

# Relationship between Multiple Forms of Plasminogen Activator in Human Breast Tumors and Plasma and the Presence of Metastases in Lymph Nodes<sup>1</sup>

Marina Colombi,<sup>2</sup> Sergio Barlati, Henri Magdelenat, and Berta Fiszer-Szafarz<sup>3</sup>

Institut Curie-Biologie, Bât. 111, Centre Universitaire, 91405 Orsay, France [M. C., B. F.-S.], Istituto di Biologia, Facoltà di Medicina, Università di Brescia, 25100 Brescia, Italy [M. C., S. B.], and Institut Curie, Section Médicale, 26 rue d'Ulm, 75231 Paris, Cedex, France [H. M.]

## ABSTRACT

Plasminogen activators (PAs), a family of proteases active in blood coagulation, may play an important role in cancer. Indeed, blood coagulation disorders, such as altered fibrinogen and fibrin metabolism and increased incidence of vascular thrombosis, are common in patients with advanced malignant disease. Different types of human tumors are known to contain high levels of PA.

The isoelectric focusing patterns of the PAs present in tumors and plasma from patients with breast cancer were compared with those of purified human urokinase and melanoma tissue PA.

The pattern of isoelectric molecular forms of PA active at pH 8 showed two groups of several bands: in plasma from tumor-bearing patients and controls, these groups were in the pI ranges of 6.6 to 6.8 and 8.0 to 8.5; in mammary adenocarcinoma tissue, the ranges were 6.8 to 7.9 and 9.0 to 9.4. These patterns were different from those obtained with purified markers; the latter were 5.8 to 9.4 and 5.9 to 7.6 for purified human urokinase and melanoma plasminogen tissue activator, respectively. PA activity in tumor-bearing patients was very high in malignant tissue and, on the contrary, very decreased in plasma; this latter decrease was correlated with the presence of metastases in the axillary lymph nodes.

These results suggest that the high PA activity in the tumor tissue might participate in the destruction of the peritumoral tissue, thus allowing its invasion by tumor cells, whereas the low activity of PA in the plasma might increase plasma fibrin, reflecting thus an early disorder in blood coagulation which would enhance the formation of metastases.

## INTRODUCTION

PAs<sup>4</sup> constitute a family of proteases which are involved in not only the fibrinolytic cascade but also cancer. Blood coagulation disorders have been known for over 100 years (43) to be common in patients with advanced malignant disease [mainly altered fibrinogen and fibrin metabolism and increased incidence of vascular thrombosis (20, 37)]. Tumor cells enhance platelet aggregation *in vitro* (17, 18, 22) and display procoagulant activity correlating with their metastatic potential *in vivo* (19). *In vitro*

transformed cells express higher PA levels than do the normal homologous ones (33, 44); PA synthesis is enhanced by tumor promoters (29, 35) and by conditioned media of transformed cells (23). Different types of human produce high levels of PA (5, 7, 9, 13, 21, 26, 30, 39), but the molecular mechanisms and the role of PA activation in tumor cells are still under investigation (12, 34, 35).

Plasma cryoproteins from cancer patients contain a fraction which displays both PA and transformation-enhancing factor activities (2, 3, 10, 11, 27, 28). The latter activity is probably related to fibronectin degradation fragments produced by the PAs (3, 4).

The metastatic process is a complex sequence of steps that may involve the presence of fibrin and blood coagulation (19). It was therefore of interest to study the types and levels of PAs present in the plasma and in the tumors of patients with mammary adenocarcinomas.

An IEF-CAS technique was developed for the separation and detection of PAs as a function of pI in whole plasma or tumor extracts. Purified human u-PA (24, 40, 45) and t-PA from human melanoma cells (38) were utilized as standards.

The isoelectric forms of PA active at pH 8 separated into 2 groups of several bands. In plasma, these groups were in the pI ranges of 6.6 to 6.8 and 8.0 to 8.5; in tumor tissues, the pI ranges were from 6.8 to 7.9 and 9.0 to 9.4. These patterns were different from those obtained with purified markers; the latter were 5.8 to 9.4 and 5.9 to 7.6 for u-PA and melanoma t-PA, respectively.

PA activity was very high in the tumor tissue; on the contrary, it was very decreased in the plasma. Moreover, a remarkable correlation was found between the decrease of plasma PA activity and the existence of metastases in the axillary lymph nodes.

We suggest that the high PA activity in the tumor tissue might participate in the destruction of the peritumoral tissue, allowing thus the invasion by the tumor, whereas its lack in the plasma of cancer patients would increase plasma fibrin and fibrin deposits, reflecting thus an early disorder in blood coagulation and favoring the formation of metastases.

