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Reduction of breast carcinoma tumor growth and lung colonization by overexpression of the soluble urokinase-type plasminogen activator receptor (CD87)

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The serine protease urokinase-type plasminogen activator, uPA, when bound to its specific receptor, uPAR (CD87), plays a significant role in tumor cell invasion and metastasis. In breast cancer, enhanced uPA antigen in the primary tumor is correlated with poor prognosis of the patient. In an *in vivo* nude mouse model, we tested tumor growth and metastasis of human breast carcinoma cells that had been transfected with an expression plasmid encoding a soluble form of uPAR (suPAR). We explored, whether suPAR/uPA interaction reduces the binding of uPA to cell surface-associated uPAR, and, as a consequence, could suppress tumor growth and metastasis of the human breast cancer cell line MDA-MB-231 BAG. Overexpressed, secreted suPAR was shown to bind and thus scavenge the uPA secreted by the transfected lines suPAR3 and suPAR10. *In vitro*, an overexpression of suPAR did not alter the proliferation rate of the transfected tumor cells, nor did it affect the expression of uPA. Overexpression of suPAR led to a reduction in the plasminogen activation-related proteolytic activity of breast carcinoma cells. Primary tumor growth in the mammary fat pad of nude mice was followed up for 52 days. Overexpression of suPAR correlated with a reduction in tumor growth (from day 21, reaching 30% by day 34) as well as lung colonization (lung metastasis-positive mice in suPAR3: 4 of 17; suPAR10: 3 of 10; parental MDA-MB-231 BAG: 13 of 18). We conclude that suPAR overexpression leading to effective scavenge of uPA impairs proteolysis as well as the tumor growth and metastatic potential of breast carcinoma cells *in vivo*. **Cancer Gene Therapy (2000) 7, 292–299**

Key words: uPA; soluble uPA receptor; CD87; plasminogen; tumor; metastasis; breast cancer.

The ability of breast cancer cells to invade the surrounding tissue and form distant metastases is closely related to their ability to disintegrate components of the surrounding extracellular matrix. Several types of proteases contribute to the degradation of the extracellular matrix, namely, serine proteases (e.g., plasmin, urokinase-type plasminogen activator (uPA)),[1] cysteine proteases (e.g., cathepsins B and L),[2] and matrix metalloproteinases (MMPs).[3] Increased expression of uPA and its membrane-bound receptor (uPAR; CD87) is closely correlated with an increase in disease recurrence and with early death of breast cancer patients.[4] We and others have found previously that uPA as well as uPAR are expressed by breast cancer cells themselves.[5–8] Cell membrane-associated uPAR is a key molecule for the induction of pericellular proteolysis, as plasminogen is efficiently activated to plasmin by cell surface-associated interactions with uPAR-bound uPA.[9] Plasmin is a broad-range serine protease that cleaves a variety of extracellular matrix proteins. In addition, when activated by cell-bound uPA, plasmin may also activate MMPs such as MMP-2 and MMP-9.[10,11] Interference of the membrane-bound function of uPAR should result in a reduction in plasminogen activation, which would decrease tumor cell proliferation, invasion, and metastasis.[12] In this context, it is important to note that the membrane-anchoring of uPAR is not a prerequisite for uPA activation, but is necessary for plasminogen activation.[9,13] In addition to the proteolytic function of uPA, uPA/uPAR interaction induces downstream intracellular signaling, resulting in the induction of cell proliferation, adherence, migration, and chemotaxis.[14–17]

The concept of suPAR serving as a scavenger for pericellular uPA was first studied *in vitro* by Wilhelm et al.[18] It was shown that recombinant suPAR blocked the binding of uPA to cancer cell uPAR and inhibited the *in*

Received March 1, 1999; Accepted April 24, 1999.

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vitro proliferation and Matrigel invasion of an ovarian carcinoma cell line.[18] The aim of the present study was to impair the proteolytic activity of a breast carcinoma cell line by gene transfer and by expression of the suPAR cDNA, and to evaluate the effect of overexpressed suPAR protein on tumor growth and metastatic potential *in vivo*.

MATERIALS AND METHODS

Cell lines and cultures

lacZ-tagged MDA-MB-231 BAG human breast carcinoma cells[19] were grown in Dulbecco's modified Eagle's medium (Life Technologies, Karlsruhe, Germany) supplemented with 10% fetal calf sera (Life Technologies), 1000 U/mL penicillin/streptomycin, and 0.01 M *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid in a 5% CO_2 atmosphere at 37°C.

