Journal of Cellular Biochemistry 53:135–144 (1993)

Expression and Localization of Elements of the Plasminogen Activation System in Benign Breast Disease and Breast Cancers

Jerzy Jankun, Hollis W. Merrick, and Peter J. Goldblatt

Department of Chemistry, University of Toledo, Toledo, Ohio 43606 (J.J.); Department of Surgery (H.W.M.) and Department of Pathology (P.J.G.), Medical College of Ohio, Toledo, Ohio 43699

Abstract The malignant potential of solid tumors is related to the ability to invade adjacent tissue and to metastasize. These properties of cancer cells depend on the synthesis of proteolytic enzymes which are able to digest adjacent connective tissue and basement membranes. We hypothesized that all elements of the plasminogen activation system might be overexpressed in malignant human breast tumors, functioning as an essential element in tumor invasion and metastasis. As determined by histopathological methods, the malignant tumors showed statistically significantly higher expression of urokinase plasminogen activator (uPA), type-1 plasminogen activator inhibitor (PAI-1), and especially urokinase plasminogen activator receptor (uPAR) than benign tissues. All those elements were present in higher amounts in the cancer cells than in the cells of benign or normal breast tissues. High exhibition of tissue plasminogen activator (tPA) found in cancer seems to be random and not related to the malignant or benign state, since benign and malignant tumors show overexpression of tissue plasminogen activator with similar frequency. When the tumors express high amounts of uPA, they express a high amount of uPAR in 50% of cases and PAI-1 in 57.3% of cases. When urokinase is expressed in low amount, the receptor is low in 28.6% and inhibitor in 21.4% of malignant breast tumors. This statistically significant consensus, 78.6% in the case of urokinase and its receptor and 78.6% in case of urokinase and its inhibitor, suggests that these activities may be the result of a unique mechanism of control, activated in the last steps of malignant transformation.

Key words: urokinase plasminogen activator, urokinase plasminogen activator receptor, plasminogen activator inhibitor, tissue plasminogen activator, breast cancer

The malignant potential of solid tumors is related to their proliferation rate as well as to their capacity for invasion and metastasis. The potential of cancer cells for tissue invasion and hematogenous spread is related to their capacity to dissolve the structures in their vicinity. Since the structure of the penetrated tissues consists mainly of proteins, e.g., fibronectin, fibrin, proteoglycans, or collagen, the primary substances used by a tumor cell for invasion and metastasis are proteases [Schmitt et al., 1992a]. Tissues of primary cancer and/or metastases of the breast, ovary, cervix uteri, bladder, kidney, prostate, liver, lung, and gastrointestinal tract have been reported to contain high levels of cathepsins, collagenase IV, or urokinase plasminogen activator (uPA) if compared with benign tumors or normal tissues [Danø et al., 1985; Duffy et al., 1990; Evers et al., 1982; Mangel, 1990; Schmitt et al., 1992a; Tandon et al., 1990]. Elevated levels of cathepsin D and uPA are of clinical significance. It has been reported that increased concentrations of those proteases in breast tumor tissue may predict a high risk of metastasis and shorten overall survival if compared to patients with smaller amounts of those factors [Duffy et al., 1990; Schmitt et al., 1992a; Tandon et al., 1990]. Not only patients with high uPA in their breast tumors, but also patients with an elevated plasminogen activator inhibitor type-1 (PAI-1) content are at high risk for relapse or shorter overall survival [Graeff et al., 1992]. The involvement of urokinase plasminogen activator receptor (uPAR) in extracellular matrix degradation was reported recently also [Quax et al., 1992].

Two types of plasminogen activators, uPA and tPA (tissue plasminogen activator) are

Received March 5, 1993; accepted June 15, 1993.

Address reprint requests to Jerzy Jankun, Chemistry Department, University of Toledo, Toledo, OH 43606-3390.

known to convert nonactive plasminogen to plasmin. This enzyme is able to attack a broad spectrum of matrix and basement membrane proteins. The uPA and tPA are present in blood and tumor tissues. tPA, rather than uPA, is mainly involved in intravascular physiological thrombolysis. The uPA predominantly mediates pericellular proteolysis during cell migration and tissue remodeling. Although uPA is initially secreted as an enzymatically inactive proenzyme (pro-uPA) by normal and tumor cells, it exerts its proteolytic functions as an active enzyme after binding to a specific high-affinity cell surface receptor (uPAR) [Blasi, 1988; Schmitt et al., 1992a]. The uPA/uPAR complex on the cell surface is the reaction site for uPA mediated plasminogen activation [Blasi, 1988]. The receptor bound uPA can remain on the cell surface for prolonged periods of time. The complexed uPA can be inactivated by PAI-1. The bindings trigger the PAI-1 mediated internalization of the PAI-1/uPA/uPAR complex.

The proposed mechanisms of uPA secretion, binding, and internalization are somewhat contradictory to clinical observations [Blasi, 1988; Duffy et al., 1990; Graeff et al., 1992; Jänicke et al., 1991; Schmitt et al., 1992a]. High levels of the inhibitor should act protectively by blocking the enzymatic activity of receptor-bound uPA, but clinical data show an association of the inhibitor with a high risk of metastasis [Graeff et al., 1992; Schmitt et al., 1992a]. Most of the studies of breast cancer have involved biochemical and immunologic detection of the elements of the plasminogen activation system in cell or tissue homogenate, and only a few have focused on the localization of some elements within tissues [Chucholowski et al., 1992; Duffy et al., 1990; Jänicke et al., 1991]. Data from clinical and in vitro studies suggest that uPA and uPAR are localized in the cancer or cancer cells [Duffy et al., 1990; Jänicke et al., 1991; Jankun et al., 1991]. However, other workers report that both proteins can be located in fibroblasts or macrophages adjacent to the invasive cancer cells [Danø et al., 1992]. This may indicate that cancer invasion is the result of concerted action of a cell population consisting of malignant as well as non-malignant cells.