Some of the preliminary results were presented in poster form at the 13th International Cancer Congress (8).

## MATERIALS AND METHODS

**Chemicals.** Acrylamide, *N,N'*-methylenebisacrylamide, Trizma base, and human urokinase (EC 3.4.21.31) were purchased from Sigma Chemical Co. (St Louis, MO); agarose (Indubiose A 37) was from IBF (Ville-neuve-la-Garenne, France); ampholines were from LKB (Stockholm, Sweden); 3-dimethylaminopropionitrile was from Eastman Kodak (Roch-

<sup>1</sup> Supported by the Conseil de Coordination of the Curie Institute, by the Italian National Research Council (Progetto finalizzato Oncologia), and by the Italian Ministry of Education.

<sup>2</sup> Recipient of a fellowship from the Association Française pour l'Etude du Cancer.

<sup>3</sup> To whom requests for reprints should be addressed.

<sup>4</sup> The abbreviations used are: PA, plasminogen activator; ADC, adenocarcinoma; FA, fibroadenoma; IEF, isoelectrofocusing; PG, plasminogen; t-PA, plasminogen tissue activator; u-PA, urokinase; IEF-CAS, isoelectrofocusing-caseinolysis.

Received August 25, 1983; accepted April 6, 1984.

ester, NY); Triton X-100 and ammonium persulfate were from Merck (Darmstadt, Germany); PG was from AB Kabi (Stockholm, Sweden); and purified t-PA from human melanoma was kindly supplied by Professor D. Collen (Leuven, Belgium).

Plasmin (EC 3.4.21.7) was obtained by incubation at 37° for 15 min of 800 µg of PG/ml with 30 million units of urokinase.

**Biological Materials.** Blood samples from 58 randomly chosen breast cancer patients without any prior medication were collected at the Curie Hospital in Paris, whereas blood from 38 allegedly healthy controls came from the Medical Control of the personnel of the Curie Institute. Blood samples from 6 patients with benign tumors were also collected. Blood was collected in the presence of disodium EDTA and centrifuged for 15 min at 4000 rpm. Small aliquots were kept at -20°.

Invasive adenocarcinoma samples from the same patients were examined histologically and classified according to: (a) the WHO Histological Typing of Breast Tumors (1) [ductal (8500/3), lobular (8520/3), mucinous (8480/3), medullary (8510/3), and apocrine (8573/3)]; (b) the Scarf-Bloom-Richardson system (6). For each tumor, the number of invaded axillary lymph nodes and the occurrence of lymph node rupture were determined.

Tumors (malignant adenocarcinomas and benign fibroadenomas) were frozen at -80° immediately after surgery and kept at -20°. Specimens were quickly thawed to room temperature and rinsed with 0.15 M NaCl. All microscopically recognizable fat and connective tissue were removed, and only portions free of visible necrosis were used. The samples were then homogenized in 9 volumes of 0.2% (w/v) Triton X-100 with a Polytron blender (type PTA 10-35) for 10 sec (4 times). The homogenates were centrifuged in a Beckman J-21B centrifuge with JA-20 rotor at 12,000 rpm for 20 min at 4° and distributed into small aliquots stored at -20°.

Protein content of the supernatants was determined according to the method of Lowry et al. (25). DNA was determined in aliquots of the homogenates according to the method of Fiszer-Szafarz et al. (16).

**Analytic IEF on Thin-Layer Polyacrylamide Gel.** This was performed with an LKB 2117 Multiphor on horizontal slabs prepared by 2-hr polymerization of a solution containing 5.3% acrylamide:bisacrylamide (30:0.8) and 2% ampholine (pH 5 to 8, 0.72%; pH 3.5 to 10, 1.1%; pH 9 to 11, 0.18%), 0.22% (v/v) 3-dimethylaminopropionitrile, and 0.025% (w/v) ammonium persulfate. The electrode solutions were 1 M phosphoric acid (anode) and 1 M sodium hydroxide (cathode). Samples were loaded in wells near the anode: 10 µl of plasma and 6 µg of protein or an amount corresponding to 1 µg of DNA for tissue extracts. Electrophoresis was run for 3 hr at 3 watts. At the end, the pH gradient was determined with pl markers supplied by Pharmacia (Stockholm, Sweden).

