Plasminogen Activator Inhibitor-1 in Chronic Kidney Disease: Evidence and Mechanisms of Action

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In 1984, Loskutoff et al. (1) purified plasminogen activator inhibitor-1 (PAI-1) from conditioned media of cultured endothelial cells. This 50-kd glycoprotein is the primary physiologic inhibitor of the serine proteases tissue-type and urokinase-type plasminogen activators (tPA and uPA, respectively). It now is known to mediate important biologic activities that extend far beyond fibrinolysis through interactions with its co-factor, vitronectin (also known as protein S), and with the urokinase receptor (uPAR) and its co-receptors (2,3). Plasma PAI-1 levels increase in response to stress as an acute-phase protein. Usually present in trace amounts, plasma PAI-1 levels increase in several chronic inflammatory states that are associated with chronic kidney disease (CKD), and it may contribute to the pathogenesis of the accelerated vascular disease in this patient population (4–7). Liver and adipose tissue seem to be the primary sources of plasma PAI-1 (8,9). Other inhibitors of plasminogen activation exist; protease nexin-1 and H9251 anti-plasmin can be produced by the kidney.

Although PAI-1 normally is not produced in kidneys, synthesis by both resident and intrarenal inflammatory cells occurs in several acute and chronic disease states (Table 1). In the past decade, a growing body of experimental evidence that has derived largely from animal models supports the view that PAI-1 is a powerful fibrosis-promoting molecule and is a promising therapeutic target for new drugs and biologics to combat the current CKD epidemic (Table 2). Exactly how PAI-1 promotes renal fibrosis is not understood completely. Recent studies suggest that in addition to its ability to inhibit serine protease activity within vascular and extracellular compartments, PAI-1 directly modulates cellular behavior, leading to a vicious cycle of inflammatory cell recruitment, fibroblast activation, and scar tissue accumulation.

PAI-1 Is Present in Most Aggressive Kidney Diseases

Acute/Thrombotic Diseases

Thrombotic microangiopathy (TMA) is a pathologic lesion that is characterized by fibrin deposition in the microvasculature, often involving glomeruli and renal arterioles. TMA characterizes renal diseases that are caused by hemolytic uremic syndrome, preeclampsia, scleroderma, malignant hypertension, and the antiphospholipid antibody syndrome. Glomerular PAI-1 deposition is a feature of TMA (10). In children who have Escherichia coli 0157:H7 infection and later develop hemolytic uremic syndrome (11), plasma PAI-1 levels increase before the onset of renal disease. Plasma PAI-1 activity correlates with renal disease severity and long-term outcome (12–15). One pediatric study found that PAI-1 activity also increases during acute renal failure of other causes (16). Animal hemolytic uremic syndrome models that should provide specific insights into the pathogenetic role of PAI-1 are under development (17,18). The extent to which renal PAI-1 is produced locally or derived from the plasma pool has not been determined.

PAI-1 expression is a feature of preeclampsia (19) and scleroderma, although experimental data suggest that PAI-1 is not essential in the latter (20,21). Radiation nephropathy is characterized in its early phase by TMA with PAI-1 generation, and it often progresses to sclerosis. Angiotensin or aldosterone inhibition reduces PAI-1 levels and the severity of glomerular sclerosis in experimental radiation nephropathy (22,23).

Crescentic Glomerulonephritis

Crescents develop as a result of segmental breaks of the glomerular basement membrane (GBM), often in association with fibrinoid necrosis. In human crescentic glomerulonephritis, PAI-1 is detected both in areas of glomerular necrosis and in crescents (24,25). Parietal epithelial cells are a source of PAI-1 and uPA in human crescentic glomerulonephritis (26).

In experimental models of anti-GBM crescentic nephritis, PAI-1 is produced, whereas tPA levels are suppressed, leading to decreased net glomerular fibrinolytic activity and prolonged fibrin deposition (27). A functional role for PAI-1 in the pathogenesis of anti-GBM crescentic glomerulonephritis was established by elegant studies in genetically engineered mice (Table...
Table 1. Human diseases with intrarenal PAI-1 expression

<table>
<thead>
<tr>
<th>Disease</th>
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<tr>
<td>Diabetic nephropathy (41, 146)</td>
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<tr>
<td>Focal necrotizing glomerulonephritis (32)</td>
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<tr>
<td>Crescentic glomerulonephritis (24–26)</td>
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<tr>
<td>Focal segmental glomerulosclerosis (32, 147)</td>
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<tr>
<td>Membranous nephropathy (32, 148)</td>
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<tr>
<td>Chronic allograft nephropathy (124, 149, 150)</td>
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<tr>
<td>Thrombotic microangiopathy (10, 24)</td>
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<td>Arterionephrosclerosis (41, 151)</td>
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*PAI-1, plasminogen activator inhibitor-1.

