Virchows Archiv A Pathol Anat (1992) 421:79–86

Virchows Archiv A
Pathological Anatomy and Histopathology
© Springer-Verlag 1992

Immunohistological detection of tissue factor in normal and abnormal human mammary glands using monoclonal antibodies

Uwe Sturm, Thomas Luther, Sybille Albrecht, Conrad Flössel, Heinz Großmann, and Martin Müller

Institute of Pathology, Medical Academy "Carl Gustav Carus", Fetscherstrasse 74, 0-8019 Dresden, Federal Republic of Germany

Received September 28, 1991 / Received after revision February 25, 1992 / Accepted February 26, 1992

Summary. Tissue factor (TF) is the primary cell-bound initiator of the coagulation protease cascade. The cytological distribution of TF in various tissues may be described on the basis of immunohistochemistry with epitope-defined monoclonal antibodies and the extravascular distribution of TF apparently represents a haemostatic envelope ready to activate coagulation when vascular integrity is disrupted. The present study localized TF in human breast cancer tissues when compared with normal breast gland tissues and benign disorders of the mammary gland. By use of a cocktail of three epitope-defined monoclonal antibodies, TF was detected only in the myoepithelia of the resting breast gland. In proliferating disorders like fibrocystic disease or in fibroadenomas, both myoepithelia and luminal epithelia showed TF expression. Of 115 breast cancers 93 reacted with anti-TF, in an inhomogeneous manner in terms of intensity and number of positive cells. There was a tendency for more positive and intensely stained cells to be found in well-differentiated structures such as tubules. Invasive ductal carcinomas exhibiting more positive and more strongly stained cells were less commonly metastatic to lymph nodes when compared with the tumours with no detectable or very low TF immunostaining. A semiquantitatively recorded score of TF immunostaining correlated with the procoagulatory activity measured (7 fibroadenomas and 24 carcinomas). The results of this study suggest that proliferation and differentiation of the mammary gland is associated with enhanced TF expression in the epithelia which are negative for TF staining in the resting gland. Malignant growth is characterized by randomly expressed epithelial TF, which expression is enhanced and more frequent in well-differentiated tumour cells.

Key words: Tissue factor – Immunohistology – Breast cancer

Correspondence to: M. Müller

Introduction

Tissue factor (TF) is a 47 kDa transmembrane glycoprotein which plays a central role in triggering the extrinsic pathway of fibrin coagulation. TF is a high-affinity cell surface receptor and essential co-factor for clotting factors VII/VIIa. The bimolecular complex of TF and factors VII/VIIa results in catalytic enhancement of the factor VIIa catalytic domain and activates the coagulation cascade in association with factor X, leading to thrombin generation and fibrin sponge formation (Nemerson and Bach 1982). TF is believed to have a key function in haemostasis and thrombogenesis (Nemerson 1988; Weiss and Lages 1988). Edgington et al. (1991) first reported the primary human structure of the TF gene, mRNA and protein. Whereas TF is constitutively expressed in various cells of extravascular tissues, it can be induced to become expressed in monocytes and endothelia by various mediators (Edwards et al. 1979; Levy et al. 1981; Bevilacqua et al. 1984; Gregory and Edgington 1985; Nawroth and Stern 1986; Drake et al. 1989).

It has only recently become possible to localize TF at the cellular level by means of immunohistological techniques. It has been demonstrated in the cells of a number of different normal human tissues, such as epidermis, kidney, brain, blood vessels, heart muscle etc. (Drake et al. 1989; Fleck et al. 1990). TF has also been detected in human atherosclerotic plaques by immunohistochemistry. It is possible that the deposition of TF in atherosclerotic plaques contributes to thrombogenicity of atherosclerotic vessels (Wilcox et al. 1989), although it has been reported by Hartzell et al. (1989) that TF may also play a role in cell growth. Furthermore, the results of Hu et al. (1990) have given further support to the latter hypothesis. To date, there are no data on TF expression and cellular distribution in tumour tissues at the histological level. The expression of TF on cancer cells may be of interest in view of tumour cell differentiation and interactions between cancer cells and extracellular environment.

Therefore, the aim of the present work was to study the expression of TF in human breast cancer tissues when compared with a series of normal human mammary glands and benign breast lesions.

