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

Allison A. Eddy* and Agnes B. Fogo[†]

*Children's Hospital and Regional Medical Center, Department of Pediatrics, University of Washington, Seattle, Washington; and [†]Department of Pathology, Vanderbilt University Medical Center, Nashville, Tennessee

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 α-2 antiplasmin 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[a]

Diabetic nephropathy (41,146)
Focal necrotizing glomerulonephritis (32)
Crescentic glomerulonephritis (24–26)
Focal segmental glomerulosclerosis (32,147)
Membranous nephropathy (32,148)
Chronic allograft nephropathy (124,149,150)
Thrombotic microangiopathy (10,24)
Arterionephrosclerosis (41,151)

[a]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 membranous nephropathy (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 tPA 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

Table 2. Manipulation of PAI-1 and serine proteases in experimental kidney diseases[a]

Genetic or Therapeutic Manipulation	Reduced Fibrosis	Increased Fibrosis	No Change in Fibrosis
PAI-1−/−	Crescentic anti-GBM nephritis (28) Streptozotocin-induced diabetes (43,44) *db/db* diabetes (44) 5/6 nephrectomy (55) Protein overload (60) Obstructive nephropathy (61) TGF-β transgenic mice (63)	Passive anti-GBM nephritis (30)	
PAI-1 overexpressing mice		Crescentic anti-GBM nephritis (28) Obstructive nephropathy (62)	
PAI-1 mutant	Anti–Thy-1 nephritis (37)		
PAI-1 neutralizing antibody	LPS-induced endotoxemia (152)		
Plasminogen−/− mice	Obstructive nephropathy (93,95)	Crescentic anti-GBM nephritis (29)	
tPA−/− mice	Obstructive nephropathy (87)	Crescentic anti-GBM nephritis (29)	
tPA recombinant protein	Crescentic anti-GBM nephritis (153) Anti–Thy-1 nephritis (36)		
uPA−/−			Crescentic anti-GBM nephritis (28) Obstructive nephropathy (109)
uPAR−/− mice		Obstructive nephropathy (71)	Crescentic anti-GBM nephritis (28)

[a]*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.

reduces PAI-1 expression and glomerular matrix accumulation and is more effective than either therapy alone (35).

Studies in the β6 knockout mice provide additional insights. The αvβ6 integrin is involved in TGF-β activation. β6−/− mice are protected from fibrosis after unilateral ureteral obstruction (UUO) and do not show increased active TGF-β and PAI-1 like the wild-type mice (50,51). However, PAI-1 expression and fibrosis, but not TGF-β, 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-β–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). *In vitro*, PAI-1−/− podocytes synthesize less collagen in response to AngII than wild-
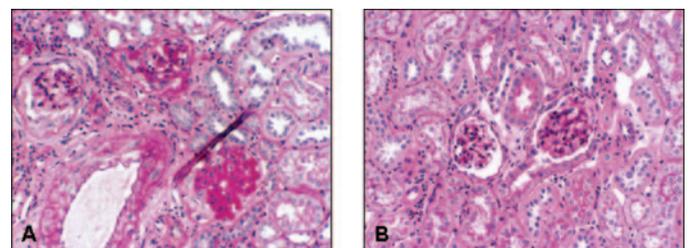

Figure 1. Plasminogen activator inhibitor-1 (PAI-1) deficiency attenuates glomerulosclerosis in mice with remnant kidneys. Ten weeks after sclerosis-prone 129Sv mice underwent 5/6 nephrectomy, glomerulosclerosis and interstitial fibrosis were evident (A). These lesions failed to develop in 129Sv /PAI-1−/−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

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

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.

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.
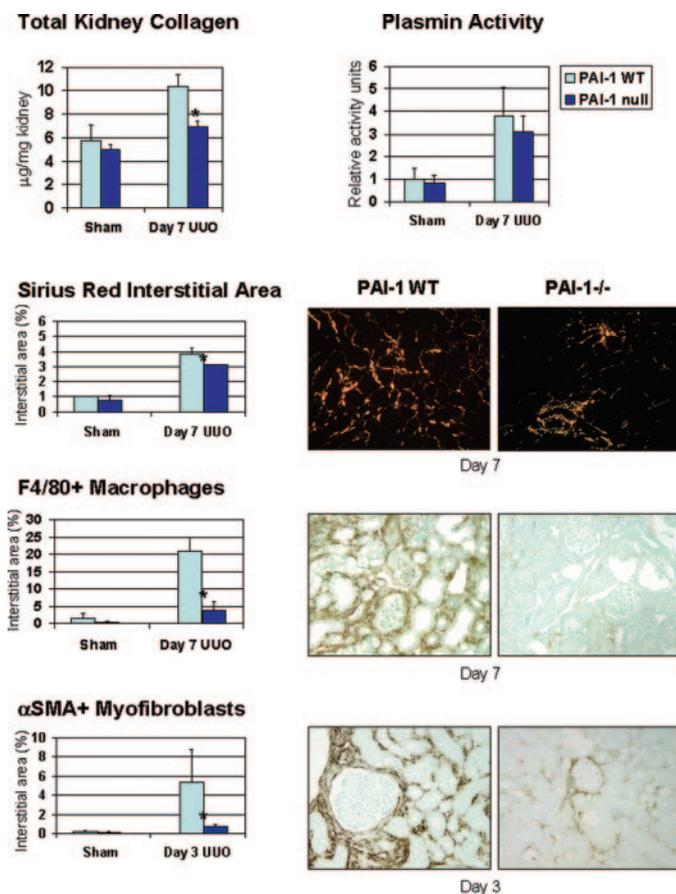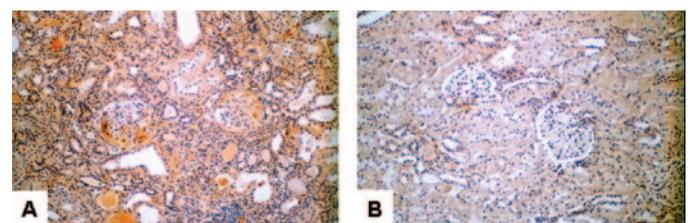

