

Theme Issue Article

Serine proteases, inhibitors and receptors in renal fibrosis

Allison A. Eddy

Seattle Children's Hospital Research Institute, Center for Tissue and Cell Sciences, and Department of Pediatrics, University of Washington, Seattle, Washington, USA

Summary

Chronic kidney disease (CKD) is estimated to affect one in eight adults. Their kidney function progressively deteriorates as inflammatory and fibrotic processes damage nephrons. New therapies to prevent renal functional decline must build on basic research studies that identify critical cellular and molecular mediators. Plasminogen activator inhibitor-1 (PAI-1), a potent fibrosis-promoting glycoprotein, is one promising candidate. Absent from normal kidneys, PAI-1 is frequently expressed in injured kidneys. Studies in genetically engineered mice have demonstrated its potency as a pro-fibrotic molecule. Somewhat surprising, its ability to inhibit serine protease activity does not appear to be its primary pro-fibrotic effect in CKD. Both tissue-type plasminogen activator and plasminogen deficiency significantly reduced renal fibrosis severity after ureteral obstruction, while genetic urokinase (uPA) deficiency had no effect. PAI-1 ex-

pression is associated with enhanced recruitment of key cellular effectors of renal fibrosis – interstitial macrophages and myofibroblasts. The ability of PAI-1 to promote cell migration involves interactions with the low-density lipoprotein receptor-associated protein-1 and also complex interactions with uPA bound to its receptor (uPAR) and several leukocyte and matrix integrins that associate with uPAR as co-receptors. uPAR is expressed by several cell types in damaged kidneys, and studies in uPAR-deficient mice have shown that it serves a protective role. uPAR mediates additional anti-fibrotic effects – it interacts with specific co-receptors to degrade PAI-1 and extracellular collagens, and soluble uPAR has leukocyte chemoattractant properties. Molecular pathways activated by serine proteases and their inhibitor, PAI-1, are promising targets for future anti-fibrotic therapeutic agents.

Keywords

Plasminogen activator inhibitor-1, urokinase, fibrosis, urokinase receptor (uPAR)

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Renal fibrosis: clinical relevance

Chronic kidney disease (CKD) has emerged as a silent killer. Recent studies estimate that one of every eight adults has CKD as defined as a glomerular filtration rate (GFR) less than 60 ml/min/1.73m², with normal being greater than 90 ml/min/1.73m² (1). Over time, their renal function progressively deteriorates until the GFR falls below 15 ml/min/1.73m², the point defined as “end-stage kidney disease” when dialysis or kidney transplantation is necessary to sustain life. Most CKD patients never reach this latter stage, as they die prematurely due to cardiovascular disease, the risk of which is increased five-fold compared to individuals without CKD. This process of a predictable and relentless decline in kidney function is caused by a fibrotic reaction that is especially destructive in the interstitial compartment of

the kidney, gradually replacing functional nephron units with a scar composed of a variety of extracellular matrix proteins (2). Remarkable is the fact that this renal fibrotic response is a final common pathway of CKD that occurs no matter what the primary kidney disease process. This includes diabetes, hypertension, and chronic glomerulonephritis that together account for 79% of adult end-stage kidney disease in the USA (3). Furthermore, a very similar sequence of pathological events occurs as part of the normal aging process, although at a much slower tempo. From the age of 20 years onward, GFR gradually declines, even without evidence of a prior kidney disease, such that the normal mean GFR at 85 years of age is 60 ml/min/1.73m² (4, 5). Very similar cellular and molecular mechanisms mediate fibrosis in other solid organs exposed to chronic injury. For patients with CKD who face deteriorating renal function due to fi-

Correspondence to:
Allison A. Eddy, MD
The Robert O. Hickman Endowed Chair in Pediatric Nephrology
Professor of Pediatrics
University of Washington
Head, Division of Pediatric Nephrology
Seattle Children's Hospital and Research Institute
1900 Ninth Avenue
Seattle, Washington, 98101–1309, USA
Tel.: +1 206 987 3030, Fax: +1 206 987 7660
E-mail: allison.eddy@seattlechildrens.org

