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Multiple activities of a multifaceted receptor: roles of cleaved and soluble uPAR

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1. ABSTRACT

The urokinase-type plasminogen activator receptor (uPAR) is a GPI-anchored cell-surface receptor involved in many physiological and pathological events that include cell migration and tissue invasion. uPAR traditional role was considered the focusing of uPA proteolytic activity on the cell surface; however, different uPAR activities have been demonstrated in the last years. In fact, cell surface uPAR functionally interacts with integrins, fMLP-receptors (fMLP-Rs) and growth factor receptors, thus regulating cell adhesion, migration and proliferation. uPAR also exists in a soluble form (suPAR) that has been detected in human body fluids. Both cell surface and suPAR can be proteolytically cleaved, thus generating truncated forms lacking the N-terminal domain and exposing the specific sequence able to interact with the fMLP-Rs. The cleaved form of suPAR binds and activates the fMLP-Rs and regulates the activity of MCP-1, RANTES and SDF1 receptors. Here, we review the role that shedding and cleavage could play in regulating uPAR structural/functional interaction with other cell-surface receptors and in uPAR-mediated biological and pathological processes.

2. INTRODUCTION

Degradation of the extracellular matrix (ECM) by cell surface associated proteolysis plays an important role in various processes, including morphogenesis, angiogenesis, mammary gland involution, inflammation, wound healing and dissemination of cancer. The urokinase (uPA)-mediated plasminogen activation system, which generates the potent serine-protease plasmin, is involved in all these events (1-2). The key molecule of this system, the uPA receptor (uPAR), possesses an impressive range of functions; indeed, it regulates not only extracellular proteolysis, but also cell adhesion to ECM, cell migration toward uPA and fMLP, and cell proliferation and survival. All these activities can be realized by uPAR ability to interact with cell-surface and ECM molecules and independently of the uPA proteolytic activity (3-4).

uPAR also exists in a soluble form (suPAR) that has been detected in biological fluids. Both cell surface and suPAR can be proteolitically cleaved, thus generating truncated forms lacking the N-terminal domain (5).

This review is focused on the regulation of uPAR-mediated functions in biological and pathological processes by receptor shedding and cleavage.

3. THE uPA RECEPTOR

The existence of a cellular binding site for uPA was reported in 1985 (6-7), but the isolation of the purified protein and the sequencing of its cDNA did not occur until 1990 (8-9). The cDNA-derived sequence revealed the human uPAR to be 313 amino acid residues long without any obvious transmembrane sequence. Accordingly, it was later shown that uPAR is anchored to the plasma membrane by a glycosylphosphatidylinositol (GPI) moiety; during the glycolipid modification of uPAR, a C-terminal signal sequence is excised, resulting in a fully processed uPAR that spans residues 1–283 (10).

The presence of a GPI anchor has important implications for the functional properties of uPAR. First, the GPI-anchor confers extreme mobility to uPAR along the cell-membrane and enables the receptor to associate to specialized microdomains of the plasma membrane (lipid rafts), capability that can play an important role in its biological functions. Second, the absence of a cytoplasmic tail implies the requirement of additional lateral interactions with integral membrane proteins to activate possible uPAR-dependent cell-signaling pathways. Finally, cell-surface uPAR expression and the levels of soluble forms of uPAR (see below) can be regulated by specific phospholipases, beside proteases.

Mature cell-surface uPAR is formed by three homologous, differently folded, domains of approximately 90 aminoacids (DI, DII and DIII, from the N-terminus). The main uPA-binding domain is located in the DI domain, but the full length molecule is required for an efficient binding to uPA (11-12). Recently, the crystal structure of the soluble form of uPAR bound to an antagonist peptide (13) and to the amino-terminal fragment (ATF) of uPA (14) has been solved, thus confirming that uPAR consists of three domains with a typical three finger fold. The three domains of uPAR delimit a central cavity which accomodates the ligand peptide. This model suggests that uPA is embedded in the central cavity and that the large outer receptor surface is available to bind additional ligands (13). In fact, uPAR can bind other ligands, such as vitronectin (VN) (15), an ECM component, and the cleaved high molecular mass kininogen (HKa) lacking the vasodilator peptide bradykinin (16). uPA positively regulates VN binding to uPAR, likely by inducing formation of uPAR dimers that exhibit an higher affinity for VN as compared to the monomers (17). uPA, indirectly, may also contribute to drive uPAR into lipid rafts, since dimeric uPAR partitions preferentially to these microdomains of cell membrane (18). Indeed, uPA increases uPAR localization to lipid rafts and modifies the composition of the lipid membrane microdomain of the receptor (19).