Figure 3. PAI-1 immunostaining in 5/6 nephrectomized rats. At 8 wk after 5/6 nephrectomy, sclerosis was moderately advanced with corresponding increased PAI-1 immunostain in sclerotic glomeruli and in the tubulointerstitium (brown stain; A). Regression of sclerosis was induced by week 12 in some rats 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
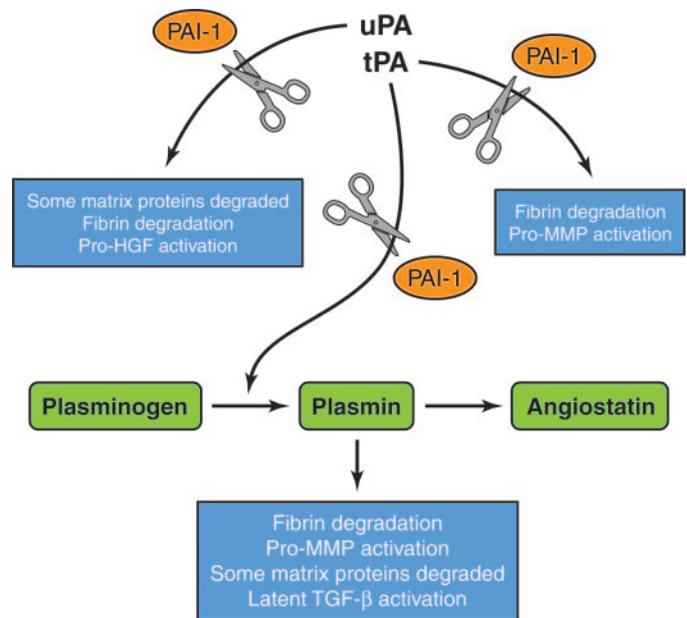
Figure 4. Schematic summary of protease inhibition–dependent extracellular PAI-1 effects that may influence fibrosis severity. The latent zymogen plasminogen is a major substrate for urokinase-type plasminogen activator (uPA) and tissue-type plasminogen activator (tPA), although each serine protease has additional cleavage targets that have been implicated in renal fibrosis. The protease plasmin also cleaves several proteins that are expressed during fibrogenesis. Plasminogen cleavage also may lead to the liberation of kringle domains as angiostatin, an angiogenesis inhibitor. MMP, matrix metalloproteinase; HGF, hepatocyte growth factor. Illustration by Josh Gramling—Gramling Medical Illustration.

matrix-producing transdifferentiated tubular epithelia into the interstitium (87). Furthermore, through interactions with $\alpha v \beta 3$ 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 plasmin(ogen) co-receptor, and the mannose receptor (102).

uPA

Synthesized 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 LRP 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
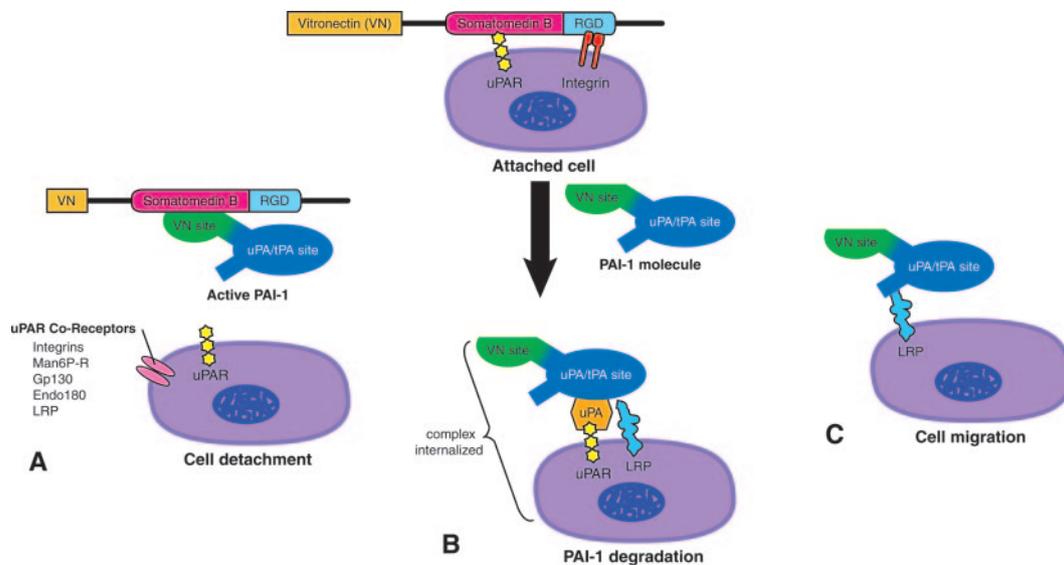
Figure 5. Schematic summary of cellular receptor–dependent effects of PAI-1 that may modulate fibrosis severity. The extracellular matrix protein vitronectin (VN) anchors cells by binding that occurs between its somatomedin B domain and the urokinase receptor (uPAR). Immediately adjacent to this vitronectin domain is an arginine-glycine-aspartate (RGD) sequence that binds the $\alpha v\beta 3$ 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 *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.

vitronectin (2,3,111). This domain is adjacent to an RGD sequence that binds $\alpha v\beta 3$ integrin. PAI-1 has "de-adhesive" actions, as it blocks uPAR–vitronectin interactions and subsequently impairs a cell's ability to bind to $\alpha v\beta 3$ integrins (112,114). Depending on the cellular and matrix milieu within which PAI-1 deposits, PAI-1 may either facilitate or impair cell migration. For example, for several carcinomas, PAI-1 expression predicts a highly invasive and metastatic phenotype (114). When PAI-1 is expressed in early fibrotic lesions that are rich in fibronectin, its ability to detach $\alpha v\beta 3$-bearing cells (*e.g.*, fibroblasts) from vitronectin anchors liberates cells, allowing them to migrate along fibronectin matrices and advance scar formation. From the UUO studies that were performed in PAI-1 engineered mice, PAI-1 levels predicted interstitial myofibroblast density and fibrosis severity: Greater in PAI-1–overexpressing mice and less in PAI-1–deficient mice compared with wild-type mice (61,62). Vitronectin (also known as S protein) also inhibits formation of complement C5b-9 membrane attack complex. Because the terminal complement cascade has been implicated in the progression of proteinuric renal disease, it is plausible PAI-1 might influence this function, although such a role has yet to be demonstrated (115).

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, *e.g.*, integrins) *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).

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-converting 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 oxidant 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 FPRL1/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
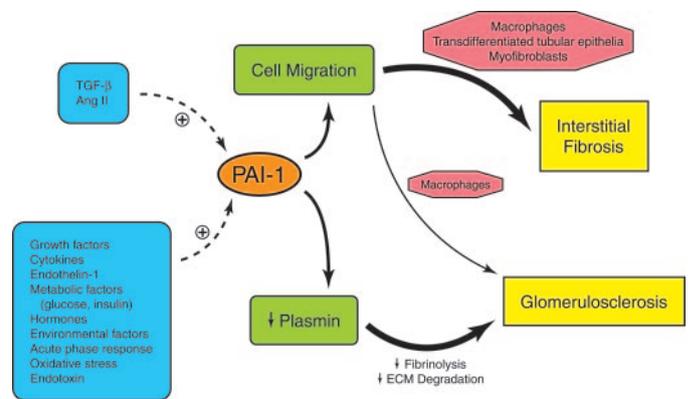

Figure 6. Schematic summary of the primary effects of PAI-1 in chronic kidney disease (CKD). Renal PAI-1 expression can be induced by a number of factors involved in disease pathogenesis. TGF-β and angiotensin II are widely recognized inducers of PAI-1, but many other factors stimulate PAI-1 expression 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.