MATERIALS AND METHODS

The breast tissues were from resected specimens retained in the pathology department, archives at Medical College of Ohio from 1991 to 1992. Representative tissue blocks were selected and were fixed in 10% phosphate-buffered formaldehyde (pH 7.4) and embedded in paraffin wax. Tissue sections, 5 μm thick, were stained with hematoxylin and eosin for routine histological evaluation. [Slides were reviewed by a surgical pathologist and were classified as: 3, normal breast; 3, fibroma; 2, infiltrating adenocarcinoma; 1, ductal carcinoma in-situ; 2, ductal carcinoma; 3, infiltrating ductal carcinoma.]

Adjacent serial sections were mounted on poly-L-lysine coated glass slides, deparaffinized in xylene followed by chloroform, and used for immunohistology, employing the peroxidase/diaminobenzidine procedure described by Wordinger et al. [1987]. Briefly, the tumor sections were blocked for endogenous peroxidase activity in 0.3% H_2O_2 methanol for 5 min. Next, sections were rehydrated in descending ethanol washes, washed in phosphate buffered saline, pH 7.4, and blocked in blocking solution for 30 min (blocking solution contains carrier solution: 96 ml of PBS, 1 g of crystalline bovine serum albumin, 3 ml of 10% Tween 20, and 20% of normal goat serum). Next, sections were incubated with primary antibodies in carrier solution for 60 min at 37°C. Affinity purified murine monoclonal antibody against uPA, class IgG_1 (8 μg/ml), was used. The antibody 3689 is directed against an epitope within the β-chain of uPA and recognizes pro-uPA, high molecular weight uPA (HMW uPA), and low molecular weight uPA (LMW uPA). This antibody recognizes free and complexed uPA, and does not crossreact with tPA. Additionally in some experiments two different monoclonal antibodies against uPA were used: 377 and 394. To insure authenticity of uPA binding, the monoclonal antibodies 3689 and 377 were preincubated with uPA (24 μg/ml) and incubated with three samples of malignant breast tissue. No staining was observed. Murine antibody 3936, class IgG_{2a} (40 μg/ml), was used to detect uPAR. The antibody recognizes noncomplexed and complexed uPAR; however, saturation receptor with HMW uPA and pro-uPA might result in drastic reduction (approximately 50%) of binding monoclonal antibody 3936 to the receptor [Chucholowski et al., 1992]. Murine antibody 3785, class IgG_1 (40 μg/ml), was used to detect free and complexed PAI-1. In some other experiments we used monoclonal antibody 3780 directed against PAI-1. To insure authenticity of PAI-1 binding, the monoclonal antibody 3785 was preincubated with PAI-1 (120

μg/ml) and incubated with three samples of malignant breast tissue. No staining was observed. To detect t-PA antibody 374B, class IgG_1 (8 μg/ml), was used, and in some other experiments antibody 373 was used. To insure authenticity of tPA binding, the monoclonal antibody 374B was preincubated with tPA (24 μg/ml) and incubated with three samples of malignant breast tissue. No staining was observed. All primary antibodies were supplied by American Diagnostica, Inc., (Greenwich, CT). The reactivity of these antibodies in immunohistochemical procedures has been verified independently [Chucholowski et al., 1992; Jensen and Wheelock, 1992; Schmitt et al., 1992a; Sier et al., 1991]. Subsequently the sections were incubated with goat anti-mouse antibody (40 μg/ml, product number 31432X from Pierce, Rockford, IL), for 30 min at 37°C. Antigen staining was identified by the brown reaction products of peroxidase with diaminobenzidine. Sections from normal kidney, in which tubule cells are positive for uPA and uPAR, served as positive controls. Negative controls consisted of incubation with nonimmune mouse serum and incubation with secondary antibody only, with the remaining steps unchanged. Second negative control samples include preincubation of malignant tissue samples with antibodies preadsorbed with antigens (uPA, PAI-1, tPA), with remanding steps unchanged. The preincubation abolishes staining of malignant breast cancers. Immunohistochemical staining of antigens was evaluated blindly for intensity and semiquantitative evaluation of the percentage of positive (neoplastic) epithelial cells as follows: 1 = no positive cells; 2 = few cells with weak positivity; 3 = few cells with distinct positivity; 4 = weak positivity of all cells; 5 = weak to distinct positivity in most cells (with focal negative areas); 6 = weak to distinct positivity in all cells; and 7 = distinct positivity in all cells [Sier et al., 1991]. Statistical analysis of immunohistochemical values and clinical data were performed using Student's t test and the χ^2 test of the independence of categorical variables [Runyon and Haber, 1983]. Differences were considered significant when $P \leq 0.05$.

RESULTS

Immunohistological uPA Staining

To insure the authenticity of staining patterns as positive controls we used the following antibodies: 377, 394, 3689 for at least two different samples of normal breast, normal kidney, benign breast, and malignant tissues. All of them showed the same pattern, but with different intensity. The antibody 3689 showed the strongest intensity of stain and was chosen for future study. Preadsorbtion of antibodies 3689 and 377 with uPA, abolishing staining of malignant breast tissues, served as negative control. Distinct differences were observed between the immunohistological uPA staining intensities of benign and malignant tumors. The benign tumors, as well as normal breast tissue, showed a range of weak positivity to distinct positivity in the ductal epithelium, most prominent on the apical surface of the cell (Fig. 1a). The malignant breast tumors showed a wide spectrum of staining intensities. In general, we found diffuse cytoplasmic staining, sometime localized in the nucleus, and in some cases strong cell membrane staining for uPA (Fig. 1b,c). No nuclear staining was observed in benign tumors. Patchy and diffuse staining of adipose and/or fibrous connective tissues was observed when malignant tumors stained very intensely for uPA. Some distinct staining of scattered macrophages occasionally was detected also. For benign tumors the mean uPA score was 2.5 ± 1.0 (SD), while for malignant tumors the mean uPA score was 6.1 ± 0.8 (see Fig. 6a). The immunohistochemical staining intensity of uPA was found to differ significantly in benign and malignant tumors (t = 14.365, P = 0.0005).