2) (28). The PAI-1−/− mice developed fewer glomerular crescents and glomerular fibrin deposits and reduced collagen accumulation long term. In contrast, mice that were engineered to overexpress PAI-1 formed more crescents along with more extensive fibrin deposits and extensive collagen accumulation. Genetic manipulations that reduce serine protease activity, either plasminogen deficiency or combined uPA and tPA deficiency, also lead to aggressive injury with more extensive crescents, necrosis, and fibrin deposition (29). Isolated tPA deficiency causes glomerular injury that is intermediate between wild-type and combination PA knockout mice, whereas isolated uPA deficiency results in fewer glomerular macrophages, but, otherwise, disease severity is similar to the wild-type mice, suggesting that tPA is the primary glomerular plasminogen activator. A different outcome was observed in a passive model of anti-GBM glomerulonephritis, in which PAI-1−/− mice developed more severe renal injury that was attributed to plasminogen activator–dependent activation of TGF-β (30). It was postulated further that enhanced TGF-β activity influenced CD4+ T cell responses, leading to disease exacerbation. It is possible that differences in the mouse strain may account for some of these divergent observations in the genetically engineered mouse studies. It is remarkable, however, that whenever renal plasmin activity is measured in these mice, it is not found to differ significantly from that of wild-type mice.

**Proliferative Glomerulonephritis**

PAI-1 mRNA and protein are increased in both human and animal models of proliferative glomerulonephritis, particularly when lesions are severe with fibrin and crescent formation (31). In human disease, increased PAI-1 levels correlate with level of proteinuria rather than with degree of proliferation. In a study of 80 patients, the highest glomerular PAI-1 levels were found in FSGS and membranoproliferative glomerulonephritis (32).

In the rat Habu snake venom proliferative glomerulonephritis model, PAI-1 is increased and localized to mesangial lesions at early time points, suggesting a possible role of PAI-1 in cell migration (33). In the rat mesangial proliferative model of anti-Thy-1 antibody–mediated glomerulonephritis, PAI-1, along with growth factors such as TGF-β and PDGF, are increased, and interventions that decrease injury also lower PAI-1 (34, 35). Conversely, injecting recombinant IPA in this rat model increases plasmin generation, with subsequent decrease in matrix accumulation (36). PAI-1 inhibition by a mutant PAI-1 that binds matrix vitronectin but does not inhibit plasminogen activator results in significant reduction in extracellular matrix (ECM) accumulation (37). This treatment also was linked to increased glomerular plasmin activity and enhanced ECM degradation. However, mRNA levels for genes encoding ECM proteins also were decreased, perhaps as a consequence of decreased macrophage infiltration and plasmin-independent effects.

**PAI-1 in Chronic Progressive Renal Disease**

In addition to its effects on fibrinolysis that may promote thrombotic and necrotizing renal lesions, PAI-1 has complex interactions with matrix proteins that enhance matrix accumulation in several glomerular disease states (38–40). In humans, PAI-1 is prominent in atherosclerotic lesions and in sclerotic glomeruli that are damaged as a consequence of hypertensive nephrosclerosis, diabetic nephropathy, and chronic allograft nephropathy. Plasma PAI-1 levels are increased in patients with insulin resistance and obesity as well as overt diabetes (38–40). Within the kidney, PAI-1 protein is prominent in Kimmelstiel-Wilson nodules, often associated with fragmented red blood cells in regions of local injury and mesangiolysis (41). Because adipose tissue is an important source of PAI-1, it also may be important in the genesis of the nephropathy of obesity that may develop even in the absence of diabetes (42).

Although rodent models of diabetes do not develop robust glomerular sclerosis or interstitial fibrosis, there is evidence to support a role for PAI-1 in the pathogenesis of diabetic nephropathy. In the mouse model of mild diabetic injury that is induced by streptozotocin injection, PAI-1−/− mice have reduced albuminuria and fibronectin levels compared with wild-type diabetic mice (43). In the db/db mouse model, PAI-1 deficiency also was associated with reduced albuminuria and kidney collagen levels, but a high mortality rate was observed (44). In early streptozotocin-induced nephropathy in rats, spironolactone therapy reduced PAI-1 and TGF-β expression levels and matrix deposition (45). In another study, PAI-1−/− mice failed to develop the diabetic phenotype that was induced in wild-type mice by feeding a high-fat diet (46). Furthermore, PAI-1−/− adipocytes were functionally distinct. They produced lower levels of several hormones that have been identified as key mediators of the dysmetabolic syndrome, including resistin, peroxisome proliferator–activated receptor-γ, and adiponectin (47). These studies suggest complex roles for PAI-1 in the pathogenesis of diabetes and its complications.

