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# Binding of Tissue-Type Plasminogen Activator to Human Melanoma Cells

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**Abstract** We have shown (Bizik et al., Cell Regul 1:895–905, 1990) that tPA can activate plasminogen on the surface of human melanoma cells in the presence of $\alpha_2$-macroglobulin ($\alpha_2$M) secretion. In the present study, we investigated the binding of tPA on the surface of Bowes melanoma cells, selected since they lacked production of PAI-1 and $\alpha_2$M. Elution of tPA from the cell layers indicated that polylysine (5 μg/ml) and tranexamic acid (10 mM), an analog of lysine, were the most efficient agents for disrupting the interaction between tPA and cell surface component(s). Using a panel of monoclonal antibodies against individual domains of tPA revealed that an antibody directed to the kringle-2 domain of tPA interfered most significantly with cell-surface plasmin generation. As tPA is a glycoprotein, interactions between the tPA sugar moieties and cell surface were also tested by the use of a series of monosaccharides. N-acetyl-D-glucosamine (100 mM) was the most potent sugar to release tPA from melanoma cells, but the results indicated that the oligosaccharides of tPA play only a supportive role in the binding of tPA to the cell surface. Quantitative comparison of the cell surface localized tPA, which was eluted by tranexamic acid, with the total cellular tPA showed that cell surface bound tPA could represent up to 10%. We conclude that tPA interacts with the melanoma cell surface in a similar manner as has been described for binding of tPA to fibrin and to the putative endothelial cell surface receptor. 

**Key words:** cell surface, lysine, melanoma, receptor, tissue-type plasminogen activator

Human tissue-type plasminogen activator (tPA), a glycoprotein with a molecular weight of about 70,000 (Danø et al., 1985; Pöllänen et al., 1991), is a key physiological regulator of fibrinolysis (Collen, 1980). It converts the zymogen plasminogen into plasmin, a proteolytic enzyme of broad substrate specificity.

The tPA synthesized by endothelial cells and secreted into the bloodstream is in a single-chain form (Binder et al., 1979; Rijken et al., 1980). This form is cleaved by plasmin into the two-chain form, consisting of an amino-terminal heavy chain and a carboxyterminal light chain held together by a disulfide bond (Rijken et al., 1982). The heavy chain is composed of distinct structural and functional domains (see Fig. 1) that have homology to other plasma proteins (Patthy, 1985). From the amino terminus, a "finger" domain may be distinguished, homologous to the finger structure in fibronectin, next an EGF-like domain homologous to the epidermal growth factor (Banyai et al., 1983), followed by two so-called kringle structures, kringle-1 and kringle-2, homologous to structures in plasminogen, urokinase and prothrombin (Sottrup-Jensen et al., 1975). The light chain contains the trypsin-like serine protease moiety that interacts with the substrate plasminogen (MacDonald et al., 1986) showing varying degrees of homology with numerous other serine proteases (Steffens et al., 1982; Patthy, 1985). This part of the molecule also constitutes the major target for the specific plasminogen activator inhibitor PAI-1 (Van Zonneveld et al., 1986a).

The function of tPA is related primarily to intravascular thrombolysis, whereas urokinase is thought to be involved in the generation of pericellular proteolysis during cell migration and tissue remodeling (Danø et al., 1985; Pöllänen et al., 1991). According to studies on cultured cells, human tumor cells of different histological ori-

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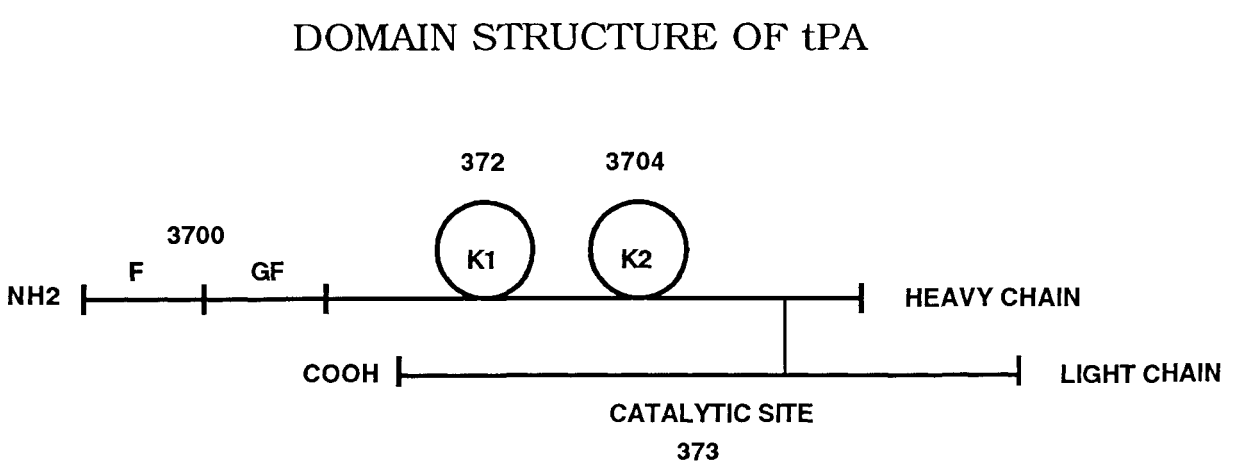