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

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.

Acknowledgments

References

1. Loskutoff DJ, Edgington TS: An inhibitor of plasminogen activator in rabbit endothelial cells. *J Biol Chem* 256: 4142–4145, 1981

2. Lijnen HR: Pleiotropic functions of plasminogen activator inhibitor-1. *J Thromb Haemost* 3: 35–45, 2005

3. Dellas C, Loskutoff DJ: Historical analysis of PAI-1 from its discovery to its potential role in cell motility and disease. *Thromb Haemost* 93: 631–640, 2005

4. Shik J, Parfrey PS: The clinical epidemiology of cardiovascular disease in chronic kidney disease. *Curr Opin Nephrol Hypertens* 14: 550–557, 2005

5. Menon V, Gul A, Sarnak MJ: Cardiovascular risk factors in chronic kidney disease. *Kidney Int* 68: 1413–1418, 2005

6. Wali RK, Henrich WL: Chronic kidney disease: A risk factor for cardiovascular disease. *Cardiol Clin* 23: 343–362, 2005

7. Vaughan DE: PAI-1 and atherothrombosis. *J Thromb Haemost* 3: 1879–1883, 2005

8. de Boer JP, Abbink JJ, Brouwer MC, Meijer C, Roem D, Voorn GP, Lambers JW, van Mourik JA, Hack CE: PAI-1 synthesis in the human hepatoma cell line HepG2 is increased by cytokines: Evidence that the liver contributes to acute phase behaviour of PAI-1. *Thromb Haemost* 65: 181–185, 1991

9. Pandey M, Loskutoff DJ, Samad F: Molecular mechanisms of tumor necrosis factor-alpha-mediated plasminogen activator inhibitor-1 expression in adipocytes. *FASEB J* 19: 1317–1319, 2005

10. Xu Y, Hagege J, Mougenot B, Sraer JD, Ronne E, Rondeau E: Different expression of the plasminogen activation system in renal thrombotic microangiopathy and the normal human kidney. *Kidney Int* 50: 2011–2019, 1996

11. Chandler W, Jelacic S, Boster D, Ciol M, Williams G, Watkins S, Igarashi T, Tarr P: Prothrombotic coagulation abnormalities during *Escherichia coli* 0157:H7 infections. *N Engl J Med* 346: 23–32, 2002

12. Nevard CH, Jurd KM, Lane DA, Philippou H, Haycock GB, Hunt BJ: Activation of coagulation and fibrinolysis in childhood diarrhoea-associated haemolytic uraemic syndrome. *Thromb Haemost* 78: 1450–1455, 1997

13. Martin AA, Woolven BL, Harris SJ, Keeley SR, Adams LD, Jureidini KF, Henning PH: Plasminogen activator inhibitor type-1 and interleukin-6 in haemolytic uraemic syndrome. *J Paediatr Child Health* 36: 327–331, 2000

14. Bergstein JM, Riley M, Bang NU: Role of plasminogen-activator inhibitor type 1 in the pathogenesis and outcome of the hemolytic uremic syndrome. *N Engl J Med* 327: 755–759, 1992

15. Chant ID, Milford DV, Rose PE: Plasminogen activator inhibitor activity in diarrhoea-associated haemolytic uraemic syndrome. *QJM* 87: 737–740, 1994

16. Van Geet C, Proesmans W, Arnout J, Vermylen J, Declerck PJ: Activation of both coagulation and fibrinolysis in childhood hemolytic uremic syndrome. *Kidney Int* 54: 1324–1330, 1998

17. Taylor CM, Williams JM, Lote CJ, Howie AJ, Thewles A, Wood JA, Milford DV, Raafat F, Chant I, Rose PE: A laboratory model of toxin-induced hemolytic uremic syndrome. *Kidney Int* 55: 1367–1374, 1999

18. Sugatani J, Igarashi T, Munakata M, Komiyama Y, Takahashi H, Komiyama N, Maeda T, Takeda T, Miwa M: Activation of coagulation in C57BL/6 mice given verotoxin 2 (VT2) and the effect of co-administration of LPS with VT2. *Thromb Res* 100: 61–72, 2000

19. Estelles A, Gilabert J, Grancha S, Yamamoto K, Thinnes T, Espana F, Aznar J, Loskutoff DJ: Abnormal expression of type 1 plasminogen activator inhibitor and tissue factor in severe preeclampsia. *Thromb Haemost* 79: 500–508, 1998

20. Matsushita M, Yamamoto T, Nishioka K: Plasminogen activator inhibitor-1 is elevated, but not essential, in the development of bleomycin-induced murine scleroderma. *Clin Exp Immunol* 139: 429–438, 2005

21. Bandinelli F, Bartoli F, Perfetto E, Del Rosso A, Moggi-Pignone A, Guiducci S, Cinelli M, Fatini C, Generini S, Gabrielli A, Giacomelli R, Maddali Bongi S, Abbate R, Del Rosso M, Matucci Cerinic M: The fibrinolytic system components are increased in systemic sclerosis and modulated by Alprostadil (alpha1 ciclodestryn). *Clin Exp Rheumatol* 23: 671–677, 2005

22. Oikawa T, Freeman M, Lo W, Vaughan DE, Fogo A: Modulation of plasminogen activator inhibitor-1 in vivo: A new mechanism for the anti-fibrotic effect of renin-angiotensin inhibition. *Kidney Int* 51: 164–172, 1997

23. Brown NJ, Nakamura S, Ma L, Nakamura I, Donnert E, Freeman M, Vaughan DE, Fogo AB: Aldosterone modulates plasminogen activator inhibitor-1 and glomerulosclerosis in vivo. *Kidney Int* 58: 1219–1227, 2000

24. Rondeau E, Mougenot B, Lacave R, Peraldi MN, Kruithof EK, Sraer JD: Plasminogen activator inhibitor 1 in renal fibrin deposits of human nephropathies. *Clin Nephrol* 33: 55–60, 1990

25. Grandaliano G, Gesualdo L, Ranieri E, Monno R, Schena FP: Tissue factor, plasminogen activator inhibitor-1, and thrombin receptor expression in human crescentic glomerulonephritis. *Am J Kidney Dis* 35: 726–738, 2000

26. Lee HS, Park SY, Moon KC, Hong HK, Song CY, Hong SY: mRNA expression of urokinase and plasminogen activator inhibitor-1 in human crescentic glomerulonephritis. *Histopathology* 39: 203–209, 2001

27. Malliaros J, Holdsworth SR, Wojta J, Erlich J, Tipping PG: Glomerular fibrinolytic activity in anti-GBM glomerulonephritis in rabbits. *Kidney Int* 44: 557–564, 1993

