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Review

The urokinase receptor: a ligand or a receptor? Story of a sociable molecule

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Abstract. In this last decade, the structure and functions of the receptor for the urokinase-type plasminogen activator have been extensively studied and characterized. This interesting receptor plays a key role in cell adhesion, migration and proliferation. It was identified 20 years ago as the specific cell-surface molecule that could bind and concentrate urokinase on the cell membrane, thus initiating the proteolytic cascade promoted by the activation of plasminogen. The identification of new extracellular ligands, such as vitronectin, and of cell-surface interactors, such as integrins and fMet-Leu-Phe receptors, shed new light on its possible roles, totally independent of the enzymatic properties of its ligand. uPAR ligands and interactors and the functional consequences of the multiple binding capability of this intriguing receptor are reviewed here.

Keywords. Urokinase receptor, uPAR, cleaved uPAR, integrins, fMLP receptor.

Introduction

The plasminogen activation system

The cellular receptor for the urokinase-type plasminogen activator (uPAR) is a key component of the plasminogen activation (PA) system, a well-characterized system of serine-proteases. The targeted gene disruption of the PA system components showed their involvement in thrombolysis, inflammation, fertility, cell migration, tissue remodelling, cancer invasion and vascularization [1].

Plasminogen is present in plasma and extracellular fluids; it can associate with fibrin via lysine-binding sites located in the noncatalytic region. The cleavage at the Arg_{561}-Val_{562} bond converts the single polypeptide chain plasminogen into plasmin, which consists of two polypeptide chains held together by a disulfide bond. Plasmin is a broad-spectrum serine-protease; it promotes the degradation of the extracellular matrix (ECM) by activating several metalloproteases and by degrading ECM components, including fibrinogen, fibronectin and vitronectin [2]. Plasminogen can be activated by different proteases, but its specific physiological activators are the tissue-type (tPA) and the urokinase-type (uPA) plasminogen activators. They are serine-proteases that share the same substrate, but exhibit different functional and structural characteristics. Both uPA and tPA are secreted as single chains and are activated by a single cleavage, that yields two chains held together by a disulfide bond. Single-chain uPA is a zymogen, which lacks proteolytic activity, unlike single-chain tPA, which exerts significant activity [3].

tPA binds fibrin with high affinity through the specific kringle 2 domain, and its activity is strongly enhanced upon fibrin binding; it plays a central role in fibrinolysis [3]. instead uPA is involved in processes that include cell migration and tissue invasion, such as inflammation, angiogenesis and tumor invasion [1]. However, both uPA- and tPA-deficient mice show increased thrombotic susceptibility, and only combined uPA/tPA-deficient mice show severe spontaneous thrombosis similar to plasmin-deficient mice, thus suggesting that these enzymes can substitute for each other in thrombolysis [4].

The PA system represents a very efficient proteolytic machinery, and its activation leads to dramatic degradation effects on the ECM. Therefore, its activity must be strictly regulated. The primary plasmin inhibitor is the α2-antiplasmin, whose effect is reduced when plasmin lysine-binding sites are occupied, i.e. when plasmin interacts with fibrin or with its cellular receptors [2]. The most efficient inhibitor of plasminogen activators is the type-1 inhibitor (PAI-1); it is secreted in an active form but is rapidly inactivated, unless it binds plasma or ECM vitronectin (VN), which stabilizes its active conformation [5]. The other specific PA inhibitor is the type-2 inhibitor (PAI-2). Its lower inhibitory efficiency, as compared with PAI-1, and the intracellular detection of nonglycosylated forms suggested a role in the regulation of the activity of other enzymes; its involvement in apoptosis, independent of uPA inhibition, has been reported [6–7].

Plasminogen, tPA and uPA can bind specific cellular receptors. The binding to receptors represents another regulatory step, since the enzyme and substrate focusing on the cell surface strongly enhances the proteolytic cascade [8]. Native, circulating plasminogen binds in a lysine- and/or carbohydrate-dependent manner to the cell surface, with low affinity but high capacity. A heterogeneous group of plasminogen receptors has been identified; they can be subdivided into three classes, based on their mode of interaction with plasminogen [9–10]. Cell-bound plasmin is protected from inactivation by its natural inhibitors, since both receptors and inhibitors utilize plasminogen/plasmin C-terminal lysines [10]. Different cellular binding sites for tPA have been described. Some of these are involved in its clearance [11]; others localize tPA activity on the cell surface, for instance in endothelial cells and neurons [12–13].

The cellular receptor for uPA (uPAR) was identified in 1985 on monocyte-like cells [14–15], and, since then, it has been extensively studied and characterized.

The urokinase receptor

uPAR structure

uPAR is synthesized as a single polypeptide chain of 313 amino acid residues, with a 21-residue signal peptide. Post-translational cleavage and removal of the last 30 C-terminal residues allow the attachment of a glycosyl-phosphatydil-inositol (GPI) tail to Gly 283, which anchors the receptor to the cell surface (GPI-uPAR) [16]. uPAR is organized into three homologous, differently folded domains of approximately 90 amino acids, with four to five disulphide bonds (D1, D2 and D3, as numbered from the N-terminus). The three domains are members of the Ly-6/uPAR/α-neurotoxin protein domain family, which are predicted to adopt a three-finger fold; only uPAR and the metastasis-associated C4.4A contain multiple domains [17–18].

Recently, the crystal structure of a uPAR-soluble form bound to an antagonist peptide was solved, thus confirming that uPAR consists of three domains with a typical three-finger fold, each domain containing three adjacent loops rich in β-pleated sheets and a small C-terminal loop [19]. The three domains of uPAR form an almost globular receptor, with a breach between D1 and D3, thus generating a central cavity (19 angstroms deep) where the ligand peptide is located. The top of the cavity is quite large and progressively narrows toward the bottom. The peptide establishes multiple contacts with the walls of the cavity; D1 plays a predominant role in this ligand interaction by providing half of the binding interface, consistent with previous results [18, 20]. uPAR exhibits a large outer surface that harbors interdomain linker regions and five possible N-linked glycosylation sites (N52, N162, N172, N200 and N233). This model suggests that uPA is embedded in the central cavity and that the large outer receptor surface is available to bind additional ligands.

The three domains of the GPI-anchored uPAR are joined by linker sequences. The D1-D2 linker region exhibits an extreme proteolytic sensitivity; in fact, it can be cleaved *in vitro* by several enzymes, such as trypsin, chymotrypsin, elastase, cathepsin G, metalloproteases and, interestingly, by plasmin and uPA itself [21–25]. The cleavage generates truncated forms of GPI-uPAR (c-uPAR), lacking D1; such cleaved forms have been detected on the surface of different cell lines and in normal and cancer tissues [26]. c-uPAR forms can vary at the N-terminus, according to the protease that cleaves the receptor and that may disrupt or not a specific sequence, corresponding to amino acids 88–92, involved in cell migration [22, 27]. uPA could be required in cell-surface uPAR cleavage *in vivo* [28].

