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# Urokinase and its receptors in chronic kidney disease

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## 1. ABSTRACT

This review focuses on the role of the serine protease urokinase-type plasminogen activator and its high affinity receptor uPAR/CD87 in chronic kidney disease (CKD) progression. An emerging theme is their organ- and site-specific effects. In addition to tubules, uPA is produced by macrophages and fibroblasts in CKD. By activating hepatocyte growth factor and degrading fibrinogen uPA may have anti-fibrotic effects. However renal fibrosis was similar between uPA wild-type and knockout mice in experimental CKD. The uPAR is expressed by renal parenchymal cells and inflammatory cells in a variety of kidney diseases. Such expression appears anti-fibrotic based on studies in uPAR-deficient mice. In CKD uPAR expression is associated with higher uPA activity but its most important effect appears to be due to effects on cell recruitment and migration that involve interactions with a variety of co-receptors and chemoattractant effects of soluble uPAR. Vitronectin and high molecular weight kininogen are alternate uPAR ligands, and receptors in addition to uPAR may also bind directly to uPA and activate cell signaling pathways.

## 2. INTRODUCTION

Accumulation of extracellular matrix proteins in the interstitium of the kidney begins as a dynamic process that is preceded by an increase in microvascular permeability, an influx of inflammatory cells, and activation/transformation of resident kidney cells – fibroblasts and tubular epithelial cells in particular (1). Interstitial matrix proteins may be derived from several cellular sources, but fibroblasts and myofibroblasts are thought to be the major contributors. If the inciting renal injury is sustained, over time the molecular composition of the interstitial "scar" also undergoes significant changes in both matrix composition and interactions between individual macromolecules. Studies performed almost 20 years ago suggested that decreased matrix turnover was a major feature of the renal fibrogenic response, yet precisely which endogenous matrix-degrading proteases normally regulate renal matrix remodeling remains unclear (2).

In principle, ramping up the activity of the endogenous proteases or administration of proteases as therapeutics appears to be an attractive approach to chronic

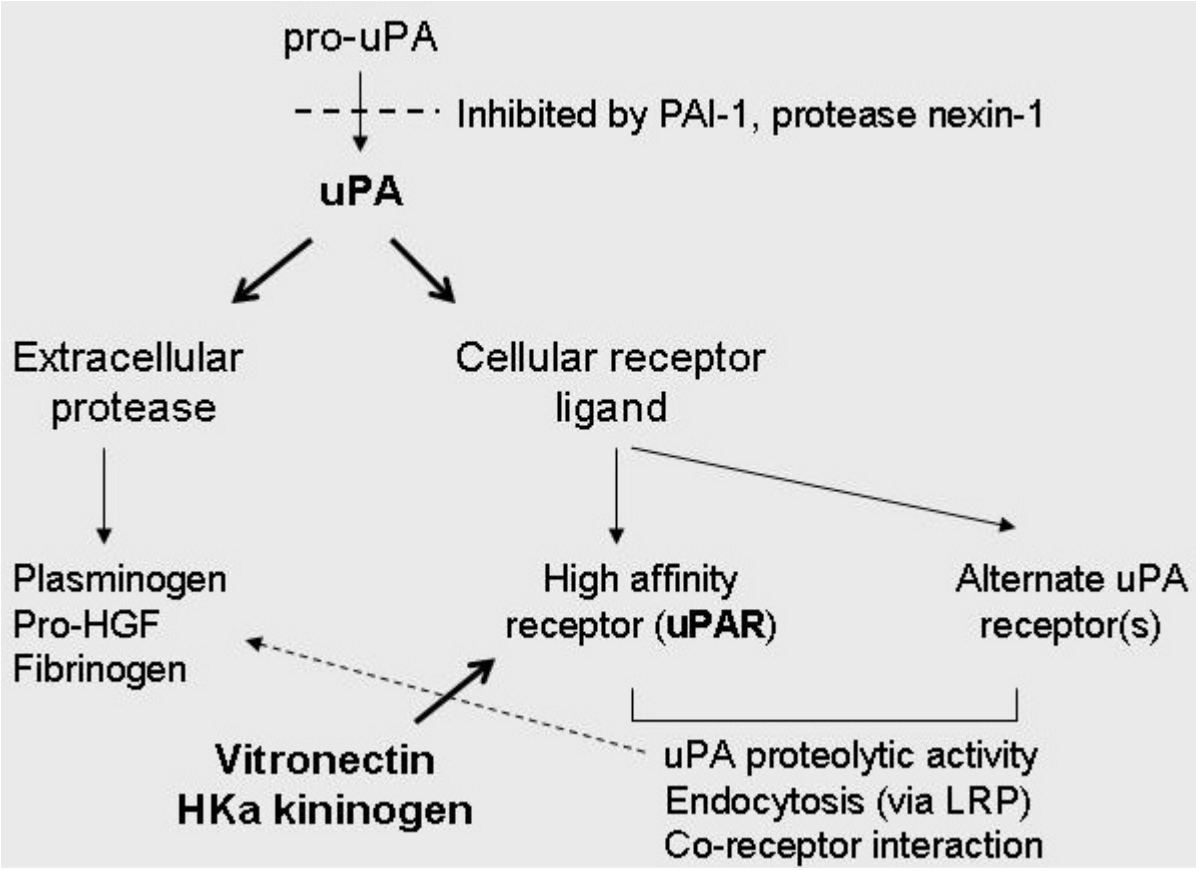


**Figure 1.** Schematic summary of uPA extracellular and cell-dependent pathways.

kidney disease. Of the four major protease families, the metalloproteinases (MMP) are considered the predominant matrix-degrading proteases. With few exceptions, the MMPs are synthesized as latent enzymes that require proteolytic cleavage for activation. Since plasmin can activate certain MMPs, at least *in vitro*, members of the serine protease family become candidates as endogenous regulators of renal fibrosis severity. Interest in the serine protease family strengthened when it was shown that plasminogen activator inhibitor-1 (PAI-1) is produced in large quantities by damaged kidneys, that fibrosis severity is significantly decreased in the absence or inhibition of PAI-1, and that fibrosis is significantly worse when PAI-1 is genetically over-expressed (3).

However, it now appears that the conceptual model of renal fibrosis as tightly regulated by a thermostat of matrix-degrading proteases is too simplistic because protease activity is not limited to the extracellular space (1). Furthermore, many of these proteases bind to cellular receptors, activate intracellular signaling pathways, and have a profound effect on cellular behavior. These effects may influence not only the early "inflammatory" phase of renal fibrogenesis but also the important later phase of cellular destruction, characterized by peritubular capillary rarefaction and tubular atrophy as the interstitial matrix expands and consolidates. This latter phase marks the point of no return in chronic kidney disease when functional nephron units are destroyed. Prior to this point, the reversal of renal scarring, as has been described in recent animal studies, is a desirable therapeutic goal and identifying the endogenous matrix-degrading pathways is an important area of ongoing investigation (4-6). This review will focus on what is currently known about the serine protease urokinase-type plasminogen activator (uPA) and its cellular receptors in chronic kidney disease. This appears to be another Pandora's box that we have only recently opened and begun to understand.