Immunohistochemical uPAR Staining

There is no commercially available antibody directed against uPAR, except 3936. Sections of normal kidney served as positive controls. The tubule cells of kidney are known to contain high amounts of uPAR [Chucholowski et al., 1992]. Intensive staining was observed within the tubule cells, when the other surrounding cells remained unstained. No staining was observed in 33% of benign tumors or normal breast (Fig. 2a), and weak to distinct positivity was detected in the rest for uPA receptor. When benign tumors stain for the receptor, the highest intensity was observed on the duct epithelium (Fig. 2b), and much weaker intensity was seen within the rest of the lobules. In contrast, malignant tumors showed a variety of uPAR intensity, mostly distinct or strong intensity of staining within the tumors. Distinct staining was observed on the cell membrane and occasionally in the nucleus of cancer cells (Fig. 2c). The phenomenon of nuclear staining for uPAR was not ob-

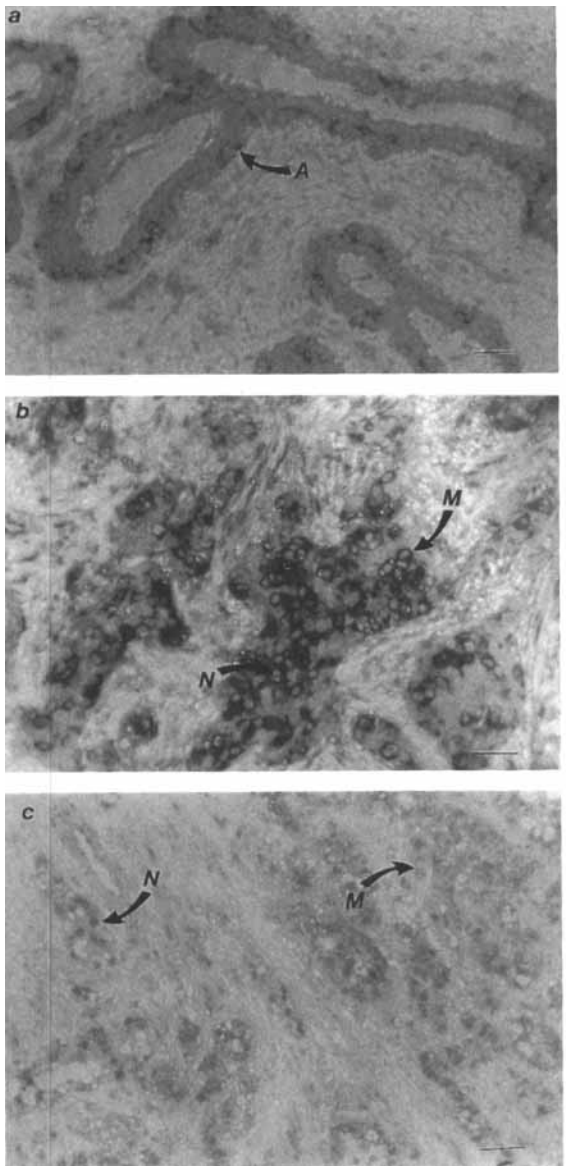

Fig. 1. Immunolocalization of uPA by MoAb 3689 in: (a) normal breast tissue, (b,c) malignant tumors. Up-regulation of uPA exhibition was observed in cancer cell membrane (M), nucleus (N), and in extracellular spaces of tumor mass. In contrast to breast cancers, normal breast tissue or benign tumor (a) showed weak staining at the apical surface of duct or lobule epithelium (A). Bar represents 45 μm.

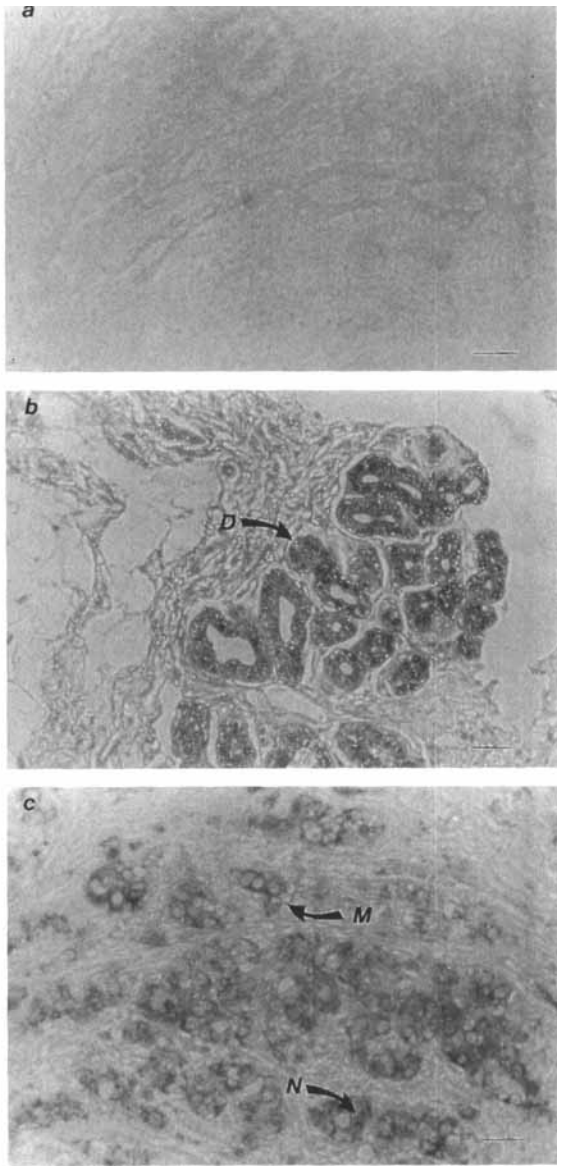

Fig. 2. Immunolocalization of uPAR by MoAb 3936 in: (a) normal breast tissue, (b) up-regulation has been observed in ductal (D) area of breast tissue, (c) breast cancer. Up-regulation of uPAR has been observed on the membrane (M) and occasionally in the nucleus (N) of the cancer cells. Weak but distinct staining (b) or no stain has been observed on some normal or benign breast tissues. Bar represents 45 μm.

served in the benign tumors. Virtually no positivity was detected in extracellular spaces. The positivity for uPAR of benign tumors was weak with the immunohistochemical score rather low, mean 2.2 ± 1.3; while the malignant tumors scored high, mean 5.3 ± 1.3 (see Fig. 6b). Those two score populations were statistically different ($t = 3.520, P = 0.005$).

