PAI-1 inhibits urokinase-induced chemotaxis by internalizing the urokinase receptor

Bernard Degryse, Cornelis F.M. Sier, Massimo Resnati, Massimo Conese[1], Francesco Blasi*

Molecular Genetics Unit, DIBIT, Department of Cell Biology and Functional Genetics, University Vita-Salute San Raffaele, Via Olgettina 58, 20132 Milan, Italy

Received 26 July 2001; accepted 5 August 2001

First published online 27 August 2001

Edited by Julio Celis

Abstract PAI-1 (plasminogen activator inhibitor-1) binds the urokinase-type plasminogen activator (uPA) and causes its degradation via its receptor uPAR and low-density lipoprotein receptor-related protein (LRP). While both uPA and PAI-1 are chemoattractants, we find that a preformed uPA–PAI-1 complex has no chemotactic activity and that PAI-1 inhibits uPA-induced chemotaxis. The inhibitory effect of PAI-1 on uPA-dependent chemotaxis is reversed when uPAR internalization is inhibited by the 39 kDa receptor-associated protein or by anti-LRP antibodies. Under the same conditions, the uPA–PAI-1 complex is turned into a chemoattractant causing cytoskeleton reorganization and extracellular-regulated kinase/mitogen-activated protein kinases activation. Thus, uPAR internalization by PAI-1 regulates cell migration. © **2001 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.**

1. Introduction

Mice lacking urokinase plasminogen activator (uPA) and its receptor uPAR have a deficient migration of various cell types. The phenotypes of these mice are related to this deficiency: sensitivity to infections, resistance to aneurisms formation and restenosis, deficient muscle regeneration [1–6]. Also plasminogen activator inhibitor type 1 (PAI-1) regulates cell migration: in fact, PAI-$1^{-/-}$ mice have a deficient recruitment of host endothelial cells upon tumor implantation [20]. Moreover, PAI-1 interaction with vitronectin prevents its binding to integrins modifying cell adhesion and migration [21–23].

uPA, uPAR and PAI-1 regulate plasmin formation and fibrin degradation, but also display direct signaling properties on cell proliferation, adhesion and migration [7–12]. uPA stimulates migration by uncovering a chemotactic epitope present at residues 88–92 of uPAR [13] which then cooperates with a transmembrane adapter to stimulate migration [14].

PAI-1 forms a covalent uPA–PAI-1 complex which inhibits uPA activity [7] and is rapidly internalized and degraded [15,16]. This process requires low density lipoprotein receptor-related protein (LRP) and uPAR ([7–19].

Since PAI-1 can induce uPAR internalization, it should also regulate uPA-dependent migration. We now show that PAI-1 indeed blocks uPA-induced cell migration and that the inhibition is reverted when uPAR and LRP internalization are blocked.

2. Materials and methods

2.1. Materials

Human ATF was obtained from Jack Henkin (Abbott Park, IL, USA); human uPA, L. Nolli (BioSearch, Italy); receptor-associated protein (RAP) and rabbit LRP antiserum by M. Nielsen and A. Nykjaer (Aarhus, Denmark); rat pro-uPA by S.A. Rabbani (Montreal, QC, Canada). Recombinant PAI-1 is purified from *Escherichia coli* strain BL21[DE3]pLysS transformed with a His-tagged PAI-1 construct (pPAI-HIS), supplied by Dr. R. Gerard (Dallas, TX, USA). PAI-1 was extracted with lysozyme (0.5 μg/ml) purified on nickel agarose (Qiagen) and heparin Sepharose CL-6B, eluted with 1 M NaCl, 10% glycerol, 50 mM Na_2HPO_4, pH 6.0 and dialyzed against phosphate-buffered saline. Recombinant PAI-1 contains 3.3 IU/μg endotoxin (Lymulus Amoebocyte Lysate Pyrotest, Difco Laboratories, Detroit, MI, USA), i.e. 1.2 IU for 10 nM PAI-1, a concentration that has no biological effect in cell culture. Non-specific rabbit polyclonal immunoglobulins, non-specific monoclonal IgG1 (MOPC-21) and formyl-methionyl-leucyl-proline are from Sigma, St. Louis, MO, USA. Collagen I, fibronectin and the secondary FITC-F(ab) 2 fragments of anti-mouse immunoglobulins are from Roche. The monoclonal anti-phosphorylated extracellular-regulated kinase (ERK) antibody and the MEK/mitogen-activated protein kinase inhibitor PD98059 are from New England Biolabs.

2.2. Immunopurification of uPA/PAI-1 complexes

Complexes of uPA and recombinant PAI-1 are formed as described [24] and purified on uPA antibody AD #394 Protein A Sepharose 4B column. Fractions are tested on 10% SDS–PAGE.

2.3. Cell culture

Mouse LB6 clone 19 [25] and rat smooth muscle cells (RSMC) [26] are cultured in Dulbecco's modified Eagle's medium (DMEM) plus 10% fetal calf serum (FCS) while U937 PR9 [27] in RPMI medium plus 10% FCS. LB6 clone 19 cells express 500 000 human uPAR/cell, no plasminogen activators or metalloproteases [25,28]. RSMC express uPAR, uPA and PAI-1 ([26] and data not shown).