## 3. UROKINASE-TYPE PLASMINOGEN ACTIVATOR (uPA)

### 3.1. Functional Overview

The uPA is produced in significant quantities by proximal and distal tubular epithelial cells and secreted across the apical membrane into the urinary space. Other sources include monocytes/macrophages, fibroblasts and myofibroblasts. Initially synthesized as a latent single chain proenzyme, uPA is proteolytically cleaved into a two chain active enzyme. Several enzymes can serve as uPA activators, including plasmin, kallikrein, cathepsin B, and the matrix metalloproteinase PUMP. Despite high uPA levels, its primary physiological function in the kidney is still unknown. One suggestion is that it may prevent renal stone formation (7). Plasminogen is the preferred uPA substrate (Figure 1), although it may also degrade

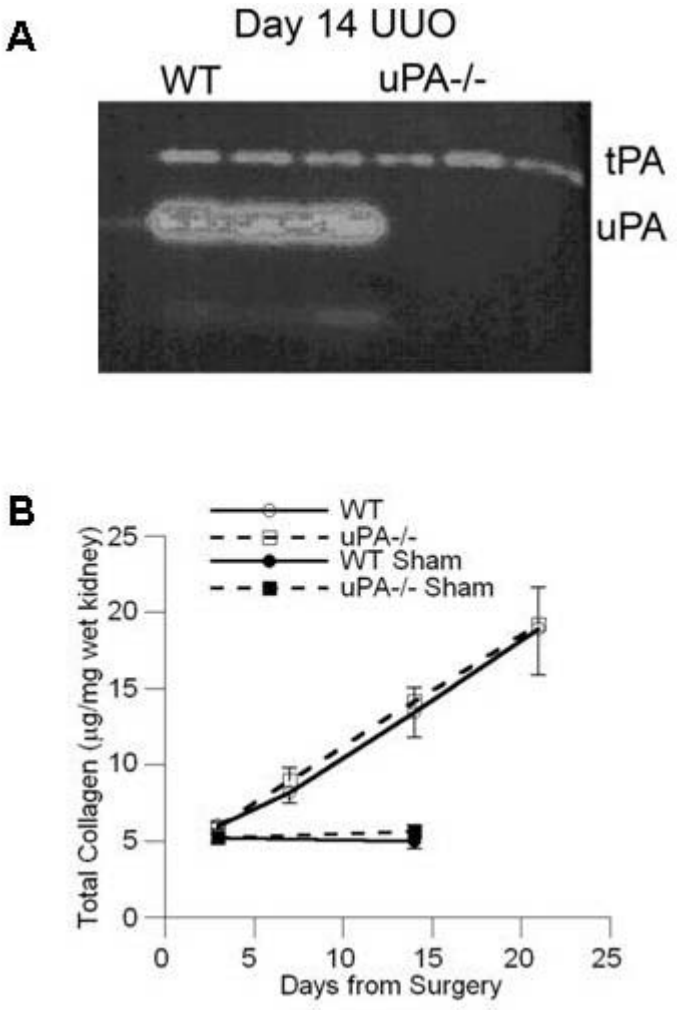


**Figure 2.** Genetic uPA deficiency does not alter renal fibrosis severity in response to unilateral ureteral obstruction (UUO) (A) A casein-plasminogen zymogram demonstrates high kidney uPA activity in wild-type (WT) mice 14 days after UUO while activity is absent in uPA-/- mice. Tissue plasminogen activator (tPA) activity was similar between the genotypes. (B) After 7, 14 and 21 days of UUO, the kidney collagen content measured as hydroxyproline levels was significantly increased compared to sham kidney levels (solid symbols) but were similar in both genotypes (open symbols) Data reproduced with permission from Yamaguchi *et al* (13) .

fibronectin and fibrin and it activates latent hepatocyte growth factor (HGF) and membrane-type MMP (8-9).

### 3.2. uPA in chronic kidney disease

The role of fibrinolytic pathways in the pathogenesis of acute glomerular disease has been of interest for decades. Most evidence suggests that endogenous activity is due to tissue-type plasminogen activator (tPA) (10). Until recently it has been unclear if uPA is involved in the pathogenesis of glomerulosclerosis and/or interstitial fibrosis. In experimental models of chronic kidney disease, uPA expression and activity increase, as has been shown in the model of unilateral ureteral ligation (UUO) (Figure 2) (11-13). Based on the known biological functions of uPA, it could be argued that it may have pro-fibrotic effects as a consequence of plasminogen (via enhanced TGF-β activation and PAR-1-dependent epithelial-to-mesenchymal transition) and FGF activation or anti-fibrotic effects by activating HGF, MT-MMP and by limited direct matrix protein (fibronectin) degradation. Although tPA is the primary physiological mediator of fibrin/fibrinogen degradation, uPA also has fibrinolytic activity and it has been used effectively in clinical settings for this purpose. Fibrin may serve as an early provisional matrix and in chronic lung disease; molecules with fibrinolytic activity usually attenuate pulmonary fibrosis. This includes studies that have administered uPA by gene therapy or recombinant protein to animals with bleomycin-induced pulmonary fibrosis (Table 1) (14-17). Despite the popular view of fibrin as a key early provisional matrix in the lung, when fibrosis severity was compared between bleomycin-treated wild-type and fibrinogen-deficient mice, no difference was found (18, 19). Similar studies have not been performed to determine if fibrinogen plays a role in chronic kidney disease.

**Table 1.** uPA and uPAR: Role in experimental fibrosis models

| Organ | Model | Manipulation | Fibrosis | Inflammation | Reference |
|---|---|---|---|---|---|
| **Urokinase** | | | | | |
| Kidney | Obstruction | uPA-/- mice | No change | No change | 13 |
| | Crescentic GN | uPA-/- mice | No change | ↓ Mϕ | 22 |
| Lung | Bleomycin | uPA-/- mice | ↑ fibrosis | ↓ early Mϕ | 113 |
| | | Inducible uPA gene | ↓ fibrosis | N.D. | 151 |
| | | uPA gene therapy | ↓ fibrosis | N.D. | 16 |
| | | uPA protein | ↓ fibrosis | N.D. | 14, 15, 17 |
| | Silica particles | uPA-/- mice | ↑ fibrosis | No change | 152 |
| Liver | $CCl_4$ | uPA-/- mice | Delayed clearance of necrotic hepatocytes | N.D. | 153 |
| | | uPA gene therapy | ↓ fibrosis | N.D. | 119-121 |
| Heart | Pressure overload | uPA-/- mice | ↓ fibrosis | ↓ leukocytes | 118 |
| | Myocarditis | uPA-/- mice | ↓ fibrosis | ↓ neutrophils | 117 |
| | Cardiac fibrosis | uPA-over-expressing macrophages | ↑ fibrosis | ↑ Mϕ | 122 |
| **uPAR** | | | | | |
| Kidney | Obstruction | uPAR-/- mice | ↑ fibrosis | ↓ Mϕ | 50 |
| | Crescentic GN | uPAR-/- mice | no change in proteinuria or fibrosis | No change Mϕ | 22 |
| | Pyelonephritis | uPAR-/- mice | ↑ inflammation | No change neutrophils | 27 |
| | Endotoxemia | uPAR-/- mice | ↑ proteinuria | N.D. | 49 |
| Lung | Bleomycin | uPAR-/- mice | No change in fibrosis; ↓ hemorrhage | ↓ early Mϕ | 113 |
| Liver | $CCl_4$ | uPAR-/- mice | No change | N.D. | 153 |
| Heart | Cardiac fibrosis in uPA-over-expressing mice | uPAR-/- mice | No change | No change Mϕ | 126 |