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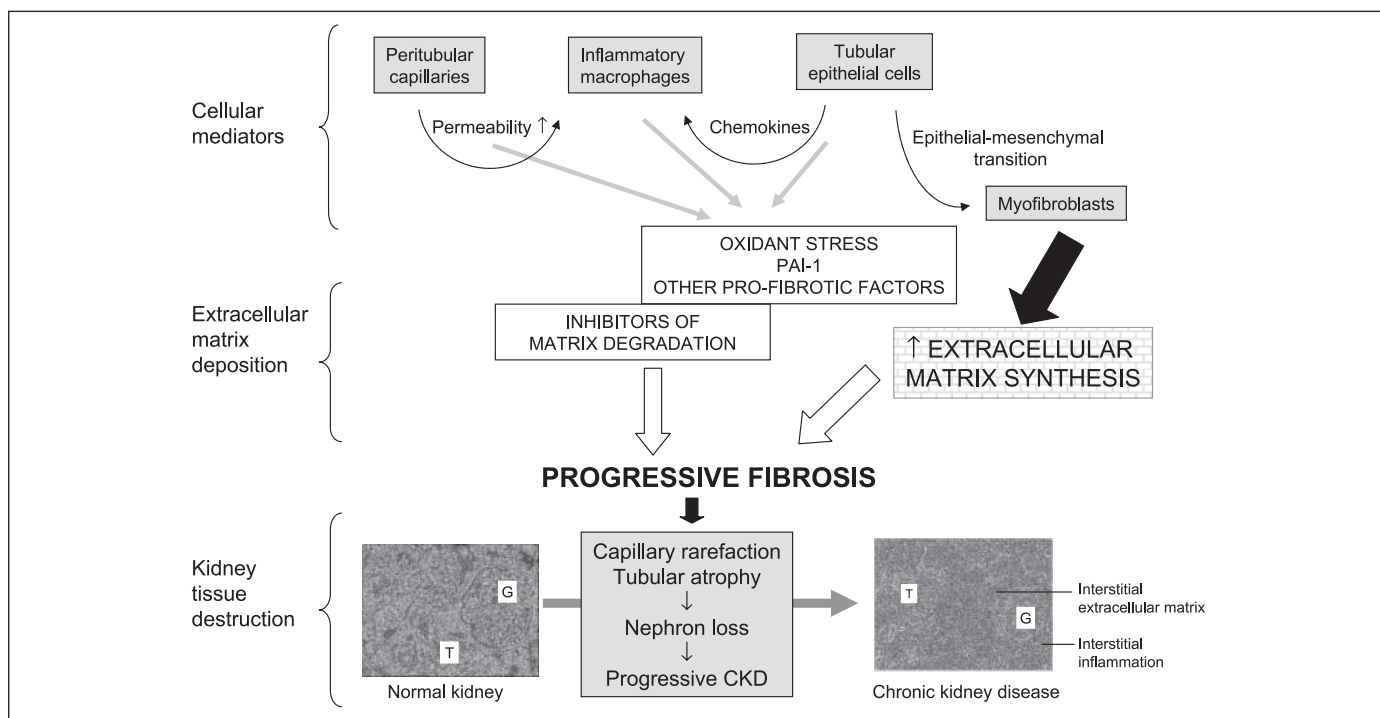


Figure 1: Schematic overview of the major pathways involved in the pathogenesis of chronic kidney disease (CKD). The initial phase is characterised by an influx of macrophages and (myo)fibroblasts into the interstitium. Macrophage recruitment is facilitated by increased permeability of the interstitial capillaries and by release of chemoattractant molecules from activated tubular epithelial cells. Locally macrophages produce a variety of biologically active products that can propagate injury and stimulate synthesis of extracellular matrix proteins by myofibroblasts. These proteins are the precursors of the scar tissue that begins to form within the interstitium. The interstitial myofibroblasts that actively secrete extracellular matrix appear to have several possible origins – resident kidney fibroblasts, circulating fibrocytes, vascular pericytes/fibroblasts and damaged tubular epithelial cells that transdifferentiate

into mesenchymal cells. In addition to enhanced synthesis rates, endogenous matrix turnover pathways or often inhibited to accelerate fibrosis. The photomicrographs are the consequences of these pathological events which convert normal kidney architecture (left) to regions of interstitial inflammation and fibrosis and tubular loss (right). The normal kidney, characterized by intact glomeruli (G) and tubules (T) that sit back-to-back with minimal intervening interstitial space are transformed into a kidney characterised by a prominent and abnormal interstitial region consisting of extracellular matrix proteins and inflammatory cells. These changes have detrimental consequences on the number of functional intact nephrons, as illustrated by the shrunken glomerulus and atrophic tubules (right).

brosis, effective therapies remain unknown. There is a compelling need to delineate specific cellular and molecular mediators that might be manipulated therapeutically.

Renal fibrosis: mechanistic overview

Based on our current understanding of the basic mechanisms of renal fibrosis, several synergistic pathways collaborate and culminate in renal scarring and nephron destruction (Fig. 1). Due to the fact that renal tubules and the adjacent interstitial space occupy most of the kidney volume (~80%), the degree of tubulointerstitial fibrosis is the best histological predictor of CKD severity and has become synonymous with “renal fibrosis.” Similar but not identical mechanisms also lead to glomerulosclerosis (6). Several key fibrogenic events work together to execute the fibrogenic response.

Inflammatory cells infiltrating the interstitium are a characteristic histological feature of CKD. Especially important are macrophages that are recruited from the pool of blood-borne monocytes. Activated macrophages secrete a diverse array of pro-inflammatory and pro-fibrotic products that perpetuate in-

jury and promote scarring. Macrophage-derived transforming growth factor- β (TGF- β) in particular is considered prominent in renal fibrosis. The importance of macrophage functional diversity has been increasingly recognized, especially in mouse models (7, 8). Macrophages may be polarized toward either pro-inflammatory “M1” or reparative “M2” phenotypes. Tissue repair has been associated with macrophage scavenging functions, mediated by cellular receptors such as the angiotensin 1 receptor and uPAR (9, 10).

