Mechanisms of Tubulointerstitial Fibrosis

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Progressive tubulointerstitial fibrosis is the final common pathway for all kidney diseases leading to chronic renal failure.1,2 Its presence correlates with impaired excretory function1 and the degree of fibrosis3,4 or fibroblast number5 are robust pathologic markers of progression. The histopathology of tubulointerstitial fibrosis features deposition of interstitial matrix in association with inflammatory cells, tubular cell loss, fibroblast accumulation, and rarefaction of the peritubular microvasculature.6 There is broad agreement that each of these hallmarks contributes to relentless progression, although priority and interrelationship among the various components including their genetic predisposition are still muddled by unresolved complexity (Figure 1).

Humans lose about 4500 nephrons per year per kidney,7 and although not all agree,8,9 classic inulin clearance studies suggest GFR falls by 10 ml/min per decade of life.10 Therefore, the calculus for conceptualizing normal loss of renal function over time begins with a starting number of nephrons at birth integrated by the natural history of tissue involution from a lifetime of subclinical vascular disease, metabolic stress, cica-trization, and nephrosclerosis;11–14 hence, the privilege of aging provides a natural window into basic mechanisms of renal fibrogenesis.

Aging disturbs normal structure-function relationships in the kidney for many reasons,15,16 including the influence of telomere shortening on cell senescence,16–18 aberrant DNA methylation destabilizing the epigenome of renal cells,19 loss of self-renewing stem cells,20–22 and diminished rates of tubular cell proliferation.23 These nuclear consequences of aging are compounded by dysregulation of cellular energy sensors,24 oxidative stress,13,25,26 and mitochondrial dysfunction26,27 fogging the integrity of nephron structure with progressive glomerular and tubulointerstitial fibrosis.12,13,28 PPARγ agonists attenuate some of these aging effects by engaging klotho,29 stabilizing mitochondrial deterioration,29 and reducing the activity of TGFβ,29,30 all to suggest that aging renal tissues are susceptible to the longitudinal affects of profibrotic signals.19,23,28,31 Persistent subclinical injury to the tubulointerstitium also affects the normal physiology of many nephrons at once, which explains the sensitivity of GFR to the spread of peneurous fibrogenesis.1

Whereas the molecular mechanisms driving fibrogenesis are better understood from 20 years ago1 and a variety of preclinical strategies to inhibit or even reverse tubulointerstitial fibrosis are effective in rodents, only a few antifibrotic therapies are in clinical use today.2,32–34 Finding better targets for future therapy in humans will require deeper understanding of the molecular signals modulating fibrogenic events.

THE EXTRACELLULAR MATRIX

Excessive deposition of extracellular matrix, particularly the presence of collagenous fibers, is the most striking and name-lending feature of tubulointerstitial fibrosis.9 By definition, fibrosis associates with both quantitative and qualitative...

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tive changes in matrix. In fibrotic kidneys the widened interstitial spaces fill with fibrillar material consisting of predominantly collagens type I and III and fibronectin. This fibrotic matrix also contains residual fragments of collagen type IV, which are normally found in basement membranes otherwise supporting intact endothelia or tubular epithelia as well as several fibronectin splice variants that modulate fibrogenic potential. The actual amount of collagen produced by renal fibrogenesis seems to attenuate fairly soon after it starts, whereas the relative extent of fibrosis seems to increase with time. This dynamic incongruity can be reconciled by recognizing that the volume of inflammatory cells, fibroblasts, tubular epithelial cells (TECs), and endothelial cells actively contribute to fibrogenesis has evolved considerably during the past 20 years and sustained growth factor pressure within the microenvironment and increased susceptibility to growth factor-mediated stimulation emerge as the principal driving force of fibrosis. The molecular systems that determine choice between pathologic fibrosis and physiologic repair however are still evolving. Epidemiologic studies identify genetic polymorphisms, epigenetic modifications, and aging as risk factors for chronic kidney disease. Linking these risk factors mechanistically to fibrosis is a task for the future.

