

## RENAL FIBROSIS

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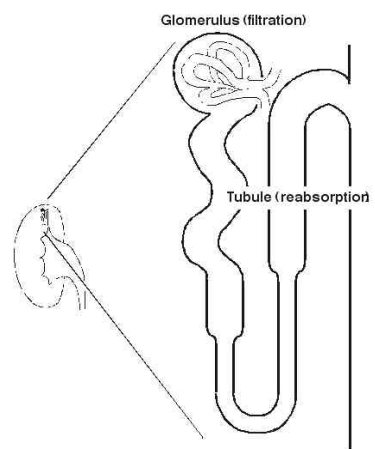
### 1. ABSTRACT

Renal fibrosis causes significant morbidity and mortality as the primary acquired lesion leading to the need for dialysis or kidney transplantation. Fibrosis can occur in either the filtering or reabsorptive component of the nephron, the functional unit of the kidney. Experimental models have identified a number of factors that contribute to renal scarring, particularly derangements of physiology involved in the autoregulation of glomerular filtration. This in turn leads to replacement of normal structures with accumulated extracellular matrix (ECM). A spectrum of changes in the physiology of individual cells leads to the production of numerous peptide and non-peptide fibrogens that stimulate alterations in the balance between ECM synthesis and degradation to favor scarring. Other proteins and small molecules may have anti-fibrotic effects. Manipulation of these opposing systems holds the promise of effective treatments for chronic progressive kidney disease.

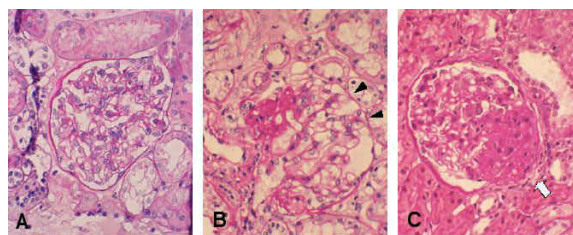
### 2. INTRODUCTION: RENAL FIBROSIS AND THE FUNCTIONAL ANATOMY OF THE KIDNEY

Renal fibrosis is the final common pathway by which acquired kidney disease leads to the need for dialysis or transplantation. End stage renal disease affects over 300,000 people in the United States, costing the economy 8 to 10 billion dollars annually. Despite the prevalence of chronic kidney failure, we are only beginning to understand the biology of renal fibrosis. A number of review articles have been published elsewhere regarding various aspects of disease progression (1-4). This chapter will review our understanding of the physical, cellular, genetic and biochemical processes that contribute to disease.

Fibrosis of the kidney can be categorized in several ways. A common approach to etiology is whether or not it can be attributed to classical inflammatory mechanisms. Other, finely balanced systems regulating fibrosis determine extracellular matrix production vs. degradation, cell proliferation vs. apoptosis, increased circulation vs. vasoconstriction, or functional adaptation vs. excessive workload and hypertrophy. Fibrosis also can be approached based upon the renal compartment in which the sclerotic process initiates. The kidney can be considered to have two functional or structural components: a filtering function accomplished in the renal *glomerulus* and a reabsorptive function via the *renal tubule*. Together, the glomerulus and tubule comprise the *nephron* (Figure 1). There are approximately  $10^6$  nephrons in the human kidney. In a 24-hour period, the glomeruli filter the equivalent of 4- to 5 times the total body water. To prevent dehydration, modulate the fluid volume, and achieve electrolyte and acid-base balance, the tubules reabsorb about 99% of the glomerular filtrate. Although the vast majority of the renal mass is in the tubules and interstitium, significant fibrosis of either the glomerular or tubulointerstitial compartment will impair kidney function. However, the stimuli for fibrosis, the potential effector mechanisms, and the biology of the cells involved are likely to be different in the two compartments. Moreover,



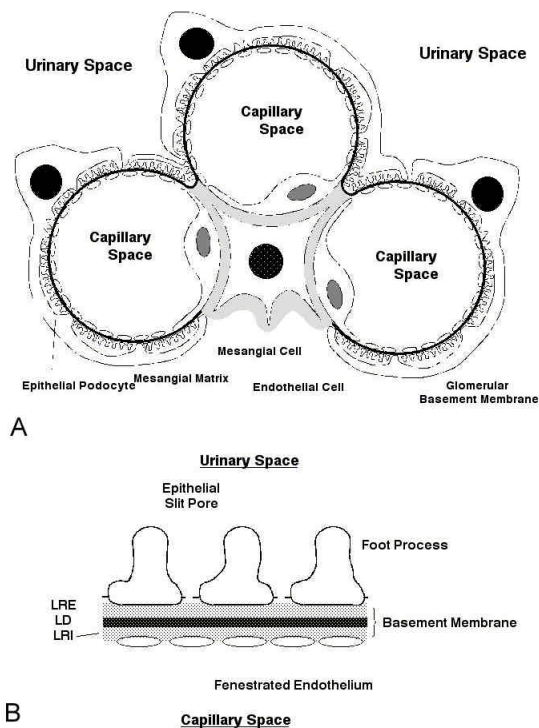
**Figure 1.** Schematic depiction of the nephron, the functional unit of the kidney. After being extruded from the capillaries in the glomerulus, 99% of the filtrate is reabsorbed in the tubule.



**Figure 2.** Histopathology of the glomerulus in health and glomerulosclerosis. (A) Cross-section of a normal glomerulus. The capillaries are clustered around a mesangial stalk, creating the impression of a “bunch of grapes” structure. (B) Mild FSGS. There is an area of extracellular matrix accumulation at 11 o’clock and two fibrous adhesions (synechiae) are denoted by the dark arrowheads. (C) Moderate FSGS. The area of matrix accumulation and collapse (white arrow) occupies more than half of the glomerulus. The matrix is predominant in a centripetal distribution centered at the hilus, the area where the small vessels enter and leave the glomerulus. Note that, despite the severity of the lesion in some areas, other capillaries in the same glomerulus appear unaffected. Photomicrographs courtesy of Dr. W. Hsueh, Children's Memorial Hospital, Chicago, IL.

because of the requirement for strict balance between the amount filtered and the amount reabsorbed, a sensitive system of tubuloglomerular feedback ensures that any process that has an impact on one compartment will have implications for the other.

When fibrosis impairs the filtering activity of the glomerulus, this process is termed *glomerulosclerosis*. Figure 2 shows normal glomerular histology and typical lesions from patients with one form of glomerulosclerosis, termed focal segmental glomerulosclerosis (FSGS). The glomerulus is a tuft of capillaries that undergo collapse and replacement with extracellular matrix in glomerulosclerosis. Under normal conditions the



**Figure 3.** Arrangement of the structures within the glomerulus. The capillaries are adjacent to a mesangial cell stalk (A). A fenestrated endothelium lines the capillaries, and a specialized epithelial cell called the podocyte provides external support but its interdigitations provide sufficient space for the passage of filtrate. Filtration occurs from the capillaries into the urinary space. A basement membrane surrounds all of the cells (gray line in figure 3A). In the case of the capillary basement membrane, it has a trilaminar structure (B) since it is produced by both the podocyte and the endothelial cell. *LRE*, lamina rara externa; *LD*, lamina densa; *LRI*, lamina rara interna. The epithelial slit pore is the final steric barrier to the filtration of macromolecules.

capillaries are surrounded by a structure of epithelial cells that have long, interdigitating processes that serve to regulate glomerular filtration of macromolecules through a system of pores (Figure 3). These pores begin to hinder the passage of molecules about 20 Å in effective radius, reaching complete restriction at about 50 Å. Thus, the larger proteins in the plasma are retained in the blood, despite the freer passage of water and electrolytes. This entire structure is supported by a stalk-like *mesangium*. The mesangial cell is of mesenchymal origin and has some functional and structural similarities to smooth muscle cells and macrophages. A basement membrane separating the capillary endothelium and the visceral epithelial cells is trilaminar, probably due to its derivation from both cell layers. The mesangium also contains an extracellular matrix that is unusual in that it has characteristics more like basement membrane (including primarily collagen IV) than matrices found in most interstitial compartments in the body. In glomerulosclerosis, any one of these cell types can contribute to fibrosis (5-7), depending upon the

initiating stimulus; interestingly, much of the accumulated matrix appears to be produced by cells native to the glomerulus.

