Metabolism of tumour-derived urokinase receptor and receptor fragments in cancer patients and xenografted mice

Cornelis F. M. Sier1, Ines Nicoletti3, Maria Lisa Santovito1, Thomas Frandsen5, Giovanni Aletti2, Augusto Ferrari2, Andrea Lissoni4, Raffaella Giavazzi3, Francesco Blasi1, Nicolai Sidenius1

1Department of Molecular Biology and Functional Genomics and FIRC Institute of Molecular Oncology, 2Department of Gynaecology and Obstetrics, San Raffaele Scientific Institute and Hospital, Milan, Italy 3Department of Oncology, Mario Negri Institute for Pharmacological Research, Bergamo, Italy 4Department of Gynaecology and Obstetrics, S. Gerardo Hospital, Monza, Italy 5H:S Hvidovre Hospital, Pediatric Department, Hvidovre, Denmark

Thromb Haemost 2004; 91: 403–11

Summary
The urokinase-type plasminogen activator receptor (uPAR) is involved in cell migration and tissue remodelling, as a receptor for pro-uPA, as a cell adhesion component, and in a soluble form as a chemoattractant. We have analyzed the presence and the molecular forms of uPAR and uPAR-fragments in urine of ovarian cancer patients in comparison with tumour tissue, ascites, and serum. Carcinoma tissue contained high levels of uPAR, but more abundantly the D2D3-fragment. Ascitic fluid contained similar ratios of suPAR fragments as corresponding tumour tissue, but serum only contained intact suPAR. Interestingly, urine contained predominantly the uPAR-fragments D1 and D2D3, and the pattern of these fragments was different in cancer patients as compared to healthy individuals. To confirm the hypothesis that circulating and urinary suPAR and suPAR-fragments originate from the tumour tissue, the presence of human suPAR (fragments) was analyzed in mice xenografted with human tumours. Indeed, high levels of urinary D1 fragment were found in mice carrying a tumour displaying cleaved uPAR on the cell surface, but little or no D1 was found in the urine from mice carrying a tumour with full-length uPAR. Mouse serum contained only intact suPAR. Our data demonstrate that the enhanced levels of suPAR fragments in the urine of cancer patients is likely to originate from uPAR expressed in the tumour tissue. Considering the biological activities that uPAR fragments display, the level and typing of uPAR fragments in urine might therefore be clinically more relevant than the plain serum uPAR content.

Keywords
uPAR, cleavage, fragments, cancer, urine

Introduction
Numerous studies indicate that the receptor for urokinase type plasminogen activator (uPAR) is involved in cancer (1). In brief, uPAR is present in increased concentrations in different types of tumours like ovarian, breast, lung and colon (2, 3), and blocking of uPAR in tumour models in vitro and in vivo leads to decreased tumour growth and metastasis (4-6). Recent studies show that enhanced levels of a soluble form of uPAR are present in serum of colon, breast and ovarian cancer patients and in even higher concentrations in ascites (3, 7-10). In general approximately 50% of the patients show normal suPAR serum values, like has been found for tumour tissue uPAR levels (2, 11).
A clear correlation between serum SUcPAR levels and prognosis has been established in colon cancer (12). Since we have recently shown that the urinary and serum levels of SUcPAR are strongly correlated (13), we have concentrated in determining the significance and the usefulness of the latter measurement in ovarian cancer. In this study we now show that urinary SUcPAR levels of ovarian carcinoma patients are also enhanced in about 50% of the patients. We confirm the hypothesis that the tumour tissue is the origin of the enhanced serum and urinary SUcPAR by analyzing the serum and urine for human SUcPAR in mice xenografted with human tumours. Not only did we find human SUcPAR in both body fluids of the mice, but we also found that the presence of cleaved forms of SUcPAR in urine and tumour tissue were correlated. Because certain soluble forms of SUcPAR (suSUcPAR) might function as a potent chemoattractant or as a scavenger of SUcPA and vitronectin (14-16) and therefore might play an additional role in cancer besides being a receptor for SUcPA, we investigated the fragmentation pattern of SUcPAR in body fluids of ovarian carcinoma patients in comparison with the tumour.

