

MINI REVIEW

CD87 (urokinase-type plasminogen activator receptor), function and pathology in hematological disorders: a review

MC Béné¹, G Castoldi², W Knapp³, GM Rigolin², L Escribano⁴, P Lemez⁵, W-D Ludwig⁶, E Matutes⁷, A Orfao⁸, F Lanza² and M van't Veer⁹ on behalf of EGIL, European Group on Immunological Classification of Leukemias

¹GEIL, Groupe d'Etude Immunologique des Leucémies, Immunology Laboratory, University Hospital of Nancy, Vandoeuvre les Nancy, France; ²Institute of Hematology, St Anna Hospital, Ferrara, Italy; ³Institute of Immunology, University of Vienna, Vienna, Austria; ⁴Department of Hematology, Hospital Ramon y Cajal, Madrid, Spain; ⁵Department of Hematology and Blood Transfusion, Hospital Jihlava, Czech Republic; ⁶HELIOS Clinic Berlin, Robert Rössle Clinic, Charité, Humboldt-University, Berlin, Germany; ⁷Department of Hematology, Royal Marsden Hospital, London, UK; ⁸Servicio de Citometria and Departamento de Medicina and Centro de Investigacion del Cancer, Hospital Universitario de Salamanca, Salamanca, Spain; and ⁹Daniel Den Hoed Cancer Center, Rotterdam, The Netherlands

The analysis of CD87 (urokinase-type plasminogen activator receptor – uPAR) expression has a potential role in the diagnostic or prognostic work-up of several hematological malignancies, particularly acute leukemia and multiple myeloma. The distribution of CD87 in acute myeloid leukemia (AML) varies according to the FAB subtype (highest expression in M5 and lowest in M0). Functionally, it is conceivable that the expression of CD87 could contribute to the invasive properties of the leukemic cells towards the skin and mucosal tissues as reflected by the clinical behavior of CD87 high cases. The lack of or weaker expression of CD87 on blast cells from ALL patients supports the concept that CD87 investigation might help in the distinction of AMLs from lymphoid malignancies. Among lymphoproliferative disorders, the expression of CD87 is exclusively found in pathological plasma cells. Since plasma cells also coexpress some adhesion molecules such as CD138 and CD56, this observation is consistent with the capacity of these cells to home in the bone compartment. High levels of soluble uPAR appear to represent an independent factor predicting worse prognosis and extramedullary involvement in multiple myeloma.

Leukemia (2004) 18, 394–400. doi:10.1038/sj.leu.2403250
Published online 11 December 2003

Keywords: CD87; uPAR; suPAR; flow cytometry

General aspects

The urokinase-type plasminogen activator (uPA) system consists of a proteinase (the uPA), its receptor (the urokinase-type plasminogen activator receptor – uPAR or CD87) and two major inhibitors, the plasminogen activator inhibitor 1 (PAI 1) and PAI 2.^{1,2}

uPA is a specific serine protease, which converts plasminogen into its active form, plasmin, a broad-spectrum serine protease involved in the digestion of basement membranes and of various protein substrates in the extracellular matrix. Therefore, it plays a crucial role in cell migration and extravasation.^{3–6} Two major functional domains have been identified in the uPA molecule: an N-terminal domain also known as 'growth factor domain' due to its homology with the epidermal growth factor and a C-terminal domain that displays protease activity. The N-terminal domain has no enzymatic activity but binds with high affinity to the cell-surface uPA receptor, uPAR or CD87.

uPAR is a heavily glycosylated glycosyl-phosphatidylinositol (GPI)-anchored cell-surface receptor, composed of 274 amino-acid residues, which binds uPA produced endogenously or released from surrounding cells, and focuses plasmin proteolytic activity on the relevant cell's surface. uPAR belongs to the Ly6/neurotoxin receptor family and consists of three internally disulfide-bonded domains (D1, D2 and D3). It is attached to the cell surface by a GPI anchor⁷ (Figure 1). The receptor has neither transmembrane nor cytoplasmic domains. The ligand-binding activity resides in the N-terminal domain, but all three domains are necessary to achieve a high-affinity binding of uPA. By binding to uPAR through its N-terminal domain, the catalytic C-terminal domain of (pro)-uPA gets close to membrane-bound plasminogen. This process results in the enzymatic activation of (pro)-uPA into uPA, which subsequently activates more plasminogen to generate additional plasmin in a mechanism that is referred to as 'reciprocal zymogen activation'.⁸ Vitronectin represents another important ligand for uPAR; the binding of uPA and vitronectin to uPAR is not mutually exclusive and uPA stimulates the vitronectin binding to uPAR.⁹

uPA and uPAR functions are modulated by the two specific inhibitors, PAI 1 and PAI 2. These two proteins belong to the serpin family and they bind and inhibit both free and receptor-bound uPA. The binding of PAI 1 to uPAR changes the properties of the receptor. Indeed, the uPAR–uPA–PAI 1 complex displays a binding site for the alpha-2-macroglobulin receptor, leading to an increased rate of internalization and degradation of this complex. uPAR is then recycled to the cell surface while the uPA–PAI 1 pair is degraded. Therefore, PAI 1 controls both the cell-surface proteolytic activity and the cellular distribution of uPAR in the plasma membrane.

uPAR-related cell functions

uPAR exerts multiple regulatory effects on cell migration, leukocyte adhesion, chemotaxis and signal transduction during leukocyte recruitment from the circulation to extravascular sites of inflammation.