N.D. – not determined; Mϕ - macrophages

Studies performed in uPA wild-type and knock-out mice have yielded surprising results (13). In the UUO model, the degree of fibrosis was identical in mice of both genotypes (Table 1, Figure 3). Compensatory changes in tPA activity and PAI-1 or urokinase receptor (uPAR) expression levels were not detected. While in the lung intratracheal administration of recombinant uPA increased levels of active HGF and reduced fibrosis in the bleomycin model, differences in active α-HGF and phosphorlyated HGF receptor levels were not detected between the kidneys of uPA+/+ and uPA-/- mice 7 days after UUO (14). These remarkable differences between the effect of uPA on lung and kidney fibrosis suggest that this serine protease may have significant tissue-specific effects. Alternatively, the anti-fibrotic effects of HGF may be site-specific. It is known that renal tubule-derived uPA is secreted apically, but whether endogenously generated uPA gains access to the interstitial space to modulate local HGF activity and interstitial fibrosis is unclear (20). Exogenous uPA as used in the lung fibrosis studies may have greater access to the sites of matrix protein deposition. We attempted to increase interstitial uPA activity by investigating the UUO model in mice genetically engineered to over-express uPA in macrophages (21). Differences in fibrosis severity compared to wild-type mice were not detected, but due to technical limitations we have not yet documented that interstitial uPA activity was significantly different between these genotypes (unpublished data). In a model of crescentic glomerulonephritis, acute disease severity was similar between uPA +/+ and uPA -/- mice while the severity of chronic glomerulosclerosis was not examined (22). It remains a possibility that the biological effects of uPA are redundant, although tPA does not appear to serve that role. Definitive studies still need to be performed to determine if exogenous uPA or ectopically expressed uPA can modulate responses to injury in the kidney as has been demonstrated in other organs such as the liver, heart, and lung (see section 6 below).

## 4. THE CLASSICAL HIGH AFFINITY UROKINASE RECEPTOR (uPAR/CD87)

### 4.1. Functional overview

Since its discovery on the surface of monocytes in 1985 as a receptor that binds to the amino terminal fragment (ATF) of uPA and concentrates protease activity (contained within the C-terminal fragment) on cell membranes, uPAR has become a very interesting molecule with diverse biological functions (23-25). The uPAR colocalizes with uPA at focal contacts in the leading edge of migrating cells (26). The uPAR has now been identified on a variety of other cell types, including inflammatory cells (monocytes, macrophages, neutrophils, activated T cells), vascular endothelial cells, epithelial cells (both glomerular and tubular), mesenchymal cells (fibroblasts, myofibroblasts, mesangial cells), and neurons (23, 27-32). The uPAR, a highly glycosylated 50-kD to 65-kD protein, is a transmembrane receptor with three extracellular domains (D1, D2 and D3) and a single membrane-inserted domain connected to a very short glycosyl-phosphatidylinositol (GPI)-anchored cytoplasmic tail. The crystal structure of uPAR in complex with a peptide antagonist was solved in 2005 (33). The subsequent identification of additional extracellular ligands and cell surface co-receptor suggests its possible roles independent of the enzymatic properties of its ligand urokinase (26, 34, 35). The currently known ligands are uPA ± PAI-1, vitronectin, and kininogen (23, 34, 36). The complex molecular interactions between uPAR and its ligands or co-receptors regulate key events during cell adhesion, migration, proliferation, and survival. The most extensive studies performed to date pertain to its role in tumor invasion and metastasis (37-40).

The uPAR has pleomorphic functions (41-43). Studies in genetically engineered mice suggest that in

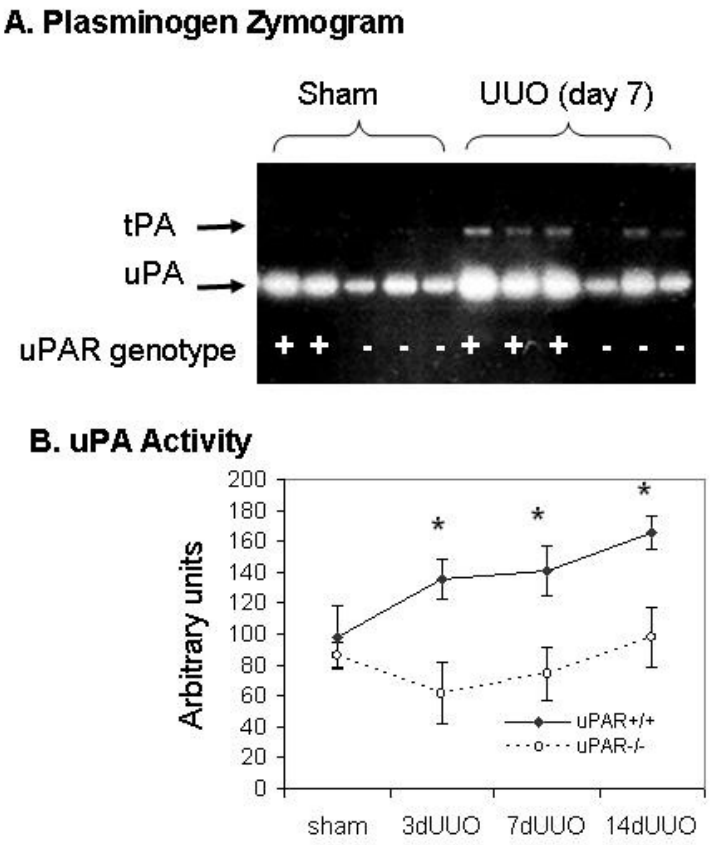


**Figure 3.** uPAR deficiency reduces kidney uPA activity. (A) Casein-plasminogen zymogram illustrates uPA and tPA activity 7 days after UUO. (B) Analysis of the mean size of the lytic bands found significantly higher uPA activity in the uPAR-expressing kidneys (uPAR+/+, closed diamonds) 3, 7, and 14 days after UUO compared to uPAR-deficient kidneys (uPAR-/-, open circles) *$P < 0.05$. Data reproduced with permission from Zhang *et al* (50).

addition to interactions with the amino terminal fragment (ATF) of uPA (both the single chain and the proteolytically active two chain enzyme), uPA and uPAR may also function independently. Three unique aspects of uPAR function underlie its great diversity. (1) In addition to uPA, vitronectin and high molecular weight kininogen are specific uPAR ligands. (2) uPAR can engage in lateral interactions with several other transmembrane cellular receptors. Together they elicit changes in cell motility and other signaling-dependent cellular functions. (3) uPAR domains D2 and D3 may be shed from the cell membrane as a soluble peptide that has significant chemotactic properties. While it is reasonable to suggest that some of these unique uPAR functions account for its ability to modulate the fibrogenic response of the kidney during chronic injury, the relative contribution of each specific function has yet to be determined.