In contrast, the origin of *tubulointerstitial fibrosis* is less well characterized and could be derived from cells intrinsic to the region or migrating in from other tissues. In addition to the tubules, which comprise the bulk of the kidney, this anatomical compartment includes numerous blood and lymph vessels that serve to transport reabsorbed fluid and electrolytes back to the general circulation. Cells native to the tubule (8) or interstitium (9) can contribute to fibrosis in this compartment.

### 3. GLOMERULOSCLEROSIS AND INTERSTITIAL FIBROSIS: LESSONS FROM HUMAN DISEASES

#### 3.1. Glomerulosclerosis

Normally, the glomerulus in cross section has a “bunch of grapes” appearance with multiple open loops representing capillaries cut in cross section (Figure 2). In glomerulosclerosis, this pattern is altered. Accumulating matrix proteins, and the collapse of normal structures, often occurs first in the area nearest the mesangial stalk. In focal segmental glomerulosclerosis (FSGS), not all of the glomeruli are affected (that is, the process is *focal*) and the spread of the lesion from the mesangium accentuates the lobular appearance of the glomerulus (hence, the *segmental* nature of the lesion). This picture can present with increased or decreased cellularity. If the process initiates in the periphery of the glomerulus (in particular if it initiates from the glomerular epithelial cell), the segmental nature may not be apparent.

Glomerulosclerosis denotes increased deposition of extracellular matrix proteins within the glomerulus. The critical final event of extracellular matrix accumulation may result from a variety of chronic renal diseases, including chronic glomerulonephritis (inflammatory process in the filter), chronic obstructive kidney disease or transplant nephropathy (Table 1) (10). This common result suggests that a variety of factors may contribute to the development of glomerulosclerosis, and that local disruptions of physiology may lead to conditions that support the accumulation of extracellular matrix in fibrosis. The FSGS lesion shown in Figure 2 occurs as part of several distinct clinical syndromes, including idiopathic FSGS, HIV-associated FSGS and hyperfiltration injury due to reduced numbers of functioning nephrons.

Several clues regarding mechanisms involved in glomerulosclerosis can be derived from clinical observations. One is that the presence of unremitting, massive proteinuria (urinary protein excretion) is associated with progressive renal scarring (11). This observation could indicate that heavy proteinuria is in itself fibrogenic, but patients with some forms of heavy proteinuria do not necessarily develop FSGS (12). Thus, it is equally possible that patients with more severe (and therefore more progressive) diseases also are more likely to have large amounts of proteinuria.

**Table 1.** Diseases associated with FSGS

Idiopathic (primary) FSGS
Secondary glomerulosclerosis
<ul style="list-style-type: none"> <li>• Drugs               <ol style="list-style-type: none"> <li>1. Analgesics</li> <li>2. Heroin</li> </ol> </li> <li>• Reflux/ obstructive nephropathy</li> <li>• Loss of renal mass/hyperfiltration               <ol style="list-style-type: none"> <li>1. Unilateral renal agenesis</li> <li>2. Oligomeganephronia</li> <li>3. Segmental hypoplasia</li> </ol> </li> <li>• Obesity</li> <li>• Pre-eclampsia</li> <li>• Human immunodeficiency virus</li> <li>• Hereditary disorders               <ol style="list-style-type: none"> <li>1. Glycogen storage disease (type I)</li> <li>2. Familial dysautonomia</li> <li>3. Spondyloepiphyseal dysplasia</li> <li>4. Epidermolysis bullosa</li> <li>5. Sickle cell disease</li> </ol> </li> <li>• Aging</li> <li>• Malignancy</li> <li>• Transplantation               <ol style="list-style-type: none"> <li>1. Recurrence</li> <li>2. De novo</li> </ol> </li> <li>• Miscellaneous               <ol style="list-style-type: none"> <li>1. Radiation nephritis</li> <li>2. Schistosomiasis</li> <li>3. Tuberculosis</li> <li>4. Infantile spasms</li> </ol> </li> </ul>
Glomerular diseases
<ol style="list-style-type: none"> <li>1. Alport's</li> <li>2. Membranous nephropathy</li> </ol>
Other progressive renal diseases

Adapted from (10).

A second source of potential mechanistic insight is to be found in recent progress in identifying genetic determinants of glomerulosclerosis. For some time, anecdotal reports have described a familial incidence. Some HLA-related predispositions have been identified, including HLA-DR4 in all American patients and HLA-A28 in African Americans (13); -B8 in children (14); -DRw8 in nephrotic Hispanic children (15), and -B8 combined with -DR3 and -DR7 in German children (16). HLA-Bw53 has been related to heroin nephropathy-associated FSGS (17). Not all studies have found such associations (18-20), but the data suggest a predisposition related to immune responsiveness. Another analysis suggests that there is a gene or gene cluster predisposing to progressive scarring regardless of disease. African American and Hispanic children appear more likely than Caucasian children to progress to renal failure with a diagnosis FSGS (21), and certain Pima Indian families with type II diabetes mellitus show much higher rates of progression to chronic renal failure compared to patients with diabetes in other PIMA families (22).

Most recently, specific gene mutations have been associated with glomerulosclerosis. These include the *nephrin* gene (*NPHS1*) associated with Finnish-type congenital nephrotic syndrome (23), and the *podocin* gene (*NPHS2*) associated with FSGS (24). Both of these genes encode proteins expressed by the glomerular visceral epithelial cell, also called the podocyte because of its characteristic foot-processes. Nephrin, which is localized to the slit diaphragm, mediates podocyte cell-cell interactions and likely contributes to the filtration barrier in the kidney. Podocin is a molecule that is associated with the cytoskeleton and cell shape. Another mutation associated with FSGS is in the *ACTN4* ( $\alpha$ -actinin 4) gene, which binds actin filaments to adhesive structures located in the cell membrane (25). Together, the involvement of these genes suggests a role for deranged glomerular epithelial cell structure and function in glomerular sclerosis. Mutations of the WT1 (Wilms' tumor suppressor) gene, particularly in exon 8 or 9, are associated with glomerulosclerosis in children with Denys-Drash or Frasier syndromes (26). These two syndromes include varied forms of glomerular scarring and gonadal dysgenesis. Interestingly, the differences in the syndromes are not explained by differences in mutations, and there is variability of organ involvement even among family members with identical mutations. Thus, disease expression is likely influenced by other factors, and multiple genes can affect the development of renal fibrogenesis.