Materials and methods

Unfixed specimens from human mammary glands were obtained from biopsies. One hundred and fifteen breast tissue specimens from patients with carcinomas and 17 mammary gland biopsies from patients with a variety of other breast diseases, including fiboradenoma ($n=10$), mastopathy ($n=6$), and cystosarcoma phyllodes ($n=1$), were tested. Normal human breast tissue (13-, 26-, and 30-year-old individuals) and 1 mammary gland from early pregnancy (gestational age 6 weeks) were obtained at necropsy (24 h post mortem). The numbers of cases and the tissues investigated are see in Tables 1 and 2. Representative parts of the respective specimens were sectioned (cryostat section 5 μm), and fixed in buffered formalin at 4° C for 4 min. The glass slides had been coated before with poly-L-lysine (0.1%; Sigma, St. Louis, Mo., USA). Corresponding tissue parts were fixed in 4% neutral formalin and embedded in paraffin. Other corresponding parts were processed for the measurement of procoagulant activity (see below). The histological typing was performed according to the World Health Organization nomenclature (Sobin 1981). The lymph node status of breast carcinoma patients was verified by histopathological examinations of all detectable axillary lymph nodes after mastectomy.

Balb/c mice were immunized with tissue factor apoprotein purified by factor VII affinity chromatography according to Rao and Rapaport (1987). Hybridomas were produced by standard procedure (Campbell 1987). Briefly, 4 days after last immunization, spleen cells were fused with mouse myeloma cells P3 Ag 8.653.1 with polyethylene glycol MW 3–37 kDa (Sigma). The fused cells were cultured in HAT (1×10^{-4} M hypoxanthine, 4×10^{-7} M aminopterine, 3×10^{-5} M thymidine; Serva, Heidelberg, FRG) supplemented RPMI 1640 (Serva) with 20% fetal calf serum (SIFIN, Berlin, FRG) in 96-well culture plates (Flow Laboratories, Irvine, Scotland).

Hybridomas were tested against tissue factor apoprotein in a solid-phase enzyme-linked immunosorbent assay (ELISA) after 2 weeks. Positive hybridomas were cloned twice. Seven clones were expanded and characterized. Antibodies were determined as IgG_1 with subclone antibodies (Sigma) by an ELISA.

The characteristics of the antibodies used in the study are shown in Table 1. In the present investigation, we used a well-characterized cocktail of monoclonal antibodies to examine tissue factor expression in comparison with our own monoclonal antibodies against TF. In normal tissues, such as kidney and epidermis, the immunostaining with both cocktails of antibodies was generally identical. No substantial differences were noted. Epithelial and mesangial cells of the renal glomeruli reacted very intensely.

The tissue slices from mammary glands were incubated overnight in humidified chambers at 4° C. The ABC technique was applied using biotinylated anti-mouse immunoglobulins and the avidin-biotin-peroxidase complex (Vectastain ABC Kit, Vector Laboratories, Burlingame, Calif., USA), according to the manufacturer's information sheet (Hsu et al. 1981). For the streptavidin method (Bonnard et al. 1984), biotinylated sheep anti-mouse immunoglobulins [diluted 1:500 in phosphate-buffered saline (PBS)] and biotinylated peroxidase streptavidin complex (diluted 1:200 in PBS) were obtained from Amersham International (Buckinghamshire, England). Endogenous peroxidase was blocked by a solution of 5% dry milk solids, 1% normal swine serum, 0.1% sodium

Table 1. Antibodies used in this study for immunostaining of tissue factor (TF)

Specificity	Animal source	Hybridoma culture supernatants	Dilution	Reference
TF	Mouse	Cocktail of three monoclonal antibodies: VI C12/B8/D8 VI C7/D3/G8 V D8/H7/G11	1:5	Own
TF	Mouse	Cocktail of three monoclonal antibodies: TF9-9C3 TF9-9B4 TF9-10H10	undiluted 1:10	Drake et al. (1989)

Table 2. Immunostaining results for TF in benign tumours and mastopathy

Histological type	Number	Immunohistochemical detection of TF in benign tumours and mastopathy (intensity/case tested)			
		None	Weak	Moderate	Strong
Intracanalicular fibroadenoma	7		5	1	1
Pericanalicular fibroadenoma	3		2	1	
Cystosarcoma phyllodes	1		1		
Mastopathy	6		2	3	1

azide in solution of 0.01 M/l TRIS-HCl, 0.14 M/l NaCl, pH 7.4 (for 30 min, prior to incubation with the monoclonal antibodies). Controls consisted of sections with the primary antibody replaced by PBS, pH 7.6. All further incubations were performed in humidified chambers at 37° C for 30 min. After treatment with each the first and second antibody the slides were thoroughly washed in three changes of PBS (pH 7.6) under stirring. Peroxidase activity was developed by use of 3,3′-diaminobenzidine (Serva) and hydrogen peroxide.