Both full-length and cleaved uPAR can be shed, thus generating soluble uPAR forms (suPAR and c-suPAR, respectively). Soluble uPAR forms have been found in biological fluids, both *in vitro* and *in vivo* [26]. uPAR can be released from the plasma membrane by glycosyl-phosphatidyl-inositol-specific phospholipase C or D [16, 29]. Although a juxtamembrane proteolytic cleavage of uPAR has often been suggested, proteases involved in this process have been identified only recently [30–31]. suPAR could also generate c-suPAR by proteolytic cleavage mediated by metalloproteases, cathepsin G and elastase [26].

Regulation of uPAR expression

Human and mouse uPAR genes have been cloned [32–33]. The human gene is located on the long arm of chromosome 19 (19q13) and contains seven exons and six introns extending over 23 kb of genomic DNA [34]. The maximal promoter activity has been identified within 188 bp from the major transcription start site of the human

uPAR gene, between –141 and +47 bp relative to the transcription initiation site [35]. This region lacks TATA and CAAT boxes and contains GC-rich sequences and consensus elements for SP1, AP1, AP2, PEA3 and NFκB [35–41]. Deletion of PEA3/Ets motif impairs the β3-integrin mediated downregulation of uPAR expression, thus suggesting that this site acts as a silencing element [40]. Recently, the Kruppel-like transcription factor has been reported to drive uPAR transcription in colon cancer cells by binding multiple sites in the uPAR promoter [42].

uPAR expression can be also regulated at a post-transcriptional level. In fact, several sequence elements regulating uPAR messenger RNA (mRNA) decay have been identified throughout the transcript [43]. These sequences are able to bind proteins that promote stability or degradation of uPAR mRNA. Functional AU-rich elements (AREs) are present in the 3′ untranslated region (UTR) of the uPAR transcript and confer it marked instability [44]. By contrast, the ELAV protein HuR and heterogeneous nuclear ribonucleoprotein C (hnRNPC) are able to bind uPAR ARE and to stabilize uPAR mRNA [45–46].

A regulatory element capable of destabilizing uPAR mRNA has been identified also within its coding region [47]. This specific mRNA sequence is able to bind phosphoglycerate kinase (PGK) in bronchial epithelial cells, thus promoting uPAR mRNA degradation [48]. The uPAR mRNA coding region can also bind a stabilizing factor that is upregulated in non-small cell lung carcinoma [49–50].

Interestingly, uPA regulates the expression of its receptor both at the transcriptional and post-transcriptional levels; indeed, uPA upregulates uPAR expression in MDA-MB-231 breast carcinoma cells by increasing SP1 binding activity and in lung carcinoma cells by increasing uPAR mRNA stability [50–51].

uPAR ligands

uPA is secreted as a 54-kDa single-chain pro-enzyme that can be converted into the two-chain active form by a single cleavage at Lys_{158}-Ile_{159}. The noncatalytic amino-terminal A chain contains the epidermal growth factor (EGF)-like domain (residues 4–43, GFD) and the triple-disulphide containing structure called kringle domain (residues 47–135); this chain, or its amino terminal fragment (ATF, residues 1–135), binds uPAR with high affinity and specificity. The C-terminal B chain contains the serine-protease domain and is unable to bind the receptor [14–15].

uPA binds the N-terminal uPAR domain 1 (D1), as also confirmed by the recent structure analysis [18–19]. Nevertheless, the ligand-binding affinity of the single D1 is strongly reduced as compared with that of the full-length receptor, since secondary binding sites are located in domains 2 and 3 (D2 and D3); therefore, the full-length molecule is required for an efficient uPA binding [20, 52–53].

In spite of its name, uPAR acts also as a receptor for other extracellular molecules, such as vitronectin (VN), an ECM component, and the cleaved high molecular mass kininogen (HKa) lacking the vasodilator peptide bradykinin [54–55]. In both cases the binding is mediated by specific sites in D2+D3 [54–56]. However, an efficient binding to VN requires full-length uPAR, as in the case of uPA binding, since truncated uPAR forms, lacking D1, fail to mediate cell adhesion to VN [57–58]. uPA positively regulates VN binding to uPAR, likely by inducing formation of uPAR dimers, which exhibit an higher affinity for VN compared with monomers [59]. Dimeric uPAR partitions preferentially to lipid rafts, the detergent-resistant microdomains of cell membrane; therefore, uPAR dimerization not only favors VN binding to uPAR but also drives it into lipid rafts [60]. By contrast, uPA binding to uPAR is independent of receptor dimerization and raft association [60]. HKa, unlike uPA, inhibits uPAR-mediated cell adhesion to VN because it probably binds and blocks the VN-binding site in uPAR D2-D3 [61]. uPAR binding to VN can also be inhibited by PAI-1, because uPAR and PAI-1 bind overlapping regions on VN, close to the integrin binding site [5].

uPAR concentrates uPA enzymatic activity on the cell surface and, for several years, that was considered to be the only uPAR function. However, extensive evidence showed that both uPA and VN induce proteolysis-independent intracellular signalling that regulates cell adhesion, migration and proliferation [1, 62]. uPA- and VN-dependent transmembrane signalling cannot be mediated directly by GPI-anchored uPAR, thus suggesting the requirement of functional uPAR partners provided with cytosolic domains and capable of signal transduction. At the moment, the best candidates to be uPAR ‘co-workers’ are integrins and receptors for the fMet-Leu-Phe (fMLP) peptide, a formylated peptide of bacterial origin.

uPAR ‘receptors’

Integrins

Integrins represent one set of molecules that are able to interact with uPAR on the cell surface (Fig. 1). Fluorescence resonance energy transfer analysis (FRET), immunolocalization and co-immunoprecipitation have identified uPAR in complex with several integrin families, such as β1, β2, β3 and β5 [63–66].

Physical association of uPAR with a β2 integrin was first demonstrated in resting neutrophils by uPAR and complement receptor type 3 (CR3, CD11b/CD18, Mac-1) co-capping [66]. uPAR was found to dissociate with spontaneous cell polarization for migration, since Mac-1 caps transformed into uropods, while uPAR accumulated at la-

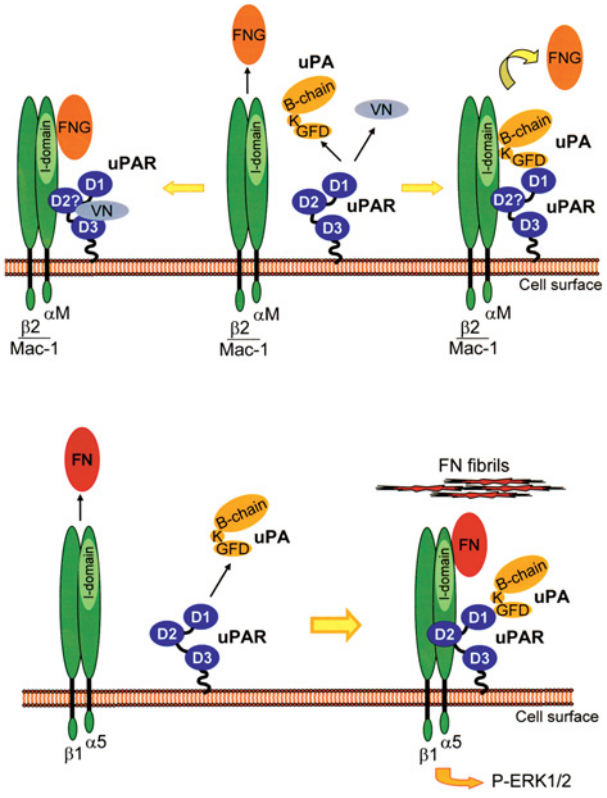

Figure 1. uPAR interactions with β2 and β1 integrins. Upper panel: uPAR can bind urokinase (uPA) and vitronectin (VN); Mlc-1 binds fibrinogen (FNG). uPAR associates with the αM chain of Mlc-1; VN promotes Mac-1 binding to FNG (left), which is reduced by uPA, most likely because of uPA interactions with the ligand-binding site (I-domain) of Mlc-1 (right). uPAR may interact with Mlc-1 through D2 as in the interaction with β1 integrins. Lower panel: uPAR binds uPA; α5β1 integrin binds fibronectin (FN) (left). uPAR interacts with both α5 and β1 integrin chains, probably through D2 (right). uPA-uPAR promotes integrin activation, formation of insoluble FN fibrils and ERK1/2 activation.