Materials and methods

Patients, urine, serum and tissue
Urine and serum from twenty-five patients (median age 62, range 20-78) admitted for surgery of ovarian carcinoma were sampled at the Departments of Gynaecology and Obstetrics, San Raffaele Hospital, Milan and Ospedale Sacra Famiglia, Erba. The FIGO (International Federation of Gynaecology and Obstetrics) stages of the tumours comprised 14 women with stage I/II, and 11 with stage III/IV respectively. Urine samples from 20 age-matched healthy women (median age 56, range 36-78) were used as controls. All samples were snap frozen and kept at -80°C. Before analysis, the samples were quickly thawed at 37°C and centrifuged at 10,000 g for 10 min. From four of the patients also the tumour tissue, ascites and serum was obtained. Biopsies from 46 patients with ovarian carcinoma were obtained from the Frozen Tumour Repository of Mario Negri Institute, San Gerardo Hospital (Milan, Italy). All tumour specimens were homogenized in phosphate buffered saline (10 ml/g of tissue) containing 1% Triton X-100 and protease inhibitors (Complete™, Roche), incubated on ice for 1 hour, centrifuged at 14,000 rpm for 10 minutes at 4°C, and the supernatant was kept at -80°C until analysis. All human derived samples were obtained after informed consent.

Tumour lines and animals
The HOC8 xenograft was established and maintained as ascites in female NCr nu/nu mice (NCI, Charles River, Frederick, MD) as described previously (7, 17). Serum and tumour cells were obtained four weeks after the i.p. injection of a tumour suspension (10 million cells) from nude mice, all bearing ascites and metastatic tumours in the peritoneal cavity (7). Urine was collected on days 0, 7, 14, 21 and 28 after tumour implantation, from mice kept in a metabolic cage for 24 hours; it was centrifuged at 3000 rpm and stored frozen at -80°C until analysis. Blood was obtained by intracardiac puncture from anesthetized mice and serum collected and frozen after centrifugation. Ascites were harvested with 3 ml of 0.9% NaCl, centrifuged and cells processed as described below. As controls, blood and urine were collected from non tumour bearing nude mice and processed in the same way. For the MDA-MB-435-BAG (MDA435) xenografts (18), cells were cultured in Eagle MEM containing glutamax (Life Technologies) and 5% foetal bovine serum. Semi-confluent cell cultures were harvested by scraping and the concentration was adjusted to 10 x 10⁶ cells/ml. Six to eight weeks old male META-Bom-nu/nu mice (Bomholdgaard, Denmark) were injected subcutaneously and bilaterally with 0.1 ml MDA435 cell suspension. After 50 days, urine, plasma, and tumour tissue were collected from tumour-bearing mice. Cell and tumour lysates were prepared by Dounce homogenization in PBS (10 ml / gram of tissue) containing 1% Triton X-100 and protease inhibitors (Complete™) for 1 hour on ice. The lysates were centrifuged at 14,000 rpm for 20 min. at 4°C and the supernatants were stored at -80°C. Total protein was determined by the DC protein assay (Bio-Rad) with bovine serum albumin as a standard. Procedures involving animals and their care are conducted in conformity with the institutional guidelines that are in compliance with Italian (D.L. n.116, G.U., suppl. 40, 18 February 1992, Circolare No. 8, G.U., July 1994), Danish (Danish Animal Care Committee #1998 / 561-146) and international (EEC Council Directive 86/609, OJ L358,1, Dec. 12, 1987; Guide for the Care and Use of Laboratory Animals, US. National Research Council, 1996) laws and policies.

uPAR ELISA
Two different uPAR ELISA were used in this study. The first was used to quantitate SUcPAR antigen in human urine. This ELISA contained a polyclonal rabbit anti-human uPAR as catching antibody and a mixture of three monoclonal antibodies (mAb R2, R3 and R5) as detecting antibodies. It detects full size uPAR, D1 and D2D3 fragments, as well as complexes with uPA and has been described previously (9). The second ELISA was designed to quantitate human SUcPAR antigen in samples of murine origin. This ELISA is an “inverted” version of the previous and uses the monoclonal R2 as catching antibody and the polyclonal anti-uPAR antibody for detection. This ELISA detects full size SUcPAR, D2D3 fragments, complexes with uPA, but not D1 fragment, and is highly specific for human uPAR (19). All samples were measured in duplicate.

uPA-activity measurement
The uPA activity in urine samples was determined using a spectrophotometric assay using a chromogenic plasmin substrate (20).
Creatinine measurement
Urinary suPAR levels were normalized for dilution by the creatinine content of the samples as described elsewhere (13). Creatinine was measured with a modification of the Jaffé-method according to the manufacturer’s instructions (Boehringer Mannheim), using a BM/Hitachi 747 analyzer. uPAR/creatinine ratios were expressed as ng/ml uPAR divided by mg/dl creatinine.