Figure 1. Interactive relationships producing fibrosis. Renal fibrosis constitutively involves inflammation, fibroblast activation, injury to the tubular epithelium, and microvascular rarefaction. Our understanding of how inflammatory cells, fibroblasts, tubular epithelial cells (TECs), and endothelial cells actively contribute to fibrogenesis has evolved considerably during the past 20 years and sustained growth factor pressure within the microenvironment and increased susceptibility to growth factor-mediated stimulation emerge as the principal driving force of fibrosis. The molecular systems that determine choice between pathologic fibrosis and physiologic repair however are still evolving. Epidemiologic studies identify genetic polymorphisms, epigenetic modifications, and aging as risk factors for chronic kidney disease. Linking these risk factors mechanistically to fibrosis is a task for the future.
Tissue Proteases

Removal of extracellular matrix from the interstitium hinges on a well-worn belief in the role of various endogenous proteases. Most studies in this area focus on two families of proteases: MMPs and members of the plasmin-dependent pathway. Both classes of proteases have the potential to fragment some extracellular matrix for removal, but also cleave nonmatrix substrates, releasing profibrotic growth factors that paradoxically trigger unwelcome consequences.

MMPs are a family of zinc-dependent endopeptidases that currently includes 25 members with varying substrate specificities controlled by tissue inhibitors of metalloproteinases 1-4. The overlapping activity and specificity of MMPs make it difficult to dissect individual actions in vivo. Most studies in the kidney focus on MMP-2 and MMP-9 (both degrade collagen type IV84 and perhaps collagen types I and III85), but fail to demonstrate a consistent antifibrotic effect in the interstitium.86,87 For example, combined pharmacologic inhibition of MMP-2, MMP-3, and MMP-9 at advanced stages of disease in Alport mice accelerates renal fibrogenesis, whereas combined MMP inhibition before onset of tubulointerstitial fibrosis is protective.88 More confusing, studies utilizing MMP null mice show unaccelerated fibrogenesis, and overexpression of MMP-2 in tubular epithelial cells is sufficient to induce tubulointerstitial fibrosis.87–89 The profibrotic effects of MMP-2 and MMP-9 on renal fibrosis, particularly the activation of MMP-2 and MMP-14 (MT1-MMP) by TGFβ associated with the degradation of basement membrane stimulating EMT,87 seem to outweigh their antifibrotic potential; in fact, mice deficient in TIMP-3 spontaneous develop interstitial fibrosis.90 MMP-14, a membrane-bound activator of secreted MMP-2 and MMP-9 in fibroblasts, is critical for invasiveness into the interstitial environment containing collagen types I and III90 and its expression is under the control of Snail, which is a key regulator of the EMT program.91,92 Thus, epithelial cells, engaged by TGFβ, express essential MMPs for both basement membrane degradation and interstitial invasion that are ultimately required for successful completion of EMT or detachment and loss into the tubular lumen (Figure 2). The effects of the plasminogen-plasmin system on fibrosis are equally complex. Active plasmin is derived from proteolytic cleavage of plasminogen by tissue-type plasminogen activator (tPA) or urokinase-type plasminogen activator (uPA), which in turn are inhibited by PAI-1.93 Plasmin degrades some matrix constituents, including laminin, entactin, perlecan, and fibronectin,93,94 whereas fibrillar collagen seems more resistant.95 Plasmin, perhaps more importantly, also affects cell behavior and function in the fibrotic environment and promotes fibrogenesis by activating MMPs96 and stimulating EMT.97 tPA null mice with ureteral obstruction have reduced local expression of MMP-9 and preserved tubular basement membrane with less EMT, and less interstitial fibrosis,98 whereas urokinase cellular receptor (uPAR) null mice develop worse renal fibrosis with lower expression levels of the antifibrotic cytokine, hepatocyte growth factor (HGF).99 Angiotensin II stimulates the PAI-1 promoter in tubular cells100 and tubulointerstitial fibrosis associates with TGFβ-stimulated PAI-1 expression,100 whereas the genetic deletion of PAI-1 ameliorates renal fibrogenesis in mice.72,102,103 The small heterodimer partner (SHP) represses the transcription of TGFβ/Smad3-regulated PAI-1 expression, and SHP null mice develop more renal fibrosis, whereas SHP overexpression inhibits its development;101 SHP may turn out to be an interesting therapeutic candidate depending on its molecular specificity.
Long-term renal fibrosis after ischemia-reperfusion injury also depends on persistent inflation of effector-memory T lymphocytes, which correlate with onset of fibrosis in later stages. Transfer of T lymphocytes from mice with prolonged ischemia-reperfusion injury to naïve recipients is sufficient to induce renal injury, further highlighting the relevance of memory lymphocytes in the progression of tubulointerstitial fibrosis. In contrast, FoxP3+ regulatory T cells (Tregs) blunt kidney injury and are required for physiological kidney regeneration. Whereas recent studies suggest the presence of CD20−B cells as are common as CD3+T lymphocytes in established fibrosis, the functional contribution of B cells to renal fibrogenesis is uncertain. When monocytes are recruited to damage the interstitium, they transdifferentiate into macrophages. There is a strong correlation between macrophage infiltration and the extent of fibrosis. Activation of TLR9 on macrophages by CpG-oligodeoxynucleotides is an accelerator for interstitial fibrogenesis, suggesting the dispersion of nuclear fragments after local cell death may enhance inflammation. Depletion of macrophages also ameliorates fibrogenesis in mice, highlighting the traditional profibrotic view of macrophages. Macrophages however can be categorized into classically activated M1 macrophages or alternatively activated M2 macrophages. Unlike the M1 macrophages, M2 macrophages are anti-inflammatory and provide cues for tissue repair. Infusion of cells enriched for M2 macrophages ameliorates renal fibrosis in mice. Finally, in addition to macrophages, dendritic cells are critical to antigen processing in tubulointerstitial injury; their proteasomal processing of albumin, for example, creates new antigenic targets. The balance or relative presence of these subsets of cells in renal inflammation over time needs more study.