Myofibroblasts are an absolutely critical population of activated interstitial cells, commonly identified as cells expressing alpha smooth muscle actin (α SMA). α SMA⁺ cells are not present in the normal renal interstitium. They appear to originate primarily from a pool of resident interstitial fibroblasts but other sources may also contribute including, pericytes and perivascular fibroblasts, circulating fibrocytes, and tubular epithelial cells transdifferentiated via a process of epithelial-to-mesenchymal transition (EMT). Interstitial myofibroblasts are considered the primary site of matrix protein synthesis during the active phase of renal fibrogenesis. Fibroblasts are also functionally heterogeneous and may be an important source of proteases and an in-

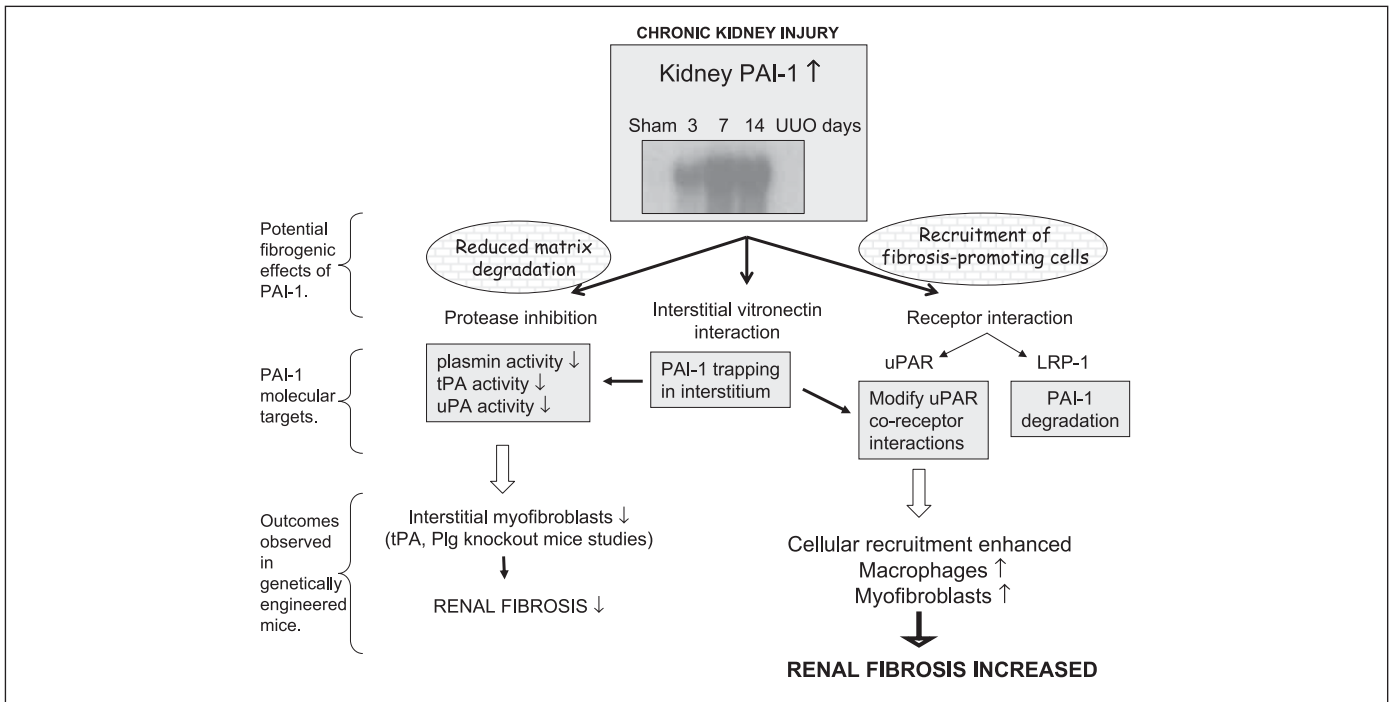


Figure 2: PAI-1 biological effects that may enhance renal fibrosis. PAI-1 expression is markedly increased in damaged kidneys, as illustrated for the mouse unilateral ureteral obstruction (UUO) model. PAI-1 is a glycoprotein that normally has a short half-life due to intracellular degradation after binding to the tertiary complex of uPA + uPAR + LRP-1. High affinity binding to vitronectin competes with uPAR binding and prolongs PAI-1 survival, as may occur when vitronectin accumulates in damaged renal interstitial regions. PAI-1 regulates the activity of two distinct molecular pathways, each of which may potentially be involved with kidney fibrosis: the protease activity-dependent pathway (leading to inhibition of tPA, uPA and plasmin activity) and a cellular pathway that involves PAI-1 interactions with uPAR and its co-receptors that is largely protease-activity-independent. Although PAI-1 blocks tPA and uPA acti-

vation, which has downstream effects on plasmin generation from plasminogen, these effects do not appear to enhance renal fibrosis based on the data that are currently available from experimental CKD models. In fact, the number of interstitial myofibroblasts and fibrosis severity are significantly reduced in tPA- and plasminogen-deficient mice compared to wild-type mice. In contrast, the ability of PAI-1 to alter interactions between uPAR and its co-receptors (such as several integrin family members) leads to enhanced recruitment of fibrosis-promoting cells, including macrophages and myofibroblasts. The PAI-1 northern blot was reproduced from Oda et al. PAI-1 deficiency attenuates the fibrogenic response to ureteral obstruction, *Kidney Int* 2001; 60: 587–596 with copyright permission (28).

tracellular site of cathepsin-dependent protein degradation (11, 12).

