FEBS Letters 584 (2010) 1923–1930

FEBS Letters

journal homepage: www.FEBSLetters.org

Review

# The urokinase receptor: Focused cell surface proteolysis, cell adhesion and signaling

Francesco Blasi [a,b,*], Nicolai Sidenius [c,*]

[a] Università Vita Salute San Raffaele, via Olgettina 60, 20132 Milano, Italy
[b] Unit on Transcriptional Regulation in Development and Cancer, IFOM (Fondazione Istituto FIRC di Oncologia Molecolare), IFOM-IEO-Campus, Via Adamello 16, 20139 Milano, Italy
[c] Unit of Cell Matrix Signaling, IFOM (Fondazione Istituto FIRC di Oncologia Molecolare), IFOM-IEO-Campus, Via Adamello 16, 20139 Milano, Italy

ARTICLE INFO

*Article history:*
Received 20 November 2009
Revised 21 December 2009
Accepted 21 December 2009
Available online 27 December 2009

Edited by Sandro Sonnino

*Keywords:*
Urokinase receptor uPAR
Cell migration
Cell adhesion
Cell proliferation
Signaling

ABSTRACT

**Plasma membrane urokinase-type plasminogen activator (uPA)-receptor (uPAR) is a GPI-anchored protein that binds with high-affinity and activates the serine protease uPA, thus regulating proteolytic activity at the cell surface. In addition, uPAR is a signaling receptor that often does not require its protease ligand or its proteolytic function.**

**uPAR is highly expressed during tissue reorganization, inflammation, and in virtually all human cancers. Since its discovery, in vitro and in vivo models, as well as retrospective clinical studies have shown that over-expression of components of the uPA/uPAR-system correlates with increased proliferation, migration, and invasion affecting the malignant phenotype of cancer. uPAR regulates the cells–extracellular matrix interactions promoting its degradation and turnover through the plasminogen activation cascade.**



## 1. Introduction

### 1.1. Identification of uPAR

The urokinase-type plasminogen activator (uPA) receptor (uPAR), was identified, isolated and cloned as the plasma membrane high-affinity binding-site of the serine protease uPA [1–4].

### 1.2. Protein synthesis and structure of uPAR

The human uPAR cDNA encodes a polypeptide of 335 amino acids including a N-terminal 22-residue secretion signal peptide and a C-terminal segment (30 amino acids) removable with the attachment of a glycosyl phosphatidylinositol (GPI)-anchor [5]. The mature protein (283 residues) is highly glycosylated and composed of three similarly sized (about 90 residues each) homologous domains (here referred to as DI, DII and DIII) and belonging to the Ly-6/uPAR protein domain family [6]. The biochemical and structural aspects of uPAR have been extensively investigated and reviewed in detail [7,8] and are summarized in Fig. 1.

### 1.3. uPAR expression

Although uPAR is expressed constitutively in many cell lines, the uPAR gene is inducible, for example in T cells [9], keratinocytes [10–12] and colon [13]. Typical inducers in culture are phorbol esters, growth factors and integrin-mediated signals. The transcription factors that have been shown to bind to the uPAR promoter and to regulate its expression are AP1, PEA3/Ets, Sp1 and Ap2 [14–17]. The expression of uPAR is also regulated at the post-transcriptional level [18–20], possibly through the action of mRNA binding proteins [21] and maybe also by micro RNA's [22].

In the healthy organism, uPAR is moderately expressed in various tissues including lungs, kidneys, spleen, vessels, uterus, bladder, thymus, heart, liver and testis. Strong uPAR expression is observed in organs undergoing extensive tissue remodeling, such as trophoblast cells and migrating, but not resting, keratinocytes at the edge of wounds [11]. In these tissues, macrophages, neutrophils, endothelial cells as well as keratinocytes seem to be the predominant uPAR-expressing cell types. In blood, the expression of uPAR is strongly increased upon activation of neutrophils [23], monocytes [24], T cells [25]. uPAR is also expressed by hematopoietic stem/progenitor cells [26].

A wide variety of human and mouse cancers and most transformed cells overexpress uPAR [27,28]. It is striking that uPAR expression is increased in many pathological conditions, in particular cancer, inflammation and infections [29]. While in most cases this is probably due to activation of transcription factors, for

* Corresponding authors. Address: Unit on Transcriptional Regulation in Development and Cancer, IFOM (Fondazione Istituto FIRC di Oncologia Molecolare), IFOM-IEO-Campus, Via Adamello 16, 20139 Milano, Italy. Fax: +39 0226434844 (F. Blasi).
*E-mail addresses:* Francesco.blasi@ifom-ieo-campus.it (F. Blasi), nicolai.sidenius@ifom-ieo-campus.it (N. Sidenius).

0014-5793/$36.00 © 2009 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.febslet.2009.12.039

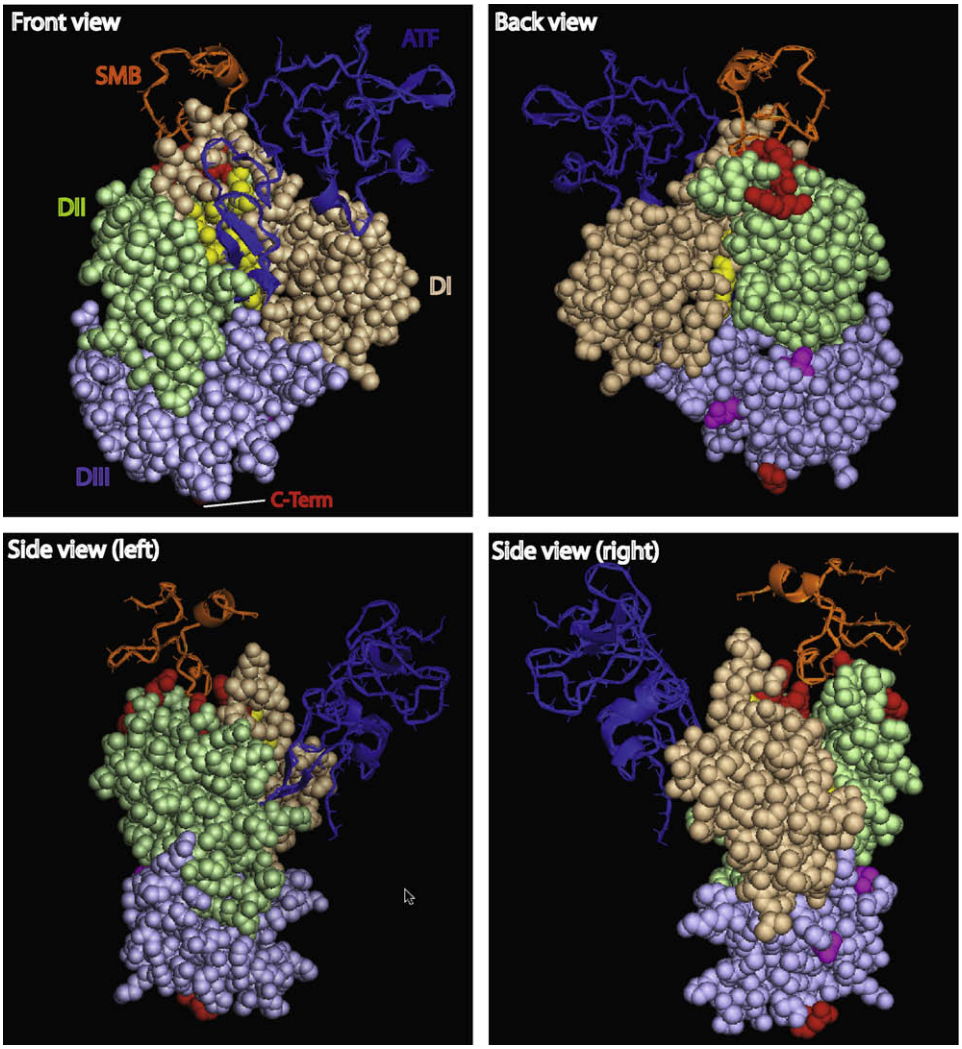


**Fig. 1.** Topology of the ternary complex between uPAR, uPA and vitronectin. The crystal structure of uPAR (atoms shown as spheres) with residues belonging to domains DI, DII and DIII color-coded wheat, pale-green and pale-blue, respectively. The amino-terminal fragment of uPA (ATF) and the somatomedin B domain of vitronectin (SMB) are shown as ribbons and colored blue and orange. Selected residues in uPAR important for VN binding (W32, R58, I63, R91 and Y92) and uPA-binding (L31, R53, L55, Y57, T64 and L66) are colored red and yellow, respectively. Two residues implicated in the interaction between uPAR and integrins (S245 and D262) are shown in purple. The structure has been oriented so that the C-terminal residue in the uPAR structures points downward and the SMB domain upwards (i.e. towards the ECM). Note that the interaction sites for ATF and SMB are entirely non-overlapping and that there is no molecular contact between these two polypeptides. The images were constructed using the coordinates deposited in the Protein Data Bank (PDB) with the code number 3BT2 and the MacPyMOL software (http://www.pymol.sourceforge.net).