Sections from all specimen were stained also for the presence of alpha-1-antitrypsin and lysozyme as described earlier using the indirect immunoperoxidase technique (Zotter et al. 1985). Deparaffinized 5-µm sections were covered with normal swine serum (24° C for 20 min), incubated with anti-alpha-1-antitrypsin (DAKO, Copenhagen, Denmark; diluted 1:500 in PBS containing 1% normal swine serum, at 24° C for 4 hours) or anti-lysozyme (Behring, Marburg, FRG; diluted 1:50 in PBS containing 1% normal swine serum at 24° C for 4 h). Endogenous peroxidase was blocked by methanol with 0.1% hydrogen peroxide (for 5 min; after incubation with polyclonal antibodies). Peroxidase-labelled goat anti-rabbit immunoglobulin (DAKO) was applied as second antibody (diluted 1:200 at 24° C for 30 min). For negative controls, PBS was used instead of the polyclonal antibodies. Washings were done in PBS, pH 7.6, under stirring. The substrate used was 3,3′-diaminobenzidine.

Tissue standard slices known to be positive for TF (normal human kidney and skin from fresh autopsy materials), alpha-1-antitrypsin (cirrhosis in alpha-1-antitrypsin deficiency), and lysozyme (malignant fibrous histiocytoma) were included in each of the tests as positive controls. Counterstaining was carried out with haematoxylin.

Staining intensity of the immunohistochemical reaction was recorded as none, minute, weak, moderate or strong. For the evaluation of the relative number of stained cells a semi-quantitative score was set up: a mixed pattern of TF positive and negative tumour cells was subdivided into less than 10%, 10–50%, and more than 50% of the cells expressing TF.

Numerical score values were assigned in relation to the staining intensity and the semi-quantitative score, namely, 0 for none, 0.25 for minute and less than 10%, 0.50 for minute and 10–50%, 0.75 for minute and more than 50% of tumour cells, 1 for weak and less than 10%, 2 for moderate and less than 10%, 3 for strong and less than 10%, 4 for weak and 10–50%, 5 for moderate and 10–50%, 6 for strong and 10–50%, 7 for weak and more than 50%, 8 for moderate and more than 50%, and 9 for strong and more than 50% of tumour cells positive.

In a limited study (7 benign and 24 malignant tumours of the breast), the immunohistologically revealed numerical score of TF expression was compared with the procoagulant activity of cell suspensions made from the very same samples. Preparation of cell suspensions and measurement of procoagulant activity (PCA) were done in a separate laboratory. PCA values and numerical score values were not compared until the whole study had been finished.

From part of the fresh tumour specimens a cell suspension was prepared by mechanical mincing in PBS. Large fragments were removed by sedimentation in a centrifugation tube. The cells were pelleted, resuspended, adjusted to a concentration of 1×10^6/ml and again centrifugated at 1400 *g* for 10 min. The pellets were frozen at −25° C. To measure the PCA, 30 µl of cell disruption buffer (15 mM/l Octyl-Glycopyranoside (Sigma) in 0.85% NaCl +25 mM/l HEPES (Sigma)) were added to the thawed cell pellets. After incubation for 15 min at 37° C the cell pellets were diluted with 70 µl cell suspension buffer (25 mM/l HEPES in 0.85% NaCl). The 100 µl of cell samples were mixed with 100 µl of citrated platelet-poor human plasma and prewarmed at 37° C for 1 min. The clotting reaction was started by adding 100 µl of 25 mM/l CaCl. The time to form a visible clot under constant stirring was recorded and converted to milliunits (mU) per millilitre per 10^6 cells of PCA by reference to a coagulation standard curve derived from serial dilutions of human brain thrombokinase (AWD, Dresden, FRG). A value of 10^5 mU/ml × 10 cells (= 50 s) was assigned to thromboplastin at 200 µg (dry weight)/ml. All probes were set up and measured as triplets.