Immunohistochemical PAI-1 Staining

As positive controls we used the following antibodies: 3780, 3785 for at least two different samples of normal breast, benign breast, and malignant tissues. All of them showed the same pattern of staining, but with different intensity. The antibody 3785 showed the strongest intensity of stain and was chosen for future study. Preadsorbtion of antibody 3785 with PAI-1, abol-

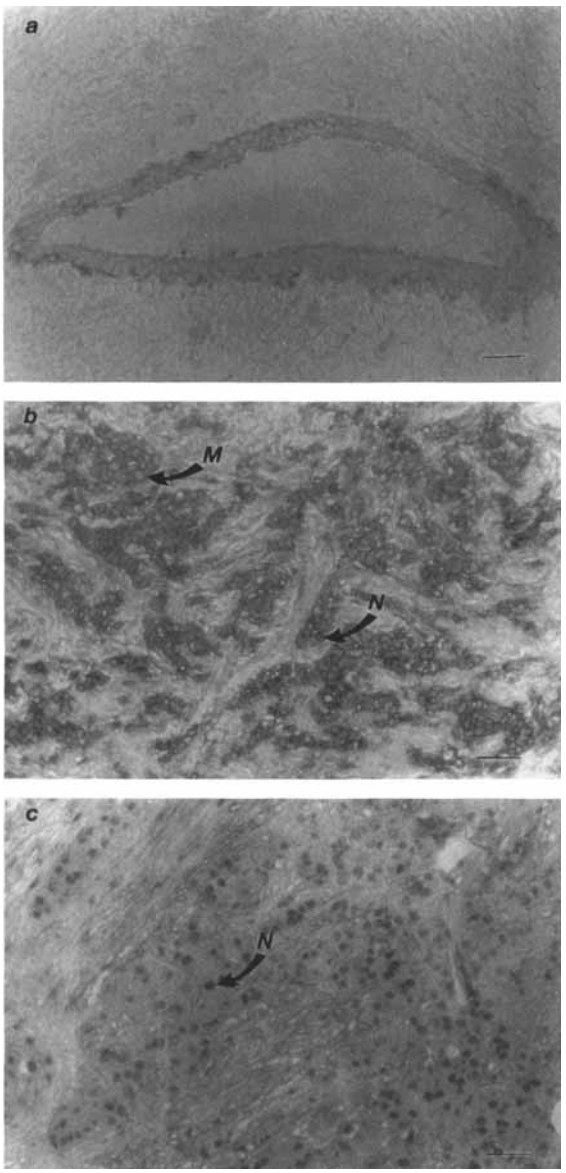

Fig. 3. Immunolocalization of PAI-1 by MoAb 3785 in: (a) normal breast tissue, (b,c) breast cancer. The antigen was detected on the cell membrane (M) and/or in the nucleus (N) of the cancer cells. Weak staining was detected on the normal or benign tissues. Bar represents 45 μm.

ishing staining of malignant breast tissues, served as negative control. Diffuse and weak staining was observed sometimes for normal tissues, especially when strong positivity was observed within the tumor for PAI-1. In 50% of benign tumors and normal breast a relatively strong positivity (4 and 5) was observed. Localization of PAI-1 was very similar to localization of uPA and uPAR (Fig. 3a). In malignant tumors different staining intensity was observed, ranging from 3 to 7. The PAI-1 can be detected in the cytoplasm, on the cell surface, and in the nucleus of the cancer cells (Fig. 3b). In two malignant tumors, the staining for PAI-1 was observed predominantly in the nucleus of the neoplastic cells (Fig. 3c). No such phenomenon was observed in normal or benign tissues. The mean immunohistochemical score of benign tumors was 3.7 ± 1.1, while the mean score of malignant tumors was 5.4 ± 1.3 (see Fig. 6c). The scores of benign and malignant tumors were statistically different ($t = 3.320, P = 0.005$).

Immunohistochemical tPA Staining

As positive controls we used the following antibodies: 373, and 374B, for at least two different samples of normal breast, benign breast, and malignant tissues. The antibodies 373 and 374B showed the same pattern of staining, but with slightly different intensity. Preadsorbtion of antibody 374B with tPA, abolishing staining of malignant breast tissues, served as negative control. The antibody 374B showed the strongest intensity of stain and was chosen for future study. Different weak and strong stain intensity was observed for tPA regardless of the benign or malignant origin of the tumor. The tPA was detected in the cytoplasm and on the cell membrane of normal and cancer cells (Fig. 4a,b,c). The mean immunohistochemical score for benign and malignant tumors was respectively: 3.0 ± 1.9 and 3.9 ± 1.7 (Fig. 6d).

The samples of benign and malignant tumors were divided into two groups: those with low scores (1–3), and those with high scores (4–7) for a given antigen. Next, we analyzed how many samples coexpress uPAR, PAI-1, or tPA in agreement with uPA (immunohistochemical score high/high or low/low). For uPA/uPAR, 4 samples scored low/low (28.6%) and 7 scored high/high (50%). For uPA/PAI-1 antigens 3 samples scored low/low (21.4%) while 8 samples scored high/high (57.2%). No such correlation could be found for uPA/tPA activators. The low/low score was found in 2 samples (18.2%), and high/high score in 3 samples (27.3%).