example Sp1 [30], it should be noted that in at least two types of cancer, ductal pancreatic cancer and breast carcinomas, the uPAR gene is frequently amplified [31,32].

### *1.4. uPAR function*

#### *1.4.1. Proteolytic functions*

Coherent with uPA being a protease, uPAR is involved in the regulation of extracellular proteolysis because it promotes cell-surface activation of plasminogen, generating plasmin [33]. Connected to its role in extracellular proteolysis, uPAR also mediates the internalization of inactive complexes between uPA and the inhibitory serpins PAI-1 and PN-1 [34,35] in cooperation with members of the low-density lipoprotein receptor family [36]. This leads to the degradation of the uPA:inhibitor complexes in the lysosome and the subsequent recycling of uPAR to the cell surface [36]. This allows the generation and regeneration (after uPAR recycling) of active cell surface-bound plasmin and hence the spatial focusing of extracellular proteolysis. For this reason uPAR was immediately proposed as an important regulator of the invasive properties of cancer cells [37].

#### *1.4.2. Non-proteolytic uPAR functions*

In addition to extracellular proteolysis, many biological activities of the receptor occur independently of the protease activity of uPA and/or are activated by over-expression of the receptor even in the absence of uPA. These functions are largely related to the regulation of the interactions between the cells and the surrounding extracellular matrix (ECM).

uPAR interacts functionally with matrix vitronectin (VN), adhesion receptors of the integrin family and G protein-coupled receptors. uPAR and integrins cooperate in migration of monocytes [38], fibrosarcoma HT1080 [39], melanoma [40], MCF-7 breast cancer [41], fibroblasts [42] and many other cells. Moreover, both anti-uPAR and anti-integrins antibodies inhibit cell migration induced by uPA [42]. Finally, inhibitors of G protein-coupled receptors, such as pertussis toxin, also inhibit uPA-induced migration [43]. This activity may be at least in part due to the cleavage between do-

mains I and II of uPAR, which generates an SRSRY amino-terminus. This peptide has chemotactic, pertussis-toxin sensitive, activity, induces ERK1/2 phosphorylation and might be expected to be a ligand of a G protein-coupled receptor. Indeed, it has been shown that the family of formyl peptide G proteins-coupled receptors (FPR and FPRL) is involved in mediating uPA-induced migration and is highly sensitive to SRSRY-peptides [44]. Likewise, the DIIDIII-fragment of uPAR is a potent chemoattractant for several different cell lines [43,45], most likely via p56/59hck and ERK1/2 phosphorylation. Inhibitors of tyrosine kinases or of heterotrimeric G proteins block the chemotactic response to DIIDIII and the induction of phosphorylation of p56/59hck. Indeed, DIIDIII has been reported to interact directly with, and to signal through the FPRL1 chemokine receptor inducing p56/59hck phosphorylation [44]. Likewise, FPR receptors appear to respond in chemotaxis to DIIDIII-derived peptides in human hematopoietic stem cells [46].

Over-expression of uPAR promotes cell spreading, migration and invasion in fibroblasts and several different tumor cell lines, and is mediated by the extracellular matrix protein VN [47–49]. This activity is triggered by a direct interaction between uPAR and matrix VN [48], requires integrin dependent signaling and results in p130Cas-Crk and DOCK180 dependent Rac activation [47,49]. It has been concluded that the interaction between uPAR and VN may be necessary and sufficient for uPAR to modulate cell shape changes and signaling. Indeed, all alanine substitutions which affect this biological activity of the receptor also display reduced VN binding [48]. Furthermore, a chimeric membrane-anchored PAI-1 molecule mimics uPAR function recapitulating VN-adhesion and uPAR signaling activity, even though these two proteins display no structural homology [48].

As regulator of proliferation, uPAR over-expression constitutively activates the EGFR pathway in many human cancer cell lines [50]. This correlates well with the over-expression of uPAR in many human cancers [51]. In these cell lines, uPAR over-expression activates EGF Receptor in the absence of EGF and induces an unbalance between p38 and p42/44. The balance between pro-apoptotic p38MAPK and the proliferation activating ERK1/2 is shifted in favor of the ERK1/2, and results in constitutive cycling. On the contrary, down-regulation of uPAR reduces the malignancy of cancer cell lines and induces a state of dormancy [52,53]. These data agree with the phenotype of the uPAR Ko mouse keratinocytes in which the EGFR cannot be activated by EGF, resulting in deficient proliferation [54]. However, the overall role of uPAR in proliferation must be more complex and may be cell-type specific, since, unlike keratinocytes, uPAR Ko embryo fibroblasts proliferate faster than wt and display a stronger tumorigenic activity upon transformation with Ras and Myc [55].

## 2. Regulation of uPAR activity

### 2.1. Regulation of uPAR activity by receptor shedding and cleavage

Two types of post-translational modifications are believed to regulate uPAR location and activity globally and irreversibly: uPAR ‘‘shedding” and uPAR ‘‘cleavage”. These events affect uPAR activity as a whole as they completely, and irreversibly, change the location and/or destroy or activate a given activity of the receptor. Whereas uPAR shedding releases the entire protein moiety from the cell surface generating soluble uPAR (suPAR), uPAR cleavage causes the release of the N-terminal domain (DI) from the rest of the receptor (DIIDIII). So, uPAR shedding solubilizes uPAR reducing the number of receptors on the cell surface, while uPAR cleavage removes the essential D1, inactivating the binding to most ligands. These two modifications may occur individually or together on a single uPAR molecule generating (at least) 4 distinct forms of uPAR in addition to the native receptor: suPAR, GPI-anchored DIIDIII, soluble DIIDIII (sDIIDIII) and the free DI fragment. Each of these forms of uPAR has different biological activity and has been found both in vitro and in vivo [56,57].

uPAR shedding occurs either by the action of a phospholipase such as phosphatidylinositol-specific phospholipase D (GPI-PLD) [58], or by proteolytic cleavage of the polypeptide chain close to the GPI-anchor. Several proteases, including plasmin, tissue kallikrein 4 and bacterial metalloproteinases, are able to cleave synthetic peptides derived from the juxtamembrane region of uPAR [59–61]. All cell surface activities of uPAR (i.e. plasmin generation, internalization of the uPA:serpin complexes, cell adhesion to VN, regulation of integrin-function, etc.) are reduced by uPAR shedding. Furthermore, released soluble uPAR and uPAR fragments can be biologically active and may function in a remote paracrine way. Soluble uPAR displays intact uPA binding and may act as an uPA-scavenger. Moreover, suPAR can interfere with cellular uPAR functions, for example with integrins, inhibiting the activity of cell surface uPAR [62]. Other forms of soluble uPAR, like the DIIDIII-fragment, display potent chemotactic activities [43,63].