PAI-1 also is increased in most experimental models of glomerulosclerosis, including cyclosporin-induced glomerulosclerosis, radiation nephropathy, various models of FSGS, chronic anti-Thy-1 nephritis, diabetic nephropathy, aging, and chronic allograft nephropathy (38, 48). Two powerful fibrogenic systems are potent PAI-1 inducers: TGF-β and the renin-angiotensinaldosterone system. Because angiotensin, PAI-1, and TGF-β often are coexpressed in chronic renal disease, it has been challenging to decipher the independent contributions of each molecule to the sclerotic process (49). However, in the anti-Thy-1 model, combined TGF-β and angiotensin inhibition
reduces PAI-1 expression and glomerular matrix accumulation and is more effective than either therapy alone (35).

Studies in the \( \beta 6 \) knockout mice provide additional insights. The \( \alpha v \beta 6 \) integrin is involved in TGF-\( \beta \) activation. \( \beta 6 -/- \) mice are protected from fibrosis after unilateral ureteral obstruction (UUO) and do not show increased active TGF-\( \beta \) and PAI-1 like the wild-type mice (50,51). However, PAI-1 expression and fibrosis, but not TGF-\( \beta \), are restored to wild-type levels by infusing angiotensin, supporting a direct link among angiotensin, PAI-1, and fibrosis. Molecular studies indicate that angiotensin-dependent, TGF-\( \beta \)-independent induction of PAI-1 gene expression involves the AT1 receptor and a glucocorticoid response element in the PAI-1 promoter (40,52). AT1 receptor blockade but not nonspecific antihypertensive treatment reduces renal PAI-1 expression that is induced by angiotensin II (AngII) infusion (53). Aldosterone also enhances angiotensin-induced PAI-1 expression. In a small study of hypertensive human kidney transplant recipients, elevated plasma PAI-1 levels were decreased by treatment with angiotensin receptor blockers but not by nifedipine (54).

Additional CKD models are remarkably attenuated when PAI-1 is absent (Table 2). Sclerosis-prone 129Sv mice that are bred to generate a PAI-1 null genotype are completely protected from glomerular scarring and tubulointerstitial fibrosis after 5/6 nephrectomy (Figure 1) (55).

![Figure 1. Plasminogen activator inhibitor-1 (PAI-1) deficiency attenuates glomerulosclerosis in mice with remnant kidneys. Ten weeks after sclerosis-prone 129Sv mice underwent 5/6 nephrectomy, glomerulosclerosis and interstitial fibrosis were evident (A). These lesions failed to develop in 129Sv/PAI-1 \(-/-\) mice (B). Magnification, \( \times 200 \) (periodic acid-Schiff).](image)

### Table 2. Manipulation of PAI-1 and serine proteases in experimental kidney diseases

<table>
<thead>
<tr>
<th>Genetic or Therapeutic Manipulation</th>
<th>Reduced Fibrosis</th>
<th>Increased Fibrosis</th>
<th>No Change in Fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAI-1 (-/-)</td>
<td>Crescentic anti-GBM nephritis (28)</td>
<td>Passive anti-GBM nephritis (30)</td>
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<td></td>
<td>Streptozotocin-induced diabetes (43,44)</td>
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<td>( db/db ) diabetes (44)</td>
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<td>5/6 nephrectomy (55)</td>
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<td>Protein overload (60)</td>
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<td>Obstructive nephropathy (61)</td>
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<td>TGF-( \beta ) transgenic mice (63)</td>
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<td>PAI-1 mutant</td>
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<td>Anti–Thy-1 nephritis (37)</td>
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<td>PAI-1 neutralizing antibody</td>
<td>LPS-induced endotoxemia (152)</td>
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<tr>
<td>Plasminogen (-/-) mice</td>
<td>Obstructive nephropathy (93,95)</td>
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<td>tPA (-/-) mice</td>
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<td>tPA recombinant protein</td>
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<td>Anti–Thy-1 nephritis (36)</td>
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<tr>
<td>uPA (-/-)</td>
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<td>uPAR (-/-) mice</td>
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\( db/db \), spontaneously diabetic mouse as a result of a genetic mutation in the leptin receptor; GBM, glomerular basement membrane; tPA, tissue-type plasminogen activator; uPA, urokinase-type plasminogen activator; uPAR, uPA receptor.
type cells (56). PAI-1 deficiency also reduces vascular sclerotic lesions that develop in wild-type mice that are exposed to AngII and salt loading or nitric oxide inhibition by L-NAME (57–59). PAI-1 deficiency protects against interstitial fibrosis that is induced by protein overload (60). In the UUO model, PAI-1−/− mice have reduced renal collagen, whereas PAI-1 overexpressing mice develop worse fibrosis (Figure 2) (61,62). PAI-1 levels are increased in TGF-β-overexpressing mice that develop progressive glomerulosclerosis (63). When these TGF-β transgenic mice are bred with PAI-1−/− mice, both glomerular and interstitial matrix deposition are reduced (63). Several of these in vivo studies uncovered an unexpected finding, namely that the fibrosis-reducing effects that are associated with PAI-1 deficiency often occurred without measurable differences in renal protease activity, whereas the number of inflammatory macrophages and myofibroblasts were reduced, especially during the early response to injury.

