

Autocrine regulation of growth stimulation in human epithelial ovarian carcinoma by serine-proteinase-catalysed release of the urinary-type-plasminogen-activator N-terminal fragment

David A. FISHMAN*, Alicia KEARNS*, Susan LARSH*, Jan J. ENGHILD‡ and M. Sharon STACK*†¹

*Department of Obstetrics and Gynecology, Northwestern University Medical School, Chicago, IL 60611, U.S.A., †Department of Cell and Molecular Biology, Northwestern University Medical School, Chicago, IL 60611, U.S.A., and ‡Department of Pathology, Duke University Medical Center, Durham, NC 27710, U.S.A.

Ovarian carcinomas secrete single-chain urinary-type plasminogen activator (scuPA) and expression of uPA is up-regulated relative to normal ovarian epithelium, leading to an enhanced proteolytic capacity which may facilitate invasion. Furthermore, the uPA receptor (uPAR) is present on ovarian carcinoma cells and is occupied in tumour tissues. In the present study, incubation of scuPA with serum-free conditioned medium from ovarian carcinoma cells resulted in release of a 14 kDa polypeptide. N-terminal sequence analysis identified this fragment as the uPA N-terminal fragment (NTF), which contains a growth-factor and a kringle domain. NTF generation was abolished by serine-proteinase inhibitors, but not inhibitors of matrix metallo-proteinases, and was not enhanced by the addition of plasminogen or plasmin. To determine whether ovarian carcinoma-cell growth is altered by uPA, the effect of exogenous scuPA or

NTF on proliferation was analysed. Both NTF and scuPA induced a dose-dependent increase in proliferation, with maximal stimulation obtained at 10–20 nM. Furthermore, blocking the interaction of endogenous uPA with uPAR using anti-NTF antibodies significantly inhibited proliferation. Together these data indicate that, in addition to enhancing the invasive activity of ovarian carcinoma cells via increased pericellular proteolysis, uPA also acts as a mitogen for ovarian carcinoma cells, suggesting a biochemical mechanism whereby uPA may contribute to ovarian carcinoma progression by modulating both cell invasion and proliferation.

Key words: amino-terminal fragment, cell proliferation, urokinase, ovarian cancer.

INTRODUCTION

Ovarian carcinoma is characterized by widespread intra-abdominal metastases mediated primarily by surface shedding of tumour cells, peritoneal implantation and proliferation [1]. As a discrete precursor lesion for ovarian cancer has not been described, the initiating events in development of ovarian cancer are poorly understood. However, ovarian cancers are unique compared with other solid malignancies by initially using peritoneal rather than haematogenous or lymphatic dissemination to transport metastatic cells. Although extracellular-matrix-degrading proteinases have been implicated in the invasion and spread of haematogenously metastasizing tumours, the role of extracellular proteinases in intraperitoneal metastasis remains unclear. Cultured ovarian carcinoma cells overexpress the serine proteinase urinary-type plasminogen activator (uPA, urokinase) relative to normal ovarian epithelial cells (reviewed in [2]) and uPA is present in conditioned medium in both the single-chain proenzyme form (scuPA) and active (two-chain uPA) forms [2,3]. Furthermore, the uPA receptor (uPAR) is also present on ovarian carcinoma cells and immunohistochemical analyses of frozen sections of primary tumours demonstrate receptor occupancy in tumour tissues, with enhanced immunoreactivity at the invasive edge [2,4].