**Fig. 1.** Schematic diagram of the domain structures of tPA. F, finger domain; GF, growth factor domain; K1, K2, two kringle domains, and the catalytic site in the light chain. The numbers refer to the monoclonal antibodies with specificities as shown.

gin differ in the mechanism of pericellular plasmin activation (Vaheri et al., 1992). Most types of human adherent tumor cells use urokinase to generate cell-bound plasmin (Stephens et al., 1989). Human melanoma (Rijken and Collen, 1981; Hoal-Van Helden et al., 1986; Bizik et al., 1990) and neuroblastoma cells (Neuman et al., 1989) form an exception among adherent human tumor cells in that they synthesize and secrete predominantly tPA. Our previous study showed that tPA can generate plasmin bound to the surface of melanoma cells (Bizik et al., 1990). These findings prompted us to characterize the interaction of tPA with the melanoma cell surface.

We now present evidence that the kringle-2 domain of tPA may play a dominant role in the interaction of the enzyme with pericellular protein(s) on melanoma cells. The interaction of tPA with the melanoma cell surface was inhibited by polylysine and tranexamic acid, indicating a role for protein(s) with appropriately presented lysine.

## MATERIALS AND METHODS

### Cell Cultures

The human melanoma lines $M_3$Dau, $M_1$Do, $M_4$Beu, and $M_2$Ge were originally provided by Dr. J.F. Doré, Centre Leon Berard, Lyon, France (Jakubovich et al., 1985). The Bowes cell line was a gift from Dr. D. Collen, Katholieke Universiteit, Leuven, Belgium. The melanoma cell line HMB-2 was kindly provided by Dr. J. Svec, Cancer Research Institute, Bratislava, Czechoslovakia (Siracky et al., 1982). The human HT-1080 fibrosarcoma cell line was obtained from the American Type Culture Collection (Rockville, MD). All these cell lines were monitored for mycoplasma contamination and were found to be negative. The cultures were grown in Eagle's minimal essential medium (MEM), supplemented with 5% FCS and antibiotics and were subcultured weekly by brief exposure to 0.25% trypsin/0.02% EDTA. The Bowes cells were continuously cultured in serum-free MEM (Kruithof et al., 1985; Dr. E.L. Wilson, New York University, New York, personal communication), as indicated in the experiments.

### Materials

Reagents and their sources were tranexamic acid (Cyklokapron, KabiVitrum, Stockholm, Sweden), poly-D-lysine hydrobromide ($M_r$ 150,000–300,000), heparan sulfate (Na salt, from bovine kidney), protamine chloride, poly-L-arginine hydrochloride ($M_r$ 70,000–150,000; Sigma, St. Louis, MO), dextran sulfate $M_r$ 500,000 (Pharmacia, Uppsala, Sweden).

Monoclonal IgG antibodies against tPA were obtained from American Diagnostica (Greenwich, CT): anti-finger/growth factor domain (#3700), anti-kringle-1 domain (#372), anti-kringle-2 domain (#3704), and anti-protease region domain (#373).

The sugars used were: L-fucose, D-mannose (Sigma), N-acetyl-D-galactosamine (Aldrich-Chemie, Steiheim, Germany), and N-acetyl-D-glu-

cosamine (Janssen Pharmaceutica, Beerse, Belgium).

### Assay of tPA

Cell culture supernatants or cell lysates were assayed for tPA activity by a modification of an immunocapture method (Stephens et al., 1987) described elsewhere (Bizik et al., 1990). Briefly, microtiter wells of polystyrene immunoplates (Nunc, Roskilde, Denmark) coated with a solution of goat IgG to human tPA (#387; American Diagnostica) were treated with the test sample (50 μl). To assay the enzyme activity bound to the wells, 40 μl of a solution of plasminogen (Deutsch and Mertz, 1970) 50 μg/ml in an assay buffer containing 50 mM Tris–HCl, pH 7.8, 60 mM NaCl, 0.01% Triton X-100, and 20 μg/ml poly-D-lysine was added per well. The plasmin produced was then assayed by its thioesterase activity (Green and Shaw, 1979) after the addition of 150 μl of a solution containing 200 mM potassium phosphate, pH 7.5, 200 mM KCl, 0.1% Triton X-100, and 200 μM Z-lysine thiobenzyl ester (Peninsula Laboratories, San Carlos, CA). The absorbances of the wells were measured in a Labsystems Multiskan MCC/340 plate reader at 405 nm. A standard of two-chain tPA (700,000 IU/mg) was obtained from American Diagnostica.