### 3.2. Tubulointerstitial fibrosis

Glomerular scarring may not represent the most significant form of fibrosis in progressive renal disease. Two lines of evidence suggest a critical role for tubulointerstitial fibrosis. First, progression may occur primarily as the result of chronic tubulointerstitial disease of an inflammatory nature (most directly apparent in chronic pyelonephritis or reflux of urine from the lower urinary tract into the kidney with voiding) or toxic nature (chronic exposure to heavy metals or certain inherited disorders involving tubular cell protein accumulation). A probable functional scenario for this sort of injury leading to loss of kidney mass is that irreversible damage to the reabsorptive part of the nephron causes atrophy of the filtration apparatus. This process may involve tubuloglomerular feedback, where decreased capacity of the tubule leads to feedback "shutting down" of filtration. Alternatively, fibrosis of the tubule may cause a poorly understood process that leads to sclerosis of the corresponding glomerulus, either directly or through some sort of alteration in vascular supply. A second line of evidence supporting a critical role for tubulointerstitial fibrosis is derived from the observation that the single finding on renal biopsy that best predicts progression of glomerular disease is the presence of interstitial infiltrates of inflammatory cells (27, 28). It is not clear whether these cells are present in order to reabsorb damaged tubules or are themselves agents of an inflammatory effector mechanism leading to tubular damage and loss. Moreover, if the latter is true, the stimulus for this effector mechanism is not known. It has been postulated that the delivery of toxins, cytokines, lipids or reactive oxygen species to the

tubule increases as a result of increased glomerular passage of macromolecules. These materials could then lead to further tissue damage and nephron loss by stimulating inflammation.

### 4. ANIMAL MODELS OF RENAL FIBROSIS

#### 4.1. Stimuli of fibrogenesis

To understand how renal fibrosis occurs, investigators have studied a number of animal models of renal scarring. There are no true models of primary FSGS, so the goal of animal studies has been to identify conditions that lead to this histopathology in order to define the factors that contribute to glomerulosclerosis and renal fibrosis. Some of these models involve the “spontaneous” occurrence of fibrosis. Several are related to hypertension, including the spontaneously hypertensive rat (SHR) (29) and the Dahl salt-sensitive rat (30). Aging leads to sclerosis in the Wistar rat (31), a process that is accelerated in the fawn-hooded rat (32). In some of these models, the rat may develop severe arteriolar lesions rather than true glomerulosclerosis. Another model in which rats develop glomerulosclerosis is the obese Zucker rat (33). This model suggests that either increased filtered load due to body mass, or hyperlipidemia itself, could stimulate fibrogenesis.

Altering systemic or local physiology experimentally can lead to glomerulosclerosis. Streptozotocin has been used to induce insulin-dependent diabetes mellitus in rats (34), mice and hamsters (35), leading eventually to glomerulosclerosis (see also section 4.4.2.1). Feeding of high-cholesterol diets to rats (36) or guinea pigs (37) also may cause sclerosis. Given the increasing incidence of obesity and diabetes in the industrial world, these are important models for study. Inducing glomerulosclerosis by direct manipulation of the kidney also represents a major experimental approach. Sclerosis can be stimulated by nephron “overload” through either excessive protein administration (38) or reduction in total renal mass (39). Either approach increases the amount of filtration per nephron unit. Inflammation-induced glomerulosclerosis occurs after the administration of anti-thymocyte antibody (anti Thy-1) to rats or mice. This treatment causes mesangiolysis, which is followed by a transient expansion of the glomerular extracellular matrix. The lesion can be rendered permanent and progressive by a second dose of antibody at the height of the initial episode (40). Biochemically, FSGS-like histopathology can be induced by treatment with adriamycin or toxins such as puromycin aminonucleoside (41). In puromycin injury, acute nephrotic syndrome heals over a few weeks and is followed months later by FSGS. Bromoethyamine induces acute papillary necrosis in rats, followed by focal interstitial fibrosis. Cyclosporine induces interstitial fibrosis in rats and mice, at least in part due to ischemia consequent to endothelial dysfunction. Unilateral ureteral obstruction stimulates both glomerulosclerosis and tubulointerstitial fibrosis (42, 43). Finally, transgenic models have implicated a number of cytokines and growth factors, including growth hormone (44), interleukin-6 (45),

Src-related tyrosine kinases (46), transforming growth factor (TGF)- $\beta$  (47), and reactive oxygen species (48).

#### 4.2. Pathophysiological events

##### 4.2.1. Intraglomerular hypertension, hyperfiltration and hypertrophy

Increased systemic blood pressure is transmitted imperfectly to the glomerulus as a result of the defense mechanism of autoregulation. Thus, increased blood pressure within the glomerular afferent arteriole is a potent stimulus to vasoconstriction. Renal autoregulation may not be entirely successful, because of host (possibly genetic) limitations or severe systemic hypertension, leading to glomerular capillary hypertension. Decreasing intrarenal hypertension (more than systemic hypertension) decreases the amount of glomerulosclerosis or its rate of progression in humans (49) and in animal models (50). A corollary of this observation is the possibility that the critical variable is not blood flow or pressure *per se*, but the increased filtered load per nephron that results. This increased flow is accomplished at the cost of glomerular capillary hypertension and glomerular enlargement, which are associated with increased production of mesangial matrix. As mentioned previously, excessive proteinuria has been associated with renal scarring in human disease and in animal models. Maneuvers that reduce glomerular capillary hypertension, such as angiotensin antagonist therapy, reduce glomerulosclerosis (51). The precise signaling pathways linking hemodynamic forces to mesangial cell activation are not well understood, although one clue may be that cyclic stretch increases collagen and TGF- $\beta$  production by cultured mesangial cells (52). Interestingly, in many animal models, a moderate reduction of renal mass without an additional insult to the kidney is insufficient to induce scarring (consistent with the observation that most humans who lose a kidney due to trauma or transplant donation show no untoward effects). But humans and rats with more severe reductions in renal mass are at risk for progressive renal failure, suggesting that the degree of functional compensation that is required is an important determinant of scarring.

A potential mediator of this fibrotic effect is the resulting hypertrophy of the nephron. It is known that enlarged glomeruli are a harbinger of glomerulosclerosis in humans (53). Addressing the potential direct effects of nephron overload vs. subsequent hypertrophic effects, Fogo and colleagues removed 2/3 of the left kidney from rats and diverted the ureter from the right kidney so that it released the urine back into the rats' abdominal cavities. These rats showed less scarring in the left kidney than did rats in which the right kidney had been removed. Since both groups of rats had identical levels of net renal function, the study was interpreted as demonstrating a role for hypertrophy, stimulated by the greater loss of renal mass in the group undergoing right nephrectomy (54). To further support the possibility that hypertrophic stimuli may cause fibrotic changes independent of direct filtration load in response to subtotal nephrectomy, the anatomical location of nephrons where sclerosis first occurs differs from that where the greatest increase in filtration occurs (55).