**Detection of PA Activity.** The run gels were equilibrated in 0.1 M Tris-HCl buffer (pH 8) and covered with a 1-mm-thick layer made of 15% lyophilized milk, 6 µg of human PG/ml, and 1.7% agarose in 0.1 M Tris-HCl buffer (pH 8) at 40°. After solidification, the double gel was incubated at 37° for various times in a moist box. PA activity was detected as clear caseinolysis areas in the cloudy background of unhydrolyzed casein and photographed; it was semiquantitatively rated from 0 to +++ according to the relative intensity of the dark background seen through the transparent spot on the photograph. Control agarose overlays without PG were used to detect PG-independent protease activities or activities due to the simultaneous presence of PG and PA at the same pl.

**Statistical Analysis.** This analysis of differences was performed with the  $\chi^2$  test.

**RESULTS**

**IEF-CAS Analysis of Purified u-PA and t-PA.** Analysis of u-PA shows (Fig. 1) 6 bands of enzymatic activity at pH 8 with pl ranging between 7.2 and 9.4 and a diffuse activity in the pl range of 6.2 to 7.1. IEF-CAS analysis of t-PA at pH 8 reveals 8 bands with pl between 5.9 and 7.6.

The purified u-PA and t-PA tested did not contain any PG-

independent caseinolytic activity.

**IEF-CAS Analysis of Human Plasma PA.** Human plasma from the controls analyzed by IEF-CAS showed various forms of PA activity. Pictures taken after different times of hydrolysis are reported in Fig. 2. At 12 hr, 2 bands with pl of 6.6 and 6.8 are visible, while after 28 hr, a band in the pl range of 8.0 to 8.5 appears. The PA forms with a pl in the range of 4.8 to 5.2 near the site of sample application were not taken into account, since they are probably due to technical artifacts (aggregated enzymes?).

Fig. 3 shows the comparison between 2 normal plasmas and 4 plasmas from tumor-bearing patients (2 benign FA and 2 malignant ADC). Control plasma (Lanes 1 and 4) contains PA activity with pl values of 6.6 to 6.8 and to a lesser extent with pl between 8.0 and 8.5. Plasma from FA-bearing patients (lanes 2 and 5) contains only PA activities with pl values of 6.6 to 6.8. As to plasma from ADC-bearing patients (Lanes 3 and 6), it contains variable PA activity with pl between 6.6 and 6.8 and very weak or no PA activity with pl values of 8.0 to 8.5. A slight caseinolytic activity was observed in the absence of added PG; it was probably due to the low level of PG normally present in human plasma; these forms focused at the same pl range as did the plasma PA forms (36).

Table 1 is a compilation of all cases studied: 38 plasmas from allegedly healthy controls with 6 from FA- and 58 from ADC-bearing patients. It shows that the control plasmas contain high levels of PA activity, the majority being the 4+ and 5+ groups, whereas the plasmas from ADC-bearing patients contain rather lower levels of that activity, most of them being in the range 1+ to 3+. The low number of samples of FA plasmas seems to indicate that they present intermediary levels of PA activity (3+ and 4+).

Table 2 gives evidence for the correlation between the level of PA activity in the plasma of ADC patients and the existence of invaded axillary lymph nodes. It appears quite clearly that the lower the PA activity, the higher the probability of metastases in the lymph nodes.

Table 1

Percentage of distribution of patients according to PA activity in plasma

The activity rating was obtained by adding the ratings of the groups having a pl of 6.6 to 6.8 and of 8.0 to 8.5. The differences of distribution in the ADC patients and the other 2 classes are significant ( $p < 0.001$ ). The difference between controls and FA patients is significant ( $p < 0.01$ ).

	Activity rating (total no. of +)					
	0	1	2	3	4	5
Controls (n = 38)				5.3	26.3	68.4
FA (n = 6)			16.7	33.3	33.3	16.7
ADC (n = 58)	3.5	34.5	34.5	25.9	1.7	

Table 2

Correlation between plasma PA activity and existence of lymph node metastases

The activity rating was obtained by adding the ratings of the groups having a pl of 6.6 to 6.8 and of 8.0 to 8.5. The difference of distribution is significant ( $p < 0.05$ ).

	Plasma PA activity rating (total no. of +)					
	0	1	2	3	4	5
No. of patients	2	20	20	15	1	0
Patients with lymph node metastases in each group (%)	100	70.0	45.0	26.7	0	

Table 3

Percentage of distribution of patients according to PA activity in tumor tissue

The activity rating was obtained by adding the ratings of the groups having a pI of 6.8 to 7.9 and of 9.0 to 9.4. The difference of distribution of the ADC and FA patients is significant ( $p < 0.001$ ).