28. Kitching AR, Kong YZ, Huang XR, Davenport P, Edgtton KL, Carmeliet P, Holdsworth SR, Tipping PG: Plasminogen activator inhibitor-1 is a significant determinant of renal injury in experimental crescentic glomerulonephritis. *J Am Soc Nephrol* 14: 1487–1495, 2003

29. Kitching AR, Holdsworth SR, Ploplis VA, Plow EF, Collen D, Carmeliet P, Tipping PG: Plasminogen and plasminogen activators protect against renal injury in crescentic glomerulonephritis. *J Exp Med* 185: 963–968, 1997

30. Hertig A, Berrou J, Allory Y, Breton L, Commo F, Costa De Beauregard MA, Carmeliet P, Rondeau E: Type 1 plasminogen activator inhibitor deficiency aggravates the course of experimental glomerulonephritis through overactivation of transforming growth factor beta. *FASEB J* 17: 1904–1906, 2003

31. Rerolle J-P, Hertig A, Nguyen G, Sraer J-D, Rondeau E: Plasminogen activator inhibitor type 1 is a potential target in renal fibrogenesis. *Kidney Int* 58: 1841–1850, 2000

32. Hamano K, Iwano M, Akai Y, Sato H, Kubo A, Nishitani Y, Uyama H, Yoshida Y, Miyazaki M, Shiiki H, Kohno S, Dohi K: Expression of glomerular plasminogen activator inhibitor type I in glomerulonephritis. *Am J Kidney Dis* 39: 695–705, 2002

33. Barnes JL, Mitchell RJ, Torres ES: Expression of plasminogen activator-inhibitor-1 (PAI-1) during cellular remodeling in proliferative glomerulonephritis in the rat. *J Histochem Cytochem* 43: 895–905, 1995

34. Peters H, Wang Y, Loof T, Martini S, Kron S, Kramer S, Neumayer HH: Expression and activity of soluble guanylate cyclase in injury and repair of anti-thy1 glomerulonephritis. *Kidney Int* 66: 2224–2236, 2004

35. Yu L, Border WA, Anderson I, McCourt M, Huang Y, Noble NA: Combining TGF-beta inhibition and angiotensin II blockade results in enhanced antifibrotic effect. *Kidney Int* 66: 1774–1784, 2004

36. Haraguchi M, Border WA, Huang Y, Noble NA: t-PA promotes glomerular plasmin generation and matrix degradation in experimental glomerulonephritis. *Kidney Int* 59: 2146–2155, 2001

37. Huang Y, Haraguchi M, Lawrence DA, Border WA, Yu L, Noble NA: A mutant, noninhibitory plasminogen activator inhibitor type 1 decreases matrix accumulation in experimental glomerulonephritis. *J Clin Invest* 112: 379–388, 2003

38. Eddy AA: Plasminogen activator inhibitor-1 and the kidney. *Am J Physiol Renal Physiol* 283: F209–F220, 2002

39. Fogo AB: The role of angiotensin II and plasminogen activator inhibitor-1 in progressive glomerulosclerosis. *Am J Kidney Dis* 35: 179–188, 2000

40. Brown NJ, Vaughan DE, Fogo AB: The renin-angiotensin-aldosterone system and fibrinolysis in progressive renal disease. *Semin Nephrol* 22: 399–406, 2002

41. Paueksakon P, Revelo MP, Ma LJ, Marcantoni C, Fogo AB: Microangiopathic injury and augmented PAI-1 in human diabetic nephropathy. *Kidney Int* 61: 2142–2148, 2002

42. Bagby SP: Obesity-initiated metabolic syndrome and the kidney: A recipe for chronic kidney disease? *J Am Soc Nephrol* 15: 2775–2791, 2004

43. Nicholas SB, Aguiniga E, Ren Y, Kim J, Wong J, Govindarajan N, Noda M, Wang W, Kawano Y, Collins A, Hsueh WA: Plasminogen activator inhibitor-1 deficiency retards diabetic nephropathy. *Kidney Int* 67: 1297–1307, 2005

44. Collins SJ, Alexander SL, Lopez-Guisa JM, Cai X, Maruvada M, Chua SC, Zhang G, Okamura DM, Matsuo S, Eddy AA: Plasminogen activator inhibitor-1 deficiency has renal benefits but some adverse systemic consequences in diabetic mice. *Nephron Exp Nephrol* 104: 23–34, 2006

45. Fujisawa G, Okada K, Muto S, Fujita N, Itabashi N, Kusano E, Ishibashi S: Spironolactone prevents early renal injury in streptozotocin-induced diabetic rats. *Kidney Int* 66: 1493–1502, 2004

46. Ma LJ, Mao SL, Taylor KL, Kanjanabuch T, Guan Y, Zhang Y, Brown NJ, Swift LL, McGuinness OP, Wasserman DH, Vaughan DE, Fogo AB: Prevention of obesity and insulin resistance in mice lacking plasminogen activator inhibitor 1. *Diabetes* 53: 336–346, 2004

47. Liang X, Kanjanabuch T, Mao SL, Hao CM, Tang YW, Declerck PJ, Hasty AH, Wasserman DH, Fogo AB, Ma LJ: Plasminogen activator inhibitor-1 modulates adipocyte differentiation. *Am J Physiol Endocrinol Metab* 290: E103–E113, 2006

48. Takeshita K, Yamamoto K, Ito M, Kondo T, Matsushita T, Hirai M, Kojima T, Nishimura M, Nabeshima Y, Loskutoff DJ, Saito H, Murohara T: Increased expression of plasminogen activator inhibitor-1 with fibrin deposition in a murine model of aging, "Klotho" mouse. *Semin Thromb Haemost* 28: 545–554, 2002

49. Huang Y, Wongamorntham S, Kasting J, McQuillan D, Owens RT, Yu L, Noble NA, Border W: Renin increases mesangial cell transforming growth factor-beta1 and matrix proteins through receptor-mediated, angiotensin II-independent mechanisms. *Kidney Int* 69: 105–113, 2006

50. Munger JS, Huang X, Kawakatsu H, Griffiths MJ, Dalton SL, Wu J, Pittet JF, Kaminski N, Garat C, Matthay MA, Rifkin DB, Sheppard D: The integrin alpha v beta 6 binds and activates latent TGF beta 1: A mechanism for regulating pulmonary inflammation and fibrosis. *Cell* 96: 319–328, 1999

51. Ma LJ, Yang H, Gaspert A, Carlesso G, Barty MM, Davidson JM, Sheppard D, Fogo AB: Transforming growth factor-beta-dependent and -independent pathways of induction of tubulointerstitial fibrosis in beta6(−/−) mice. *Am J Pathol* 163: 1261–1273, 2003

