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Defective cell migration in an ovarian cancer cell line is associated with impaired urokinase-induced tyrosine phosphorylation

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Abstract The urokinase receptor (u-PAR), a protein anchored to cell membrane by a glycosyl phosphatidylinositol, plays a central role in cancer cell invasion and metastasis by binding urokinase plasminogen activator (u-PA), thereby facilitating plasminogen activation. Plasmin can promote cell migration either directly or by activating metalloproteinases that degrade some of the components of the extra cellular matrix. However, the IGR-OV1-Adria cell line contains the u-PAR but does not migrate even in the presence of exogenous u-PA, although the parental IGR-OV1 cell line migrates normally in the presence of u-PA. We therefore investigated the role of cell signalling for u-PA induced cell locomotion. We show that cell migration induced by u-PA–u-PAR complex is always associated with tyrosine kinase activation for the following reasons: (1) the blockade of the u-PAR by a chimeric molecule (albumin-ATF) inhibits not only the u-PA-induced cell migration, but also the signalling in IGR-OV1 line; (2) the binding of u-PA to u-PAR on non-migrating IGR-OV1-Adria cells was not associated with tyrosine kinase activation; (3) the inhibition of tyrosine kinase also blocked cell migration of IGR-OV1. Therefore tyrosine kinase activation seems to be essential for the u-PA-induced cell locomotion possibly by the formation of a complex u-PAR–u-PA with a protein whose transmembrane domain can ensure cell signalling. Thus, IGR-OV1 and IGR-OV1-Adria cell lines represent a good model for the analysis of the mechanism of u-PA–u-PAR-induced cell locomotion.

Key words: Ovarian cancer cell line; Urokinase; Tyrosine phosphorylation; Cell migration

1. Introduction

Remodelling of extra cellular matrix (ECM) by plasminogen activation is believed to play a prominent role in cancer cell migration and infiltration in the surrounding tissue [1,2]. Urokinase plasminogen activator (u-PA) converts plasminogen into plasmin, which activates a cascade of proteolytic events, leading to collagen, laminin and fibronectin degradation [3]. Receptor for u-PA (u-PAR) captures u-PA at the cell surface. U-PAR is a glycosyl phosphatidylinositol (GPI)-linked cell surface protein [4] that is expressed in many tumour cells while u-PA appears to be synthesised by the cells in the stroma and is then bound to cancer cells which express the u-PAR [5]. Binding of u-PA and plasminogen to their receptors increases the rate of plasminogen activation by u-PA. The simultaneous presence of u-PAR and plasminogen receptor on the cell surface results in the assembly of a highly efficient system of plasmin generation and protection from plasmin inhibition [6]. The formation of plasmin initiates thereafter a pericellular and oriented proteolysis thereby enhancing migration potential [7,8].

U-PA–u-PAR complex could also act in a plasminogen-independent pathway in various ways. For instance, u-PA is said to activate the hepatocyte stimulating factor (HSF) [9]. Finally, recent evidence indicates that u-PAR is part of molecular complex capable of mediating adhesion to matrix components as well as transcription of transmembrane signalling, suggesting a cooperative interaction among receptors involved in cell migration, adhesion and matrix degradation [10]. In this work, we present a model which provides evidence that the presence of u-PAR–u-PA complex on the cell surface is not by itself enough to promote cell migration. In this model, we show that the migration of ovarian cancer cell line (IGR-OV1) is a u-PA dependent event while the derived cell line (IGR-OV1-Adria) loses its ability to migrate despite an effective binding of u-PA to u-PAR and plasmin generation on cell surface, possibly due to an absence of signal transduction associated with u-PA binding to u-PAR.

2. Material and methods

ATF-HSA chimeric protein is a Kluyveromyces-secreted u-PAR antagonist which has been previously described and characterised [11]. It is a genetic conjugate comprising human serum albumin (HSA) linked to the 1–135 amino-terminal fragment (ATF) of human urokinase.