### RNA Hybridization and DNA Probe

Total cellular RNA was extracted using the urea-LiCl method (Auffray and Rougeon, 1980), poly(A)$^+$ RNA was selected by oligo(dT)-cellulose (Type 77F; Pharmacia), electrophoresed in 1% agarose gels containing 2.2 M formaldehyde, transferred to nitrocellulose (Schleicher & Schuell, Dassel, Germany), and hybridized to $^{32}$P DNA probes according to established procedures (Sambrook et al., 1989). When the hybridization was completed, the filters were washed under nonstringent (1 × NaCl/citrate, 0.5% SDS, +65°C) and/or stringent (0.1 × NaCl/citrate, 0.5% SDS, 65°C) conditions. Filters were exposed to Kodak X-Omat AR film at −75°C. The DNA probe was labeled by nick translation (Sambrook et al., 1989) using a nick-translation kit (Amersham, Arlington Heights, IL). The PAI-1 probe used in this study represented the Bam HI fragment (Andreasen et al., 1986). It was a kind gift from Dr. Leif Lund of the Finsen Institute, Copenhagen, Denmark.

### Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Samples dissolved in 12.5 mM Tris-HCl, pH 6.8, containing 4% SDS, 20% glycerol, 10% 2-mercaptoethanol and 0.005% bromphenol blue were heated for 2 min in boiling water and analyzed by the use of vertical 9% polyacrylamide slab gels with a 3.5% acrylamide spacer gel as described (Laemmli, 1970).

### Zymography

After electrophoresis in 9% polyacrylamide slab gels under nonreducing conditions, and without heating of the specimens, SDS was removed from the gels by extensive washing on a gyratory shaker for 6 h in PBS with 2.5% Triton X-100 (Granelli-Piperno and Reich, 1978), and an indicator agarose gel containing casein and plasminogen was placed in contact with each polyacrylamide gel. An agarose gel lacking plasminogen was used as a control, and the gel sandwich was incubated overnight at 37°C in a humidified atmosphere to develop lysis zones. The size of the lysis zones was quantified by densitometric scanning of the photograph taken with dark ground illumination with an attachment to a Beckman 2202 Ultroscan laser densitometer.

### Immunoblotting

The procedure was as described previously in detail (Bizik et al., 1986). Aliquots of serum-free conditioned media were frozen immediately and lyophilized. Then the samples were dissolved in sample buffer for SDS-PAGE and separated as described above. After separation, the proteins were transferred electrophoretically to nitrocellulose sheets (Schleicher-Schuell) (Towbin et al., 1979). For immunological staining of the transferred proteins, a rabbit antiserum to tumor-associated $\alpha_2$M (Bizik et al., 1986) was used at a dilution of 1:1,000 and, after binding of peroxidase-conjugated swine anti-rabbit IgG (DAKO, Copenhagen, Denmark), at a dilution of 1:200, the bands were visualized using 3,3′-diaminobenzidine tetrahydrochloride (Sigma) as substrate. To estimate the concentration of $\alpha_2$M in analyzed samples, standards of commercially available $\alpha_2$M (Sigma) were run simultaneously on the gels. The intensity of the corresponding bands were quantified by densitometric scanning using a Beckman 2202 Ultroscan laser densitometer.

For immunoblotting analysis of PAI-1, rabbit anti-human melanoma PAI-1 (#395R; American Diagnostica) was used at a dilution of 1:500. PAI-1 isolated from a cell line (#105; American Diagnostica) was used as a positive control.

## RESULTS

### Tissue-Type Plasminogen Activator and PAI-1 Production in Melanoma Cell Cultures

All the melanoma cell lines analyzed in our study constantly secreted tPA at rates characteristic of each cell line (Bizik et al., 1990). The rate at which tPA accumulated in the conditioned medium was not linearly related to the number of cells in the microplate wells (Fig. 2). As cell densities of $M_3$Dau, $M_2$Ge, $M_1$Do, $M_4$Beu, and HMB-2 cell lines increased, so the rate of accumulation of enzyme per $10^6$ cells exponentially decreased. The Bowes cell line was an exception in our set, as the cells showed only a very slight decrease in the rate of tPA accumulation with increased cell densities.