### Renal Fibrosis Regression

It was suggested recently that early renal “scars” remodel and may even disappear. Regression has been clearly demonstrated in certain glomerular diseases that are associated with expansion of the mesangial matrix (human diabetes, the acute Thy-1 rat glomerulonephritis model) and in models of sclerosis (5/6 nephrectomy, aging) (64,65). In the 5/6 nephrectomy glomerulosclerosis model inhibitors of angiotensin or aldosterone given alone or in combination significantly decrease and sometimes reverse existing glomerulosclerosis. This beneficial effect is strongly linked to decreased glomerular PAI-1 expression (Figure 3) (66,67). Similar results are seen in aging rats, where existing aortic and glomerular sclerosis are reversed by high dose AT1 receptor blockade and are associated with decreased PAI-1 levels (68). In the 5/6 nephrectomy model, glomerulosclerosis regression is associated with increased plasmin activity (67).

The molecular composition of sclerotic glomeruli differs from that of the normal mesangial matrix and seems to be more resilient to proteolytic degradation, perhaps explaining why glomerulosclerosis regression is not achieved in all 5/6 nephrectomized rats that are treated with high-dose renin-angiotensin system blockers. The same distinction also applies to the interstitium, where interstitial matrix may expand transiently during acute self-limited diseases such as acute tubular necrosis and nephrotic syndrome, whereas interstitial fibrosis that is induced by chronic and progressive injury is more resistant to remodeling and regression (69). Nonetheless, a recent mouse UUO study reported convincing evidence of interstitial matrix remodeling when UUO was released after 7 d (70). The specific molecular mechanisms that reverse interstitial and glomerular deposits of fibrotic matrix proteins remain to be determined, but it is tempting to speculate that PAI-1 might be one molecular switch: On during fibrosis and off again during regression.

**Figure 2.** PAI-1 deficiency attenuates interstitial fibrosis in mice with obstructive nephropathy. After complete ureteral obstruction, interstitial fibrosis rapidly develops. Compared with C57BL/6 PAI-1 wild-type (WT) mice, the obstructed kidneys of PAI-1−/− mice demonstrate less fibrosis (total collagen and Sirius red–stained interstitial area). Disease attenuation in the PAI-1−/− mice was associated with fewer F4/80+ interstitial macrophages and α-smooth muscle actin (α-SMA)-positive interstitial myofibroblasts but not with detectable differences in renal plasmin activity. Graphs show the quantitative data; representative photomicrographs are shown to the right for Sirius red collagen, F4/80, and α-SMA staining, respectively. *P < 0.05. Magnification, ×400. Reprinted from reference (61), with permission.

**Figure 3.** PAI-1 immunostaining in 5/6 nephrectomized rats. At 8 wk after 5/6 nephrectomy, sclerosis was moderately advanced with corresponding increased PAI-1 immunostain in sclerotic glomeruli and in the tubulointerstitium (brown stain; A). Regression of sclerosis was induced by week 12 in some rats that were treated with high-dosage angiotensin receptor blocker starting at 8 wk, with corresponding marked decrease in PAI-1 immunostain (B). Magnification, ×200 (PAI-1 immunostain). Reprinted from reference (67), with permission.
Fibrosis-Promoting Consequences of PAI-1

PAI-1 is synthesized as a single-chain glycopeptide that is secreted rapidly; platelets seem to be the only cell that is capable of its intracellular storage. In CKD, PAI-1 accumulates in the interstitium (71,72). PAI-1 spontaneously converts to a more stable inactive molecule unless it interacts with one of its binding partners: The matrix molecule vitronectin, tPA or uPA, or possibly LDL receptor–associated protein (LRP), a multiligand scavenging and signaling receptor also known as the α-2 macroglobulin receptor (73). PAI-1 converts to its inactive form when a flexible joint in the reactive center loop region bends, thereby making tPA and uPA binding sites inaccessible (74). Vitronectin accumulation at sites of renal injury may trap active PAI-1 as a result of high-affinity binding. The function of PAI-1 in tissue pathology is likely to depend on whether vitronectin is present. PAI-1 expression is highly regulated and may be induced in a variety of cells. Numerous growth factors, coagulation factors, metabolic factors, hormones, and environmental factors that are implicated in kidney disease pathogenesis have been shown to induce PAI-1 expression (reviewed in reference [38]). More recently identified PAI-1 agonists include C-reactive protein (75), thymosin β4 (76), CD40L (77), and sterol regulatory element–binding proteins (78). Prostaglandin E1 and vitamin D have been reported to decrease PAI-1 expression (79,80).