Immunoprecipitation and immunoblotting
Immunoprecipitation and immunoblotting of full-size uPAR and uPAR fragments was performed using a previously described protocol (21). In brief, indicated volumes of urine, ascites, serum and tissue homogenate samples were diluted to one milliliter using RIPA buffer (0.1% sodium dodecyl sulfate (SDS), 1% Triton X-100, 1% deoxycholate, 0.15 M NaCl and 0.05 M Tris-HCl pH 7.6) containing a cocktail of protease inhibitors (Complete™). The samples were immunoprecipitated for 16 hours at 4°C using biotinylated monoclonal antibodies R2 and R3, previously immobilized on streptavidin coated beads. After washing with RIPA buffer the absorbed material was eluted by boiling in non-reducing sample buffer and the proteins were separated by 12% SDS-PAGE. Proteins were transferred to PVDF membranes (Immobilon-P™, Millipore) and probed with a rabbit polyclonal anti-uPAR antibody. The immune-complexes were visualized by incubation with a peroxidase conjugated donkey anti-rabbit antibody (Amersham) and chemoluminescent detection (SuperSignal Ultra™, Pierce Chemicals). This technique detects all known forms of uPAR including the D2D3 and D1 fragments (21).

Calculations and statistics
The immunoblots were scanned and subsequently analyzed with NIH-Image version 1.63 densitometry software using 2 different exposure times to avoid overexposure in some samples. To evaluate differences between group mean and median values all data were analyzed with the Students’ t test or the Mann-Whitney rank test respectively. P values below or equal to 0.05 were considered significant.

Results
Urine from ovarian cancer patients contains elevated levels of suPAR antigen
To establish if the increased expression of uPAR in solid human tumours not only results in higher levels of suPAR in the circulation but also in the urine, we assayed urine samples obtained from ovarian cancer patients by ELISA. The urinary suPAR/creatinine ratios of 25 ovarian cancer patients and in control groups of sex and age matched healthy volunteers are presented in Figure 1. The ratio of suPAR and creatinine was used because it eliminates differences due to dilution (13). All urine samples contained suPAR, but the median level of urinary suPAR was significantly elevated in carcinoma patients as compared to control individuals (p=0.004). Levels above the median of controls plus twice the standard deviation was diagnostic of ovarian cancer with a sensitivity of 43% and a specificity of 93%. The urinary suPAR levels correlated with disease progression as patients with higher grade tumours (filled circles in Fig. 1) had significantly higher levels of urinary suPAR (FIGO stage I and II (n=14) versus stage III (n=11), p=0.010).

Urinary suPAR is derived from tumour tissue
To test the hypothesis that suPAR in the urine from cancer patients derives from the tumour tissue, we analyzed suPAR in urine obtained from mice xenografted with the human ovarian carcinoma HOC8 (22) or the human metastatic breast carcinoma MDA435 (23). The ELISA used is specific for human uPAR without interference of endogenous uPAR from the mice. Figure 2A presents the time dependence of human suPAR concentration in urine averaged from 6 mice xenografted with the HOC8 tumour, showing a correlation between time of tumour growth and urinary suPAR levels. Urine from MDA435 xenografted mice also contained soluble human suPAR (7.4 ng/ml, 50 days after xenografting, n=2, data not shown), whereas no suPAR was detected in urine from non-xenografted mice (n=6, data not shown).
Next, we analyzed uPAR in tumour lysates, serum and urine of mice xenografted with HOC8 and MDA435 (Fig. 2B) using immunoabsorption and blotting. While the HOC8 tumour lysate only contained full-length uPAR, the MDA435 tumour lysate also contained a lower MW band representing cleaved uPAR (the D2D3 fragment). Both tumours apparently released full-length receptor because human full-length suPAR was detected in the serum of mice xenografted with both carcinoma types. In urine however, no full-size suPAR was found but D2D3 fragments were present in mice xenografted with both tumour types. High levels of D1 fragment were observed only in urine from mice bearing the MDA435 but not the HOC8 tumour, even after long exposure of the films (not shown). The difference between the urinary D1 levels of the two tumour models thus correlates with the presence of cleaved uPAR on the tumour cells, indicating that the urinary D1 fragment originates from cleavage of cell surface uPAR in the distant tumour tissues. It is to note that the size of the D2D3 fragment is smaller in urine than in the tissue, likely due to removal of the GPI-anchor and/or progressive proteolysis.