Simultaneously measured values of $\alpha_2$M, another characteristic protein secreted by human melanoma cells (Bizik et al., 1989; 1990), showed a linear decline with cell densities. This effect was not seen in the case of Bowes cells, which did not produce this wide spectrum proteinase inhibitor (Fig. 2). PAI-1, primarily a tPA inhibitor, is produced by many types of cultured cells, including some melanoma cells (Wagner and Binder, 1986). It has also been established that the interaction of PAI-1 with tPA occurs on the surface of endothelial cells (Sakata et al., 1988). In order to evaluate the presence of PAI-1 in our selection of melanoma cell lines, mRNAs from all cells were analyzed by Northern hybridization using a human PAI-1 cDNA. None of the cell lines analyzed expressed detectable PAI-1 transcripts (Fig. 3). PAI-1 transcripts of 2.4 kb and 3.2 kb were found however in the HT-1080 fibrosarcoma cell line used as a positive control (Fig. 3, lane 7). The result of Northern blot analyses were confirmed by immunoblotting of melanoma cell conditioned media. As can be seen in Figure 4, the cell lines used in our study did not secrete detectable amounts of PAI-1.

Based on these preliminary results, we took advantage of the Bowes cell line, and used it in the following studies, since (1) the amount of secreted tPA was related to cell numbers, and (2) the cells did not produce $\alpha_2$M or PAI-1, which could interfere with the activity of tPA and plasmin on the cell surface. The Bowes cell line exhibited the same characteristics of tPA production when cultured continuously in a completely serum-free medium (data not shown).

### Effect of Polyionic Substances and a Lysine Analog on the Interaction of tPA With Bowes Melanoma Cell Surface

tPA is a basic protein with an isoelectric point of 8 (Binder et al., 1979), containing 122 charged amino acids (Bennett et al., 1991). It was therefore reasonable to analyze the ionic interactions of tPA with the components of the melanoma cell surface. Both types of charged polymers, polyanions and polycations were tested. The polyanion dextran sulfate (40 μg/ml) did not affect surface-bound tPA (Fig. 5). Two different polycations, polyarginine and protamine chloride (40 μg/ml), did not release significant amounts of tPA from the cell surface. However, by contrast, polylysine (5 μg/ml), i.e. at a concentration 8 times lower, released nearly 3-fold the amount of tPA compared to control treatment with buffer. A similar amount of tPA was eluted from the cell surface of Bowes cells by 10 mM tranexamic acid, an analog of lysine. These results indicate that binding of tPA to the surface of melanoma cells involves a lysine binding site.

In these experiments highly charged polyionic substances were present in the samples tested by immunocapture assay; it was therefore necessary to verify that these substances did not interfere in the actual assay. The appropriate concentrations of the agents (see Fig. 5) were therefore added to aliquots of Bowes cells conditioned media and the tPA activity analyzed by immunocapture assay. All samples exhibited slightly decreased tPA activity (up to 10%) as compared to the control sample (Fig. 6). However, the results indicated that polyionic substances did not influence significantly the assay used for screening of tPA activity and the differences detected among polyionic substances as shown in Figure 5 really reflect the effect of the agents on cell surface-bound tPA.

### Effect of Sugars on the Binding of tPA to the Surface of Bowes Melanoma Cells

Melanoma-derived tPA contains a high mannose-type oligosaccharide in kringle 1, a complex-type in kringle-2 and another complex-type oligosaccharide in the protease domain (Pohl et al., 1987; Parekh et al., 1989; Spellman et al., 1989). Mannose and galactose have been reported to diminish the rate of clearance of tPA from the