uPAR binding to VN can be inhibited by the specific type-1 uPA inhibitor (PAI1), because uPAR and PAI1 bind overlapping regions on VN, close to the integrin binding site (20). It has been recently suggested that a direct uPAR-VN interaction is both required and sufficient to initiate a signal transduction, without the requirement of lateral interactions with transmembrane proteins, which, in contrast, have been proposed by several and different reports (21-22).

Up-regulation of the cell surface uPAR has been observed in host response to infection, in inflammatory disorders and in several human diseases, including numerous cancers (3, 5, 22). The administration of inactive uPA, uPAR peptide antagonists or antisense uPAR, prevents growth, invasiveness and metastasis of different mouse tumors *in vivo* (23). However, the overexpression of both uPA and uPAR in basal epidermis induces only epidermal thickening and sub-epidermal blisters (24). Moreover, uPAR-/- mouse embryonic fibroblasts grow faster at low serum, produce more colonies in agar and produce tumors *in vivo* in nude mice with a lower latency period (25). Thus, further studies are needed to elucidate the role of uPAR in cancer.

4. uPAR PARTNERS AND ACTIVITIES

Interactions of membrane-bound uPAR with other cell surface receptors have been considered crucial for uPAR-dependent activities. In fact, uPAR stimulation regulates cell adhesion, migration and proliferation (3, 22), protects from apoptosis and anoikys (4, 26-28) and induces epithelial mesenchymal transition (EMT), independently of uPA enzymatic activity (29). At present, a large body of evidence suggests that uPAR is likely organized into a dynamic multiprotein signaling complex and that the expression and assembly of specific uPAR interactors determine the response to uPA in a specific cell type. (22, 30). A recent *in vivo* study highlighted this aspect in the regulation of bone homeostasis in which uPAR shows the ability to exert contrasting activities in different cell types. Indeed, uPAR -/- osteoblasts show a proliferative advantage without difference in apoptosis as compared to wild type (wt) controls. On the resorptive side, the number of osteoclasts formed *in vitro* from uPAR -/- monocytes is decreased (31).

Various reports demonstrated uPAR capability to associate to integrins of the beta1, beta2 and beta3 families. uPAR interactions with integrins lead to important functional consequences on integrin activity that, again, can be different in the different cell types (22, 32). Recently, integrin binding sites have been identified in uPAR domain DII (residues 130-142, peptide D2A) (33) and in uPAR domain DIII (residues 240-248) (34).

D2A abolishes uPAR-alphav-beta3 and uPAR-alpha5-beta1 co-immunoprecipitation, indicating that it can bind both of these integrins; in addition, D2A has chemotactic activity that requires alphav-beta3 and activates alphav-beta3-dependent signaling pathways. Interestingly, mutating two glutamic acids into two alanines generates the peptide D2A-Ala, which not only lacks chemotactic activity but also inhibits vitronectin, fibronectin and collagen-dependent cell migration (33).

The specific sequence identified in the uPAR domain DIII binds the alpha5-beta1 integrin; substituting a single amino acid in that region (S245A) impairs uPAR binding to the purified integrin (34). In the recently solved crystal structure of uPAR, the Ser-245 is confined to the large external surface of the receptor, a location that is well separated from the central urokinase plasminogen binding cavity and that could be available for the binding to other molecules, such as integrins (13-14).

However, uPAR binding to integrins requires the full length receptor, including domain I (35), as well as for uPAR-uPA and uPAR-VN interactions (12, 36). Interestingly, uPA, can simultaneously bind uPAR and specific integrins, through different domains of its molecule, thus initiating and potentiating intracellular signaling and migration (37-38).

The importance of uPAR-integrins interactions has been documented also *in vivo* by a synthetic peptide (p25), which interferes with the formation of uPAR-integrin complexes. Administration of p25 to nude mice resulted in significantly reduced tumor progression of MDA-MB-231 cells in bone when compared to the scrambled control peptide (39).

uPAR interaction with integrins seems to play also a critical role in the regulation of kidney permeability (40). In fact, induction of uPAR signaling in podocytes leads to foot process effacement and urinary protein loss via a mechanism that includes lipid-dependent activation of alphav-beta3 integrin.