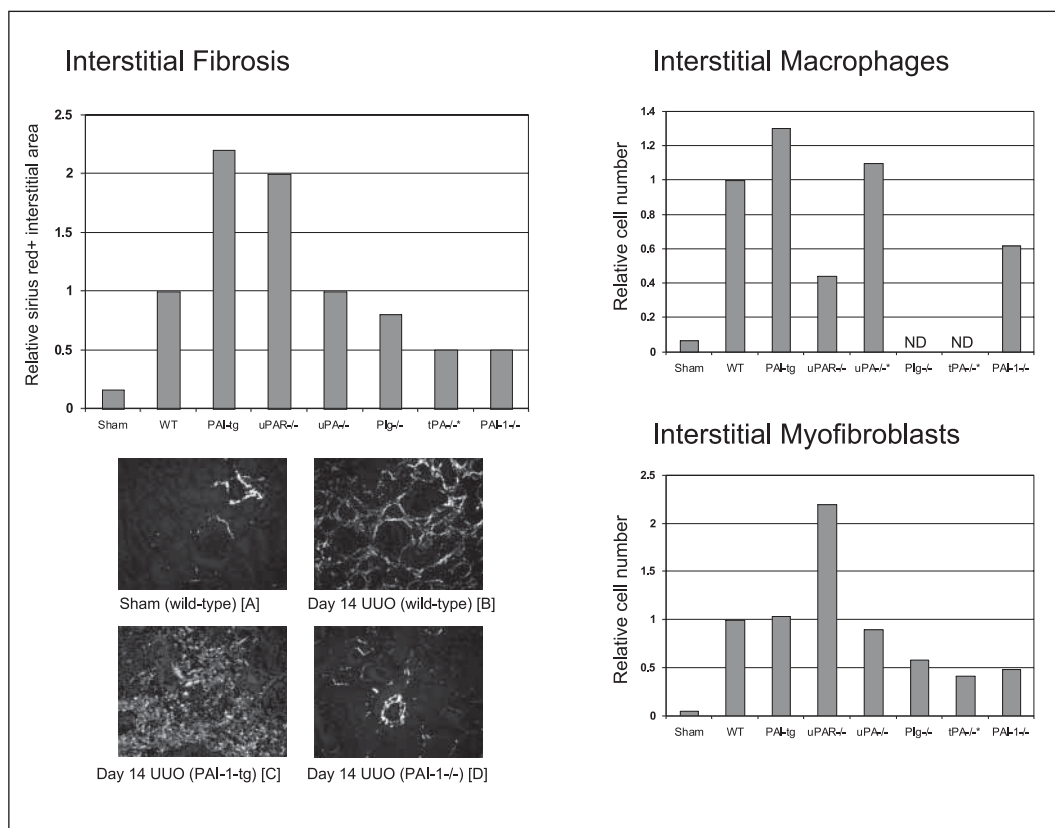
Microvascular endothelial cells are actively involved in the early phase of kidney injury, promoting interstitial cell recruitment by producing chemoattractant and adhesion molecules, and altering permeability which allows plasma proteins (such as fibrinogen and oxidized albumin) to leak into an edematous interstitium. Whether acutely dysfunctional or chronically damaged, disruption of the interstitial capillary network leads to tubular cell hypoxia and a state of oxidant stress that accentuates renal fibrosis (13, 14). Changes in the expression of pro- and anti-angiogenic factors and their receptors are an incompletely understood aspect of the renal fibrogenic response. Impaired angiogenesis contributes to the loss of interstitial capillaries and oxidant stress in chronic kidney disease.

Tubular epithelial cells produce a vast repertoire of molecules that may be modulated by injury and others that are expressed *de novo* in response to injury. Tubules may promote interstitial inflammation via their ability to synthesize chemokines and chemoattractants (15). Together with the tubular basement membrane (TBM) and adjacent endothelial cells of the intersti-

tial capillaries, they normally form a water-tight barrier between the urinary space and the interstitium. As kidney injury transitions from an acute inflammatory to a chronic fibrotic phase, the tubular epithelia assume new roles as they begin to synthesize a variety of pro-fibrotic molecules and some matrix proteins and they may even transform into myofibroblast precursor cells (16, 17). Tubular cell apoptosis is a common feature of CKD. As disease progresses, tubular epithelia lose their ability to self-renew and tubular atrophy ensues. This later phase is possibly the single most important factor that determines the rate of renal functional decline. Tubular epithelial cells also function as endocytic cells. On their apical membranes the megalin-cubulin-amnionless receptor complex plays an important role in the reabsorption of urinary proteins (18). It has recently been recognized that they also express certain scavenger receptors traditionally associated with macrophages (19, 20). We have recently found that renal tubules express the class B scavenger receptor CD36. Using bone marrow transplantation strategies, we found that like macrophage CD36, tubular epithelial cell CD36 plays a significant pro-fibrotic role (unpublished observations).