Previous studies have demonstrated that high-molecular-mass uPA (HMMuPA) is mitogenic for epithelial cancer cells, and the growth-stimulatory activity was localized to the N-terminal

fragment (NTF; amino acids 1–143; Figure 1) which contains a growth-factor and a kringle domain [6,7]. In the present study we report that human ovarian epithelial carcinoma cells produce an enzyme which catalyses the cleavage of scuPA, releasing a 14 kDa fragment which was identified by N-terminal sequence analysis

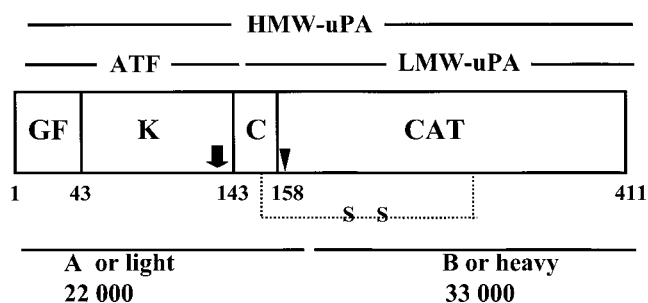


Figure 1 Diagram showing scuPA domain structure and cleavage sites

NTF, residues 1–143; HMMuPA ('HMW-uPA'), residues 1–411; LMMuPA ('LMW-uPA'), residues 144–411; growth-factor domain (GF), residues 1–43; kringle domain (K), residues 44–143; connecting peptide (C), residues 144–158; catalytic domain (CAT), residues 159–411; ▼, K¹⁵⁸-I¹⁵⁹ plasmin cleavage site; S■■■■S designates the C¹⁴⁸-C²⁷⁹ disulphide bond; ↓, K¹³⁵-K¹³⁶ ovarian carcinoma serine-proteinase cleavage site. Note that the diagram is not to scale. The data on which the diagram is based were obtained from references [7,13] and references cited therein.

Abbreviations used: APMA, aminophenylmercuric acetate; NTF, N-terminal fragment; DCI, dichloroisocoumarin; iPr₂P-F, di-isopropyl fluoro-phosphate; uPA, urinary-type plasminogen activator (urokinase); tPA, tissue-type plasminogen activator; scuPA, single-chain uPA; uPAR, uPA receptor; HMMuPA and LMMuPA, high- and low-molecular-mass uPAs; Tos-Lys-CH₂Cl, N²-p-tosyl-L-lysylchloromethane ('TLCK'); Tos-Phe-CH₂Cl, N-tosyl-L-phenylalanylchloromethane ('TPCK'); TAT-2, tumour-associated-trypsinogen 2.

¹ To whom correspondence should be sent.

as NTF. NTF generation is abolished by serine-proteinase inhibitors, but is not affected by inhibitors of matrix metallo-proteinases. Furthermore, NTF is mitogenic for ovarian carcinoma cells, and blocking the interaction of endogenous NTF with uPAR significantly inhibits proliferation, suggesting an autocrine mechanism for regulation of ovarian-carcinoma-cell proliferation.

EXPERIMENTAL

Materials

Single-chain uPA (scuPA) and purified NTF were generously given by Dr. Jack Henkin, Abbott Laboratories, Abbott Park, IL, U.S.A. Di-isopropyl fluorophosphate (iPr₂P-F)-inactivated uPA was generously given by Tammy Moser, Duke University Medical Center, Durham, NC, U.S.A. Plasminogen was purified from human plasma as previously described [8]. Human α_2 -antiplasmin was purified by affinity chromatography on plasminogen-Sepharose as described [8]. Low-molecular-mass uPA (LMMuPA), which lacks the NTF (see Figure 1), and antibodies directed against amino acids 17–34 in the uPA NTF (#3471) and against the uPA B chain (catalytic domain, #3689; see Figure 1) were purchased from American Diagnostica, Greenwich, CT, U.S.A. Alkaline phosphatase-conjugated secondary antibodies, goat IgG, 5-bromo-4-chloroindol-3-yl phosphate/NitroBlue Tetrazolium, cell-culture reagents, aprotinin, dichloroisocoumarin (DCI), *N*^z-*p*-tosyl-L-lysylchloromethane (Tos-Lys-CH₂Cl; 'TLCK'), *N*-tosyl-L-phenylalanyl-chloromethane (Tos-Phe-CH₂Cl; 'TPCK'), aminophenyl-mercuric acetate (APMA), and 1,10-phenanthroline were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. PVDF membranes (Immobilon) were purchased from Millipore, Bedford, MA. HMMuPA was the product of Calbiochem, San Diego, CA, U.S.A. The Cell Titer 96 AQ cell proliferation assay kit was purchased from Promega, Madison, WI, U.S.A.