2.4. Chemotaxis assays

2.4.1. Cell treatment. Trypsin-detached cells are neutralized with FCS, washed and resuspended twice in 10 ml serum-free DMEM. The final pellet is resuspended in 1 ml of serum-free DMEM and cell concentration determined. U937 PR9 cells are pelleted by centrifugation, washed thrice in serum-free RPMI, and cell concentration determined.

2.4.2. Boyden chambers. Chemotaxis is measured in Boyden chambers as described before [26]. Filters (5 μm, Corning) are coated with collagen I. For assays with adherent cells, the filters are further coated with fibronectin [26]. Attractants are added in serum-free medium to the lower chamber. A total volume of 200 μl serum-free

*Corresponding author. Fax: (39)-2-2643 4844.
E-mail address: blasi.francesco@hsr.it (F. Blasi).

[1] Present address: Institute for Experimental Treatment of Cystic Fibrosis, H.S. Raffaele Scientific Institute, Via Olgettina 58, 20132 Milan, Italy.

0014-5793/01/$20.00
PII: S0014-5793(01)02797-1

DMEM or RPMI is added to the upper chamber, and the cells layered on top. Inhibitors of migration (i.e. antibodies, RAP) are added to both lower and upper chambers. 20 000–40 000 cells are allowed to migrate at 37°C in 10% CO_2 for either 3 (adherent LB6 clone 19, RSMC) or 1 h (U937 PR9). Experiments are performed in triplicate. Results are the mean ± S.D. of the number of cells counted in 10 high power fields per filter. Migration in the absence of chemoattractant is considered 100%. Data were analyzed with the Student's *t*-test, and values of $P < 0.05$ are considered significant.

2.5. Immunofluorescence microscopy

2.5.1. Cytoskeletal rearrangements. Cells are incubated with appropriate stimulators for 30 min at 37°C and filamentous actin visualized with TRITC-phalloidin for 30 min at 37°C and viewed by conventional immunofluorescence microscopy as described before [29].

2.5.2. uPAR internalization. LB6 clone 19 cells are treated with 10 nM uPA plus 500 nM PAI-1 for 1 h at 4°C and then incubated at 37°C for 0 or 60 min. uPAR is visualized by immunofluorescence with monoclonal antibody R2 followed by recognition with fluoresceinated anti-mouse FITC IgG as described [24].

2.5.3. Nuclear localization of ERKs. Cells stimulated for 30 min with 10 nM uPA, 10 nM PAI-1, or 10 nM uPA–PAI-1 complex are treated with primary anti-phosphorylated ERK antibodies for 24 h at 4°C, according to the manufacturer's instructions, and secondary FITC antibodies. DAPI (4,6-diamidino-2-phenylindole, Roche) is used to label the nucleus [31].

2.5.4. Cell surface expression of uPAR and LRP. Cytofluorimetry is performed as previously described [24].

2.6. Ligand degradation assay

LB6 clone 19 cells, $0.1–0.2 \times 10^6$/2 cm^2 well, are incubated with benzamidine Sepharose 6B-purified [I^{125}]uPA ($2.5–7.5 \times 10^5$ cpm/nmol) [30] in binding buffer (DMEM plus 1 mg/ml bovine serum albumin, 25 mM HEPES, pH 7.4) for 2 h on ice, as described [19]. Non-bound ligand is eliminated by washing with binding buffer, cells reincubated at 37°C for 3 h, the medium removed, and trichloro-acetic acid (TCA) solubility used to quantitate degraded ligand. Cells incubated at 4°C are used to estimate total binding. Experiments are performed in triplicate. Results represent the mean ± S.D.

3. Results

3.1. PAI-1 induces chemotaxis

As shown in Fig. 1A, PAI-1 stimulates LB6 clone 19 migration in a dose-dependent manner reaching a plateau at 2–20 nM. The effect is not due to an endotoxin contaminant as its concentration (see Section 2) is too low. Anti-PAI-1 antibodies inhibit PAI-1-, but not fMLP-dependent chemotaxis (Fig. 1B). This chemotactic activity cannot involve uPA, since LB6 clone 19 cells do not produce uPA [28].

3.2. PAI-1 inhibits uPA-dependent chemotaxis

Migration of LB6 clone 19 cells is stimulated 2.8-fold by 10 nM uPA, 2.5-fold by 10 nM ATF, the receptor-binding domain of uPA, and 2.3-fold by 10 nM PAI-1 (Fig. 2A). The chemotactic activity of uPA and PAI-1 is not additive since the [uPA+PAI-1] is not more active than PAI-1 or uPA alone (Fig. 2A) even at 500 nM PAI-1 (not shown). In contrast, the combination of 10 nM PAI-1 and ATF, which does not bind PAI-1, has an additive effect (Fig. 2A). Since PAI-1 and uPA form a covalent complex, we have tested the activity of a preformed, purified uPA–PAI-1 complex. Indeed, unlike free uPA and PAI-1, the preformed and purified uPA–PAI-1 complex at the same concentration has no chemotactic effect (Fig. 2B).