Figure 3: PAI-1 and serine proteases: effects of genetically altered expression levels on renal fibrosis severity (sirius red+ interstitial collagen), F4/80+ interstitial macrophages and α SMA+ interstitial myofibroblasts in the mouse unilateral ureteral obstruction (UUO) model.

The data are expressed as the percent interstitial area stained relative to levels in wild-type mice 14 days after UUO (except for the tPA^{-/-} study and the uPA^{-/-} study for which day 7 data for total kidney collagen levels and macrophage area are respectively shown, and indicated by the *). Data are taken from the following publications: (10, 28, 29, 37, 44, 47). The photomicrographs A, B and D are reproduced from Oda et al. PAI-1 deficiency attenuates the fibrogenic response to ureteral obstruction, *Kidney Int* 2001; 60: 587–596 with copyright permission (28). ND – not done.



Molecular mediators of fibrosis

Each of the cells discussed above may secrete molecules that promote fibrosis (2). Particularly important in the kidney are reactive oxygen/nitrogen species, TGF- β , members of the renin-angiotensin-aldosterone system, and plasminogen activator inhibitor-1 (PAI-1). Others shown to play a role include platelet-derived growth factor, fibroblast growth factor, epidermal growth factor, tumor necrosis factor- α , and parathyroid hormone-related protein. Down-regulation of certain endogenous inhibitors of fibrotic pathways may also enhance fibrosis. Examples of these include hepatocyte growth factor, bone morphogenic protein 7, relaxin, and heme oxygenase.

Plasminogen activator inhibitor-1 (PAI-1) as a key fibrosis mediator

This review will focus on the role of PAI-1 in the pathogenesis of CKD (Fig. 2) (6). PAI-1 is a 50 kd glycoprotein that is produced in the liver as an acute phase protein and by adipocytes (21). It circulates in the plasma in low concentration with a half-life measured in minutes unless it forms a stable complex with vitronectin. Most studies agree that PAI-1 cannot be detected in normal kidneys, but its expression can be induced in several cells (all intrinsic glomerular cells, tubular epithelial cells, macrophages, and fibroblasts) in response to acute and chronic injury. Numerous molecules have been identified as PAI-1 agonists (22).

Studies of human kidney specimens have documented PAI-1 protein expression in virtually all chronic diseases that have been examined. Gene expression studies confirm significant intrarenal PAI-1 expression. What remains unclear is whether circulating PAI-1 protein also serves as a kidney disease mediator. Polymorphisms in the PAI-1 promoter exist and determine protein levels – the four guanine (4G) variant at position –675 correlates with higher plasma protein levels. Clinical studies have produced conflicting data, possibly due to population heterogeneity, but the PAI-1 4G/4G genotype has been associated with increased CKD risk in some studies (23, 24). Enhanced PAI-1 expression is also a feature of obesity, the metabolic syndrome and cardiovascular disease, raising the possibility of shared pathogenetic mechanisms with CKD (21, 25–27).

Studies of experimental CKD models in genetically engineered mice have clearly shown that PAI-1 expression is associated with worse fibrosis. In the unilateral ureteral obstruction (UUO) model, 14 days after ureteral ligation total kidney collagen levels were >3-fold higher in wild-type mice compared to PAI-1 knockout mice, and were >1.8-fold higher in PAI-1 over-expressing mice compared to wild-type mice (Fig. 3) (28, 29). Other models in which genetic PAI-1 deficiency has been protective include diabetic nephropathy, 5/6 nephrectomy, crescentic anti-GBM nephritis, protein overload nephropathy and the spontaneous renal fibrosis that develops in TGF- β over-expressing mice (6, 30, 31). PAI-1 inhibition by mutant recombinant PAI-1 protein or a neutralizing antibody has also been shown to attenuate experimental kidney fibrosis (30–32).

Alternative plasminogen activator inhibitors in renal fibrosis

In addition to PAI-1, PAI-2 and protease nexin-1 are known to inhibit the plasminogen activators. The only published studies of PAI-2 in chronic kidney disease report that it is undetectable, which suggests that PAI-2 is not mechanistically involved in CKD. Protease nexin-1 has not been extensively investigated in the kidney, but its expression has been reported in the UUO model and in association with certain glomerular diseases (29, 33, 34). Protease nexin-1 has been implicated in the pathogenesis of fibrotic skin disease (35). Because protease nexin-1 mice are infertile, investigating experimental models is challenging and not yet reported for kidney disease. Protease nexin-1 is one of the multiple ligands that binds to low-density lipoprotein receptor-associated protein-1 (LRP-1) (36).