Fibroblasts

Fibroblasts in the renal interstitium are considered the principal source of fibrillar matrix (collagen types I and III), and tubulointerstitial fibrosis inevitably associates with a robust accumulation of fibroblasts, displaying increased intrinsic proliferative activity or, in some fibroblasts, what is called an activated phenotype expressing α-smooth muscle actin (αSMA). Tissue fibroblasts exhibit phenotypic heterogeneity, more likely based on origin, but this heterogeneity in tissue is also bimodal: those cells that are RhoA-dependent αSMA+ and those that are not. One generic dilemma with αSMA is that when used as a fibroblast marker in injury, it is not distinguishable from αSMA+ mural cells—vascular smooth muscle cells or venular pericytes; nor does this marker detect the larger population of αSMA+ fibroblasts. The view of myofibroblasts as principal mediators of renal fibrosis is also based on the suggestion that fibrosis associates with de novo accumulation of αSMA+ cells. This notion, however, has to be tempered by observations that αSMA+ fibroblasts do not move, renal fibrogenesis persists despite decreasing

The profibrotic action of these proteases is a paradigm shift in thinking about the dynamics of matrix deposition or its dissolution (Figure 2), illustrating the complexity of balancing modifier effects, timing and context, and weighing what seems to be true in vitro with what is not observed in vivo. Whereas current evidence argues against MMPs or plasmin to degrade only complex matrixes in vivo, murine studies reporting regression of tubulointerstitial fibrosis do demonstrate less matrix deposition. How this matrix is removed biochemically, or fails to form, is still unclear. Involvement of other proteases, perhaps the lysosomal family of cysteine protease cathepsins, may be important. The whole approach to the regulation of collagen deposition in renal tissue is ripe for new insight.