### 4.2.2. Other pathophysiological mechanisms

Additional physical events may contribute to scarring. Lipid nephrotoxicity is suggested by similarities between atherosclerosis and glomerulosclerosis (56). It should be noted that glomerulosclerosis occurs in capillaries, whereas atherosclerosis occurs primarily in larger arterial vessels. Nonetheless, it has been proposed that the mesangial cell, which takes up and metabolizes lipid (57), could play a role in glomerulosclerosis similar to that of the vascular smooth muscle cell in atherosclerosis. Modulation of hyperlipidemia alters rates of progression in animal models (58). Direct effects of lipids on renal cell function also may be important (57). In addition, the effects of lipids could be hemodynamic (59) or immunostimulatory (60). Immunostimulation is reflected by increased mononuclear cells in both the glomerulus (61) and the tubulointerstitial compartment (62).

Another potential mechanism takes into account the anatomy of the glomerular lesion. Many authors now believe that an early event after injury is loss of podocytes or podocyte activation, leading to adhesion of the glomerular capillary to Bowman's capsule, which surrounds the glomerulus (5). An example of such an adhesion is shown in Figure 2. Kriz has further proposed that this could allow glomerular filtrate, with its plasma content of proinflammatory molecules, to be delivered directly into the tubulointerstitium. A reactive process could be initiated, leading to tubulointerstitial fibrosis and nephron loss (63).

## 5. CELLULAR AND MOLECULAR MECHANISMS

### 5.1. The fibrogenic process

A characteristic of idiopathic FSGS, and of many other forms of glomerulosclerosis encountered both clinically and in experimental models, is its focality. Even within a single glomerulus, there may be areas of intense involvement and areas that appear to be spared. Thus, the development of this lesion does not represent a diffuse and nonspecific manifestation of injury, but rather a highly regulated process that reflects a number of systemic and local factors. A hypothetical model can be constructed invoking a cascade of biological events. Recent advances in the genetic analysis of FSGS have identified a number of gene mutations that involve proteins that govern the structure and function of the epithelial cell (23-25). Thus, a change in the epithelial cell structure permitting hyperfiltration, ballooning of the capillary and/or epithelial podocyte dysfunction appears to initiate a lesion. It is possible that the mesangial cell plays a supporting role in matrix accumulation, and in some models such as radiation nephritis, the endothelial cell plays an important role (64). These complex paracrine interactions (or, in the case of overload proteinuria, perhaps simply the effect of massive proteinuria (38)) lead to changes in the charge or composition of the ECM, altering the ability of the matrix to bind growth factors or to serve as a substrate to the adjacent cells. These alterations may change the cell behavior in ways that, in turn, likely cause further changes in the nature of the ECM that these cells produce. Increased ECM synthesis, decreased degradation, or both

(65) lead to matrix accumulation and capillary obliteration, with apoptosis making the process irreversible. This spiral of events results in a pathogenic process that is amplifying, accelerating and sustaining.

From this schema, it is apparent that two types of pathogenic event are necessary for the progression of glomerular disease. The first is a series of cellular activities that stem from abnormal glomerular physiology such as intraglomerular hypertension or hyperfiltration, with resulting changes in cell function that include both initiating events such as cytokine production and terminal events leading to glomerular involution. The second is the matrix-accumulating process itself, with increased production and/or decreased turnover causing fibrosis.

### 5.2. Balance in extracellular matrix (ECM) turnover

#### 5.2.1. ECM synthesis

The extracellular matrix in the kidney is comprised of three different compartments. The ECM proteins found in the typical basement membranes surrounding capillaries and other tubular structures include collagen IV, laminin, nidogen and heparan sulfate proteoglycans. There are kidney-specific laminins, primarily  $\alpha$ -laminin in the glomerulus (66). The mature glomerular capillary basement membrane collagen IV is comprised of the  $\alpha$ 3(IV),  $\alpha$ 4(IV) and  $\alpha$ 5(IV) chains, whereas the mesangial collagen is primarily  $\alpha$ 1(2)(IV). Under normal conditions, the turnover rate for the ECM proteins is relatively low. Mesangial cells make collagen I only in culture and during the extended response to injury. Increased renal cortical expression of a variety of matrix molecules occurs soon after injury that leads to glomerulosclerosis, and may include both proteins normally found in the glomerulus and atypical matrices in response to injury (reviewed in (65)). In experimental interstitial fibrosis, expression of collagen I, -III and -IV, fibronectin and laminin were all increased (67).

#### 5.2.2. ECM proteases

Two major families of extracellular matrix proteases are expressed in the kidney: the matrix metalloproteinases (MMPs) and the plasminogen activators (PAs). The MMPs are a large family of neutral zinc proteases that are secreted as zymogens and then activated catalytically to expose a zinc-containing active site (68). They have varying substrate specificities, although these have been determined primarily *in vitro* and may show different selectivity *in vivo* in health and disease. The second family, the plasminogen activators, also are produced in pro-forms (69). Originally characterized as a part of the thrombolytic (anticoagulant) pathway, they have subsequently been found to modulate ECM turnover as well. Tissue-type PA is produced by most of the cells in the kidney, whereas urokinase-type PA is not usually associated with mesangial cells but is produced by surrounding endothelial cells and epithelial cells. PA activity has been associated primarily with laminin degradation (70) but it also may play a role in the catalytic activation of MMPs (71).

Under normal physiological circumstances, the expression of these molecules is tightly regulated in concert



with the ECM proteins. However, in disease, this balance may be altered. In the rodent Thy1 nephritis model, initial mesangial expansion is decreased during a remodeling phase that coincides with increased expression of MMP-2 (72). In contrast, MMP-2 expression appears to decrease in certain models of ECM accumulation (73, 74).

### 5.2.3. ECM protease inhibitors

The activity of the ECM proteases is regulated post-translationally by a series of biological antagonists. The tissue inhibitors of metalloproteinases (TIMPs) bind to the MMPs and, depending upon the site of binding, either facilitate activation of the protease or inhibit its activation or activity. TIMP-1 shows markedly increased expression in the tubulointerstitium during chronic, progressive tubulointerstitial fibrosis (67). Surprisingly, mice in which specific TIMPs have been genetically deleted do not show a marked decrease in rates of disease progression (75). One potential explanation for this observation is that there are multiple TIMPs that may show significant redundancy of function. A similar family of homologous proteins known as the plasminogen activator inhibitors (PAIs) regulates PA activity. PAI-1 expression is increased by TGF- $\beta$ , and the expression and activity of PAI-1 correlates with the matrix-accumulating phase of several glomerular and tubulointerstitial diseases (reviewed in (76)).

### 5.2.4. Matrix organization

An important and largely overlooked issue is the organization of ECM proteins into stable matrices. This is a process that requires appropriate transport, post-translational processing of the structural proteins and subsequent interaction between the matrix proteins and their receptors. For example, collagens are arranged into lattice-like or fibrillar structures in a process that requires an intact mechanism for communication between the cytoskeleton and the external environment (77). FSGS has been associated with abnormalities of either the cytoskeleton ( $\alpha$ -actinin-4, (78)) or the ECM receptors that link the cytoskeleton to the external environment ( $\beta$ 4-integrin, (79)). This is an area that is ripe for further investigation.