Vectors and cell line transfection

The cDNA encoding the soluble form of uPAR (suPAR) (amino acids 1–283) was generated by polymerase chain reaction amplification of uPAR cDNA:[20] N-terminal primer, 5′-AATATAAGCTTGAGCTGCCCTCGCGAC-3′; C-terminal primer, 5′-TTTTATCTAGATTACCCACTGCGGTACTG-3′. The amplified sequence was subcloned into the *Hin*dIII/*Xba*I sites of the pRcRSV plasmid (Invitrogen, San Diego, Calif) and verified by sequencing. suPAR was released from the pRcRSV vector by digestion with *Hin*dIII and *Bam*HI and cloned into the polylinker site of the pcDNA3.1/hygro eukaryotic expression vector (Invitrogen). MDA-MB-231 BAG cells were transfected with the resulting vector using lipofectamine™ (Life Technologies) and selected with media containing 250 μg/mL hygromycin (Sigma, München, Germany). Individual clones were isolated and expanded to cell lines, and the amount of suPAR antigen in the media was determined by uPAR enzyme-linked immunosorbent assay (ELISA) (see below).

Zymography

Zymography for gelatinases was performed according to the method of Fridman et al,[21] with some modifications. Briefly, samples of 2×10^4 cells were subjected to 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (nonreducing conditions) with 0.5 mg/mL of gelatin (Sigma) present in the resolving gel. After electrophoresis at 5°C, the gels were washed for 1 hour in 2.5% (vol/vol) Triton X-100 in 50 mM tris(hydroxymethyl)aminomethane (Tris)-HCl (pH 7.5) and subsequently incubated for 24–72 hours at 37°C in 50 mM Tris-HCl (pH 7.5) containing 5 mM $CaCl_2$ and 0.2 M NaCl to allow gelatinases to degrade the gelatin incorporated into the gel. At the end of the incubation, the gels were stained with Coomassie blue and then destained. Recombinant MMP-2 and MMP-9 (Diabor, Oulu, Finland) were used as standards.

uPA and uPAR ELISA

A total of 5×10^5 cells were suspended in 2 mL of media, plated on 35-mm Petri dishes, and incubated for 24 hours. The cell media was removed and centrifuged for 10 minutes at 2000 rpm, 4°C, in an Eppendorf centrifuge; the supernatant was collected for determination of suPAR. The cell pellet of 5×10^5 cells was resuspended, incubated in 500 μL of phosphate-buffered saline (PBS)/1% Triton X-100 overnight at 4°C with gentle shaking, and centrifuged in an Eppendorf centrifuge for 30 minutes at 14,000 rpm. The amount of uPA and uPAR in the cell supernatants and extracts was determined according to the protocols supplied by the manufacturer of the Imubind uPA ELISA kit no. 894 and the Imubind uPAR ELISA kit no. 893 (both from American Diagnostica). For isolation of the soluble form of uPAR, snap frozen (in liquid nitrogen) tissue specimens from mice were pulverized in the frozen state and suspended in Tris-buffered saline. The suspensions were centrifuged in an Eppendorf centrifuge for 30 minutes at 14,000 rpm. The supernatants, containing the soluble but not the membrane-associated form of uPAR were used for the determination of suPAR by the Imubind uPAR ELISA kit. The antibody in the Imubind uPA ELISA kit recognizes free uPA as well as uPA bound to uPAR or suPAR (our data and the product information sheet provided by the manufacturer).

Analysis of uPA/uPAR interaction

For analysis of the ligand-binding capacity of suPAR, a solid-phase uPA ligand-binding assay was used.[22] For this purpose, microtiter plates were coated with recombinant suPAR produced by Chinese hamster ovary (CHO) cells, which served as the capture molecule for uPA. uPA-containing samples from the supernatants of untransfected cells or suPAR-expressing cell lines were added to allow binding of uPA to immobilized suPAR. Supernatants of cells (8×10^5) cultured for 24 hours in 2 mL of media were used. Receptor-bound uPA was detected by biotinylated monoclonal antibody no. 377 directed to the A-chain of uPA (American Diagnostica) followed by an avidin-peroxidase reaction. Purified human amino-terminal fragment (ATF) (American Diagnostica) of uPA was used as a standard and in competition experiments.