**4.2. uPAR in experimental kidney disease**

Based on mRNA expression studies in mice, uPAR does not appear to be expressed in normal kidneys. However, *de novo* expression by glomerular and tubular epithelial cells and a variety of renal interstitial cells (fibroblasts, inflammatory cells and endothelial cells) has been confirmed in human renal diseases such as diabetic nephropathy, allograft rejection, and pyelonephritis (29, 44, 45). There is a growing list of uPAR agonists that include interleukins-1, -2, -4, -6, -7, -10, -13; tumor necrosis factor-α and -β; TGF-β; interferon-α, -γ; thrombospondin-1 and monocyte chemoattractant proteins-1, -2, -3 (46-48).

Studies comparing renal disease between uPAR wild-type (+/+) and genetically deficient (-/-) mice have provided important insights into its diverse functions (Table 1). Mice with targeted disruption of the uPAR gene are phenotypically normal and fertile. The severity of acute crescentic glomerulonephritis was similar in uPAR+/+ and uPAR-/- mice (22). However, in the absence of uPAR, mice were protected from endotoxin-induced proteinuria (49). The role of uPAR in the genesis of proteinuria is thought to involve β3 integrins and their collaborative effects on podocyte function. Acute pyelonephritis is more severe in uPAR -/- mice due to impaired bacterial phagocytosis (27). In a model of chronic kidney disease induced by UUO, induced uPAR expression in tubular and interstitial cells was associated with renoprotective effects including decreased numbers of myofibroblasts and reduced renal fibrosis compared to uPAR -/- mice (30, 50). It is of interest that the group A streptococcal surface dehydrogenase (GAPDH) also binds to uPAR (51).

### 4.3. Receptor-ligand interactions

#### 4.3.1. uPAR stabilizes membrane-associated uPA activity

When the noncatalytic uPA A chain binds to the D1 domain of uPAR, it stabilizes and concentrates proteolytic activity in pericellular zones. While uPA activation may be achieved by a variety of proteases, one interesting mechanism involves vascular endothelial growth factor receptor-2 that can activate uPAR-bound uPA via a process that involves MMP-2 (52). The receptor can also bind latent uPA, which retains its ability to be activated. In the study of the UUO model, uPA proteolytic activity (as assessed by plasminogen gel zymography) and levels of the anti-fibrotic hepatocyte growth factor (HGF) were significantly higher in the uPAR +/+ mice that had less fibrosis than the uPAR -/- mice (Figure 3) (50). HGF is known to have potent anti-fibrotic effects. Although these findings suggested that uPAR-dependent uPA catalytic activity might account for the receptor's ability to attenuate renal scarring, our subsequent studies in uPA -/- mice did not reproduce the uPAR-/- phenotype after UUO (13). Bi-transgenic mice that over-express both uPAR and uPA in the skin develop extensive alopecia due to the enhanced urokinase catalytic activity, while isolated deficiency of uPA or uPAR results in no cutaneous phenotype (53). This observation suggests a likely catalytic synergy between the receptor and its ligand.

Ligation of uPA to uPAR can initiate a variety of cellular responses that are discussed in greater detail in the subsequent sections. (1) Through transactivation of uPAR co-receptors, including the epidermal growth factor receptor, several integrins, and gp130, extracellular signal regulated kinase (ERK) or signal transducer and activator of transcription (STAT) signaling pathways may be activated (28, 54-57). (2) The uPA-ligated cell surface uPAR may laterally interact with integrins, especially those that bind to the extracellular matrix proteins vitronectin and fibronectin (58, 59). (3) When uPAR-bound uPA interacts with PAI-1, this receptor complex can cluster with the scavenger receptor low density lipoprotein receptor-related protein (LRP) leading to the endocytic degradation of uPA/PAI-1 (60).

#### 4.3.2. PAI-1/uPAR interactions

The renal anti-fibrotic effects of uPAR and uPA may also be indirect and regulated by interstitial levels of plasminogen activator inhibitor-1 (PAI-1) (3). Although PAI-1 has a very short half-life, it binds with high affinity to vitronectin. This interaction stabilizes PAI-1 and may account for the accumulation of PAI-1 within the renal interstitium during fibrosis. PAI-1 is one of the most potent pro-fibrotic molecules known to be expressed in the kidney. PAI-1 binds and inhibits uPA whether receptor-bound or free. The trimolecular complex of uPAR + uPA + PAI-1 initiates a shuttle that internalizes and degrades PAI-1 within lysosomes. Meanwhile, uPAR is salvaged and recycled back to the cell membrane. LRP-1 is the critical uPAR partner in this shuttling process (Figure 4). It has also been reported that PAI-1 may interact directly with LRP-1 to promote cellular mobility (61). Whether the PAI-1 degrading cellular shuttle can function in the absence of uPA bound to uPAR is unclear. In the obstructed kidneys of uPAR -/- mice, PAI-1 protein levels were significantly higher than kidneys from uPAR +/+ with less fibrosis, suggesting a potential link between PAI-1 and uPAR during renal fibrosis (30). Ongoing and future studies need to address the role of uPAR in the context of its other known cellular and molecular functions.

Interactions with PAI-1 may have additional effects on uPAR function. PAI-1 enhances uPAR-mediated, uPA-induced ERK signal activation (62). It has also been suggested that PAI-1 may directly bind to uPAR, activating ERK signaling and stimulating TGF-β expression (63). The uPAR-mediated cellular effects of PAI-1 are supported by the inhibitory effect of anti-uPAR antibody (64). By confocal microscopy it has been observed that the addition of PAI-1 alone induces significant cell surface LRP clustering in uPAR+/+ but not uPAR-null primary kidney fibroblast cultures, suggesting a possible direct interaction between PAI-1 and uPAR (65, 66). PAI-1 binds with high affinity to vitronectin that can bind directly to uPAR, and is also a ligand for certain integrin receptors known to function as uPAR co-receptors (see section 4.3.3).