	PA activity rating (total no. of +)						
	0	1	2	3	4	5	6
FA (n = 5)	80		20				
ADC (n = 20)				25	25	30	20

**IEF-CAS Analysis of Malignant and Benign Breast Tumor Tissue PA.** The same analysis of PA was performed on extracts from breast tumors of different histological types. It revealed the presence of 2 different types of PA with pI from 6.8 to 7.9 and from 9.0 to 9.4 (Fig. 4). While the former PA forms were present in malignant and benign tissues, the latter ones were found in malignant tissues only. Table 3 gives a compilation of all the results obtained. It seems that most benign tumors lack any PA activity whatsoever, whereas, on the contrary, malignant tumors are characterized by high levels of all forms of PA.

## DISCUSSION

The role of hydrolytic enzymes and particularly proteases in malignant tumor invasiveness and metastasis spreading could be of fundamental importance. Among the many proteases known, PA has been extensively studied in tumor tissue (5, 7, 9, 12, 13, 21, 26, 30, 32-34, 38, 39, 44). We studied this activity in human plasma and breast tumors by IEF-CAS. This technique yields tissue-specific band patterns corresponding to the various forms of PA; the activity is determined semiquantitatively; it separates the enzyme activity from possible activators and/or inhibitors. We studied the PA enzymatically active at pH 8.0. Our observations thus do not exclude the possibility of some other forms active at other pHs.

Our results essentially show (a) that the isoelectric patterns of PA forms in human breast tumors (either malignant or benign) are different from those present in the plasma. They also differ from the u-PA and from the melanoma t-PA patterns. This would suggest that the various PA groups observed might be tissue specific, but nevertheless, this does not exclude the possibility that some forms might exist in more than one tissue; however, even if the protein moiety could be the same, it is still very interesting that the presence of different sugar groups could allow plasma PAs to differentiate from the tumor ones; (b) that the malignant tumor tissue groups are very active, whereas the plasma groups from tumor-bearing patients have a very low or no activity at all. The high level of activity of malignant tumor tissue PA is compatible with a role in the spreading of the tumor (42), inasmuch as the benign noninvading tumors have vanishing levels of PA activity. We found no correlation between the WHO classification of mammary malignant tumors and their level of extractable PA activity. The Scarf-Bloom-Richardson classification, too, gives no clear correlation with the PA activity levels of the tumor (data not shown because of complexity). The difficulty in finding any such correlation might in fact be due to the difficulty in finding a classification of value for the prognosis of the individual patient (41) and to the statistically very large number of cases necessary in each class. Tarin *et al.* (42) also observed no significant difference in the amount of PA produced by tumors

with a high or low pulmonary colonization potential.

The decreased PA activity in cancer plasma might reflect a diminished synthesis of the plasma-type enzyme(s) or the presence of inhibitor(s) (31), which would have to be covalently linked to the enzyme molecule as, in spite of the IEF electrical field, no high activities could be detected. Similarly, hyaluronidase, another hydrolytic enzyme, displays the same pattern; it is very active in the extracellular space of tumors (15), whereas a potent inhibitor is present in the plasma of cancer patients (14).

This decreased PA activity would produce a perturbation in the fibrin metabolism; indeed, a perturbed clotting activity has been described in tumor-bearing patients (20). The thus increased fibrin in plasma could serve as an anchoring for metastatic cells (19).

This loss of plasma PA activity seems to be related to the existence of metastases in axillary lymph nodes, as almost all invaded lymph nodes occur in patients with very low levels of plasma PA activities (Table 2).

Among the allegedly normal plasmas, we observed 2 of them lacking PAs in the 8.0 to 8.5 pI range. We cannot decide whether these plasmas are really normal or express some unknown disease, as we have not studied any other pathology than breast tumor.

We do not know the determinants of the decrease in PA activity of the plasma of cancer patients. However, as this enzyme takes part in the blood coagulation process, the disturbance produced by cancer results in perturbation of that process. This perturbation, in turn, may favor conditions for production and development of metastases.

We have shown that the perturbations in blood coagulation are readily detectable in patients whose tumor has just been discovered.

We have also established a correlation between the decreased PA activity in plasma and the presence of metastases in axillary lymph nodes in mammary cancer patients. The low plasma PA activity in patients with tumors in an early stage of cancer points to the existence of a perturbation of the blood coagulation, which will be greatly amplified in the later stages of the disease (20, 37). We believe that further investigation of this relationship in other types of cancer may throw some light on the mechanism of metastasis and its therapy.

In summary, we suggest that the malignant tumor tissue PA might play a role in the invasion of the peritumoral space, whereas the lack of plasma PA activity facilitates the formation of metastases. The high activity of ADC tissue PA is probably related to the lack of plasma PA activity in the same host by a complex sequence of steps, in which the host reaction is certainly very important.