2.1. Cancer cells

The ovarian cancer parental cell line (IGR-OV1) was grown in tissue culture as previously reported [12]. The derived cell line (IGR-OV1-Adria) was selected from IGR-OV1 cells, following acquisition of resistance to adriamycin due to an over expression of the *MDR1* gene [13]. The cells were then cultured in RPMI medium supplemented with 10% foetal calf serum (FCS), 2 mmol/l L-glutamine, 10^5 IU/l penicillin, and 100 mg/l streptomycin (Gibco, Paisley, Scotland, UK). The cells were cultured without FCS during 24 h prior to experimental manipulation. For cell migration assays, the cells were incubated for the 2 days of migration in the presence of 2% cell growth supplement Ultrozer (Biosepra, Courbevoie-F), instead of FCS. The rate of cell proliferation was identical for the two cell lines irrespective for the presence or the absence of FCS.

Two cell lines, resistant to chemotherapy, were used as controls: parental HL 60 and daunorubicin resistant HL 60, parental K 562 and adriamycin resistant K 562.

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2.2. Cell lysate

Cells from confluent monolayers were washed with PBS and then scrapped in PBS containing 1 mM EDTA. The cell pellets were then re suspended (0.5×10^7 cells) in 0.5 ml of lysis buffer (10 mM Tris-HCl, pH 8.1, 140 mM NaCl, 2 mM EDTA, 1 mM PMSF, 100 KIU aprotinin, 0.01 mg/ml leupeptin and 1% Triton X-100). For further detail see in Busso et al. [14]. Protein in the lysate was quantitated by the DC protein assay kit (BioRad laboratories, USA) which does not give an overestimation for samples containing detergents.

2.3. Determination of u-PA level

Several methods were used for u-PA evaluation.

2.3.1. U-PA activity. To the 96-well microtiter plate-attached confluent cells (membrane-associated PA) or to 100 μl of supernatant, or cell lysate (3 cycles of freezing-thawing) were added 100 μl of plasmin-free plasminogen at 250 μg/ml (Diagnostica Stago, France) (or 100 μl of phosphate buffer for control) and 50 μl of the plasmin-sensitive chromogenic substrate S2251 (Kabi, Sweden). Plasmin generated was evaluated by its amidolytic activity on S-2251. The paranitroaniline released from the substrate was measured by its absorbance at 405 nM. In order to differentiate between t-PA and u-PA, the same experiment was carried out in the presence of amiloride (1 mM final concentration), which inhibits only u-PA activity [15]. U-PA activity was expressed as the difference in the absorbance obtained in the presence and in the absence of plasminogen.

2.3.2. Antigenic assay of u-PA. The antigenic assay of u-PA was done in the cell lysates, by ELISA, technique previously described for u-PA determination in ovarian tumour [16]. The results have been expressed in O.D.

2.3.3. Immuno-histochemical determination on intact cells. The cells were cultured in a glass chamber slide Lab-Tek (Nunc Inc., Naperville, IL) and then successively incubated for 1 h at 4°C first with 1:100 diluted polyclonal anti-serum anti-u-PA (kindly given by Pr. R. Lijnen, Leuven, Belgium), second with 1:50 diluted biotinylated Ig anti-rabbit Ig (Amersham, les Ulis, France) and third with 1:50 diluted streptavidin-fluorescein (Amersham). Computer-assisted image analysis of immuno histochemical fluorescence was performed using a confocal scanning Laser microscope (ACAS 570, Meridian Instruments, Okemos, MI) with a laser power of 20 W (wavelength excitation: 488 nM, emission: 525 nM).

2.3.4. Zymography. Zymography was done as described by Granelli and Piperno [17].

2.4. Determination of u-PAR level

2.4.1. Biological evaluation. Cells were cultured on a 96-well microtiter plates, as described above. When the cells are confluent, the wells are carefully washed and then 200 μl of urokinase at concentrations ranging from 0.5 to 2 U/ml (or buffer as control) were added to each well. After an incubation period of 30 min at 4°C, the supernatants were discarded and the cells were carefully washed 3 times with PBS containing 1% bovine albumin. U-PAR level was evaluated by measuring the u-PA bound to the cells, using synthetic substrate, as described above.