DISCUSSION

These studies were undertaken to test the hypothesis that uPA, uPAR, PAI-1, and tPA activity would be present in high levels in malignant human breast tumors, suggesting that the plasminogen activator system functions in the tumor invasion and/or metastatic processes. The results support the hypothesis that uPA, uPAR,

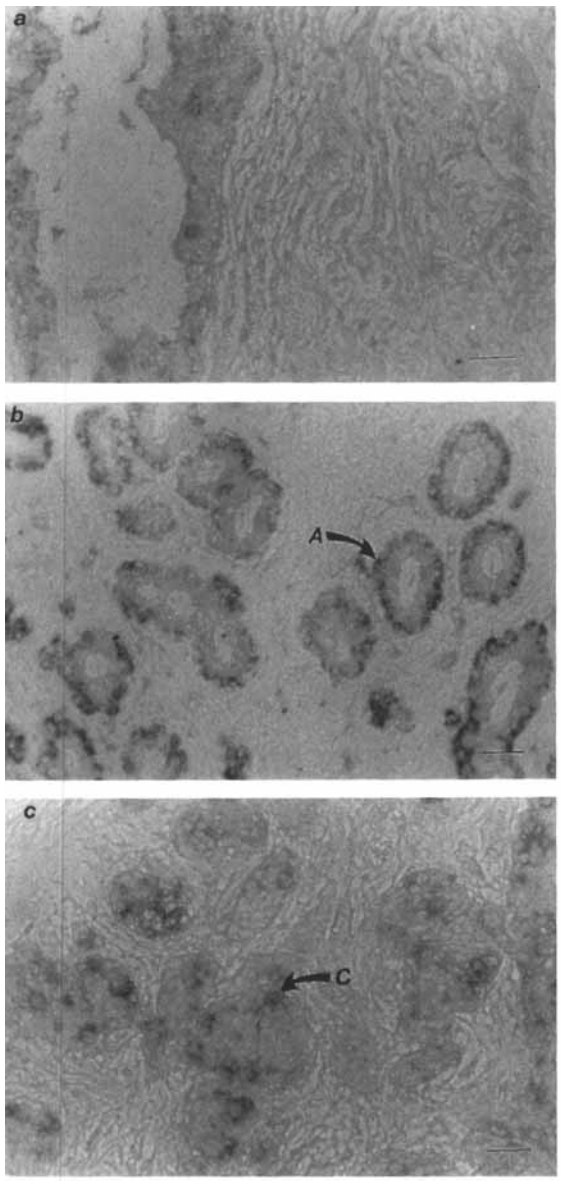

Fig. 4. Immunolocalization of tPA by MoAb 374B in: (a) normal breast tissue, (b) benign breast tissue, (c) malignant breast tissue. Diffuse staining has been observed within the tumor mass and in the cytoplasm of the cancer cells. Visualization of uPA, uPAR, PAI-1, tPA, was done on different tumors (Figs. 1–4). Bar represents 45 μm.

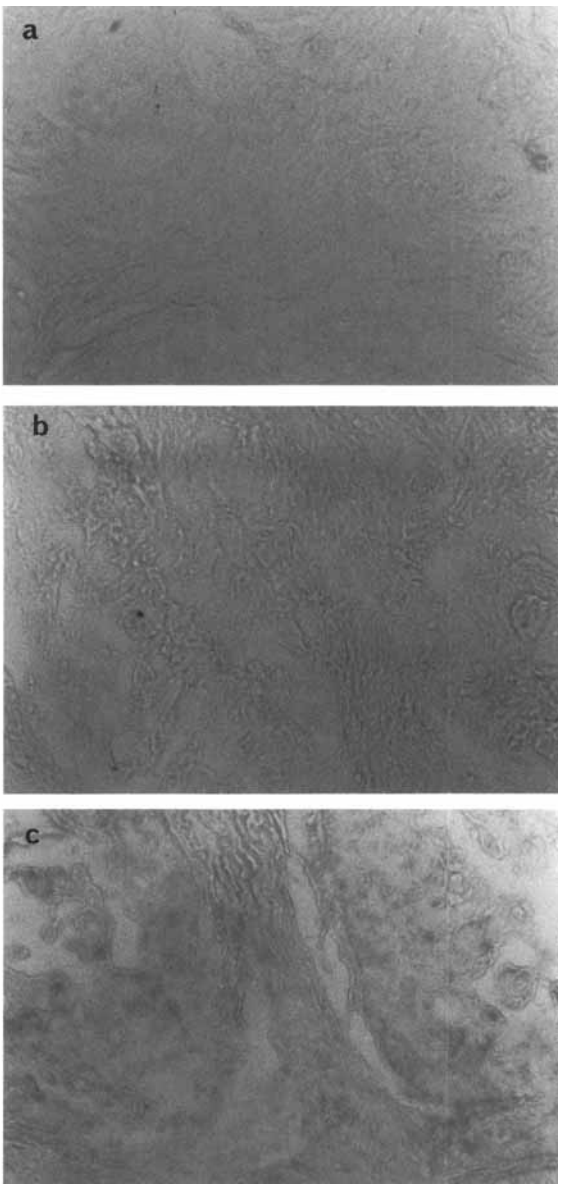

Fig. 5. Negative controls of malignant breast tissues incubated with monoclonal antibodies preincubated with its ligands: (a) monoclonal antibody 3689 preincubated with uPA, (b) monoclonal antibody 3785 preincubated with PAI-1, (c) monoclonal antibody 374B preincubated with tPA.

PAI-1, but not tPA, are overexpressed as determined by immunohistology using monoclonal antibodies 3689, 3936, 3785, and 374B. Neoplastic tissues of breast were found to exhibit elevated levels of uPA, uPAR, PAI-1, and tPA. Only overexpression of uPA, uPAR, and PAI-1 have been found to correlate well with the malignant state of tumor cells. High exhibition of tPA found in cancer seems to be random and not related to the malignant or benign state, since benign and malignant tumors show overexpression of tPA with similar frequency.