Protease-Dependent Effects of PAI-1

Plasmin

Although plasminogen is not produced in the kidney, plasma plasminogen readily can escape to extravascular sites (81). Active plasmin is generated by tPA- or uPA-dependent cleavage of the latent zymogen plasminogen (Figure 4). The active disulfide-linked homodimer is required to degrade fibrin and remodel thrombotic clots. Plasmin activity is particularly important within vascular spaces. Fibrin also may be required for crescent formation (82). What remains unclear is the extent to which fibrin(ogen) accumulates within renal mesangial and interstitial matrices and whether its presence in such sites facilitates scar formation. In contrast, fibrin as an early “provisional matrix” has been considered an important precursor to pulmonary fibrosis. However, the severity of pulmonary fibrosis is not attenuated in fibrinogen-deficient mice, indicating that fibrin does not seem to be essential for fibrogenesis in the lung (83,84). Similar studies have not yet been performed to determine whether fibrinogen plays a role in progressive kidney disease.

Although fibrin is the preferred plasmin substrate, plasmin also cleaves other proteins that are thought to be involved in fibrosis. First, plasmin activates several latent metalloproteinases (MMP) (85). The MMP are a large family of matrix-degrading enzymes. The collagenase IV enzymes or gelatinases are abundant in the kidney, MMP-9 in particular. In response to chronic injury that is induced by UUO, MMP-9 activity declines, a change that was predicted to impair matrix turnover and promote fibrosis (86). However, other studies demonstrate that MMP-9 also degrades tubular basement membranes, an effect that may promote fibrosis by facilitating the migration of matrix-producing transdifferentiated tubular epithelia into the interstitium (87). Furthermore, through interactions with ανβ3 integrins, the gelatinases may influence cellular behavior directly (88). When transgenic mice were generated to overexpress MMP-2 in renal proximal tubules, the mice developed interstitial fibrosis and tubular atrophy (89). Therefore, at this time, it is unclear whether renal MMP activation by plasmin (or other proteases) attenuates or promotes renal fibrosis. Plasmin also can activate prourokinase (90).

Second, plasmin has limited ability to degrade certain matrix proteins directly, including fibronectin, laminin, entactin, tenascin-C, thrombospondin, and perlecan (38). By degrading ECM, plasmin also may release sequestered growth factors that modulate fibrosis severity. Studies by Noble et al. support the view that intraglomerular plasmin activity promotes matrix degradation (91). Third, in vitro, plasmin is a potent activator of latent TGF-β (92). However, the primary pathway(s) by which TGF-β is activated during progressive renal damage remains unknown. Plasmin remains a candidate. Fourth, in addition to thrombin, the protease-activated receptor-1 can be activated by plasmin. When plasmin activates tubular cell protease-activated receptor-1, it may initiate their transdifferentiation and promote fibrosis (93). This interaction also inhibits monocyte apoptosis (94).
With so many possible activities, it was unclear whether increases in renal plasmin activity promote or attenuate CKD. This question was complicated further by results of studies of CKD models in PAI-1 genetically manipulated mice. Although PAI-1 levels correlate with disease severity, there is no detectable difference in renal plasmin activity (61,62). For clarification of the role of plasmin, UUO was induced in plasminogen wild-type and knockout mice. Renal fibrosis was less severe in the plasminogen-deficient mice, associated with lower levels of active TGF-β (93,95). These findings do contrast with outcomes in the bleomycin-induced lung injury model, in which fibrosis is worse in plasminogen-deficient mice, suggesting that the fibrogenic effects of plasminogen might be organ specific (96).

One additional effect of plasminogen deserves mention. The angiogenesis inhibitor angiostatin is a plasminogen cleavage product that comprises three to five of its kringle domains. Angiostatin binds to several endothelial cell surface receptors, but it also may interact with other cells. Diabetic rats that were treated with an adenoviral vector–expressing angiostatin developed less albuminuria and glomerular hypertrophy associated with downregulation of TGF-β and vascular endothelial growth factor levels (97).

On the basis of currently available data, it seems that renal PAI-1 expression does not necessarily alter renal plasmin activity. Other regulators, such as α-2 anti-plasmin, might be more critical (60,98). Plasmin has several proteolytic targets and can trigger receptor-dependent effects, and, together, its activities favor fibrosis development, at least in the UUO model. It is possible that many of the profibrotic effects of PAI-1 align more closely with its ability to block plasmin-independent proteolytic actions of tPA and uPA.