2.4.2. Immuno-histochemical determination. U-PA bound to the cells was eluted by 3 min incubation at room temperature with 0.1 M glycine-HCl buffer, pH 3, (for better availability of the epitopes recognised by the antibody against u-PAR), and then rinsed with several changes of PBS.

U-PAR was detected as previously described for u-PA using successive incubations for 1 h at 4°C with polyclonal anti-u-PAR obtained in the goat (Product 399 G, American Diagnostics, Greenwich, CT), biotinylated Ig anti-goat Ig (Amersham) and streptavidin-fluorescein (Amersham).

2.4.3. Antigenic assay. ELISA was performed in the cell lysates by the Imubind u-PAR Strip-well ELISA kit from American Diagnostica (Greenwich, CT), according to the procedure indicated by the manufacturer. The results are expressed in ng u-PAR/mg protein.

2.5. Determination of PAI-1 in cell lysate and in the supernatant of the cells

This was evaluated by ELISA using Asserachrom PAI-1 (Diagnostica Stago).

2.6. Cell migration

After reaching confluence, cells were dislodged by a cell scraper, on a standardised surface, washed 3 times with the culture medium in the absence of foetal calf serum and then cultured with 2% Ultrozer G containing the required growth factors (Biosepra, Courbevoie-F). The cell migration was determined by measuring the displacement of the edges after 24 and 48 h incubation [18]. It was expressed by the number of cells which migrate into 10 mm^2, 48 h after introducing 'the wound' into the confluent monolayer.

Migration assays were also performed, using the IGR OV1 cells incubated with two different tyrosine kinase inhibitors (either tyrphostin at 50 μM (final concentration) for 18 h or genistein at 10 μM for 48 h) and with an inhibitor of protein kinase C (calphostin C at 40 nM for 18 and 48 h), as described by Klemke et al. [19].

Migration of IGR-OV1-Adria was also performed using cells incubated with u-PA at 2 IU/ml (final concentration) during cell migration.

2.7. Analysis of signal transduction after u-PA addition to the cells

Two techniques were used. It was also done on cells incubated for various periods of time from 5 to 60 min with u-PA at 2 IU/ml.

2.7.1. Immuno-histochemical determination on intact cells. Cells were cultured in glass chamber slide and then fixed and permeabilized with 0.5% triton X-100 and 3% paraformaldehyde for 5 min followed by 30 min post-fixation with 3% paraformaldehyde [20]. Tyrosine phosphorylation was then analysed by immunocytochemistry by incubating the cells at 4°C for 2 h using the monoclonal antibody against phosphotyrosine from Santa Cruz Biotechnology (Santa Cruz, CA). Immunoconjugate was detected as described above by

Table 1
U-PA and u-PAR evaluations in IGR-OV1 and IGR-OV1-Adria cell lines

	IGR-OV1 (mean ± SD)	IGR-OV1-Adria (mean ± SD)
u-PA		
u-PA activity (OD)[a]		
Intact cells	0.869 ± 0.105	0.029 ± 0.005
Lysed cells	1.594 ± 0.307	0.030 ± 0.004
Conditioned medium	0.393 ± 0.057	0.056 ± 0.023
u-PA antigen (OD)[b]		
Lysed cells	1.235 ± 0.212	0.129 ± 0.012
u-PAR		
Immunohistochemistry (fluorescence units)[a]		
Intact cells	1 919 ± 396	2 470 ± 337
ELISA (ng/mg of protein)[b]		
Lysed cells	103 ± 14	119 ± 12

U-PA activity was determined by adding plasminogen and S2251. Plasmin generation was measured as the OD after 2 h incubation at 37°C as described in Section 2. U-PA antigen was determined by ELISA. U-PAR was determined by computer-assisted image analysis of immunohistochemical fluorescence and by ELISA.