Serine proteases, uPA, tPA, and plasmin do not attenuate renal fibrosis

A key fundamental question is the mechanism by which PAI-1 promotes fibrosis. It was initially assumed that its role as a serine protease inhibitor would be its critical effect, but this has not been confirmed in experimental kidney disease models in genetically engineered mice. Plasminogen is cleaved to generate the active protease plasmin by urokinase-type plasminogen activator (uPA) and tissue-type plasminogen activator (tPA), both of which are inhibited by PAI-1. Plasmin cleaves numerous substrates, but relevant to potential antifibrotic functions, it can directly degrade some matrix proteins (fibronectin, laminin, entactin, tenascin, thrombospondin and perlecan), indirectly degrade several matrix proteins by activating latent metalloproteinases (MMP), and it also degrades fibrin, which can serve as a provisional matrix scaffold to initiate a fibrotic response (22, 37). Although plasminogen mRNA is difficult to detect in the kidney (suggesting that its synthesis may not occur locally) (38), plasminogen activity can be demonstrated in kidney tissue. When a UUO study was performed in plasminogen-deficient mice, fibrosis severity was actually worse in comparison to the wild-type mice (37). Two significant differences were identified that likely contributed to the fibrosis-promoting effects of plasminogen. (i) TGF- β activity was significantly higher in the plasminogen wild-type mice. TGF- β is known to be efficiently activated *in vitro* by plasmin (39). (ii) Following the finding that the number of matrix-producing α SMA⁺ interstitial cells was significantly higher in the wild-type mice, studies *in vitro* established that plasmin interacts with kidney fibroblasts to promote their migration and transform their phenotype to E-cadherin-negative, fibroblast-specific protein-1 positive “myofibroblast-like” cells. These effects were shown to involve protease-activated receptor 1 (PAR1) and extracellular signal-regulated kinase (ERK) signalling.

The possibility that PAI-1 promotes fibrosis through its ability to inhibit the activity of uPA and/or tPA has also been ruled out based on studies in genetically engineered mice. uPA is abundantly expressed in normal renal tubules. Other cells recruited to sites of injury such as macrophages and fibroblasts can also syn-

thesize uPA. Renal uPA activity increases after UUO, but studies comparing activity levels in PAI-1 wild-type and knockout mice surprisingly did not detect significant differences in uPA activity (40). Certain direct proteolytic effects of uPA might be expected to attenuate renal fibrosis. These include its fibrinolytic activity, its ability to degrade some matrix proteins (such as fibronectin), and to activate latent hepatocyte growth factor (HGF), which has been shown to have impressive anti-fibrotic effects (41–43). However, when the mouse UUO model was compared between uPA wild-type and knockout mice, the degree of renal fibrosis, fibrinogen levels, and active HGF were similar between the wild-type and uPA-deficient mice (44). uPA may interact with several distinct cellular receptors, some that promote and others that inhibit renal fibrosis (45, 46). The combined effects on fibrosis may thus vary depending upon which receptors are available. Evidence is accumulating that uPA may have organ-specific effects during fibrotic responses, as a variety of experimental interventions to alter uPA activity have reported decreased fibrosis in the lung and liver and increased fibrosis in the heart when mice with significantly higher uPA were examined (45).

Endogenous tPA is localized primarily to intravascular compartments, where it plays a critical role as a fibrinolytic protease. Compared to uPA activity, tPA activity is barely detectable in normal kidneys; modest increases occur after UUO (45). Nonetheless, this activity has been shown to enhance fibrosis by several mechanisms in studies comparing wild-type and tPA knockout kidneys and fibroblasts. After UUO, tPA-deficient mice develop less severe fibrosis (47). This protective effect was ascribed to two important mechanisms. (i) tPA-expressing kidneys were shown to have higher MMP-9 levels and more extensive tubular basement membrane damage, thought to facilitate formation and migration of transdifferentiated tubular epithelial cells into the interstitium, where they contribute to ongoing fibrosis. (ii) tPA interacting with its cellular receptor, LRP-1, inhibits fibroblast apoptosis, increasing the survival of these profibrotic interstitial cells. Interaction of tPA with myofibroblasts has also been shown to activate LRP-1-mediated integrin signalling (48, 49).

The results of these studies raise an important question that still remains to be answered, that is, which matrix-degrading proteases are activated during renal fibrosis to balance the rate of intracellular matrix accumulation? Impaired collagenolytic activity is known to be an important mechanistic pathway leading to renal fibrosis. A large family of matrix-degrading metalloproteinases have been considered the primary effectors of extracellular matrix degradation. The gelatinases MMP-2 and MMP-9, best known as collagen IV-degrading proteases, are the most abundant intrarenal members of the MMP family. However, emerging evidence suggests that the gelatinases actually promote rather than prevent tubulointerstitial fibrosis. This was convincingly shown in a transgenic mouse that was engineered to over-express MMP-2 in proximal tubular cells. As these mice aged, they developed accelerated interstitial fibrosis and increased tubular cell apoptosis (50).

More studies are needed to identify which extracellular matrix-degrading proteases work constructively to remodel the interstitial matrix proteins before they accumulate and organize to form scars that destroy intact nephrons. With increasing evi-

dence that the early stages of renal fibrosis can regress, identifying the molecular pathways that reverse fibrosis remains an important mission (51–54).