## ACKNOWLEDGMENTS

The authors gratefully acknowledge the help from physicians and scientists from the Curie Hospital and Institute: Dr. J. P. Thiéry for his financial contribution; many surgeons for providing the plasmas and the surgical tissue specimens; Dr. C. Chieq for histological determinations; Dr. D. Szafarz for very fruitful discussions; and Dr. D. Collen for supplying purified t-PA.

## REFERENCES

1. Azzopardi, J. G., Chepik, O. F., Hartmann, M. W. H., Jafarey, N. A., Lombart-Bosch, A., Ozzello, L., Rilke, F., Sasano, N., Sobin, L. H., Sommers, S. C., Stalsberg, H., Sugar, J., and Williams, A. O. The World Health Organization Histological Typing of Breast Tumors, Ed. 2, Vol. 78, pp. 806-816. Chicago:



- American Society of Clinical Pathologists, 1982.
2. Barlati, S., De Petro, G., Vartio, T., and Vaheri, A. Transformation-enhancing factor markers. In: *Biological and Biochemical Tumor Markers*, pp. 295–306. New York: Plenum Publishing Corp., 1982.
  3. Barlati, S., and Mignatti, P. Transformation-enhancing factors in the plasma of cancer patients. In: L. Jimenez de Asua (ed.), *Control Mechanisms in Animal Cells*, pp. 269–277. New York: Raven Press, 1980.
  4. Barlati, S., Mignatti, P., Brega, A., De Petro, G., and Ascari, E. Utilization of Rous sarcoma virus for the detection of transformation-enhancing and inhibiting factors in human plasma. In: S. Barlati and C. de Gluli-Morghen (eds.), pp. 331–348. Padua, Italy: Piccin Medical Books, 1978.
  5. Barrett, J. C., and Sheela, S. Plasminogen activator in metastasis. In: L. A. Liotta and I. R. Hart (eds.), *Tumor Invasion and Metastasis*, pp. 359–374. The Hague: Martinus Nijhoff, 1982.
  6. Bloom, H. J. G., and Richardson, W. W. Histological grading and prognosis in breast cancer. A study of 1409 cases, of which 359 have been followed for 15 years. *Br. J. Cancer*, 11: 359–376, 1957.
  7. Camiolo, S. M., Markus, G., Evers, J. L., Hobika, G. H., DePasquale, J. L., Beckley, S., and Grimaldi, J. P. Plasminogen activator content of neoplastic and benign human prostate tissues; fibrin augmentation of an activator activity. *Int. J. Cancer*, 27: 191–198, 1981.
  8. Colombi, M., Szafarz, B., and Barlati, S. Expression of multiple forms of plasminogen activators in human mammary tumors and plasma (abstract). *Proceedings of the 13th International Cancer Congress*, PS64, 1982.
  9. Corasanti, J. G., Celik, C., Camiolo, S. M., Mittelman, A., Evers, J. L., Barbasch, A., Hobika, G. H., and Markus, G. Plasminogen activator content of human colon tumors and normal mucosae: separation of enzymes and partial purification. *J. Natl. Cancer Inst.*, 65: 345–351, 1980.
  10. De Petro, G., Barlati, S., Vartio, T., and Vaheri, A. Transformation-enhancing activity of gelatin-binding fragments of fibronectin. *Proc. Natl. Acad. Sci. USA*, 78: 4965–4969, 1981.
  11. De Petro, G., Barlati, S., Vartio, T., and Vaheri, A. Transformation-enhancing activity in plasma of tumor patients: relationship with fibronectin fragments. *Int. J. Cancer*, 31: 157–162, 1983.
  12. Dvorak, H., Dvorak, A. M., Manseau, E. J., Wiberg, L., and Churchill, W. H. Fibrin gel investment associated with line 1 and line 10 solid tumor growth, angiogenesis, and fibroplasia in guinea pigs. Role of cellular immunity, myofibroblasts, microvascular damage, and infarction in line 1 tumor regression. *J. Natl. Cancer Inst.*, 62: 1459–1472, 1979.