**tPA**

tPA is produced primarily by endothelial cells, but it also is synthesized by many other cells, including monocytes and fibroblasts. It traditionally has been viewed as an intravascular protease because of its need for fibrin to optimize its plasminogen activator activity. Other important actions of tPA were identified recently. Basal renal tPA activity is low (detected in glomerular cells and collecting duct epithelia). Decreased glomerular tPA activity has been implicated in the pathogenesis of crescentic glomerulonephritis; genetic tPA deficiency worsened disease, whereas recombinant tPA has been reported to reduce glomerular matrix expansion (29,36). Renal tPA activity increases after UUO (61,71). During chronic tubulointerstitial disease, the predominant effect of tPA seems to enhance renal fibrosis (87). One relevant mechanism is MMP-9 activation in the proximity of tubular basement membranes. Although total renal MMP-9 activity declines after UUO, preservation of its activity by tPA at specific sites of chronic damage may be harmful (86). In addition to protease activity, tPA promotes monocyte adhesion and activates fibroblast intracellular signaling pathways by binding to LRP (99,100). tPA mediates vascular smooth muscle cell contraction via interactions that involve LRP and αvβ3 integrin (101). It also may bind to other cellular receptors such as annexin 2, a plasminogen co-receptor, and the mannose receptor (102).

**uPA**

Sythesized by tubular epithelial cells and secreted apically into the urinary space, the kidney is a rich source of uPA. At sites of damage, uPA also may be produced by inflammatory cells and activated fibroblasts. The primary physiologic role of renal uPA is unknown; it might play a role in nephrolithiasis prevention (103). In response to chronic damage that is induced by UUO, renal uPA mRNA levels and protease activity increase despite significant increases in PAI-1 (61,71). uPA activity has been associated with several proteolytic effects that should decrease fibrosis. Although plasminogen is the preferred uPA substrate, uPA also degrades some matrix proteins, such as fibronectin (104), and it activates latent hepatocyte growth factor (HGF) (105) and membrane-type metalloproteinases (106). HGF has been shown to attenuate fibrosis in several experimental models (107). In experimental pulmonary fibrosis, intratracheal uPA therapy increased active HGF levels and reduced scarring (108). However, we recently found that the severity of inflammation, interstitial myofibroblast infiltration, and renal fibrosis were identical in uPA wild-type and knockout mice after UUO (109).

Although studies in genetically engineered mice have proved to be useful tools for delineating the mechanism of action of PAI-1 in CKD, it needs to be acknowledged that men and mice may not be the same and that only a limited number of kidney disease models have been investigated thus far. Evidence is emerging slowly in favor of the concept that the roles of the serine proteases and their inhibitors are cell specific and distinctly different in the glomerular and tubulointerstitial regions where levels of tPA, uPA, uPAR, and LRPI in particular may differ. For example, in contrast to the results of the studies in the chronic UUO model, in vitro (110) and in vivo studies (36) have shown that glomerular plasmin activity that is generated by plasminogen activators is associated with reduced matrix accumulation. It also remains plausible that undisclosed consequences of gene ablation, such as compensatory upregulation of other genetic programs (e.g., protease nexin-1) may account for some of the findings. Nonetheless, current evidence suggests that biologic effects beyond protease inhibition likely contribute to the striking ability of PAI-1 to promote fibrosis. PAI-1 also influences the recruitment and/or behavior of several cell types that are involved in the fibrogenic response. In particular, although a unique PAI-1 receptor has not been identified, PAI-1 modifies the function of the uPAR and several of its co-receptors.

**Receptor-Dependent PAI-1 Effects**

PAI-1 influences cellular behavior in several ways, often independent of its ability to block plasminogen activation (Figure 5).

**Vitronectin/αvβ3 Interactions**

PAI-1 regulates cellular adhesion and migration via high-affinity interactions with its co-factor vitronectin. The function of PAI-1 in tissue remodeling and fibrosis likely differs in the presence and absence of vitronectin. uPAR and PAI-1 compete for a common binding site in the somatomedin B domain of
vitronectin (2,3,111). This domain is adjacent to an RGD sequence that binds the \( \alpha \beta 3 \) integrin receptor. In the presence of PAI-1, several outcomes are possible. (A) Cells may detach from their vitronectin anchors. PAI-1 has higher affinity than uPAR for the somatomedin B domain of vitronectin; this interaction also may disrupt RGD-integrin binding. uPAR-bearing cells thus are detached from their vitronectin matrix connections and liberated to participate in interactions that may involve uPAR and any of its co-receptors: Integrins, mannose-6-phosphate/insulin growth factor II receptor (Man6P-R), gp130, Endo 180, or the LDL receptor-associated protein (LRP). (B) Alternatively, PAI-1 might bind to cells \( \text{via} \) uPAR-bound uPA. Then this entire complex usually is degraded by LRP-dependent endocytosis, a process that leads to PAI-1 degradation. (C) PAI-1 can interact directly with LRP and thereby direct the movement of cells such as monocytes toward PAI-1. Illustration by Josh Gramling—Gramling Medical Illustration.