## 5.3. Cellular events

### 5.3.1. Regulation of ECM turnover at the cellular level

Several cellular models for regulation of ECM turnover have been described. The most common glomerular cell that has been studied is the mesangial cell. In part, the rationale for this choice is that it is the cell that is specific to the initial localization of most forms of focal segmental glomerulosclerosis. Moreover, it is the cell most easily propagated from glomerular tissue. Mesangial cells are responsive to a number of mediators (see next section) such as transforming growth factor (TGF)- $\beta$ , platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF), angiotensin II and prostaglandins. These mediators and others stimulate mesangial cell proliferation, apoptosis or changes in ECM turnover. The stimuli for specific cellular events depend upon the initial pathogenic event. For example, mesangial cell proliferation may result from activity of bFGF (80) or PDGF (81) in different models. Angiotensin II may stimulate mesangial cell

contraction (82) and also stimulates the production of TGF- $\beta$  (83), which in turn could act in an autocrine or paracrine fashion to decrease net cellular ECM turnover at the level of ECM production (84) or ECM protease inhibitor activity (85).

The mechanisms of action of these mediators is currently under intensive investigation. A number of cellular signaling pathways have been identified as activated and potentially leading to changes consistent with renal fibrosis. These include the activities of protein kinase C and the ERK MAP kinase pathway during hyperglycemia (86) and the Smad signal transduction pathway stimulated by TGF- $\beta$  (87). Although many of these pathways are characterized as acting at the level of gene transcription in regulating ECM turnover, other mechanisms may contribute as well. For example, an effect on laminin mRNA expression has been postulated to enhance production of that molecule in high-insulin states (88). The regulation of apoptosis occurs in the cytoplasm at the level of MAP kinase and ceramide signaling, as well as in the nucleus via regulation of expression of pro- and anti-apoptotic proteins.

### 5.3.2. Transdifferentiation of renal cells into those with fibrogenic function

Increasing attention has been paid in renal disease to the concept of resident tissue cells differentiating into fibroblasts as a critical event in fibrogenesis. In the blood vessel, the fibrous plaque appears to require the differentiation of the vascular smooth muscle cell into a "myofibroblastoid phenotype." In the kidney, this has been best characterized as a transition from an epithelial cell phenotype (tubular epithelium) to a fibroblastoid cell that produces and secretes ECM (89). This epithelial-to-mesenchymal transdifferentiation is felt to represent a transition to a less-differentiated state since the cell's function is less specific than it was when it was acting as a tubular epithelial cell, regulating transport. Recent data suggest that this process involves a change in the adhesion of the cell to both its underlying basement membrane and neighboring cells. The amount of smooth muscle  $\alpha$ -actin that the cell produces increases, and the cell migrates into the interstitium where it begins to secrete excess ECM (89). This model also may apply to the glomerulus, since mouse mesangial cells begin to produce smooth muscle  $\alpha$ -actin before they produce collagen, and blocking smooth muscle  $\alpha$ -actin expression inhibits collagen production (90).

The potential contribution to fibrosis of cells that migrate into the kidney is not known. Clearly, macrophages play an important role by promoting inflammation and activating profibrotic cascades (91). In addition, it is uncertain whether these cells also undergo transition to a fibroblastoid state and contribute to the net accumulation of ECM.

### 5.3.3. Interaction among cells specific to the kidney

Investigators studying the pathogenesis of progressive kidney disease have differed regarding the primary cell involved in the pathogenesis of ECM accumulation. Human genetic data implicating a variety of

podocyte proteins that connect the cytoskeleton to its external milieu suggest that the epithelial cell is paramount in progressive glomerular disease. Additional data in support of this notion include the enhancing effect of stretch and hypertension on the cell synthesis of, and response to, TGF- $\beta$  (92) (*in vivo*, likely to be transmitted through the capillary to the podocyte) and the finding of shed podocytes in the urine of patients (93). Data attributing sclerosis to a hypertrophic stimulus also suggest a feedback mechanism, from the glomerular or tubular epithelium to the other cells of the glomerulus. In contrast, those who favor a critical role for the mesangial cell point to its central location in the glomerulus where much of the ECM accumulates, its role as a phagocytic cell and possibly as a regulator of the microcirculation, and its ready transformation to a matrix-accumulating phenotype. It is likely that the mesangial cell and the podocyte interact via paracrine mechanisms, modulating each other's function. It is possible that the epithelial cell interaction with its ECM could be disrupted by inflammation, hydrostatic pressure, matrix derangements or cytokines and respond with detachment or the production of factors that stimulate mesangial cell ECM accumulation.

### 5.4. Mediators of fibrosis

Numerous mediators of tissue fibrosis have been described. Space is too limited here for a comprehensive review. Therefore, the focus in this section will be briefly discuss selected recent developments, and will emphasize for references recent review articles. For simplicity, we will offer the term fibrogen to define a peptide or non-peptide mediator of the fibrotic process, defined as an agent which directly or indirectly stimulates the proliferation or activation of fibroblasts, the transdifferentiation of cells into fibroblasts, the conversion of mesenchymal cells (eg., mesangial cells) into an activated producer of fibrotic matrix protein, or the production of fibrotic matrix protein by mesenchymal cells.

#### 5.4.1. Peptide fibrogens

##### 5.4.1.1. Transforming growth factor- $\beta$

Transforming growth factor- $\beta$  (TGF- $\beta$ ) is secreted as a latent protein and, following activation to mature TGF- $\beta$ , serves as the prototypical fibrogen. TGF- $\beta$  expression in the kidney is increased in both infiltrating cells and most parenchymal cells in one or more models of experimental renal disease, and increased circulating levels of TGF- $\beta$  also induce renal fibrosis (94). TGF- $\beta$  receptor stimulation is associated with activation of the Smad pathway as well as MAP kinase pathways (95), resulting in production of other mediators such as PAI-1, as well as matrix proteins including collagens and fibronectin.

##### 5.4.1.2. Connective tissue growth factor

Connective tissue growth factor (CTGF) is a member of the CCN protein family, named for members Cyr61 (cysteine rich protein), CTGF, and Nov (nephroblastoma overexpressed gene) (96, 97). A major regulator of CTGF expression is TGF- $\beta$ , leading to the suggestion that CTGF serves to mediate TGF- $\beta$  action. However, ablation of the TGF- $\beta$ /Smad signaling pathway inhibits collagen promoter activation, indicating a direct

link that obviates a role for CTGF as the sole mediator of TGF- $\beta$ -stimulated fibrosis. CTGF expression is increased in cultured mesangial cells exposed to TGF- $\beta$ , high glucose medium, or cyclic stretch (98) and *in vivo* in interstitial fibroblasts in the remnant nephron model (99).

#### 5.4.1.3. Angiotensin II

Angiotensin II (AII) has come of age as a driver of tissue fibrosis in heart, blood vessels, and kidney (100-104). AII may be delivered via the circulation or produced locally, and promotes fibrosis both directly, acting on parenchymal cells, and indirectly, acting via inflammatory cells. Mechanical strain and oxidized low-density lipoproteins increase the expression of angiotensin type I receptors, suggesting that angiotensin-mediated stimulatory effects may not require an absolute increase in AII levels. AII stimulates production of TGF- $\beta$  by mesangial cells and possibly other renal cell types as well. It also stimulates cultured interstitial fibroblast proliferation; converts fibroblasts to activated, smooth muscle actin-positive myofibroblasts (a property shared with PDGF and TGF- $\beta$ ); and increases matrix production by these cells. AII stimulates production of PAI-1, thereby inhibiting activation of matrix degrading enzymes. As mentioned above, AII stimulates chemotaxis of inflammatory cells and by increasing production of MCP-1 by mesangial cells and osteopontin by tubular epithelial cells.