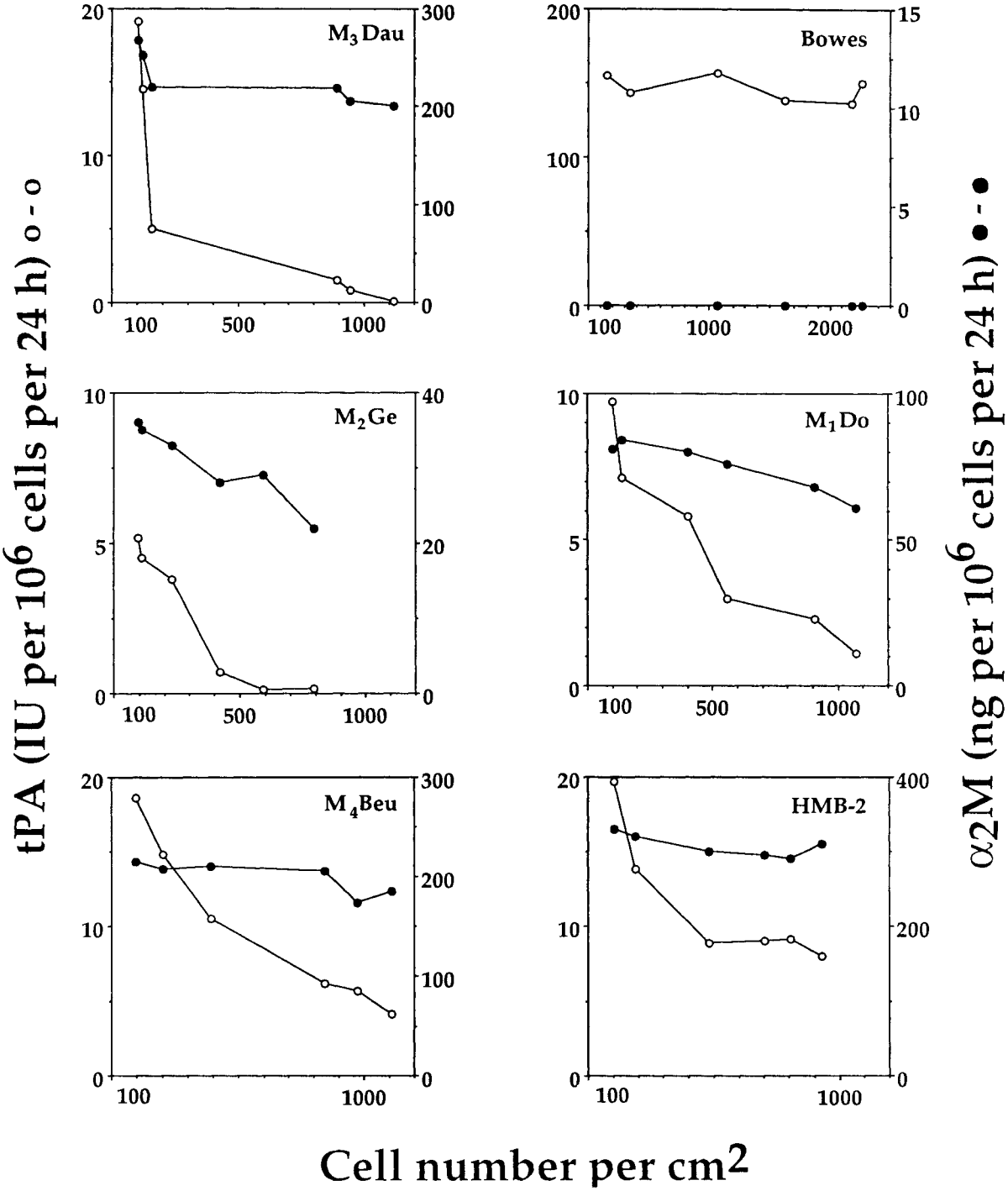


**Fig. 2.** Tissue-type plasminogen activator and $\alpha_2$M secretion as a function of cell density in cultures of six human melanoma cell lines At the cell densities indicated, replicate cultures were used to collect serum-free harvest fluids from 24 hr of incubation Plasminogen activator and $\alpha_2$M measurements were then carried out as described in Materials and Methods The cells were harvested by trypsinization for counting Similar results were obtained in three separate experiments

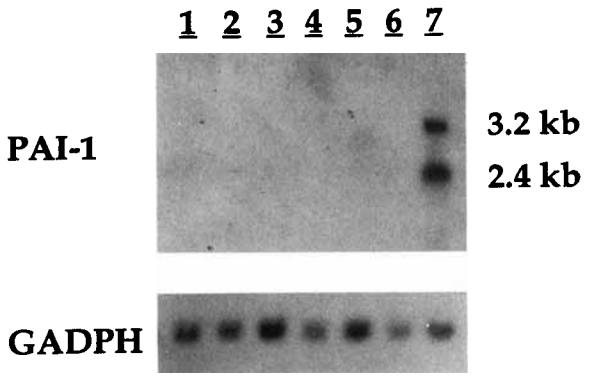


**Fig. 3.** Northern blot analysis of melanoma mRNAs probed with the cDNA for human PAI-1 Ten micrograms of poly(A)$^+$ RNA from the melanoma cell lines 1, $M_3$Dau, 2, Bowes, 3, $M_2$Ge, 4, $M_1$Do, 5, $M_4$Beu, 6, HMB-2, and 7, HT-1080 fibrosarcoma cell line were size-fractionated on agarose gels, blotted to a nitrocellulose membrane filter and hybridized to $^{32}$P-labeled PAI-1 cDNA probe Hybridization ($10^6$ cpm/ml) was performed in 50% formaldehyde, 5× SSC, 5 mM EDTA, 0 5% SDS, 1× Denhardt's, 250 μg/ml denatured salmon sperm DNA at 42°C overnight The filter was then washed in 1× SSC, 0 5% SDS at 65°C and subsequently in 0 1× SSC, 0 5% SDS at 65°C The specificity of the hybridization was confirmed using HT 1080 mRNA as a positive control The amounts of mRNAs loaded on each lane were controlled by rehybridization of the same blot with a cDNA probe specific for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) Ribosomal RNAs were separated on the same gel and were used as markers