INFLAMMATORY CELLS

Tubulointerstitial fibrosis is typically conditioned by the infiltration of inflammatory cells including dendritic cells, lymphocytes, and mast cells. Whereas inflammation as a whole contributes significantly to the engagement of fibrogenesis, evidence in recent years also highlights the antifibrotic effects of various subsets of lymphocytes and macrophages, providing novel potential avenues for antifibrotic therapies.

Innate immunity through the activation of toll-like receptors 4 (TLR4) and TLR9, unlike TLR2 receptors, is an important early mediator of renal fibrogenesis. Lymphocytes precede the influx of macrophages. Rag-2 null mice, which lack both mature B and T lymphocytes, are protected from fibrosis, demonstrating that effector lymphocytes are an essential early component of fibrogenesis. Adoptive transfer of CD4+ cells into Rag-2−/− mice, but not CD8+ cells so much, increases fibrogenesis. The contribution of CD3+T cells to renal fibrogenesis is well established, as activated cytotoxic T cells attack tubular epithelial cells. Administration of PGE1 inhibits the effector arm of T lymphocytes in interstitial nephritis that is overcome by competitive exposure to IL-1.
numbers of αSMA+ myofibroblasts,31,121 αSMA− fibroblasts contain and likely express interstitial collagens in vivo,31,61,152 and mice deficient of αSMA develop more fibrosis and their fibroblasts produce more collagen type I than αSMA+ fibroblasts.156 Unequivocal functional data to support the idea that nonmotile αSMA+ myofibroblasts principally mediate fibrogenesis is lacking.

The most durable fibroblast marker in the kidney is fibroblast-specific protein-1 (FSP1; S100A4).38,146,147,151 The proclivity for FSP1 in fibroblasts, a member of the S100 family of calcium-binding proteins, was discovered in a differential genetic screen between fibroblasts and epithelial cells,157 and renal fibrosis associates with a robust accumulation of FSP1+ fibroblasts.5,61,157 A few studies suggest FSP1 is expressed by macrophages;55,158,159 however, this notion persists from studies using underdetection methods for FSP1/S100A4 that may enhance nonspecific staining and/or from improper application of so-called anti-macrophage antibodies that share target specificities with fibroblasts and are not macropage-specific;158–160 FSP1 promoter activity in fibroblasts driving a GFP reporter60,161 or Cre recombinase162 clearly discriminate tissue fibroblasts from F4/80+ macrophages. FSP1+ F4/80+ fibroblasts are also the only population in the kidney proven to contribute to fibrogenesis, as their ablation in FSP1−/− mice ameliorates fibrosis substantially.61 This kind of functional deletion experiment is critical evidence not yet reported in FSP1−/− cells expressing αSMA, particularly pericytes.5,64 For the moment, it is best to think of all tissue fibroblasts as potentially fibrogenic.5,80,147,152

The true heterogeneity of fibroblasts in the kidney may be understood by examining the different cellular origins of these cells.147 Pools of fibrogenic fibroblasts coming either by proliferation of resident fibroblasts,163 some of which may be subject to local or time-dependent epigenetic changes;68,164 from local tubular epithelial cells51,59,157,165 or endothelial cells (simple squamous epithelia)51,166 after EMT or EndMT during tissue injury; after recruitment of FSP1+, CD34− bone marrow–derived fibrocytes;59,144 and perhaps from blood vessel shedding of αSMA+ pericytes54,150 that derive developmentally from mesothelium through EMT,167,168 It is not yet clear whether FSP1−, αSMA+ pericytes54,55 should be considered true fibroblasts or a separate and unique phenotype of collagen–producing cells.190 As opposed to tissue-derived fibroblasts, CD34− bone marrow–derived fibrocytes59 may also have a unique lineage from CD11b+, CD115+, Gr1+ leukocyte progenitors under the control of CD4+ T cells;56 some studies suggest these circulating fibrocytes do not contribute to tissue fibrogenesis,53,57 whereas other experiments suggest they can to a limited extent.50,56,59 For the moment, it is probably best to view multiple lineages as contributing to the final mix of fibroblast populations in tissue. It is entirely unknown if these various lineages have priority among individuals or different diseases or if there is individual variation or preference as fibrogenesis progresses.