Proteolysis

The ovarian epithelial carcinoma cell lines DOV13, OVCA429, and OVCA 433 were generously provided by Dr. Robert Bast, Jr., MD Andersen Cancer Center, Houston, TX, U.S.A., and were maintained as previously described [9]. Cells (1.5×10^5) were cultured for 18 h in serum-free medium, and conditioned medium was concentrated 10-fold using a Centricon concentrator using a YM10 membrane (Amicon). To determine the effect of ovarian-carcinoma-cell-conditioned medium on scuPA, concentrated conditioned medium was incubated with scuPA at 37 °C for the indicated times. Reaction products were analyzed by electrophoresis under reducing or non-reducing conditions on SDS/15% polyacrylamide gels and electrotransferred to Immobilon. Blots were then stained with Coomassie Blue or Western-blotted using an anti-NTF or anti-(uPA B-chain) antibody (1:500) and an alkaline phosphatase-conjugated secondary antibody (1:1000), and developed using 5-bromo-4-chloroindol-3-yl phosphate/NitroBlue Tetrazolium [3]. In studies involving proteinase inhibitors, conditioned medium was incubated with the inhibitor as indicated, followed by addition of scuPA. The reaction was allowed to proceed for 3–18 h at 37 °C, followed by electrophoretic analysis as described above. For N-terminal-sequence analysis, reaction products were electrophoresed, electroblotted on to Immobilon, stained with Coomassie Blue, and the bands of interest sequenced using an Applied Biosystems 477A pulse liquid-phase sequencer with online phenylthiohydantoin analysis.

Proliferation

To assess the effect of NTF and scuPA on proliferation, cells were subcultured in serum containing medium into 96-well microtitre plates at a density of 2500 cells/well. Following cell attachment (2 h), the medium was replaced with serum-free medium and cells were serum starved for 48 h. Test compounds were then added in fresh medium containing 0.5% serum, and cells were cultured for 24–72 h. Positive control wells contained 20% serum to stimulate proliferation. Proliferation was monitored using the Cell Titer 96 AQ cell proliferation kit according to manufacturer's specifications and measuring absorbance at 490 nm. Proliferation index (PI) was calculated as described by Rabbani [10] as:

$$PI = (A_E - A_B) / (A_S - A_B)$$

where A_E is the absorbance obtained in the experimental sample, A_B is the basal (unstimulated) absorbance and A_S is the absorbance resulting from cells stimulated with 20% serum. Antibody inhibition of proliferation stimulated by endogenous uPA species was assessed by culturing cells as described above for 48 h in the presence of anti-NTF or control IgG followed by analysis of proliferation. Cell viability was monitored by Trypan Blue exclusion. Statistical analyses (*t* test) were performed using SigmaPlot 4.0 (Statistical Product and Service Solutions Inc., Chicago, IL, U.S.A.).

