lead to scar formation, the process is known as *glomerulosclerosis*. In addition to inflammation, glomerulosclerosis can result from other diseases of a structural, functional, or genetic nature, or it may be idiopathic.

Filtered antigens, biologically active proteins, or lipids are trapped by the renal tubular cells in the process of pinocytosis. At times, these antigens or biologically active molecules may change the structure of the renal tubular cells or are themselves chemokinetic, initiating an inflammatory process in which macrophages are recruited and exacerbate the degree of tubular damage. This inflammation generates *tubulointerstitial nephritis* (TIN). TIN, too, may become chronic, leading to tubulointerstitial fibrosis (2). TIN may result from toxic or allergenic effects of drugs, viral infections, genetic abnormalities of the tubulointerstitium, or metabolic activation of the tubular epithelia.

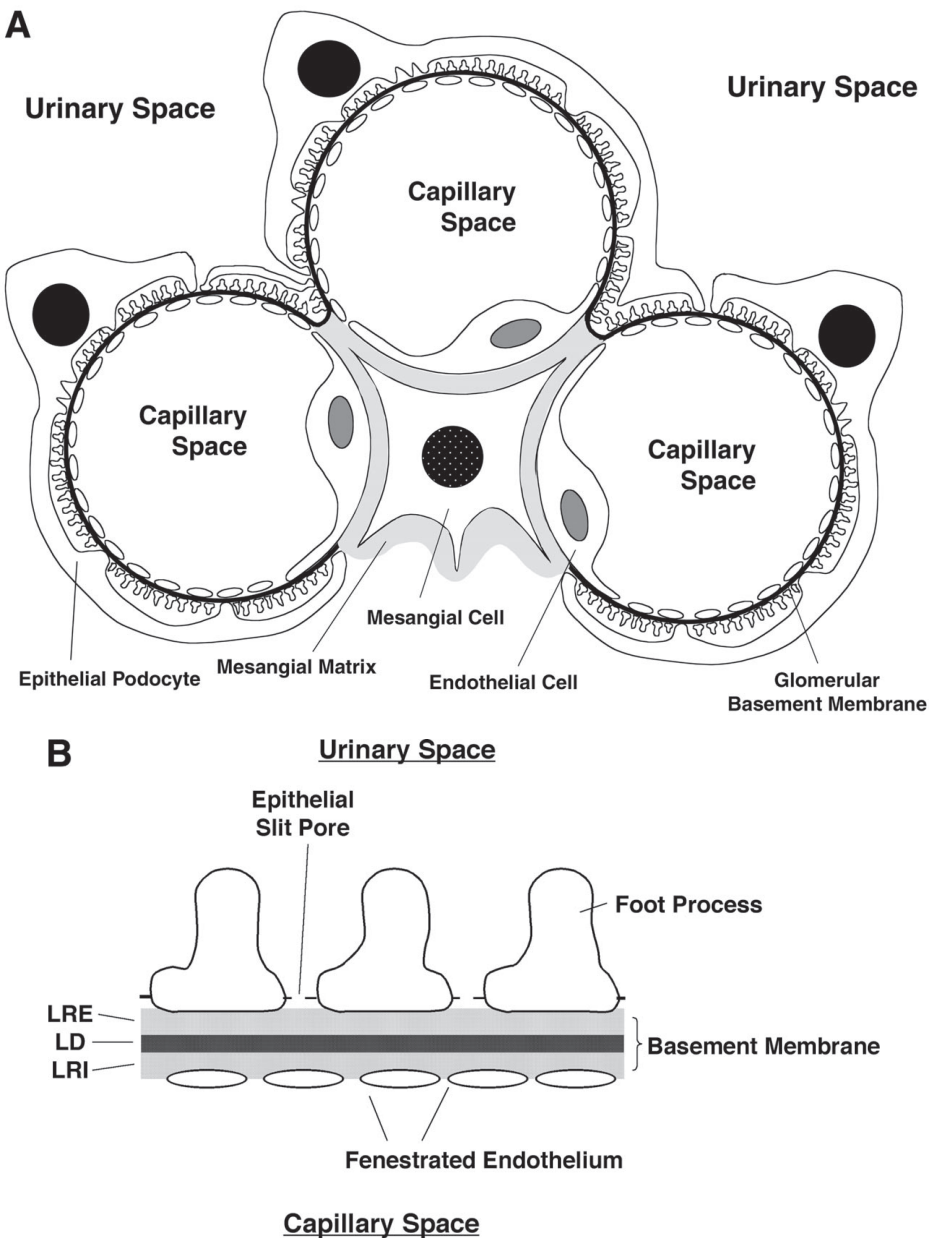


Fig. 2. Microscopic structure and ultrastructure of the glomerulus. (A) A cross-section of a glomerular tuft, with the blood flow running perpendicular to this page. Filtration occurs from the capillaries into the urinary space. A fenestrated endothelium lines the capillaries. The podocyte provides external support, while its interdigitations provide sufficient space for the passage of filtrate. A basement membrane is adjacent