Determination of proteolytic activity of living cells

The method applied is based on the use of DQ™-collagen type IV and DQ™-casein (Molecular Probes, Eugene, Ore), which serve as fluorescein-conjugated but fluorescence-quenched protease substrates. On degradation of the substrates by proteases, released fluorescent peptides are quantified by a fluorescence reader (HTS 7000 Bio Assay Reader, Perkin Elmer, Überlingen, Germany) at excitation and emission wavelengths of 492 nm and 535 nm, respectively. The increase in fluorescence is proportional to caseinolytic and collagenase activity (own data and product information sheet, Molecular Probes D-12052). A total of 100 μL of phenol red-free Dulbecco's modified Eagle's medium (Life Technologies) containing either 5 μg of DQ™-collagen or 0.1 μg of DQ™-casein was added to the wells of a 96-well microtiter plate. A total of 2.5×10^5 cells in 100 μL of media were added to each well, and, where indicated, supplemented with 1.8 μg/mL of human plasminogen (Roche Diagnostics, Penzberg, Germany). The plate was incubated at 37°C in 5% CO_2 for 72 hours. The fluorescence of each well was measured in the fluorescence microtiter plate reader every 12 hours.

Mice and tumor cell inoculation

Pathogen-free, female athymic (*nu*/*nu*, CD1) mice of 4–6 weeks of age were obtained from Charles River (Sulzfeld, Germany). For orthotopic primary tumor growth, mice were injected in the mammary fat pad with 2×10^6 parental or transfected cells in 100 μL of PBS. Tumor size was measured twice a week with a caliper and expressed as the mean of the widest and smallest diameters. For induction of experimental metastasis, 1×10^6 cells per 200 μL of PBS were inoculated

Table 1. Secretion of uPAR into the Supernatants of Parental and Six suPAR-Transfected MDA-MB-231 BAG Cell Lines Determined by uPAR ELISA

Tumor cells	uPAR in cell supernatants (ng/10^6 cells ± SD)	Increase (x-fold)
MDA-MB-231 BAG (parental)	3.6 ± 0.6	—
suPAR2	1723 ± 99	478.6
suPAR3	1884 ± 45	523.3
suPAR4	540 ± 35	150.0
suPAR6	52 ± 11	14.4
suPAR10	752 ± 33	208.9
suPAR14	28 ± 8	7.8

into the tail vein of each mouse. Mice were sacrificed at 52 days postinoculation, and tumors, lungs, and liver were removed. Half of the tumors and half of the lobes of lungs and liver were snap frozen in liquid nitrogen for analysis by ELISA. The other halves of the tumors and organs were stained with the β-galactosidase substrate 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal) (Roche Diagnostics) as described previously.[23]

Statistical analyses

For normal distributions, the Student's *t* test was employed. For the data on lung colonization, the normality test failed; therefore, a Kruskal-Wallis one-way analysis of variance on ranks was performed to assess any differences between the groups MDA-MB-231 BAG, suPAR3, and suPAR10. In the case of a statistically significant difference ($P < .05$), pairwise comparisons of groups were done by Dunn's method.

RESULTS

Transfection and isolation of suPAR-overexpressing MDA-MB-231 BAG cells

MDA-MB-231 BAG parental cells were transfected with the pcDNA3.1/hygro/suPAR construct, coding for suPAR, overexpressed under the control of the cytomegalovirus promoter. Cells were selected with hygromycin, and individual clones were isolated, expanded to cell lines, and tested by ELISA for suPAR secretion into the supernatants. The resulting cell lines secreted variable amounts of suPAR (Table 1), ranging from a 7-fold excess to a >500-fold excess over the parental line. For each individual cell line, the detectable amount of suPAR remained stable upon repetitive passaging of the cells (20 passages). For further studies, we used the cell line suPAR3 (expressing an ~500-fold excess of suPAR) and suPAR10 (expressing an ~200-fold excess of suPAR). The doubling rates of suPAR transfectants *in vitro* were unaltered compared with the parental cell line (data not shown).

Secretion of uPA and MMP-2/-9 by MDA-MB-231 BAG breast cancer cells

The secretion of uPA and gelatinases MMP-2 and MMP-9 of MDA-MB-231 BAG breast cancer cells and the suPAR-transfected cells was determined. Approximately equal amounts of uPA were detectable in the supernatants of parental and suPAR-transfected cells by ELISA (Fig 1A). The active and latent forms of MMP-9 and the latent form of MMP-2 were detected in the supernatants of the tumor cells by zymography. Levels in the suPAR transfected cells did not differ from those of the parental line (Fig 1B).