Regression analysis was performed by a bivariate method. The results were compared statistically by the *t*-test for correlation. Various proportions were compared using the chi-squared test.

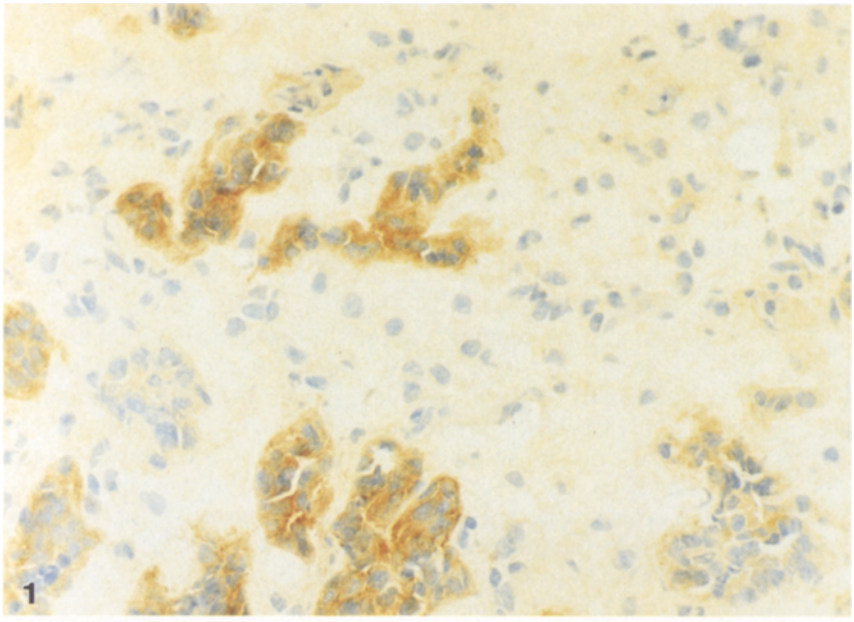
Results

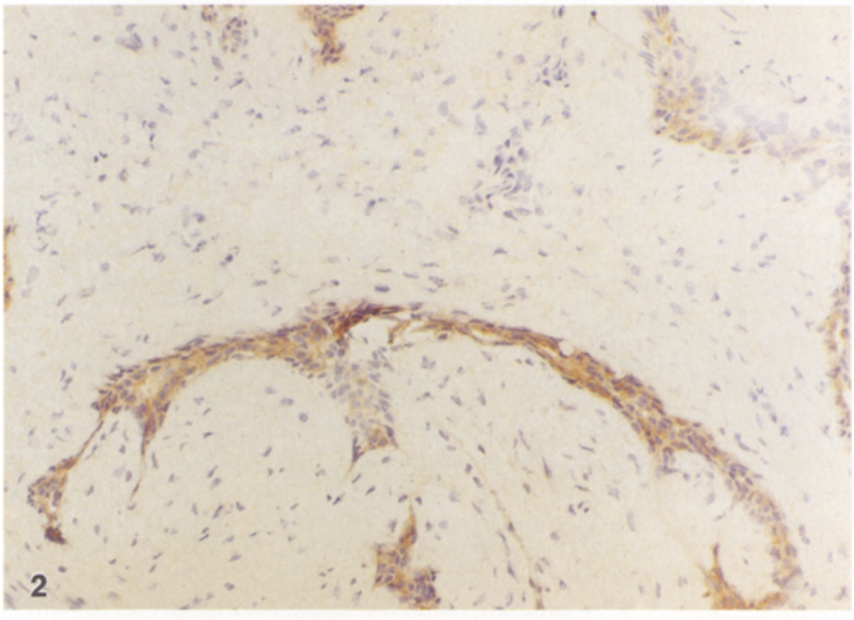
In resting mammary gland the ductal myoepithelium was weakly positive for TF. No reactivity was observed in ductal epithelia or acini. The pregnancy-stimulated breast showed a strongly positive reaction in the epithelial cells of the inner layer and in the myoepithelial cells of the ducts. The proliferating acini of this gland exhibited a different TF distribution pattern: strongly diffuse cell staining was seen in about 40% of acinar cells, a moderate reaction was found in 50%, and weak cytoplasmic staining was seen in about 10% of all acini (Fig. 1).