Migration

uPAR expression is strictly linked to cellular migration through its capacity to promote pericellular proteolysis. In response to uPA binding, the uPAR–uPA complex has been shown to cluster

Correspondence: G Castoldi, Hematology Section, Department of Biomedical Sciences, University of Ferrara, Corso Giovecca, 203, Ferrara 44100, Italy; Fax: +390532 212142; E-mail: sse@dns.unife.it
Received 7 August 2003; accepted 18 November 2003; Published online 11 December 2003

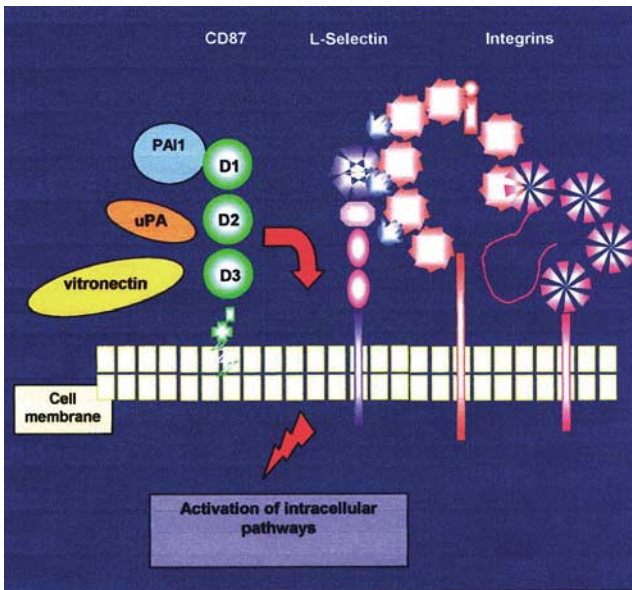


Figure 1 Schematic representation of the uPA system. uPAR is a glycosylated GPI-anchored cell-surface receptor composed of three extracellular domains (D1, D2 and D3). The receptor has neither transmembrane nor cytoplasmic domains. By binding to uPAR, (pro)-uPA is enzymatically activated into uPA, which subsequently activates plasminogen to generate plasmin in a mechanism that is referred to as ‘reciprocal zymogen activation’. Vitronectin (VN) represents another important ligand for uPAR: the binding of uPA and VN to uPAR is not mutually exclusive. uPA and uPAR functions are modulated by two specific inhibitors PAI 1 and PAI 2. The same region of VN is required for interaction with PAI 1, uPAR and integrins. The uPAR molecule after uPAR–uPA–PAI 1 complex internalization and degradation is recycled to the cell surface. In addition, the uPAR molecule interacts with the L-selectin and integrins and activates intracellular signaling pathways.

and polarize at focal sites of the cell-substratum or intercellular contacts.^{10,11} uPAR clustering favors the concentration of uPA and plasmin activity on the cell surface, a property likely to facilitate pericellular proteolysis and cell movement across tissue barriers.

Chemotaxis

uPAR is directly involved in the chemotaxis of monocytes and neutrophils. Chemotaxis is induced through a uPA-dependent conformational change of uPAR, which uncovers very potent chemotactic sequences residing in the linker connecting domains D1 and D2;¹² such a chemotactic role of uPA may also be independent of uPA activity.^{13,14}

Adhesion

Apart from inducing cell migration, uPA and uPAR can regulate cell adhesion to extracellular matrix proteins including vitronectin and fibronectin. The adhesion of myeloid cells to vitronectin might relate to uPAR occupancy, since uPAR itself binds vitronectin and mediates adhesion processes.^{15,16} The binding of uPAR to vitronectin also inhibits adhesion to other proteins, such as fibronectin or fibrinogen, and inhibits fibrinogen internalization via the CD11b/CD18 integrin. This

suggests that uPAR competition is involved in adhesion as well as in internalization/degradation processes.^{6,17}

Signal transduction

Recently, it has been documented that uPAR, in spite of lacking an intracytoplasmic domain, is involved in signal transduction pathways.^{7,18} uPAR aggregation triggers activation signals through its association with such membrane spanning proteins as $\beta 1$, $\beta 2$ and $\beta 3$ integrins, specialized in relating the intracellular and extracellular environments of the cells. It has been suggested that uPAR may act as a ligand rather than as an integrin-associated protein.¹⁹

Tissue remodeling

Recent results indicate that the activation of plasminogen into plasmin focuses the proteolytic activity on the surface of multiple myeloma (MM) plasma cells and may contribute to the removal of bone matrix noncollagenous proteins in this disease.²⁰ In addition, plasmin can be involved in the activation of latent MMPs,^{21–23} which, by degrading type I collagen, may also participate in bone remodeling.²⁴

The uPA system is also involved in the activation of prohepatocyte growth factor into its active form, which in turn induces the secretion of IL-11 by osteoblasts, a cytokine with a potent stimulatory effect on osteoclastogenesis.^{25,26} The expression of uPA and uPAR could therefore represent a pathway by which normal and MM plasma cells interact with the bone marrow (BM) structure and might influence or trigger biological events such as bone matrix degradation, plasma cell invasion and homing, and potentially disease progression, in MM.

Soluble uPAR (suPAR)

A suPAR has been characterized in the plasma of normal healthy subjects as well as in the plasma and body fluids of cancer patients.^{27–33}

suPAR is released from the plasma membrane by cleavage of the GPI anchor. suPAR can be further cleaved in the region that links domain D1 to domain D2 to yield two fragments, respectively, composed of D1 and D2D3. The latter exhibits direct chemotactic activity.⁷ Cleaved uPAR is unable to bind uPA/PAI 1 complexes, neither is it internalized nor does it have high affinity for vitronectin,³⁴ and may be unable to act as a mediator of cell adhesion.¹⁴

It has been suggested that in cancer patients, peripheral blood levels of suPAR confer a poor prognosis.^{30–32} In fact, although suPAR is actively released from cancer cells, the rate of receptor shedding does not correlate with the intensity of uPAR expression or with the amount of tumor cells in the BM.³¹ Recent observations suggest that suPAR fragments display chemokine-like activities, and that, *in vitro*, suPAR could be capable of modulating different processes such as cell adhesion, migration and proliferation.^{4,35–37}

Expression of uPAR (CD87) in normal hematopoietic cells and hematologic disorders

While the biochemical and functional properties of the uPA/uPAR system have been largely investigated,⁷ few reports have been published so far in which the distribution of the uPAR

molecule has been assessed in hematological disorders. At the Fifth International Workshop on Leukocyte Differentiation Antigens,³⁸ six monoclonal antibodies (MoAbs) were verified as recognizing uPAR and were clustered as CD87.