RESULTS AND DISCUSSION

Cleavage of scuPA releases the NTF

To determine whether ovarian carcinoma cells can process scuPA, carcinoma-cell-conditioned-medium samples were incubated with exogenous scuPA and the reaction products analysed by electrophoresis and Western blotting. Following incubation of scuPA with conditioned medium from either DOV13 or OVCA429 cells and electrophoresis under non-reducing conditions, a 14 kDa fragment was observed which was cross-reactive with anti-NTF antibodies (Figure 2, upper panel, lanes 2–3), but did not react with an anti-(B chain) antibody. Identical results were obtained using the ovarian epithelial carcinoma cell line OVCA433 and with a primary culture of human ovarian epithelial carcinoma designated OVET1, which does not secrete uPA (results not shown; [11,12]). N-terminal-sequence analysis of the 14 kDa fragment indicated a sequence of S¹NELHQVP, identical with that of the scuPA N-terminus. On the basis of immunological cross-reactivity, molecular mass and N-terminal sequence, the fragment was identified as the NTF. Analysis of the sample under reducing conditions demonstrated the presence of the uPA B chain (Figure 1; Figure 2, upper panel, lanes 4–5). N-terminal-sequence analysis confirmed that this band contained two B-chain sequences (K¹⁵⁸PPSSPPEE and I¹⁵⁹IGGEFTT) discussed below. To analyse the time course of NTF generation, conditioned medium from DOV13 cells was incubated with scuPA for various time periods and the reaction products analysed by electrophoresis. Densitometric scanning of the Coomassie Blue-stained blots indicated that NTF was generated after as little as 30 min incubation (Figure 2, lower panel). Appearance of the uPA B chain shows a similar time course, suggesting that NTF release may accompany further processing of scuPA (Figure 2, lower panel).

The conversion of the zymogen plasminogen to the active proteinase plasmin is catalysed by uPA. Plasmin in turn cleaves scuPA at K¹⁵⁸–I¹⁵⁹, generating the active, two-chain proteinase [13]. To determine the effect of plasminogen and plasmin on NTF generation, scuPA was incubated with DOV13-cell-con-

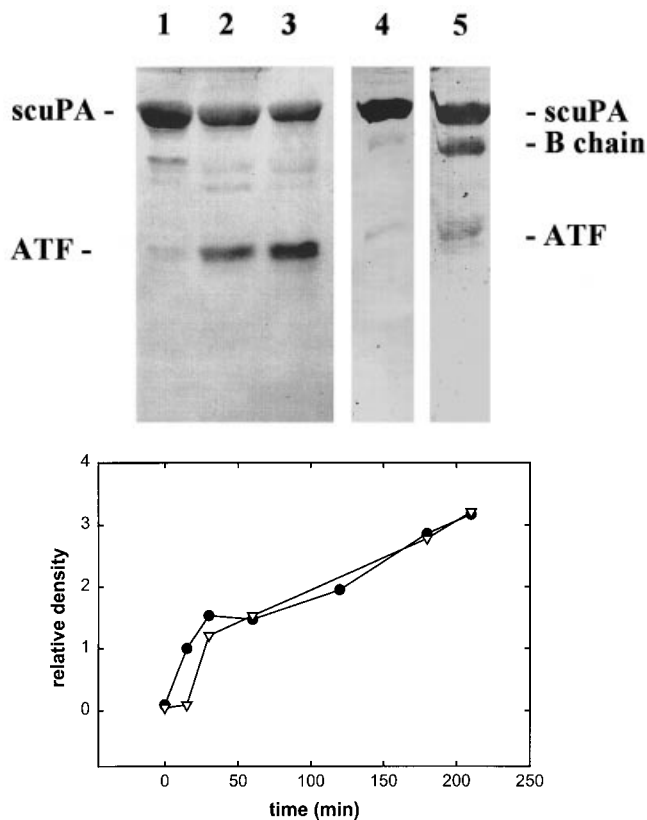


Figure 2 Cleavage of scuPA by ovarian-carcinoma-cell-conditioned medium

Upper panel: ovarian-carcinoma-cell-conditioned medium catalyses cleavage of NTF from scuPA. Epithelial ovarian carcinoma cells (DOV13; OVCA429; 1.5×10^5 cells) were cultured overnight in serum-free medium. The conditioned medium was harvested, concentrated 10-fold, and a 20 μ l aliquot was incubated with scuPA (10 μ g) at 37 °C for 18 h (lanes 1–3). Reaction products were analysed by electrophoresis under non-reducing conditions on SDS/15% polyacrylamide gels, electrotransferred to Immobilon and Western-blotted using an antibody directed against the NTF and an alkaline phosphatase-conjugated secondary antibody (lanes 4–5). Samples were prepared as described, electrophoresed under reducing conditions, electrotransferred to Immobilon and stained with Coomassie Blue. Lanes 1 and 4, scuPA only; lanes 2 and 5, scuPA + DOV13-cell-conditioned medium; lane 3, scuPA + OVCA429-cell-conditioned medium. Lower panel: time course of NTF generation. Conditioned medium from DOV13 cells was incubated with scuPA as described above for the indicated times and reaction products were analysed by electrophoresis and Western blotting (NTF) or Coomassie Blue staining (B chain). NTF release (●) was analysed under non-reducing conditions, and B-chain generation (▽) was determined under reducing conditions. Bands were scanned using a densitometer, and relative band intensities are shown as a function of time.