In fibrocystic diseases, reactions for TF were observed in the luminal layer cells in more heterogeneous fashion and in the myoepithelial cells of ductal structures. Most of the ductal luminal cells were negative. The positive cells exhibited cytoplasmic staining. Pink cells were always completely negative. In 2 of 4 cases, acini showed focal TF expression which was not associated with particular histological patterns. Mesenchymal tissue present in mastopathy and mammary glands was TF-negative.

In fibroadenomas, TF was expressed in the epithelial component only. In almost all of them staining was observed in epithelium and myoepithelium though the intensity of the reaction varied in different tumour regions (Fig. 2). Some lesions exhibited a weak cytoplasmic staining. No immunostaining was noted in the mesenchymal component of fibroadenomas and the cystosarcoma phyllodes. The results obtained for benign tumours and mastopathy are listed in Table 2.

As summarized in Table 3, 93 out of 115 breast carcinomas exhibited some staining for TF. Staining intensity and patterns of distribution were independent of tumour localization, tumour size, and the age of the patients. TF-positive cells were observed in well-differentiated carcinomas as well as in poorly differentiated ones. Twelve and 32 of the 93 TF-positive breast cancers gave minute and weak immunostaining in up to 50% of the tumour cells only. Twenty-seven and 20 of the TF-positive carcinomas showed moderate or strong staining. In only 7 of these samples did the tumour parenchyma exhibit TF in more than 50% of the cells (Fig. 3). In no case was TF expression seen in all neoplastic cells. Occasionally, distinct areas of these tumours were found to be completely negative. Tubular structures formed in invasive ductal carcinomas were almost always positive. Solid structures were predominantly negative. Scirrhous patterns were characterized by TF-negative as well as positive cells. In invasive ductal carcinomas with intraductal components, the staining pattern appeared interesting, as the myoepithelial remnants of the ducts were most strongly positive for TF. In some of these cases,





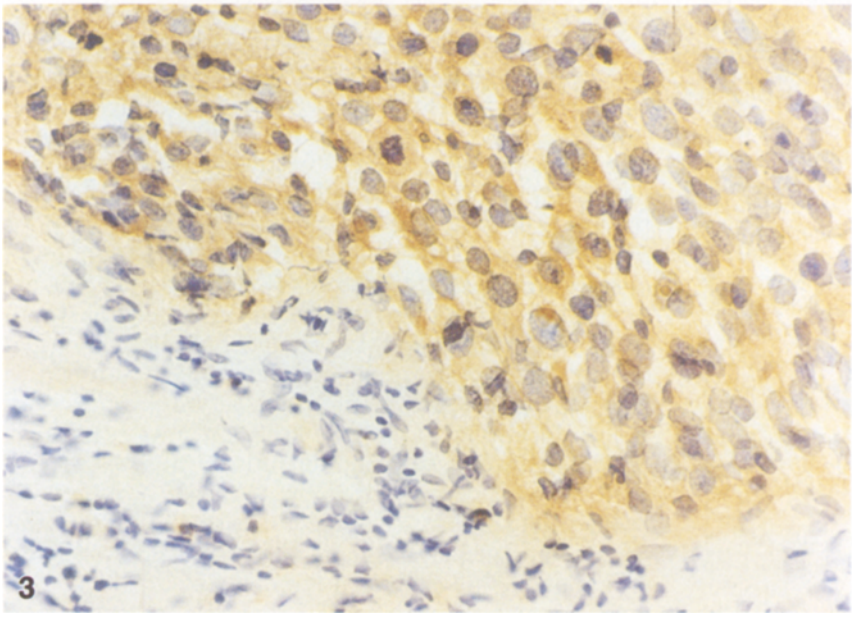


Fig. 1. Pregnancy-stimulated mammary gland. Anti-tissue factor (TF). The acinous cells show a strong and diffuse cytoplasmic immunostaining. ×40