## 2. Kidney Diseases Causing Fibrosis

Any disease that leads to chronic kidney damage may result in fibrosis. Given the complexity of renal structure, the compartmentalization delineated by normal basement membranes is essential for maintaining the functional integrity of the nephron. Thus, in conditions involving acute toxicity, the ability of the renal tubular epithelial cell to rapidly regenerate assures that recovery can occur as long as the tubular basement membrane remains intact. However, if this, too, is destroyed, then the likelihood of regeneration of a normal nephron may decrease. Therefore, the factors regulating both the balance and timing of extracellular matrix (ECM) turnover are critical (3). For example, in some models of inflammatory glomerulonephritis, infiltrating macrophages produce proteolytic enzymes that destroy the structure (4). In contrast, in the rat anti-Thy1 nephritis model, an injected antibody against a thymic antigen cross-reacts with a similar antigen in the rat mesangial cell. The resulting disease involves mesangiolysis and ECM accumulation that is self-limited, with resolution beginning to occur by 1 wk. Interestingly, resolution is preceded by increased expression of matrix metalloproteinase (MMP)-2 (5), suggesting that this ECM protease activity is, in this case, important for resolving a potential scar rather than for the degree of damage it might cause to glomerular structures.

Human diseases associated with fibrosis can be divided roughly into three categories. As described previously, a number of glomerular diseases, such as those associated with inflammation of the basement membrane or cells comprising the filter (glomerulonephritis), or diabetes mellitus, can lead to scarring of the glomerulus (6). In addition, *idiopathic focal segmental glomerulosclerosis* (FSGS) is a disease in which scarring of this filter occurs in a focally distributed pattern and without any apparent antecedent cause (7). **Figure 3** illustrates three different types of glomerular disease to emphasize the importance of structural considerations in the type of fibrosis that occurs. The first shows a glomerulus from a kidney with membranous nephropathy (MN). Immune complexes trapped in the glomerular filter cause a thickening of the capillary wall, leading to an accentuation of the capillary loops. Whereas the epithelial and endothelial cell likely contribute to this thickened membrane, the process clearly is different from that in FSGS. In FSGS, the central location

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Fig. 2. (*continued*) to all of the cells (black line for glomerular basement membrane [GBM], gray shading for mesangial matrix). **(B)** Ultrastructure of the capillary basement membrane. Note the trilaminar structure of the GBM and the pores in the epithelial slit diaphragm that are the ultimate determinant of steric selectivity for the filtration of macromolecules. LRE, lamina rara externa; LD, lamina densa; LRI, lamina rara interna. Reprinted with permission from **ref. 37**.

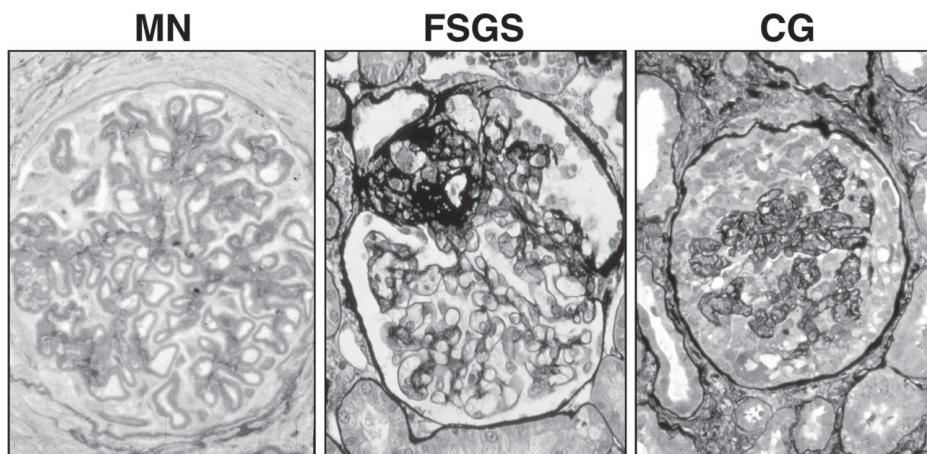


Fig. 3. Glomeruli from patients with membranous nephropathy (MN), focal segmental glomerulosclerosis (FSGS), or collapsing glomerulopathy (CG). The darkest areas show staining for collagen. MN shows thickened basement membranes; FSGS involves the segmental accumulation of collagen; and CG manifests similar accumulation of collagen with collapsed capillary structures. Microphotographs courtesy of Drs. Charles Alpers and Stuart Shankland, University of Washington.

of ECM accumulation and the segmental appearance of the lesion contrast with the diffuse, finely structured involvement of the capillary loops in MN. The likely source of this ECM is the mesangial cell. In collapsing glomerulopathy (CG), FSGS is accompanied by additional events involving the podocyte, leading to a different pattern of matrix accumulation. Thus, the nature of the glomerular scar is likely determined by the structural pattern of the cells that are involved.