ditioned medium in the presence of plasminogen or plasmin. Generation of NTF was not enhanced by either plasminogen or plasmin, indicating that NTF is not released as a result of plasmin-mediated cleavage of scuPA (results not shown). This is supported by N-terminal-sequence data, which indicate that the new N-terminus generated after DOV13-cell-conditioned-medium-catalysed scuPA cleavage is K^{136} PSSPPEE, demonstrating that the NTF is released as a result of a serine-proteinase-catalysed cleavage at K^{135} – K^{136} (Figure 1, arrow), rather than the plasmin cleavage site, K^{158} – I^{159} (Figure 1, arrowhead, and [7, 13]). Furthermore, conditioned media from three breast epithelial carcinoma cell lines which also secrete uPA (MCF-7, MDA-MB-231 and MDA-MB-435) do not produce NTF when incubated under identical conditions with scuPA (results not shown), providing further evidence that neither plasmin cleavage nor

uPA autolysis are responsible for the observed scuPA cleavage. This is in agreement with the data described above using OVET1 cells, which do not produce uPA, but nevertheless catalyse NTF release from scuPA.

To determine the mechanistic class of the proteinase which initiates cleavage of scuPA, conditioned medium from DOV13 cells was incubated with scuPA in the presence of the various small-molecule and proteinaceous inhibitors. Generation of NTF was neither inhibited nor stimulated by 1,10-phenanthroline or APMA, respectively (results not shown), demonstrating that the cleavage is not matrix-metalloproteinase-mediated. To characterize the involvement of serine proteinases in NTF release, scuPA was incubated with ovarian-carcinoma-cell-conditioned medium in the presence of Tos-Lys- CH_2Cl , Tos-Phe- CH_2Cl , DCI, aprotinin or α_2 -antiplasmin (Figure 3). Only DCI blocked generation of NTF (Figure 3, lanes 7–8), effectively inhibiting at concentrations as low as 5 μ M. Lack of inhibition by Tos-Phe- CH_2Cl indicates that the serine proteinase is not chymotrypsin-like. Although the cleavage-site sequence (K^{135} – K^{136}) is suggestive of an enzyme with trypsin-like specificity, the reaction was not blocked by Tos-Lys- CH_2Cl , indicating that the cleavage enzyme exhibits preference for a dibasic-amino-acid sequence. The lack of inhibition by either aprotinin or α_2 -antiplasmin provides additional evidence supporting the observation that the cleavage is not catalysed by plasmin.

Although the identity of the serine proteinase which catalyses scuPA cleavage is currently unknown, serine-proteinase activities distinct from uPA and plasmin have been reported in association with ovarian carcinoma cells. For example, tumour-associated-trypsinogen 2 (TAT-2), an enzyme immunologically indistinguishable from pancreatic trypsinogen 2, is present in ovarian-cyst fluid and is elevated in ovarian-carcinoma patients [14]. Similar to results reported above, TAT-2 is not inhibited by aprotinin [15]. The membrane-bound serine proteinase hepsin is also elevated in ovarian cancer tissues [16]. However, activity of this enzyme is blocked by aprotinin, and it has not been reported in conditioned media [17]. Although it is interesting to speculate that one of these enzymes may be involved in scuPA processing, the existence of a novel proteolytic activity cannot be definitively ruled out.