  13. Evers, J. L., Patel, J., Madeja, J. M., Schneider, S. L., Hobika, G. H., Camiolo, S. M., and Markus, G. Plasminogen activator activity and composition in human breast cancer. *Cancer Res.*, 42: 219–226, 1982.
  14. Fiszer-Szafarz, B. Demonstration of a new hyaluronidase inhibitor in serum of cancer patients. *Proc. Soc. Exp. Biol. Med.*, 129: 300–302, 1968.
  15. Fiszer-Szafarz, B., and Gullino, P. M. Hyaluronidase activity of normal and neoplastic interstitial fluid. *Proc. Soc. Exp. Biol. Med.*, 133: 805–807, 1970.
  16. Fiszer-Szafarz, B., Szafarz, D., and Guevara de Murillo, A. A general, fast, and sensitive micromethod for DNA determination: application to rat and mouse liver, rat hepatoma, human leukocytes, chicken fibroblasts, and yeast cells. *Anal. Biochem.*, 110: 165–170, 1981.
  17. Gasic, G. J., Gasic, T. B., and Stewart, C. C. Antimetastatic effects associated with platelet reduction. *Proc. Natl. Acad. Sci. USA*, 61: 46–52, 1968.
  18. Gastpar, H. Platelet aggregation inhibitors and cancer metastasis. *Ann. Chir. Gynaecol.*, 71: 142–150, 1982.
  19. Gilbert, L. C., and Gordon, S. G. Relationship between cellular procoagulant activity and metastatic capacity of B16 mouse melanoma variants. *Cancer Res.*, 43: 536–540, 1983.
  20. Gordon, S. G. A proteolytic procoagulant associated with malignant transformation. *J. Histochem. Cytochem.*, 29: 457–463, 1981.
  21. Harvey, S., Minowada, J., Takita, H., Kover, L., and Markus, G. Urokinase-like plasminogen activators of unusually high molecular weight secreted by a cell line derived from a human lung cancer case. *J. Biol. Chem.*, 257: 5645–5651, 1982.
  22. Honn, K. V., Cavanaugh, A., Evens, C., Taylor, J. D., Sloane, B. F., and Mayevsky, P. Tumor cell-platelet aggregation: induced by cathepsin B-like proteinase and inhibited by prostacyclin. *Science (Wash. DC)*, 217: 540–542, 1982.
  23. Krycève-Martinerie, C., Biquard, J. M., Lawrence, D., Vigier, P., Barlati, S., and Mignatti, P. Transformation-enhancing factor(s) released from chicken Rous sarcoma cells: effect on some transformation parameters. *Virology*, 112: 436–449, 1981.
  24. Lesuk, A., Terminiello, L., and Traver, J. H. Crystalline human urokinase: some properties. *Science (Wash. DC)*, 147: 880–881, 1965.
  25. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, 193: 265–275, 1951.
  26. Markus, G., Takita, H., Camiolo, S. M., Corasanti, J. G., Evers, J. L., and Hobika, G. H. Content and characterization of plasminogen activators in human lung tumors and normal lung tissue. *Cancer Res.*, 40: 841–848, 1980.
  27. Mignatti, P., Ascari, E., and Barlati, S. Potential diagnostic and prognostic significance of the transformation-enhancing factor(s) in the plasma cryoprecipitate of tumor patients. *Int. J. Cancer*, 25: 727–734, 1980.
  28. Mignatti, P., and Barlati, S. Enhancement of plasminogen activator activity by the culture medium of Rous sarcoma virus-transformed cells. *J. Gen. Virol.*, 63: 365–373, 1982.
  29. Miskin, R., and Reich, E. Plasminogen activator: induction of synthesis by DNA damage. *Cell*, 19: 217–224, 1980.