A third form of renal fibrosis starts in the tubulointerstitium. In its most common form, diseases like chronic recurrent kidney infections combine with reflux of urine into the renal parenchyma to stimulate fibrogenesis, even in the glomeruli. Another example is the sclerosing effect of chronic tubular toxins. It is important to emphasize that glomerular and tubular processes cannot be entirely distinguished as a cause of end-stage kidney disease. For example, loss of nephrons as a result of tubular damage from reflux or of certain progressive genetic disorders of the tubule causes involution of the glomeruli that are at the proximal portion of the same tubules. Conversely, progression of FSGS is predicted by the identification of lymphocytic infiltration of the tubulointerstitium (8), suggesting that excessive delivery of serum components to the tubule initiates an inflammatory cascade that plays an important part in

nephron loss and fibrogenesis. Even in diseases that typically have been thought of as glomerular (such as protein-overload proteinuria, in which repeated intraperitoneal injections of albumin into rats eventually causes glomerulosclerosis), the critical pathogenetic event in the progression towards end-stage disease may be increased expression of tissue inhibitor of metalloproteinase (TIMP)-1 in the peritubular space (9). Together, glomerulosclerosis and tubulointerstitial fibrosis may be the necessary twin hallmarks of ECM accumulation and scarring in progressive kidney disease.

### 3. Epidemiology and Genetics of Renal Fibrosis

Several factors suggest an interplay of environmental factors and genes in the advent of renal fibrosis. The incidence of FSGS, which already is the most common acquired cause of chronic renal failure in children, is increasing in both children and adults (7). FSGS has been associated with numerous syndromes and a number of sibling pairs have been reported to have the disease (6). A striking aspect of this familial incidence is that cases may be clustered temporally within families, suggesting that a genetic predisposition was present in those families and that a common event precipitated the disease.

Recently, a series of genes has been identified whose mutations define apparently Mendelian inheritance patterns for FSGS. The Wilms tumor suppressor gene, *WT1*, is one such gene. Mutation of exons 8 or 9, which encode the zinc finger region of the protein, has been associated with two syndromes (10). Denys–Drash syndrome includes congenital nephrotic syndrome (massive proteinuria leading to severe edema), XY pseudohermaphroditism, and Wilms tumor (11). The histopathology in these patients is diffuse mesangial sclerosis, characterized by increased ECM production and cellular proliferation in the mesangium. Other patients with *WT1* mutations have Frasier syndrome (12). These patients manifest histopathology more like that of FSGS, in which the cellularity of the glomerulus is unaffected and ECM accumulation has a characteristic focal and segmental (rather than diffusely distributed) pattern. They also have associated gonadal abnormalities. It is striking that the nature and severity of clinical findings may vary even among family members (13), suggesting the important influence of additional genes or environmental factors.

Single-gene mutations for glomerulosclerosis have been identified in *NPHS1*, *NPHS2*, or *ACTN4*. All of these genes encode for podocyte structural proteins. *NPHS1* is the gene for nephrin, a protein that helps form the epithelial slit diaphragm that is the final restrictive component regulating the size of molecules permitted to pass through the glomerular filtration barrier. It is mutated in congenital nephrotic syndrome of the Finnish type (14), a progressive and treatment-resistant condition in which infants are born with massive proteinuria. *NPHS2* is the gene for podocin (15), a protein that is found in the foot-



processes of the podocyte. Homozygous and single-allele mutations have been found in some cases of FSGS in children who manifest a variety of clinical presentations (16). *ACTN4* is the gene for  $\alpha$ -actinin4, a protein that helps mediate attachments between the cytoskeleton and adhesion molecules on the surface of the podocyte. Mutations inherited in an autosomal dominant pattern are found most commonly in patients who present with FSGS in early adulthood (17).

An intriguing consideration in the genetics of renal fibrosis is the possible existence of a so-called “renal failure gene.” Conjecture regarding such a gene is based on two considerations. One is the observation that patients in families with renal disease are much more likely than those in the general population to require dialysis for end-stage kidney disease (18). The second consideration is the increased incidence of end-stage renal disease (19), or more aggressive rates of the progression of fibrosis (20), in African-American patients compared with Caucasians. This increased incidence in African-Americans has been attributed to hypertension but, in the families with a high incidence of close relatives on dialysis, most of the cases were not the result of hypertensive kidney disease. These observations of familial and racial predilections may intersect in the classic studies of the Pima Indian tribe in the American southwest. This group has an extremely high incidence of type 2 diabetes mellitus, but not all of the patients have progressive kidney disease. Instead, there is a familial distribution of severe renal involvement (21), suggesting that the genetic trait responsible for the renal failure may be distinct from that for the type 2 diabetes.