#### 5.4.1.4. Plasminogen activator inhibitor-1

Plasminogen activator inhibitor-1 (PAI-1) is upregulated in fibrotic renal disease and may contribute to impaired matrix degradation (105). PAI-1 knockout mice have reduced interstitial collagen and TGF- $\beta$ 1 content and reduced interstitial fibrosis following unilateral ureteral obstruction, although there was no change in plasmin activity (105). The authors concluded that the favorable effects of the absence of PAI-1 were due to the role of PAI-1 in the recruitment and activation of macrophages and fibroblasts.

#### 5.4.1.5. Insulin

Insulin increases mesangial cell expression of collagens I and IV and fibronectin (106). It enhances renal proximal tubular epithelial cell translation of laminin mRNA into protein through a PI-3-kinase- and mTOR-dependent mechanism (88), suggesting a novel mechanism for ECM accumulation. In view of the exploding incidence of non-insulin-dependent diabetes mellitus in the developed countries, this mechanism may have increasing importance for progressive renal disease.

#### 5.4.1.6. Growth hormone and insulin-like growth factor-1

Mice transgenic for the overexpression of growth hormone (GH) develop glomerulosclerosis (107). Interestingly, although many GH effects are mediated through insulin-like growth factor-1 (IGF-1), which is present in glomerular filtrate under conditions of proteinuria (108), mice transgenic for IGF-1 do not develop glomerulosclerosis spontaneously. However, mice transgenic for IGF-binding protein-1 do develop sclerosis (109). GH effects could be indirect, related to



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hyperfiltration (110). Further, GH antagonism prevents glomerular hypertrophy and albuminuria in nonobese diabetic mice (111).

### 5.4.1.7. Basic fibroblast growth factor

Basic fibroblast growth factor (FGF2) has prominent angiogenic and mitogenic activity. FGF-2 is up-regulated in the mesangial proliferative phase of fibrotic renal disease (80) and chronic administration to rats accelerates glomerulosclerosis initiated by other stimuli (112).

### 5.4.1.8. Epidermal growth factor

Epidermal growth factor (EGF) supplementation attenuates the course of acute renal failure, but interruption of EGF signaling in a transgenic mouse bearing an EGF dominant negative receptor was associated with reduced interstitial fibrosis in 3/4 nephrectomized mice (113). This suggests that EGF may play some role in fibroblast recruitment or differentiation. In contrast, in congenital obstructive uropathy, EGF appears to protect against fibrosis (114).

### 5.4.1.9. Platelet-derived growth factor

Platelet-derived growth factor (PDGF) is potent mitogen for mesenchymal cells, in particular mesangial cells. It has been shown to be active in mesangial cell migration in glomerular sclerosis (80).

### 5.4.1.10. Endothelin

Endothelin is a potent vasoconstrictor that may also have direct pro-fibrotic effects. Pharmacologic antagonism reduces fibrosis in experimental models including remnant nephron (115), and endothelin transgenic mice develop glomerulosclerosis and interstitial fibrosis (116).

### 5.4.1.11. Tumor necrosis factor- $\alpha$ (TNF- $\alpha$ )

TNF- $\alpha$  has a major role in inflammation; its role as a fibrogen has not been extensively investigated. Mice lacking either of the TNF- $\alpha$  receptors, p55 or p75, manifest reduced fibrosis following unilateral ureteral ligation (117).

### 5.4.1.12. Osteopontin

Osteopontin is a secreted extracellular matrix protein bearing an RGD motif (classic for integrin binding). Its functions include promoting cell-matrix adhesion and a cytokine regulating inflammation and tissue repair (118). Osteopontin is produced by macrophages and also by renal parenchymal cells, including tubular epithelial cells (119). Osteopontin knockout mice manifest reduced inflammation and fibrosis following ureteral ligation, supporting a pathogenic role (120).

### 5.4.1.13. Leptin

Leptin is a peptide product of adipocytes that binds a hypothalamic receptor, thereby regulating satiety. Leptin stimulates production of TGF- $\beta$ 1 and collagen IV production by cultured mouse glomerular endothelial cells, as well as cellular proliferation (121). Leptin also stimulates expression of the type II TGF- $\beta$  receptor and collagen I by cultured mouse mesangial cells (122). These

data suggest a possible role for leptin in diabetes and obesity-related FSGS.

### 5.4.1.14. Osteonectin

Osteonectin (also known as SPARC, secreted protein rich in cysteine) is a counter-adhesive protein, in a family that includes thrombospondin and tenascin. Osteonectin induces TGF- $\beta$  in glomerulonephritis (123). It has been proposed that osteonectin and TGF- $\beta$  constitute an autocrine feedback loop, in which each stimulates the other and both stimulate the activation of mesangial cells to a collagen I-producing phenotype (124).

### 5.4.1.15. Transcription factors

Ets family transcription factors promote epithelial-mesenchymal transition (125), and thereby may have important roles in renal fibrosis. Ets family members heterodimerize with AP1 to increase transcription of MMP, which increases matrix degradation, and Ets heterodimerizes with Sp1 to increase transcription of tenascin-C and collagen I, promoting matrix accumulation. This area is a fruitful one for future development.

## 5.4.2. Non-peptide fibrogens

### 5.4.2.1. Glucose and related molecules

A sizable literature documents that renal cell culture in the presence of increasing concentrations of glucose alters cellular metabolism toward matrix accumulation, suggesting that ambient glucose level may contribute to fibrosis. Mesangial cells and tubular epithelial cells cultured in high glucose medium have increased collagen I and -IV mRNA levels, possibly due to increased gene transcription (126). Mesangial cells exposed to high glucose produce large amounts of CTGF (127).

Chronic hyperglycemia increases advanced glycosylation end-products (AGE) and these are potential mediators of glycemia-associated pathology (128). AGE increase production of PDGF by mesangial cells (129) and production of CTGF by fibroblasts (130), and induce proteinuria and glomerulosclerosis in rats (131). Glycosylation of ECM may make it less susceptible to degradation by MMPs (132), altering the balance between synthesis and breakdown to favor accumulation. Inhibition of AGE accumulation with aminoguanidine ameliorates glomerulosclerosis in diabetic mice, more (albeit indirect) evidence for the role AGE in matrix expansion. Whereas most murine models of diabetic nephropathy have shown limited histopathologic change, mice that have insulin deficiency and over-express the AGE receptor develop diabetic glomerulosclerosis, adding weight to the argument that AGE are important as mediators of diabetic glomerulopathy (133).

### 5.4.2.3. Reactive oxygen species (ROS)

Several mechanisms may contribute to generation of oxidant species that act to promote fibrosis. First, ischemia-reperfusion injury may enhance the production of ROS by two enzyme families. NADPH-oxidase dependent enzymes are expressed in phagocytic cells (including macrophages and neutrophils) and in renal parenchymal

cells (the recently-described NADPH-oxidase 4 (NOX4) is present in mesangial cells and tubular epithelial cells (134)). Xanthine oxidase is present and active in endothelial cells. Second, oxidized low-density lipoproteins are endocytosed by monocytes and mesangial cells, serving to activate those cells and promote fibrosis. Oxysterols increase production of TGF- $\beta$  by mouse macrophages (135). Toxic free radicals include superoxide anion ( $O_2^{\bullet-}$ ), reactive nitrogen species such as nitric oxide ( $NO^{\bullet}$ ) and hydroxyl radicals ( $OH^{\bullet}$ ); all of these are unstable and attack a plethora of cellular targets which have been the subject of extensive study. One such product is an aldehyde derived from oxidation of membrane lipids, 4-hydroxy-nonenal (HNE). HNE serves as a marker of oxidative stress and stimulates production of TGF- $\beta$  (135). Markers of oxidative stress, including expression of heme oxygenase-1 and the isoprostane 8-iso prostaglandin F $2\alpha$ , rise in kidneys subjected to unilateral ureteral obstruction, prior to the development of fibrosis (136).