The second type of uPAR hydrolysis, referred to as uPAR ‘‘cleavage”, is a proteolytic event in the linker region connecting domains I and II of uPAR resulting in the generation of two uPAR fragments known as DI and DIIDIII. The cleavage releases the DI fragment from the cells, but the DIIDIII-fragment may either remain associated with the cell membrane or be released from the cell surface by receptor shedding as described above. The linker region connecting DI and DIIDIII in uPAR is prone to hydrolysis by a variety of proteases including uPA, plasmin, neutrophil elastase, and by a number of different matrix metalloproteinases (MMPs) [64–66]. While cleavage of purified soluble uPAR by uPA is an inefficient process that does not require the high-affinity interaction between the two molecules [64], cleavage of cell surface uPAR by uPA is accelerated and requires binding of uPA to uPAR [67]. Two explanations for the accelerated cleavage of cell surface uPAR by uPA have been proposed. First, it has been suggested that the exposure of the linker region connecting DI and DII is different in soluble and GPI-anchored uPAR [66,68]. Second, dimerization and/or clustering of uPAR in specific lipid membrane domains known as lipid rafts may position the catalytic domain of bound uPA in a favorable position for the cleavage of flanking receptor molecules [69]. Independently of the responsible enzyme and mechanism, cleavage strongly affects the biological activity of uPAR. On one hand, the physical separation of the DI and DIIDIII-fragments practically abolishes uPA and VN binding, the lateral association with integrins and consequently the biological activity of uPAR in both extracellular proteolysis and cell signaling via cell adhesion. On the other hand, uPAR cleavage generates fragments endowed with strong, *bona-fide* chemokine-like activities in a variety of cell systems.

### 2.2. The uPAR interactome and its regulation

As a GPI-anchored receptor molecule, the signaling activity of uPAR relies on its interaction with other proteins. Since its discovery about twenty years ago, a wide variety of uPAR interactors have been reported in the literature. Based on the level of evidence available, these interactors may be divided into two groups. The first group is formed by the serine protease uPA and the extracellular matrix protein VN, which may be considered the ‘‘core” uPAR ligands for which extensive, independent and coherent biological, biochemical and structural evidence is available. Recently, it has been proposed that this ‘‘ménage à trois” between uPAR, uPA and VN may be sufficient to explain most, or all, of the pleiotropic cellular effect of uPAR [48,70].

The second group of interactors encompasses a long series of proteins for which the directness of the interactions as well as their structural basis is poorly understood. Nevertheless, much evidence has accumulated that the physical and functional interaction of uPAR with this group of proteins is an essential part of the biology of uPAR (reviewed in [29,71,72]). In brief, uPAR functionally interacts with a variety of receptor tyrosine kinases, like EGFR and PDGFR [50,54,73,74]; a series of integrins (reviewed in [75]); caveolin [76]; and receptors of the low-density lipoprotein receptor family including the LDL receptor-related protein (LRP) and LRP1B [36,77,78]. Certain forms of uPAR (i.e. the soluble DIIDIII-fragment) interact with the G-protein-coupled receptors FPR, FPRL1 and FPRL2 [44,79]. In addition, uPAR has been shown to associate with the cation-independent Mannose 6-phosphate/insulin-like growth factor-II receptor (CIMPR/IGF-II receptor) that has been implicated in the targeting of uPAR to lysosomes [80]. Finally, by chemical cross-linking uPAR has also been shown to associate with the collagen receptor uPARAP/Endo180 [81] in a process that requires the contemporary binding of pro-uPA.

As a consequence of the above listed interactions, uPAR activates various intracellular signaling molecules such as the tyrosine kinase Src, the serine kinase Raf, focal adhesion kinase (FAK), p130Cas and extracellular-signal-regulated kinase (ERK)/mitogen-activated protein kinase (MAPK), among others. Activation of these proteins results in profound changes in cell proliferation, adhesion and migration.

### 2.2.1. The uPA/uPAR interaction

*2.2.1.1. Structural basis for the interaction between uPA and uPAR.* The high-affinity binding (Kd in the low nanomolar range) of uPA to cells [1,2] led to the identification, purification and cloning of uPAR [3,4]. This interaction has been extensively studied at the biochemical level on purified proteins (reviewed in [7]). Crystal structures of uPAR in complex with an antagonistic peptide or with the receptor-binding part of uPA (the amino-terminal fragment, ATF) have been solved [82–84]. The two proteins interact through the N-terminal growth-factor-like domain (GFD) of uPA [85] and a large hydrophobic binding pocket involving residues from all three domains of uPAR (recently reviewed in [8]). The extended nature of the uPA/uPAR-interface renders the affinity of the interaction relatively insensitive to single amino-acid substitutions in uPAR, but highly dependent upon the intact three-domain structure of the receptor [86,87]. The structure of uPAR and its interaction with uPA is presented in Fig. 1.

*2.2.1.2. Regulation of the uPA/uPAR interaction.* As a receptor for uPA, uPAR may be considered "constitutively active" as high-affinity binding occur without the need for any additional co-factors. The interaction however requires the intact three-domain structure of uPAR explaining why cleavage of uPAR in the linker region connecting DI and DII is an irreversible inhibitory event. Cleavage of uPAR by uPA might act as a negative-feedback mechanism in extracellular proteolysis, although the actual occurrence and relevance of this feedback still has to be determined. In addition to uPAR cleavage, the affinity of the interaction with uPA is moderately dependent upon expression levels and on the type and degree of uPAR glycosylation [88,89]. The existence of intact cell surface uPAR incapable of uPA binding [90] has been reported, suggesting that poorly understood "cryptic" forms of the receptor may also exist.

### 2.2.2. The uPAR/VN interaction

*2.2.2.1. Structural basis for the uPAR/VN interaction.* The discovery of VN as a ligand for uPAR came from the observation that the adhesion of stimulated monocytes to serum-coated surfaces is enhanced by ligand-occupancy of uPAR [91,92]. Fractionation of serum identified VN as the component responsible for the increased adhesion [92], and several lines of evidence confirmed uPAR to be the responsible membrane receptor [93]. In contrast to integrin binding, the interaction of uPAR with VN does not require divalent cations and does not involve the RGD-motif present in this extracellular matrix protein.

The X-ray structure of the ternary complex between uPAR, ATF and the somatomedin B (SMB) domain of VN has been determined [94] and is in good accordance with the major findings of two independent and complete, alanine scans of uPAR [48,95]. Although initial experimentation pointed towards an interaction between regions within DII/DIII of uPAR [93,96] and the heparin binding domain of VN [97] there is now compelling evidence that the interaction is entirely mediated by a composite epitope exposed on the DI/DII interface of uPAR and the N-terminal somatomedin B domain of VN (reviewed in [8]). Although more than 30 different alanine substitutions noticeably impair uPAR-mediated cell binding to VN only a handful of these do so also in the presence of uPA [48]. Two of these residues, W32 and R91, are located in the uPAR:SMB interface of the crystal structure [94] (see Fig. 1) and their substitution with alanine results in particularly low VN binding [48,95]. The W32A and R91A mutations both display normal uPA binding affinity [48,87] and thus represent excellent candidate mutations for use in structure function analyses aimed at understanding the physiological importance of the uPAR/VN interaction. Both the W32A and R91A uPAR mutants do however display some residual VN binding [48,95] and care should be taken in using these mutants to document the existence of VN independent uPAR functions [98].

Importantly, the SMB domain of VN also harbors an overlapping high-affinity binding site for PAI-1 and is located adjacent to the RGD motif mediating integrin binding [99]. Indeed, several alanine substitutions in the SMB domain of VN impair not only uPAR [87,100] but also PAI-1 binding to the same domain, rendering them of little use in structure–function studies in biological systems where PAI-1 may be present. The identification of mutations in the SMB domain that selectively impair uPAR and/or PAI-1 binding would greatly facilitate future studies aimed at addressing the relative importance of these two interactions in the biology of uPAR.

*2.2.2.2. Regulation of the uPAR/VN interaction.* As for uPA, the binding of VN to uPAR requires the intact three-domain structure of the receptor [101,102]. This is explained by the fact that the binding epitope for VN in uPAR involves residues in both DI and DII [94]. The binding of soluble recombinant uPAR to immobilized VN is a high-affinity interaction (Kd in the low nanomolar range) and is strongly dependent upon concomitant uPA binding [93,95,103]. On the contrary the binding of VN to immobilized uPAR is rather low affinity (1 μM range) and only moderately affected by uPA binding [95].