**Figure 5: Schematic summary of cellular receptor–dependent effects of PAI-1 that may modulate fibrosis severity.** The extracellular matrix protein vitronectin (VN) anchors cells by binding that occurs between its somatomedin B domain and the urokinase receptor (uPAR). Immediately adjacent to this vitronectin domain is an arginine-glycine-aspartate (RGD) sequence that binds the \( \alpha \beta 3 \) integrin receptor. In the presence of PAI-1, several outcomes are possible. (A) Cells may detach from their vitronectin anchors. PAI-1 has higher affinity than uPAR for the somatomedin B domain of vitronectin; this interaction also may disrupt RGD-integrin binding. uPAR-bearing cells thus are detached from their vitronectin matrix connections and liberated to participate in interactions that may involve uPAR and any of its co-receptors: Integrins, mannose-6-phosphate/insulin growth factor II receptor (Man6P-R), gp130, Endo 180, or the LDL receptor-associated protein (LRP). (B) Alternatively, PAI-1 might bind to cells \( \text{via} \) uPAR-bound uPA. Then this entire complex usually is degraded by LRP-dependent endocytosis, a process that leads to PAI-1 degradation. (C) PAI-1 can interact directly with LRP and thereby direct the movement of cells such as monocytes toward PAI-1. Illustration by Josh Gramling—Gramling Medical Illustration.

**Urokinase Receptor/LDL Receptor–Associated Protein Interactions**

The urokinase receptor (uPAR or CD87), a highly glycosylated 50- to 65-kD protein, binds to both latent and active uPA, providing a unique mechanism to concentrate proteolytic activity in pericellular regions, where it potentially facilitates cell migration (112). The uPAR is expressed by many cells, including monocytes, neutrophils, activated T cells, endothelial cells, glomerular epithelial and mesangial cells, renal tubular epithelial cells, fibroblasts, and myofibroblasts (10,116–120). When PAI-1 binds to receptor-bound uPA, it triggers internalization and degradation of both uPA and PAI-1 (along with uPAR and possibly uPAR co-receptors, \( \text{e.g.} \), integrins) \( \text{via} \) endocytic LRP receptors. This seems to be the primary cellular pathway for PAI-1 degradation. In uPAR–/- mice, renal fibrosis is more severe, possibly due in part to enhanced PAI-1 interstitial accumulation (71). This endocytic mechanism also may account for some of the cell-detachment effects that are orchestrated by the presence of PAI-1 (121).

Although uPAR itself is a nonsignaling receptor that is anchored to the plasma membrane by glycosylphosphatidylinositol, its ectodomain interacts with several co-receptors that confer diverse biologic properties, including fibrotic reactions. Exactly how the presence of PAI-1 might alter uPAR co-receptor activities requires further investigation. Furthermore, recent data suggest that uPA itself may bind directly to some of the co-receptors. Known uPAR co-receptors include several integrins, L-selectin, LRP, the IL-6 gp130 receptor, uPAR-associated protein (or Endo180), and the mannose-6-phosphate/insulin growth factor II receptor (CD222).

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uPAR does not seem to be expressed in normal kidneys, but it is present in several renal disease states, including endotoxemia, acute tubular necrosis, thrombotic microangiopathy, crescentic glomerulonephritis, pyelonephritis, acute and chronic allograft rejection, diabetic nephropathy, and obstructive nephropathy (10,71,118,122–126). Compared with wild-type mice, uPAR−/− mice develop more severe fibrosis after UUO (71); uPAR deficiency does not alter the severity of crescentic glomerulonephritis (29). In addition to uPA, uPAR has two ligands: Vitronectin and high molecular weight kininogen (Hka) (127). Hka and bradykinin are generated by kallikrein-mediated kininogen cleavage. Bradykinin, signaling via its B2 receptor, elicits important antifibrotic effects that may contribute to the renoprotective effects of angiotensin-converted enzyme inhibitors (128,129). Whether specific interactions between Hka and uPAR influence fibrosis severity is not yet clear.

An important feature of CKD is the progressive rarefaction of the interstitial capillary network, which aggravates hypoxia and oxidative stress, thereby contributing to the spiral of worsening renal damage. Angiogenesis failure characterizes the renal interstitial environment in CKD (130). Recent animal studies have reported that administration of proangiogenic factors such as vascular endothelial growth factor or angiopoietin-1 reduce renal fibrosis (131,132). PAI-1 modulates angiogenesis, although its primary in vivo effect remains controversial: Inhibition has been observed in many experimental conditions (133–135). In CKD, PAI-1 accumulates primarily within the interstitial matrix that surrounds interstitial capillaries. Although the specific mechanisms involved remain to be clarified, cellular receptors may play a role as angiogenesis is enhanced in the presence of uPAR (71,136).