The flow cytometric evaluation of CD87 expression proved to be particularly suitable for both a qualitative and quantitative analysis of this receptor in different hematological conditions, and it has become the method of choice to assess CD87 expression in normal and neoplastic peripheral blood cells.

In the following sections of this review, we summarize EGIL's experience³⁹ together with the most relevant data reported in the literature on the expression of CD87 in normal hematopoietic cells as well as in different hematological disorders. We will also discuss the potential diagnostic and prognostic utility of this molecule.

CD87 expression on normal hematopoietic cells

uPA and uPAR are expressed by leukocytes (including polymorphonuclear neutrophils, monocytes, macrophages, eosinophils and activated T lymphocytes), endothelial cells and fibroblasts. Among normal BM cells, uPAR is expressed on a fraction of myeloid precursors (promyelocytes, myelocytes and metamyelocytes), monocytes and their precursors, but not on circulating dendritic cell precursors or CD34+ hematopoietic stem or progenitor cells.⁴⁰⁻⁴² During short-term liquid culture in the presence of cytokines, the number of CD34+ cells decreases in parallel concomitant to an increase in the number of uPAR+ cells, which is also associated with a more differentiated morphology and phenotype.⁴³

CD87 expression in clonal hematopoietic disorders

Table 1 summarizes the distribution and expression of uPAR (CD87) in different hematological disorders reported in the literature and from EGIL's experience through the use of different MoAbs. As it may be seen in this table, most studies in which CD87 expression was evaluated have focused either on acute leukemia or on MM and only limited information is available in other clonal hematological disorders.

Acute leukemias: Knapp *et al*⁴⁴ initially reported CD87 expression in 41% of a series of 50 acute leukemia cases using the VIM5 MoAb. CD87 was most frequently expressed in acute myeloid leukemias (AMLs) with monocytic differentiation (FAB

M4 and M5), and it was coexpressed with CD14 in nine out of 14 positive cases. A weak CD87 expression was also observed by these authors in 3/16 precursor B-cell acute lymphoblastic leukemia (ALL) cases.

Plesner *et al*,⁴³ using two different MoAbs, reported a similar reactivity for CD87 in normal monocytes and AML blasts. In their series of AML patients, CD87 was negative on blasts from six M1 cases, while this molecule was expressed in 3/4 M2 cases, 2/7 M4 and 7/10 M5 AML cases. uPAR was also present in 13/13 cases of Langerhans' cell histiocytosis and 3/4 cases of histiocytic sarcoma. Using the same antibodies, the expression of CD87 could not be demonstrated by the same authors on neoplastic cells from neither B- nor T-cell lymphomas, and it was also absent in Hodgkin's disease.

Using a panel of five different MoAbs, Jardí *et al*⁴⁵ reported that only a minority of AML patients, those with blasts showing differentiation features, presented the surface expression of uPAR (>20% of positivity), while permeabilized blast cells expressed CD87 in all seven cases tested. Based on these results, Jardí *et al*⁴⁵ suggest that a mechanism of translocation of uPAR to the cell surface may regulate the expression of this molecule.

More recently, Lanza *et al*⁴⁰ showed that AML blasts displayed a heterogeneous pattern of expression of CD87, with reactivity strictly dependent on the type of cell involved (granulocytic or monocytic) and its degree of maturation. The highest rate of uPAR expression was observed in this study in monoblastic AML (20/20 uPAR+ patients), while the lowest was found among patients with poorly differentiated AML (M0 subtype). Overall, 60/74 AML cases showed CD87 expression. In addition, two out of three biphenotypic acute leukemias showed CD87 expression, while blasts from only 3/24 ALL cases (12.5%) studied were CD87+. However, the degree of positivity was considerably weaker in ALL blasts than in AML cells.⁴⁰

A comparative analysis between peripheral blood and BM blasts at diagnosis and relapse revealed that the intensity of uPAR expression was significantly higher in circulating blast cells and at relapse, supporting the notion that the cellular uPAR content positively correlates with invasive manifestations of AML.⁴⁰

Similar findings were reported by Mustjoki *et al*⁴⁶ who found CD87 expression in 28/29 AML patients, 2/3 biphenotypic acute leukemias and in only 2/9 ALL cases.

Interestingly, in AML patients whose blast cells show monocytic differentiation, uPAR+ blasts were also CD11c+, CD14+, CD13 low, CD33 high, lysozyme high and MPO low/negative. By contrast, in cases having myeloid/granulocytic

Table 1 Flow cytometric expression of CD87 in different hematologic malignancies

CD87 clone	AML	ALL	MM	MGUS	NHL	SMCD	Author
VIM5	14/34 (M4-M5)	3/16 (B-ALL)	—	—	—	—	Knapp <i>et al</i> ⁴⁴
R2 and R4	12/27	0/5	0/10	—	0/22	—	Plesner <i>et al</i> ⁴³
Mo3f, VIM5, Mo3f109, Mo3e68, Mo3c100	2/16 ^a 7/7 ^b	0/6 ^a 1/1 ^b	—	—	—	—	Jardí <i>et al</i> ⁴⁵
Mo3f	2/15	0/6	—	—	—	—	Lopez-Pedraza, <i>et al</i> ⁴⁷
3B10 and VIM5	60/74	3/24	—	—	—	—	Lanza <i>et al</i> ⁴⁰
R3 and R4	28/29	2/9	—	—	—	—	Mustjoki <i>et al</i> ⁴⁶
3937	—	—	7/7	—	—	—	Hjertner <i>et al</i> ²⁰
VIM5	72/98	5/32	—	—	—	—	Castoldi, unpublished data
VIM5	—	0/3	49/49	10/16	4/10 ^c	—	Rigolin <i>et al</i> ⁴⁸
VIM5	—	—	—	—	—	3/7	Escribano (personal communication)

^a>20% surface expression.