Depletion of interstitial fibroblasts by selectively corrupting their DNA replication is one classic strategy for experimenting antifibrotic therapy.61 Conditional expression of an IκB dominant-negative transgene in fibroblasts also attenuates fibrogenesis by inhibiting NF-κB.60 But because specific targeting of fibroblasts has not yet been achieved without genetic manipulation, current antifibrotic therapies center on various other approaches; these approaches include blocking PDGF-C,165 PDGF-D,170 or TGFβ171,172 with neutralizing antibodies or using pirfenidone,173–175 pyridoxamine,176 ALK5 receptor kinase inhibitors,177 paricalcitol,178 Ras inhibitors,68,179 or PPARγ agonists.30 Blockade of AT1R by the small molecule inhibitor CGP-48933 also attenuates interstitial fibrosis,180 as does inhibition of angiotensin II3 or aldosterone action.3,33

TUBULAR EPITHELIUM

Tubular epithelial cells spark the progression of fibrogenesis to their own detriment. They do this by modulating their proliferation,23,181 or by providing proinflammatory chemokines and cytokines that induce mononuclear inflammation and the formation of fibroblasts (Figure 3).59,182–184 These local cytokines provide critical signals mediating primary interstitial injury.185 Secondary interstitial damage after glomerulonephritis also depends on the tubular toxicity of glomerular proteinuria,1,186 the filtration of protein-bound cytokines from plasma to the tubular microenvironment,184,187 or possible cytokine leakage through bulk fluid flow from the different arteriole directly into the interstitial spaces associated with the juxtaglomerular apparatus.188 Hydrodynamic forces along the tubules themselves are also profibrotic.189 Tubular stretch from obstruction induces TGFβ and NF-κB; increases in single nephron GFR and compensatory increases in fluid shear stress also decrease tPA and increase TGFβ.

The epicenter for early molecular action by tubular epithelial cells is through NF-κB.190 Tubular cells toggle on NF-κB after exposure to proteins from tubular fluid,134,191 activation of CD36 scavenger receptors,25 or exposure to connective tissue growth factor,192 CD40 ligand,193 angiotensin II,194,195 or aldosterone.32,33 Activation of NF-κB is critical for initiating the downstream release of PAPI-1 or chemokines and growth factors,196 particularly IL-1,137 IL-6,197 MCP-1/CCL2,198,199 RANTES/CCL5,132,200 or TNFα.202 In opposition, administration of Smad7,201,202 paricalcitol,203 truncated IκBα,204 HGF,205 spironolactone,32,206 and decoy NF-κB oligodeoxynucleotides207 all attenuate tubulointerstitial injury by inhibiting the activity of NF-κB. Experimental inhibition of proinflammatory chemokines such as TNFα or CCR1 has also been successful in preventing progressive interstitial injury.113,138

With time, persistent tubulointerstitial injury inevitably associates with mitochondrial dysfunction or loss,26,208 formation of agglomerular tubuli,209 tubular atrophy,2,7 and rampant interstitial fibrosis,146 creating the classic pathologic picture of renal progression.210 Major epithelial mechanisms contributing to
Figure 3. The cytokine milieu in fibrogenesis. NF-κB activators and inhibitors compete for the engagement of mammalian target of rapamycin and release of chemokines and proinflammatory cytokines that draw macrophages, dendritic cells, and T lymphocytes to the tubulointerstitium. These mononuclear cells injure the tubular epithelia and activate fibroblasts. The transcriptional activation of EMT and EndMT is engaged by hypoxia and various cytokines, particularly TGFβ, EGF, and ILK. EMT inhibitors block the development of fibroblasts that deposit extracellular matrix into the fibrogenic interstitium.