[a] $n = 10$.

[b] $n = 5$.

incubating the cells at room temperature for 1 h at 4°C successively first with 1:50 activated biotinylated Ig anti-mouse Ig (Amersham) and second with 1:50 diluted streptavidin-fluorescein (Amersham).

To analyse the effect of PAR blockade on signal transduction, the same experiment was performed after incubating the IGR-OV1 cells for 30 min at 37°C with ATF-HSA (100 nM).

2.7.2. Western blot analysis. Samples (75 μl) of cell lysates (2×10^6 cells) were electrophoresed on 10% SDS-PAGE under non-reducing conditions and electroblotted to nitro-cellulose sheets which were subsequently blocked with 1% bovine serum albumin in TBST buffer (10 mM Tris-HCl, pH 8.1, 150 mM NaCl, 0.05% Tween-20). Phosphotyrosyl-containing proteins were detected by incubating the nitro-cellulose sheets for 2 h at room temperature successively with (1) monoclonal antibody against phosphotyrosine at 2 μg/ml, (2) 1:50 activated biotinylated Ig anti-mouse Ig (Amersham) and (3) 1:50 diluted streptavidin-peroxidase (Amersham). All antibodies and streptavidin peroxidase were diluted in PBS containing 1% albumin. Before each incubation, the blots were washed with 4 changes of PBS for 5 min each. After washing, immunoperoxidase was detected by diaminobenzidine (DAB) (6 mg of tetrahydrochloride DAB in 10 ml of 50 mM Tris buffer, pH 7.6, containing 10 μl of 30% perhydrol). The blots were developed at room temperature for 5 min and the reaction was stopped by rinsing the gels several times with PBS.

3. Results and discussion

The results presented in Table 1 show that the u-PA expressed on the derived ovarian cancer cell line (IGR-OV1-Adria) was much lower as compared to the parental cell line, whatever the technique used (biological activity or immunological assay). This was also confirmed by immunohistochemical analysis (not shown). Amiloride inhibited the PA activity by more than 90%, indicating the specificity for the u-PA. These results were also confirmed by zymography which clearly showed that the parental cell line produced significantly more u-PA than the derived IGR-OV1-Adria cells. This difference was not due to varying amounts of PAI-1 secretion since no u-PA–PAI complex was detected in the two cell lines by zymography (Fig. 1) and PAI-1 was not detected by ELISA in either the conditioned medium or the cell lysate of IGR-OV1 and IGR-OV1-Adria cells. We did not find any relationship between resistance to chemotherapy and the loss of u-PA in cells, since other cell lines (HL 60 resistant to daunorubicin, K562 resistant to adriamycin) contained the same levels of u-PA as the corresponding parental cell lines (results not shown).

However, IGR-OV1-Adria cells expressed u-PAR just as well as IGR-OV1 cells, as demonstrated by both immunohistochemical measurements using computer analysis image on cell membrane and by ELISA in the lysed cells (Table 1). Similarly, they exhibited almost identical capacity to bind exogenous u-PA, demonstrating that u-PAR in both cell lines is functional: after subtracting the basal level of u-PA, the addition of exogenous u-PA induced a similar increase in PA activity in the two cell lines, as evaluated by plasminogen activation (results not shown).

Table 2
Migration of IGR-OV1 and IGR-OV1-Adria: dependence of cell signalling (tyrosine phosphorylation)

Cell tested	Number of cells which migrated in 10 mm^2 of wounded area in 48 h
	Mean ± SD ($n=4$)
IGR-OV1	300 ± 120
IGR-OV1+Aprotinin 1 μM	130 ± 40
IGR-OV1+Tyrphostin[a]	74 ± 20
IGR-OV1+Genistein[a]	20 ± 7
IGR-OV1+Calphostin[a]	280 ± 100
IGR-OV1-Adria	60 ± 40
IGR-OV1-Adria+u-PA[b]	70 ± 50

[a]Concentration and incubation time are indicated in Section 2.
[b]2 IU/ml.