mellipodia of polarized cells [67]. Subsequently, the presence of uPAR and β2 integrins was identified in large receptor complexes that included signalling molecules [68]. uPAR association with Mac-1 facilitates the adhesive functions of Mac-1, whereas uPAR occupancy with uPA negatively influences cell adhesion [69]. In agreement with these observations, induction of Mac-1 and uPAR expression on monocytes by transforming growth factor-β 1 and vitamin D3 confers them uPAR-dependent adhesion to VN, which is further promoted by engagement of Mac-1; vice versa, vitronectin attachment promotes subsequent Mac-1-mediated fibrinogen degradation (Fig. 1, upper panel, left). By contrast, the other uPAR ligand, uPA, inhibits Mac-1-mediated fibrinogen binding and degradation [70] (Fig. 1, upper panel, right). A binding site for uPAR on Mlc-1 integrin has been identified by homology with a peptide capable of binding uPAR (P-25); it corresponds to amino acids 424–440 (peptide M25) in the region between the ligand-binding I-domain and highly conserved divalent cation repeats of CD11b, the α(M) subunit of Mac-1. M25 affects uPAR-β2 integrin association by competing with Mac-1 for the binding to uPAR. Disruption of complexes impairs β2 integrin activity, thus indicating a positive role of uPAR in β2 integrin functions [71].

Mac-1 can bind also uPA. In fact, uPA directly interacts with the ligand-binding I-domain of Mac-1 α(M) subunit [72] and could compete with fibrinogen in binding to Mac-1, thus inhibiting integrin function, as previously observed [69].

uPA may bind simultaneously with both uPAR and Mac-1, since different binding sites are involved; uPA interacts with uPAR through its GFD in the amino terminal fragment (ATF), whereas it recognizes Mac-1 through the kringle and proteolytic domains. Mac-1 is also able to bind simultaneously uPA and uPAR, through its I and non-I domains, respectively [72] (Fig. 1, upper panel, right). The resulting multicontact trimolecular complex regulates cell adhesion and migration, and fibrinolysis; in fact, Mac-1 supports uPA-dependent cell adhesion and migration and enhances uPA-mediated plasminogen activation [72–73].

A major uPAR role in regulating β2 integrin activity has also been shown *in vivo*; in fact, the β2 integrin-dependent recruitment of leukocytes to inflamed peritoneum is significantly reduced in uPAR-deficient mice, since the absence of uPAR affects leukocyte adhesion to endothelium [74]. uPAR is also required for the recruitment of neutrophils to the lung in response to *Pseudomonas aeruginosa* pneumonia infection, which recruits neutrophils to the pulmonary parenchyma by a β2 integrin-dependent mechanism [75].

The first evidence of uPAR interaction with β1 integrins was reported in uPAR-transfected HEK-293 cells, in which uPAR and integrins form stable complexes that inhibit cell adhesion to fibronectin (FN) and, by contrast, increase RGD-independent cell adhesion to VN [63].

A uPAR-binding peptide (P-25), identified by phage display library, disrupts uPAR-β1 integrin complexes and abolishes uPAR effects on cell adhesion to FN and VN [63]. uPAR-induced impairment of cell adhesion to FN has not been ascribed to a direct effect of uPAR on integrin activity but to the limited expression of caveolin, which forms complexes with uPAR and is able to bind several signalling molecules linked to integrin functions, including Src family kinases [76]. Nevertheless, uPAR-dependent HEK-293 cell adhesion to VN is mediated by α3 integrin, a major β1 integrin partner for uPAR in these cells, thus indicating that integrin-dependent signalling is not impaired by uPAR overexpression. In MDA-MB-231 breast cancer cells, uPA-induced uPAR-α3β1 association enhances spreading and focal adhesion kinase (FAK) phosphorylation also on FN or collagen type I (CG) [77]. By contrast, a different report shows that, in the same cell line, P-25, which is expected to dissociate uPAR-integrin

complex, increases cell adhesion to FN, as in HEK-293 cells, thus suggesting a negative effect of uPAR on the activity of FN-specific integrins [78].

Also uPAR association with $\alpha5\beta1$ integrin in human epidermoid carcinoma HEp-3 cells expressing high uPAR levels promotes cell adhesion to FN, accompanied by ERK1/2 activation and serum-independent growth stimulation (Fig. 1, lower panel). Disruption of uPAR-$\alpha5\beta1$ complexes with antibodies or P-25 reduces FN-dependent ERK1/2 activation [79]. uPAR-activating interactions with the $\alpha5\beta1$ integrin also facilitates the formation of insoluble FN fibrils that suppresses p38 activity (Fig. 1, lower panel). Low uPAR-expressing HEp3 cells, even if expressing a similar level of $\alpha5\beta1$ integrin, show a low ERK/p38 activity ratio and do not assemble FN fibrils [80]. By contrast, P-25 exerts an opposite effect in human skin fibroblasts as compared with HEp-3 cells, since it increases FN matrix assembly by promoting the activation state of the $\alpha5\beta1$ integrin. Nevertheless, anti-uPAR antibodies partially inhibit P-25 effects in these cells, even though uPAR is expected to be dissociated from $\beta1$ integrins by the peptide and unable to interfere with integrin functions [81]. Therefore, uPA-uPAR are required for $\alpha5\beta1$ activation and cell adhesion to FN. On the other hand, the same integrin is required for cell adhesion to uPA and for uPA-induced cell migration, i.e. to transduce signals initiated by uPA in the same cells [82].

uPAR-binding sites have been identified on both α- and $\beta1$-integrin chains (Fig. 1, lower panel, left). $\alpha3$-$\beta1$ integrin associates with uPAR via a surface loop within the $\alpha3$ β-propeller (residues 242–246) but outside the ligand-binding region; cell treatment with a 17 mer $\alpha3\beta1$ integrin peptide (peptide $\alpha325$) or Ala mutations within the uPAR interacting loop of $\alpha3$ (H245A) chain disrupts uPAR-$\alpha3\beta1$ integrin complexes and impairs uPA-uPAR-dependent signals [77, 83]. Recently, a uPAR-binding site has also been identified in a $\beta1$-chain loop (residues 224–232) of $\alpha5\beta1$ integrin; this loop is very close to the β-propeller of $\alpha5$ chain in the energy-minimized model of the integrin structure [84] (Fig. 1, lower panel, right). The synthetic peptide corresponding to the uPAR-binding site of $\beta1$ or a $\beta1$ chain Ser227Ala point mutation abrogate functional uPAR effects on $\alpha5\beta1$ integrin. Interestingly, uPAR association with $\alpha5\beta1$ integrin induces the integrin to bind an additional site on FN, in addition to the RGD site bound by $\alpha5\beta1$ integrin not complexed with uPAR [84].