Analysis of the uPA-binding capacity of suPAR

To test whether the overexpressed suPAR is produced and secreted as an active receptor molecule, a solid-phase uPA ligand-binding assay was applied to test for binding of secreted suPAR to uPA.[18] MDA-MB-231 BAG cells secreted relatively high amounts of nonoccupied uPA (~64 ng/10^6 cells), detectable even in the 1/125 dilution of the supernatant in the ligand-binding assay (Fig 2A). Very little uPA binding to solid-phase-associated recombinant CHO-suPAR was detected when supernatants from suPAR3 and suPAR10 were used, strongly indicating that endogenous uPA formed a uPA/suPAR complex in these cases. Next, 1/2 serial dilutions of the supernatants from both suPAR3 and suPAR10 were made, and a fixed amount (0.5 ng/mL) of the ATF of uPA was added (Fig 2B). The supernatant of

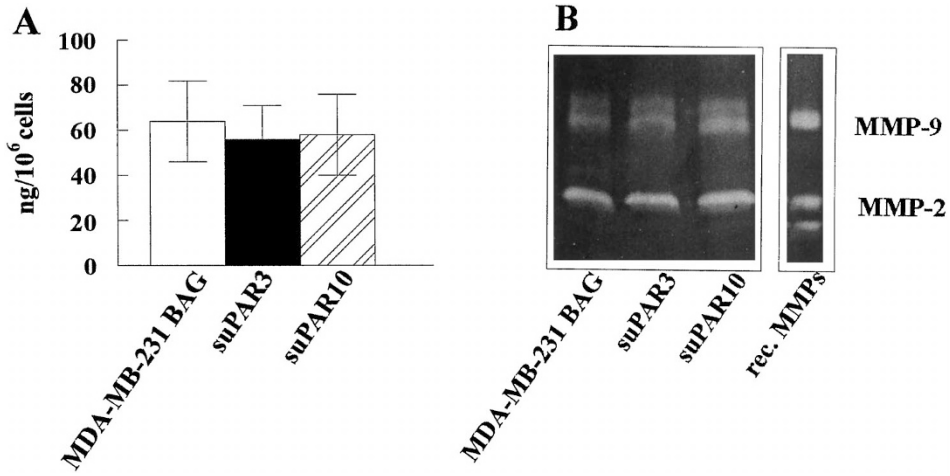


Figure 1. Secretion of uPA and MMP-2/-9 by MDA-MB-231 BAG breast cancer cells. **A:** uPA antigen was determined by ELISA in supernatants of the cells; the SD of three determinations is indicated. **B:** Zymography with supernatants of 2 × 10^4 cells revealed expression of the latent and activated forms of MMP-9 and the latent form of MMP-2. Recombinant MMP-9 and MMP-2 standards (rec MMPs) were used.