### 5.4.2.4. Hypoxia

Exposure of cultured tubular epithelial cells and renal interstitial fibroblasts to a low-oxygen environment (<1%  $O_2$ ) induces a pro-fibrotic phenotype characterized by increased production of collagen I, TIMP-1 and TGF- $\beta$  production and decreased production of MMP-1 and MMP-2 (137). Antibodies to TGF- $\beta$  did not neutralize this effect, suggesting that this mediator was not responsible for the cell transformation. Instead, the authors suggest that an oxygen sensor increases production of the transcription factor hypoxemia inducible factor-1 (HIF-1), which in turn activates particular genes.

### 5.4.2.5. Aldosterone

Aldosterone has recently been recognized as a potent fibrogen in the heart and kidney. Pharmacologic antagonists (spironolactone or the more selective aldosterone receptor antagonist eplerenone) ameliorates glomerulosclerosis in the salt-sensitive hypertensive rat model, even without a reduction in blood pressure (reviewed in (138)). The mechanisms of aldosterone action may include upregulation of AII receptors, increased synthesis of TGF- $\beta$  and PAI-1, generation of reactive oxygen species, and endothelial dysfunction.

## 6. ANTI-FIBROTIC THERAPIES

There are many potential fibrogen targets and one must consider carefully which targets are likely to be therapeutically important (139). We propose that anti-fibrotic agents only be tested in clinical studies when all of the following criteria are met. (This approach is modified from criteria proposed for anti-cytokine therapy in pulmonary fibrosis by Coker *et al.* (140)).

1. The fibrogen must stimulate *in vitro* mesenchymal cell proliferation or activation (as manifested by increased matrix production), or promote transdifferentiation of epithelial cells into fibroblastic cells.
2. Fibrogen protein expression or activity must be increased *in vivo* in a relevant animal model of tissue fibrosis, if available, and in human disease tissue.

3. Inhibitors of fibrogen expression or function must reduce fibrosis in a relevant animal model, if available.

## 6.1. Peptide inhibitors of fibrosis

### 6.1.1. Interferon- $\gamma$

Interferon- $\gamma$  (IFN- $\gamma$ ) and TGF- $\beta$  act in opposing manners on fibroblasts and lymphocytes, suggesting the possibility of an interaction. The molecular mechanism responsible for this observation appears to be induction by IFN- $\gamma$  of Smad 7, an antagonistic Smad which prevents the interaction of the Smad 3 with the TGF- $\beta$  receptor complex and thereby prevents phosphorylation and activation of Smad 3 (141). In cultured renal interstitial fibroblasts stimulated by TGF- $\beta$ , IFN- $\gamma$  reduces FGF-2 mRNA and inhibits fibronectin production and, to a lesser extent, collagen I production (142). *In vivo*, IFN- $\gamma$  inhibits fibroblast conversion to the activated, myofibroblast phenotype and reduces collagen production in the remnant nephron model (143). In clinical trials, IFN- $\gamma$  reduces idiopathic pulmonary fibrosis (144). While it has the disadvantage of requiring parenteral injection, IFN- $\gamma$  is an attractive candidate for future clinical trials of progressive renal disease.

### 6.1.2. Hepatocyte growth factor

Hepatocyte growth factor (HGF) and TGF- $\beta$  may constitute a counter-regulatory feedback system, with HGF acting to down-regulate TGF- $\beta$ , prevent the transdifferentiation of tubular epithelial cells, and limit progressive interstitial fibrosis (145, 146). *In vitro*, TGF- $\beta$  increases  $\alpha$ -smooth muscle actin expression and decreases E-cadherin expression, while HGF reverses these effects (147). Exogenous HGF, or HGF-expressing plasmid, reduces fibrosis in two mouse models of interstitial fibrosis, genetic and ureteral obstruction (148, 149). On the other hand, over-expression of HGF in the proximal tubule is associated with tubular injury, suggesting that the quantity or timing of HGF expression may influence whether HGF acts to stabilize or compromise epithelial cell phenotype (150).

### 6.1.3. Bone morphogenetic protein-7

Bone morphogenetic protein-7 (BMP7) is a member of the TGF- $\beta$  superfamily which is required for normal metanephric development (reviewed in (151)). In cultured chondroblasts, TGF- $\beta$  and BMP7 have opposing roles in matrix production; this important work has not been extended to renal parenchymal cells. Exogenous BMP7 reduces fibrosis unilateral ureteral obstruction (152, 153).

### 6.1.4. Relaxin

Relaxin, a protein associated with increased ligament flexibility during pregnancy, has been shown to reduce collagen accumulation in bleomycin-induced pulmonary fibrosis in mice and to reduce collagen production by lung fibroblasts and hepatic stellate cells, the latter a modified pericyte resembling mesangial cells (154, 155). Relaxin administration by minipump over 28 days reduced macrophage infiltration, TGF- $\beta$  immunostaining, and interstitial fibrosis in the rat bromoethylamine

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interstitial fibrosis model (156); studies in other experimental models would be of interest.

### 6.1.5. Vascular endothelial growth factor

Vascular endothelial growth factor (VEGF), a potent angiogenic factor, is expressed by podocytes and tubular epithelial cells, and VEGF receptors are present on endothelial cells and, in the setting of proliferative glomerulonephritis, mesangial cells. VEGF administration improves function and reduces fibrosis in the remnant nephron model, possibly by increasing interstitial capillary growth and thereby reducing hypoxia (157).

### 6.1.6. Small leucine rich proteoglycans

The Small leucine rich proteoglycan (SLRP) family includes decorin, which has been shown to bind TGF- $\beta$ . Parenteral administration of decorin and decorin gene therapy have each been shown to reduce the transient glomerulosclerosis that accompanies anti-Thy1 nephritis in rats (158, 159).

## 6.2. Small molecule inhibitors of fibrosis

### 6.2.1. Angiotensin antagonists

Angiotensin converting enzyme (ACE) inhibitors have emerged as widely-used inhibitors of fibrosis in the cardiovascular tissues and kidney. Numerous studies document reduction of fibrosis in animal models of kidney disease (for review see Table 1 in (1)). Angiotensin receptor blockers (ARB) have effects limited to blocking the binding of AII to its major receptor. ACE inhibitors, by contrast, inhibit degradation of several peptides, including not only AII but also bradykinin. Presumably, both ACE inhibitors and ARB decrease intraglomerular hypertension and the hyperfiltration of potentially fibrogenic serum components to the tubulo-interstitium. However, other antihypertensive agents do not have this effect, and it has been postulated that ACE inhibition interferes with hypertrophic effects of the renin-angiotensin system, independent of its effects on hydrostatic pressure. Cardiac fibroblasts have both AII and bradykinin receptors, and the bradykinin receptor blocker HOE140 antagonizes the therapeutic effects of ACE inhibitors on cardiac remodeling (160). While it is unknown as yet whether the same is true for renal fibrosis, the effects of ARB are of considerable interest.