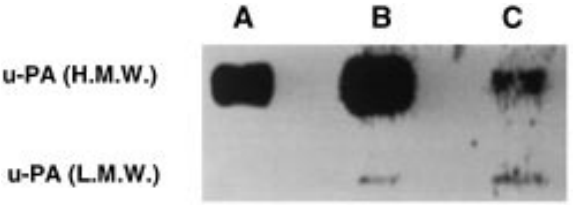

Fig. 1. Zymography of cell extracts for plasminogen activator detection. Lane A: u-PA. Lane B: IGR-OV1 cell extract. Lane C: IGR-OV1-Adria cell extract. 10^9 cells/ml were lysed in the sample buffer containing 4 M urea and 2% SDS. 20 μl of the different extracts were applied to the gel and run for 4 h at 20 mA. At the end of the electrophoresis, gels were incubated for 1 h with 2.5% Triton-X100 under gentle agitation and then extensively washed in water. The gels were applied to a plasminogen-rich fibrin plate and incubated for 16 h in moist atmosphere and then the gels were photographed. The PA were identified according to their molecular weights. HMW u-PA: high molecular weight u-PA. LMW u-PA: low molecular weight u-PA.

U-PA/u-PAR is involved in cancer cell migration and invasion. Since, in the absence of u-PA secretion, exogenous u-PA provided by stromal cells could be implicated in cancer cell invasion by binding to u-PAR, we analysed in this work the cell migration of parental and derived cell line. After wounding the confluent culture, cell displacement was measured by following the wound repair after 48 h. With IGR-OV1, a total wound repair was observed in 48 h, partially due to plasmin generation since addition of aprotinin (1 μM), a potent plasmin inhibitor, inhibited 57% cell migration. IGR-OV1-Adria did not migrate even after 48 h (Table 2). Interestingly, the inability of IGR-OV1-Adria cells to migrate was not corrected by the addition of exogenous u-PA, even at high concentrations (2–20 U/ml), despite the concomitant increase in u-PA activity expressed on the cell surface. This observation suggests that u-PA/u-PAR mediated extracellular proteolysis is not sufficient for inducing cell migration. Therefore we hypothesised that u-PA binding to u-PAR can also induce cell locomotion by inducing a cytoskeletal rearrangement related to a cell signalling via tyrosine kinase activation. This hypothesis was supported by our results shown in Fig. 2, on both migrating IGR-OV1 (Fig. 2A) and non-migrating IGR-OV1-Adria cell lines (Fig. 2B): an expression of phosphotyrosine was noted on IGR-OV1 in basal conditions which increased further after u-PA addition; interestingly, the expression of phosphotyrosine was very low in IGR-OV1-Adria cells even when u-PA was added. As presented in Fig. 2C, the expression of the phosphotyrosine on IGR-OV1 was inhibited by adding the u-PAR blocker — ATF-HSA — at 100 nM, concentration which was known to inhibit cell migration [11].

The absence of expression of phosphotyrosine in IGR-OV1-Adria was also evidenced by Western blot analysis since phosphotyrosine was undetectable in cell extracts obtained from IGR-OV1-Adria cells even after the incubation of cells with u-PA, while in IGR-OV1 cell extracts, the expression of phosphotyrosine was evident in untreated cells and increased after incubation with u-PA (Fig. 3). The difference observed in the basal tyrosine phosphorylation pattern in both cell lines was