chronic tubulointerstitial fibrosis are G2/M cell cycle arrest,181 cellular apoptosis,211 and EMT-forming fibroblasts;147,151 all three processes are modulated by the transcriptional repressor GLIS2.212 EMT is a fundamental process to create cells that will move,155 a modification in lineage maturation that disturbs the state of nuclear diapause in epithelial and endothelial cells.59,213 Although not all agree,54,159,214,21511 studies confirm these transitions to fibroblasts in a variety of lineage-tracing experiments from different tissues, including kidney,50,59,60,165 intestine,216 liver,217 heart,166 lung,218–220 and endothelium50,166,221 using 10 different cell fate reporter constructs.

The role of EMT in forming renal fibroblasts has been recently reviewed19 and debated;260 several of the lineage-tracing studies unable to detect EMT or FSP1+ fibroblasts are likely confounded by use of nonpreferred anti-FSP1/S100A4 antibodies and/or inadequate antigen-retrieval methods during tissue preparation.54,159,213213 by relying on a collagen promoter driving green fluorescent protein used to detect fibroblasts that perhaps may not express in all fibroblasts because of methylation or enhancer bias,160 and by overlooking lineage evidence that αSMA+ mural cells/pericytes may have emerged previously from EMT.167,168,222 It is increasingly clear that fibroblasts derive from multiple parental lineages, including epithelial cells, endothelial cells, and bone marrow endosteal cells. The status of pericytes in fibrogenesis remains an area of great interest that will require more functional evidence of their role one way or the other. Finally, although EMT is difficult to assess in humans who are not biopsied until there is clinical evidence of disease,3 early renal allograft biopsies suggest changes in epithelial phenotype supportive of transitions preceding fibrosis,223–225 although not all agree.226 Glis2 mutations producing autosomal recessive NPHP7, a rare form of nephronophthisis, may be the first suggestive evidence of spontaneous or derepressed EMT causing renal fibrosis in humans.212,227

EMT induces a variety of intermediate cell phenotypes, not all of which complete their transition to fibroblasts.147,225 This intermediacy and its reversibility may depend on the variable persistence of endogenous perturbagens: hypoxia,165,228–230 TGFβ,231 EGF,231,232 PTHR,233 and plasmin227 all induce EMT, as does activation of ILK+ or RAGE.234 Vitamin D receptors downregulate the renin-angiotensin system driving EMT and TGFβ and receptor absence accelerates fibrogenesis.235

After induction, a hierarchy of transcription factors program EMT, including GLIS2212 and CBF-A;236 a master regulator of Snail,237 Twist,238 HMGA2, LEF-1, and Ets-1.236 Overexpression of Snail1237 induces EMT and fibrosis and recessive mutations in Glis2 spontaneously invoke EMT in tubular cells in mice with renal fibrosis;212 short interfering RNA knockdown of Snail1 also blocks EMT during vascular mural cell differentiation.222 There appears to be a gradualness to EMT events in the kidney,223 as indicated by the variably active signaling of β-catenin, Snail, or Twist in watershed epithelial cells along chronically injured tubules that is based on the time-dependent permissiveness of local cytokine action. Whereas activation of the EMT program is a means for parental cells to resist proapoptotic stimuli,211,239 such tenuous protection from cell death also results in loss of parental cell phenotype.59,240 The true frequency of tubular epithelial cells that complete EMT and become fibroblasts is unknown, but likely depends on the persistence of inflammation, epigenetic control of fibrotic gene regulation,67 and ultimately the emergence of tubular atrophy with acellular scars that are the consequence of profibrotic cytokine exhaustion and tubular cell disappearance.