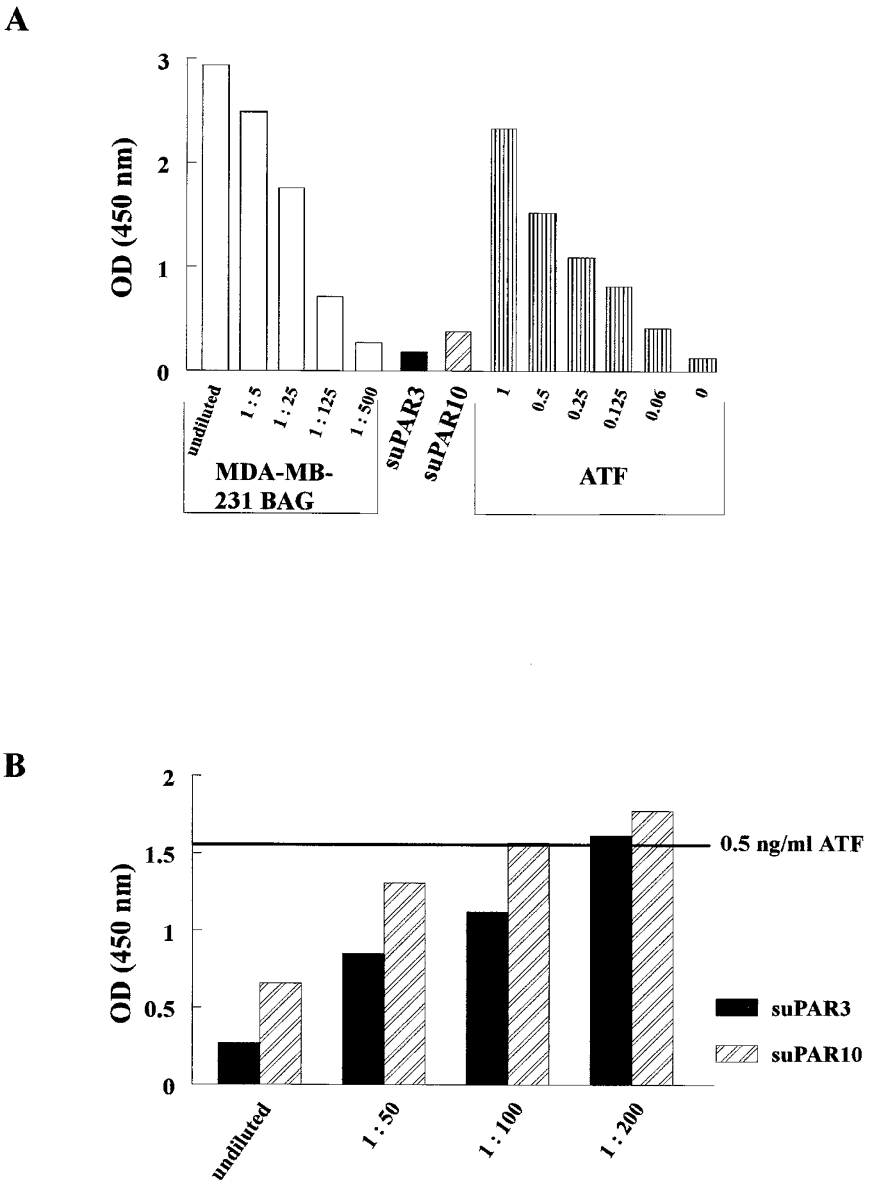


Figure 2. Binding capacity of suPAR secreted by stably transfected MDA-MB-231 BAG cells. **A:** Binding of endogenously synthesized and secreted uPA to suPAR. A total of 100 μL of supernatants of untransfected cells (undiluted and in serial 1/5 dilutions), suPAR3, and suPAR10 (both undiluted) was used in the solid-phase uPA ligand-binding assay. In addition, 100 μL of a solution containing serial 1/2 dilutions (from 1 to 0.06 ng/mL) of ATF was used for comparison; 0 indicates buffer only. **B:** Inhibition of ATF binding to solid-phase-associated CHO-uPAR$_{1-277}$ by suPAR secreted by suPAR3 and suPAR10. ATF was added to dilutions of the supernatants at a final concentration of 0.5 ng/mL. One representative result of several experiments is shown.

suPAR3 displayed competitive binding of ATF up to a dilution of 1/100, and the supernatant of suPAR10 displayed binding up to 1/50, demonstrating that both the suPAR3 and the suPAR10 lines overexpress and secrete active suPAR with uPA-binding potential.

suPAR overexpression leads to reduction of proteolytic activity of breast cancer cells in vitro

We subsequently examined whether the overexpression of suPAR and scavenging of uPA had an effect on the proteolytic activity of living tumor cells. For this purpose, we incubated untransfected MDA-MB-231 BAG cells and the transfected suPAR3 and suPAR10 cell lines with fluorescent DQ^{TM}-casein and DQ^{TM}-collagen type IV substrates (Fig 3A). Overexpression of suPAR led to a 60% (suPAR3) and 56% (suPAR10) inhibition of the proteolytic activity of the tumor cells in the presence of plasminogen (a 100% inhibition was observed in the presence of aprotinin, a serine-protease inhibitor; our unpublished observation). In the absence of plasminogen, the caseinolytic activity of the parental cells was reduced by 70%, and the suPAR-overexpressing lines suPAR3 and suPAR10 showed further reduction by another 48% and 45%, respectively. In addition, we tested for the consequences of affecting the plasmin system with suPAR on the collagenolytic activity of the tumor cells by incubation with fluorescently labeled type IV collagen (Fig 3B). Overexpression of suPAR led to ~30% (suPAR3) and 27% (suPAR10) inhibition of the collagenolytic activity of the tumor cells in the presence of plasminogen. In the absence of plasminogen, collag-