Losartan reduces glomerulosclerosis and renal collagen content (predominantly an index of interstitial fibrosis) in aging rats (161) and has similar effects in radiation nephropathy (162). Recent clinical studies suggest a benefit in nephropathy in type 2 diabetes by reduced progression to macroalbuminuria and subsequent impaired glomerular filtration, although morphologic studies have not been performed (163, 164).

### 6.2.2. Aldosterone antagonists

Aldosterone antagonism has emerged as a potent anti-fibrotic approach to slowing or reversing the progression of cardiac fibrosis, and is the subject of considerable interest in renal fibrosis. The aldosterone antagonist spironolactone reduced glomerulosclerosis in a rat radiation nephropathy model, although to a lesser extent

than losartan (162). Studies in human patients have not been reported.

### 6.2.3. Pirfenidone

Pirfenidone is a potent anti-fibrotic whose mechanism of action is unknown. Possible actions include suppression of TGF- $\beta$  production, suppression of PDGF production (165), inhibition of TNF- $\alpha$  signaling (166), suppression of ICAM-1 expression in cultured fibroblasts (167) and scavenging of ROS (168). Pirfenidone has an anti-fibrotic effect in the various rat models including remnant nephron (169) and ureteral obstruction (170). Human clinical trials are in progress for patients with FSGS and patients with nephropathy associated with type 1 diabetes.

### 6.2.4. Pentosan polysulfate

Pentosan polysulfate is a non-anticoagulant heparinoid that shares with heparin the ability to bind many growth factors. Therapy with pentosan polysulfate reduces matrix production by cultured mesangial cells and prevents glomerulosclerosis in the remnant nephron model in rats (171).

### 6.2.5. PPAR- $\gamma$ ligands

The peroxisome proliferator-activated receptor- $\gamma$ , one of a family of nuclear receptors, binds physiologic ligands (particularly linoleic acid derivatives) and pharmacologic ligands (including the hypoglycemic agents troglitazone, rosiglitazone, and piaglitazone). Besides experimental diabetic nephropathy, it has been recently shown that these agents have efficacy in nondiabetic glomerulosclerosis, specifically the remnant nephron model (172). This was associated with decreased PAI-1 and TGF- $\beta$  expression, although the molecular pathways remain to be dissected.

### 6.2.6. Methyl-xanthines

Pentoxifylline (and its precursor, pentafylline) are substituted methyl-xanthines have been used for their rheologic effects on erythrocytes, to improve blood flow. These agents have other effects, including scavenging free radicals (173) and antifibrotic activity. Pentoxifylline inhibits matrix protein production by cultured renal interstitial fibroblasts and mesangial cells (174). The drug attenuates Thy-1 nephritis (175); studies in progressively fibrotic renal models have not been reported. Pentoxifylline reduces proteinuria in patients with membranous nephropathy (176) and diabetic nephropathy (177), although it is unclear whether this is associated with anti-fibrotic effect.

### 6.2.7. Anti-oxidants

While it is likely that oxidant species play a role in tissue fibrosis, it is less clear that anti-oxidant therapy with the agents currently available retards fibrosis. Anti-oxidants typically exist in a dual system composed of anti-oxidant and pro-oxidant, which are inter-convertible. For example, ascorbic acid is paired with its oxidation product, dehydroascorbic acid. A potent anti-oxidant is associated with a weak pro-oxidant, and vice versa. Thus, anti-oxidant therapy does have the potential for a deleterious

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effect. Agents include ascorbic acid (vitamin C),  $\alpha$ -tocopherol (vitamin E), selenium, lazaroids (21-aminosteroids), and probucol. ProbucoL was approved for clinical use for a number of years as a cholesterol lowering agent; it also is somewhat weak anti-oxidant. Fluvastatin inhibits HMG-coA and also acts as a hydroxyl radical scavenger.

Vitamin E reduced glomerulosclerosis in the remnant nephron model (178-181). On the other hand, in the puromycin model of FSGS, neither probucol nor lovastatin therapy reduced interstitial fibrosis (182).

Other anti-oxidant agents appear to act as enzyme inhibitors. Allopurinol inhibits xanthine oxidase, a source of free radicals. Resveratrol (a phytoalexin present in red wine) is a tyrosine kinase inhibitor, although its mechanism of anti-oxidant activity is not well defined. Given the wide therapeutic indications for anti-oxidant therapies in many diseases, including tissue fibrosis, it is hoped that further pharmaceutical development will make new compounds available.

### 6.2.8. Tamoxifen and estrogen

The anti-estrogen tamoxifen has been shown to reduce dermal scarring, in association with reduced TGF- $\beta$  and FGF-2 (183). The mechanisms are not well understood, although it was shown that tamoxifen reduces the production of TGF- $\beta$  by cultured keloid fibroblasts (184). Little is known about the role of anti-estrogens in renal fibrosis, although these agents reduce mesangial collagen production *in vitro* (185).

Somewhat paradoxically, estradiol also has been shown to inhibit collagen production by murine mesangial cells (186) and to enhance MMP-9 expression by mouse mesangial cells (187). The literature regarding estrogen effects on renal fibrosis is controversial. One study of human diseases raises the possibility of protective effects (188). In animal studies, female sex appears to be protective in some cases and exacerbating in others.

### 6.2.9. Prolyl hydroxylases inhibitors

Prolyl-hydroxylases act on collagen, converting proline to hydroxyproline, and also act on nuclear factors, including hypoxia-inducible factor (189). Small molecule inhibitors of prolyl-hydroxylases (190) reduce fibrosis in liver and heart; they have not been tested in kidney models (191-193).

### 6.3. Clinical approach to fibrotic kidney disease

At present, the therapy for renal fibrosis has two components. First, the underlying disease process is treated, when this is possible. Thus, inflammatory disease may be susceptible to glucocorticoids or other immunosuppressants, including cyclosporine (e.g., lupus nephritis or FSGS). Certainly, it has not been formally excluded that these agents may also be of benefit due to direct effects on the fibrotic process. In the case of diabetes, improved control of plasma glucose slows the progression of renal disease and, remarkably, pancreatic transplantation is associated with reversal of established

diabetic nephropathy. This holds out the hope that fibrosis may be reversed in other diseases as well (194). Hypertension is an important progression factor for fibrotic renal disease, and aggressive therapy is warranted to lower blood pressure below approximately 130/80 mmHg.

Second, the fibrotic process may be targeted directly by therapies. Thus, ACE inhibitors have been shown to slow progression (as measured by renal function, glomerular basement membrane thickening, glomerulosclerosis and interstitial fibrosis) of both diabetic and non-diabetic glomerular disease, although not all studies confirm this result—perhaps due to small sample size (179-181, 195).

Unquestionably, the development of additional therapies that halt the progression of renal fibrosis, or even reverse established fibrosis, are of great importance. Demonstration of reduced fibrosis may require serial renal biopsy. Nevertheless, approval of such therapies for renal fibrosis by the U.S. FDA will require evidence of clinical benefit, as measured by reduced numbers of patients progressing to a doubling of serum creatinine and/or end-stage disease.

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