^b>20% intracellular expression.

^c3/4 Waldenstrom macroglobulinemia.

SMCD: adult indolent systemic mast cell disorders.

AML, uPAR+ blasts showed a coexpression of CD34, CD117, HLA-DR and CD13, and were MPO high, lysozyme low⁴⁰ in greater than 60% of cases. In particular, CD14, an antigen, strongly expressed on the surface of monocytes and macrophages, appeared to be differently distributed according to the intensity of expression of uPAR: 87% of CD14 positivity for bright CD87+ blasts and 34% for CD87- or -dim blast cells. Furthermore, a bright CD87 expression positively correlated, in the different FAB subtypes, with the intensity of expression of CD116 (the GM-CSF receptor), but not with CD117 (Figures 2 and 3).

In addition to these phenotypic associations, uPAR expression has also been correlated with cytogenetic findings in AML. Clonal alterations have been observed in 93% of AML patients with a bright uPAR expression, but only in 71% of AML patients having either low or no uPAR expression.⁴⁰ Half of the patients with bright uPAR expression had a complex karyotype, while this was seen in only 11% of those patients with low or negative uPAR expression. A normal karyotype was present in 7 and 29% of uPAR bright and uPAR neg/low blasts, respectively. A complex karyotype, including rearrangements of chromosome 11 at band 11q23, and a bright uPAR expression were almost exclusively associated with M5 AML, suggesting that these characteristics may identify a peculiar variant of AML patients with a worse prognosis.⁴⁰

In line with these observations, bright CD87 expression has also been correlated in AML patients with mucocutaneous infiltration, hepatosplenomegaly, lymphadenopathy and CNS involvement.⁴⁰ In addition, AML patients expressing uPAR manifested a higher frequency of bleeding complications.⁴⁷ These observations suggest that uPAR expression is associated with clinical features predicting a more aggressive course of the disease.

Multiple myeloma: As already outlined above, preliminary data suggest that primary malignant plasma cells from MM patients do express uPA and uPAR and that this functionally active proteolytic system may be involved in bone matrix degradation in this disease.²⁰

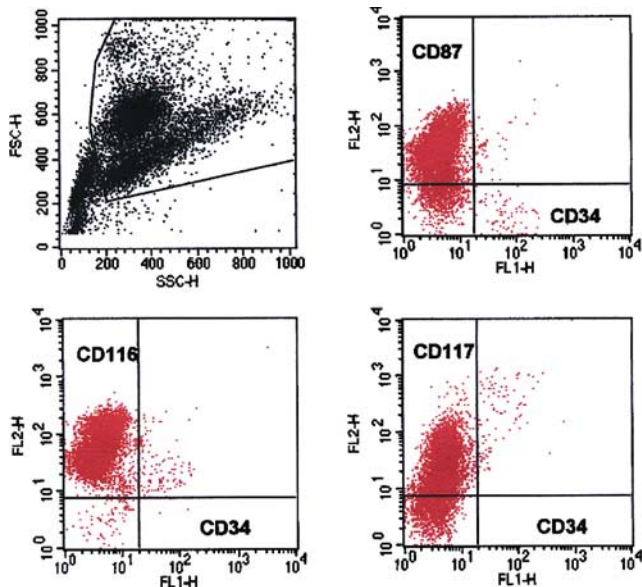
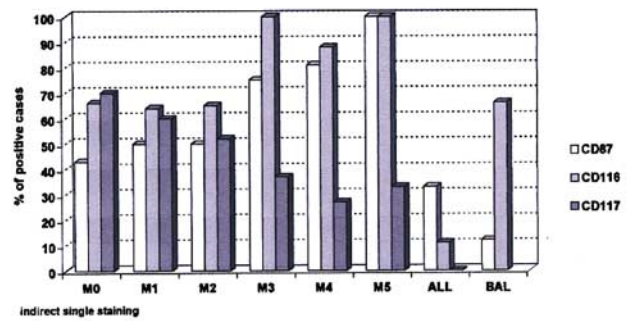


Figure 2 Flow cytometric expression of CD87, CD116 and CD117 in a patient with AML M5 FAB.

a Expression of CD87, CD116, CD117 in acute leukemias (74 cases)



b Expression of CD87, CD116, CD117 in acute leukemias (74 cases)

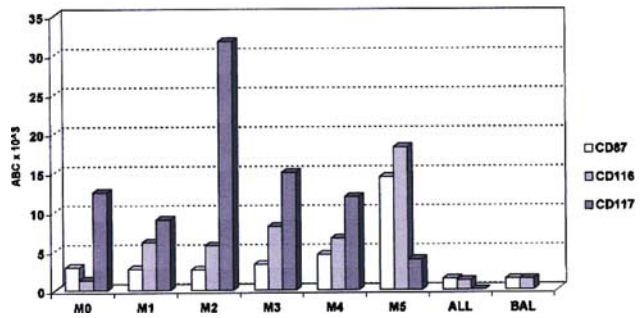


Figure 3 Expression of CD87, CD116 and CD117 in AML patients.

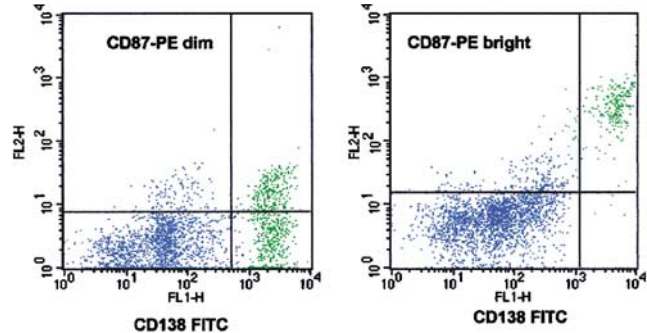


Figure 4 Flow cytometric expression of CD87 in two MM patients.