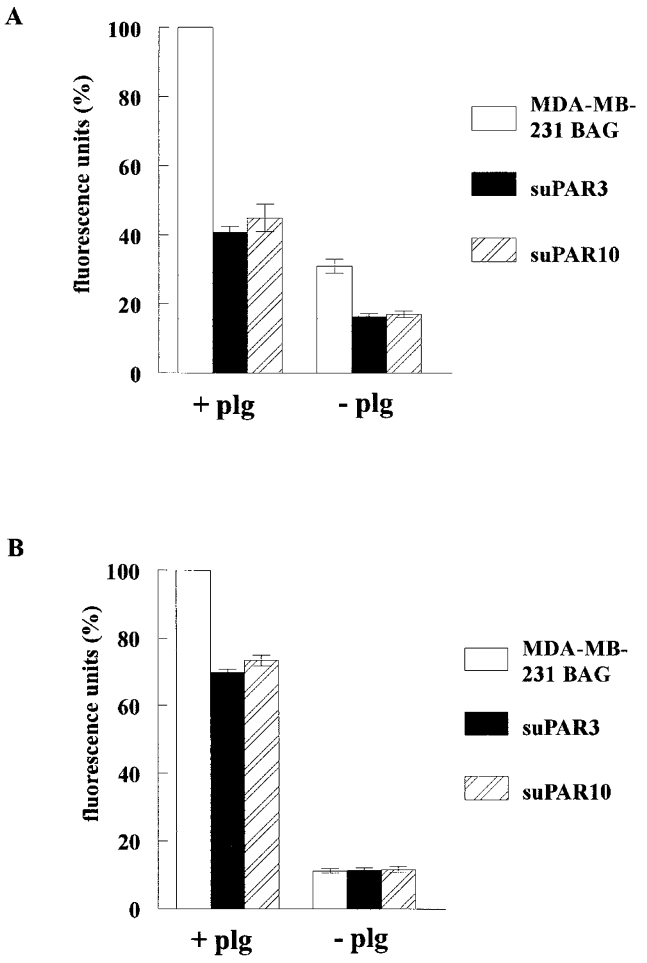


Figure 3. suPAR overexpression leads to reduction of the proteolytic activity of living MDA-MB-231 BAG cells. Fluorescence after a 24-hour incubation of living cells with substrates DQ™-casein and DQ™-collagen type IV of three different experiments, respectively, is shown; SD are indicated. **A:** Caseinolytic activity determined in the presence (+ plg) **(left)** and absence (− plg) **(right)** of plasminogen. **B:** Collagenolytic activity determined in the presence (+ plg) **(left)** and absence (− plg) **(right)** of plasminogen.

enolytic activity was reduced by 90%; further inhibition of collagenolytic activity was not observed with either of the suPAR-overexpressing cell lines.

Overexpression of suPAR by MDA-MB-231 BAG breast cancer cells impairs orthotopic primary tumor growth and metastasis

The effect of suPAR overexpression on primary tumor growth was investigated in an experimental animal model in which human MDA-MB-231 BAG breast cancer cells or the suPAR-overexpressing cell lines were injected orthotopically into the mammary fat pads of nude mice. Two independent sets of experiments were performed. In the initial experiment, mice were inoculated with 2×10^6 parental MDA-MB-231 BAG cells (injected mice: $n = 7$) or suPAR3 cells (injected mice: $n = 7$), respectively. At 30 days after tumor cell inoculation, the tumor diameter was reduced in the suPAR3 group by 40% (4.6 mm ± 0.42 vs. 7.8 mm ± 0.6 in the MDA-MB-231 BAG group); at the end of the experiment (at day 51), tumor diameter was reduced by 21% (7.9 mm ± 1.45 vs. 9.9 mm ± 1.1 in the parental 231 BAG group).

In the second experiment, we also included the suPAR10 cell line to determine whether the ~2.5 times lower expression level of suPAR compared with suPAR3 would have an effect *in vivo*. After a latency period of 10 days, tumor diameters could be determined in all injected mice (Fig 4). Overexpression of suPAR inhibited the orthotopic primary tumor growth of breast cancer cells; this inhibition was visible starting at day 21 (a 13% and 19% reduction in mice with suPAR3 and suPAR10, respectively), reaching 30% reduction for suPAR3 and suPAR10 at day 35. At the end of the experiment (day 52), a reduction in primary tumor growth of 39% (suPAR3) and 24% (suPAR10) was observed (Fig 4). Examination of tumor biopsies by uPAR ELISA at the end of the experiment revealed highly elevated uPAR antigen levels in the tumor specimens of suPAR3 (280.9 ± 76.6 ng/mg total protein ($n = 9$) versus 1.46 ± 0.3 ng/mg total protein ($n = 7$) in tumors derived from parental MDA-MB-231 BAG), confirming suPAR overexpression within the tumor. X-Gal staining of lungs and liver did not reveal any spontaneous metastases at the endpoint of the experiments (52 days after tumor cell inoculation). There was no histological difference between control and suPAR-expressing tumors.

We investigated the effect of suPAR overexpression on lung colonization by an experimental metastasis assay. Tumor cells were inoculated into the tail vein to generate lung metastases. Lungs were isolated and stained with X-Gal at 52 days postinoculation (to visualize the *lacZ*-tagged MDA-MB-231 BAG tumor cells[15] and the transfected cell lines), and metastases were counted. suPAR-overexpressing tumor cells colonized the lung less often (lung metastasis-positive mice in suPAR3: 4 of 17 (i.e., 24%); suPAR10: 3 of 10 (i.e., 30%)) than parental cells (231 BAG: 13 of 18 (i.e., 72%)). The Kruskal-Wallis test for nonuniform distribution was applied, and the three groups were found to be significantly different ($P = .0032$) (Fig 5). The suPAR3 ($P < .05$) and suPAR10 ($P < .05$) groups were significantly different from the parental cell control as tested by a nonparametrical pairwise comparison procedure (Dunn's method).