The high-affinity interaction between uPAR and VN has been suggested to require uPAR dimerization and/or oligomerization [69,104,105]. Although binding experiments using purified proteins strongly suggest that uPA regulates VN binding by controlling uPAR dimerization [104] the data are not entirely conclusive. First, while the model used to explain the uPA dose-dependence of uPAR binding to VN predicts that dimerization is a high-affinity reaction, complexes containing dimeric uPAR cannot readily be detected by gel filtration [104]. Second, the model, as well as the experimental evidence, indicate that the high-affinity VN binding complex between uPAR and uPA has a 2:1 stoichiometry [104] and not the 1:1 ratio observed in the uPAR:ATF:SMB crystal structure [94].

In contrast to uPA, VN binding to uPAR is thus a highly regulated and complex process. In its native state uPAR displays no or little

VN binding. However, uPA binding, receptor oligomerization and partitioning to discrete membrane domains trigger VN binding.

*2.2.3. The interaction between uPAR and integrins*

*2.2.3.1. Identification of the uPAR–integrin interaction.* The interaction of uPAR with integrins was originally demonstrated by the co-immunoprecipitation of uPAR and integrins in cell extracts [106]. The isolation of an uPAR-binding peptide from a phage library [107] that disrupted both co-immunoprecipitation with integrins and VN-adhesion, provided functional significance to the interaction [108]).

*2.2.3.2. Structural basis of the uPAR/integrin interaction.* The original phage derived uPAR:integrin antagonistic peptide P25 [108] displays some homology to a linear sequence present in the propeller domain of the $\alpha_M$ chain of Mac-1. An integrin peptide (called M25) derived from this sequence was likewise found to bind uPAR and block uPAR:integrin co-immunoprecipitation [62]. The corresponding peptide from the α3 integrin chain (called α325) was also found to bind uPAR and block its interaction with this integrin [109]. Comparisons of the three peptide sequences reveal that even though there is clear homology between P25 and M25, as well as between M25 and α325, there is only *one* residue which is conserved in all three peptides. This is remarkable as all three peptides are reported to bind uPAR and have essentially the same biological activity. Coherently with the predicted importance of the histidine residue common to the three peptides [110], a single alanine substitution (H245A in α3) is sufficient to abolish the biological activity of uPAR in α3β1-dependent mesenchymal transition [110]. Several studies have also evidenced a strong functional interaction between uPAR and the fibronectin (FN) receptor α5β1 [111,112] as well as with the VN receptors αvβ3 [49,113] and αvβ5 [114]. However, both α5 and αV chains lack this critical histidine residue [109], suggesting that uPAR may interact with these integrins in a different way. In support of such alternative interactions, peptides derived from the β1 integrin sequence, as well as a specific β1 mutant (S227A), impair both the physical and functional association between uPAR and α5β1 [112].

Attempts to identify the regions of uPAR involved in the interaction with integrins have been published [113,115,116] and uPAR mutants with deficient integrin interaction(s) have been reported [115,116]. The uPAR residues implicated in the interaction with integrins are: E134, E135, S245, H249 and D262. The residues identified in these three studies however do not point towards a single coherent binding site in uPAR but rather suggest the existence of multiple and diverse binding sites. In this context it should be noted that a comprehensive study aimed specifically at the unbiased functional identification of the integrin binding site in uPAR failed to detect any such site and also excluded all the previously identified sites [48]. Hence, the wealth of evidence underlying the concept of integrin–uPAR interaction is still in need of a convincing structural basis.

*2.2.3.3. Regulation of the uPAR/integrin interaction.* Little is known about how uPAR–integrin interactions are regulated. As for VN binding the association between these receptors requires the intact 3-domain structure of uPAR [117] and is promoted by uPA binding [62,112,118]. Binding of ligand to the integrin also seems to favor the interaction [50,112].

*2.2.4. The homotypic uPAR interaction*

The existence and functional relevance of uPAR dimerization was initially deduced from the peculiar biphasic uPA dose-dependence of suPAR binding to immobilized VN [95,104] which can be accurately explained only if the high-affinity VN binding form of uPAR is a dimer [104]. Indeed, on the surface of living cells uPAR dimerizes as evidenced by chemical cross linking [69], photon counting histogram (PCH, [105,119] and fluorescence energy transfer (FRET, [105]).

Self-association of uPAR can be demonstrated in vitro by co-immunoprecipitation experiments using differentially tagged suPAR molecules. Under these conditions the process is regulated by uPA binding and displays a dose-dependence very similar to that observed for VN binding. In living cells dimeric uPAR is preferentially located in detergent insoluble membrane domains, i.e. lipid rafts, suggesting that membrane partitioning may also regulate dimerization [69]. The cause/consequence connection between uPAR dimerization and lipid raft association is however not clear.

Although the structural basis for uPAR oligomerization is still unknown, some data suggest that the hydrophobic uPA binding cavity of the receptor may be involved. Indeed, complete saturation of the receptors with uPA actually reduces binding of suPAR to VN [95,104] as well as uPAR-uPAR co-immunoprecipitation [104]. However, the VN:uPAR:uPA high-affinity complex is no longer inhibited by excess uPA, suggesting that the binding cavity on both uPAR molecules in this complex are occupied [104]. In agreement with this possibility, a large number of the residues implicated in uPA-independent uPAR-mediated cell binding to VN (L31, R53, L55, Y57, T64, L66 and E68) have their side chains exposed in the uPA binding cavity of uPAR [48].

## 3. Dynamics of uPAR membrane localization

### *3.1. uPAR internalization and recycling*

As a cell surface receptor, uPAR is normally located at the external leaflet of the plasma membrane [11]. However, in certain cell types, namely neutrophils, uPAR may be predominantly present in intracellular secretory vesicles and is exposed at the cell surface only upon cell activation [23]. Although predominantly found on the plasma membrane, uPAR localization is regulated in a highly dynamic way by interactions with ligands and other membrane receptors. Binding of uPA:serpin complexes to uPAR results in the formation of quaternary complexes with members of the LRP family [36], which are internalized by clathrin-mediated endocytosis [77]. In this process, the uPA:serpin complexes are degraded in the lysosomes while uPAR recycles back to the plasma membrane [120]. Also in the absence of uPA:serpin complexes the location of uPAR on the cell surface is modulated by at least LRP1b [78] as well as by the cation-independent Mannose 6-phosphate/insulin-like growth factor-II receptor (CIMPR/IGF-II receptor) which may target uPAR to lysosomes [80].

It has recently been found that internalization and recycling of uPAR also takes place constitutively in the absence of ligands, through a pathway that is independent of LRP-1 and clathrin but shares some properties with macropinocytosis. The ligand-independent route does not require uPAR partitioning into lipid rafts, is amiloride-sensitive, independent of the activity of small GTPases RhoA, Rac1 and Cdc42, and does not require PI3K. Constitutively endocytosed uPAR is found in EEA1 positive early/recycling endosomes but does not reach lysosomes in the absence of ligands. Electron microscopy analysis reveals the presence of uPAR in ruffling domains at the cell surface, within macropinosome-like vesicles, and in endosomal compartments [121].

### *3.2. uPAR membrane partitioning*

In the plasma membrane, uPAR partitions in both lipid rafts and more fluid membrane regions [69]. While monomeric uPAR is mainly located in detergent soluble (DS) membrane domains,

dimeric uPAR is preferentially associated with detergent resistant (DRS) membranes or lipid rafts [69]. In detergent resistant membrane (DRM) fractions, uPAR is associated with an environment whose glycosphingolipid composition is different from the average composition of the plasma membrane, as shown by glycosphingolipid analysis of immunoprecipitated uPAR [122]. Moreover, the amount of uPAR found in the DRM changes in the presence of ligands along with the nature of the lipid environment. Indeed, in the absence of ligands the environment is very similar to that of total DRM, enriched in sphingomyelin and glycosphingolipids. However, after treatment of cells with uPA the lipid environment is strongly impoverished of neutral glycosphingolipids [122]. Unlike signaling, however, lipid rafts association is not involved in ligand-dependent or constitutive uPAR internalization.