Recently, an integrin-binding site on uPAR has also been identified; it corresponds to the amino acids 130–142 (peptide D2A) located in the uPAR domain 2 (D2) (Fig. 1, lower panel, right). D2A abolishes uPAR co-immunoprecipitation with $\alpha v\beta3$ and $\alpha5\beta1$ integrins, indicating that it can bind both these molecules. D2A activates $\alpha v\beta3$ integrin-dependent signalling pathways and stimulates cell migration, whereas peptide D2A-Ala, generated by mutating two glutamic acids into two alanines, lacks chemotactic activity, and, in addition, inhibits VN-, FN- and CG-dependent cell migration [85]. However, it seems likely that other sites of interaction between uPAR and integrins exist, besides the D2A sequence [85]. uPAR binding to integrins requires the full-length receptor, as in the case of uPAR binding to uPA and VN [23, 27].

fMLP receptors

fMLP is a formylated peptide of bacterial origin that stimulates chemotaxis by activating seven transmembrane domain G-protein-coupled receptors. Three fMLP receptors have been identified and cloned: the high-affinity *N*-formyl-peptide receptor (FPR) and its homologues FPR-like 1 (FPRL1) and FPR-like2 (FPRL2). FPR is a high-affinity receptor for fMLP; cyclosporin H (CsH) is a specific FPR antagonist. FPRL1 has a much lower affinity for fMLP, whereas it is efficiently activated by several different molecules, such as lipoxin A_4, serum amyloid A, the prion peptide $PrP_{106–126}$, human immunodeficiency virus-type 1 (HIV-1) envelope peptides, the *Helicobacter pylori* Hp(2–20) peptide and various synthetic peptides [86–87]. FPRL2 shows a high homology with the other two fMLP receptors but does not bind fMLP and shares some ligands with FPRL1, as Hp(2–20) and synthetic peptides. fMLP receptors were first identified in leukocytes, and subsequently in several different cell types, including epithelial cells [87].

uPAR can be cleaved by different proteases in the D1-D2 linker region [26]. The cleavage promotes the release of D1 and the exposure, in the linker region, of the specific sequence SRSRY, corresponding to the amino acids 88–92 [22, 88]. The soluble cleaved uPAR (c-suPAR), or its derived peptides containing the SRSRY sequence, bind and activate FPRL1 in monocytes, thus inducing their migration [89] (Fig. 2, upper panel, left). Interestingly, c-suPAR does not induce calcium mobilization [89–90], unlike fMLP and other FPRL1 ligands [86]. Recently, the ability of c-suPAR to activate other fMLP receptors in different cell types was reported. In fact, c-suPAR induces basophil migration by activating both FPRL1 and FPRL2 [91] and binds and activates FPR in hematopoietic stem cells and in epithelial HEK-293 cells [92–93]. Thus, c-suPAR can be considered as a ligand of fMLP receptors, whereas it lacks the capability to bind both the extracellular uPAR ligands, uPA and VN [18, 57].

c-suPAR has been detected in biologic fluids from patients affected by different pathologies; it could be generated either by the shedding of cleaved cell-surface uPAR or directly by suPAR cleavage. *In vivo* c-suPAR could mediate cell migration, even though a biological *in vivo* role has not yet been reported [26].

Full-length soluble uPAR does not bind fMLP receptors, [22, 88–89], even though it is able to interact with integrins and VN [57, 63]. By contrast, full-length GPI-an-

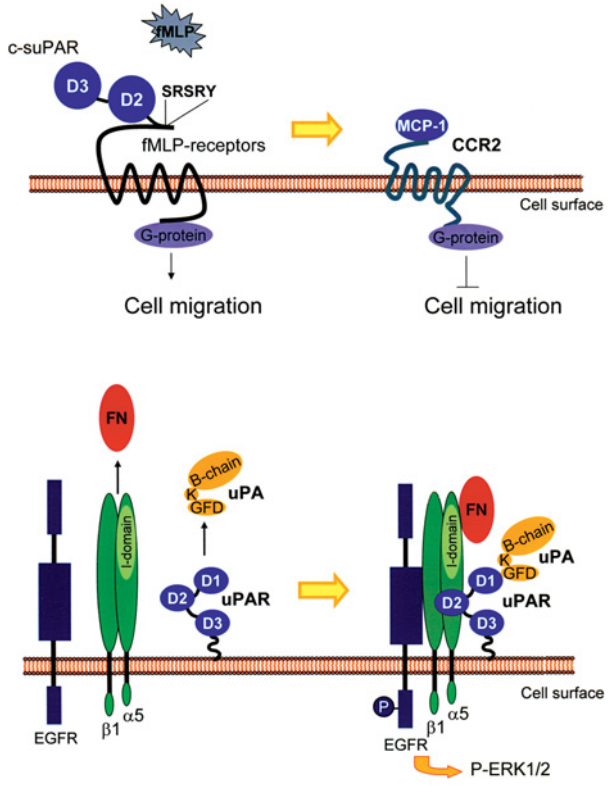

Figure 2. uPAR interactions with chemokine and growth factor receptors. Upper panel: The soluble form of cleaved uPAR (c-suPAR), which lacks D1 but still contains the specific SRSRY sequence in the D1-D2 linker region, interacts with fMLP receptors, thus inducing cell migration (left). c-suPAR binding to fMLP receptors inhibits MCP-1-dependent cell migration (right). Lower panel: uPAR binds urokinase (uPA); $\alpha 5\beta 1$ integrin binds fibronectin (FN) (left). High uPAR expression promotes $\alpha 5\beta 1$ integrin association with EGF receptor (EGFR) and EGFR phosphorylation. uPA strongly increases integrin-EGFR association and induces activation of EGFR and ERK1/2, independently of EGFR ligands (right).

chored uPAR interacts with FPR through the specific SRSRY sequence in the D1-D2 linker region [27]; indeed, its expression is required for fMLP-dependent migration in monocytes and HEK-293 epithelial cells [27, 94]. The different behavior of soluble and cell-surface receptor could be due to a different conformation, since antibodies directed against the SRSRY sequence react only with the GPI-anchored receptor and not with suPAR [95]. fMLP receptors are also involved in cell migration induced by uPA or its amino terminal fragment (ATF) [89, 91, 93]. GPI-uPAR can thus be considered as an endogenous ligand for fMLP receptors [89, 91].