Flow cytometry assays have shown that uPAR was expressed by neoplastic plasma cells in 49/49 MM patients, although important variability with respect to the intensity of uPAR expression was observed^{20,43,48} (Figure 4). This heterogeneous pattern of expression probably reflects the fact that uPAR, stored in the cytoplasmic compartment, is translocated to the cell surface upon cell activation and later recycled to the cell surface after internalization and degradation of the uPA-PAI 1 complex.^{1,2} In MM patients, CD87 expression appears to correlate inversely with the maturation stage of the malignant plasma cells.⁴⁸ CD45+ immature plasma cells^{49,50} showed the highest levels of expression of CD87, and also displayed a stronger reactivity for both CD138 and CD56, two molecules known to play an important role in cell adhesion processes.⁵¹ These findings suggest that, as CD56,⁵¹ CD87 might be involved in the regulation of plasma cell homing, and therefore participate in the pathogenesis of this disease.

Of interest is the observation that plasma cells from Monoclonal Gammopathy of Underdetermined Significance also appear to be CD87+, indicating that CD87 expression might not be a unique characteristic of MM plasma cells, but rather a marker of clonal plasma cell proliferation.⁴⁸

Among MM patients, CD87 expression did not show any significant association with a deletion of the long arm of chromosome 13.⁴⁸

Chronic lymphoproliferative disorders: Conflicting results have been reported with respect to the CD87 expression in B-cell chronic lymphoproliferative disorders. While cells from chronic lymphocytic leukemia patients do not express CD87, in B-cell lymphoma (B-NHL) patients, CD87 seems to be present mainly in Waldenström macroglobulinemia, a disease characterized by the proliferation of clonal lymphoplasmocytic cells and plasma cells, and thus closer to MM than other B-NHL.^{43,48} Additional studies on a larger series of patients with chronic lymphoproliferative disorders are necessary to determine the level of CD87 expression in this heterogeneous group of diseases.

Adult indolent systemic mast cell disorders (SMCD): Clonal BM mast cells from patients with SMCDs show variable expression of CD87. Accordingly, while four out of seven adult patients suffering from indolent systemic mastocytosis were CD87-, the other three cases showed dim CD87 expression on the clonal mast cells (L Escribano, personal communication).

suPAR levels in patients with different hematological malignancies: It has been recently shown that, in AML, suPAR levels correlate with the number of circulating tumor cells and with a poor response to chemotherapy, and that suPAR levels decrease rapidly during chemotherapy.³¹ This study also showed that fragmented uPAR (D2D3) was present on the blast cells from AML patients, but not in peripheral blood leukocytes from normal healthy controls, which expressed only small amounts of the full-length receptor. According to Mustjoki *et al*³¹, some peculiar functions of the uPAR fragments can be further postulated. In particular, an excess of uPA, which is considered a possible candidate for uPAR cleavage,³³ could explain uPAR fragmentation on the blast cells' surface leading to a decreased proteolytic activity because the ligand-binding domain D1 would be released.³¹ Altogether, these data suggest that not only uPAR but also its soluble fragments may play a role in the pathophysiology of AMLs.

In MM patients, the intensity of CD87 expression on plasma cells does not correlate with the levels of circulating suPAR.⁴⁸ However, suPAR levels have been found to correlate with a number of factors known to predict a more aggressive clinical course of the disease, including disease stage, plasma creatinine level, sCD138 and beta-2-microglobulin serum levels. By contrast no association between C-reactive protein serum levels or 13q deletion and suPAR levels has been observed. In a preliminary multivariate linear regression analysis, high suPAR levels were, along with disease stage, an independent parameter predicting extramedullary involvement in MM. In addition, suPAR levels in this study were an independent prognostic factor for predicting survival. These preliminary results suggest that suPAR may be a useful parameter to be prospectively investigated in MM patients, as it might provide additional prognostic information related to a more aggressive and fatal course of the disease.

The precise functional role of suPAR is not yet completely understood but it is likely that full size suPAR as well as its fragments may compete with cell membrane uPAR and therefore affect uPAR occupancy and availability. High levels of suPAR can be associated with a defective adhesion of plasma cells to the extracellular matrix, possibly through competition with plasma cell membrane-bound uPAR for the interaction with vitronectin and integrins.^{48,52} High levels of suPAR could therefore represent an indicator of a reduced capacity for MM plasma cells to interact with the bone matrix and of their capacity to disseminate to extramedullary sites. This is supported by our observations in MM patients.⁴⁸

In addition, with regard to a possible pathogenetic role of uPAR and suPAR in MM, it has been shown that functional uPAR is involved in angiogenesis⁵³ and that the cleavage of uPAR by metalloprotease-12 (MMP12) decreases its angiogenic properties, which in turn are restored by the inhibition of MMP12 activity.⁵⁴ These observations suggest that uPAR could represent a new target for such antiangiogenic factors as thalidomide and that high levels of suPAR could characterize a phase of the disease in which angiogenesis is decreased and the progression of the disease requires a different therapeutical approach. Further studies are warranted to clarify the interaction between uPAR and MMPs and their contribution to the pathogenesis and treatment of MM.

Conclusions

The uPAR system is involved in important physiological processes, including proteolysis, adhesion, signal transduction, cell migration and chemotaxis. However, the uPA system, and particularly uPAR, is also expressed by neoplastic cells in a variety of hematological and nonhematological malignancies. The expression of uPAR and its soluble form may play a relevant role in the pathophysiology of these disorders.