**Evaluation of the presence of different forms of uPAR in tumour tissue and corresponding body fluids of ovarian cancer patients**

We next analyzed the structure and distribution of uPAR antigen in the tumour tissue and different fluids of ovarian cancer patients. Ovarian cancer is especially convenient for this type of study because this tumour type allows the analysis of molecules directly released from the tumour into the surrounding ascites. In figure 3 an immunoblot analysis illustrating the presence and structure of uPAR in tumour tissue, ascites, blood, and urine obtained from four ovarian carcinoma patients is presented. In all four patients, tumour tissue detergent lysates showed two major bands indicating both cell-bound full-size uPAR and the fragment D2D3. The cleaved form was present often in higher amounts, implicating that cleavage occurs already on the cell surface. Also the ascitic fluid from the same patients revealed soluble full-size uPAR and D2D3 in about the same ratio as found in the corresponding tumour, in agreement with both forms of the receptor being released from the tumour tissue. The D1 fragment, which is released upon cleavage of cell-bound uPAR (24, 25) was not detected in the tumour tissue nor in the ascitic fluid suggesting that this fragment is released from the tumour tissue and rapidly cleared from the ascites. The corresponding urine of three of the four patients contained detectable amounts of D1 next to full-size suPAR and D2D3 (Fig. 3). Indeed, in the serum from 25 patients and 15 healthy controls only full-length suPAR could be detected (data partially shown in Fig. 5C). Control experiments in which five times more serum was immunoprecipitated did not reveal the presence of any D1 fragment in serum (not shown).

**uPAR cleavage in ovarian tumour tissue**

To more precisely determine the prevalence and extent of uPAR-cleavage in tumour tissue we analyzed and quantified the degree of uPAR-cleavage in detergent lysates of tumour biop-
sies from a larger and separate group (n=46) of patients suffering from ovarian cancer. To improve the scanning resolution of the bands on the films, the separation of full-length uPAR and the D2D3 fragments was optimized by using a deglycosylation step prior to SDS-PAGE and immunoblotting (21). Deglycosylation converts the smeary bands of uPAR, caused by heterogeneous glycosylation, into discrete bands (Fig. 4A). Quantitation of these blots for 46 tumour biopsies is presented in figure 4B. The densitometry values of the bands corresponding to D2D3 were divided by the sum of D2D3 and uncleaved uPAR, resulting in a ratio that indicates cleavage. Cleavage of uPAR was observed in all samples and on average 51% (range: 7%-92%) of total uPAR-antigen was present in the D2D3 form. A trend towards a lower degree of uPAR-cleavage in tissue from higher stage tumours (FIGO 3) was observed in approximately 50% of these patients (Fig. 4B), but did not lead to significant differences compared with the lower FIGO stage patients. Cleavage of uPAR has been suggested to occur preferentially when high concentrations of both uPAR and uPA are present (26) and we therefore measured the total levels of uPA and uPAR antigen in the lysates by ELISA and correlated the levels to the degree of uPAR-cleavage. No significant correlation was found between the degree of uPAR cleavage and the total levels of uPA or uPAR-antigen (results not shown).