We performed an ELISA of lung extracts of the suPAR3 group (bearing 26 metastases) and found elevated suPAR levels (27.06 ng/mg total protein) compared with lungs bearing 30 metastases from mice of the MDA-MB-231 BAG control group (0.32 ± 0.06 ng/mg total protein, $n = 3$). No liver metastases were found in either group of mice.

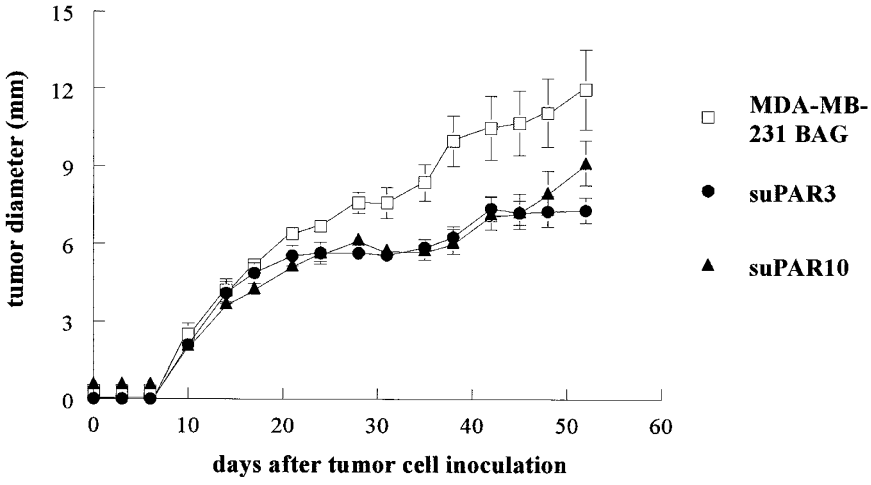
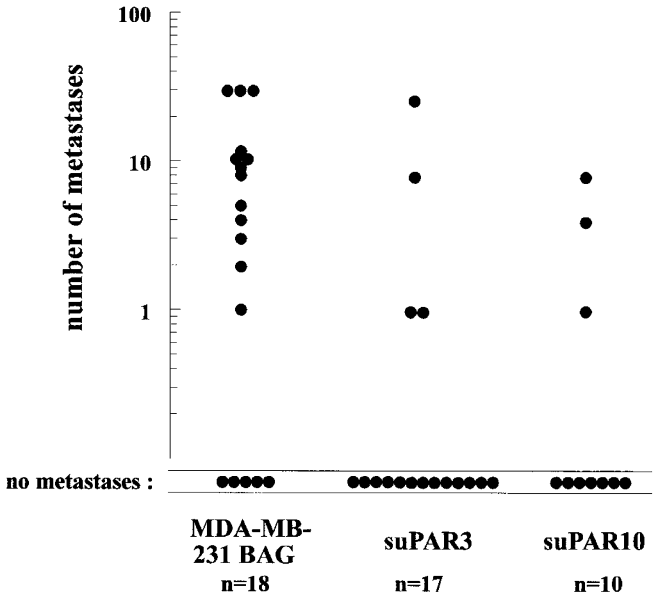


Figure 4. Overexpression of suPAR impairs orthotopic primary tumor growth. The tumor growth kinetic of the second experiment, including parental MDA-MB-231 BAG cells (n = 7 mice) and the transfected cell lines suPAR3 (n = 9) and suPAR10 (n = 9), is shown. Values are expressed ± SD.

DISCUSSION

In the present study, we show for the first time the reduction of orthotopic primary tumor growth and lung colonization *in vivo* of a human breast carcinoma cell line engineered to overexpress suPAR. The concept of impairing the function of a tumor-associated factor by overexpression of a soluble form of its receptor was



Kruskal-Wallis-test:

	Median	25 % quartile	75 % quartile	
MDA-MB-231 BAG	4.5	0	11.0	p = 0.0032
suPAR3	0	0	0.25	
suPAR10	0	0	1.0	

Dunn's method:

suPAR3 *vs* MDA-MB-231 BAG: $p < 0.05$

suPAR10 *vs* MDA-MB-231BAG: $p < 0.05$

Figure 5. Effects of suPAR overexpression on experimental lung metastasis. Numbers of X-Gal-stained metastases were plotted on a logarithmic scale. Each point represents the result from half a lung of each mouse. The table indicates the results of the statistical analyses.