52. Brown NJ, Kim KS, Chen YQ, Blevins LS, Nadeau JH, Meranze SG, Vaughan DE: Synergistic effect of adrenal steroids and angiotensin II on plasminogen activator inhibitor-1 production. *J Clin Endocrinol Metab* 85: 336–344, 2000

53. Nakamura S, Nakamura I, Ma L, Vaughan DE, Fogo AB: Plasminogen activator inhibitor-1 expression is regulated by the angiotensin type 1 receptor in vivo. *Kidney Int* 58: 251–259, 2000

54. Ishikawa A, Ohta N, Ozono S, Kawabe K, Kitamura T: Inhibition of plasminogen activator inhibitor-1 by angiotensin II receptor blockers on cyclosporine-treated renal allograft recipients. *Transplant Proc* 37: 994–996, 2005

55. Ma LJ, Naito T, Han JY, Fogo AB: Plasminogen activator inhibitor-1 (PAI-1) deficiency prevents the development of glomerulosclerosis in the subtotal nephrectomy (5/6 Nx) in the mouse [Abstract]. *J Am Soc Nephrol* 16: 653A, 2005

56. Naito T, Rodriguez G, Borza D-B, Ma L, Pozzi A, Fogo AB:

Podocyte PAI-1 affects angiotensin II (AngII)-induced ECM accumulation [Abstract]. *Lab Invest* 86: 264A, 2006

57. Weisberg AD, Albornoz F, Griffin JP, Crandall DL, Elokdah H, Fogo AB, Vaughan DE, Brown NJ: Pharmacological inhibition and genetic deficiency of plasminogen activator inhibitor-1 attenuates angiotensin II/salt-induced aortic remodeling. *Arterioscler Thromb Vasc Biol* 25: 365–371, 2005

58. Kaikita K, Fogo AB, Ma L, Schoenhard JA, Brown NJ, Vaughan DE: Plasminogen activator inhibitor-1 deficiency prevents hypertension and vascular fibrosis in response to long-term nitric oxide synthase inhibition. *Circulation* 104: 839–844, 2001

59. Kaikita K, Schoenhard JA, Painter CA, Ripley RT, Brown NJ, Fogo AB, Vaughan DE: Potential roles of plasminogen activator system in coronary vascular remodeling induced by long-term nitric oxide synthase inhibition. *J Mol Cell Cardiol* 34: 617–627, 2002

60. Oda T, Kim H, Wing D, Lopez-Guisa J, Jernigan S, Eddy AA: Effects of genetic PAI-1 deficiency in mice with protein-overload proteinuria [Abstract]. *J Am Soc Nephrol* 10: 578A, 1999

61. Oda T, Jung YO, Kim H, Cai X, Lopez-Guisa J, Ikeda Y, Eddy AA: PAI-1 deficiency attenuates the fibrogenic response to ureteral obstruction. *Kidney Int* 30: 587–596, 2001

62. Matsuo S, Lopez-Guisa JM, Cai X, Okamura DM, Alpers CE, Bumgarner RE, Peters MA, Zhang G, Eddy AA: Multifunctionality of PAI-1 in fibrogenesis: Evidence from obstructive nephropathy in PAI-1-overexpressing mice. *Kidney Int* 67: 2221–2238, 2005

63. Krag S, Danielsen CC, Carmeliet P, Nyengaard J, Wogensen L: Plasminogen activator inhibitor-1 gene deficiency attenuates TGF-beta1-induced kidney disease. *Kidney Int* 68: 2651–2666, 2005

64. Fioretto P, Steffes MW, Sutherland DE, Goetz FC, Mauer M: Reversal of lesions of diabetic nephropathy after pancreas transplantation. *N Engl J Med* 339: 69–75, 1998

65. Kriz W, Hahnel B, Hosser H, Ostendorf T, Gaertner S, Kranzlin B, Gretz N, Shimizu F, Floege J: Pathways to recovery and loss of nephrons in anti-Thy-1 nephritis. *J Am Soc Nephrol* 14: 1904–1926, 2003

66. Aldigier JC, Kanjanbuch T, Ma LJ, Brown NJ, Fogo AB: Regression of existing glomerulosclerosis by inhibition of aldosterone. *J Am Soc Nephrol* 16: 3306–3314, 2005

67. Ma LJ, Nakamura S, Aldigier JC, Rossini M, Yang H, Liang X, Nakamura I, Marcantoni C, Fogo AB: Regression of glomerulosclerosis with high-dose angiotensin inhibition is linked to decreased plasminogen activator inhibitor-1. *J Am Soc Nephrol* 16: 966–976, 2005

68. Ma LJ, Nakamura S, Whitsitt JS, Marcantoni C, Davidson JM, Fogo AB: Regression of sclerosis in aging by an angiotensin inhibition-induced decrease in PAI-1. *Kidney Int* 58: 2425–2436, 2000

69. Eddy A: Can renal fibrosis be reversed? *Pediatr Nephrol* 20: 1369–1375, 2005

70. Cochrane AL, Kett MM, Samuel CS, Campanale NV, Anderson WP, Hume DA, Little MH, Bertram JF, Ricardo SD: Renal structural and functional repair in a mouse model of reversal of ureteral obstruction. *J Am Soc Nephrol* 16: 3623–3630, 2005

71. Zhang G, Kim H, Cai X, Lopez-Guisa J, Carmeliet P, Eddy A: Urokinase receptor modulates cellular and angiogenic

responses in obstructive uropathy. *J Am Soc Nephrol* 14: 1254–1271, 2003

72. Duymelinck C, Dauwe SEH, De Greef KEJ, Ysebaert DK, Verpooten GA, De Broe ME: TIMP-1 gene expression and PAI-1 antigen after unilateral ureteral obstruction in the adult male rat. *Kidney Int* 58: 1186–1201, 2000

73. Herz J, Strickland DK: LRP: A multifunctional scavenger and signaling receptor. *J Clin Invest* 108: 779–784, 2001

74. Wind T, Hansen M, Jensen JK, Andreasen PA: The molecular basis for anti-proteolytic and non-proteolytic functions of plasminogen activator inhibitor type-1: Roles of the reactive centre loop, the shutter region, the flexible joint region and the small serpin fragment. *Biol Chem* 383: 21–36, 2002

75. Nakakuki T, Ito M, Iwasaki H, Kureishi Y, Okamoto R, Moriki N, Kongo M, Kato S, Yamada N, Isaka N, Nakano T: Rho/Rho-kinase pathway contributes to C-reactive protein-induced plasminogen activator inhibitor-1 expression in endothelial cells. *Arterioscler Thromb Vasc Biol* 25: 2088–2093, 2005