Monocyte/Macrophage Chemotactic Receptors

Studies in genetically engineered mice uncovered an apparent relationship between macrophage recruitment and PAI-1 levels. An in vitro chemotaxis experiment suggested that PAI-1 itself might have monocyte chemoattractant properties (61). This effect seems to be dependent on LRP expression (137). Macrophage recruitment that leads to TGF-β production seems to be an important mechanism whereby PAI-1 enhances renal fibrosis (37,61,62). In addition, uPAR can be cleaved by proteases to generate a soluble form that comprises domains 2 and 3. Soluble uPAR triggers monocyte chemotaxis by binding the FPR/L1/LXA4 (formyl-methionyl-leucyl-proline–like receptor/1 lipoxin A4 receptor) (138). How the presence of PAI-1 influences uPAR shedding by proteases remains to be determined.

PAI-1 Genotype: A Risk Factor for CKD?

One of several factors that influence plasma levels in humans is PAI-1 genotype. Higher levels correlate with polymorphic variance in the number of guanine bases (4G versus 5G) in the promoter at position −675. The 5G variant binds the E2F transcription repressor, whereas 4G fails to do so and is associated with higher plasma levels. Therefore, PAI-1 genotype might be a predictor of CKD progression. The PAI-1 4G/4G genotype was linked to increased risk for vascular complications in patients with diabetes, especially when superimposed on the angiotensin-converting enzyme D/D genotype that is associated with increased renin-angiotensin system activity (139). Increased PAI-1 plasma levels and 4G/4G PAI-1 genotype also have been linked to increased risk for chronic allograft dysfunction (140). In patients with lupus nephritis, the 4G/4G PAI-1 genotype has been associated with higher disease activity and more severe necrotizing lesions than those with the 5G genotype, suggesting that PAI-1 influences glomerular proliferative lesions as well as sclerosis (141,142). Although more studies are needed, thus far, PAI-1 genotype seems to be a better predictor of atherosclerosis (including renovascular lesions) and cardiovascular disease than CKD risk (143,144). With rare exception (141), PAI-1 genotype has not been predictive of diabetic nephropathy, perhaps not surprising because so many other PAI-1 agonists are elevated in patients with diabetes (38).

Conclusion

PAI-1 is a multifunctional glycoprotein with impressive fibrosis-promoting effects in the kidney. High renal PAI-1 levels seem to predict a bad long-term outcome, although more rigorous clinical studies still are needed to establish its prognostic predictive value in humans. In lesions that are characterized by the presence of fibrin, such as those that occur in certain glomerular and vascular diseases, inhibition of fibrinolysis closely associates with chronic damage. Switching off PAI-1 has been shown experimentally to prevent CKD progression and may even facilitate its regression. By contrast, in the renal interstitium, where PAI-1 may accumulate as a result of the presence of vitronectin, its primary fibrogenic effects align more closely

![Figure 6. Schematic summary of the primary effects of PAI-1 in chronic kidney disease (CKD). Renal PAI-1 expression can be induced by a number of factors involved in disease pathogenesis. TGF-β and angiotensin II are widely recognized inducers of PAI-1, but many other factors stimulate PAI-1 expression in CKD. The ability of PAI-1 to reduce plasmin activity seems to promote thrombotic and necrotizing glomerular lesions, many of which progress to sclerosis. In other glomerular lesions, PAI-1 inhibits extracellular matrix breakdown. Within the tubulointerstitium, the profibrotic effects of PAI-1 align more closely with its ability to promote migration of monocytes/macrophages, transdifferentiated tubular epithelia and (myo)fibroblasts in particular. Illustration by Josh Gramling—Gramling Medical Illustration.](image-url)
with its ability to facilitate cell migration (monocytes and myofibroblasts in particular), although other potential mechanisms remain to be elucidated (Figure 6). Among the many renoprotective properties of the AngII inhibitors is their ability to suppress PAI-1. Development of selective anti–PAI-1 therapeutic agents is under way, but the ideal antifibrotic agent has not yet been discovered (145). Much still remains to be disclosed about the role of PAI-1 in CKD. For example, will PAI-1 genotype or kidney expression levels prove to be useful predictors of CKD risk in humans? If AngII and TGF-β activities are therapeutically blocked, then can PAI-1 still be synthesized and promote fibrosis? Is PAI-1’s role as an inhibitor of fibrinolysis relevant to CKD pathogenesis? And much remains to be learned about the receptor-dependent biologic effects of PAI-1 that are relevant to renal fibrogenesis and regression.

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