#### 4.3.3. Alternative uPAR ligands: vitronectin and high molecular weight kininogen

Nine years after the discovery of uPAR, it was further identified as a high affinity adhesion receptor for extracellular matrix vitronectin (VN) (Figure 5) (34). VN binds to both membrane GPI-anchored uPAR and soluble uncleaved uPAR at domain D1 and the D1-D2 linker region (65, 67, 68). The VN-uPAR or VN-suPAR binding is facilitated by concurrent receptor binding to urokinase. Indeed, VN can concentrate proteolytic activity by five-fold on cell surface and extracellular matrix via the formation/trapping of a ternary uPA/suPAR/VN complex (34). This complex can be disassembled when VN is cleaved by plasmin (that may be activated by uPA-mediated cleavage of the zymogen plasminogen) or when PAI-1 competes with uPAR for the VN somatomedin B domain (69). The ability of PAI-1 to competitively inhibit uPAR's binding to the extracellular matrix VN, thereby regulating VN-uPAR and VN-integrin-uPAR interactions, plays a key role in uPAR-dependent cell adhesion and migration (70, 71). Vitronectin expression in the tubulointerstitium is known to be up-regulated in response to chronic damage (30).

In 1997 uPAR was also shown to be a cellular receptor for the cleaved high molecular weight kininogen (HKa) (36). The domain 5 of the HKa binds to uPAR at a site within domains 2 and 3 (36, 72). The uPAR-mediated cellular effect of HKa depends on the composition of the extracellular matrix. HKa selectively induces apoptosis of endothelial cells grown on VN, but not cells grown on fibronectin; under these conditions it also inhibits angiogenesis (73, 74). When HKa binds to uPAR on endothelial cells, it can physically disrupt the VN-uPAR signaling complex formed with co-receptors alphavbeta3 or alpha5beta1 integrins, caveolin, and Src kinase resulting in anti-adhesive effect and apoptosis (73). The ligation of

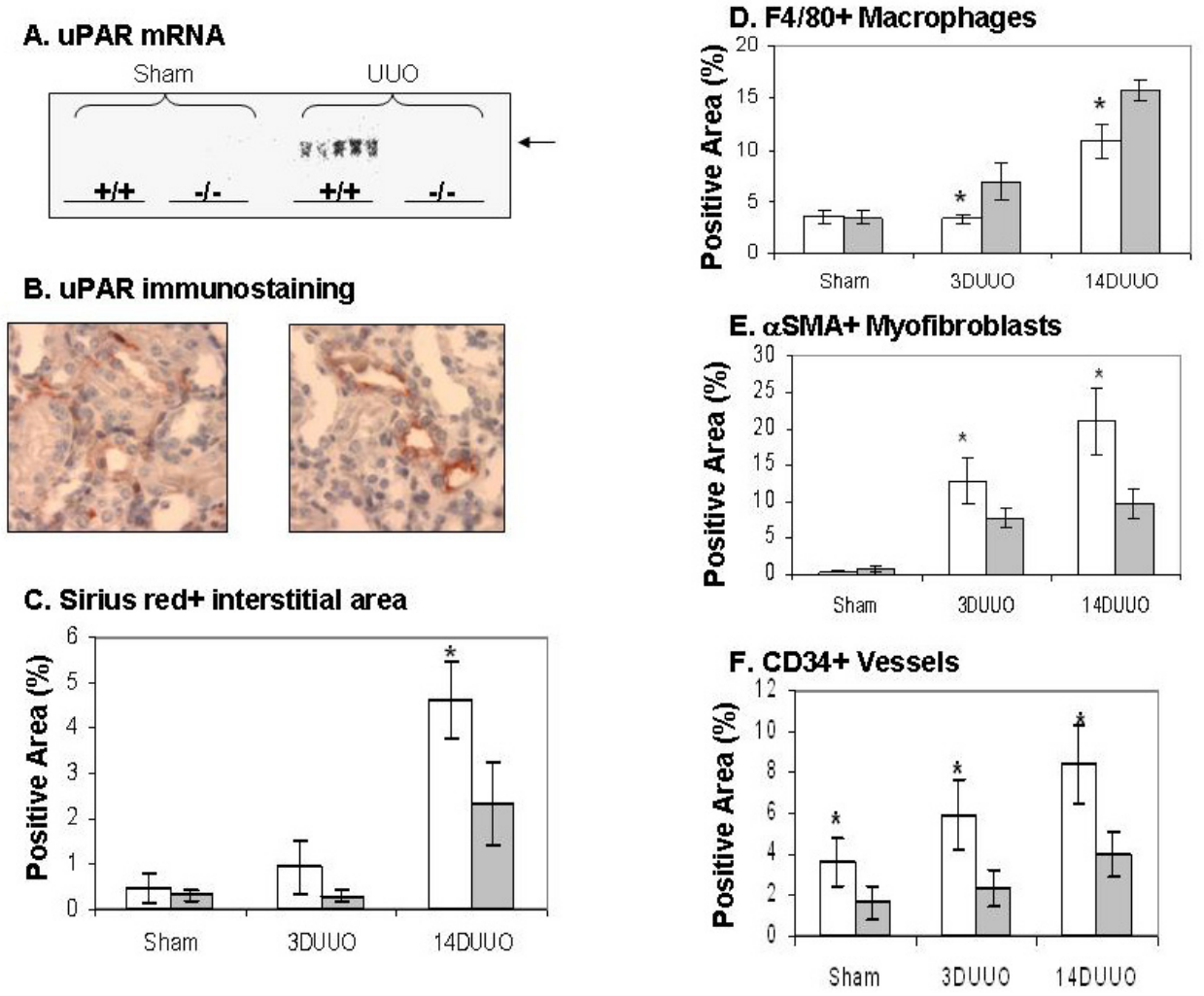


**Figure 4.** uPAR expression and function during experimental chronic kidney injury induced by UUO. (A) Northern blot analysis identifies uPAR mRNA in wild-type kidneys 7 days after UUO; no transcripts were detected in sham or uPAR-/- kidneys. (B) Kidney uPAR immunostaining identifies positive interstitial and tubular cells 7 days after UUO. (C) Measurement of interstitial area expressing mature sirius red+ collagen fibrils detected significantly more fibrosis in the uPAR-deficient mice (open bars) in comparison wth strain-identical uPAR wild-type mice (grey bars) Using immunohistochemical staining followed by computer-assisted image analysis, uPAR-deficient mice were found to have fewer F4/80+ macrophages (D), more alpha smooth muscle actin (αSMA)+ myofibroblasts (E) and more CD34+ interstitial vessels (endothelial and lymphatic) (F) compared to wild-type mice. *$P < 0.05$. Data reproduced with permission from Zhang *et al* (30).

uPAR by HKa can stimulate cytokine and chemokine gene expression in mononuclear cells via mitogen-activated protein kinases (JNK and p38) and the nuclear factor kappa B (NFκB) signaling pathway (75). Careful studies still need to be performed to determine if VN and/or HKa participate in uPAR-dependent anti-fibrotic effects in the kidney.