Membrane-bound uPAR functionally interacts also with a G-protein coupled receptor (GPCR) involved in chemotaxis, the high affinity receptor (FPR) for the fMet-Leu-Phe peptide (fMLP) (35). fMLP is a peptide of bacterial origin that is a strong leukocyte chemoattractant. fMLP receptors were firstly identified in leukocytes, and subsequently in several different cell types, including epithelial cells (41). The fMLP receptor family comprises FPR and its homologues FPRL-1 and FPRL-2. FPRL-1 has a much lower affinity for fMLP, whereas it is efficiently activated by several different molecules, including lipoxin A_4, serum amyloid A, various peptides and, as recently shown, annexin 1 (41, 42). FPRL-2 shows a high homology with the other two fMLP receptors but does not bind fMLP and shares few ligands with FPRL-1 (41). Recently a specific ligand for FPRL-2 has been identified as F2L, a peptide derived from heme-binding protein (43). fMLP-dependent cell migration requires uPAR expression (35, 44); on the other hand, uPA-dependent cell migration requires FPRL-1 expression in monocytes and involves FPRL-1 and FPRL-2 in basophils (45- 46).

uPAR can also cross-talk with growth factor receptors, such as the epidermal growth factor receptor (EGFR) and the platelet derived growth factor receptor (PDGFR) beta, which seem to mediate uPAR signaling (47-48). Indeed, uPA binding to uPAR can initiate a cell-signaling pathway that is mediated by EGFR (47, 49). Conversely, uPAR-dependent cell-signaling may prime cells to proliferate in response to EGF. The importance of this pathway depends on the c-Src level in the cell and is relevant in breast cancer cells (50, 51).

uPAR cross-talk with the platelet-derived growth factor receptor (PDGFR)-beta has been reported in human vascular smooth muscle cells (VSMC) (48). uPA induces uPAR association with PDGFR-beta, thus stimulating its phosphorylation. uPAR-PDGFR-beta association is required for uPA-induced migratory and proliferative downstream signals. However, unlike the association to EGFR, uPA strongly inhibits PDGF-induced migration.

5. THE CLEAVED FORM OF MEMBRANE-BOUND uPAR: PARTNERS AND ACTIVITIES

Various proteases, including trypsin, chymotrypsin, elastase, cathepsin G, metalloproteases, plasmin and uPA can cleave uPAR in the DI-DII linker region (5, 52). The cleavage promotes the release of the uPA-binding DI domain, thus leaving on the cell surface truncated forms of GPI-uPAR (c-uPAR) lacking DI. These DII-DIII uPAR forms, according to the site of cleavage, can unmask at the N-terminus a peculiar sequence, SRSRY, (aminoacids 88-92), that is involved in cell migration and chemotaxis (53). The SRSRY sequence is required for the functional interaction of the GPI-anchored uPAR with the high affinity fMLP-receptor, FPR; in fact, cells transfected with a truncated form of uPAR, lacking SRSRY, are unable to migrate in a fMLP gradient, as well as uPAR negative cells, albeit they express fMLP receptors (35). Most proteases able to remove DI, including metalloprotease 12, elastase, cathepsin G and uPA itself, cleave the receptor leaving the SRSRY sequence at the N-terminus of the GPI-anchored receptor (5). The cleaved forms of GPI-uPAR, independently of the presence of the SRSRY sequence, are unable to interact with integrins (35).

In conclusion, at least two forms of uPAR are present on the cell surface: full-length uPAR and cleaved uPAR, each specifically interacting with one or more transmembrane proteins. These interactions and their effects on signaling pathways activation have been recently described by exploiting a cleavage resistant (hcr) uPAR mutant with mutations in the SRSRY sequence (49). Wild-type (wt) and hcr-uPAR engage different pathways in uPA-dependent cell migration. uPA-induced association of wt-uPAR to alpha3-beta1 results in uPAR cleavage and extracellular signal-regulated kinase (ERK) activation, the process involving FPRL-1. By contrast, hcr-uPAR with the mutated SRSRY sequence does not activate ERKs and does not engage FPRL-1 or any other G protein-coupled receptor, but it activates an alternative pathway initiated by uPAR association with EGFR and the alpha3-beta1 integrin, that results in the autophosphorylation of EGFR. Thus, uPAR and c-uPAR appear to mediate different signaling pathways. This observation has been confirmed also in a report on fibroblast-to-myofibroblast differentiation (54). Indeed, fibroblasts express increased amounts of full-length cell surface uPAR as compared to myofibroblasts, which have increased expression of c-