Similar results are obtained in suspension-growing U937 PR9 cells. Like in LB6 clone 19 cells, the purified preformed uPA–PAI-1 complex has no effect, while free PAI-1 and uPA induce migration (Fig. 2C). Purified uPA–PAI-1 complex has no effect also on RSMC (data not shown) that strongly respond to uPA [26].

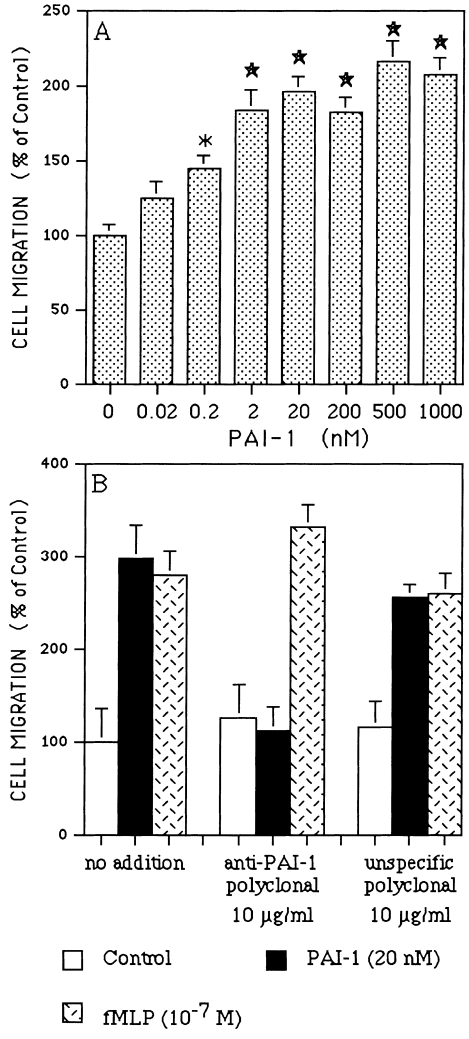


Fig. 1. PAI-1 has chemotactic activity in LB6 clone 19 cells. A: Concentration-dependent stimulation of migration by PAI-1. B: Effect of anti-PAI-1 or unspecific antibodies on PAI-1- or fMLP-induced migration. The data represent the mean ± S.D. of two experiments performed in triplicate, with (*) $P = 0.015$ or $P < 0.001$. The value of 100% indicates random (control) migration.

The uPA–PAI-1 complex is internalized along with uPAR and LRP, and degraded [15–17,24]. We have checked that in LB6 clone 19 cells, that express LRP and uPAR (immunofluorescence and cytofluorimetric analysis), uPAR is indeed internalized under the conditions of Fig. 2A (not shown). We have therefore tested the effect of internalization inhibitors on migration in the presence or absence of the uPA–PAI-1 complex. The LRP antagonist RAP (Fig. 2B,C) and anti-LRP antibodies (data not shown) do not or only moderately (in U937 PR9 cells) affect cell migration. However, in the presence of RAP the purified uPA–PAI-1 complex stimulates chemotaxis of LB6 clone 19 (Fig. 2B) and U937 PR cells (Fig. 2C). Likewise, the uPA–PAI-1 complex has chemotactic activity in the presence of anti-LRP antibodies (Fig. 2D).

RAP inhibits [^{125}I]uPA degradation in LB6 clone 19 cells induced by PAI-1 (Fig. 3A). The TCA solubility of the radioactive ligand in the incubation medium increases with increas-