## 4. Conclusions

Twenty years of intensive research by many laboratories have underscored the importance of uPAR and its ligands in a variety of biological phenomena. Interestingly the requirement for uPAR is not observed under normal conditions (for example in KO animals in a mouse facility). However, uPAR requirement and function becomes obvious under pathological circumstances, like acute and chronic inflammation, infections, tumorigenesis and induced hematopoietic stem cells mobilization, or under conditions of tissue remodeling or reconstruction. Despite the many investigations over the last 24 years, and despite the solution of uPAR tertiary structure, we are still missing crucial information necessary to understand the molecular basis of its function. Although this is surprising, our feeling is that it reflects its involvement in an hitherto unrecognized general mechanism regulating the coupling of cells to extracellular matrix and influencing cell signaling. The next years will undoubtedly solve some of these mysteries.

## References


[1] Vassalli, J.D., Baccino, D. and Belin, D. (1985) A cellular binding site for the Mr 55,000 form of the human plasminogen activator, urokinase. J. Cell Biol. 100, 86–92.

[2] Stoppelli, M.P., Corti, A., Soffientini, A., Cassani, G., Blasi, F. and Associan, R.K. (1985) Differentiation-enhanced binding of the aminoterminal fragment of human urokinase plasminogen activator to a specific receptor on U937 monocytes. PNAS 82, 4939–4943.

[3] Nielsen, L.S., Kellerman, G.M., Behrendt, N., Picone, R., Dan, K. and Blasi, F. (1988) A 55,000–60,000 Mr receptor protein for urokinase-type plasminogen activator. Identification in human tumor cell lines and partial purification. J. Biol. Chem. 263, 2358–2363.

[4] Roldan, A.L., Cubellis, M.V., Masucci, M.T., Behrendt, N., Lund, L.R., Danø, K., Appella, E. and Blasi, F. (1990) Cloning and expression of the receptor for human urokinase plasminogen activator, a central molecule in cell surface, plasmin dependent proteolysis. EMBO J. 9, 467–474 (published erratum appears in EMBO J. 9 (5) (1990) 1674).

[5] Ploug, M., Rønne, E., Behrendt, N., Jensen, A.L., Blasi, F. and Danø, K. (1991) Cellular receptor for urokinase plasminogen activator. Carboxyl-terminal processing and membrane anchoring by glycosyl-phosphatidylinositol. J. Biol. Chem. 266, 1926–1933.

[6] Ploug, M. and Ellis, V. (1994) Structure–function relationships in the receptor for urokinase-type plasminogen activator. Comparison to other members of the Ly-6 family and snake venom alpha-neurotoxins. FEBS Lett. 349, 163–168.

[7] Ploug, M. (2003) Structure–function relationships in the interaction between the urokinase-type plasminogen activator and its receptor. Curr. Pharm. Des. 9, 1499–1528.

[8] Kjaergaard, M., Hansen, L.V., Jacobsen, B., Gardsvoll, H. and Ploug, M. (2008) Structure and ligand interactions of the urokinase receptor (uPAR). Front. Biosci. 13, 5441–5461.

[9] Bianchi, E., Ferrero, E., Fazioli, F., Mangili, F., Wang, J., Bender, J.R., Blasi, F. and Pardi, R. (1996) Integrin-dependent induction of functional urokinase receptors in primary T lymphocytes. J. Clin. Invest. 98, 1133–1141.

[10] Lund, L.R., Eriksen, J., Ralfkiaer, E. and Rømer, J. (1996) Differential expression of urokinase-type plasminogen activator, its receptor, and inhibitors in mouse skin after exposure to a tumor-promoting phorbol ester. J. Invest. Dermatol. 106, 622–630.

[11] Solberg, H., Ploug, M., Hoyer-Hansen, G., Nielsen, B.S. and Lund, L.R. (2001) The murine receptor for urokinase-type plasminogen activator is primarily expressed in tissues actively undergoing remodeling. J. Histochem. Cytochem. 49, 237–246.

[12] Marschall, C. et al. (1999) UVB increases urokinase-type plasminogen activator receptor (uPAR) expression. J. Invest. Dermatol. 113, 69–76.

[13] Pyke, C., Ralfkiaer, E., Rønne, E., Høyer-Hansen, G., Kirkeby, L. and Danø, K. (1994) Immunohistochemical detection of the receptor for urokinase plasminogen activator in human colon cancer. Histopathology 24, 131–138.

[14] Lengyel, E., Wang, H., Stepp, E., Juarez, J., Wang, Y., Doe, W., Pfarr, C.M. and Boyd, D. (1996) Requirement of an upstream AP-1 motif for the constitutive and phorbol ester-inducible expression of the urokinase-type plasminogen activator receptor gene. J. Biol. Chem. 271, 23176–23184.

[15] Hapke, S. et al. (2001) Beta(3)A-integrin downregulates the urokinase-type plasminogen activator receptor (u-PAR) through a PEA3/ets transcriptional silencing element in the u-PAR promoter. Mol. Cell. Biol. 21, 2118–2132.

[16] Schewe, D.M., Biller, T., Maurer, G., Asangani, I.A., Leupold, J.H., Lengyel, E.R., Post, S. and Allgayer, H. (2005) Combination analysis of activator protein-1 family members, Sp1 and an activator protein-2alpha-related factor binding to different regions of the urokinase receptor gene in resected colorectal cancers. Clin. Cancer Res. 11, 8538–8548.

[17] Schewe, D.M. et al. (2003) Tumor-specific transcription factor binding to an activator protein-2/Sp1 element of the urokinase-type plasminogen activator receptor promoter in a first large series of resected gastrointestinal cancers. Clin. Cancer Res. 9, 2267–2276.

[18] Lund, L.R., Ellis, V., Rønne, E., Pyke, C. and Danø, K. (1995) Transcriptional and post-transcriptional regulation of the receptor for urokinase-type plasminogen activator by cytokines and tumour promoters in the human lung carcinoma cell line A549. Biochem. J. 310, 345–352.

[19] Wang, G.J., Collinge, M., Blasi, F., Pardi, R. and Bender, J.R. (1998) Posttranscriptional regulation of urokinase plasminogen activator receptor messenger RNA levels by leukocyte integrin engagement. Proc. Natl. Acad. Sci. USA 95, 6296–6301.

[20] Shetty, S., Kumar, A. and Idell, S. (1997) Posttranscriptional regulation of urokinase receptor mRNA: identification of a novel urokinase receptor mRNA binding protein in human mesothelioma cells. Mol. Cell. Biol. 17, 1075–1083.

[21] Shetty, S. and Idell, S. (2004) Urokinase receptor mRNA stability involves tyrosine phosphorylation in lung epithelial cells. Am. J. Respir. Cell. Mol. Biol. 30, 69–75.

[22] Sasayama, T., Nishihara, M., Kondoh, T., Hosoda, K. and Kohmura, E. (2009) MicroRNA-10b is overexpressed in malignant glioma and associated with tumor invasive factors, uPAR and RhoC. Int. J. Cancer 125, 1407–1413.

[23] Plesner, T. et al. (1994) The receptor for urokinase-type plasminogen activator and urokinase is translocated from two distinct intracellular compartments to the plasma membrane on stimulation of human neutrophils. Blood 83, 808–815.

[24] Min, H.Y., Semnani, R., Mizukami, I.F., Watt, K., Todd III, R.F. and Liu, D.Y. (1992) CDNA for Mo3, a monocyte activation antigen, encodes the human receptor for urokinase plasminogen activator. J. Immunol. 148, 3636–3642.

[25] Nykjaer, A., Moller, B., Todd III, R.F., Christensen, T., Andreasen, P.A., Gliemann, J. and Petersen, C.M. (1994) Urokinase receptor. An activation antigen in human T lymphocytes. J. Immunol. 152, 505–516.