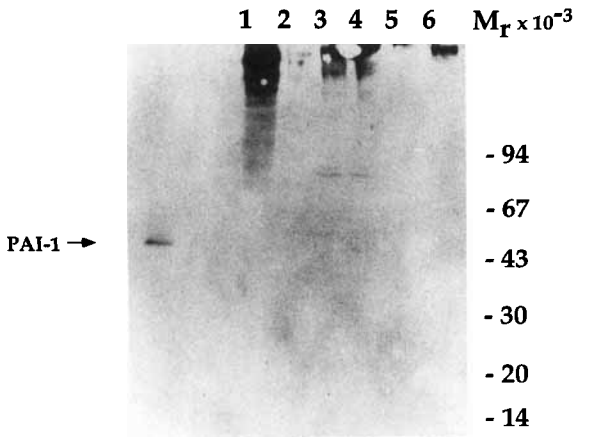


**Fig. 4.** Immunoblotting analysis of melanoma cell conditioned media using an anti-PAI-1 antibody One milliliter aliquots of conditioned medium, corresponding to 5× $10^5$ cells for each cell line were frozen and lyophilized Samples were dissolved in sample buffer and run on SDS–PAGE (5–15% acrylamide) After separation the proteins were transferred electrophoretically to nitrocellulose and stained with the antiserum to PAI-1 Staining was visualized by incubation with peroxidase–conjugated secondary antibodies followed by enzyme substrate The arrow indicates the position of commercial PAI-1 (2 μg) run in the same gel

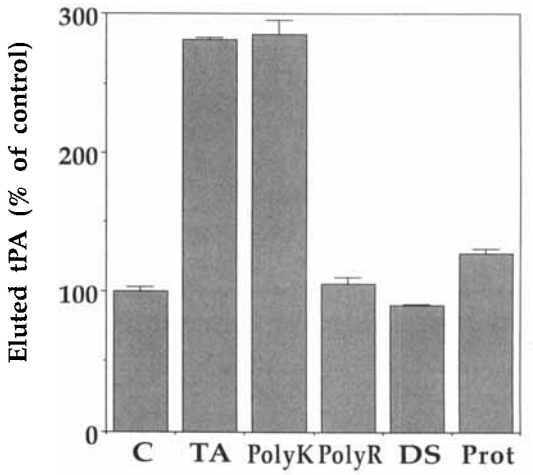


**Fig. 5.** Elution of tPA from the surface of melanoma cells by polyionic substances and a lysine analog Monolayers of Bowes cells cultivated in serum-free medium were incubated for 30 min at 37°C with the following substances in Dulbecco's PBS and the eluted tPA was assayed by immunocapture activity assay Reagents used tranexamic acid (TA, 10 mM), polylysine hydrobromide (polyK, 5 μg/ml), polyarginine (polyR, 40 μg/ml), dextran sulfate (DS, 40 μg/ml), protamine chloride (Prot, 40 μg/ml) The result for each agent is expressed as the percentage of the value obtained with control-PBS incubated cells

circulation and interfere with the binding of tPA to liver endothelial and Kupffer cells (Smedsrod and Einarsson, 1990). To establish whether the binding of tPA to Bowes cells was dependent on its carbohydrate moieties, the effect of four different monosaccharides was investigated. Figure 7 shows that N-acetyl-D-glucosamine was the most potent agent, L-fucose and D-mannose moderate ones, and N-acetyl-D-galactosamine the least potent to release cell surface-bound tPA. However, the most potent sugar, i.e., N-acetyl-D-glucosamine, still released only 25% more tPA than the control buffer. Clearly, these agents were considerably less potent than polyionic substances.

### Influence of Monoclonal tPA Antibodies on Plasminogen Activation at the Surface of Melanoma Cells

Four monoclonal antibodies against human tPA were used to probe the involvement of individual domains of tPA in the process of plasminogen activation on the surface of Bowes cells. The antibodies used had epitopes in the finger/epidermal growth factor, the kringle-1, the kringle-2, and the protease catalytic domains, respectively (Fig. 1). It is apparent from the results

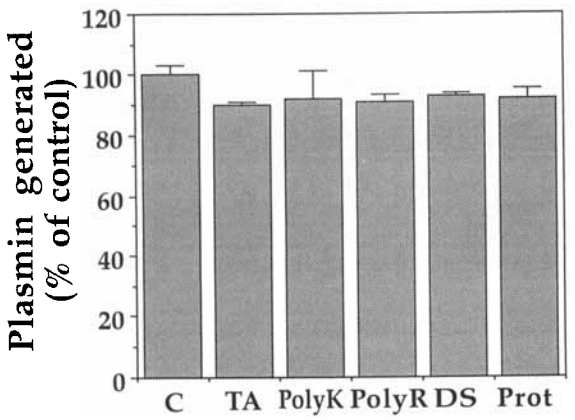


Fig. 6. Testing for interference of polyionic substances and a lysine analog in the immunocapture activity assay of tPA Conditioned medium of Bowes cells cultivated in serum-free medium was aliquoted and the indicated agents were added to the final concentration as used in the experiment shown in Fig 5 Samples were tested by immunocapture activity assay The results for individual aliquots with each agent is shown as a percentage of the control sample