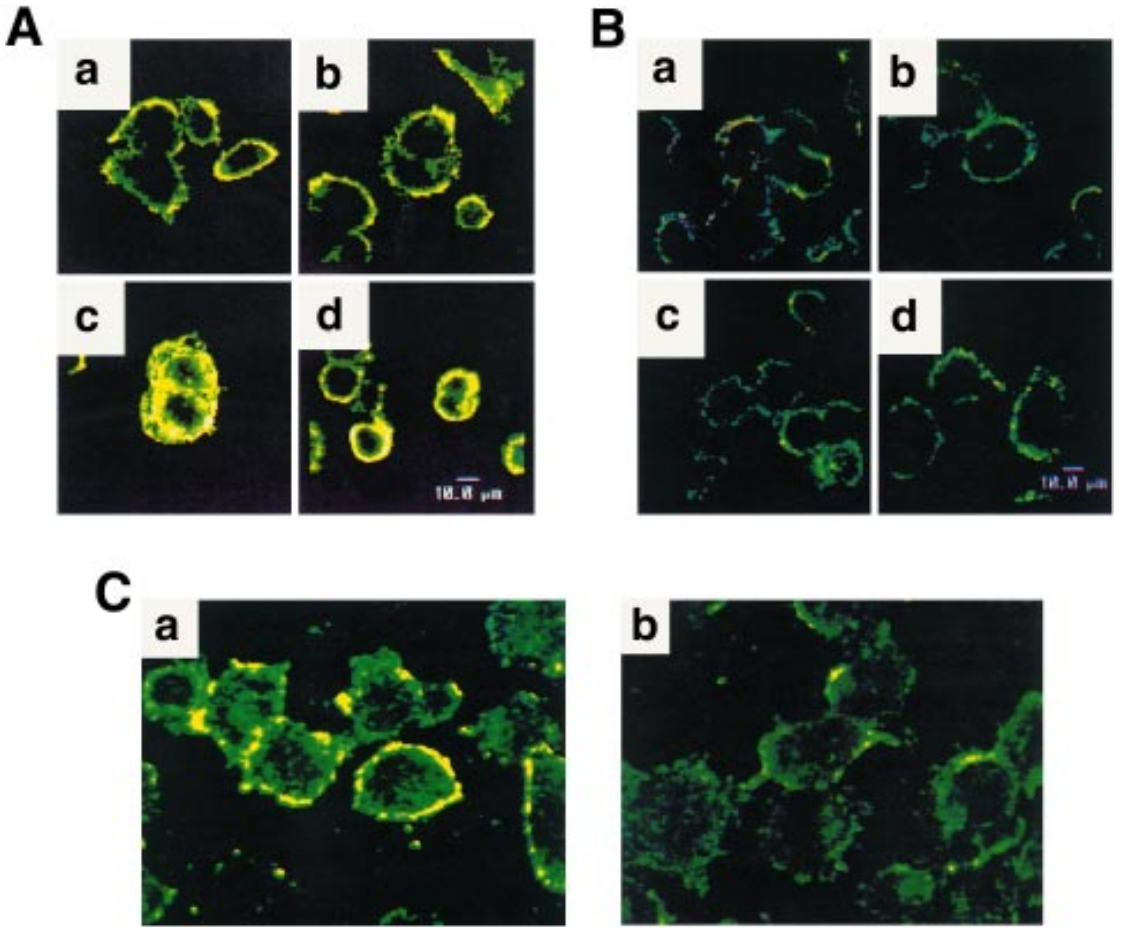

Fig. 2. Phosphotyrosine expression in IGR-OV1 and IGR-OV1-Adria cell lines. A: IGR-OV1. a: Basal level of phosphotyrosine (1980 fluorescence units). b–d: Level of phosphotyrosine in IGR-OV1 previously incubated with u-PA for 15, 30 or 60 min, respectively (b = 4212, c = 21 852, d = 15 979 fluorescence units). B: IGR-OV1-Adria. a: Basal level of phosphotyrosine (425 fluorescence units). b–d: Level of phosphotyrosine in IGR-OV1-Adria previously incubated with u-PA for 15, 30 or 60 min, respectively (b = 428, c = 412, d = 509 fluorescence units). C: u-PAR blockade in IGR-OV1 cell line. a: IGR-OV1 cell line. b: IGR-OV1 cell line incubated for 30 min with 100 nM ATF-HSA. Fixed and permeabilized cells were incubated for 90 min at room temperature with 1:300 diluted monoclonal antibody against phosphotyrosine, after extensive washing, immunoconjugate was detected by adding successively for 60 min 1:50 diluted biotinylated Ig anti-mouse Ig and 1/50 streptavidin-fluorescein. Image analysis of immunohistochemical fluorescence was performed using a confocal laser microscope (wavelength excitation: 488 nM and emission: 525 nM).