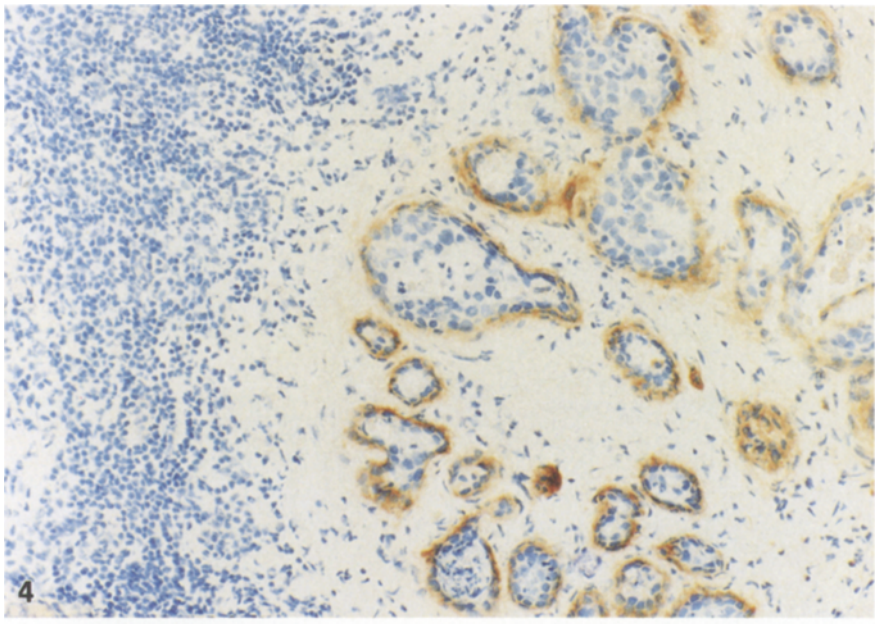
Fig. 2. Fibroadenoma incubated with antibodies to TF. Epithelial cells are TF-positive although the intensity of reaction varies. ×20

Fig. 3. Invasive ductal carcinoma. TF antibodies react with the cytoplasm of many tumour cells. ×40

Table 3. Immunostaining results of TF in breast carcinomas

Histological type	Number	Immunohistochemical detection of TF in breast cancers (cases positive with lymph-node metastases/cases tested)												
		None		Minute			Weak			Moderate			Strong	
			<10%[a]	10–50%[a]	>50%[a]	<10%[a]	10–50%[a]	>50%[a]	<10%[a]	10–50%[a]	>50%[a]	<10%	10–50%[1]	>50%[a]
Intraductal carcinoma	2	0/1					0/1							
Invasive ductal carcinoma	62	8/10	4/5	1/1		12/16	2/2		9/10	1/9		1/1	0/8	
– with intraductal component	24	1/2	2/2			0/3	2/4			0/2	1/4	0/1	1/4	0/2
– with medullary structures	5	2/2		0/2			0/1							
– with mucinous structures	1	0/1												
Invasive lobular carcinoma	15	3/5	1/2				2/4	0/1		0/1		2/2		
Invasive ductal carcinoma and invasive lobular carcinoma	3					1/1						0/1		1/1
Intraductal carcinoma and invasive lobular carcinoma	1									0/1				
Medullary carcinoma	3	0/1					0/1		1/1					
Total	115													