Table 1. Ligands and cell-surface partners of the different forms of uPAR

uPAR Forms	Ligands	Cell-surface partners
Full length GPI-uPAR	uPA, Vitronectin, HKa	Integrins, fMLP-receptors, EGFR
[1]Cleaved GPI-uPAR		fMLP-receptors
Full length suPAR	uPA, Vitronectin, HKa	Integrins
[1]Cleaved suPAR		fMLP-receptors

[1]Cleaved uPAR forms bind fMLP-receptors only if containing the ^{88}SRSRY^{92} sequence.

PAR. Protease inhibitors that inhibit uPAR cleavage prevent myofibroblast differentiation, that can be prevented also by overexpression of a noncleavable form of uPAR. Therefore, uPAR truncation is necessary to induce fibroblast transition to myofibroblast.

In Systemic Sclerosis (SSc), microvascular endothelial cells (MVECs) angiogenesis is blocked by the matrix metalloproteinase 12-dependent cleavage of uPAR (55). Indeed, in normal MVECs, full-size uPAR is connected with the actin cytoskeleton; this connection, mediated by the alphaM- and alphaX-subunits of beta2 integrin, is lost in SSc MVECs, in which cleaved uPAR cannot associate with beta2 integrins or with actin. The uncoupling of uPAR from beta2 integrins in SSc MVECs impairs the activation of Rac and Cdc42 and blocks the integrin-delivered signals to the actin cytoskeleton (56). Thus, uPAR cleavage may contribute to the reduced angiogenic properties of SSc MVECs by inhibiting integrin-cytoskeleton connections.

uPAR cleavage can obviously functions as a mechanism that negatively regulates several uPAR activities: the focusing and enhancement of uPA-dependent proteolysis on the cell surface, the adhesion to VN, the regulation of integrin functions. However, less obviously, the observations that uPAR cleavage is crucial for the fibroblast-myoblast transition and that it is able to initiate a cell-signaling pathway leading to ERK activation (49) highlight the possibility that also the cleaved GPI-membrane anchored uPAR could exert specific functions.

6. SOLUBLE uPAR: PARTNERS AND ACTIVITIES

The soluble form of the full length uPAR (suPAR) has been detected both *in vitro* and *in vivo* (5). Increased suPAR levels have been observed in sera from patients affected by various diseases, including different types of cancers (57). Recently, elevation of plasma uPAR levels associated with features of biologically aggressive prostate carcinoma, disease progression after radical prostatectomy, and metastasis have been observed (58).

Full length uPAR release from the cell surface can be due to the action of glycosyl-phosphatidyl-inositol specific phospholipase C or D (10, 59) or of proteases such as plasmin and cathepsin G (60-61). suPAR ligands are the same as the GPI-anchored receptor: extracellular molecules, such as uPA, HKa and VN, and cell-surface molecules as beta1 and beta2 integrins (5). By contrast, shed uPAR becomes unable to interact with fMLP receptors (53) (Table 1).

Therefore, suPAR, by binding uPA and VN, can reduce uPA-dependent cell-bound proteolysis and cell adhesion to VN by functioning as a potent scavenger molecule for uPA and VN. However, the soluble receptor is able to activate cell signaling pathways in uPAR negative cells (62) and can still activate beta2 and beta1 integrins, thus inducing leukocyte adhesion to endothelium (63) and ERK1/2 activation in Hep3 cells (64).

Thus, uPAR shows a dual role, it can inhibit some activities and activate others. Indeed, increased suPAR levels represent a negative prognostic factor in several tumors (57); at the same time, suPAR is a potent scavenger for uPA, thus reducing ovarian, prostate and breast carcinoma tumor growth and lung colonization by breast carcinoma cells (65-68). In addition, suPAR-dependent signals can inhibit cancer cell growth and invasion (62).