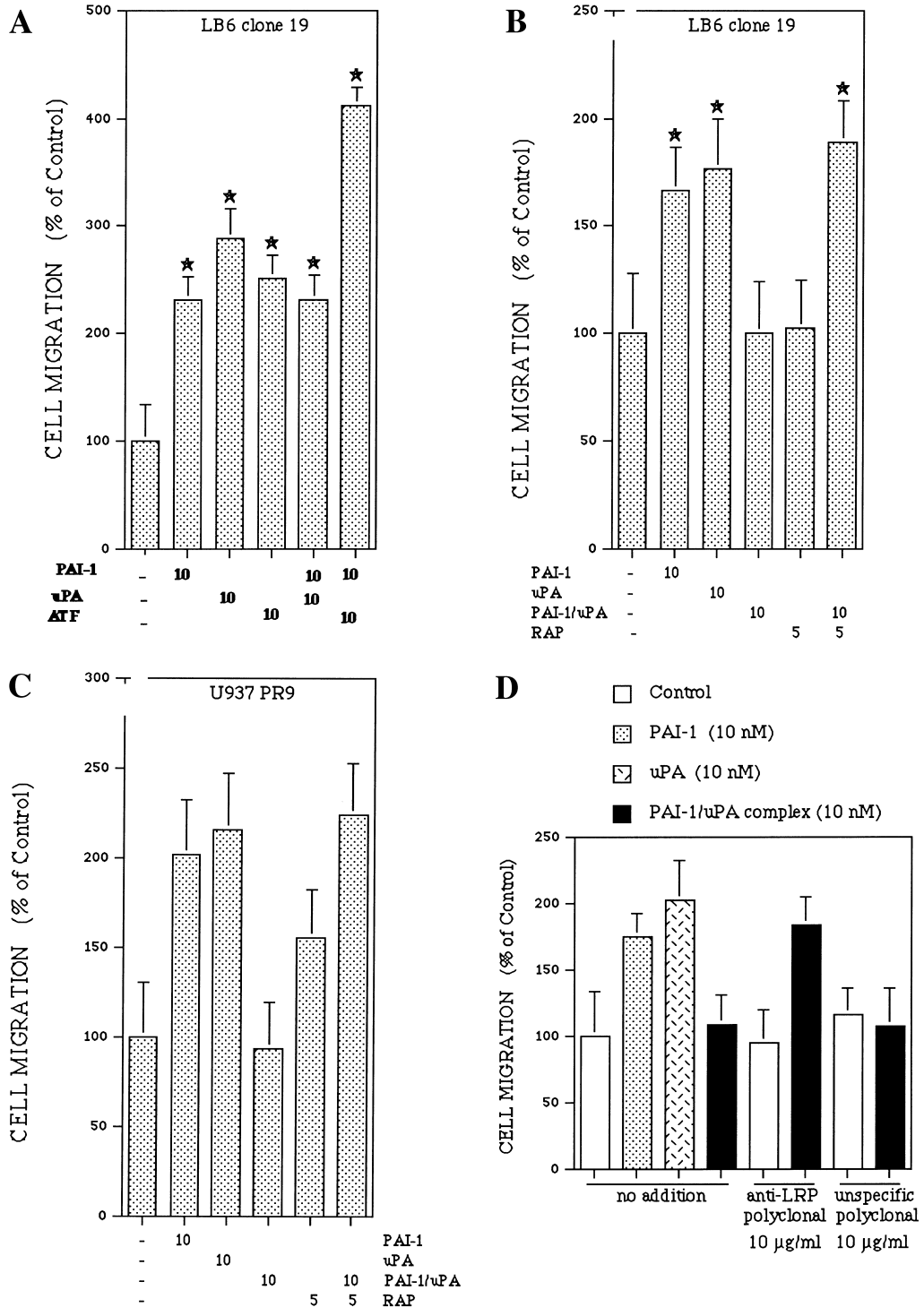


Fig. 2. PAI-1 inhibits the chemotactic activity of uPA by forming uPA–PAI-1 complexes and causing internalization of uPAR. A: PAI-1 inhibits the chemotactic effect of uPA but not of ATF in LB6 clone 19 cells. B: RAP restores the chemotactic activity of preformed, purified UPA–PAI-1 complexes in LB6 clone 19 cells. C: The preformed, purified, uPA–PAI-1 complex has no chemotactic effect in U937 PR9 cells but acquires it in the presence of RAP. D: Anti-LRP antibodies restore the chemotactic activity of preformed uPA–PAI-1 complex on LB6 clone 19 cells. Data are the average from either four (A) or two experiments (B–D); $^{*}P < 0.001$. The numbers below the abscissa represent the concentrations (nM), except for RAP (μg/ml). For other details, see Section 2 and the legend of Fig. 1.

ing PAI-1 (black bars, a measure of uPA degradation), and this is inhibited in the presence of RAP (Fig. 3B) or anti-LRP antibodies (not shown). Thus, under the conditions in which uPA–PAI-1 acquires chemotactic activity uPA–PAI-1 degradation is blocked.

3.3. *PAI-1–uPA complex induces cytoskeleton changes and nuclear translocation of ERK 1/2 when it is not internalized*

Pro-uPA induces cell morphology and cytoskeleton changes which accompany chemotaxis in RSMC [32]. We have used phalloidin-FITC fluorescence to show the changes induced by