Human carcinomas originating from colon [Nishino et al., 1988], stomach [Nishino et al., 1988], uterus [Larsson et al., 1987], ovary [Saito et al., 1990], breast [Duffy et al., 1990; Jänicke et al., 1991; Schmitt et al., 1992a], and prostate [Gaylis et al., 1989] have been found to be positive for uPA by histological evaluation. These observations, as well as an in vitro study [Jankun

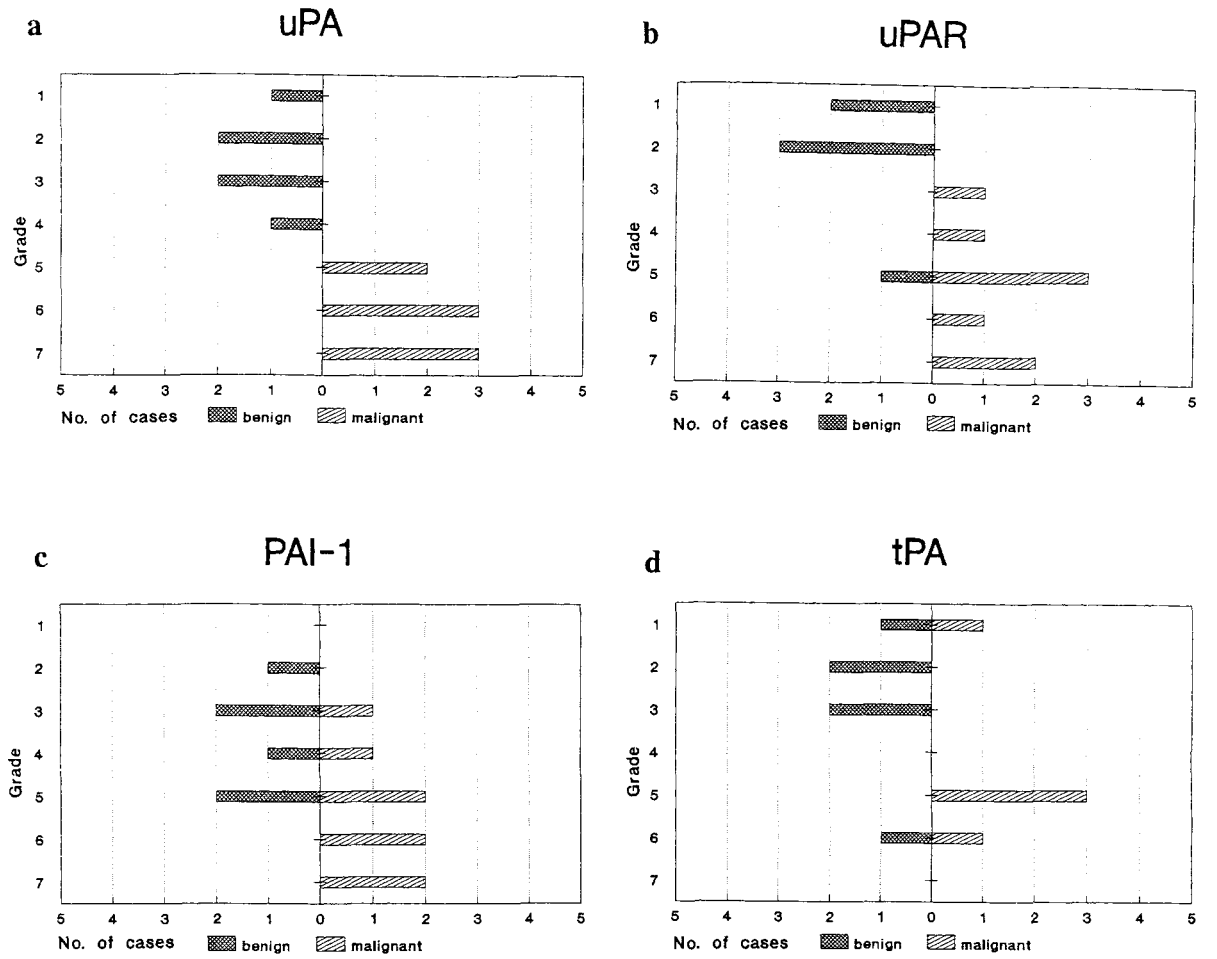

Fig. 6. Occurrence of immunohistochemical grade staining of benign and malignant breast tissues for: a, uPA; b, uPAR; c, PAI-1; d, tPA.

et al., 1991], illustrate that malignant transformation of cells is associated with an increased intracellular content of uPA, an antigen which is only sporadically observed in their normal tissue counterparts [Sier et al., 1991]. With respect to the subcellular localization of uPA, we found a diffuse cytoplasmic staining in the cytoplasm (benign and malignant tumors), and/or cell membrane staining of uPA that is in accordance with most other studies [Duffy et al., 1990; Erickson, 1991; Jänicke et al., 1991; Schmitt et al., 1992a,b]. Presence of the uPA in the cytoplasm and on the cell membrane correlates well with the theory of uPA localization and activation [Blasi, 1988; Danø et al., 1992]. Localization of uPA in the nucleus was not reported; however, appearance of anti-uPA staining is obvious from some photographs of other investigators [Erickson, 1991; Schmitt et al., 1992b]. In contrast to one report [Danø et al., 1992] we do not find any strongly positive normal cells or macrophages in tissue adjacent to the tumor. The lack of staining for uPA in this study can be associated with the type of antibody used (e.g., not able to detect uPA that is receptor-bound and/or complexed with PAI-1) and it was reported by others [Jänicke et al., 1989; Kohga et al., 1989; Sier et al., 1991]. Light occasional staining of normal tissue for uPA could be associated with uPA secreted by cancer cells, or can be positive in granulocytes [Jänicke et al., 1989], natural-killer cells [Capoen et al., 1986], and fibroblasts or macrophage-like cells [Larsson et al., 1987].