76. Al-Nedawi KN, Czyz M, Bednarek R, Szemraj J, Swiatkowska M, Cierniewska-Cieslak A, Wyczolkowska J, Cierniewski CS: Thymosin beta 4 induces the synthesis of plasminogen activator inhibitor 1 in cultured endothelial cells and increases its extracellular expression. *Blood* 103: 1319–1324, 2004

77. Pontrelli P, Ursi M, Ranieri E, Capobianco C, Schena FP, Gesualdo L, Grandaliano G: CD40L proinflammatory and profibrotic effects on proximal tubular epithelial cells: Role of NF-kappaB and lyn. *J Am Soc Nephrol* 17: 627–636, 2006

78. Jiang T, Wang Z, Proctor G, Moskowitz S, Liebman SE, Rogers T, Lucia MS, Li J, Levi M: Diet-induced obesity in C57BL/6J mice causes increased renal lipid accumulation and glomerulosclerosis via a sterol regulatory element-binding protein-1c-dependent pathway. *J Biol Chem* 280: 32317–32325, 2005

79. Kishida M, Urakaze M, Takata M, Nobata Y, Yamamoto N, Temaru R, Sato A, Yamazaki K, Nakamura N, Kobayashi M: PGE1 inhibits the expression of PAI-1 mRNA induced by TNF-alpha in human mesangial cells. *Exp Clin Endocrinol Diabetes* 113: 365–371, 2005

80. Ruth Wu-Wong J, Nakane M, Ma J, Cook AL: Vitamin D analogs downregulate plasminogen activator inhibitor-1 in human coronary artery smooth muscle cells. *J Thromb Haemost* 3: 1545–1546, 2005

81. Romer J, Bugge TH, Pyke C, Lund LR, Flick MJ, Degen JL, Dano K: Impaired wound healing in mice with a disrupted plasminogen gene. *Nat Med* 2: 287–292, 1996

82. Drew AF, Tucker HL, Liu H, Witte DP, Degen JL, Tipping PG: Crescentic glomerulonephritis is diminished in fibrinogen-deficient mice. *Am J Physiol Renal Physiol* 281: F1157–F1163, 2001

83. Ploplis VA, Wilberding J, McLennan L, Liang Z, Cornelissen I, DeFord ME, Rosen ED, Castellino FJ: A total fibrinogen deficiency is compatible with the development of pulmonary fibrosis in mice. *Am J Pathol* 157: 703–708, 2000

84. Hattori N, Degen JL, Sisson TH, Liu H, Moore BB, Pandrangi RG, Simon RH, Drew AF: Bleomycin-induced pulmonary fibrosis in fibrinogen-null mice. *J Clin Invest* 106: 1341–1350, 2000

85. Nagase H: Activation mechanisms of matrix metalloproteinases. *Biol Chem* 378: 151–160, 1997

86. Kim H, Oda T, Lopez-Guisa J, Wing D, Edwards DR, Soloway PD, Eddy AA: TIMP-1 deficiency does not attenuate interstitial fibrosis in obstructive nephropathy. *J Am Soc Nephrol* 12: 736–748, 2001

87. Yang J, Shultz RW, Mars WM, Wegner RE, Li Y, Dai C, Nejak K, Liu Y: Disruption of tissue-type plasminogen activator gene in mice reduces renal interstitial fibrosis in obstructive nephropathy. *J Clin Invest* 110: 1525–1538, 2002

88. Brooks PC, Stromblad S, Sanders LC, von Schalscha TL, Aimes RT, Stetler-Stevenson WG, Quigley JP, Cheresh DA: Localization of matrix metalloproteinase MMP-2 to the surface of invasive cells by interaction with integrin alpha v beta 3. *Cell* 85: 683–693, 1996

89. Cheng S, Pollock AS, Mahimkar R, Olson JL, Lovett DH: Matrix metalloproteinase 2 and basement membrane integrity: A unifying mechanism for progressive renal injury. *FASEB J* 20:1898–1900, 2006

90. Kasai S, Arimura H, Nishida M, Suyama T: Primary structure of single-chain pro-urokinase. *J Biol Chem* 260: 12382–12389, 1985

91. Huang Y, Border WA, Lawrence DA, Noble NA: Noninhibitory PAI-1 enhances plasmin-mediated matrix degradation both in vitro and in experimental nephritis. *Kidney Int* 70: 512–522, 2006

92. Lyons RM, Gentry LE, Purchio AF, Moses HL: Mechanism of activation of latent recombinant transforming growth factor beta 1 by plasmin. *J Cell Biol* 110: 1361–1367, 1990

93. Zhang G, Collins S, Cai X, Lopez-Guisa J, Eddy A: Plasmin(ogen) promotes renal interstitial fibrosis by inducing epithelial-to-mesenchymal transition: Role of plasminogen activate signals [Abstract]. *J Am Soc Nephrol* 15: 36A, 2004

94. Mitchell JW, Baik N, Castellino FJ, Miles LA: Plasminogen inhibits TNFalpha-induced apoptosis in monocytes. *Blood* 107: 4383–4390, 2006

95. Edgtton KL, Gow RM, Kelly DJ, Carmeliet P, Kitching AR: Plasmin is not protective in experimental renal interstitial fibrosis. *Kidney Int* 66: 68–76, 2004

96. Swaisgood CM, French EL, Noga C, Simon RH, Ploplis VA: The development of bleomycin-induced pulmonary fibrosis in mice deficient for components of the fibrinolytic system. *Am J Pathol* 157: 177–187, 2000

97. Zhang SX, Wang JJ, Lu K, Mott R, Longeras R, Ma JX: Therapeutic potential of angiostatin in diabetic nephropathy. *J Am Soc Nephrol* 17: 475–486, 2006

98. Menoud P-A, Sappino N, Boudal-Khoshbeen M, Vassalli J-D, Sappino A-P: The kidney is a major site of a2-antiplasmin production. *J Clin Invest* 97: 2478–2484, 1996

99. Cao C, Lawrence DA, Li Y, Von Arnim CA, Herz J, Su EJ, Makarova A, Hyman BT, Strickland DK, Zhang L: Endocytic receptor LRP together with tPA and PAI-1 coordinates Mac-1-dependent macrophage migration. *EMBO J* 25: 1860–1870, 2006

100. Hu K, Yang J, Tanaka S, Gonias SL, Mars WM, Liu Y: Tissue-type plasminogen activator acts as a cytokine that triggers intracellular signal transduction and induces matrix metalloproteinase-9 gene expression. *J Biol Chem* 281: 2120–2127, 2006

101. Akkawi S, Nassar T, Tarshis M, Cines DB, Higazi AA: LRP and alphavbeta3 mediate tPA-activation of smooth muscle cells. *Am J Physiol Heart Circ Physiol* 291: H1351–H1359, 2006

102. Rescher U, Gerke V: Annexins: Unique membrane binding proteins with diverse functions. *J Cell Sci* 117: 2631–2639, 2004