not attributed to a different cell proliferation rate, since it was identical for the two cell lines.

Furthermore, in the presence of specific inhibitors of tyrosine kinases (tyrphostin and genistein), IGR-OV1 cells largely lost their ability to migrate (Table 2), suggesting that tyrosine kinase activation is important for cell migration. Addition of calphostin, an inhibitor of protein kinase C, did not induce any modification in cell displacement.

Because u-PAR is anchored to the membrane by a glycosyl phosphatidylinositol (GPI) anchor [21–23], it was assumed that u-PA activates a transmembrane signal through a third molecule that is associated with u-PAR instead of transducing this signal by u-PAR itself [24]. Further study will be needed to determine if the defective tyrosine kinase activation after incubation of IGR-OV1-Adria cells with u-PA is related to a defective association of u-PAR with the protein whose transmembrane domain can ensure cell signalling.

We conclude that the absence of tyrosine phosphorylation of cytosolic proteins in IGR-OV1-Adria, when u-PA binds to u-PAR, may impair the rearrangement of cytoskeleton necessary for cell migration and could explain the absence of IGR-OV1-Adria migration even when u-PA is associated to u-

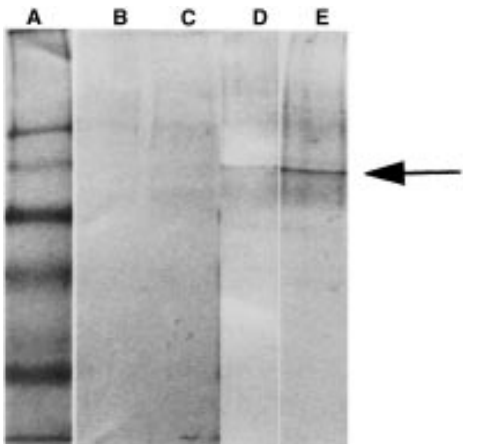

Fig. 3. Assessment of phosphotyrosine by Western blot in the two cell lines incubated in the presence or absence of u-PA. Lane A: Standard molecular weights (123 000-89 000-67 000-49 000-37 500 and 34 000). Lane B: Lysate from IGR-OV1-Adria cells in the absence of exogenous u-PA. Lane C: Lysate from IGR-OV1-Adria previously incubated for 30 min with u-PA (2 IU/ml). Lane D: Lysate from IGR-OV1 cells in the absence of exogenous u-PA. Lane E: Lysate from IGR-OV1 cells previously incubated for 30 min with u-PA (2 IU/ml).

PAR. In addition, because cytoskeletal reorganization induces u-PA gene expression [25], the absence of cell signalling observed in IGR-OV1-Adria could also explain the defective u-PA secretion of these cells.

We provide here a cell model that will be useful for identifying these molecules which activate transmembrane signal and cell locomotion in response to u-PA action on the cells.