While systematic overexpression of uPA in breast cancer has been abundantly documented, histopathological localization of uPAR in breast cancer has not been studied widely. In fact most studies were conducted on tumor extracts or cell lines [Blasi, 1988; Chucholowski et al., 1992;

Capoen et al., 1986]. Similarly, as in the case of uPA, uPAR is overexpressed by cancer cells. Benign tumors do not express uPAR at all, or express it in very small amounts. Overexpression of uPAR in a breast cancer cell line has already been documented [Chucholowski et al., 1986], but there has been no systematic histopathological study of its localization. The necessary presence of the cell surface uPA localizing molecule, uPAR, in cancer invasion and metastasis was anticipated and some evidence already exists [Jankun et al., 1991]. It appears that one of the functions of uPAR, in addition to simply providing a mechanism for confining uPA to the cell surface, is to localize uPA to discrete areas of the cell surface [Ellis and Danø 1992]. In this way uPAR would direct proteolytic activity against normal tissue and would spare the cancer cells expressing it. We analyzed all our samples to find evidence of such localization. However, only in some cases, when uPAR is present in moderate amounts, can such localization be detected. In cases of overexpression of this antigen, it was not only localized on the cell surface of the tumor's leading edge, but present in all cancer cells and sometimes in the nucleus of the cancer cell. We were not able to confirm the fact of exhibition of uPAR in the nucleus of cancer cells.

The levels of exhibition of PAI-1 in benign and malignant tumors were relatively high and exhibition of this antigen was higher in malignant breast tumors than in the benign tumors. This fact was observed previously in tumor extracts [Chucholowski et al., 1986; Jänicke et al., 1991; Schmitt et al., 1992a]. In general PAI-1 was expressed in the same extracellular compartments as was uPA or uPAR. However, in two cases of malignant tumors we find PAI-1 to be present predominantly in the nucleus of the cancer cells. This somewhat surprising finding may explain why some malignant breast tumors expressing high amounts of uPA and PAI-1 (10% of all node-negative breast cancers) are at high risk of metastasis and relapse of the disease [Jänicke et al., 1991]. As pointed out in the introduction, high coexpression of uPA and PAI-1 is correlated with a poor prognosis for patients with breast cancer. This finding is somewhat unexpected and contradictory, since coexpression of activator and inhibitor should quench activity of uPA and consequently depress proteolytic activity driven by this enzyme, thus diminishing metastasis. Clinical observations seem to show different facts. On the basis of our findings we propose the following possible explanation: in the case of exhibition of high amounts of uPA and uPAR, the uPA is bound to its receptor and remains on the cell surface for prolonged periods of time [Blasi, 1988; Cubelis et al., 1989]. When PAI-1 is available, it can bind to the uPA/uPAR receptor complex triggering the internalization of the PAI-1/uPA/uPAR complex by receptor-mediated endocytosis. The PAI-1/uPA/uPAR complex will be dissociated and PAI-1 and uPA will be digested, but receptor will be recycled to the cell surface and concentrate the uPA (if available) on the cell surface again [Kohga et al., 1989; Quax et al., 1992]. This process will lead to clearing of the PAI-1 from the vicinity of the cell surface. It is not clear why PAI-1 is concentrated in the nucleus of the cancer cell. However, many receptor-binding proteins, like protein hormones and growth factors, bind to the receptors and endocytose. It is, therefore, conceivable that such protein signaling (or their degradation products) acts directly within the cell, or cell nucleus, like the steroid hormones [Graeff et al., 1992]. If so, it may be that internalized PAI-1 or its fragment can induce some changes within the cells. Indeed, it has been reported that the receptor-mediated internalization of PAI-1/uPA/uPAR complexes may trigger the proliferation of the cancer cells [Graeff et al., 1992; Kirchheimer et al., 1987, 1989]. This hypothesis is consistent with findings that high exhibition of PAI-1 was statistically correlated with S-phase of cancer cells [Graeff et al., 1992]. Furthermore, endocytosis exposes protein to acidic proteases of lysosomes. This leads to random proteolytic cleavage and production of different protein fragments. It is possible that some undigested fragments of uPA or uPAR will still be complexed with fragments of PAI-1. If so, we should detect some positivity for uPA and/or uPAR in the cell nucleus. Indeed, uPA and uPAR occasionally have been detected in the nucleus of cells expressing strong PAI-1 positivity in the cell nucleus, but not on the cells showing no exhibition of it.

When the tumors exhibit high amounts of uPA, they exhibit a high amount of uPAR in 50% of cases and PAI-1 in 57.3% of cases. When uPA is expressed in low amount, the uPAR is low in 28.6% and PAI-1 in 21.4%. This statisti-

cally significant consensus, 78.6% in a case of uPA/uPAR and 78.6% in a case of uPA/PAI-1, suggests that activity of uPA, uPAR, and PAI-1 may be the result of a unique mechanism of control, activated in the last steps or step of carcinogenesis. This regulatory mechanism or pathway is not known yet, but is in agreement with our results and has been postulated by others [Chucholowski et al., 1992; Jankun et al., 1991].

The present findings are of importance in clinical evaluation of risk factors associated with breast cancer. We postulate that, in addition to the widely accepted risk factors of uPA and PAI-1, exhibition of the receptor for urokinase plasminogen activator should be included in future trials of risk for metastasis and relapse of the disease. The uPA, uPAR, and PAI-1 should be treated as a system and not as individual proteins, and may provide additional evidence on the basis of which to better predict the outcome of the disease.

ACKNOWLEDGMENTS

We are grateful to Dr. Richard Hart (President of American Diagnostica, Inc.) for the generous supply of antibodies used in this study. We would like to acknowledge the helpful advice and critical comments on the manuscript of Dr. Jonathan Myles, and we also thank Dr. Jim Hampton for critical comments on the manuscript (Medical College of Ohio). This work was supported in part by grants from: American Cancer Society, Inc., Ohio Division; American Diagnostica, Inc.; and NIH (BSRG 978303-SOC) to J.J.

REFERENCES

Blasi F (1988): Surface receptors for urokinase plasminogen activator receptor. Fibrinolysis 2:73–84.