#### 4.3.4. uPAR lateral interactions with co-receptors

##### 4.3.4.1. Integrins

Despite the fact that cellular uPAR lacks a signal transducing cytoplasmic tail, it is able to profoundly influence cellular behavior such as cell migration by interacting with several other cell surface molecules (Figure 5). First to be recognized was the association with the leukocyte integrin (CD11b/CD18) (76). Subsequently, it has been shown that uPAR can interact with several integrin family members, including α3, α5, αv, β1, β2, β3 and β5. Such interactions initiate cytoskeletal reorganization and activate cell signaling pathways that regulate cell migration within extracellular matrices (77, 78). When uPA binds to uPAR it promotes cell adhesion by increasing the affinity of the receptor for both VN and integrins (58, 59). VN-uPAR interactions are critical for cell adhesion and migration (67). The most commonly identified effect is one of "deadhesion", where cells can be released from one matrix footprint (often fibronectin) in order to move forward and attach to another matrix protein

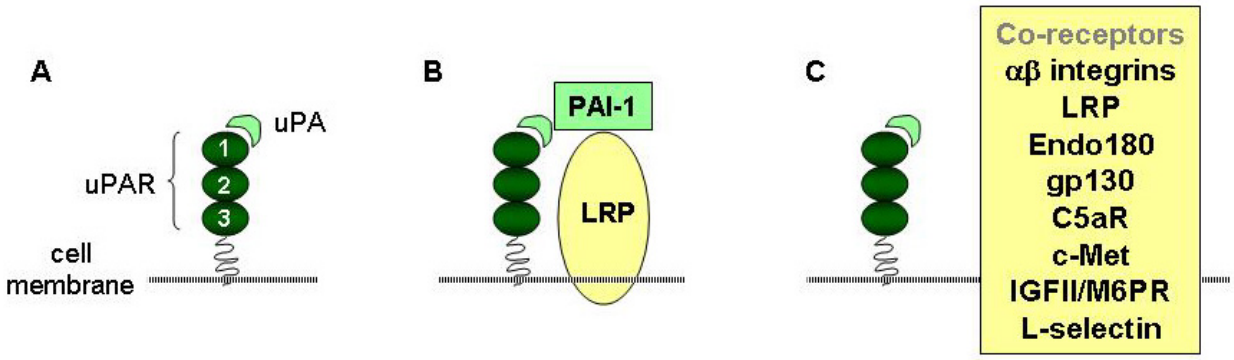


**Figure 5.** Schematic summary of uPAR and its co-receptors. (A) Extracellular uPAR (domain 1) interacts with uPA, both latent and active forms. (B) Recruitment of PAI-1 to receptor-bound uPA is an important event. Not only does it neutralize uPA activity but the trimolecular complex may co-associate with LRP leading to internalization of the entire complex, PAI-1 degradation and re-shuffling of uPAR back to the cell membrane. (C) uPAR itself a non-signaling receptor is predisposed to establishing lateral membrane interactions with several other receptors. Together they trigger proteolysis-independent intracellular signaling to modulate a variety of cellular behaviors. Many of these co-receptors also express uPA-binding motifs.

such as VN (24). PAI-1 and the locally concentrated active enzyme urokinase are regulators that promote cell detachment and migration by disrupting these VN-uPAR and VN-integrin interactions (70, 71, 79). In the obstructive uropathy model, uPAR deletion was associated with significantly fewer numbers of interstitial macrophages, perhaps due to the disruption of this pathway (30).

The uPAR appears necessary for adequate host defense during infections including acute pyelonephritis, peritonitis, pneumococcal pneumonia, and meningitis (27, 80-81). In these studies, the mice lacking uPAR manifest a blunted leukocyte response to the infection and delayed pathogen clearance. In an experimental model of allergic encephalitis in which leukocyte recruitment requires β2 integrin function, the absence of uPAR reduced neutrophil recruitment (82).

Glomerular podocytes and renal tubules express various integrins during specific disease processes, and they may also co-express uPAR (30, 49, 50). Given the importance of the integrin family for migration and crosstalk with extracellular matrix proteins, the specific role of uPAR in those processes deserve further exploration. In addition to effects on cell adhesion and migration, clustering between activated uPAR and one of its co-receptors may lead to trans-activation and initiation of intracellular signaling (83). Examples that may be relevant to renal disease include the JAK1/STAT1 described in the human kidney epithelial tumor cell line TCL-598, the gp130/Tyk2/STAT3 reported in human mesangial cells, the ERK/MAPK activated in fibroblasts, endothelial cells and tumor cells, and the JNK/MAPK and p38/MAPK pathways in mononuclear cells (28, 66, 75, 84-86).

#### 4.3.4.2. Low density lipoprotein receptor related protein-1 (LRP-1)

The uPAR has been characterized as a scavenger receptor by virtue of its ability to work in collaboration with other scavenger receptors, especially LRP-1 to degrade ligands by endocytosis (60, 87, 88). This endocytic pathway is the primary route of elimination of extracellular PAI-1. PAI-1 and uPA are subsequently degraded within lysosomes while uPAR is recycled to the cell surface. The uPAR-associated protein (uPARAP/Endo180) is another internalization receptor connected with cell surface uPAR that engages in matrix turnover during tissue remodeling (89, 90).

LRP-1 is itself a multi-ligand receptor with several important biological functions (91). These ligands include PAI-1, uPA and tPA. Recent studies have shown that LRP-1 activated by tPA triggers intracellular signaling to promote matrix metalloproteinase-9 expression, macrophage migration, and fibroblast survival. Other known LRP-1 ligands include coagulation factors (VIIa, VIIIa, IXa, anti-thrombin III, tissue factor pathway inhibitor, heparin cofactor II), metalloproteinases (MMP-9, MMP-13), complement (C3, C1 inhibitor), thrombospondin -1 and -2, apolipoprotein E, α2 macroglobulin and LRP-receptor-associated protein (RAP). Whether uPAR influences the interaction of LRP with any of these ligands is unknown.

#### 4.3.4.3. The growing list of uPAR co-receptors

Several uPAR co-receptors have themselves been implicated in fibrogenic reactions. Glycoprotein 130 (gp130) is a key member of the interleukin-6 superfamily. Homodimers serve as the signaling receptor for interleukin-6 and interleukin-11 while gp130 heterodimers constitute receptors for ciliary neurotrophic factor, cardiolipin-like cytokine, leukemia inhibitory factor, oncostatin M, and neuropoietin (92).

The receptor for complement component C5a (C5aR) is expressed in response to acute and chronic kidney injury. Studies in C5aR deficient mice reported reduced interstitial disease severity in a chronic glomerulonephritis model while a specific C5a receptor antagonist has been shown to reduce renal disease in

several experimental models (93-96). In a model of immune complex pulmonary inflammation, uPAR was necessary for C5a signaling (28). The insulin-like growth factor-II/mannose-6-phosphate (IGFII/M6P) receptor has been shown to be involved in directing uPAR to lysosomes (97). In an experimental model of partial hepatectomy the early regenerative phase was characterized by interactions between uPA, uPAR and the HGF receptor c-Met (98). Recently, uPAR has been shown to interact with platelet-derived growth factor B receptor and epidermal growth factor receptor (99-101).

#### 4.3.5. Additional uPAR activities

In addition to regulating uPA catalytic activity, promoting PAI-1 degradation and modulating various cell-cell and cell-matrix interactions, uPAR may enhance cell survival and promote angiogenesis. The uPA-uPAR interaction stimulates proliferation in most cell lines (85, 101-103). PAI-1 promotes this effect by further enhancing intracellular ERK/MAPK signaling (62). uPAR interaction with uPA can stimulate proliferation; uPAR expression also confers resistance to caspase-mediated apoptosis and anoikis (104, 105) .