7. CLEAVED FORMS OF SOLUBLE uPAR: PARTNERS AND ACTIVITIES

Metalloproteases, cathepsin G and elastase can cleave suPAR in the DI-DII linker region, as well as GPI-uPAR; interestingly, uPA does not cleave suPAR as efficiently as GPI-uPAR (5). As well as for GPI-uPAR, the cleavage promotes the release of DI and can unmask the specific sequence SRSRY, endowed with chemotactic activity (53). The cleaved form of suPAR (c-suPAR) has been detected in fluids from individuals affected by different cancers and from neurologic patients (5, 57).

c-suPAR, or its derived peptides containing the SRSRY sequence, bind and activate FPRL-1 in monocytes, thus stimulating their migration (45). In the last years, c-suPAR capability to activate other fMLP receptors in different cell types has been reported. In fact, c-suPAR binds and activates FPR in hematopoietic stem cells (HSCs) and in epithelial HEK-293 cells (69, 70) and the c-suPAR derived peptide $uPAR_{84-95}$ induces basophil migration by activating both FPRL-1 and FPRL-2 (46). Thus, c-suPAR can be considered as a ligand of fMLP receptors; in addition, it can regulate the activity of other chemoattractant receptors in a fMLP-R dependent manner. In fact, pretreatment of monocytes with increasing amounts of c-suPAR prevents cell migration in response to MCP-1 and RANTES. Heterologous desensitization of CCR2, the MCP-1 receptor, cannot account for c-suPAR-mediated inhibition of MCP-1-induced cell migration because c-suPAR does not inhibit MCP-1-receptor binding and MCP-1-induced intracellular Ca (2+) increase nor induces CCR2 internalization. Rather, c-suPAR dramatically decreases chemokine-induced integrin-dependent rapid cell adhesion, a crucial step in cell migration, by interacting with FPRL-1 (71).

c-suPAR effects on cell adhesion have been also observed in epithelial cells; indeed, the specific uPAR-

derived peptide SRSRY decreases adhesion of uPAR-negative HEK-293 cells to VN; interestingly, FPR desensitization abolishes the SRSRY-dependent adhesion effect (70), thus suggesting the involvement of fMLP receptors also in c-suPAR capability to regulate cell adhesion, independently of cell-surface uPAR expression. Recently, the functional epitope on uPAR that is responsible for its interaction with the full-length, extended form of vitronectin has been identified by using a comprehensive alanine-scanning library of purified single-site uPAR mutants. The five residues identified as "hot spots" for vitronectin binding form a contiguous epitope consisting of Trp^{32}, Arg^{58}, and Ile^{63} (uPAR domain I) and, interestingly, of Arg^{91} and Tyr^{92} (DI-DII linker region), thus including two residues of the $^{88}SRSRY^{92}$ peptide (72). Therefore, SRSRY peptides, beside regulating uPAR-independent cell migration and adhesion by a fMLP-receptor-dependent mechanism, could also reduce uPAR-dependent cell adhesion to VN by displacing uPAR from VN.

c-suPAR, as a ligand of fMLP-receptors, is also able to activate cell-signaling pathways; indeed, it induces PKC and ERK-1/2 activation, the latter involving the alphaV-beta5 integrin (62, 70).

All these observations indicate that the SRSRY-containing c-suPAR plays active roles in cell adhesion and migration, including the capability to activate cell-signaling pathways.

Recently, a role for c-suPAR in the mobilization of hematopoietic stem cell mobilization has been reported, thus ultimately suggesting biological *in vivo* functions of this uPAR-derived molecule.

7.1. c-suPAR involvement in mobilization of hematopoietic stem cells

Hematopoietic stem cells (HSCs) are clonogenic cells capable of self-renewal and multilineage differentiation; they are commonly identified on the basis of the expression of the CD34 antigen and lineage negativity. $CD34^+$ cells contain cell populations capable of both short-term and long-term reconstitution of myeloablated transplant recipients. The majority of $CD34^+$ HSCs reside in the bone marrow (BM-HSCs), being very low the percentage of circulating HSCs (PB-HSCs) (73). The finding of increased circulating HSC levels in the blood of patients following treatments with cyclophosphamide and other drugs, the subsequent technical improvements allowing large-scale harvesting and studies showing faster BM repopulation in patients transplanted with autologous mobilized PB-HSCs led to the wide use of PB-HSCs in transplantation therapy (73). G-CSF has now become the standard mobilizing agent, even though various other cytokines are able to induce mobilization (74). Mobilization implies the distruption of HSC interactions with the BM microenvironment. CXC receptor 4 (CXCR4) and its ligand, the stromal derived factor 1 (SDF1), play a crucial role in HSC retention in BM. Indeed, murine and human HSC express CXCR4 and efficiently migrate toward SDF1, which is largely produced by BM endothelium (75). AMD3100, a pharmacologic inhibitor of CXCR4, induces leukocytosis and mobilization of $CD34^+$ cells in humans and mice, thus demonstrating the crucial role of CXCR4 and its ligand in HSC retention/mobilization (76, 77).