The nature of such a genetic trait remains a subject of considerable interest. Areas of potential study include gene polymorphisms that might lead to overexpression of molecules that exacerbate the physical factors contributing to glomerulosclerosis, molecules that represent hormonal mediators that enhance renal scarring, and molecules for which no obvious connection to sclerosis exists. In order to understand how such targets might be selected, it is appropriate to first consider the physical and biochemical factors that have been associated with renal scarring.

## **4. Pathophysiology of Renal Disease**

### **4.1. Animal Models**

The first clues regarding the causes of progressive kidney disease were derived from studies of animal models, largely in the rat. These models examine the effects of excessive nephron load or mechanical injury, toxic/metabolic stimuli of fibrosis, genetic predilection (hypertensive or diabetic rats and mice, Samoyed dogs) or, most recently, gene overexpression or deletion studies. Nephron overload has been accomplished either by repeated injections of



bovine serum albumin (BSA) to cause excessive glomerular filtration of protein (22), or ablation of renal mass to cause a compensatory response in remaining nephrons (23). In both models, proteinuria is accompanied by sclerosis of both the glomeruli and the tubulointerstitium. In particular, the subtotal nephrectomy model has been used to study the hemodynamic factors that contribute to renal scarring (24). Another form of injury is that involved with unilateral ureteral obstruction (25). Together, these models can be conceptualized as involving insults delivered primarily to the glomerulus (subtotal nephrectomy), the tubule (obstruction), or both (overload), yet the effect is to stimulate fibrosis in both sites.

Biochemical stimuli of fibrogenesis include treatment with puromycin aminonucleoside (26) or doxorubicin (Adriamycin) (27), a model felt to have some elements of FSGS but also to show tubulointerstitial changes. Other common models of fibrosis in animals include immune complex diseases (28), and diabetes mellitus (29). Rats rendered hyperlipidemic by diet or other manipulations also develop glomerulosclerosis (30). Hyperlipidemia from genetically inherited traits also leads to kidney fibrosis (31). Another genetic trait that has been associated with glomerulosclerosis in the rat is hypertension (32). Finally, just as there may be a “renal failure” gene in humans, different strains of rodents show differential susceptibility to the induction of renal fibrosis. For example, mice with the ragged oligosyndactyly pintail background show enhanced susceptibility to a variety of stimuli of renal fibrosis compared with those lacking this background (33).

Finally, a number of transgenic mouse models develop glomerulosclerosis. Two involve the disruption of podocyte genes that are identical to those identified in relation to human disease, *ACTN4* (34) and *nephrin* (35). Mice with deficient expression of the podocyte structural protein CD2AP, which interacts with nephrin, develop a disease that histologically approximates FSGS and die at an early age (36), again supporting the importance of effective podocyte structure and cell–cell interactions in maintaining normal ECM expression. For other transgenic or knockout models, however, the relationship is less direct. Thus, overexpression of growth hormone, interleukin-6, or a portion of the human immunodeficiency virus genome in mice leads to glomerulosclerosis. All of these genes likely enhance glomerular cell activation or inflammation (reviewed in **ref. 37**). Less clear is the role of the peroxisomal protein Mpv-17; glomerulosclerosis develops in mice lacking this gene (38).

#### **4.2. Physical Factors That Contribute to Glomerulosclerosis**

Clinical observations have suggested that hypertension may accelerate the progression of renal fibrosis (39). However, the majority of investigators now

believe that the critical parameter is not systemic hypertension, but rather intraglomerular hypertension (39). It is possible that this reflects not just the level of pressure within the glomerulus, but a degree of glomerular hyperfiltration (40). Thus, when the renal mass is reduced, the remaining nephrons may hypertrophy and assume the work of the lost nephrons, leading to possible nephron overload (41). In humans with a mild reduction in renal mass, no significant deleterious effects are encountered (42). However, severe reductions in renal mass are frequently associated with glomerular scarring and further nephron loss (41), and even milder degrees of renal ablation can accelerate progression when superimposed upon other lesions (43).

One potential explanation of this phenomenon is that, in the remnant kidney, nephron overload causes increased passage of proteins and biologically active molecules across the glomerular filter to the tubule, with resulting activation of tubulointerstitial fibrosis as described above. In this case, nephron overload has similar effects to those of proteinuria induced by recurrent albumin injections. However, it also has been suggested that reactive hypertrophy directly causes the progressive fibrosis (43). By this model, the same signals that lead to growth of the cellular elements of the nephron also could stimulate fibrogenesis. In support of this possibility, glomerular fibrosis in FSGS often is presaged by enlargement of otherwise normal-appearing glomeruli (44). Regardless of the primary cause, the reactive inflammation and fibrogenesis are exacerbated by alterations in the local physiology. Biologically active lipids stimulate immune responses (45) and the production of inflammatory eicosanoids. Activation of the podocytes and the extrusion of plasma proteins leads to adhesion of the glomerular tuft to the surrounding epithelium (46). This may permit extrusion of plasma proteins into the interstitium, instead of the urinary space, triggering an inflammatory reaction.