uPAR cross-talking receptors

c-suPAR

c-suPAR is a ligand of fMLP receptors, thus it could regulate their activity or the activity of other chemoattractant receptors by homologous or heterologous desensitization. Homologous or heterologous desensitization of G-protein-coupled chemotaxis-receptors downregulates the cellular response to chemoattractants. Homologous desensitization occurs when the receptor is occupied by its agonist and is phosphorylated by G-protein-coupled receptor kinases; the heterologous desensitization is due to the phosphorylation induced by second messenger-triggered kinases such as PKA or PKC, which have been activated by different receptors; in both cases phosphorylation can be followed by internalization [86, 96]. FPR is a potent activator of second messengers involved in heterologous desensitization; in fact, desensitization of CXCR1, CCR5 and CXCR4 chemokine-receptors induced by FPR activation has been reported [86, 96–97]. FPRL1 ligands also induce heterologous desensitization of CCR5 and CXCR4 by phosphorylation and internalization [86, 97–98].

c-suPAR pretreatment abolishes chemotaxis of various cell types toward fMLP, but fMLP-receptor phosphorylation and/or internalization has not been investigated [89, 91–93]. Exposure to c-suPAR also inhibits monocyte migration in response to MCP-1; phosphorylation and/or internalization of CCR2, the MCP1 receptor, have not been explored. The mechanism regulating c-suPAR-mediated CCR2 inactivation seems different from those of homologous or heterologous desensitization. c-suPAR does not bind monocyte CCR2, nor does it prevent MCP-1-induced intracellular Ca(2+) increase; rather, c-suPAR strongly decreases MCP-1-induced cell adhesion to fibrinogen (FNG), which is mediated by $\beta 2$ integrins [90] (Fig. 2, upper panel, right). Accordingly, c-suPAR reduces integrin-mediated cell adhesion of HEK-293 cells plated on VN; the specific uPAR-derived peptide SRSRY decreases HEK-293 cell adhesion to VN by increasing uPAR association with $\alpha v\beta 5$ integrin [93]. Interestingly, SRSRY peptide stimulates PKC activity [93], which could be involved in heterologous desensitization.

c-suPAR or its derived chemotactic peptide (residues 84–95) also inhibit migration of human hematopoietic stem cells and KG1 leukemic cells toward the stromal derived factor 1 (SDF1), likely by affecting the activity of its receptor, CXCR4. The mechanism has not yet been elucidated [92].

GPI-uPAR

The ability of uPAR to interact with integrins and to regulate their activity could imply that it could also be involved in the activation of specific growth factor (GF) receptors. In fact, integrins and GF receptors can associate in supramolecular complexes that allow a mutual cooperation between the two signalling systems. Cell adhesion to specific substrates enhances GF-dependent responses

and, vice versa, some GFs stimulate cell adhesion. This collaboration has been demonstrated for $\alpha v\beta 3$ integrin, which associates with PDGF, IGF-I and VEGF receptors [99]. Various tyrosine kinase receptors, including EGF, PDGF, VEGF and HGF receptors, can be activated by integrin in the absence of any ligand [99]. In fact, integrins physically interact with GF receptors. Integrin clustering at the adhesion sites could increase local concentration of GF receptors, thus causing their oligomerization and transactivation; however, the mechanism seems more complicated.

Activation of the EGF receptor (EGFR) has been extensively analyzed in HEp3 cells expressing high and low levels of uPAR (H-HEp3 and L-HEp3, respectively). As previously reported, H-HEp3 cells show higher uPAR association with $\alpha 5\beta 1$ integrin, adhesion to FN and ERK1/2 activation, as compared with L-HEp3 cells [79]. Both H-HEp3 and L-HEp3 cells show equivalent expression of $\alpha 5\beta 1$ integrin but different EGFR expression, being higher in L-HEp3 cells. Unexpectedly, H-HEp3 cells show a detectable basal EGFR phosphorylation that is strongly increased by cell adhesion to FN, unlike L-HEp3 cells. uPAR expression influences EGFR activation independently of the levels of EGFR and of its ligands, rather by promoting the $\alpha 5\beta 1$ activation state that, on FN, leads to the formation of a multiprotein complex that contains at least integrins, EGFR and FAK [100]. uPA strongly increases $\alpha 5$-EGFR association and induces activation of EGFR and ERK1/2, which is an important downstream target of EGFR (Fig. 1, lower panel, right). These impressive results suggest that the uPA-uPAR system could utilize the EGFR to transduce signals. In fact, uPA-induced ERK1/2 activation in H-HEp3 cells is abolished by an EGFR-kinase inhibitor, thus indicating that uPA binding to uPAR could initiate a signal that is mediated by EGFR [100]. Accordingly, EGFR may function as a transducer of the uPA-dependent signal for ERK activation in EGFR-transfected CHO cells. However, uPA induces ERK activation also in untrasfected CHO cells, which are EGFR negative, likely by an alternative pathway [101]; nevertheless, EGFR expression is required in CHO cells to induce uPA-dependent mitogenic signals [102].

uPAR cross-talk with another GF receptor, the platelet-derived growth factor receptor (PDGFR)-β, has been reported in human vascular smooth muscle cells (VSMCs). uPA induces uPAR association with PDGFR-β, PDGF-independent PDGFR-β dimerization and phosphorylation.

uPAR-PDGFR-β association and PDGFR-β tyrosine kinase activity are required for uPA-induced migratory and proliferative downstream signals. However, uPA strongly inhibits PDGF-induced migration, in contrast with its activating effects on PDGFR [103].

Conclusion

All together these studies clearly indicate a mutual cooperation between uPAR and its interactors: the activity of integrins and of chemokine and growth factor receptors requires uPAR expression and, on the other hand, uPAR utilizes these receptors to transduce signals that it is not able to transduce. Thus, uPAR is not simply an integrin or a fMLP receptor ligand; rather, it participates in supramolecular complexes that include integrins, other receptors and signalling molecules. Most likely, fMLP receptors are also included in these complexes, even though most reports focus only on uPAR-integrin or on uPAR-fMLP receptor interactions. The identity and precise role of the different components of these large complexes as well as their relations have not yet been fully elucidated. Both uPAR and fMLP receptors are expressed in almost all cell types analyzed, suggesting that they are stable components of these functional complexes, whereas chemokine or growth factor receptors could vary and involve different specific integrins.

The results of uPAR structure analysis strongly support the hypothesis that uPAR is a molecule capable of establishing multiple contacts, since it shows a large outer surface with exposed linker region and glycosylation sites.

In conclusion, this field appears to be a very ebullient research area rich in new information but still requiring studies to elucidate the general mechanism of action, to identify new interacting molecules that could connect the different components of the complex and to explain contrasting results.