The relevance of EMT for progression of renal fibrosis is highlighted by studies in which administration of bone morphogenic protein-7 (BMP7),240 HGF,241,242 the small-molecule inhibitor ILK, QLT-0267,243 or Y-27632, an inhibitor of Rho/ROCK244 all inhibit tubular EMT and ameliorate fibrogenesis. Trps1 operates downstream of BMP7 in the kidney,245 and the availability of Smad7 controlling the phosphorylation of Smad3 is normally assured by Trps1 inhibition of ubiquitination through Arkadia;246 Trps1 haploinsufficiency raises the levels of Arkadia, decreasing the availability of Smad7, and thus facilitating fibrosis through TGFβ/Smad3–mediated EMT. Induction of heat shock protein-72 (HSP72) also protects tubular epithelia from apoptosis, EMT, and fibrosis,239 and overexpression of klotho247 or
inhibition of the mammalian target of rapamycin (mTOR) attenuates fibrogenesis in a variety of experimental models.

A pivotal determinant in the failure to recover from interstitial injury is the relative absence of neophron regeneration in kidneys that become fibrotic. One reason for this is that glomerulotubular disconnections limit repair of nephrons. The glomerulotubular neck at the antivascular pole of the glomerular tuft is an at-risk break point for the formation of agglomerular tubuli and surprisingly angiotensin inhibition protects these unwanted separations. Another reason is that cell cycle arrest of tubular epithelium during acute kidney injury may stimulate profibrotic signaling. Whereas tubular cells atrophic tubules variably increase their proliferative activity in advanced tubulointerstitial disease, this restorative potential may decline with aging through the modulation of the adipokine, zinc-α2-glycoprotein, known as ZAG.

The regenerative capacity of injured tubules absent persistent inflammation is likely the result of self-renewing tubular cells or perhaps progenitor cells within other niches along the nephron. A CD24+, CD133+ progenitor cell niche has been identified in the parietal epithelium of Bowman’s capsule near the vascular pole, and the adoptive transfer of these cells at the time of tubular injury obviates the appearance of interstitial fibrosis. The glomerulotubular neck region is also where ex vivo differentiated embryonic stem cells traffic after adoptive transfer during development and may be a tubular niche not unlike the vascular pole. Expansion of renal progenitor cells is under the control of histone deacetylases and their enzymatic inhibition may offer restorative potential to the kidney.

**THE MICROVASCULATURE**

Rarefaction of the peritubular vasculature with ensuing chronic hypoxia are hallmarks of tubulointerstitial fibrosis. In early stages of injury the peritubular vasculature is damaged by proapoptotic stimuli, particularly transient ischemia. Progressive fibrosis then remodels the peritubular endothelial network governed by imbalance among pro- and antiangiogenic stimuli or loss of peritubular endothelial cells by EndMT-forming fibroblasts.

The fibrotic tubulointerstitium is a hypoxic environment. Hypoxia results from microvascular rarefaction made worse by matrix expansion throughout the interstitium, requiring the oxygen gradient to diffuse greater distances from vasoconstricted or attenuated microvessels to adjacent ischemic tubules. Hypoxia also contributes directly to the progression of tubulointerstitial fibrosis by simulating EMT and promoting matrix accumulation from new fibroblasts.

Different strategies to improve this chronic hypoxia during renal progression show some promise in experimental models of renal fibrosis. Angiotensin II inhibition increases capillary blood flow and cortical oxygen levels in normal renal tissues, and long-acting calcium channel blockers attenuates the hypoxia of angiotensin II–induced tubulointerstitial injury. Inhibition of the vasoconstrictor, endothelin, is also renoprotective, particularly in combination with ACE inhibition. Activation of the hypoxia-inducible factor (HIF) pathway or stabilization of HIF proteins attenuates renal fibrosis as well. The ultimate goal of reversing microvascular rarefaction, however, has been difficult to achieve. Whereas the HIF pathway is an important inducer of angiogenic vascular endothelial growth factor (VEGF) proteins, systemic administration of VEGF does not improve renal microvascular disease. Whereas administration of the antibiotic molecule, BMP7, blocks EMT in the kidney, its affect on EndMT in the kidney is unknown; however, BMP7 does inhibit EndMT, endothelial loss, and fibrogenesis in experimental cardiac fibrosis.