#### **4.3. Humoral and Cellular Mechanisms**

A variety of cytokines and soluble mediators have been implicated in renal fibrosis. Angiotensin II, which plays a critical role in regulating intraglomerular hydrostatic pressure, also stimulates hypertrophy by glomerular cells and the production of additional growth factors. Insulin, growth hormone, and insulin-like growth factor (IGF)-1 stimulate both cellular hypertrophy and ECM accumulation (47). An important mediator of fibrogenesis in the glomerulus and the tubulointerstitium is transforming growth factor (TGF)- $\beta$ . This pleiotrophic cytokine enhances ECM synthesis and decreases ECM degradation (48), and also stimulates the expression of connective tissue growth factor (CTGF), another profibrotic cytokine (49). Mechanisms of TGF- $\beta$  action will be discussed later. Other peptides that are expressed locally during renal fibrogenesis

include basic fibroblast growth factor (bFGF) (50,51), epidermal growth factor (EGF) (52), platelet-derived growth factor (PDGF) (53), endothelin-1 (54), tumor necrosis factor (TNF)- $\alpha$  (55), osteopontin (56), and leptin (57) (for a more extensive review of agents that contribute to renal fibrosis, see **ref. 37**). Some of these factors contribute directly by stimulating ECM accumulation, whereas others activate cells, stimulate inflammation or angiogenesis, and induce hypertrophy.

Not all of the cells of the kidney produce all of the mediators required for fibrogenesis. Significant paracrine interactions likely contribute to ECM accumulation. For example, most of the genes for which mutations have been linked to FSGS are expressed specifically in the podocyte, and podocyte abnormalities such as defective structure and apoptosis have been associated with progressive sclerosis. However, ECM accumulation is most often prominent in the mesangium, leading to the segmental appearance of the lesion. Thus, either the podocyte manifests pathological production of cytokines that stimulate mesangial cell fibrogenesis, or the structural changes that occur in the glomerulus as a result of podocyte abnormalities stimulate profibrotic mechanisms in the mesangial cell. The latter possibility could reflect a change in the normal balance of signals that are exchanged among the podocyte, endothelial cell, and mesangial cell to maintain physiological homeostasis. Alternatively, it may reflect changes in physical forces on the cell that occur with the loss of the support structure provided by the podocytes. The resulting stretching could be similar to that observed with intraglomerular hypertension.

An important aspect of cellular responses is the role of epithelial-to-mesenchymal transition (EMT). Although fibroblasts clearly contribute to interstitial fibrosis, increasing evidence supports the notion that at least some of the ECM accumulation is derived from cells that originate in the tubular epithelium. The nephron forms initially from interaction between the branching ureteric bud and nests of mesenchymal cells that condense around the leading edge of the bud. These cells switch from a migratory phenotype to a quiescent, epithelial phenotype (**Fig. 4**) in the process of mesenchymal-to-epithelial transition (MET). This differentiated phenotype is maintained by a combination of cytokines/growth factors, cell adhesion to a basement membrane, and cell-cell interactions that lead to polarity and thus support the transport functions of the tubule. As a consequence of the disease process inflammation, TGF- $\beta$  expression and the loss of cell-ECM adhesion and cell polarity stimulate the cell to de-differentiate back to a matrix-accumulating phenotype that is defined, although not necessarily determined, by the expression of smooth muscle  $\alpha$ -actin ( $\alpha$ SMA) (58). This cell migrates into the interstitium where it has similar properties to those of the native or infiltrating fibroblast. A similar phenom-