**Urine from ovarian cancer patients displays an altered uPAR-fragment pattern**

Having established that uPAR-cleavage is a prominent phenomenon in ovarian cancer we investigated whether uPAR fragmentation in the tumour tissue results in an altered pattern of uPAR fragments in the urine compared to control women. Figure 5 shows the presence of full-size suPAR and suPAR-fragments in urine from 7 ovarian carcinoma patients (P1-P7) and 7 healthy women (C1-C7) as detected by immunoprecipitation and immunoblotting. The pattern of suPAR fragments in the urine samples from healthy volunteers was found to be remarkably consistent (Fig. 5A). The major urinary form of suPAR is in all cases the D2D3 fragment, but full-size suPAR is always present. Quantification of uPAR bands on the blots of urine samples from 19 healthy control women indicated that on average D2D3 was twice as abundant as full-size suPAR (Table 1). The D1 fragment could be detected in several urine samples, but the intensity of this particular band was always low compared to full-size suPAR (Fig. 5A, Table 1). The suPAR fragment pattern in urine from ovarian cancer patients was clearly more heterogeneous (Fig. 5B, Table 1). Some patients showed distribution patterns similar to normal urine’s (P3, P4, P5, and P6), whereas others had relatively high amounts of D2D3 (P1) or D1 (P2, and P7). Urine from patient P7 contained high amounts of all forms, but the D1 band was clearly the most intense. Quantification of the bands by densitometry of the immunoblots revealed a significant difference between the ratio of full-length suPAR and D1 fragment in urine from ovarian cancer patients and healthy individuals, with the cancer patients having a relatively higher ratio of D1 to suPAR (Table 1). As indicated before, also in these patients full-length suPAR was the only form of suPAR present in the serum (Fig. 5C). A correlation between the amount of D1 and D2D3 was not found and neither was there a relation between cleavage and the uPA activity in the urine (Table 1).

**Discussion**

Components of the plasminogen activation system are found in enhanced amounts in various types of tumours (27). High tissue and serum levels of uPA, uPAR, and inhibitor PAI-1 are associated with bad prognosis in human cancers (3, 8, 28-35). Because we previously showed that suPAR levels of ovarian
carcinoma patients are enhanced (3) and that urinary and serum suPAR levels are strongly correlated (13), we hypothesized that enhanced urinary suPAR levels might also be associated with ovarian cancer. The present study shows that the majority of ovarian cancer patients display increased urinary concentrations of suPAR, comparable to what was found previously in serum and tissue from ovarian carcinoma patients (2, 3, 36, 37). The source(s) of suPAR in serum or urine from healthy individuals has not been identified yet, but the enhanced levels of circulating suPAR in many cancer types might reflect an increase in tissue uPAR. Indeed, plasma levels of human suPAR correlate with the tumour burden in nude mice carrying xenografts of ovarian and breast cancer (19). Our results showed a similar correlation for urinary levels of suPAR (Fig. 2).

Several studies, including this, found evidence for the existence of D2D3 fragments of uPAR on the surface of tumour cells (26, 38). Here we show that no fragmented suPAR could be detected in serum from healthy volunteers, nor in serum from patients with ovarian cancer, or in serum from mice xenografted with human tumours (Figs. 2, 3, 5, and results not shown). In
Table 1: Quantitative analysis of suPAR fragments in urine. Ratios of the suPAR fragments D1 and D2D3 in the urine of ovarian carcinoma patients. The data represent the values obtained by scanning of the bands on the immunoblots (see Fig. 5B) divided by the value of the full-size suPAR. The values for the total group of carcinoma patients (n=7) and the healthy volunteers (controls, n=19) are mean ± SD. Stage and grade are according to the International Federation of Gynaecology and Obstetrics (FIGO). The activity of uPA (uPA act.) in the urine samples is given in mIU/ml. Metastasis of ovarian tumour, *Significantly different from controls (P < 0.05, Mann-Whitney U-test).