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1 Blasi F. and Carmeliet P. (2002) uPAR: a versatile signalling orchestrator. Nat. Rev. Mol. Cell Biol. **3:** 932–943
2 Castellino F. J. and Ploplis V. A. (2005) Structure and function of the plasminogen/plasmin system. Thromb. Haemost. **93:** 647–654
3 Collen D. and Lijnen H. R. (2005) Thrombolytic agents. Thromb. Haemost. **93:** 627–630
4 Carmeliet P., Bouche A., De Clercq C., Janssen S., Pollefeyt S., Wyns S. et al. (1995) Biological effects of disruption of the tissue-type plasminogen activator, urokinase-type plasminogen activator and plasminogen activator inhibitor-1 genes in mice. Ann. N. Y. Acad. Sci. **748:** 367–381
5 Dellas C. and Loskutoff D. J. (2005) Historical analysis of PAI-1 from its discovery to its potential role in cell motility and disease. Thromb. Haemost. **93:** 631–640
6 Dickinson J. L., Bates E. J., Ferrante A. and Antalis T. M. (1995) Plasminogen activator inhibitor type 2 inhibits tumor necrosis factor α-induced apoptosis. Evidence for an alternate biological function. J. Biol. Chem. **270:** 27894–27904
7 Zhou H. M., Bolon I., Nichols A., Wohlwend A. and Vassalli J. D. (2001) Overexpression of plasminogen activator in-

hibitor type 2 in basal keratinocytes enhances papilloma formation in transgenic mice. Cancer Res. **61:** 970–976
8 Ellis V., Scully M. F. and Kakkar V. V. (1989) Plasminogen activation initiated by single-chain urokinase-type plasminogen activator. Potentiation by U937 monocytes. J. Biol. Chem. **264:** 2185–2188
9 Ranson M. and Andronicos N. M. (2003) Plasminogen binding and cancer: promises and pitfalls. Front. Biosci. **8:** 294–304
10 Herren T., Swaisgood C. and Plow E. F. (2003) Regulation of plasminogen receptors. Front. Biosci. 8: d1–d8
11 Kuiper J., Otter M., Rijken D. C., van Berkel T. J. (1988) Characterization of the interaction *in vivo* of tissue-type plasminogen activator with liver cells. J. Biol. Chem. **263:** 18220–18224
12 Fukao H., Ueshima S., Takaishi T., Okada K. and Matsuo O. (1997) Enhancement of tissue-type plasminogen activator (t-PA) activity by purified t-PA receptor expressed in human endothelial cells. Biochim. Biophys. Acta **1356:** 111–120
13 Verrall S. and Seeds N. W. (1989) Characterization of 125I-tissue plasminogen activator binding to cerebellar granule neurons. J. Cell Biol. **109:** 265–271
14 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
15 Stoppelli M.P, Corti A., Soffientini A., Cassani G., Blasi F. and Assoian R. K. (1985) Differentiation-enhanced binding of the amino-terminal fragment of human urokinase plasminogen activator to a specific receptor on U937 monocytes. Proc. Natl. Acad. Sci. USA **82:** 4939–4943
16 Ploug M., Ronne E., Behrendt N., Jensen A. L., Blasi F. and Dano K. (1991) Cellular receptor for urokinase plasminogen activator. Carboxyl-terminal processing and membrane anchoring by glycosyl-phosphatidylinositol. J. Biol. Chem. **266:** 1926–1933
17 Ploug M., Kjalke M., Ronne E., Weidle U., Hoyer-Hansen G. and Dano K. (1993) Localization of the disulfide bonds in the NH2-terminal domain of the cellular receptor for human urokinase-type plasminogen activator. A domain structure belonging to a novel superfamily of glycolipid-anchored membrane proteins. J. Biol. Chem. **268:** 17539–17546
18 Behrendt N., Ploug M., Patthy L., Houen G., Blasi F. and Dano K. (1991) The ligand-binding domain of the cell surface receptor for urokinase-type plasminogen activator. J. Biol. Chem. **266:** 7842–7847
19 Llinas P., Le Du M. H., Gardsvoll H., Dano K., Ploug M., Gilquin B. et al. (2005) Crystal structure of the human urokinase plasminogen activator receptor bound to an antagonist peptide. EMBO J. **24:** 1655–1663
20 Ploug M. (1998) Identification of specific sites involved in ligand binding by photoaffinity labeling of the receptor for the urokinase-type plasminogen activator. Residues located at equivalent positions in uPAR domains I and III participate in the assembly of a composite ligand-binding site. Biochemistry **37:** 16494–16505
21 Hoyer-Hansen G., Ronne E., Solberg H., Behrendt N., Ploug M., Lund L. R. et al. (1992) Urokinase plasminogen activator cleaves its cell surface receptor releasing the ligand-binding domain. J. Biol. Chem. **267:** 18224–18229
22 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
23 Montuori N., Rossi G. and Ragno P. (1999) Cleavage of urokinase receptor regulates its interaction with integrins in thyroid cells. FEBS Lett. **460:** 32–36
24 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
25 Beaufort N., Leduc D., Rousselle J. C., Magdolen V., Luther T., Namane A. et al. (2004) Proteolytic regulation of the urokinase receptor/CD87 on monocytic cells by neutrophil elastase and cathepsin G. J. Immunol. **172:** 540–549
26 Montuori N., Visconte V., Rossi G. and Ragno P. (2005) Soluble and cleaved forms of the urokinase-receptor: degradation products or active molecules? Thromb. Haemost. **93:** 192–198
27 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. **277:** 46932–46939
28 Zhou H. M., Nichols A., Meda P. and Vassalli J. D. (2000) Urokinase-type plasminogen activator and its receptor synergize to promote pathogenic proteolysis. EMBO J. **19:** 4817–4826
29 Wilhelm O. G., Wilhelm S., Escott G. M., Lutz V., Magdolen V., Schmitt M. et al. (1999) Cellular glycosylphosphatidylinositol-specific phospholipase D regulates urokinase receptor shedding and cell surface expression. J. Cell Physiol. **180:** 225–235
30 Beaufort N., Leduc D., Rousselle J. C., Namane A., Chignard M. and Pidard D. (2004) Plasmin cleaves the juxtamembrane domain and releases truncated species of the urokinase receptor (CD87) from human bronchial epithelial cells. FEBS Lett. **574:** 89–94
31 Beaufort N., Leduc D., Rousselle J. C., Magdolen V., Luther T., Namane A. et al. (2004) Proteolytic regulation of the urokinase receptor/CD87 on monocytic cells by neutrophil elastase and cathepsin G. J. Immunol. **172:** 540–549
32 Casey J. R., Petranka J. G., Kottra J., Fleenor D. E. and Rosse W. F. (1994) The structure of the urokinase-type plasminogen activator receptor gene. Blood **84:** 1151–1156
33 Suh T. T., Nerlov C., Dano K. and Degen J. L. (1994) The murine urokinase-type plasminogen activator receptor gene. J. Biol. Chem. **269:** 25992–25998
34 Borglum A. D., Byskov A., Ragno P., Roldan A. L., Tripputi P., Cassani G. et al. (1992) Assignment of the urokinase-type plasminogen activator receptor gene (PLAUR) to chromosome 19q13.1-q13.2. Am. J. Hum. Genet. **50:** 492–497
35 Soravia E., Grebe A., De Luca P., Helin K., Suh T. T., Degen J. L. et al. (1995) A conserved TATA-less proximal promoter drives basal transcription from the urokinase-type plasminogen activator receptor gene. Blood **86:** 624–635
36 Allgayer H., Wang H., Wang Y., Heiss M. M., Bauer R., Nyormoi O. et al. (1999) Transactivation of the urokinase-type plasminogen activator receptor gene through a novel promoter motif bound with an activator protein-2α-related factor. J. Biol. Chem. **274:** 4702–4714
37 Wang Y., Dang J., Johnson L. K., Selhamer J. J. and Doe W. F. (1995) Structure of the human urokinase receptor gene and its similarity to CD59 and the Ly-6 family. Eur. J. Biochem. **227:** 116–122
38 Lengyel E., Wang H., Stepp E., Juarez J., Wang Y., Doe W. et al. (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
39 Dang J., Boyd D., Wang H., Allgayer H., Doe W. F. and Wang Y. (1999) A region between –141 and –61 bp containing a proximal AP-1 is essential for constitutive expression of urokinase-type plasminogen activator receptor. Eur. J. Biochem. **264:** 92–99
40 Hapke S., Gawaz M., Dehne K., Kohler J., Marshall J. F., Graeff H. et al. (2001) β(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
41 Wang Y., Dang J., Wang H., Allgayer H., Murrell G. A. and Boyd D. (2000) Identification of a novel nuclear factor-kappaB sequence involved in expression of urokinase-type plas-