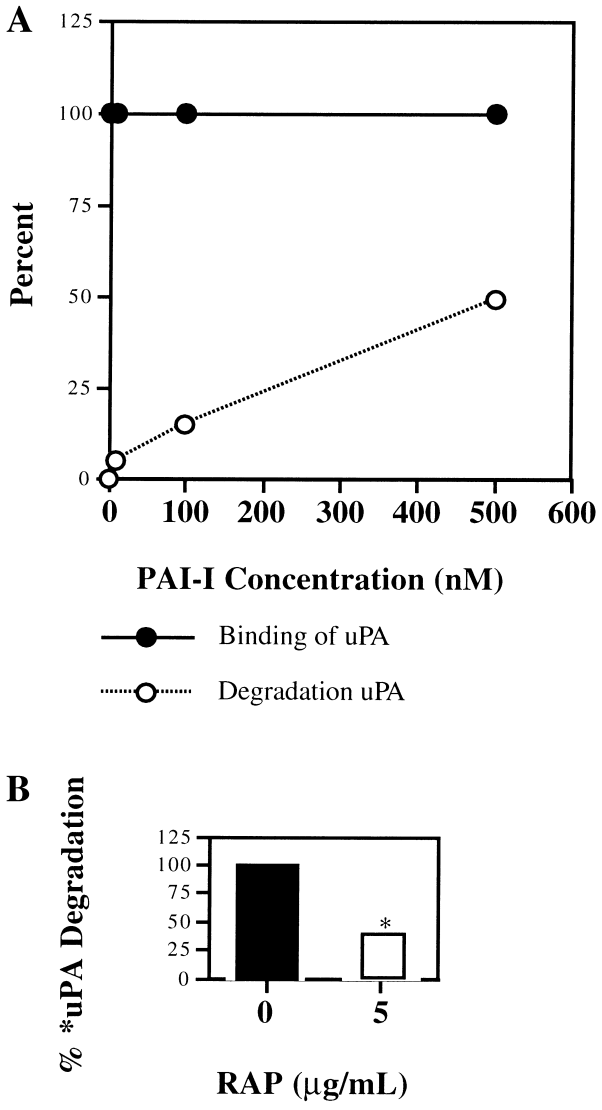


Fig. 3. In LB6 clone 19 cells, PAI-1-dependent uPA degradation is prevented in the presence of RAP. A: Cells were incubated at 4°C for 2 h with 10 nM [^{125}I]uPA. The amount bound at this time in the absence of PAI-1 (bound) is taken as 100%. Then, cells are washed free of ligand, further incubated for 3 h at 37°C with increasing doses of PAI-1 and the percent of degraded [^{125}I]uPA measured by evaluating the percent of TCA-soluble radioactivity. The TCA solubility of the ligand in the absence of cells has been subtracted. Degradation of uPA is expressed as percent of bound uPA. B: RAP inhibits PAI-1-induced degradation of [^{125}I]uPA. Cells treated as above were incubated with PAI-1+RAP. In this panel, uPA degradation obtained with 500 nM PAI-1 was taken as 100%. (*) $P < 0.001$ with respect to control.

uPA on actin cytoskeleton. After 30 min treatment human pro-uPA, but not purified uPA–PAI-1 complex, has a profound effect on actin cytoskeleton and cell shape (Fig. 4). Only in the presence of 5 μg/ml RAP, which has no effect on its own, uPA–PAI-1 induces cytoskeletal changes identical to those observed with pro-uPA. Thus the uPA–PAI-1 complex can cause cytoskeletal rearrangements but only when it cannot induce internalization of uPAR.

Pro-uPA induces uPAR-dependent phosphorylation and nuclear localization of ERK 1/2 [31,33,34]. As shown in Fig. 5, a 30 min treatment of RSMC with uPA (30 min), but not

Fig. 4. A purified, preformed uPA–PAI-1 complex induces actin cytoskeleton reorganization in RSMC but only in the presence of the internalization inhibitor RAP. Fluorescence microscopy analysis of sub-confluent cultures of RSMC treated for 30 min at 37°C with: A, none; B, preformed uPA–PAI-1 complex (10 nM); C, uPA–PAI-1 complex (10 nM) plus 5 μg/ml RAP; D, pro-uPA (10 nM). Actin filaments were stained with TRITC-phalloidin.

→

with preformed uPA–PAI-1 complex, induces nuclear staining with anti-ERK 1/2 antibodies, an effect inhibited by the specific MEK inhibitor PD98059. Free PAI-1 has no effect as previously reported [34]. In the presence of RAP, however, the uPA–PAI-1 complex induces the nuclear translocation of ERK 1/2. Again, this is inhibited by PD98059, while RAP on its own has no effect.