The analysis of the distribution of CD87 in neoplastic cells, at least in certain hematological disorders such as acute leukemia and MM, suggests different roles for this molecule. As far as the demonstration of uPAR in AML is concerned, a significant diagnostic contribution is coming from several studies. The combined use of anti-CD87, MPO and lysozyme MoAbs may provide useful information to distinguish between myeloid and monocytic leukemia. In addition, a prognostic role may arise from the observation that patients having an elevated number of UPA receptors, irrespective of the FAB subtype, have a greater tendency for cutaneous and tissue infiltration and a higher frequency of chromosome abnormalities, thus suggesting that uPAR expression positively correlates with the invasive potential of AML cells.

As far as the MM is concerned, CD87 expression clearly correlates with the maturation degree of the plasma cells and with the expression of such adhesion molecules as CD138 and CD56. In addition, soluble CD87 seems to represent a good predictor in MM for an extramedullary involvement of the disease and a worse prognosis.

Taken together, these data are consistent with a multifaceted activity of the CD87 molecule, which appears to be able to influence the clinical and prognostic pattern of these disorders by different pathophysiologic mechanisms.

The recent development of anti-urokinase receptor antibodies may further underline the possibility that the uPAR molecule could represent a suitable target for new therapeutic options. Among the various therapeutic strategies that might be envisioned, targeting the uPA system,⁵⁵⁻⁵⁷ the most interesting

approach, as far as hematological disorders are concerned, is represented by the development of such fusion proteins as the diphtheria toxin/urokinase fusion protein, which has been demonstrated, *in vitro*, to be toxic to CD87+ AML blasts.⁵⁸ Overall, these preliminary observations suggest that the uPAR molecule could represent a suitable target for new therapeutic strategies in hematological malignancies. For clinical application, these new agents may provide additional or synergistic benefits if used in combination with conventional therapies⁵⁹ or with drugs targeting more than one of the interacting proteolytic pathways.³⁷

Acknowledgements

This work was supported by MURST 60 and 40%, COFIN, AIRC coordinated project.