Capoen O, Saksela O, Saksela E (1986): Identification and localization of urokinase-plasminogen activator in human NK-cells. Int J Cancer 38:355–360.

Chucholowski N, Schmitt M, Rettenberger P, Schüren E, Moniwa N, Groetzki L, Wilhelm O, Weildle U, Jänicke F, Graeff H (1992): Flow cytofluorometric analysis of the urokinase receptor (uPAR) on tumor cells by fluorescent uPA-ligand or monoclonal antibody 3936. Fibrinolysis 6(Suppl 4):95–102.

Cubelis MV, Andreasen P, Ragno P, Mayer M, Danø K, Blasi F (1989): Accessibility of receptor-bound urokinase to type-1 plasminogen activator inhibitor. Proc Natl Acad Sci USA 86:4828–4832.

Danø K, Andreasen PA, Grondhal-Hansen J, Kristensen P, Nielsen LS, Skriver L (1985): Plasminogen activators, tissue degradation and cancer. Adv Cancer Res 44:139–266.

Danø K, Behrendt N, Ellis V, Eriksen J, Høyer-Hansen J, Kristensen P, Lund LR, Nielsen BS, Ploug M, Pyke C, Rønne E, Blasi F (1992): The role of uPA and its receptor in tissue remodeling and tumor invasion. Fibrinolysis 6(Suppl 2):10.

Duffy MJ, Reilley D, O'Sullivan C, O'Higgins N, Fennelly JJ, Andreasen P (1990): Urokinase plasminogen activator, a new and independent prognostic marker in breast cancer. Cancer Res 50:6827–6829.

Ellis V, Danø K (1992): The urokinase receptor and the regulation of cell surface plasminogen activation. Fibrinolysis 6(Suppl 4):27–34.

Erickson D (1991): Metastatic machinations. Sci Am (July) 110–111.

Evers JL, Patel J, Madeja JM, Schneider SL, Hobika GH, Camiolo SM, Markus G (1982): Plasminogen activator activity and composition in human breast cancer. Cancer Res 42:219–226.

Gaylis FD, Keer HN, Wilson MJ, Kwaan HC, Sinha AA, Kozlowski JM (1989): Plasminogen activators in human prostate cancer cell lines and tumors. Correlation with aggressive phenotype. J Urol 142:193–198.

Graeff H, Harbeck L, Pache O, Wilhelm O, Jänicke F, Schmitt M (1992): Prognostic impact and clinical relevance of tumor-associated proteases in breast cancer. Fibrinolysis 6(Suppl 4): 45–53.

Jänicke F, Schmitt M, Hafter R, Hollrieder A, Babic R, Ulm K, Gössner K, Graeff H (1990): The urokinase plasminogen activator (uPA) is a potent predictor of early relapse in breast cancer. Fibrinolysis 4:69–98.

Jankun J, Maher VM, McCormick JJ (1991): Malignant transformation of human fibroblasts correlates with increased activity of receptor-bound plasminogen activator. Cancer Res 51:1221–1226.

Jensen PJ, Wheelock MJ (1992): Regulation of urokinase plasminogen activator localization in keratinocytes by calcium ion and E-cadherin. Exp Cell Res 202(1):190–198.

Kirchheimer JC, Wojta J, Hienert G, Christ G, Heger ME, Pflüger H, Binder BR (1987): Effect of urokinase on the proliferation of primary cultures of human prostatic cells. Thromb Res 48:291–298.

Kirchheimer JC, Wojta J, Christ G, Binder BR (1989): Functional inhibition of endogenously produced urokinase decreases cell proliferation in human melanoma cell line. Proc Natl Acad Sci USA 86:5424–5428.

Kohga S, Harvey SR, Suzumiya J, Sumiyoshi A, Markus G (1989): Comparison of the immunohistochemical localization of urokinase in normal and cancerous human colon tissue. Fibrinolysis 3:17–22.

Larsson G, Larsson A, Astedt B (1987): Tissue plasminogen activator and urokinase in normal, diaplastic, and cancerous squamous epithelium and uterine cervix. Thromb Haemost 58:822–826.

Mangel WF (1990): Better reception for urokinase. Nature 344:488–489.

Nishino N, Aoki K, Tokura Y, Sakaguchi S, Takada Y, Takada A (1988): The urokinase type of plasminogen activator in cancer of digestive tracts. Tromb Res 50:527–535.

Quax PHA, van Muijen GNP, Pedersen N, de Bart ACW, Schrauwen Y, Barlowatz-Meimon G, Blasi F, Verheijen JH (1992): The plasminogen activator system in extracellular matrix degradation. Fibrinolysis 6(Suppl 4):41–44.

Runyon PR, Haber A (1983): " Fundamentals of Behavioral Statistics." London: Assison-Weley Publishing Company.

Saito K, Nagashima M, Iwata M (1990): The concentration of tissue plasminogen activator and urokinase in plasma of patients with ovarian and uterine tumors. Thromb Res 58:355–366.

Schmitt M, Jänicke F, Graeff H (1992a): Tumor associated proteases. Fibrinolysis 6(Suppl 4):3–26.

Schmitt M, Jänicke F, Pache L, Harbeck N, Graeff H (1992b): Urokinase (uPA) is a strong independent prognostic factor in node-negative breast cancer. Fibrinolysis 6(Suppl 2):57.

Sier CF, Fellbaum C, Verspaget HW, Schmitt M, Griffoen G, Graeff H, Höfler H, Lamers BH (1991): Immunolocalization of urokinase plasminogen activator in adenomas and carcinomas of colorectum. Histopathology 19:231–237.

Tandon AT, Clark GM, Chamnes GC, Chrigwin JM, McGuire WL (1990): Cathepsin D and prognosis in breast cancer. N Engl J Med 322:297–302.

Wordinger RJ, Miller GW, Nicodemus DS (1987): "Manual of Immunoperoxidase Techniques." Chicago: American Society of Clinical Pathologists Press Chicago.