<table>
<thead>
<tr>
<th>Patient</th>
<th>Cancer</th>
<th>Stage</th>
<th>Grade</th>
<th>Type</th>
<th>uPA act.</th>
<th>D1</th>
<th>D2D3</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>ovarian</td>
<td>3c</td>
<td>4</td>
<td>serous</td>
<td>98</td>
<td>0.0</td>
<td>6.7</td>
</tr>
<tr>
<td>P2</td>
<td>ovarian</td>
<td>2c</td>
<td>2</td>
<td>serous</td>
<td>645</td>
<td>1.2</td>
<td>1.1</td>
</tr>
<tr>
<td>P3</td>
<td>ovarian</td>
<td>4</td>
<td>4</td>
<td>serous</td>
<td>30</td>
<td>0.7</td>
<td>1.4</td>
</tr>
<tr>
<td>P4</td>
<td>endometrial†</td>
<td>3a</td>
<td>2</td>
<td>serous</td>
<td>74</td>
<td>1.3</td>
<td>2.6</td>
</tr>
<tr>
<td>P5</td>
<td>ovarian</td>
<td>1a</td>
<td>2</td>
<td>mucinous</td>
<td>67</td>
<td>0.2</td>
<td>3.0</td>
</tr>
<tr>
<td>P6</td>
<td>ovarian</td>
<td>3c</td>
<td>3</td>
<td>serous</td>
<td>288</td>
<td>0.3</td>
<td>2.3</td>
</tr>
<tr>
<td>P7</td>
<td>ovarian</td>
<td>3c</td>
<td>4</td>
<td>serous</td>
<td>9</td>
<td>4.7</td>
<td>2.0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The case of xenografted mice, serum suPAR derives with certainty from tumour cells, as these cells represent the only human component in those mice. Assuming that also in cancer patients the enhanced levels of serum suPAR derive from the surface of tumour cells, the absence of fragments in serum implies that either uPAR is not fragmented in the tissue or that these fragments are very rapidly eliminated from, or do not enter the circulation. We showed in this study that fragment D2D3 was present in all investigated human ovarian tumour tissues and also in the tumour of mice xenografted with human MDA435 breast carcinoma. Moreover, in ascites of these patients we found full-size suPAR as well as fragment D2D3, indicating that both forms are indeed released. It is unlikely that the fragments do not enter the bloodstream, especially for the small D1 fragment. It is possible that uPAR-fragments are indeed released from the tissue into the circulation but that their half-life in the blood is too short to generate a detectable level. In favour of the latter hypothesis we recently showed that in leukaemia, in which the tumour is in direct contact with the bloodstream, the D2D3 fragment can indeed be identified in the serum (39). The apparent lack of a correlation between levels of D1 and D2D3 fragments in the urine may have several reasons. An excess of D1 may reflect the fact that a great fraction of the D2D3 fragment remains GPI-anchored and thus associated with the cell surface. An excess of D2D3 fragment is more difficult to explain, but may possibly be caused by progressive proteolysis of the D1 fragment and a consequent destruction of the binding epitope for the R3 antibody. A possible explanation for a more stable presence of full-length suPAR in the circulation is that its interaction with other serum proteins may prevent immediate clearance. Two likely ligands for suPAR in the blood are pro-uPA and vitronectin. Association of suPAR with these molecules would bring the molecular weight of the complex well above the limit for simple diffusion into the urinary system. The uPAR fragments are unable to associate with physiological concentrations of uPA and vitronectin (24, 40, 41) and their half-life is therefore not expected to be modulated by these interactions. Off course we cannot exclude that one, or both, of these fragments interact with some, yet unidentified, component(s) of the vasculature thereby lowering the circulating levels to below our detection limit.

Our immunoblot data from healthy volunteers clearly show that in urine most of the suPAR is present as D2D3 fragment. Next to the fact that there appears to be more suPAR in patient’s urine, the most striking difference is that while in healthy volunteers the ratio between full-size suPAR and D2D3 is rather stable, this is not the case in urine from cancer patients. Therefore, changes in the urinary suPAR fragment ratio might reflect the cleavage of uPAR in distant parts of the organism. Our model with xenografted mice shows that in both human tumours suPAR fragments are present in the urine. More importantly, the urinary level of the D1 fragment correlates with the extent of surface uPAR cleavage in the xenografted tumour tissue. This result argues for a similar phenomenon in human cancer patients. Compared to the very regular pattern of suPAR fragments in urine from healthy volunteers, some patients showed relatively high urinary D1 levels.

It cannot be ruled out that in addition to cleavage on the cell surface uPAR might also be fragmented in the soluble state, for example in the kidney or urine. uPA is found ubiquitously and abundantly in all segments of the kidney’s tubular epithelium, where it is thought to prevent fibrin clot formation in the kidney.
The presence of suPAR fragments in human urine suggests that suPAR release and fragmentation may be part of a biologically relevant process. In particular, the cell adhesive/chemotactic activities (48, 49) of these fragments might be indicators of extensive cell migration and hence the enhanced presence of cleaved fragments of suPAR in urine of cancer patients might provide clinically relevant information about the tumour and should therefore be studied in larger groups of patients with various types of cancer.

Acknowledgements
Gunilla Höyer-Hansen, Nils Brünner and Ross Stephens (Finsen Laboratory, Copenhagen) are acknowledged for providing antibodies and the MDA435 xenografts tissue samples. Andrea Mariani, Anna Maria Codecioni and Valter Torri are acknowledged for providing ovarian carcinoma specimens. Vittorio Agape (Department of Clinical Biochemistry, Ospedale San Raffaele, Milan) and Anton de Bart (TNO-PG, Leiden the Netherlands) analyzed respectively the creatinine and uPA activity in the urine samples.

References