NTF stimulates ovarian-carcinoma-cell proliferation

To assess the potential functional consequences resulting from the presence of NTF in the ovarian-carcinoma-cell micro-environment, the effect of exogenous NTF on cell proliferation was analysed. Proliferation of ovarian carcinoma cells was enhanced by NTF, with maximal stimulation observed at 10–30 nM NTF (Figure 4). Proliferation was also stimulated by scuPA, although the magnitude of the stimulatory effect was only approximately half of that observed with NTF (Figure 4). LMMuPA, which lacks the NTF and does not bind the uPA receptor (Figure 1), had no stimulatory effect on ovarian-carcinoma-cell proliferation. These data are in agreement with previous results showing that proliferation of both renal carcinoma and normal renal cells is stimulated by uPA, but not tissue-type PA (tPA) or plasmin [18]. Similarly, a human epidermal tumour line (CCL20.2) displayed enhanced proliferation in the presence of both active and iPr_2P -F-inactivated HMMuPA, while LMMuPA did not affect proliferation [6]. More recent studies using prostate carcinoma and osteosarcoma cells also demonstrated that the growth-promoting activity of uPA is independent of catalytic function and is localized in the NTF [10, 19]. Interestingly, fucosylation of Thr¹¹ within the NTF is

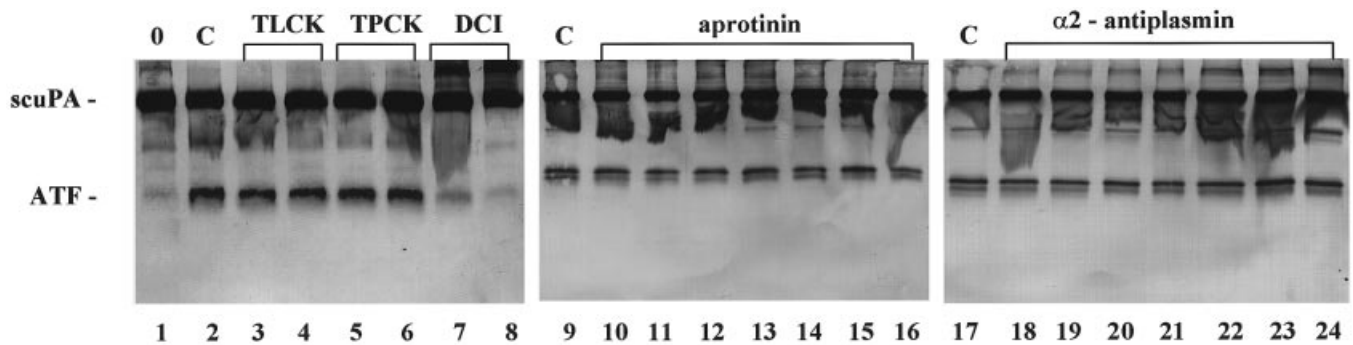


Figure 3 Effect of serine proteinase inhibitors on NTF ('ATF') generation

scuPA (10 μ g) was incubated with buffer (lane 1) or with DOV13-cell-conditioned medium (lanes 2–24) for 18 h at 37 °C, and reaction products were analysed by electrophoresis under non-reducing conditions and Western blotting. Lanes 2, 9 and 17 are controls containing only scuPA and DOV13-cell-conditioned medium; lanes 3–8, synthetic inhibitors [3, 10 μ M Tos-Lys-CH₂Cl ('TLCK'); 4, 1 mM Tos-Lys-CH₂Cl; 5, 10 μ M Tos-Phe-CH₂Cl ('TPCK'); 6, 1 mM Tos-Phe-CH₂Cl; 7, 5 μ M DCI; 8, 15 μ M DCI]; lanes 10–16 contain aprotinin at 0.005, 0.05, 0.5, 2.5, 5.0, 7.5 and 10.0 μ g respectively. Lanes 18–24 contain α_2 -antiplasmin at 0.005, 0.05, 0.5, 2.5, 5.0, 10.0 and 20.0 μ g respectively.