(Fig. 8) that there was little or no effect on plasmin generation in cell cultures in the presence of antibodies directed either to the finger/epidermal growth factor or the kringle-1 domain. The antibody against the protease catalytic domain of tPA only moderately reduced plasminogen activation (by 15%). The most marked decrease in plasminogen activation (by 60%) was in cell cultures treated with the antibody

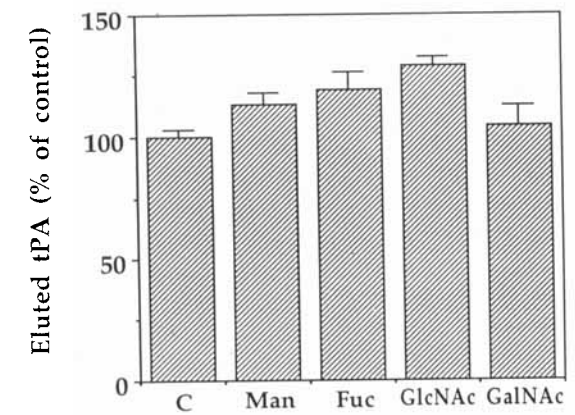


Fig. 7. Elution of tPA from monolayers of melanoma cells by various monosaccharides Bowes cells cultivated in serum-free medium were incubated for 30 min at 37°C with the following sugars in Dulbecco's PBS and released tPA was assayed by immunocapture activity assay Sugars used D-mannose (Man, 100 mM), L-fucose (Fuc, 100 mM), N-acetyl-D-glucosamine (GlcNAc, 100 mM), N-acetyl-D-galactosamine (GalNAc, 100 mM) Results are expressed as a percentage of the tPA released from monolayers by PBS

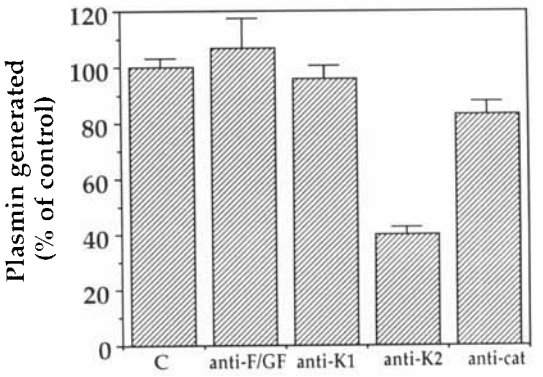


Fig. 8. Effect of monoclonal antibodies to various domains of human tPA on plasminogen activation on the surface of Bowes cells Monolayers of Bowes cells cultivated in serum-free medium were preincubated for 18 hr with different antibodies to human tPA (10 μg/ml) and the same amounts of antibodies were added again 2 hr before plasminogen was added to the cultures Plasminogen activation on preincubated cells was measured by addition of purified human plasminogen (50 μg/ml) for 30 min, followed by washing with serum-free medium The cell-bound plasmin was eluted with 1 mM tranexamic acid and the activity of released plasmin was measured by its thioesterase activity The effect of various antibodies to tPA is given as the percentage of activity obtained in a control culture in which no antibody was added C, no antibody, anti-F/GF, antibody to finger/growth factor domain, anti-K1, antibody to kringle-1 domain, anti-K2, antibody to kringle-2 domain, anti-cat, antibody to catalytic (protease) region

against the kringle-2 domain. The results suggest that the kringle-2 domain of tPA has a key role in facilitating the process of plasminogen activation on the cell surface.