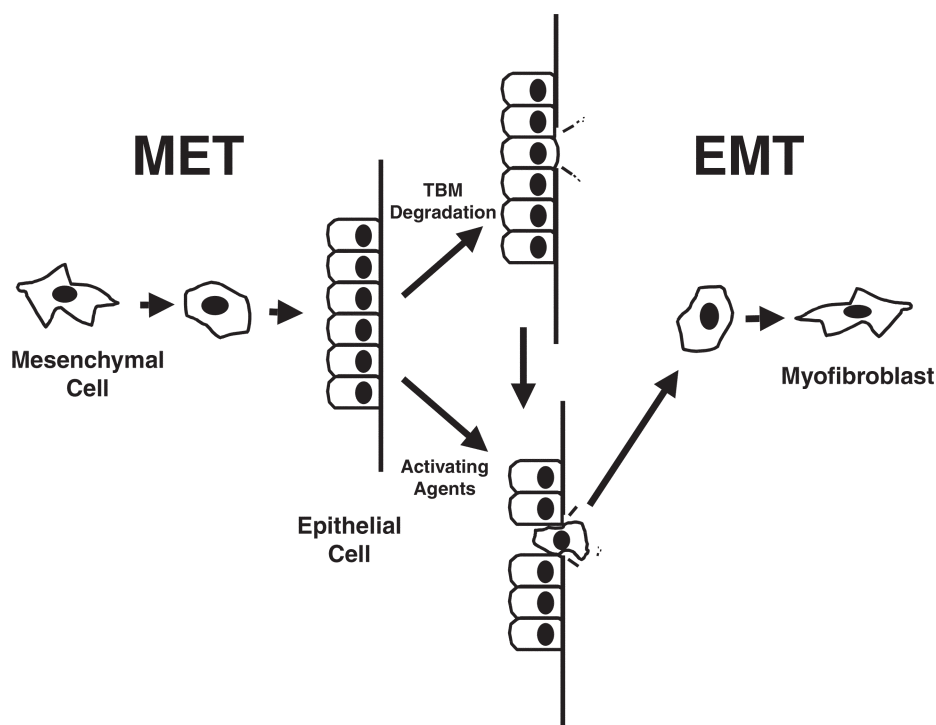


Fig. 4. Differentiation and de-differentiation of the renal tubular epithelial cell. Mesenchymal cells condense during nephrogenesis by the process of mesenchyme-to-epithelial transition (MET) to form a polarized epithelial barrier with tight junctions that permit selectivity of transport. With loss of anchorage and/or degradation of the tubular basement membrane (TBM), the cells become myofibroblasts by undergoing epithelial-to-mesenchymal transition (EMT). Alternatively, activating agents such as growth factors and cytokines may stimulate phenotypic changes that directly cause TBM degradation. In either case, the resulting loss of cell–cell or cell–matrix adhesion generates cells that likely contribute to tubulointerstitial fibrosis.

enon could occur with podocytes, and in the mesangial cell the fibrogenic response in disease (59) and to TGF- $\beta$  (60) is associated with increased  $\alpha$ SMA expression.

#### 4.4. Molecular Mechanisms in Renal Fibrogenesis

As with other systems, the critical determinant of ECM accumulation in the kidney is the balance between ECM synthesis and its degradation. The constituents of this balance in the kidney have been characterized. The glomerular

ECM surrounding the mesangial cell and that in the GBM between the endothelial cells and the podocytes differ in the isoforms of type IV collagen that are found:  $\alpha 3(\text{IV})$ ,  $\alpha 4(\text{IV})$ , and  $\alpha 5(\text{IV})$  in the GBM (61), but  $\alpha 1(\text{IV})$  and  $\alpha 2(\text{IV})$  in the mesangium. The mesangial matrix is relatively unique in that it is not a basement membrane in the true sense but still contains basement membrane collagens rather than interstitial collagens. The glomerulus also includes s-laminin, a kidney-specific laminin (62). The kidney-specific ECM proteins are critical for normal glomerular development; in Alport's disease, where  $\alpha 1/2(\text{IV})$  chains are incorporated into an abnormal GBM (63), structural abnormalities may manifest as the gradual development of renal disease (64). Alternatively, abnormal glomerular ECM may impair development sufficiently that a proper glomerulus is not formed (65). The tubular basement membrane and the small amount of interstitial matrix in the tubulointerstitium are more typical of other matrices in the body.

The major ECM proteases are expressed in the kidney: MMPs such as gelatinase A (MMP-2) (66) and meprin (67), and plasminogen activators urokinase (uPA) and tissue-type plasminogen activator (4). The regulators of these ECM proteases, the TIMPs (68) and the plasminogen activator inhibitors (PAIs) (69) also are present. Under normal conditions, the balance among the proteases, their inhibitors, and the synthesis of ECM proteins is tightly regulated. However, in disease, the regulation of the expression of these proteins, and the timing of this regulation, is crucial. For example, as described above under **Subheading 2.**, ECM proteases could play an important role in the resolution of the acute model of anti-Thy-1 nephritis (5), but it may have a critical negative effect on basement membrane integrity in the initiation phase of other diseases (70). Similarly, TIMP-1 expression appears important in the progression of several models of renal fibrosis (71), and PAI-1 appears to have an important role in the ECM accumulation of diseases mediated by TGF- $\beta$  (72). Notably, however, mice deficient in TIMP-1 do not show a decrease in rates of disease progression (23); this could reflect the redundancy of TIMP-related systems that are available to counter the effects of proteases.