[26] Tjwa, M. et al. (2009) Membrane-anchored uPAR regulates the proliferation, marrow pool size, engraftment, and mobilization of mouse hematopoietic stem/progenitor cells. J. Clin. Invest. 119, 1008–1018.

[27] Sidenius, N. and Blasi, F. (2003) The urokinase plasminogen activator system in cancer: recent advances and implications for prognosis and therapy. Cancer Metast. Rev. 22, 205–222.

[28] Rasch, M.G., Lund, I.K., Almasi, C.E. and Hoyer-Hansen, G. (2008) Intact and cleaved uPAR forms: diagnostic and prognostic value in cancer. Front. Biosci. 13, 6752–6762.

[29] Blasi, F. and Carmeliet, P. (2002) UPAR: a versatile signalling orchestrator. Nat. Rev. Mol. Cell. Biol. 3, 932–943.

[30] Zannetti, A. et al. (2000) Coordinate up-regulation of Sp1 DNA-binding activity and urokinase receptor expression in breast carcinoma. Cancer Res. 60, 1546–1551.

[31] Meng, S. et al. (2006) UPAR and HER-2 gene status in individual breast cancer cells from blood and tissues. Proc. Natl. Acad. Sci. USA 103, 17361–17365.

[32] Hildenbrand, R., Niedergethmann, M., Marx, A., Belharazem, D., Allgayer, H., Schleger, C. and Strobel, P. (2009) Amplification of the urokinase-type plasminogen activator receptor (uPAR) gene in ductal pancreatic carcinomas identifies a clinically high-risk group. J. Am. Pathol. 174, 2246–2253.

[33] Ellis, V., Pyke, C., Eriksen, J., Solberg, H. and Dano, K. (1992) The urokinase receptor: involvement in cell surface proteolysis and cancer invasion. Ann. N.Y. Acad. Sci. 667, 13–31.

[34] Cubellis, M.V., Wun, T.C. and Blasi, F. (1990) Receptor-mediated internalization and degradation of urokinase is caused by its specific inhibitor PAI-1. EMBO J. 9, 1079–1085.

[35] Conese, M., Olson, D. and Blasi, F. (1994) Protease nexin-1-urokinase complexes are internalized and degraded through a mechanism that requires both urokinase receptor and alpha 2-macroglobulin receptor. J. Biol. Chem. 269, 17886–17892.

[36] Nykjaer, A. et al. (1992) Purified alpha 2-macroglobulin receptor/LDL receptor-related protein binds urokinase plasminogen activator inhibitor type-1 complex. Evidence that the alpha 2-macroglobulin receptor mediates cellular degradation of urokinase receptor-bound complexes. J. Biol. Chem. 267, 14543–14546.