References

- 1 Bu G, Warshawsky I, Schwartz AL. Cellular receptors for the plasminogen activators. *Blood* 1994; **83**: 3427–3436.
- 2 Plesner T, Behrendt N, Ploug M. Structure, function and expression on blood and bone marrow cells of the urokinase-type plasminogen activator receptor, uPAR. *Stem Cells* 1997; **15**: 398–408.
- 3 Wei Y, Lukashov M, Simon DJ, Bodary SC, Rosenberg S, Doyle MV et al. Regulation of integrin function by the urokinase receptor. *Science* 1996; **273**: 1551–1555.
- 4 Chapman HA. Plasminogen activators, integrins, and the coordinated regulation of cell adhesion and migration. *Curr Opin Cell Biol* 1997; **9**: 714–724.
- 5 May AE, Kanse SM, Lund LR, Gislér RH, Imhof BA, Preissner KT. Urokinase receptor (CD87) regulates leukocyte recruitment via beta 2 integrins *in vivo*. *J Exp Med* 1998; **188**: 1029–1037.
- 6 Blasi F. Proteolysis, cell adhesion, chemotaxis and invasiveness are regulated by the uPA–uPAR–PAI1 system. *Thromb Haemostasis* 1999; **82**: 298–304.
- 7 Blasi F, Carmeliet P. uPAR: a versatile signalling orchestrator. *Nat Rev Mol Cell Biol* 2002; **3**: 932–943.
- 8 Petersen LC. Kinetics of pro-urokinase/plasminogen activation: stimulation by a template formed by the urokinase receptor bound to poly-D-lysine. *Eur J Biochem* 1997; **245**: 316–323.
- 9 Wei Y, Waltz DA, Rao N, Drummond RJ, Rosenberg S, Chapman HA. Identification of urokinase receptor as an adhesion receptor for vitronectin. *J Biol Chem* 1994; **269**: 32380–32388.
- 10 Pollanen J, Hedman K, Nielsen LS, Dano K, Vaheri A. Ultrastructural localization of plasma membrane-associated urokinase-type plasminogen activator at focal contacts. *J Cell Biol* 1988; **106**: 87–95.
- 11 Sitrin RG, Pan PM, Harper HA, Todd III RF, Harsh DM, Blackwood RA. Clustering of urokinase receptors (uPAR; CD87) induces proinflammatory signalling in human polymorphonuclear neutrophils. *J Immunol* 2000; **165**: 3341–3349.
- 12 Fazioli F, Resnati M, Sidenius N, Higashimoto Y, Appella E, Blasi F. A urokinase-sensitive region of the human urokinase receptor is responsible for its chemotactic activity. *EMBO J* 1997; **16**: 7279–7289.
- 13 Gyetko M, Todd III R, Wilkinson C, Sitrin R. The urokinase receptor is required for monocyte chemotaxis *in vitro*. *J Clin Invest* 1994; **93**: 2380–2388.
- 14 Gyetko M, Sitrin R, Fuller J, Todd III R, Standiford T. Function of the urokinase receptor (CD87) in PMN chemotaxis. *J Leukocyte Biol* 1995; **58**: 533–538.
- 15 Wei Y, Yang X, Liu Q, Wilkins JA, Chapman H. A role for caveolin and the urokinase receptor in integrin-mediated adhesion and signalling. *J Cell Biol* 1999; **144**: 1285–1294.
- 16 Wei Y, Eble JA, Wang Z, Kreidberg JA, Chapman HA. Urokinase receptors promote beta-1 integrin function through interactions with integrin alpha3beta1. *Mol Biol Cell* 2001; **12**: 2975–2986.
- 17 Mondino A, Resnati M, Blasi F. Structure and functions of the urokinase receptor. *Thromb Haemostasis* 1999; **82** (Suppl): 19–22.
- 18 Chapman HA, Wei Y, Simon D, Waltz DA. Role of urokinase receptor and caveolin in regulation of integrin signaling. *Thromb Haemostasis* 1999; **82**: 291–297.
- 19 Tarui T, Mazar AP, Cines DB, Takada Y. Urokinase-type plasminogen activator receptor (CD87) is a ligand for integrins and mediates cell–cell interaction. *J Biol Chem* 2001; **276**: 3983–3990.
- 20 Hjertner O, Qvigstad G, Hjorth-Hansen H, Seidel C, Woodliff J, Epstein J et al. Expression of urokinase plasminogen activator and the urokinase plasminogen activator receptor in myeloma cells. *Br J Haematol* 2000; **109**: 815–822.
- 21 Werb Z, Mainardi CL, Vater CA, Harris ED. Endogenous activation of latent collagenase by rheumatoid synovial cells Evidence for a role of plasminogen activator. *N Engl J Med* 1977; **296**: 1017–1023.
- 22 Carmeliet P, Moons L, Lijnen R, Baes M, Lemaître V, Tipping P et al. Urokinase-generated plasmin activates matrix metalloproteinases during aneurysm formation. *Nat Genet* 1997; **17**: 439–444.
- 23 Barille S, Akoundi C, Collette M, Mellerin MP, Rapp MJ, Harousseau JL et al. Metalloproteinases in multiple myeloma: production of matrix metalloproteinase-9 (MMP-9), activation of proMMP-2, and induction of MMP-1 by myeloma cells. *Blood* 1997; **90**: 1649–1655.
- 24 Daci E, Udagawa N, Martin TJ, Bouillon R, Carmeliet G. The role of the plasminogen system in bone resorption *in vitro*. *J Bone Min Res* 1999; **14**: 946–952.
- 25 Borset M, Hjorth-Hansen H, Seidel C, Sundan A, Waage A. Hepatocyte growth factor and its receptor c-met in multiple myeloma. *Blood* 1996; **88**: 3998–4004.
- 26 Hjertner O, Torgensen M, Seidel C, Hjorth-Hansen H, Waage A, Borset M et al. Hepatocyte growth factor (HGF) induces interleukin-11 secretion from osteoblasts: a possible role for HGF in myeloma-associated osteolytic bone disease. *Blood* 1999; **94**: 3883–3888.
- 27 Pedersen N, Schmitt M, Ronne E, Nicoletti M, Hayer-Hansen G, Conese M et al. A ligand-free, soluble urokinase receptor is present in the ascitic fluid from patients with ovarian cancer. *J Clin Invest* 1993; **92**: 2160–2167.
- 28 Hoist-Hansen C, Hamers MJ, Johannessen BE, Brunner N, Stephens RW. Soluble urokinase receptor released from human carcinoma cells: a plasma parameter for xenograft tumour studies. *Br J Cancer* 1999; **81**: 203–211.
- 29 Sier CF, Stephens RW, Bizik J, Mariani A, Bassan M, Pedersen N et al. The level of urokinase-type plasminogen activator receptor is increased in serum of ovarian cancer patients. *Cancer Res* 1998; **58**: 1843–1849.
- 30 Stephens RW, Nielsen HJ, Christensen IJ, Sorensen S, Dano K, Brunner N. Plasma urokinase receptor levels in patients with colorectal cancer: relationship to prognosis. *J Nat Cancer Inst* 1999; **91**: 869–874.
- 31 Mustjoki S, Sidenius N, Sier CFM, Blasi F, Elonen E, Alitalo M et al. Soluble urokinase receptor levels correlate with number of circulating tumor cells in acute myeloid leukemia and decrease rapidly during chemotherapy. *Cancer Res* 2000; **60**: 7126–7132.
- 32 Riisbro R, Christensen IJ, Piironen T, Greenall M, Larsen B, Stephens RW et al. Prognostic significance of soluble urokinase plasminogen activator receptor in serum and cytosol of tumor tissue from patients with primary breast cancer. *Clin Cancer Res* 2002; **8**: 1132–1141.
- 33 Hoyer-Hansen G, Ronne E, Solberg H, Behrendt N, Ploug M, Lund LR. Urokinase plasminogen activator cleaves its cell surface receptor releasing the ligand-binding domain. *J Biol Chem* 1992; **267**: 18224–18229.
- 34 Hoyer-Hansen G, Behrendt N, Ploug M, Dano K, Preissner KT. The intact urokinase receptor is required for efficient vitronectin binding; receptor cleavage prevents ligand interaction. *FEBS Lett* 1997; **420**: 79–85.
- 35 Wilhelm OG, Wilhelm S, Escott GM, Lutz V, Magdolen V, Schmitt M et al. Cellular glycosylphosphatidylinositol-specific phospholipase D regulates urokinase receptor shedding and cell surface expression. *J Cell Physiol* 1999; **180**: 225–235.
- 36 Blasi F. uPA, uPAR, PA-1: key interaction of proteolytic, adhesive, and chemotactic highways? *Immunol Today* 1997; **18**: 415–417.