We showed that during human HSC mobilization, uPAR expression increased significantly on $CD33^+$ myeloid precursors and on $CD14^+$ monocytic cells released from BM into the circulation. By contrast, $CD34^+$ cells, T and B lymphocytes remained uPAR-negative. Up-regulation of cell-surface uPAR in $CD33^+$ and $CD14^+$ monocytic cells coincided with the increase of the soluble form of the receptor (suPAR) in the serum, thus suggesting its release from the monocytic cell surface. Both the previously described full length and cleaved forms of shed suPAR were observed. We then showed that chemotactic c-suPAR is a potent *in vitro* chemoattractant for BM-HSCs, since they express the high affinity receptor for fMLP (FPR). Moreover, c-suPAR or the c-suPAR derived peptide $uPAR_{84-95}$ inhibited *in vitro* SDF-1 dependent migration of $CD34^+$ BM-HSCs (69). Altogether, these data suggested that during G-CSF-induced HSC mobilization, uPAR was first up-regulated on $CD33^+$ and $CD14^+$ cells and then released from the cell surface, thus leading to increased suPAR levels in the serum. suPAR could be rapidly cleaved both in the serum and in the BM. In the first case c-suPAR could chemoattract BM HSCs, inducing their migration into the circulation through a positive gradient. In the second case, c-suPAR could inactivate CXCR4, thus promoting HSC release from BM (Figure 1). Subsequently, we showed that the chemotactic c-suPAR derived peptide, $uPAR_{84-95}$, elicits mobilizing activity *in vivo* (78). Indeed, administration of human $uPAR_{84-95}$ induced migration of mouse $CD34^+$ HSCs/HPCs into the circulation to an extent similar to that observed with G-CSF.

These findings demonstrated for the first time an *in vivo* biological effect of the chemotactic epitope of c-suPAR, thus finally indicating that this molecule is an *in vivo* active molecule and not only a degradation product, and thus it could be involved in physiologic and pathologic processes.

8. SUMMARY AND PERSPECTIVE

In 1995 the first observation that uPAR, beta 2-integrins and Src-kinases associated in a single receptor complex in human monocytes was reported (79). Since then, various and multitudinous reports showed uPAR capability to transduce signals of cell adhesion, migration, proliferation, survival. The requirement of partners able to trasduce uPAR-dependent signals was obvious, because of the absence of a cytoplasmic tail of uPAR, and it was as much obvious that the GPI-tail could confer to uPAR a mobility that cannot be a feature of a transmembrane protein. Thus, that capability enables uPAR to move along the membrane and to associate to different molecules in signaling membrane complex that can activate different functions in different cell types. At present, the best candidates to partner uPAR functions are integrins and fMLP receptors. Their cooperation in the various activities of the the different forms of uPAR is undebatable, on the