PAI-1 enhances macrophage and myofibroblast recruitment to the renal interstitium: role of uPAR and LRP-1

A remarkable histopathological difference in the response to ureteral obstruction between PAI-1-deficient and over-expressing mice was significant differences in the kinetics of interstitial cell recruitment (29, 40). Both macrophages and myofibroblasts were reduced in the groups with the lowest PAI-1 expression levels. Although a unique PAI-1 receptor has not yet been identified, it is one of multiple proteins that can bind to LRP-1. This interaction is thought to promote cell chemoattraction (55). PAI-1 is also known to influence integrin-dependent cell adhesion and migration via its ability to interact with the urokinase receptor (uPAR) and its bound ligand, uPA (45).

uPAR and its co-receptors in renal fibrosis

The high-affinity receptor for urokinase (uPAR) is not expressed in normal kidneys. Its expression can be induced on some renal tubules, inflammatory cells, and interstitial myofibroblasts in response to injury (10, 56). In addition to uPA, the other uPAR ligands are vitronectin and high-molecular-weight kininogen (HK) (57, 58). Although uPAR lacks an intracellular signaling domain, it is able to mediate several important biological effects due to its interaction with a series of co-receptors that include several integrins, LRP-1, Endo180 (also known as uPAR-associated protein, or uPARAP), the receptor for complement component 5a (C5aR), the HGF receptor c-Met, insulin-like growth factor II/mannose-6-phosphate (IGFII/M6P) receptor, and leukocyte (L)-selectin (45, 59). Studies in uPAR genetically-deficient mice have demonstrated that uPAR serves a protective role during the kidney's response to injury. These studies have included endotoxin-induced proteinuria, acute pyelonephritis, and UUO (10, 56, 60, 61). Several uPAR-dependent mechanisms have been implicated.

- (i) It binds to both latent single-chain and active two-chain uPA. This interaction stabilizes cell-associated uPA activity, but the importance of this effect on renal fibrosis is unclear, given the finding that endogenous uPA does not appear to regulate renal fibrosis severity.
- (ii) The uPAR plays an important role in intracellular PAI-1 degradation. This process involves the formation of a quaternary complex with uPAR, uPA, LRP-1, and PAI-1. This entire complex is internalised and transported to lysosomes for degradation. The uPAR survives this process intact and is shuttled back to the cell membrane. In a study of UUO-induced nephropathy, kidney PAI-1 levels were higher in uPAR-deficient mice in comparison to wild-type mice, suggesting that uPAR-dependent PAI-1 turnover helps to attenuate the renal fibrogenic response (56). However, we did not find that UUO-induced renal fibrosis was less severe in

PAI-1/uPAR double-deficient mice compared to uPAR-deficient mice (unpublished data).

- (iii) uPAR may have other scavenging activities relevant to renal fibrosis. This possibility is supported by the observation that the interstitial monocyte/macrophage inflammatory response to UUO is more robust in wild-type mice, even though they develop less severe fibrosis than uPAR-deficient mice (56). uPAR may be added to a growing list of macrophage receptors that are expressed by macrophages which are polarized to a “M2” phenotype associated with tissue repair. uPAR has been reported to promote leukocyte signaling by transactivating its leukocyte integrin co-receptors (62, 63). Bone marrow transplantation studies in progress will clarify if uPAR-expressing macrophages attenuate renal injury after UUO.
- (iv) There are several mechanisms by which uPAR can facilitate the recruitment of monocytes to the kidney where they may exacerbate injury and contribute to scarring. uPAR exists in a soluble form (suPAR) consisting of its second and third domains, generated by proteolytic cleavage by enzymes that include uPA, plasmin, MMP-12, kallikrein and chymotrypsin (64). That the kidney can generate suPAR is supported by its detection in urine. SuPAR is a potent chemoattractant for leukocytes that express its receptor, the G protein-coupled receptor formyl-peptide-receptor-like 1/lipoxin A4 receptor (FPRL1/LXA4R) (65). As a transmembrane receptor, uPAR has been shown to promote leukocyte recruitment through interactions with the leukocyte integrin CD11b/CD18 and with L-selectin (62, 66, 67). Glycoprotein 130 (gp130, the key receptor for the interleukin-6 super-family) and the complement chemoattractant C5a are both known to regulate acute inflammatory responses and to have the ability to function as uPAR co-receptors (68, 69). Expression of both gp130 and C5aR has been reported in models of renal injury, but distinguishing between their independent and uPAR-associated roles during renal fibrosis remain to be determined.
- (v) The renal expression of uPAR during a fibrogenic response to injury has a significant impact on the interstitial myofibroblasts, thought to be the major site of extracellular matrix synthesis: their numbers are greatly enhanced in the absence of uPAR (56). In addition to leukocyte integrins, several of the matrix-binding integrins ($\alpha 3$, $\alpha 5$, αv , $\beta 1$, $\beta 2$, $\beta 3$ and $\beta 5$) are uPAR co-receptors that can influence cell migration (70). The mechanism is complex and not yet fully deciphered, but the prevailing view is that uPAR serves a “deadhesive” function, that liberates cells such as fibroblasts to move along new extracellular matrices that form in the interstitium during the early phase of renal fibrosis (71).
- (vi) uPAR has been reported to promote angiogenesis, stimulate proliferation, and inhibit caspase-mediated apoptosis (72).