minogen activator receptor. Eur. J. Biochem. **267:** 3248–3254
42 Wang H., Yang L., Jamaluddin M. S. and Boyd D. D. (2004) The Kruppel-like KLF4 transcription factor, a novel regulator of urokinase receptor expression, drives synthesis of this binding site in colonic crypt luminal surface epithelial cells. J. Biol. Chem. **279:** 22674–22683
43 Montuori N., Rossi G. and Ragno P. (2002) Post-transcriptional regulation of gene expression in the plasminogen activation system. Biol. Chem. **383:** 47–53
44 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
45 Tran H., Maurer F. and Nagamine Y. (2003) Stabilization of urokinase and urokinase receptor mRNAs by HuR is linked to its cytoplasmic accumulation induced by activated mitogen-activated protein kinase-activated protein kinase 2. Mol. Cell. Biol. **23:** 7177–7188
46 Shetty S. (2005) Regulation of urokinase receptor mRNA stability by hnRNP C in lung epithelial cells. Mol. Cell Biochem. **272:** 107–118
47 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
48 Shetty S., Muniyappa H., Halady P. K. and Idell S. (2004) Regulation of urokinase receptor expression by phosphoglycerate kinase. Am. J. Respir. Cell Mol. Biol. **31:** 100–106
49 Montuori N., Mattiello A., Mancini A., Santoli M., Taglialatela P., Caputi M. et al. (2001) Urokinase-type plasminogen activator upregulates the expression of its cellular receptor through a post-transcriptional mechanism. FEBS Lett. **508:** 379–384
50 Montuori N., Mattiello A., Mancini A., Taglialatela P., Caputi M., Rossi G. et al. (2003) Urokinase-mediated posttranscriptional regulation of urokinase-receptor expression in non small cell lung carcinoma. Int. J. Cancer **105:** 353–360
51 Zannetti A., Del Vecchio S., Carriero M. V., Fonti R., Franco P., Botti G. et al. (2000) Coordinate up-regulation of Sp1 DNA-binding activity and urokinase receptor expression in breast carcinoma. Cancer Res. **60:** 1546–1551
52 Behrendt N., Ronne E. and Dano K. (1996) Domain interplay in the urokinase receptor. Requirement for the third domain in high affinity ligand binding and demonstration of ligand contact sites in distinct receptor domains. J. Biol. Chem. **271:** 22885–22894
53 Gardsvoll H., Dano K. and Ploug M. (1999) Mapping part of the functional epitope for ligand binding on the receptor for urokinase-type plasminogen activator by site-directed mutagenesis. J. Biol. Chem. **274:** 37995–38003
54 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
55 Colman R. W., Pixley R. A., Najamunnisa S., Yan W., Wang J., Mazar A. at al. (1997) Binding of high molecular weight kininogen to human endothelial cells is mediated via a site within domains 2 and 3 of the urokinase receptor. J. Clin. Invest. **100:** 1481–1487
56 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
57 Hoyer-Hansen G., Behrendt N., Ploug M., Dano 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
58 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
59 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
60 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
61 Chavakis T., Kanse S. M., Lupu F., Hammes H. P., Muller-Esterl W., Pixley R. A. et al. (2000) Different mechanisms define the antiadhesive function of high molecular weight kininogen in integrin- and urokinase receptor-dependent interactions. Blood **96:** 514–522
62 Ossowski L. and Aguirre-Ghiso J. A. (2000) Urokinase receptor and integrin partnership: coordination of signalling for cell adhesion, migration and growth. Curr. Opin. Cell Biol. **12:** 613–620
63 Wei Y., Lukashev M., Simon D. I., Bodary S. C., Rosenberg S., Doyle M. V. et al. (1996) Regulation of integrin function by the urokinase receptor. Science **273:** 1551–1555
64 Xue W., Mizukami I., Todd R. F. 3rd, Petty H. R. (1997) Urokinase-type plasminogen activator receptors associate with β1 and β3 integrins of fibrosarcoma cells: dependence on extracellular matrix components. Cancer Res. **57:** 1682–1689
65 Reinartz J., Schafer B., Batrla R., Klein C. E. and Kramer M. D. (1995) Plasmin abrogates $\alpha v\beta$5-mediated adhesion of a human keratinocyte cell line (HaCaT) to vitronectin Exp. Cell Res. **220:** 274–282
66 Xue W., Kindzelskii A. L., Todd R. F. 3rd, Petty H. R. (1994) Physical association of complement receptor type 3 and urokinase-type plasminogen activator receptor in neutrophil membranes. J. Immunol. **152:** 4630–4640
67 Kindzelskii A. L., Laska Z. O., Todd R. F. 3rd, Petty H. R. (1996) Urokinase-type plasminogen activator receptor reversibly dissociates from complement receptor type 3 (αMβ2′ CD11b/CD18) during neutrophil polarization. J. Immunol. **156:** 297–309
68 Bohuslav J., Horejsi V., Hansmann C., Stockl J., Weidle U. H., Majdic O. et al. (1995) Urokinase plasminogen activator receptor, β2-integrins and Src-kinases within a single receptor complex of human monocytes. J. Exp. Med. **181:** 1381–1390
69 Sitrin R. G., Todd R. F. 3rd, Albrecht E. and Gyetko M. R. (1996) The urokinase receptor (CD87) facilitates CD11b/CD18-mediated adhesion of human monocytes. J. Clin. Invest. **97:** 1942–1951
70 Simon D. I., Rao N. K., Xu H., Wei Y., Majdic O., Ronne E. et al. (1996) Mac-1 (CD11b/CD18) and the urokinase receptor (CD87) form a functional unit on monocytic cells. Blood **88:** 3185–3194
71 Simon D. I., Wei Y., Zhang L., Rao N. K., Xu H., Chen Z. et al. (2000) Identification of a urokinase receptor-integrin interaction site. Promiscuous regulator of integrin function. J. Biol. Chem. **275:** 10228–10234
72 Pluskota E., Soloviev D. A. and Plow E. F. (2003) Convergence of the adhesive and fibrinolytic systems: recognition of urokinase by integrin αMβ2 as well as by the urokinase receptor regulates cell adhesion and migration. Blood **101:** 1582–1590
73 Pluskota E., Soloviev D. A., Bdeir K., Cines D. B. and Plow E. F. (2004) Integrin αMβ2 orchestrates and accelerates plasminogen activation and fibrinolysis by neutrophils. J. Biol. Chem. **279:** 18063–18072
74 May A. E., Kanse S. M., Lund L. R., Gisler R. H., Imhof B. A. and Preissner K. T. (1998) Urokinase receptor (CD87) regulates leukocyte recruitment via β2 integrins *in vivo*. J. Exp. Med. **188:** 1029–1037
75 Gyetko M. R., Sud S., Kendall T., Fuller J. A., Newstead M. W. and Standiford T. J. (2000) Urokinase receptor-deficient mice have impaired neutrophil recruitment in response to pul-