  30. Nagy, B., Ban, J., and Brdar, B. Fibrinolysis associated with human neoplasia: production of plasminogen activator by human tumors. *Int. J. Cancer*, 19: 614–620, 1977.
  31. Ogston, D., and Bennett, B. Biochemistry of naturally occurring inhibitors of the fibrinolytic enzyme system. In: D. Ogston and B. Bennett (eds.), *Hæmostasis: Biochemistry, Physiology, and Pathology*, pp. 230–238. London: John Wiley & Sons, Inc., 1977.
  32. Ossowski, L., and Reich, E. Antibodies to plasminogen activator inhibit human tumor metastasis. *Cell*, 35: 611–619, 1983.
  33. Ossowski, L., Unkeless, J. C., Tobia, A., Quigley, J. P., Rifkin, D. B., and Reich, E. An enzymatic function associated with transformation of fibroblasts by oncogenic viruses. II. Mammalian fibroblast cultures transformed by DNA and RNA tumor viruses. *J. Exp. Med.*, 137: 112–126, 1973.
  34. Paranjpe, M., Engel, L., Young, N., and Liotta, L. Activation of human breast carcinoma collagenase through plasminogen activator. *Life Sci.*, 78: 1223–1231, 1980.
  35. Quigley, J. P. Phorbol ester-induced morphological changes in transformed chick fibroblasts: evidence for direct catalytic involvement of plasminogen activator. *Cell*, 17: 131–141, 1979.
  36. Raum, D., Marcus, D., and Alper, C. A. Genetic polymorphism of human plasminogen. *Am. J. Genet.*, 32: 681–689, 1980.
  37. Rickles, F. R., Edwards, R. L., Barb, C., and Cronlund, M. Abnormalities of blood coagulation in patients with cancer. *Fibrinopeptide A generation and tumor growth. Cancer (Phila.)*, 51: 301–307, 1983.
  38. Rijken, D. C., and Collen, D. Purification and characterization of the plasminogen activator secreted by human melanoma cells in culture. *J. Biol. Chem.*, 256: 7035–7041, 1981.
  39. Sheela, S., and Barrett, J. C. *In vitro* degradation of radiolabelled, intact basement membrane mediated by cellular plasminogen activator. *Carcinogenesis (Lond.)*, 3: 363–369, 1982.
  40. Soberano, M. E., Ong, E. B., Johnson, A. J., Levy, M., and Schoellmann, G. Purification and characterization of two forms of urokinase. *Biochim. Biophys. Acta*, 445: 763–773, 1976.
  41. Stenkvist, B., Bengtsson, E., Eriksson, O., Jarkrans, T., Nordin, B., and Westman-Naeser, S. Histopathological systems of breast cancer classification: reproducibility and clinical significance. *J. Clin. Pathol.*, 36: 392–398, 1983.
  42. Tarin, D., Ho, B. J., and Evans, D. J. Correlation of collagenase secretion with metastatic-colonization potential in naturally occurring murine mammary tumours. *Br. J. Cancer*, 46: 266–272, 1982.
  43. Trousseau, A. *Phlegmatia alba dolens*. In: *Clinique Médicale de l'Hôtel-Dieu de Paris*, Ed. 2, Vol. 3, pp. 654–712. Paris: J. B. Baillière et Fils, 1865.
  44. Unkeless, J. C., Tobia, A., Ossowski, L., Quigley, J. P., Rifkin, D. B., and Reich, E. An enzymatic function associated with transformation of fibroblasts by oncogenic viruses. I. Chick embryo fibroblast cultures transformed by avian RNA tumor viruses. *J. Exp. Med.*, 137: 85–111, 1973.
  45. White, W. F., Barlow, G. H., and Mozen, M. M. The isolation and characterization of plasminogen activators (urokinase) from human urine. *Biochemistry*, 5: 2160–2169, 1966.