- 37 Mizukami IF, Todd III RF. A soluble form of the urokinase plasminogen activator receptor (suPAR) can bind to hematopoietic cells. *J Leukocyte Biol* 1998; **64**: 203–213.
- 38 Todd III RF, Barnathan ES, Bohuslav J, Chapman HA, Cohen RL, Pelez J et al. CD87 cluster workshop report pp 932–9. In: Schlossman SF, Boumsell L, Gilks W, Harlan JM, Kishimoto T, Morimoto C, Ritz J, Shaw S, Silverstein R, Springer T, Tedder TF, Todd RF (eds) *Leukocyte Typing V. White cell differentiation antigens. Proceedings of the Fifth international Workshop and Conference held in Boston USA 3–7 November 1993*. Oxford, New York, Tokyo: Oxford University Press, 1995.
- 39 Bené MC, Castoldi GL, Knapp W, Ludwig WD, Matutes E, Orfao A et al. Proposals for the immunological classification of leukemias. *Leukemia* 1995; **9**: 1783–1786.
- 40 Lanza F, Castoldi GL, Castagnari B, Todd III RF, Moretti S, Spisani S et al. Expression and functional role of urokinase-type plasminogen activator receptor in normal and acute leukemic cells. *Br J Haematol* 1998; **103**: 110–123.
- 41 Almeida J, Bueno C, Alguero MC, Sanchez ML, Canino MC, Fernandez ME et al. Extensive characterization of the immunophenotype and pattern of cytokine production by distinct subpopulation of normal human peripheral blood MHC+ lineage cells. *Clin Exp Immunol* 1999; **188**: 392–409.
- 42 Gadd S, Majdic O, Kasinrerck W, Stockinger H, Maurer D, Eher R et al. M5, a phosphoinositol linked human myelomonocytic activation associated antigen. *Clin Exp Immunol* 1990; **80**: 252–256.
- 43 Plesner T, Ralfkiaer E, Wittrup M, Johnsen H, Pyke C, Pedersen TL et al. Expression of the receptor for urokinase-type plasminogen activator in normal and neoplastic blood cells and hemopoietic tissue. *Am J Clin Pathol* 1994; **102**: 835–841.
- 44 Knapp W, Strobl H, Majdic O. Flow cytometric analysis of cell-surface and intracellular antigens in leukemia diagnosis. *Cytometry* 1994; **18**: 187–198.
- 45 Jard M, Ingles-Esteve J, Bungal M, Azqueta C, Velasco F, Lopez-Pedreira C et al. Distinct patterns of urokinase receptor (uPAR) expression by leukemic cells and peripheral blood cells. *Thromb Haemostasis* 1996; **76**: 1009–1019.
- 46 Mustjoki S, Alitalo R, Stephens RW, Vaheri A. Blast cell-surface and plasma soluble urokinase receptor in acute leukemia patients: relationship to classification and response to therapy. *Thromb Haemostasis* 1999; **81**: 705–710.
- 47 Lopez-Pedreira C, Jardgrave M, del Mar Malagon M, Ingles-Esteve J, Dorado G, Torres A et al. Tissue Factor (TF) and urokinase plasminogen activator receptor (uPAR) and bleeding complications in leukemic patients. *Thromb Haemostasis* 1997; **77**: 62–70.
- 48 Rigolin GM, Tieghi A, Ciccone M, Zenone Bragotti L, Cavazzini F, Della Porta M et al. Soluble urokinase-type plasminogen activator receptor (suPAR) as an independent factor predicting worse prognosis and extra bone marrow involvement in multiple myeloma patients. *Br J Haematol* 2003; **120**: 953–959.
- 49 Hata H, Xiao H, Petrucci MT, Woodliff J, Chang R, Epstein J. Interleukin-6 gene expression in multiple myeloma: a characteristic of immature tumor cells. *Blood* 1993; **81**: 3357–3364.
- 50 Schneider U, van Lessen A, Huhn D, Serke S. Two subsets of peripheral blood plasma cells defined by differential expression of CD45 antigen. *Br J Haematol* 1997; **97**: 56–64.
- 51 Rawstron A, Barrans S, Blythe D, Davies F, English A, Pratt G et al. Distribution of myeloma plasma cells in peripheral blood and bone marrow correlates with CD56 expression. *Br J Haematol* 1999; **104**: 138–143.
- 52 Yebra M, Goretzk L, Pfeifer M, Mueller BM. Urokinase-type plasminogen activator binding to its receptor stimulates tumor cell migration by enhancing integrin-mediated signal transduction. *Exp Cell Res* 1999; **250**: 231–240.
- 53 Kroon ME, Koolwijk P, Van Goor H, Weidle UH, Collen A, van der Pluijm G, van Hinsbergh VW. Role and localisation of urokinase receptor in the formation of new microvascular structures in fibrin matrices. *Am J Pathol* 1999; **154**: 1731–1742.
- 54 Koolwijk P, Sidenius N, Peters E, Sier CFM, Hanemaaijer R, Blasi F et al. Proteolysis of the urokinase-type plasminogen activator receptor by metalloproteinase-12: implication for angiogenesis in fibrin matrices. *Blood* 2001; **97**: 3123–3131.
- 55 Muehlenweg B, Sperl S, Magdolen V, Schmitt M, Harbeck N. Interference with the urokinase plasminogen activator system: a promising therapeutic concepts for solid tumor. *Expert Opin Biol Ther* 2001; **1**: 683–691.
- 56 Sato S, Kopitz C, Schmalix WA, Muehlenweg B, Kessler H, Schmitt M et al. High-affinity urokinase-derived cyclic peptides inhibiting urokinase/urokinase receptor-interaction: effects on tumor growth and spread. *FEBS Lett* 2002; **528**: 212–216.
- 57 Guo Y, Higazi AA, Arakelian A, Sachais BS, Cines D, Goldfarb RH et al. A peptide derived from the nonreceptor binding region of urokinase plasminogen activator (uPA) inhibits tumor progression and angiogenesis and induces tumor cell death *in vivo*. *FASB J* 2000; **14**: 1400–1410.
- 58 Frankel AE, Beran M, Hogge DE, Powell BL, Thorburn A, Chen YO et al. Malignant progenitors from patients with CD87+ acute myelogenous leukemia are sensitive to a diphtheria toxin-urokinase fusion protein. *Exp Hematol* 2002; **30**: 1316–1323.
- 59 Guo Y, Mazar AP, Lebrun JJ, Rabbani SA. An antiangiogenic urokinase-derived peptide combined with tamoxifen decreases tumor growth and metastasis in a syngenic model of breast cancer. *Cancer Res* 2002; **62**: 4678–4684.