Numerous studies have documented that strategies to block uPAR expression are associated with decreased angiogenesis and tumor growth and metastasis (85, 103, 106). In contrast, uPAR has been shown to promote angiogenesis in the eye (107). In the UUO model of chronic kidney disease, uPAR +/+ mice have significantly fewer numbers of CD34+ interstitial cells, either as a direct uPAR effect or as an indirect consequence of enhanced PAI-1 clearance, as PAI-1 may have anti-angiogenic properties.

## 5. SOLUBLE uPAR (suPAR): A CHEMOTACTIC PEPTIDE

GPI-anchored uPAR can be cleaved by active urokinase at its D1-D2 linker region, leaving on the cell surface a truncated receptor (D2 and D3) where the p88-92 chemotactic sequence is exposed (72, 108). With the D1 domain removed, the truncated receptor gains chemotactic properties while its ability to interact with uPA, vitronectin, and various co-receptors is lost. If the truncated D2+D3 receptor or even the intact receptor is shed from the cell surface, it is called the soluble form of uPAR (suPAR) (Figure 5) (45). SuPAR has been detected in human plasma and urine. Far from being an inactive degradation product, suPAR has important chemotactic properties due to its ability to bind to the G protein-coupled receptor formyl-peptide-receptor-like-1/lipoxin A4 receptor (FPRL1/LXA4R). Whether this truncated suPAR-induced signaling is mediated by the fMLP receptor homologue FPRL1 and FPRL2 is still unclear (55, 56). FPRL1/LXA4R is expressed by leukocytes and epithelial cells. Interaction between suPAR and its receptor appears to be biologically important for innate and adaptive immune responses. For example, plasma suPAR levels correlate with disease activity in AIDS and bacteremia (45, 109).

SuPAR also inactivates CXCR4, the chemokine receptor primarily responsible for hematopoietic stem cell retention in bone marrow (110). Following parenteral administration of suPAR to mice along with granulocyte-colony stimulating factor, leukocytosis and enhanced stem cells mobilization is observed (111, 112).

Plasma and urinary suPAR levels are increased in humans with acute pyelonephritis, an effect that could be reproduced experimentally when endotoxin was injected into a group of volunteers (45). Renal studies in uPAR genetic mice demonstrated significantly fewer interstitial macrophages in the uPAR-/- group (30). While this difference might be due to the absence of suPAR, lateral interactions of membrane-anchored uPAR with leukocyte intergrins, the anaphylatoxin C5a receptor and/or Endo180 (involved in directional sensing during chemotaxis) may also contribute to uPAR-dependent monocyte recruitment. In experimental pyelonephritis, although the absence of uPAR did not affect neutrophil recruitment, it was required for their bacterial phagocytic function (27).

## 6. ROLE OF uPA and uPAR DURING FIBROGENESIS: DISEASE AND ORGAN SPECIFICITY

The degree of overlap in fibrogenic pathways in solid organs such as the kidney, liver, heart, and lung is remarkable. However, when it comes to select members of the serine protease family and its associated receptors, a divergence of effects is often observed based on studies performed in animals (Table 3). The reason for these differences is still not clear. In some situations, worse fibrosis may be a secondary consequence of changes in inflammation severity. In the lung, where fibrin is thought to form a critical early provisional matrix during fibrosis, uPA may be more effective as an anti-fibrotic protease. However, the fact that genetic fibrinogen deficiency has no effect on the severity of bleomycin-induced pulmonary fibrosis challenges this theory (18, 113). It is possible that there are important organ-specific differences in HGF activation mechanisms. In the lung and liver, uPA plays an important role in the regulation of HGF activity, while in the kidney, an alternate pathway may serve this role – such as HGF activator and its specific inhibitors HAI-1 and HAI-2 (14, 114-116).

In the kidney, where endogenous uPA is produced in large quantities, uPA deficiency had no effect on the severity of UUO-induced renal fibrosis (13). In contrast, uPA deficiency worsened bleomycin-induced lung fibrosis and significantly reduced fibrosis in hearts that were damaged by viral myocarditis or left ventricular pressure overload (113, 117, 118). Delivery of exogenous uPA as a recombinant protein or via an uPA-expressing viral vector reduced fibrosis in the lung and liver, while mice with macrophages engineered to over-express uPA spontaneously developed cardiac fibrosis (119-122). In chronic renal tubulointerstitial disease the induction of endogenous uPAR reduces fibrosis severity, while in a model of crescentic glomerulonephritis, genetic deficiency of either uPA or uPAR did not worsen glomerulosclerosis, although the degree of glomerular inflammation was reduced in the uPAR-/- mice (22, 50). In humans, uPAR

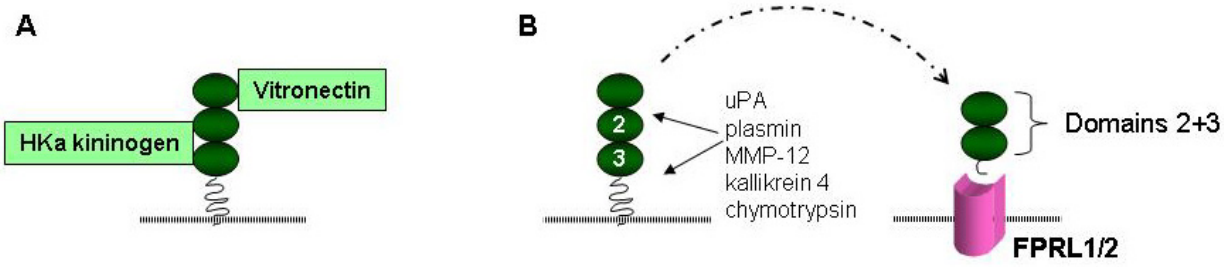


**Figure 6.** uPAR: alternative ligands and uPAR as a ligand. (A) The uPAR also expresses specific binding sites for vitronectin in domain 1 and the flanking interdomain linker region and for high molecular weight kininogen in domains 2 and 3, (B) A variety of proteases may release uPAR domains 2 and 3 which have been detected in plasma and urine in certain human diseases. This soluble peptide (suPAR) possesses chemotactic properties for cells that express receptors that are formyl-peptide-receptor-like (FPRL) FPRL1 is also the lipoxin A4 receptor.

expression levels are higher on fibroblasts isolated from fibrotic lungs compared to normal lungs (123). In human keloid compared with normal scars, uPAR expression is enhanced (124). There is evidence that membrane-bound uPAR is necessary for myofibroblast differentiation (125). Following bleomycin administration, the degree of hemorrhage and inflammation is reduced in uPAR-/- mice but no difference in fibrosis was detected compared with uPAR+/+ mice (113). In the heart, uPAR deficiency does not prevent the fibrogenic effects of uPA-over-expression (126). While a large majority of the cellular and molecular mediators of fibrosis elicit similar effects in all of these organs, the uPA-uPAR family is a clear exception which highlights the need to carefully elucidate mechanisms of fibrosis under a variety of pathological states.