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References

[1] Dano, K., Andreassen, P.A., Grondahl-Hansen, J., Kristensen, P., Nielsen, L.S. and Skriver, L. (1985) Adv. Cancer Res. 44, 139–266.
[2] Dano, K., Grondhal-Hansen, J., Eriksen, J., Schnack Nielsen, B., Romer, J. and Pyke, C. (1993) in: Barret, A.J. and Bond, J. (Eds.), Proteolysis and Protein Turnover, pp. 239–245, Portland Press, Portland, OR.
[3] Mignatti, P. and Rifkin, D.B. (1993) Physiol. Rev. 73, 161–195.
[4] Ploug, M., Rønne, E., Behrendt, N., Jensen, A.L. and Blasi, F. (1991) J. Biol. Chem. 266, 1926–1933.
[5] Pyke, C., Kristensen, P., Ralfkiaer, E., Grondahl-Hansen, J., Eriksen, J., Blasi, F. and Dano, K. (1991) Am. J. Path. 138, 1059–1067.
[6] Estreicher, A., Mühlhauser, J., Carpentier, J.L., Orci, L. and Vassali, J.D. (1990) J. Cell. Biol. 111, 783–792.
[7] Pöllanen, J., Stephens, R. and Vaheri, A. (1991) Adv. Canc. Res. 57, 273–328.
[8] Pöllanen, J., Hedman, K., Nielsen, L.S., Dano, K. and Vaheri, A. (1988) J. Cell. Biol. 106, 87–95.
[9] Naldini, L., Tamagnone, L., Vigna, E., Sachs, M., Hartmann, G., Birchmeier, W., Daikuhara, Y., Tsubouchi, H., Blasi, F. and Comoglio, P.M. (1992) EMBO J. 11, 4825–4833.
[10] Wei, Y., Lukashev, M., Simon, D.I., Bodary, S.C., Rosenberg, S., Doyle, M.V. and Chapman, H.A. (1996) Science 273, 1551–1555.
[11] Lu, H., Yeh, P., Guitton, J.D., Mabilat, C., Desanlis, F., Maury, I., Legrand, Y., Soria, J. and Soria, C. (1994) FEBS Lett. 356, 56–59.
[12] Bénard, J., Da Silva, J., De Blois, M.C., Boyer, P., Duvillard, P., Chin, E. and Riou, G. (1985) Canc. Res. 45, 4970–4979.
[13] Bénard, J., Da Silva, J., Teyssier, J.R. and Riou, G. (1989) Int. J. Cancer 43, 471–477.
[14] Busso, N., Masur, S.K., Lazega, D., Waxman, S. and Ossowski, L. (1994) J. Cell. Biol. 126, 259–270.
[15] Montesano, R., Pepper, S.M., Mohle, U., Steinlin-Risau, W., Wagner, E.F. and Orci, L. (1990) Cell 62, 435–445.
[16] Pujade-Lauraine, E., Lu, H., Mirshahi, S., Soria, J., Soria, C., Bernadou, A., Kruithof, E.K., Lijnen, A.R. and Burtin, P. (1993) Int. J. Cancer 55, 27–31.
[17] Granelli-Piperno, A. and Reich, E. (1978) J. Exp. Med. 148, 223–234.
[18] Pepper, M.S., Sappino, A.P., Stöcklin, R., Montesano, R., Orci, L. and Vassali, J.D. (1993) J. Cell. Biol. 122, 673–684.
[19] Klemke, R.L., Yebra, M., Bayna, E.M. and Cheresh, D.A. (1994) J. Cell. Biol. 127, 859–866.
[20] Volberg, T., Zick, Y., Dror, R., Sabanay, I., Gilon, C., Levitski, A. and Geiger, B. (1992) EMBO J. 11, 1733–1742.
[21] Stefanova, I., Horejsi, V., Ansotegui, I.J., Knapp, W. and Stockinger, H. (1991) Science 254, 1016–1019.
[22] Robinson, P.J. (1991) Immunol. Today 12, 35–41.
[23] Cinek, T. and Horejsi, V. (1992) J. Immunol. 149, 2262–2270.
[24] Dumler, I., Petri, T. and Schleuning, W.D. (1993) FEBS Lett. 322, 37–40.
[25] Lee, J.S., Von der Ahe, D., Kiefer, B. and Nagamine, Y. (1993) Nucleic Acids Res. 21, 3365–3372.