Fig. 1. Isoelectric forms of t-PA and u-PA. IEF gels and casein overlays containing 6 µg PG/ml were incubated at pH 8.0 and 37° for 10 hr. Lane 1, 12 ng t-PA; Lane 2, 100 Sigma milliunits u-PA.

Fig. 2. Effect of incubation time on casein degradation in IEF analysis of PA activity in human plasma. Gels with casein overlay containing 6 µg PG/ml were incubated at pH 8.0 and 37° for 12 hr (a), 20 hr (b), and 28 hr (c). Lane 1, 5 Sigma milliunits u-PA; Lane 2, 10 µl plasma from a healthy donor.

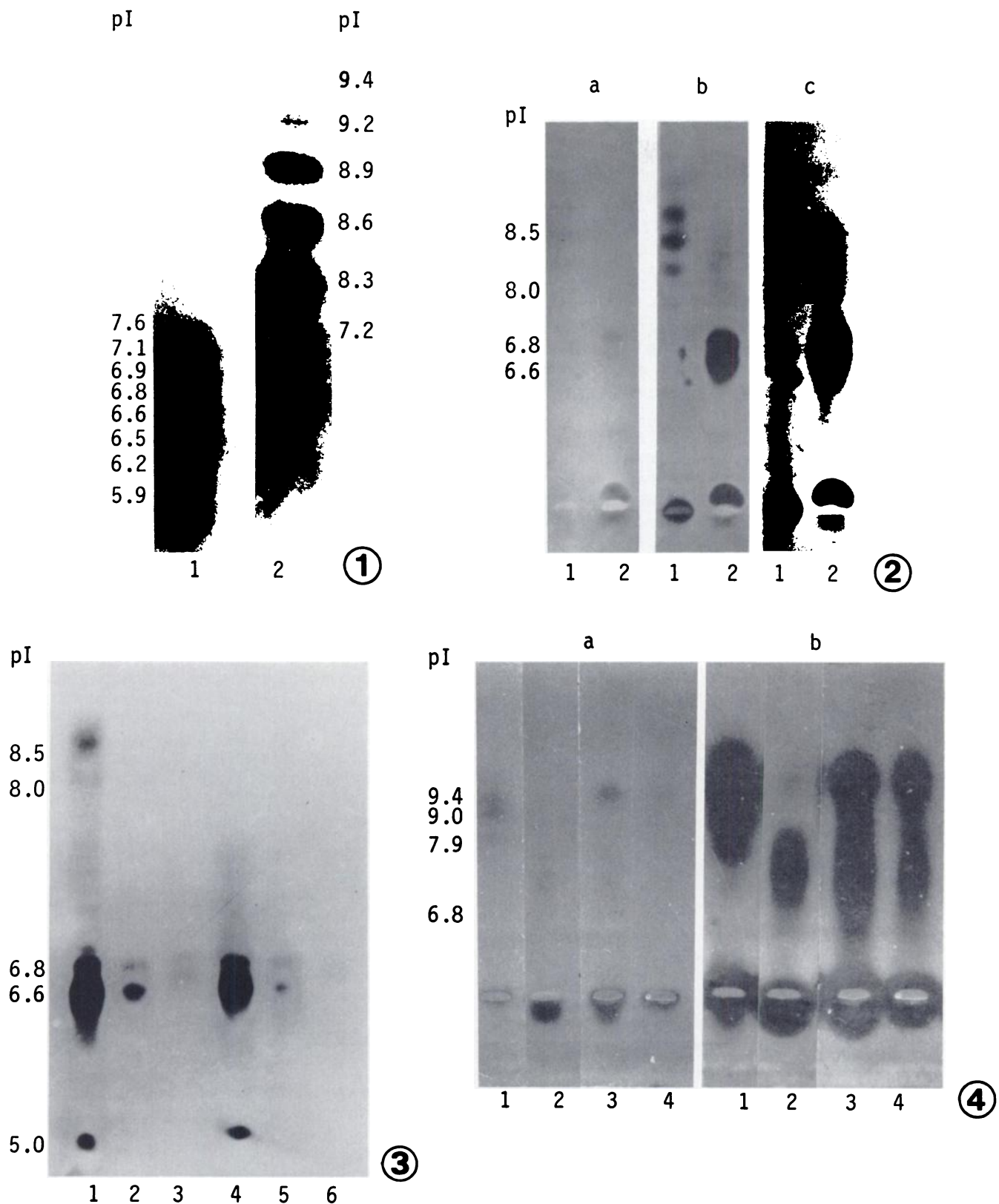


Fig. 3. PA activity in plasma from tumor-bearing patients. Gels with casein overlay containing 6  $\mu$ g PG/ml were incubated at pH 8.0 and 37° for 20 hr:

Plasma origin	Activity level	
	pi 6.6–6.8	pi 8.0–8.5
Lane 1, control plasma	+++	++
Lane 2, plasma from FA bearer (Case 32)	++	0
Lane 3, plasma from ADC bearer (Case 73)	+	0
Lane 4, control plasma	+++	0
Lane 5, plasma from FA bearer (Case 33)	++	0
Lane 6, plasma from ADC bearer (Case 88)	0	0

Fig. 4. PA activity in extracts of mammary tumors as revealed by IEF. The gels with casein overlay containing 6  $\mu$ g PG/ml were incubated at pH 8.0 and 37° for 14 hr (a) and 30 hr (b). Lane 1, 6 Sigma millionits u-PA; Lane 2, extract from FA equivalent to 1  $\mu$ g DNA or containing 6  $\mu$ g protein; Lanes 3 and 4, extract from ADC equivalent to 1  $\mu$ g DNA or containing 6  $\mu$ g protein.

# Cancer Research

The Journal of Cancer Research (1916–1930) | The American Journal of Cancer (1931–1940)

## Relationship between Multiple Forms of Plasminogen Activator in Human Breast Tumors and Plasma and the Presence of Metastases in Lymph Nodes

Marina Colombi, Sergio Barlati, Henri Magdelenat, et al.

*Cancer Res* 1984;44:2971-2975.

**Updated version** Access the most recent version of this article at:  
<http://cancerres.aacrjournals.org/content/44/7/2971>

**E-mail alerts** [Sign up to receive free email-alerts](#) related to this article or journal.

**Reprints and Subscriptions** To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at [pubs@aacr.org](mailto:pubs@aacr.org).

**Permissions** To request permission to re-use all or part of this article, use this link <http://cancerres.aacrjournals.org/content/44/7/2971>. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.