monary Pseudomonas aeruginosa infection. J. Immunol. **165:** 1513–1519
76 Chapman H. A., Wei Y., Simon D. I., Waltz D. A.. (1999) Role of urokinase receptor and caveolin in regulation of integrin signalling. Thromb. Haemost. **82:** 291–7
77 Wei Y., Eble J. A., Wang Z., Kreidberg J. A. and Chapman H. A. (2001) Urokinase receptors promote β1 integrin function through interactions with integrin $\alpha 3\beta 1$. Mol. Biol. Cell **12:** 2975–2986
78 van der Pluijm G., Sijmons B., Vloedgraven H., van der Bent C., Drijfhout J. W., Verheijen J. et al. (2001) Urokinase-receptor/integrin complexes are functionally involved in adhesion and progression of human breast cancer *in vivo*. Am. J. Pathol. **159:** 971–982
79 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 signalling. J. Cell Biol. **147:** 89–104
80 Aguirre-Ghiso J. A., Liu D., Mignatti A., Kovalski K. and Ossowski L. (2001) Urokinase receptor and fibronectin regulate the ERK(MAPK) to p38(MAPK) activity ratios that determine carcinoma cell proliferation or dormancy *in vivo*. Mol. Biol. Cell **12:** 863–879
81 Monaghan E., Gueorguiev V., Wilkins-Port C. and McKeown-Longo P. J. (2004) The receptor for urokinase-type plasminogen activator regulates fibronectin matrix assembly in human skin fibroblasts. J. Biol. Chem. **279:** 1400–1407
82 Tarui T., Andronicos N., Czekay R. P., Mazar A. P., Bdeir K., Parry G. C. et al. (2003) Critical role of integrin $\alpha 5\beta 1$ in urokinase (uPA)/urokinase receptor (uPAR, CD87) signalling. J. Biol. Chem. **278:** 29863–29872
83 Zhang F., Tom C. C., Kugler M. C., Ching T. T., Kreidberg J. A., Wei Y. et al. (2003) Distinct ligand binding sites in integrin $\alpha 3\beta 1$ regulate matrix adhesion and cell-cell contact. J. Cell Biol. **163:** 177–188
84 Wei Y., Czekay R. P., Robillard L., Kugler M. C., Zhang F., Kim K. K. et al. (2005) Regulation of $\alpha 5\beta 1$ integrin conformation and function by urokinase receptor binding. J. Cell Biol. **168:** 501–511
85 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 signalling activity: generation of a new integrin inhibitor. J. Biol. Chem. **280:** 24792–24803
86 Le Y., Li B., Gong W., Shen W., Hu J., Dunlop N. M. et al. (2000) Novel pathophysiological role of classical chemotactic peptide receptors and their communications with chemokine receptors. Immunol. Rev. **177:** 185–94
87 Le Y., Murphy P. M. and Wang J. M. (2002) Formyl-peptide receptors revisited. Trends Immunol. **23:** 541–8
88 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
89 Resnati M., Pallavicini I., Wang J. M., Oppenheim J., Serhan C. N., Romano M. et al. (2002) The fibrinolytic receptor for urokinase activates the G protein-coupled chemotactic receptor FPRL1/LXA4R. Proc. Natl. Acad. Sci. USA **99:** 1359–1364
90 Furlan F., Orlando S., Laudanna C., Resnati M., Basso V., Blasi F. et al. (2004) The soluble D2D3(88–274) fragment of the urokinase receptor inhibits monocyte chemotaxis and integrin-dependent cell adhesion. J. Cell Sci. **117:** 2909–2916
91 de Paulis A., Montuori N., Prevete N., Fiorentino I., Rossi F. W., Visconte V. et al. (2004) Urokinase induces basophil chemotaxis through a urokinase receptor epitope that is an endogenous ligand for formyl peptide receptor-like 1 and -like 2. J. Immunol. **173:** 5739–48
92 Selleri C., Montuori N., Ricci P., Visconte V., Carriero M. V., Sidenius N. et al. (2005) Involvement of the urokinase-type plasminogen activator receptor in hematopoietic stem cell mobilization. Blood **105:** 2198–2205
93 Gargiulo L., Longanesi-Cattani I., Bifulco K., Franco P., Raiola R., Campiglia P. et al. (2005) Cross-talk between fMLP and vitronectin receptors triggered by urokinase receptor-derived SRSRY peptide. J. Biol. Chem. **280:** 25225–25232
94 Gyetko M. R., Todd R. F. 3rd, Wilkinson C. C. and Sitrin R. G. (1994) The urokinase receptor is required for human monocyte chemotaxis *in vitro*. J. Clin. Invest. **93:** 1380–7
95 Hoyer-Hansen G., Pessara U., Holm A., Pass J., Weidle U., Dano K. et al. (2001) Urokinase-catalysed cleavage of the urokinase receptor requires an intact glycolipid anchor. Biochem J. **358:** 673–679
96 Ali H., Richardson R. M., Haribabu B. and Snyderman R. (1999) Chemoattractant receptor cross-desensitization. J. Biol. Chem. **274:** 6027–6030
97 Le Y., Shen W., Li B., Gong W., Dunlop N. M. and Wang J. M. (1999) A new insight into the role of 'old' chemotactic peptide receptors FPR and FPRL1: down-regulation of chemokine receptors CCR5 and CXCR4. Forum **9:** 299–314
98 Li B. Q., Wetzel M. A., Mikovits J. A., Henderson E. E., Rogers T. J., Gong W. et al. (2001) The synthetic peptide WKYMVm attenuates the function of the chemokine receptors CCR5 and CXCR4 through activation of formyl peptide receptor-like 1. Blood **97:** 2941–2947
99 Comoglio P. M., Boccaccio C. and Trusolino L. (2003) Interactions between growth factor receptors and adhesion molecules: breaking the rules. Curr. Opin. Cell Biol. **15:** 565–571
100 Liu D., Aguirre Ghiso J., 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
101 Jo M., Thomas K. S., O'Donnell D. M. and Gonias S. L. (2003) Epidermal growth factor receptor-dependent and -independent cell-signalling pathways originating from the urokinase receptor. J. Biol. Chem. **278:** 1642–1646
102 Jo M., Thomas K. S., Marozkina N., Amin T. J., Silva C. M., Parsons S. J. et al. (2005) Dynamic assembly of the urokinase-type plasminogen activator signalling receptor complex determines the mitogenic activity of urokinase-type plasminogen activator. J. Biol. Chem. **280:** 17449–17457
103 Kiyan J., Kiyan R., Haller H. and Dumler I. (2005) Urokinase-induced signalling in human vascular smooth muscle cells is mediated by PDGFR-β. EMBO J. **24:** 1787–1797