**GENETIC PREDISPOSITION TO RENAL PROGRESSION**

Whereas revelations regarding new cellular mechanisms of renal fibrogenesis continue to flourish, the identification of key risk factors underlying the true biologic fate of renal tissue is far from clear. Risk factors for chronic kidney disease are rapidly emerging from epidemiologic studies, including age, nephron mass, gender, levels of calcium, sympathetic activity, obesity and diabetes, cardiovascular disease, genetic loci and polymorphisms, and epigenetic modifications. But how these risk factors modulate fibrogenesis is still uncertain.

Genetic polymorphisms in cytokine risk profiles in some instances can be explained using existing knowledge of fibrotic signaling pathways. In other cases, it is unclear why genetic polymorphisms in uromodulin or methylenetetrahydrofolate synthetase increase susceptibility to fibrogenesis. Insights into the role of epigenetics in renal progression and fibrogenesis are also just starting to emerge. For example, aberrant hypermethylation of selected genes including RASAL1, a suppressor of Ras signaling, contributes to perpetual fibroblast activation and fibrogenesis. Furthermore, there are large families of small, naturally occurring, noncoding RNAs that interfere post-transcriptionally with the stability or translation of coding mRNA. Three prototypical families of interfering RNAs include short interfering RNAs (siRNA), microRNAs (miRNAs), and piwi-interacting RNAs (piRNA). miRNA-192, for example, promotes the activation of TGFB/Smad3-mediated fibrosis in ureteral obstruction and 5/6 nephrectomy, and in diabetic glomeruli, TGFB stimulates miRNA-192 to synergize with deltaEF1 short hairpin RNAs that increase Colla2 E-box activity in mesangial cells. Oddly enough, low levels of miRNA-192 in human kidney biopsies of diabetic nephropathy correlate with late presentation and less fibrosis. Thus, the interfering RNA story becomes complicated quickly, largely because the network effect of multiple families is not addressed by studying the action of a single family member.

**CONCLUSIONS**

Major progress has been made during the last 20 years in characterizing the cel-
lular and molecular mechanisms of interstitial injury. The molecular events determining choice between self-limited repair of the tubulointerstitium or progressive fibrogenesis suggest that persistent cytokine pressure and the accumulation of unwanted inflammatory cells are the principal effectors of chronicity. There remains a gap in understanding the genetic drivers of this cytokine pressure or sensitivity, and our knowledge of how genetic polymorphisms and epigenetic modifications facilitate fibrogenesis are in their infancy.

Modulating the pivotal role of tubular epithelial cells in their provision of profibrotic stimuli, and understanding the molecular basis for fibroblast expansion and collagen accumulation also requires more work. New therapies to abrogate EMT are on the horizon but the chromatin biology modulating these phenotypic changes are unclear; determining the point of no return for intermediate states of transition is still an open question.

Today, the distinction between profibrotic and antiﬁbrotic stimuli also seems increasingly muddled—some macrophages, T cell subpopulations, and mast cells, many of which were once viewed as unequivocally pernicious, now seem renoprotective. Tissue proteases, also once considered renoprotective, in many respects now look as if they encourage fibrogenesis. The role of progenitor cells in regeneration and the depletion of stem cell niches along the adult nephron is also an underdeveloped area of investigation.

Most importantly, although the presence of tubulointerstitial fibrosis has always implied an untenable fate, this perception is changing in new preclinical studies using unique biologic modifiers. We have renewed optimism that in the not too distant future some of these novel insights will become clinically relevant for patients with chronic kidney disease.

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DISCLOSURES

None.

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