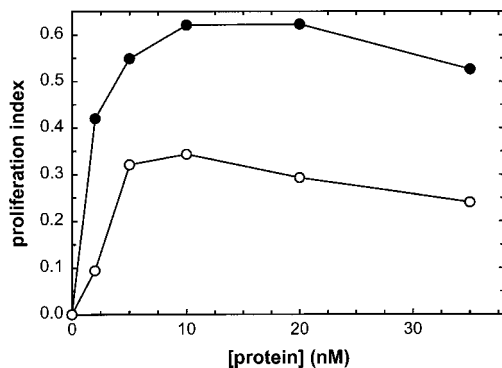


Figure 4 Effect of NTF on ovarian-carcinoma-cell proliferation

DOV13 cells (2500) were serum-starved as described in the Experimental section and incubated for 48 h in medium containing 0.5% fetal-bovine serum in the presence of increasing concentrations of NTF (●) or scuPA (○). Proliferation was analysed and the proliferation index (PI) was calculated as described in the text.

required for mitogenic activity [19]. In contrast, both active uPA and tPA were mitogenic for normal human fibroblasts [20], suggesting that the proliferative effect of uPA may be cell-type-specific. To determine whether blocking the interaction of endogenous NTF (whether free, or as a component of intact scuPA or HMMuPA) with uPAR affects basal proliferation, ovarian carcinoma cells were cultured in the presence of anti-NTF antibodies, which inhibit binding of NTF to uPAR. Proliferation was significantly inhibited in cultures containing anti-NTF relative to control IgG (Figure 5), indicating that binding of NTF to uPAR functions as an autocrine mechanism for the control of ovarian carcinoma proliferation. Furthermore, these results extend previous observations that proliferation of cells of epithelial origin is regulated by NTF in a manner independent of uPA proteolytic activity.

In summary, our laboratory and others have previously demonstrated that ovarian carcinoma cells produce scuPA and contain the uPAR [2–5, 21–22]. The present results demonstrate that the scuPA produced, in addition to enhancing the invasive activity of ovarian carcinoma cells via increased pericellular

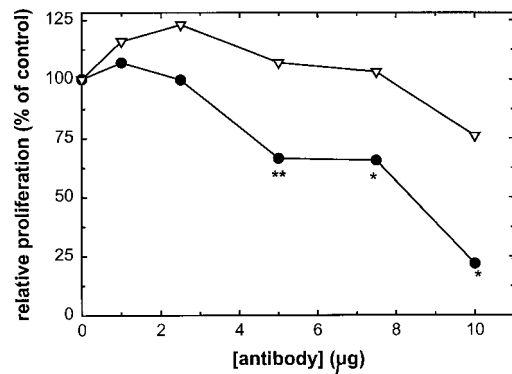


Figure 5 Inhibition of proliferation by anti-NTF antibodies

DOV13 cells (2500) were cultured for 48 h in the presence of increasing concentrations of (●) anti-NTF or (▽) control antibodies and proliferation analysed as described in the text. Basal proliferation in the absence of antibodies is designated 100%. Statistical significance: * $P < 0.05$; ** $P < 0.005$ (as compared with samples treated with control antibodies).

proteolysis, also acts as a mitogen for ovarian cancer cells. Furthermore, as blocking the interaction of endogenous NTF with uPAR inhibits cell growth, our data suggest an autocrine pathway through which ovarian carcinoma proliferation is regulated. Together these data suggest a potential mechanism whereby uPA may potentiate the intraperitoneal metastasis of epithelial ovarian cancer by affecting both tumour-cell invasion and proliferation.

This work was supported by Research Grant CA 58900 (to M.S.S.) from the National Cancer Institute, the Stenn Fund for Ovarian Cancer Research and Research Grant HL 49542 (to J.J.E.) from the National Heart, Lung and Blood Institute.