recently used by Goldman et al.[24] They demonstrated inhibition of tumor growth and metastasis by overexpression of the soluble vascular endothelial growth factor receptor sFLT-1 in human fibrosarcoma cells. Scavenging of uPA by suPAR interferes with the binding of uPA to its cellular receptor and leads to a reduction in the proteolytic activity of breast carcinoma cells and to a reduction in the orthotopic tumor growth and lung colonization of these tumor cells *in vivo*. So far, experimental[4,25–28] and also preliminary clinical approaches[29] to achieve inhibition of the proteolytic activity of tumor cells to inhibit tumor progression have concentrated on the use of natural[25–27] or synthetic[4,28,29] inhibitors that are capable of interacting directly with the enzymatic center of the proteases.

Our *in vitro* experiments indicate that both the receptor-binding capacity of uPA and its ability to induce protease-mediated extracellular matrix degradation were effectively scavenged by overexpression of suPAR, which was secreted by the stably transfected breast carcinoma cells suPAR3 and suPAR10. Antibodies against uPA and uPAR were also used with MDA-MB-231 BAG cells, and were shown to inhibit *in vitro* invasiveness.[30]

Although the expression levels of the endogenous serine protease uPA and MMP-2 and MMP-9 were not affected by the expression of suPAR and expression of the plasminogen-activator inhibitor-1 and tissue inhibitors of metalloproteinases was unaltered in suPAR3 and suPAR10 compared with the parental line (data not shown), the plasminogen-dependent proteolytic activity of these cells toward collagen type IV or casein was effectively impaired by suPAR. In the presence of exogenously added plasminogen, interaction of uPA with suPAR reduced plasmin-mediated casein degradation by half and reduced the collagenolytic activity of the cells as well. These observations are in agreement with findings of others that cell surface-bound plasmin may degrade collagen type IV.[31] Moreover, the lack of plasmin activity or uPA/plasminogen binding to the cell may inhibit the activation of MMP-2 and MMP-9.[10,11,32] The residual caseinolytic and collagenolytic activities are most likely not a consequence of inefficient scavenging of uPA by suPAR, because we have shown that all of the endogenous uPA produced by the parental MDA-MB-231 BAG tumor cells was bound to suPAR in the suPAR-overexpressing lines (Fig 2A).

Although administration of a rather high amount of recombinant suPAR on human ovarian carcinoma led to an inhibition of cell proliferation,[18] suPAR-transfected MDA-MB-231 BAG breast cancer cell proliferation was not affected. Nevertheless, at the level of suPAR achieved in our model, tumor growth was reduced. This may indicate that the tumor growth of MDA-MB-231 BAG cells in nude mice is not only proliferation-dependent but could also involve other mechanisms (e.g., impact on cell adhesion, migration, chemotaxis, proteolysis, and signal transduction).[16]

suPAR-overexpressing breast carcinoma cells colonized the lungs of nude mice less efficiently than the parental cells, also pointing to the fact that the uPA/uPAR system is not only involved in tumor-associated proteolysis but also in cell/matrix interaction and receptor-integrin crosstalks.[33] The lungs of mice inoculated with suPAR transfectants displaying metastases still exerted increased levels of suPAR, indicating that suPAR overexpression *per se* is not a required to completely block the adhesion and growth of breast cancer cells in the lungs.

suPAR10 cells secreted <40% of the amount of soluble receptor secreted by suPAR3 cells; nevertheless, the two lines were indistinguishable with regard to the reduction in tumor growth and lung colonization in the nude mouse. Even lower amounts of suPAR may be sufficient to impair tumor spread.

The present study provides "proof of principle" for the use of overexpressed suPAR cDNA in tumor inhibition. The concept of introducing and overexpressing suPAR could be applied for the patient's benefit, provided efficient viral or nonviral gene delivery vectors are developed. Then transduction of primary tumors, tumor-adjacent stroma cells, or target organs of metastasis with suPAR cDNA may be a feasible gene therapy approach to inhibit tumor growth and metastatic dissemination in human breast cancer.

ACKNOWLEDGMENTS

We thank Dr. R. Senekowitsch-Schmidtke (Munich, Germany) for allowing us to perform the animal studies in her facility, Dr. U. Kliebsch (Munich, Germany) for expert help in the statistical analysis, and Dr. N. Brünner (Copenhagen, Denmark) for the generous gift of the MDA-MB-231 BAG cell line. We also thank S. Baur and K. Honert for their expert technical assistance. This work was supported by Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie (BMBF)/Deutsche Forschungsanstalt für Luft und Raumfahrt e. V. (DLR) Grant GF-GFGE01-01GE96194 (Somatic Gene Therapy).

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