[37] Blasi, F., Vassalli, J.D. and Danø, K. (1987) Urokinase-type plasminogen activator: proenzyme, receptor, and inhibitors. J. Cell Biol. 104, 801–804.
[38] Gyetko, M.R., Todd III, R.F., Wilkinson, C.C. and Sitrin, R.G. (1994) The urokinase receptor is required for human monocyte chemotaxis in vitro. J. Clin. Invest. 93, 1380–1387.
[39] Xue, W., Mizukami, I., Todd III, R.F. and Petty, H.R. (1997) Urokinase-type plasminogen activator receptors associate with beta1 and beta3 integrins of fibrosarcoma cells: dependence on extracellular matrix components. Cancer Res. 57, 1682–1689.
[40] Yebra, M., Parry, G.C.N., Strömblad, S., Mackman, N., Rosenberg, S., Mueller, B.M. and Cheresh, D.A. (1996) Requirement of receptor-bound urokinase-type plasminogen activator for integrin alphavbeta5-directed cell migration. J. Biol. Chem. 271, 29393–29399.
[41] Nguyen, D.H., Hussaini, I.M. and Gonias, S.L. (1998) Binding of urokinase-type plasminogen activator to its receptor in MCF-7 cells activates extracellular signal-regulated kinase 1 and 2 which is required for increased cellular motility. J. Biol. Chem. 273, 8502–8507.
[42] Degryse, B., Resnati, M., Rabbani, S.A., Villa, A., Fazioli, F. and Blasi, F. (1999) Src-dependence and pertussis-toxin sensitivity of urokinase receptor-dependent chemotaxis and cytoskeleton reorganization in rat smooth muscle cells. Blood 94, 1–15.
[43] Resnati, M., Guttinger, M., Valcamonica, S., Sidenius, N., Blasi, F. and Fazioli, F. (1996) Proteolytic cleavage of the urokinase receptor substitutes for the agonist-induced chemotactic effect. EMBO J. 15, 1572–1582.
[44] Resnati, M., Pallavicini, I., Wang, J.M., Oppenheim, J., Serhan, C.N., Romano, M. and Blasi, F. (2002) The fibrinolytic receptor for urokinase activates the G protein-coupled chemotactic receptor FPRL1/LXA4R. Proc. Natl. Acad. Sci. USA 99, 1359–1364.
[45] Fazioli, F., Resnati, M., Sidenius, N., Higashimoto, Y., Appella, E. and Blasi, F. (1997) A urokinase-sensitive region of the human urokinase receptor is responsible for its chemotactic activity. EMBO J. 16, 7279–7286.
[46] Selleri, C. et al. (2006) In vivo activity of the cleaved form of soluble urokinase receptor: a new hematopoietic stem/progenitor cell mobilizer. Cancer Res. 66, 10885–10890.
[47] Kjøller, L. and Hall, A. (2001) Rac mediates cytoskeletal rearrangements and increased cell motility induced by urokinase-type plasminogen activator receptor binding to vitronectin. J. Cell Biol. 152, 1145–1157.
[48] Madsen, C.D., Ferraris, G.M., Andolfo, A., Cunningham, O. and Sidenius, N. (2007) UPAR-induced cell adhesion and migration: vitronectin provides the key. J. Cell Biol. 177, 927–939.
[49] Smith, H.W., Marra, P. and Marshall, C.J. (2008) UPAR promotes formation of the p130Cas-Crk complex to activate Rac through DOCK180. J. Cell Biol. 182, 777–790.
[50] Liu, D., Ghiso, J.A., Estrada, Y. and Ossowski, L. (2002) EGFR is a transducer of the urokinase receptor initiated signal that is required for in vivo growth of a human carcinoma. Cancer Cell 1, 445–457.
[51] Hoyer-Hansen, G. and Lund, I.K. (2007) Urokinase receptor variants in tissue and body fluids. Adv. Clin. Chem. 44, 65–102.
[52] Kook, Y.H., Adamski, J., Zelent, A. and Ossowski, L. (1994) The effect of antisense inhibition of urokinase receptor in human squamous cell carcinoma on malignancy. EMBO J. 13, 3983–3991.
[53] Yu, W., Kim, J. and Ossowski, L. (1997) Reduction in surface urokinase receptor forces malignant cells into a protracted state of dormancy. J. Cell Biol. 137, 767–777.
[54] D'Alessio, S., Gerasi, L. and Blasi, F. (2008) UPAR-deficient mouse keratinocytes fail to produce EGFR-dependent laminin-5, affecting migration in vivo and in vitro. J. Cell Sci. 121, 3922–3932.
[55] Mazzieri, R., D'Alessio, S., Kenmoe, R.K., Ossowski, L. and Blasi, F. (2006) An uncleavable uPAR mutant allows dissection of signaling pathways in uPA-dependent cell migration. Mol. Biol. Cell 17, 367–378.
[56] Sidenius, N., Sier, C.F.M. and Blasi, F. (2000) Shedding and cleavage of the urokinase receptor (uPAR): identification and characterisation of uPAR fragments in vitro and in vivo. FEBS Lett. 475, 52–56.
[57] Sier, C.F. et al. (2004) Metabolism of tumour-derived urokinase receptor and receptor fragments in cancer patients and xenografted mice. Thromb. Haemost. 91, 403–411.
[58] Wilhelm, O.G. et al. (1999) Cellular glycosylphosphatidylinositol-specific phospholipase D regulates urokinase receptor shedding and cell surface expression. J. Cell Physiol. 180, 225–235.
[59] Beaufort, N., Leduc, D., Rousselle, J.C., Magdolen, V., Luther, T., Namane, A., Chignard, M. and Pidard, D. (2004) Proteolytic regulation of the urokinase receptor/CD87 on monocytic cells by neutrophil elastase and cathepsin G. J. Immunol. 172, 540–549.
[60] Beaufort, N., Debela, M., Creutzburg, S., Kellermann, J., Bode, W., Schmitt, M., Pidard, D. and Magdolen, V. (2006) Interplay of human tissue kallikrein 4 (hK4) with the plasminogen activation system: hK4 regulates the structure and functions of the urokinase-type plasminogen activator receptor (uPAR). Biol. Chem. 387, 217–222.
[61] Leduc, D., Beaufort, N., de Bentzmann, S., Rousselle, J.C., Namane, A., Chignard, M. and Pidard, D. (2007) The *Pseudomonas aeruginosa* LasB metalloproteinase regulates the human urokinase-type plasminogen activator receptor through domain-specific endoproteolysis. Infect. Immun. 75, 3848–3858.
[62] Simon, D.I. et al. (2000) Identification of a urokinase receptor–integrin interaction site. Promiscuous regulator of integrin function. J. Biol. Chem. 275, 10228–10234.
[63] Montuori, N. and Ragno, P. (2009) Multiple activities of a multifaceted receptor: roles of cleaved and soluble uPAR. Front. Biosci. 14, 2494–2503.
[64] Høyer-Hansen, G., Rønne, E., Solberg, H., Behrendt, N., Ploug, M., Lund, L.R., Ellis, V. and Danø, K. (1992) Urokinase plasminogen activator cleaves its cell surface receptor releasing the ligand-binding domain. J. Biol. Chem. 267, 18224–18229.
[65] Koolwijk, P., Sidenius, N., Peters, E., Sier, C.F., Hanemaaijer, R., Blasi, F. and van Hinsbergh, V.W. (2001) Proteolysis of the urokinase-type plasminogen activator receptor by metalloproteinase-12: implication for angiogenesis in fibrin matrices. Blood 97, 3123–3131.
[66] Andolfo, A., English, W.R., Resnati, M., Murphy, G., Blasi, F. and Sidenius, N. (2002) Metalloproteases cleave the urokinase-type plasminogen activator receptor in the D1–D2 linker region and expose epitopes not present in the intact soluble receptor. Thromb. Haemost. 88, 298–306.
[67] Høyer-Hansen, G., Ploug, M., Behrendt, N., Rønne, E. and Danø, K. (1997) Cell-surface acceleration of urokinase-catalyzed receptor cleavage. Eur. J. Biochem. 243, 21–26.
[68] Høyer-Hansen, G., Pessara, U., Holm, A., Pass, J., Weidle, U., Danø, K. and Behrendt, N. (2001) Urokinase-catalysed cleavage of the urokinase receptor requires an intact glycolipid anchor. Biochem. J. 358, 673–679.
[69] Cunningham, O., Andolfo, A., Santovito, M.L., Iuzzolino, L., Blasi, F. and Sidenius, N. (2003) Dimerization controls the lipid raft partitioning of uPAR/CD87 and regulates its biological functions. EMBO J. 22, 5994–6003.
[70] Madsen, C.D. and Sidenius, N. (2008) The interaction between urokinase receptor and vitronectin in cell adhesion and signalling. Eur. J. Cell Biol. 87, 617–629.
[71] Ragno, P. (2006) The urokinase receptor: a ligand or a receptor? Story of a sociable molecule. Cell. Mol. Life Sci. 63, 1028–1037.
[72] Tang, C.H. and Wei, Y. (2008) The urokinase receptor and integrins in cancer progression. Cell. Mol. Life Sci. 65, 1916–1932.
[73] Kiyan, J., Kiyan, R., Haller, H. and Dumler, I. (2005) Urokinase-induced signaling in human vascular smooth muscle cells is mediated by PDGFR-beta. EMBO J. 24, 1787–1797.
[74] Jo, M., Thomas, K.S., Takimoto, S., Gaultier, A., Hsieh, E.H., Lester, R.D. and Gonias, S.L. (2007) Urokinase receptor primes cells to proliferate in response to epidermal growth factor. Oncogene 26, 2585–2594.
[75] Kugler, M.C., Wei, Y. and Chapman, H.A. (2003) Urokinase receptor and integrin interactions. Curr. Pharm. Des. 9, 1565–1574.
[76] Wei, Y., Yang, X., Liu, Q., Wilkins, J.A. and Chapman, H.A. (1999) A role for caveolin and the urokinase receptor in integrin-mediated adhesion and signaling. J. Cell Biol. 144, 1285–1294.
[77] Czekay, R.P., Kuemmel, T.A., Orlando, R.A. and Farquhar, M.G. (2001) Direct binding of occupied urokinase receptor (uPAR) to LDL receptor-related protein is required for endocytosis of uPAR and regulation of cell surface urokinase activity. Mol. Biol. Cell 12, 1467–1479.
[78] Li, Y., Knisely, J.M., Lu, W., McCormick, L.M., Wang, J., Henkin, J., Schwartz, A.L. and Bu, G. (2002) LDL receptor-related protein 1B impairs urokinase receptor regeneration on the cell surface and inhibits cell migration. J. Biol. Chem. 22, 22.
[79] Selleri, C. et al. (2005) Involvement of the urokinase-type plasminogen activator receptor in hematopoietic stem cell mobilization. Blood 105, 2198–2205.
[80] Nykjaer, A. et al. (1998) Mannose 6-phosphate/insulin-like growth factor-II receptor targets the urokinase receptor to lysosomes via a novel binding interaction. J. Cell Biol. 141, 815–828.
[81] Behrendt, N., Jensen, O.N., Engelholm, L.H., Mortz, E., Mann, M. and Dano, K. (2000) A urokinase receptor-associated protein with specific collagen binding properties. J. Biol. Chem. 275, 1993–2002.
[82] Llinas, P., Helene Le Du, M., Gardsvoll, H., Dano, K., Ploug, M., Gilquin, B., Stura, E.A. and Menez, A. (2005) Crystal structure of the human urokinase plasminogen activator receptor bound to an antagonist peptide. EMBO J. 24, 1656–1663.
[83] Huai, Q. et al. (2006) Structure of human urokinase plasminogen activator in complex with its receptor. Science 311, 656–659.
[84] Barinka, C. et al. (2006) Structural basis of interaction between urokinase-type plasminogen activator and its receptor. J. Mol. Biol. 363, 482–495.
[85] Appella, E. and Blasi, F. (1987) The growth factor module of urokinase is the binding sequence for its receptor. Ann. N.Y. Acad. Sci. 511, 192–195.
[86] Behrendt, N., Ploug, M., Patthy, L., Houen, G., Blasi, F. and Danø, K. (1991) The ligand-binding domain of the cell surface receptor for urokinase-type plasminogen activator. J. Biol. Chem. 266, 7842–7847.
[87] Gardsvoll, H., Gilquin, B., Ledu, M.H., Menez, A., Jorgensen, T.J. and Ploug, M. (2006) Characterization of the functional epitope on the urokinase receptor. Complete alanine scanning mutagenesis supplemented by chemical crosslinking. J. Biol. Chem. 281, 19260–19272.
[88] Picone, R. et al. (1989) Regulation of urokinase receptors in monocytelike U937 cells by phorbol ester phorbol myristate acetate. J. Cell Biol. 108, 693–702.
[89] Gardsvoll, H., Werner, F., Sondergaard, L., Dano, K. and Ploug, M. (2004) Characterization of low-glycosylated forms of soluble human urokinase receptor expressed in Drosophila Schneider 2 cells after deletion of glycosylation-sites. Protein Expr. Purif. 34, 284–295.
[90] Bass, R. and Ellis, V. (2009) Regulation of urokinase receptor function and pericellular proteolysis by the integrin alpha(5)beta(1). Thromb. Haemost. 101, 954–962.