103. du Toit PJ, Van Aswegen CH, Steinmann CM, Klue L, Du Plessis DJ: Does urokinase play a role in renal stone formation? *Med Hypotheses* 49: 57–59, 1997

104. Gold LI, Schwimmer R, Quigley JP: Human plasma fibronectin as a substrate for human urokinase. *Biochem J* 262: 529–534, 1989

105. Naldini L, Vigna E, Bardelli A, Follenzi A, Galimi F, Comoglio PM: Biological activation of pro-HGF (hepatocyte growth factor) by urokinase is controlled by a stoichiometric reaction. *J Biol Chem* 270: 603–611, 1995

106. Kazes I, Delarue F, Hagege J, Bouzhir-Sima L, Rondeau E, Sraer JD, Nguyen G: Soluble latent membrane-type 1 matrix metalloprotease secreted by human mesangial cells is activated by urokinase. *Kidney Int* 54: 1976–1984, 1998

107. Liu Y: Hepatocyte growth factor in kidney fibrosis: Therapeutic potential and mechanisms of action. *Am J Physiol Renal Physiol* 287: F7–F16, 2004

108. Hattori N, Mizuno S, Yoshida Y, Chin K, Mishima M, Sisson TH, Simon RH, Nakamura T, Miyake M: The plasminogen activation system reduces fibrosis in the lung by a hepatocyte growth factor-dependent mechanism. *Am J Pathol* 164: 1091–1098, 2004

109. Yamaguchi I, Cai X, Collins SJ, Lopez-Guisa JM, Okamura DM, Eddy AA: Endogenous urokinase plasminogen activator, unlike its receptor, does not affect renal interstitial fibrosis in mice with unilateral ureteral obstruction [Abstract]. *J Am Soc Nephrol* 16: 426A, 2005

110. Baricos WH, Cortez SL, El-Dahr S, Schnaper HW: ECM degradation by cultured human mesangial cells is mediated by a PA/plasmin/MMP-2 cascade. *Kidney Int* 47: 1039–1047, 1995

111. Declerck PJ, De Mol M, Alessi MC, Baudner S, Paques EP, Preissner KT, Muller-Berghaus G, Collen D: Purification and characterization of a plasminogen activator inhibitor 1 binding protein from human plasma. Identification as a multimeric form of S protein (vitronectin). *J Biol Chem* 263: 15454–15461, 1988

112. Preissner KT, Kanse SM, May AE: Urokinase receptor: A molecular organizer in cellular communication. *Curr Opin Cell Biol* 12: 621–628, 2000

113. Kjoller L: The urokinase plasminogen activator receptor in the regulation of the actin cytoskeleton and cell motility. *Biol Chem* 383: 5–19, 2002

114. Bajou K, Noel A, Gerard RD, Masson V, Brunner N, Holst-Hansen C, Skobe M, Fusenig NE, Carmeliet P, Collen D, Foidart JM: Absence of host plasminogen activator inhibitor 1 prevents cancer invasion and vascularization. *Nat Med* 4: 923–928, 1998

115. Nangaku M, Pippin J, Couser WG: C6 mediates chronic progression of tubulointerstitial damage in rats with remnant kidneys. *J Am Soc Nephrol* 13: 928–936, 2002

116. Rondeau E, Ochi S, Lacave R, He CJ, Medcalf R, Delarue F, Sraer JD: Urokinase synthesis and binding by glomerular epithelial cells in culture. *Kidney Int* 36: 593–600, 1989

117. Nguyen G, Li X-M, Peraldi M-N, Zacharias U, Hagege J, Rondeau E, Sraer J-D: Receptor binding and degradation of urokinase-type plasminogen activator by human mesangial cells. *Kidney Int* 46: 208–215, 1994

118. Almus-Jacobs F, Varki N, Sawdey MS, Loskutoff DJ: En-

dotoxin stimulates expression of the murine urokinase receptor gene in vivo. *Am J Pathol* 147: 688–698, 1995

119. Shetty S, Kumar A, Johnson AR, Pueblitz S, Holiday D, Raghu G, Idell S: Differential expression of the urokinase receptor in fibroblasts from normal and fibrotic human lungs. *Am J Respir Cell Mol Biol* 15: 78–87, 1996

120. Wagner SN, Atkinson MJ, Wagner C, Hofler H, Schmitt M, Wilhelm O: Sites of urokinase-type plasminogen activator expression and distribution of its receptor in the normal human kidney. *Histochem Cell Biol* 105: 53–60, 1996

121. Czekay RP, Aertgeerts K, Curriden SA, Loskutoff DJ: Plasminogen activator inhibitor-1 detaches cells from extracellular matrices by inactivating integrins. *J Cell Biol* 160: 781–791, 2003

122. Florquin S, van den Berg JG, Olszyna DP, Claessen N, Opal SM, Weening JJ, van der Poll T: Release of urokinase plasminogen activator receptor during urosepsis and endotoxemia. *Kidney Int* 59: 2054–2061, 2001

123. Xu Y, Berrou J, Chen X, Fouqueray B, Callard P, Sraer JD, Rondeau E: Induction of urokinase receptor expression in nephrotoxic nephritis. *Exp Nephrol* 9: 397–404, 2001

124. Tang WH, Friess H, di Mola FF, Schilling M, Maurer C, Graber HU, Dervenis C, Zimmermann A, Buchler MW: Activation of the serine proteinase system in chronic kidney rejection. *Transplantation* 65: 1628–1634, 1998

125. Kenichi M, Masanobu M, Takehiko K, Shoko T, Akira F, Katsushige A, Takashi H, Yoshiyuki O, Shigeru K: Renal synthesis of urokinase type-plasminogen activator, its receptor, and plasminogen activator inhibitor-1 in diabetic nephropathy in rats: Modulation by angiotensin-converting-enzyme inhibitor. *J Lab Clin Med* 144: 69–77, 2004

126. Roelofs JJ, Rowshani AT, van den Berg JG, Claessen N, Aten J, ten Berge IJ, Weening JJ, Florquin S: Expression of urokinase plasminogen activator and its receptor during acute renal allograft rejection. *Kidney Int* 64: 1845–1853, 2003

127. Colman R: Contact activation pathway: Inflammation, fibrinolytic, anticoagulant, antiadhesive and angiogenic activities. In *Hemostasis and Thrombosis. Basic Principles and Clinical Practice*, edited by Colman R, Hirsh J, Marder V, Clowes A, George J, Philadelphia, Lippincott Williams & Wilkins, 2001, pp 103–121

128. Schanstra JP, Neau E, Drogoz P, Arevalo Gomez MA, Lopez Novoa JM, Calise D, Pecher C, Bader M, Girolami JP, Bascands JL: In vivo bradykinin B2 receptor activation reduces renal fibrosis. *J Clin Invest* 110: 371–379, 2002