Studies of the regulation of this balance have centered on models of kidney diseases that are mediated by TGF- $\beta$  or by diabetes-related effects of high glucose and insulin. TGF- $\beta$  effects have been related to the Smad signaling pathway. In the mesangial cell, collagen I expression is mediated by Smad3 and Smad4 interacting with the *COL1A1/2* promoters (73). Phosphorylation of Smad3 is enhanced by activity of the extracellular signal regulated kinase (ERK) mitogen-activated protein (MAP) kinase (74) and is further dependent on other mediators including protein kinase C (PKC)  $\delta$  (75) and phosphatidylinositol-3-kinase (76). TGF- $\beta$  also stimulates cytoskeletal rearrangement in the mesangial cell (60), an event associated with increased  $\text{Ca}^{2+}$  flux (77). Nuclear

activity of the Smad complex requires the Sp1 transcription factor binding to its sites on the collagen promoter (78). Type IV collagen expression also is activated by TGF- $\beta$  through Smad signaling. Phosphodiesterases appear to play a role in this process (79). Finally, laminin- $\gamma$ 1 expression is regulated in mesangial cells by several transcription factors, including the basic helix-loop-helix/leucine zipper transcription factor, TFE3 (80), and the gut-enriched Kruppel-like factor (GKLF) that bind to the bcn-1 element on the *LAMC1* gene (81), in a manner that requires synergy with Sp1. Although some of these studies have not yet been linked with TGF- $\beta$  stimulation, experiments in other systems indicate that TGF- $\beta$ /Smad signaling may interact with and/or activate these transcription factors.

Although many of the same pathways mediate collagen expression in renal tubular cell culture, there are some important differences. One is that the activation of ERK MAP kinase decreases collagen expression in the renal tubular epithelium. Although there may be opposite effects of ERK on Smad signaling in renal tubular epithelial cells (82), the main mechanism by which collagen expression is inhibited may be the blockage of EMT by ERK in these cells (83); this is a much more complex response than direct activation of collagen genes, and the effects of ERK may therefore be more subtle or complicated. Regardless of the effects on specific events, fibrosis in the kidney is clearly Smad-mediated, since Smads show signs of activation in the kidneys of diabetic mice (84). Further, diabetic nephropathy (85) and obstructive uropathy (86), both of which have major tubulointerstitial components, are ameliorated in Smad3-null mice.

In diabetes, in addition to TGF- $\beta$ /Smad signaling, other pathways have been demonstrated to play a role in fibrogenesis. Insulin activates the signaling pathway mediated by Janus kinase (JAK) and signal transducers and activators of transcription (STAT). High local glucose concentrations also activate JAK/STAT signaling in mesangial cells, leading to cell activation and the production of TGF- $\beta$  and fibronectin (87). Antisense to JAK2 blocks angiotensin II-stimulated type IV collagen expression (88). Because angiotensin II may stimulate TGF- $\beta$  production, and JAK is upstream of TGF- $\beta$  expression, the role of JAK2 could relate to an effect mediated by TGF- $\beta$ . However, it is possible that JAK/STAT directly signals ECM expression as well. In studies with significance for type II diabetes mellitus, excess insulin has been found to phosphorylate the protein translation repressor 4E-BP1, an eIF4E-binding protein, in renal epithelial cells. The result is enhanced translation of laminin proteins that could explain the paradox of decreased laminin mRNA expression but increased laminin protein expression in db/db mice, a model of type II diabetes (89). Finally, the peroxisome proliferator-activated receptors (PPARs) may have diverse roles in fibrogenesis (90). PPAR $\gamma$  is an insulin-sensitizing agent

that also affects the kidney. Troglitazone, a PPAR $\gamma$  agonist of the thiazolidinedione family, reduces collagen I production by mesangial cells in response to hyperglycemia (91), and PPAR $\gamma$  agonists also may ameliorate non-diabetic glomerulosclerosis (92). PPAR $\alpha$  may have deleterious effects on the kidney through its effects on lipid metabolism. Direct effects on renal scarring currently are a subject of investigation.

## 5. Treatment of Renal Fibrosis

The present therapy for kidney fibrosis is comprised of three approaches: strategies to combat inflammation, treatments directed at the physiology of fibrosis, and more novel therapies that have been identified by empirical means. The only accepted therapies directed at the disease process as it is traditionally understood involve anti-inflammatory strategies. Patients whose disease responds to such treatment usually have a better prognosis than those who do not respond. Thus, very high-dose corticosteroids have been used to treat FSGS in children (93) and long-term oral treatment with high-dose corticosteroids has been reported to be efficacious in adults (94). A more standard approach to FSGS is the use of cyclosporine (95). This is the only treatment that has been shown to be efficacious with controlled, prospective trial (96). A significant side effect of cyclosporine is nephrotoxicity, so its long-term use requires cautious monitoring of renal function. Anecdotal reports indicating that other anti-inflammatory drugs such as FK506 (tacrolimus) or mycophenolate mofetil have not yet been tested in larger studies.