[91] Waltz, D.A., Sailor, L.Z. and Chapman, H.A. (1993) Cytokines induce urokinase-dependent adhesion of human myeloid cells. J. Clin. Invest. 91, 1541–1552.
[92] Waltz, D.A. and Chapman, H.A. (1994) Reversible cellular adhesion to vitronectin linked to urokinase receptor occupancy. J. Biol. Chem. 269, 14746–14750.
[93] Wei, Y., Waltz, D.A., Rao, N., Drummond, R.J., Rosenberg, S. and Chapman, H.A. (1994) Identification of the urokinase receptor as an adhesion receptor for vitronectin. J. Biol. Chem. 269, 32380–32388.
[94] Huai, Q. et al. (2008) Crystal structures of two human vitronectin, urokinase and urokinase receptor complexes. Nat. Struct. Mol. Biol. 15, 422–423.
[95] Gardsvoll, H. and Ploug, M. (2007) Mapping of the vitronectin-binding site on the urokinase receptor: involvement of a coherent receptor interface consisting of residues from both domain I and the flanking interdomain linker region. J. Biol. Chem. 282, 13561–13572.
[96] Li, Y., Lawrence, D.A. and Zhang, L. (2003) Sequences within domain II of the urokinase receptor critical for differential ligand recognition. J. Biol. Chem. 278, 29925–29932.
[97] Waltz, D.A., Natkin, L.R., Fujita, R.M., Wei, Y. and Chapman, H.A. (1997) Plasmin and plasminogen activator inhibitor type 1 promote cellular motility by regulating the interaction between the urokinase receptor and vitronectin. J. Clin. Invest. 100, 58–67.
[98] Hillig, T. et al. (2008) A composite role of vitronectin and urokinase in the modulation of cell morphology upon expression of the urokinase receptor. J. Biol. Chem. 283, 15217–15223.
[99] Deng, G., Curriden, S.A., Wang, S., Rosenberg, S. and Loskutoff, D.J. (1996) Is plasminogen activator inhibitor-1 the molecular switch that governs urokinase receptor-mediated cell adhesion and release? J. Cell Biol. 134, 1563–1571.
[100] Okumura, Y., Kamikubo, Y., Curriden, S.A., Wang, J., Kiwada, T., Futaki, S., Kitagawa, K. and Loskutoff, D.J. (2002) Kinetic analysis of the interaction between vitronectin and the urokinase receptor. J. Biol. Chem. 277, 9395–9404.
[101] Høyer-Hansen, G., Behrendt, N., Ploug, M., Danø, K. and Preissner, K.T. (1997) The intact urokinase receptor is required for efficient vitronectin binding: receptor cleavage prevents ligand interaction. FEBS Lett. 420, 79–85.
[102] Sidenius, N. and Blasi, F. (2000) Domain 1 of the urokinase receptor (uPAR) is required for uPAR-mediated cell binding to vitronectin. FEBS Lett. 470, 40–46.
[103] Sidenius, N. et al. (2004) Expression of the urokinase plasminogen activator and its receptor in HIV-1-associated central nervous system disease. J. Neuroimmunol. 157, 133–139.
[104] Sidenius, N., Andolfo, A., Fesce, R. and Blasi, F. (2002) Urokinase regulates vitronectin binding by controlling urokinase receptor oligomerization. J. Biol. Chem. 277, 27982–27990.
[105] Caiolfa, V.R. et al. (2007) Monomer dimer dynamics and distribution of GPI-anchored uPAR are determined by cell surface protein assemblies. J. Cell Biol. 179, 1067–1082.
[106] Bohuslav, J. et al. (1995) Urokinase plasminogen activator receptor, beta 2-integrins, and Src-kinases within a single receptor complex of human monocytes. J. Exp. Med. 181, 1381–1390.
[107] Goodson, R.J., Doyle, M.V., Kaufman, S.E. and Rosenberg, S. (1994) High-affinity urokinase receptor antagonists identified with bacteriophage peptide display. Proc. Natl. Acad. Sci. USA 91, 7129–7133.
[108] Wei, Y., Lukashev, M., Simon, D.I., Bodary, S.C., Rosenberg, S., Doyle, M.V. and Chapman, H.A. (1996) Regulation of integrin function by the urokinase receptor. Science 273, 1551–1555.
[109] Wei, Y., Eble, J.A., Wang, Z., Kreidberg, J.A. and Chapman, H.A. (2001) Urokinase receptors promote beta1 integrin function through interactions with integrin alpha3beta1. Mol. Biol. Cell 12, 2975–2986.
[110] Zhang, F., Tom, C.C., Kugler, M.C., Ching, T.T., Kreidberg, J.A., Wei, Y. and Chapman, H.A. (2003) Distinct ligand binding sites in integrin alpha3beta1 regulate matrix adhesion and cell–cell contact. J. Cell Biol. 163, 177–188.
[111] Aguirre Ghiso, J.A., Kovalski, K. and Ossowski, L. (1999) Tumor dormancy induced by downregulation of urokinase receptor in human carcinoma involves integrin and MAPK signaling. J. Cell Biol. 147, 89–104.
[112] Wei, Y. et al. (2005) Regulation of alpha5beta1 integrin conformation and function by urokinase receptor binding. J. Cell Biol. 168, 501–511.
[113] Degryse, B., Resnati, M., Czekay, R.P., Loskutoff, D.J. and Blasi, F. (2005) Domain 2 of the urokinase receptor contains an integrin-interacting epitope with intrinsic signaling activity: generation of a new integrin inhibitor. J. Biol. Chem. 280, 24792–24803.
[114] Gargiulo, L. et al. (2005) Cross-talk between fMLP and vitronectin receptors triggered by urokinase receptor-derived SRSRY peptide. J. Biol. Chem. 280, 25225–25232.
[115] Chaurasia, P., Aguirre-Ghiso, J.A., Liang, O.D., Gardsvoll, H., Ploug, M. and Ossowski, L. (2006) A region in urokinase plasminogen receptor domain III controlling a functional association with alpha5beta1 integrin and tumor growth. J. Biol. Chem. 281, 14852–14863.
[116] Wei, Y., Tang, C.H., Kim, Y., Robillard, L., Zhang, F., Kugler, M.C. and Chapman, H.A. (2007) Urokinase receptors are required for alpha5beta1 integrin-mediated signaling in tumor cells. J. Biol. Chem. 282, 3929–3939.
[117] Montuori, N., Carriero, M.V., Salzano, S., Rossi, G. and Ragno, P. (2002) The cleavage of the urokinase receptor regulates its multiple functions. J. Biol. Chem. 23, 23.
[118] Tarui, T. et al. (2003) Critical role of integrin alpha 5beta 1 in urokinase (uPA)/urokinase receptor (uPAR, CD87) signaling. J. Biol. Chem. 278, 29863–29872.
[119] Malengo, G., Andolfo, A., Sidenius, N., Gratton, E., Zamai, M. and Caiolfa, V.R. (2008) Fluorescence correlation spectroscopy and photon counting histogram on membrane proteins: functional dynamics of the glycosylphosphatidylinositol-anchored urokinase plasminogen activator receptor. J. Biomed. Opt. 13, 031215.
[120] Nykjaer, A., Conese, M., Christensen, E.I., Olson, D., Cremona, O., Gliemann, J. and Blasi, F. (1997) Recycling of the urokinase receptor upon internalization of the uPA:serpin complexes. EMBO J. 16, 2610–2620.
[121] Cortese, K., Sahores, M., Madsen, C.D., Tacchetti, C. and Blasi, F. (2008) Clathrin and LRP-1-independent constitutive endocytosis and recycling of uPAR. PLoS One 3, e3730.
[122] Sahores, M., Prinetti, A., Chiabrando, G., Blasi, F. and Sonnino, S. (2008) UPA binding increases UPAR localization to lipid rafts and modifies the receptor microdomain composition. Biochim. Biophys. Acta 1778, 250–259.