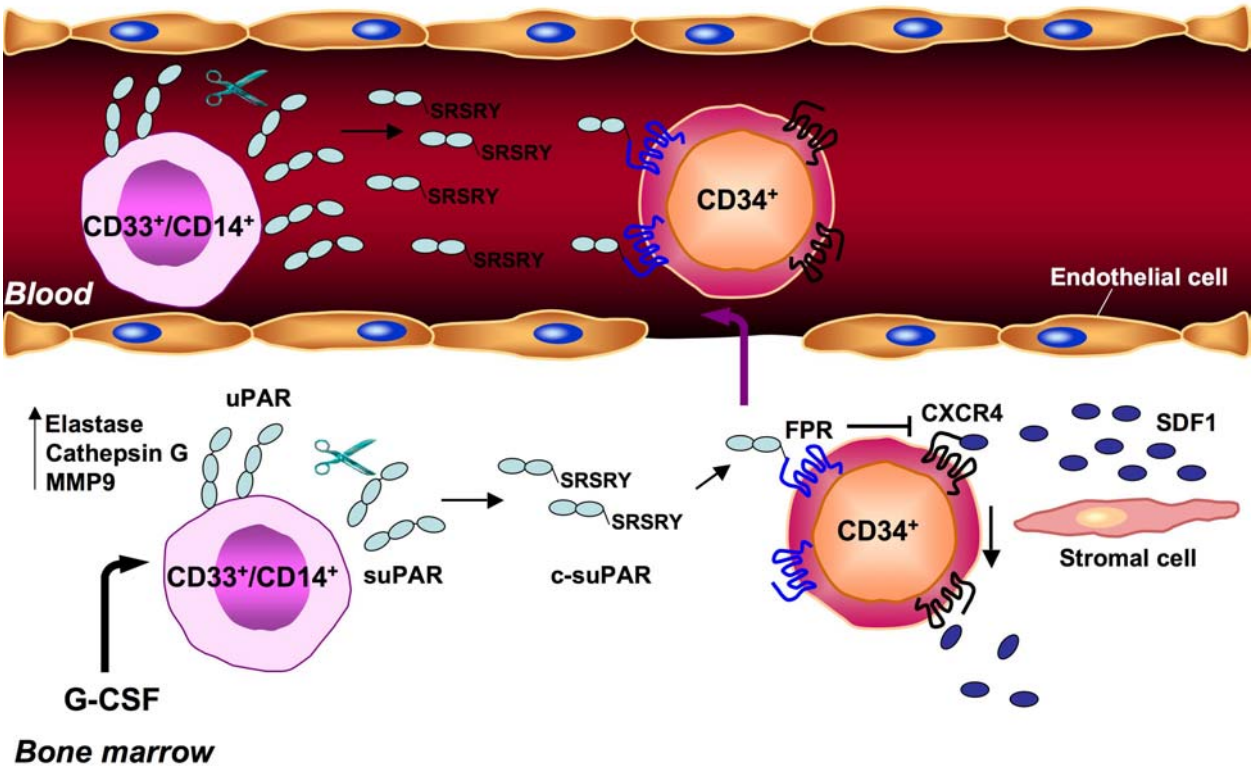

Figure 1. uPAR role in HSC mobilization. Following G-CSF administration, uPAR is up-regulated on $CD33^+$ and $CD14^+$ cells and then released from the cell surface, thus leading to increased suPAR levels in the serum. suPAR could be rapidly cleaved both in the serum and in the BM by various up-regulated proteases, thus generating c-suPAR. Serum c-suPAR may chemoattract BM HSCs, inducing their migration into the circulation through a positive gradient. BM c-suPAR may also inactivate CXCR4, thus promoting HSC release from BM.

basis of many significant and outstanding reports. However, the mechanism by which uPAR cooperates with these partners, on our point of view, is still unclear. The first point is the requirement of a physical association of uPAR to its partners, in particular to integrins, that recently has been questioned (21). The second important point is whether both GPCR and integrins are simultaneously required for uPAR-dependent activities, and, in this case, what is the mechanism linking uPAR to integrins and to GPCR. Finally, it is also still unclear how the EGFR, that also has been shown to play a function in uPAR-dependent activities, can participate to this mechanism, and the role of vitronectin, that, according a recent report, seems to play a crucial role in all uPAR activities.

The identification of the distinct roles of the involved interactors, and of their relationships, could contribute to elucidate the roles of the different uPAR forms in various diseases, in particular in cancer, in which they often seem to exert contrasting effects.

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Abbreviations: EGFR: epidermal growth factor receptor, PDGFR: platelet-derived growth factor receptor, FPR and FPRL-1: formyl peptide receptor and formyl peptide receptor-like, HKa: high molecular mass kininogen, PAI-1: plasminogen activator inhibitor-1, uPA: urokinase-type plasminogen activator, uPAR: uPA receptor, ERK: extracellular signal-regulated kinase, VSMC: human vascular smooth muscle cells, SSc: Systemic Sclerosis, MVEC: microvascular endothelial cells, HSCs: hematopoietic stem cells, G-CSF: granulocyte-colony stimulating factor; BM: bone marrow.

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