In addition to matrix accumulation, another histological feature of chronic kidney disease is the presence of an interstitial infiltrate of mononuclear cells. Interstitial monocytes/macrophages in particular have been implicated in fibrogenic pathways, making the mediators of monocyte recruitment and activation of interest as potential therapeutic targets (1). Several known uPA functions suggest that it may also promote inflammation. As a protease, uPA may liberate fibrin and suPAR, both known monocytes chemoattractants. It may activate proinflammatory cytokines and through interactions with PAI-1 and LRP, uPA may influence the movement of monocytes (61). The uPAR is known to facilitate cell migration via interactions with the β2 family of leucocyte integrins and with the fibronectin- or vitronectin-binding integrins, while suPAR is a leukocyte chemoattractant.

In animal models of acute inflammatory diseases such as bacterial pneumonia or meningitis, endotoxin-induced peritonitis, immune complex-induced pneumonia and allergic encephalitis, inflammation (primarily neutrophils) is attenuated in uPAR-deficient mice (80, 81, 82, 127, 128). In a model of acute muscle injury, accumulation of monocytes was shown to involve uPA but not its uPAR receptor (129). However, in chronic disease processes associated with fibrosis, the effects of uPA and uPAR deficiency on the associated inflammatory response are less consistent, as summarized in Table 1. The phenotypic heterogeneity of tissue macrophages has recently been appreciated (130-132). Classically activated M1 macrophages are typically associated with tissue injury, while M2 alternatively activated macrophages are usually associated with tissue repair and remodeling. Administration of M2 macrophages has recently been reported to reduce fibrosis in obstructive nephropathy and adriamycin nephrosis (133, 134). Although more studies are necessary, it is tempting to speculate that macrophage uPAR expression is associated with the reparative M2 phenotype as renal fibrosis is worse in uPAR-/- mice despite less interstitial inflammation compared to uPAR+/+ mice (30).

## 7. uPAR AS A THERAPEUTIC TARGET

uPAR has become a therapeutic target of interest for cancer and other pathologic conditions, including bone homeostasis (osteoporosis), neuron development, and atherosclerosis (32, 135-137). Pharmaceutical strategies of uPAR inactivation or blockade for *in vivo* evaluation have included RNA interference, adenovirus-mediated transfer of anti-sense uPAR, uPAR signaling blocked with anti-sense oligonucleotides or other small molecule inhibitors, adenovirus-mediated delivery of an uPA/uPAR antagonist, inhibitory monoclonal antibodies, and thalidomide (85, 107, 138, 139, 140, 141). Soluble uPAR has also been investigated as a potential inhibitor of tumor growth (142). These intervention strategies have been shown to decrease tumor invasion/metastasis, angiogenesis, and growth in breast cancer, ovarian cancer, brain tumor, gliomas, glioblastoma multiform, osteosarcoma, non-small cell lung cancer, prostate cancer, and melanoma; to reduce retinal neovascularization in retinopathy; and to suppress macrophage infiltration into the vascular wall of ApoE-deficient mice (85, 107, 138, 139, 141, 142, 143, 144).

## 8. OTHER uPA RECEPTORS

In addition to high-affinity binding of the growth factor-like uPA domain to uPAR, specific uPA binding to an alternative, unidentified low-affinity cellular receptor has been reported (145-147). We have found that urokinase signals renal fibroblast activation via the MAPK pathway (30). This regulation appears to be mediated at least in part by an alternative urokinase receptor as uPA can

initiate mitogenesis in uPAR-/- fibroblasts. Using phage display technology, Liang *et al* identified a putative uPA-binding consensus sequence in 12 membrane proteins, further supporting the existence of additional urokinase receptors (148). Among these candidate receptors, several are already known as uPAR co-receptors: LRP, gp130, integrins, Endo180, and the IGF-II/M6P receptor. This suggests that different uPA sites might simultaneously bind to uPAR and one of its co-receptors (149). In other words, urokinase may serve as a bridge to cluster and coordinate the functions of uPAR and co-receptors.

We have recently identified the muscle type nicotinic receptor alpha1 (nAChR$\alpha$1) as an alternative urokinase cellular signaling receptor for renal fibroblasts (150). It controls a complex of signaling proteins by tyrosine-phosphorylation, including calcium-binding proteins annexin II and AHNAK, cytoskeletal proteins actin, actinin and vimentin, and nucleoprotein histone 4. This new "outside-in" signaling pathway appears to initiate important profibrotic effects in the kidney (manuscript in preparation).

## 9. PERSPECTIVE

uPAR and suPAR are multifunctional proteins. The final outcome of the intricate interactions between uPAR and its ligands and co-receptors is a significant change in cellular phenotype that modulates cell adhesion, migration, proliferation, phagocytosis, and survival. These uPAR-regulated cellular functions have been shown to be crucial in a variety of physical and pathologic processes *in vivo* including inflammation, wound healing and fibrogenesis, neoangiogenesis, tumor growth and metastasis, and tissue destruction and regeneration (28, 30, 85, 124). During fibrogenesis, current evidence suggests that the specific role of uPA and uPAR are organ- and site-specific. In the kidney, *de novo* uPAR expression during acute and chronic injury appears to represent a beneficial defense response. The recent recognition of several alternative uPA receptor candidates introduces a new level of complexity to uPA pathophysiology that has yet to be explored.

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**Abbreviations:** AHNAK: nucleoprotein also known as desmoyokin, C5aR: complement component C5a receptor, CKD: chronic kidney disease, CXCR4: alpha-chemokine receptor specific for stromal-derived-factor-1, ERK: extracellular signal-regulated kinase , FGF: fibroblast growth factor, FPRL1: formyl peptide receptor-like-1, HGF: hepatocyte growth factor, IGFII: insulin-like growth factor II, JAK: Janus kinase, JNK: c-jun N-terminal kinase, LXA4R: lipoxin A4 receptor, LRP: low density lipoprotein receptor-related protein, MAPK: mitogen-activated protein kinase, M6PR: mannose-6-phosphate, MMP : matrix metalloproteinase, MT-MMP: membrane-type matrix metalloproteinase, PAI-1: plasminogen activator inhibitor-1, RAP: receptor-associated protein, STAT: signal transducer and activator of transcription: , suPAR: soluble uPAR, tPA: tissue-type plasminogen activator, Tyk2: tyrosine kinase 2, uPA : urokinase-type plasminogen activator, uPAR : high affinity urokinase receptor, CD87, uPARAP: uPAR-associated protein also known as Endo180, UUO : unilateral ureteral obstruction, VN: vitronectin

**Key Words:** Urokinase, Urokinase Receptor, Serine Protease, Plasminogen Activator Inhibitor-1, Low Density Lipoprotein Receptor-Related Protein, Fibrosis, Vitronectin, Integrin, Review

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