uPAR-associated protein (uPARAP/Endo180)

uPARAP was first recognized as a protein that associates with uPAR in 1993 (73). This receptor is a member of the mannose receptor family and is typically expressed in tissues undergoing remodeling. Levels are low in normal kidneys; its expression is in-

duced 10-fold in the experimental UUO kidney disease model (74). Its expression was first recognized on fibroblasts, but in areas of tissue injury uPARAP expression has been reported on (myo)fibroblasts, macrophages, and epithelial cells. We have recently observed that UUO-induced renal fibrosis is significantly worse in uPARAP-deficient mice (74). What remains to be determined is whether the uPAR-uPARAP interaction is required for this renal protective effect. uPARAP has recently been reported to serve as a cellular receptor for several collagens (I, IV, V) that are known to accumulate in the interstitium during fibrosis (75–77). Once collagen binds to uPARAP, the ligand is endocytosed via clathrin-coated pits and transported to endolysosomes for degradation. This mechanism of collagen turnover appears to serve an important role in regulating the degree of renal fibrosis and nephron loss in chronic kidney disease.

The other uPAR ligands: vitronectin and kininogen

Vitronectin (also known as S protein of the complement regulatory pathway) is an extracellular matrix protein that has not received great attention in chronic kidney disease. Vitronectin is one of the extracellular matrix proteins that accumulates in the interstitium during renal fibrosis (56). Vitronectin cellular receptors include the integrins $\alpha\beta 3$ and $\alpha\beta 5$ that are also uPAR co-receptors. Binding between vitronectin and uPAR is significantly increased in the presence of uPA (57). PAI-1 inhibits this interaction, as it shares a common vitronectin-binding domain (somatomedin B domain) with uPAR but binds to it vitronectin with much higher affinity (21, 78). It seems likely that vitronectin will be involved in cellular migration during renal fibrosis, but this has yet to be documented by vitronectin inhibitors or in genetically deficient animals.

HK has binding sites within uPAR domains 2 and 3. This interaction has been shown in a variety of systems *in vitro* to regulate cell migration, apoptosis, and chemokine production (79–81). HK is a precursor of bradykinin, a molecule that has been shown to have anti-fibrotic effects in the kidney (82, 83). Bradykinin is degraded by angiotensin converting-enzyme inhibitor (ACE). Elevated bradykinin levels in CKD patients treated with ACE drugs may be involved in the renoprotective effects of these drugs (84). Whether uPAR modulates any of these activities during renal fibrosis awaits investigation.

Alternative uPA receptors

There is increasing evidence that uPA can bind to receptors in addition to the high-affinity uPAR. Based on phage display technology, 12 membrane receptors were identified that express a putative uPA-binding motif (85). The list includes many of the uPAR co-receptors: LRP-1, gp130, uPARAP, c-Met, IGFII/MGPR, and several integrins. Studies from our laboratory comparing uPAR wild-type and knockout kidney fibroblasts suggested that uPA interactions with an alternative receptor could be

biologically important in CKD. In particular, uPAR-deficient kidney fibroblasts were observed to proliferate faster than uPAR wild-type fibroblasts, consistent with the observation *in vivo* of significantly more interstitial myofibroblasts (Fig. 3) and worse fibrosis after UUO in the uPAR-deficient mice (86). The ability of uPA to stimulate uPAR-deficient fibroblast proliferation suggested the possibility that these cells expressed an alternative uPA receptor that might stimulate their proliferation. We recently pursued the nicotine acetylcholine receptor alpha 1 (nAChR $\alpha 1$), one of the original 12 candidates, as a candidate for this putative alternative fibroblast uPA receptor that might be active during renal fibrosis (46). Studies *in vitro* established that uPA binds to nAChR $\alpha 1$ and stimulates tyrosine phosphorylation of the receptor and a complex of signalling proteins. nAChR $\alpha 1$ was also shown to function as an uPA-gated ion channel to stimulate calcium influx and promote fibroblast proliferation and migration. Using a hydrodynamic strategy to deliver silencing RNA to the kidney, NACHR $\alpha 1$ down-regulation was associated with a significant reduction in renal fibrosis severity after UUO supporting the notion that uPA-NACHR $\alpha 1$ interactions are pro-fibrotic while uPA-uPAR interactions were previously shown to have the opposite effect. Thus cell-specific differences in the relative level of expression of various uPA receptors may determine whether local uPA is pro-fibrotic (in the heart), anti-fibrotic (in the lung and liver) or has no net effect (as in normal kidneys) (45).

Conclusion

PAI-1 has important fibrogenic effects in CKD. Serine protease inhibition does not appear to account for its profibrotic effects in the kidney; in fact, tPA and plasmin are profibrotic. These effects may be organ specific, as uPA has anti-fibrotic effects in other solid organs undergoing a fibrogenic response. This might be due to tissue-specific differences in the expression of uPA receptors, including some that enhance and others that reduce fibrosis. PAI-1 and the uPA high-affinity receptor uPAR have important effects that regulate interstitial inflammatory cell and myofibroblast recruitment, with the net effect that renal fibrosis is worse in PAI-1 expressing mice and attenuated in uPAR-expressing mice. Although uPAR itself is a non-signalling receptor, several co-receptors appear to be involved, though whether their effects require interactions with uPA/uPAR/PAI-1 or if they also function independently remains to be clarified. Several molecular pathways triggered by serine proteases and their inhibitors are promising candidates as new therapeutic targets for treating CKD patients.

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