REFERENCES

- Hoskins, W. J. (1995) *J. Cell. Biochem. (suppl.)* **23**, 189–199
- Stack, M. S., Ellerbroek, S. M. and Fishman, D. A. (1998) *Int. J. Oncol.* **12**, 569–576

- 3 Moser, T. L., Young, T. N., Rodriguez, G. C., Pizzo, S. V., Bast, R. C. and Stack, M. S. (1994) *Int. J. Cancer* **56**, 552–559
- 4 Young, T. N., Rodriguez, G. C., Moser, T. L., Bast, Jr., R. C., Pizzo, S. V. and Stack, M. S. (1994) *Am. J. Obstet. Gynecol.* **170**, 1285–1296
- 5 Pujade-Lauraine, H., Lu, H., Mirshahi, S., Soria, J., Soria, C., Bernadou, A., Kruithof, E. K.O., Lijnen, H. R. and Burtin, P. (1993) *Int. J. Cancer* **55**, 27–31
- 6 Kirchheimer, J. C., Wojta, H., Christ, G. and Binder, B. R. (1987) *FASEB J.* **1**, 125–128
- 7 Marcotte, P. A., Kozan, I. M., Dorwin, S. A. and Ryan, J. M. (1992) *J. Biol. Chem.* **267**, 13803–13806
- 8 Enghild, J. J., Valnickova, Z., Thogersen, I. B., Pizzo, S. V. and Salvesen, G. (1993) *Biochem. J.* **291**, 933–938
- 9 Bast, Jr., R. C., Feeney, M., Lazarus, H., Nadler, L. M., Colvin, R. B. and Knapp, R. C. (1981) *J. Clin. Invest.* **68**, 1331–1337
- 10 Rabbani, S. A., Desjardins, J., Bell, A. W., Banville, D., Mazar, A., Henkin, J. and Goltzman, D. (1990) *Biochem. Biophys. Res. Commun.* **173**, 1058–1064
- 11 Moser, T. L., Pizzo, S. V., Bafetti, L. M., Fishman, D. A. and Stack, M. S. (1996) *Int. J. Cancer* **67**, 695–701
- 12 Fishman, D. A., Bafetti, L. M. and Stack, M. S. (1997) *Invasion Metastasis* **16**, 150–159
- 13 Lijnen, H. R., Van Hoef, B. and Collen, D. (1987) *Eur. J. Biochem.* **169**, 359–364
- 14 Koivunen, E., Itkonen, O., Halila, H. and Stenman, U. H. (1990) *Cancer Res.* **50**, 2375–2378
- 15 Figarella, C., Negri, G. A. and Guy, O. (1975) *Eur. J. Biochem.* **53**, 457–463
- 16 Tanimoto, H., Yan, Y., Clarke, J., Korourian, S., Shigemasa, K., Parmley, T. H., Parham, G. P. and O'Brien, T. J. (1997) *Cancer Res.* **57**, 2884–2887
- 17 Kazama, Y., Hamamoto, T., Foster, D. C. and Kiesel, W. (1995) *J. Biol. Chem.* **270**, 66–72
- 18 Kirchheimer, J. C., Wojta, J., Christ, G., Hienert, G. and Binder, B. B. (1988) *Carcinogenesis* **11**, 2121–2123
- 19 Rabbani, S. A., Mazar, A. P., Bernier, S. M., Haq, M., Bolivar, I., Henkin, J. and Goltzman, D. (1992) *J. Biol. Chem.* **267**, 14151–14156
- 20 DePetro, G., Copeta, A. and Barlati, S. (1994) *Exp. Cell Res.* **213**, 286–294
- 21 Kobayashi, H., Ohi, H., Sugimura, M., Shinohara, H., Fujii, T. and Terao, T. (1992) *Cancer Res.* **52**, 3610–3614
- 22 Chambers, S. K., Gertz, R. E., Ivins, C. M. and Kacinski, B. M. (1995) *Cancer* **75**, 1627–1633

Received 26 March 1999/10 May 1999; accepted 31 May 1999