To address the physiology of chronic renal disease, clinicians have sought to decrease intraglomerular hypertension or hyperfiltration. Thus, inhibition of angiotensin-converting enzyme (ACE) or blockade of the angiotensin II receptor has been used to delay the progression of diabetic nephropathy (97). Although ACE inhibition has been utilized in other diseases as well, the results have not been as clear-cut. Nonsteroidal anti-inflammatory agents have been used to reduce glomerular filtration rate in order to reduce proteinuria, although such reports are anecdotal and the treatment is potentially nephrotoxic. Studies in rats indicate that severe protein restriction, likely causing decreased glomerular filtration, also may slow the progression of renal failure. Multicenter clinical trials of protein restriction in humans have met with equivocal results (98,99). Anticoagulants or antiplatelet agents also have been tried as treatments for some forms of chronic glomerulonephritis (100), although this approach has not proven broadly successful. It should be noted that some investigators believe that ACE inhibition or angiotensin receptor blockade affects the biology of kidney cells directly, in addition to its hemodynamic effects. Angiotensin II stimulates TGF- $\beta$  expression by several renal cell types (101), suggesting that it stimulates autocrine fibrogenic pathways. Moreover, angio-



tensin II expression has been related to glomerular hypertrophy. Thus, inhibition of this mediator could retard disease progression through multiple mechanisms. Finally, in diabetes mellitus, the best “therapy” is tight control of glucose levels to prevent the deleterious effects of hyperglycemia.

Recently identified empirical treatments are under study in animal models and human trials. Pirfenidone may suppress growth factor production, signal transduction, or reactive oxygen species propagation (*102*) and presently is in clinical trials. Heparinoids such as pentosan polysulfate bind to growth factors and prevent progression in rats that have experimental reductions in renal mass to stimulate fibrogenesis (*103*). These properties were originally identified in heparin and were determined to reflect effects on growth factor activity, not the coagulation pathway (*104*). Other treatments that have been used experimentally in animal models with less clear effects include anti-oxidants and estrogens.

## 6. New Directions for the Study of Renal Fibrosis

Significant challenges remain in the study of renal fibrosis. Two critical issues in the pathogenesis of fibrosis are the process of ECM organization and the mechanism(s) by which physiological events such as intraglomerular hypertension modulate signal transduction pathways to effect scarring. With regard to matrix organization, *in vitro* and *in vivo* studies have demonstrated that integration of collagens into a stable basement membrane requires ascorbic acid. Indeed, in scorbutic mesangial cell cultures, collagen protein can be remarkably short-lived, with more than 80% of recently synthesized collagen degraded within 2 h (*105*). ECM organization is directed largely by the cells that are adjacent to the matrix, in a process that requires integrin binding (*106*). Although it is established that the diabetic milieu may make the ECM more resistant to degradation by proteolytic enzymes (*107*), in general the issue of how organization and stability of the renal ECM are accomplished has not been addressed.

A second issue is that of relating the physiological events to the cell and molecular biology of renal fibrogenesis. How does the replacement of  $\alpha 3(4)/5(IV)$  collagen with  $\alpha 1(2)(IV)$  collagen lead to the development of kidney disease in patients with Alport’s syndrome? How does glomerular hypertension accelerate the progression of glomerular sclerosis? What are the specific proteins, delivered to the tubule via pathological filtration of proteins through the glomerulus, that stimulate tubulointerstitial fibrosis? Are some of these proteins critical effectors of EMT, and, if so, how do they stimulate this process? Understanding how physiology relates to biochemistry will be an important step in understanding how to prevent the loss of kidney function.

The development of new treatments, informed by our improved understanding of disease pathogenesis, remains the ultimate challenge. Given the structural complexity of the nephron and the functional complexity of fibrogenic signals, the elucidation of essential stimuli regulating the development and maintenance of nephron integrity will provide one key to treatment. Another will depend upon the identification of critical nodes of interaction between signaling pathways; interrupting signals at these points will be the most effective way of interrupting disease progression. Finally, several agents have been identified as blocking specific cellular responses to fibrogenic signals. Thus, hepatocyte growth factor (HGF) antagonizes renal tubular cell EMT (82), and bone morphogenetic protein (BMP)-7 similarly antagonizes EMT (108) and perhaps other effects of TGF- $\beta$ /Smad signaling, as it activates an alternative Smad pathway to that activated by TGF- $\beta$  (109). Recent studies suggest that a construct expressing the anti-Smad, Smad7, can be delivered directly to the kidney by infusion and focused ultrasound pulses (25). In addition to gene therapy, the efficacy of biochemical inhibitors related to newer insights regarding fibrogenesis is under study. Thus, because reactive oxygen species have been determined to play a role in at least some models of kidney disease (110), the use of anti-oxidants such as ascorbic acid,  $\alpha$ -tocopheryl (vitamin) E, and selenium may be of benefit. Given the broad number of avenues for investigation and potential treatments, the future can be regarded with significant optimism.

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