### Analysis of tPA Distribution in Bowes Cells

In this experiment we addressed the question of how much tPA is elutable from the cell surface by 10 mM tranexamic acid as compared to the total cellular content of the enzyme. The pattern of zymographic analysis and densitometric screening of the zymogram is shown in Figure 9. The amount of tPA released by tranexamic acid from the cell surface represented approximately 8% of the total tPA present in Bowes cells. Tranexamic acid elutable tPA accounted for one-third of the tPA amount secreted by the same number of Bowes cells for 24 hr. Unlike in the experiment shown in Figure 5, the cells were treated with tranexamic acid for only 5 min to reduce the background level of spontaneously secreted tPA in the sample of cell surface bound tPA. Also the quantitation of tPA in the relatively highly viscous samples of cell

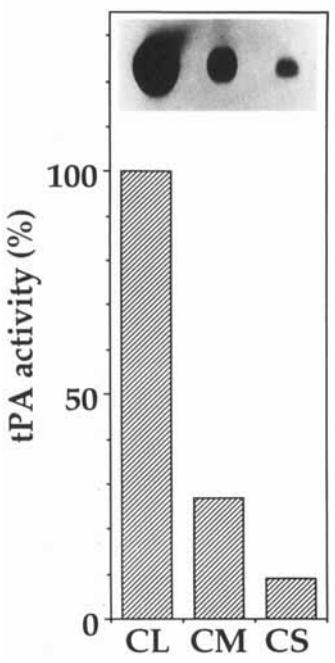


**Fig. 9.** Zymographic analysis of tPA content in Bowes cells. Parallel cultures of Bowes cells cultivated in serum-free medium were processed for analysis and samples represented: CL, cell monolayer dissolved in lysis buffer (50 mM glycine pH 7.8, 5 mM iodoacetamide, 5 mM EDTA, 0.5% Triton X-100); CM, conditioned medium after 24 hr incubation of Bowes cells with fresh serum-free medium; CS, the cell surface eluate of a monolayer of Bowes cells with 10 mM tranexamic acid for 5 min at +37°C. The samples were adjusted to equivalent volume, then centrifuged at 5000*g* for 10 min, and an aliquot of each supernatant was used for PAGE followed by zymography. Intensity of lysis zones (shown in the upper part of the figure) was quantified by densitometric scanning. The total cellular amount of tPA represents 100%.

lysates was more reliably assayed by zymography than by immunocapture assay.

## DISCUSSION

Activation of plasminogen on the surface of some tumor cells of neuroectodermal origin, e.g., melanomas mostly depends on the presence and appropriate deposition of tPA on the cell surface (Bizik et al., 1990). Binding of tPA has been documented previously for both endothelial cells (Hajjar et al., 1987; Barnathan et al., 1988) and human fibroblasts (Hoal-Van Helden et al., 1983). A clear characterization of a specific receptor protein, as in the case of uPA (Roldan et al., 1990) has not been accomplished. However, a tPA receptor $M_r$ 40,000) has been isolated recently from human placental tissue, which bound both tPA and Lys-plasminogen (Hajjar, 1991). The receptor is thought to represent a unique double-ligand endothelial cell receptor, functioning to support plasmin generation at the cell surface.

Activation of plasminogen by tPA is strongly promoted by fibrin interaction, which serves to align and concentrate tPA and plasminogen within the fibrin clot to facilitate fibrinolysis. It has been shown that the binding of tPA to fibrin is mediated by two functional domains, the finger and kringle-2 domains (van Zonneveld et al., 1986b). Since the binding mediated by the kringle-2 domain is inhibited in the presence of ϵ-aminocaproic acid, the binding sites in the fibrin molecule for the kringle-2 domain appear to be specific lysine residues in fibrin molecules. Bennett et al. (1991) demonstrated that kringle-1 does not have appreciable lysine binding affinity.

In this study, we showed that tPA could be removed most efficiently from the surface of melanoma cells by polylysine and a lysine analog, tranexamic acid. Experiments with monoclonal antibodies against individual domains of tPA suggested that kringle-2 was the most important domain of tPA in facilitating the process of plasminogen activation on the surface of melanoma cells. It can be speculated that the antibody against kringle-2 blocked the appropriate binding of tPA on the cell surface. Interestingly, our results indicate that tPA may interact with a cell surface component(s) of human melanoma cells in a similar manner to that described for its binding to fibrin or a putative receptor on endothelial cells.

Binding of tPA to melanoma cells exhibited different characteristics when it was compared to the binding of tPA to the mannose receptor present on macrophages and liver cells. The mannose receptor is considered to be a physiological ligand for tPA that could be involved in the rapid clearance of tPA from plasma (Otter et al., 1991). In the study cited, the most potent inhibitors of the monosaccharides tested were D-mannose and L-fucose; N-acetyl-D-glucosamine was a moderate inhibitor. In our experiments, D-mannose and L-fucose were weak effectors, but N-acetyl-D-glucosamine was the most potent agent to release tPA from its binding to the melanoma cell surface. The characteristics of the carbohydrate-mediated tPA binding to melanoma cells, as documented in our study, does not correspond with the characteristics of the mannose receptor, and it seems only to support binding of

tPA on the cell surface. Specific lectins may take part in this process.

We conclude therefore that melanoma cells possess on their surface a tPA-binding component, which efficiently mediates plasminogen activation and depends on a lysine-binding mechanism. Our current experiments are focused on identification of the binding protein(s) involved.

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