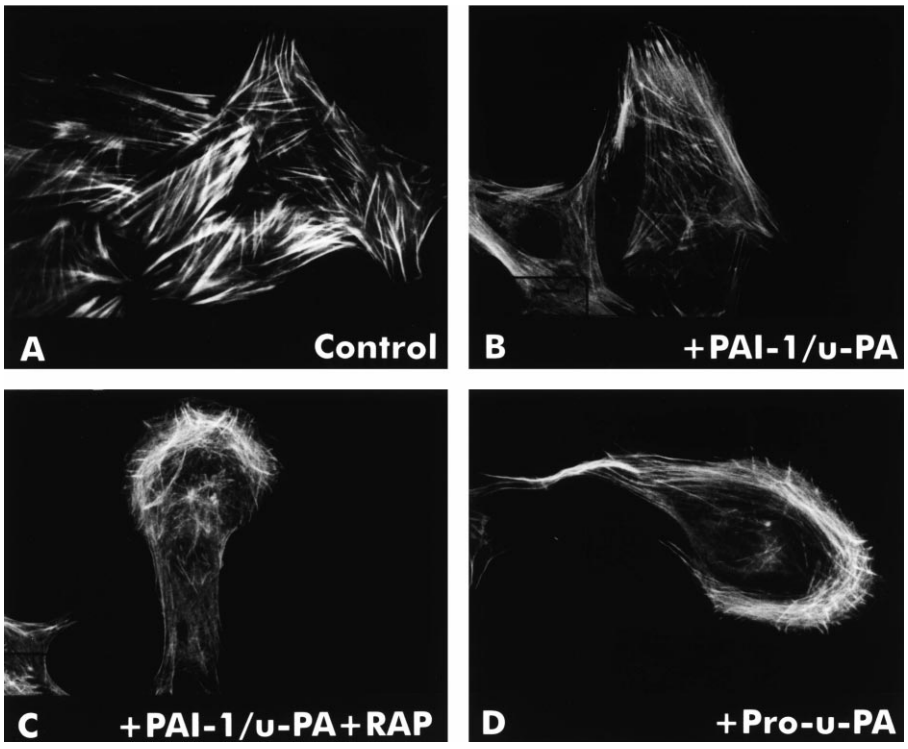
4. Discussion

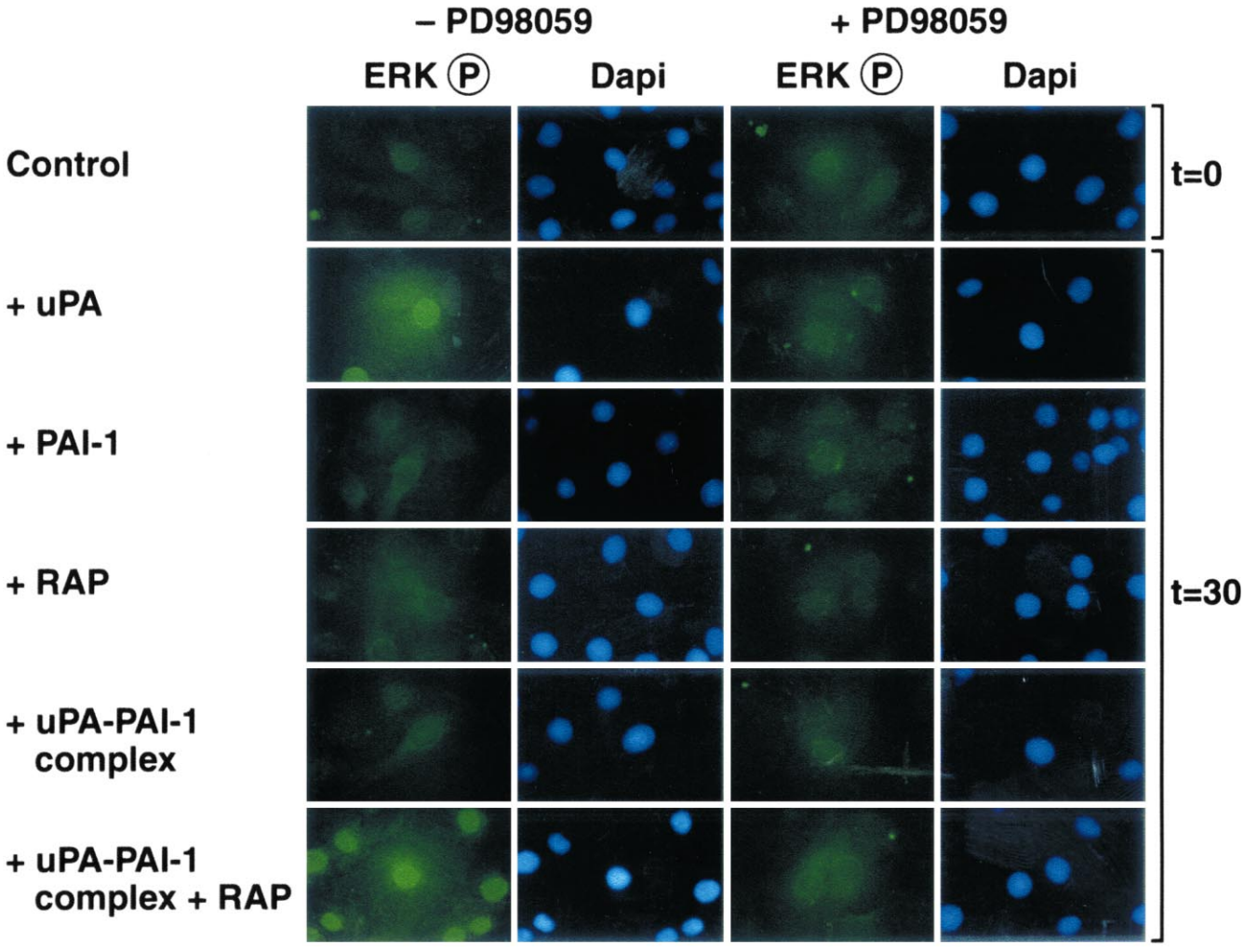
The chemotactic activity of pro-uPA, active uPA and ATF requires binding to uPAR. Indeed, binding uncovers the chemotactic epitope present in the linker region between domains D1 and D2 [13]. Also PAI-1 has migration regulating activity [22,23,30] but no information is available on the activity of the uPA–PAl-1 complex.