129. Okada H, Watanabe Y, Kikuta T, Kobayashi T, Kanno Y, Sugaya T, Suzuki H: Bradykinin decreases plasminogen activator inhibitor-1 expression and facilitates matrix degradation in the renal tubulointerstitium under angiotensin-converting enzyme blockade. *J Am Soc Nephrol* 15: 2404–2413, 2004

130. Nangaku M: Chronic hypoxia and tubulointerstitial injury: A final common pathway to end-stage renal failure. *J Am Soc Nephrol* 17: 17–25, 2006

131. Kang DH, Hughes J, Mazzali M, Schreiner GF, Johnson RJ: Impaired angiogenesis in the remnant kidney model: II. Vascular endothelial growth factor administration reduces renal fibrosis and stabilizes renal function. *J Am Soc Nephrol* 12: 1448–1457, 2001

132. Kim W, Moon S-O, Lee SY, Jang KY, Cho C-H, Koh GY, Choi K-S, Yoon K-H, Sung MJ, Kim DH, Lee S, Kang KP,

Park SK: COMP-angiopoietin-1 ameliorates renal fibrosis in a unilateral ureteral obstruction model. *J Am Soc Nephrol* 17: 2474–2483, 2006

133. Brodsky S, Chen J, Lee A, Akassoglou K, Norman J, Goligorsky MS: Plasmin-dependent and -independent effects of plasminogen activators and inhibitor-1 on ex vivo angiogenesis. *Am J Physiol Heart Circ Physiol* 281: H1784–H1792, 2001

134. Stefansson S, Petitclerc E, Wong MK, McMahon GA, Brooks PC, Lawrence DA: Inhibition of angiogenesis in vivo by plasminogen activator inhibitor-1. *J Biol Chem* 276: 8135–8141, 2001

135. Balsara RD, Castellino FJ, Ploplis VA: A novel function of plasminogen activator inhibitor-1 in modulation of the AKT pathway in wild-type and plasminogen activator inhibitor-1-deficient endothelial cells. *J Biol Chem* 281: 22527–22536, 2006

136. Schnaper HW, Barnathan ES, Mazar A, Maheshwari S, Ellis S, Cortez SL, Baricos WH, Kleinman HK: Plasminogen activators augment endothelial cell organization in vitro by two distinct pathways. *J Cell Physiol* 165: 107–118, 1995

137. Degryse B, Neels JG, Czekay RP, Aertgeerts K, Kamikubo Y, Loskutoff DJ: The low density lipoprotein receptor-related protein is a motogenic receptor for plasminogen activator inhibitor-1. *J Biol Chem* 279: 22595–22604, 2004

138. Resnati M, Pallavicini I, Wang JM, Oppenheim J, Serhan CN, Romano M, Blasi F: The fibrinolytic receptor for urokinase activates the G protein-coupled chemotactic receptor FPRL1/LXA4R. *Proc Natl Acad Sci U S A* 99: 1359–1364, 2002

139. Kimura H, Gejyo F, Suzuki Y, Suzuki S, Miyazaki R, Arakawa M: Polymorphisms of angiotensin converting enzyme and plasminogen activator inhibitor-1 genes in diabetes and macroangiopathy1. *Kidney Int* 54: 1659–1669, 1998

140. Reis K, Arinsoy T, Derici U, Gonen S, Bicik Z, Soylemezoglu O, Yasavul U, Hasanoglu E, Sindel S: Angiotensinogen and plasminogen activator inhibitor-1 gene polymorphism in relation to chronic allograft dysfunction. *Clin Transplant* 19: 10–14, 2005

141. Wang AY, Poon P, Lai FM, Yu L, Choi PC, Lui SF, Li PK: Plasminogen activator inhibitor-1 gene polymorphism 4G/4G genotype and lupus nephritis in Chinese patients. *Kidney Int* 59: 1520–1528, 2001

142. Hertig A, Rondeau E: Role of the coagulation/fibrinolysis system in fibrin-associated glomerular injury. *J Am Soc Nephrol* 15: 844–853, 2004

143. Kohler HP, Grant PJ: Plasminogen-activator inhibitor type 1 and coronary artery disease. *N Engl J Med* 342: 1792–1801, 2000

144. Reis KA, Onal B, Gonen S, Arinsoy T, Erten Y, Ilgit E, Soylemezoglu O, Derici U, Guz G, Bali M, Sindel S: Angiotensinogen and plasminogen activator inhibitor-1 gene polymorphism in relation to renovascular disease. *Cardiovasc Intervent Radiol* 29: 59–63, 2006

145. Gils A, Declerck PJ: Plasminogen activator inhibitor-1. *Curr Med Chem* 11: 2323–2334, 2004

146. Yamamoto T, Nakamura T, Noble NA, Ruoslahti E, Border WA: Expression of transforming growth factor beta is elevated in human and experimental diabetic nephropathy. *Proc Natl Acad Sci U S A* 90: 1814–1818, 1993

147. Yamamoto T, Noble NA, Cohen AH, Nast CC, Hishida A,

Gold LI, Border WA: Expression of transforming growth factor-beta isoforms in human glomerular diseases. *Kidney Int* 49: 461–469, 1996

148. Nakamura T, Tanaka N, Higuma N, Kazama T, Kobayashi I, Yokota S: The localization of plasminogen activator inhibitor-1 in glomerular subepithelial deposits in membranous nephropathy. *J Am Soc Nephrol* 7: 2434–2444, 1996

149. Wang Y, Pratt JR, Hartley B, Evans B, Zhang L, Sacks SH: Expression of tissue type plasminogen activator and type 1 plasminogen activator inhibitor, and persistent fibrin deposition in chronic renal allograft failure. *Kidney Int* 52: 371–377, 1997

150. Shihab FS, Yamamoto T, Nast CC, Cohen AH, Noble NA, Gold LI, Border WA: Transforming growth factor-beta and matrix protein expression in acute and chronic rejection of human renal allografts. *J Am Soc Nephrol* 6: 286–294, 1995

151. Marcantoni C, Ma LJ, Donnert E, Federspiel C, Fogo AB: Plasminogen activator inhibitor-1 (PAI-1) and peroxisome proliferator-activated receptor-gamma (PPAR-gamma) in hypertensive nephrosclerosis [Abstract]. *J Am Soc Nephrol* 11: 351A, 2000

152. Montes R, Declerck PJ, Calvo A, Montes M, Hermida J, Munoz MC, Rocha E: Prevention of renal fibrin deposition in endotoxin-induced DIC through inhibition of PAI-1. *Thromb Haemost* 84: 65–70, 2000

153. Zoja C, Corna D, Macconi D, Zilio P, Bertani T, Remuzzi G: Tissue plasminogen activator therapy of rabbit nephrotoxic nephritis. *Lab Invest* 62: 34–40, 1990