Even though PAI-1, uPA and ATF stimulate chemotaxis, the combination of PAI-1 plus uPA is less active than PAI-1 or uPA alone. In contrast, the combination of ATF plus PAI-1 exerts an additive effect on cell migration (Fig. 2A). ATF and uPA do not differ with respect to the mechanisms by which they induce migration [13,14,26], both requiring uPAR, but ATF does not bind PAI-1 and does not induce uPAR internalization [15,16,24]. The results of Fig. 2, therefore, suggest that the inhibitory role of PAI-1 on uPA-dependent migration depends on formation of an uPA–PAI-1 complex, its degradation and the internalization of uPAR and LRP. Indeed, a purified, preformed uPA–PAI-1 complex has no chemotactic activity in LB6 clone 19 or U937 PR9 cells (Figs. 2 and 3) and does not induce morphological changes in RSMC (Fig. 5) nor the nuclear translocation of ERK 1/2 (Fig. 5). Under the conditions of our experiments, the purified uPA–PAI-1 complex binds uPAR with high affinity, induces uPAR internalization and is degraded, as expected (Fig. 5, data not shown and [15,17,24]). However, the block of uPAR internalization by RAP or LRP antibodies allows uPA–PAI-1 complex to induce, just like uPA, cell migration (Figs. 2 and 3), cytoskeleton reorganization (Fig. 4) nor ERK 1/2 nuclear localization (Fig. 5). In these experiments, the purified uPA–PAI-1 complex binds uPAR, induces its internalization and is degraded as expected (Fig. 3 and data not shown). However, the block of uPAR internalization by RAP or LRP antibodies allows the uPA–PAl-1 complex to induce,

→

Fig. 5. Nuclear translocation of ERK 1/2 requires the presence of uPAR on the cell surface. Immunofluorescence analysis of RSMC treated for 30 min with the inducers indicated on the left and stained with antibodies recognizing phosphorylated ERK 1/2 (ERK-P) or with the nuclear fluorescent probe DAPI. The experiment is also carried out with cells pre-treated for 1 h with the MEK inhibitor PD98059 (indicated on top). UPA, PAI-1 and the covalent, purified uPA–PAI-1 complex are used at 10 nM. RAP is used at 5 μg/ml.


A
Control
B
+PAI-1/u-PA
C
+PAI-1/u-PA+RAP
D
+Pro-u-PA


– PD98059
+ PD98059
ERK (P)
Dapi
ERK (P)
Dapi
Control
+ uPA
+ PAI-1
+ RAP
+ uPA-PAI-1 complex
+ uPA-PAI-1 complex + RAP
t=0
t=30

just like uPA, cell migration (Figs. 2 and 3). Thus, inhibitors of uPAR internalization turn the inactive uPA–PAI-1 complex into a stimulator of cell migration in three different cell lines, RSMC, LB6 clone 19 and U937 cells. Since PAI-1 on its own does not induce ERK 1/2 phosphorylation and translocation (Fig. 5) [34], the lack of signaling is likely due to a combination of uPAR internalization and/or ligand degradation.

Several reports indicate that uPAR internalization via LRP plays a key role in signal transduction and regulation of cell migration. For example, RAP-treated MCF-7 cells or LRP-deficient cells display an increase in both cell surface uPAR and cell migration [35,36]. The effect of PAI-1 can be exerted not only through LRP, but also through VLDL-R, which also binds RAP and can cause uPAR internalization [19]. Indeed, neutralization of the VLDL-R with RAP decreases uPAR catabolism and increases cell migration [36]. Thus PAI-1 is an important regulator of cell migration.

Acknowledgements: We are deeply grateful to all our colleagues who provided reagents and to the Italian Association for Cancer Research (A.I.R.C.), the Italian Ministry of University and Scientific and Technological Research (PRIN 1999) and the AIDS fund of the Istituto Superiore di Sanit for support.

References

[1] Carmeliet, P., Moons, L., Dewerchin, M., Mackman, N., Breier, L.T., Breier, G., Ploplis, V., Muller, M., Nagy, A., Plow, E., Gerard, R., Edgington, R., Ridsau, W. and Collen, D. (1997) Ann. N.Y. Acad. Sci. 811, 191–206.
[2] Carmeliet, P., Moons, L., Dewerchin, M., Rosenberg, S., Herbert, J.M., Lupu, F. and Collen, D. (1998) J. Cell Biol. 140, 233–245.
[3] Gyetko, M.R., Chen, G.-H., McDonald, R.A., Goodman, R., Huffnagle, G.B., Wilkinson, C.C., Fuller, J.A. and Toews, G.B. (1996) J. Clin. Invest. 97, 1818–1826.
[4] Gyetko, M.R., Sud, S., Kendall, T., Fuller, J.A., Newstead, M.W. and Standiford, T.J. (2000) J. Immunol. 165, 1513–1519.
[5] May, A.E., Kanse, S.M., Lund, L.R., Gisler, R.H., Imhof, B.A. and Preissner, K.T. (1998) J. Exp. Med. 188, 1029–1037.
[6] Lluis, F., Roma, J., Suelves, M., Parra, M., Aniorte, G., Gallardo, E., Illa, I., Rodriguez, L., Hughes, S.M., Carmeliet, P., Roig, M. and Munoz-Canoves, P. (2001) Blood 97, 1703–1711.
[7] Bajou, K., Noel, A., Gerard, R.D., Masson, V., Brunner, N., Holst-Hansen, C., Skobe, M., Fusenig, N.E., Carmeliet, P., Collen, D. and Foidart, J.M. (1998) Nat. Med. 4, 923–928.
[8] Deng, G., Curriden, S.A., Wang, S., Rosenberg, S. and Loskutoff, D.J. (1996) J. Cell Biol. 134, 1563–1571.
[9] Stefansson, S. and Lawrence, D.A. (1996) Nature 383, 441–443.
[10] Kjoeller, L., Kanse, S.M., Kirkegaard, T., Rodenburg, K.W., Roenne, E., Goodman, S.L., Preissner, K.T., Ossowski, L. and Andreasen, P.A. (1996) Exp. Cell Res. 232, 420–429.
[11] Andreasen, P.A., Kjoeller, L., Christensen, L. and Duffy, M.J. (1997) Int. J. Cancer 72, 1–22.
[12] Blasi, F. (1997) Immunol. Today 18, 415–417.
[13] Chapman, H.A. (1997) Curr. Opin. Cell Biol. 9, 714–724.
[14] Johnsen, M., Lund, L.R., Roemer, J., Almholt, K. and Dano, K. (1998) Curr. Opin. Cell Biol. 10, 667–671.
[15] Loskutoff, D.J., Curriden, S.A., Hu, G. and Deng, G. (1999) Acta Pathol. Micr. Scand. 107, 54–61.
[16] Ossowski, L. and Aguirre Ghiso, J.A. (2000) Curr. Opin. Cell Biol. 12, 613–620.
[17] Fazioli, F., Resnati, M., Sidenius, N., Higashimoto, Y., Appella, E. and Blasi, F. (1997) EMBO J. 16, 7279–7286.
[18] Resnati, M., Guttinger, M., Valcamonica, S., Sidenius, N., Blasi, F. and Fazioli, F. (1996) EMBO J. 15, 1572–1582.
[19] Cubellis, M.V., Wun, T.-C. and Blasi, F. (1990) EMBO J. 9, 1079–1085.
[20] Jensen, P.H., Christensen, E.I., Ebbesen, P., Gliemann, J. and Andreasen, P.A. (1990) Cell Regul. 1, 1043–1056.
[21] Nykjaer, A., Petersen, C.M., Møller, B., Jensen, P.H., Moestrup, S.K., Holtet, T.L., Etzerodt, M., Thoegersen, H.C., Munch, M., Andreasen, P.A. and Gliemann, J. (1992) J. Biol. Chem. 267, 14543–14546.
[22] Nykjaer, A., Conese, M., Christensen, E.I., Olson, D., Cremona, O., Gliemann, J. and Blasi, F. (1997) EMBO J. 16, 2610–2620.
[23] Heegaard, C.W., Simonsen, A.C.W., Oka, K., Kjoeller, L., Christensen, A., Madsen, B., Ellgaard, L., Chan, L. and Andreasen, P.A. (1995) J. Biol. Chem. 270, 20855–20861.
[24] Conese, M., Nykjaer, A., Petersen, C.M., Cremona, O., Pardi, R., Andreasen, P.A., Gliemann, J., Christensen, E.I. and Blasi, F. (1995) J. Cell Biol. 131, 1609–1622.
[25] Roldan, A.L., Cubellis, M.V., Masucci, M.T., Behrendt, N., Lund, L.R., Dano, K. and Blasi, F. (1990) EMBO J. 9, 467–474.
[26] Degryse, B., Resnati, M., Rabbani, S.A., Villa, A., Fazioli, F. and Blasi, F. (1999) Blood 94, 649–662.
[27] Grignani, F., Ferrucci, P.F., Testa, U., Talamo, G., Fagioli, M., Alcalay, M., Mencarelli, A., Grignani, F., Peschle, C., Nicoletti, L. and Pelicci, P.G. (1993) Cell 74, 423–431.
[28] Ossowski, L., Clunie, G., Masucci, M.T. and Blasi, F. (1991) J. Cell Biol. 115, 1107–1112.
[29] Degryse, B., Bonaldi, T., Scaffidi, P., Muller, S., Resnati, M., Sanvito, F., Arrigoni, G. and Bianchi, M.E. (2001) J. Cell Biol. 152, 1197–1206.
[30] Holmberg, L., Bladu, B. and Aastedt, B. (1976) Biochim. Biophys. Acta. 445, 215–222.
[31] Degryse, B., Orlando, S., Resnati, M., Rabbani, S.A. and Blasi, F. (2001) Oncogene 20, 2032–2043.
[32] Aguirre Ghiso, J., Kovalski, A.K. and Ossowski, L. (1999) J. Cell Biol. 147, 89–104.
[33] Webb, D.J., Nguyen, D.H. and Gonias, S.L. (2000) J. Cell Sci. 113, 123–134.
[34] Webb, D.J., Thomas, K.S. and Gonias, S.L. (2001) J. Cell Biol. 152, 741–752.
[35] Weaver, A.M., Hussaini, I.M., Mazar, A., Henkin, J. and Gonias, S.L. (1997) J. Biol. Chem. 272, 14372–14379.
[36] Webb, D.J., Nguyen, D.H., Sankovic, M. and Gonias, S.L. (1999) J. Biol. Chem. 274, 7412–7420.