[a] Percentage of tumour cells expressing TF



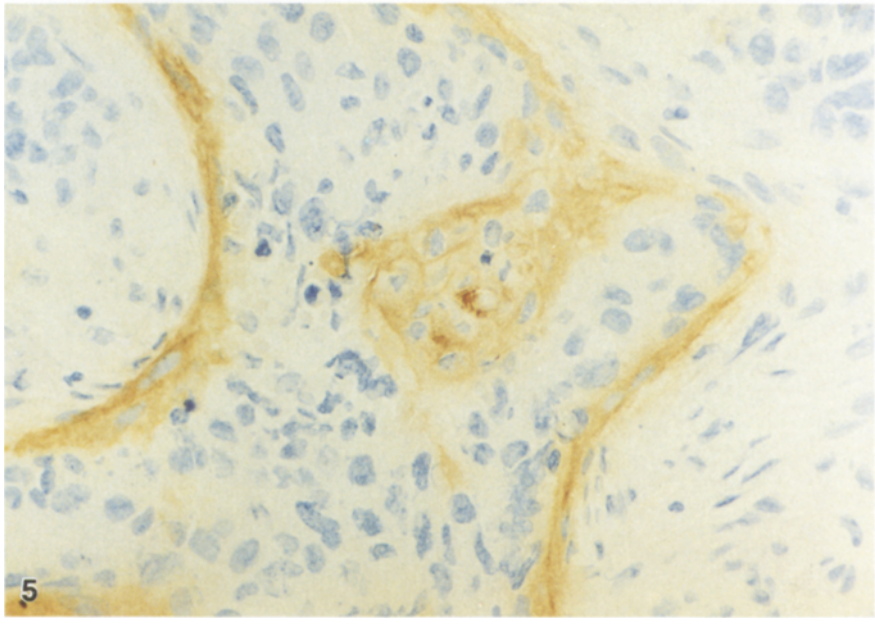


Fig. 4. Invasive ductal carcinoma. Foci of cancerization. A TF-positive reaction is seen in myoepithelial cells. ×20

Fig. 5. TF expression in an invasive ductal carcinoma with predominant intraductal component. Ductal remnants are positive (most likely myoepithelia). A group of intraductal tumour cells is TF-positive. ×40

single tumour cells of groups of intraductal neoplastic cells were often faintly positive (Fig. 4). Furthermore, in the periphery of these tumours, few acinar foci of cancerization were seen. The carcinomatous cells were mostly TF negative. They were lined by myoepithelial cells which were most positive for TF (Fig. 5).

Those cases of invasive ductal carcinomas with and without intraductal components, which reacted moderately and strongly in more than 50% of the tumour cells, rarely showed regional lymph node metastases (4 of 31 cases, 12.9%). However, metastases were found in axillary lymph nodes in 41 of 55 carcinomas (74.5%) of the same histological type with absent or faint TF expression (Table 4). Statistical analysis revealed a significant correlation between expression of TF and lymph node metastases ($P<0.01$) in this group.

In sections of invasive lobular carcinomas with Indian file patterns and targetoid patterns (classical type) TF was detected in 10 of 15 cases. Only 10 of these tumours showed a weakly or moderately positive staining in 10–50% of the tumour cells, whereas less than 10% of the cells were strongly positive in 2 cases.

In most of the breast carcinomas variable numbers of macrophages and histiocytes were observed in the peripheral tissue, as well as in the tumour stroma. Macrophages and histiocytes showed strong cytoplasmic immunoreactivity for lysozyme, whereas only some mononuclear cells were positive for alpha-1-antitrypsin. Re-

Table 4. TF expression in invasive ductal carcinomas with and without intraductal component; regional lymph node involvement related to proportion of immunohistochemical detection of TF

Immunostaining	Axillary lymph node status			
	Lymph node positive		Lymph node negative	
	Number	%	Number	%
None, minute, weak and moderate up to 10% of tumour cells	41	74.5	14	25.5
Moderate more than 10% of tumour cells, strong	4	12.9	41	87.1

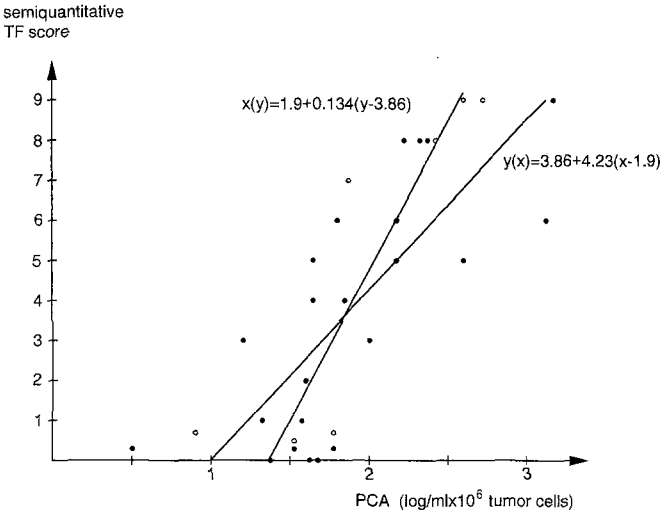


Fig. 6. Schematic representation of the correlation between TF expression (numerical score values) and the determination of tumour-cell-derived procoagulant activity (PCA outlined logarithmically as mU/ml × 10 tumour cells). ○, Fibroadenomas ($n=7$); ●, carcinomas ($n=24$). Numerical score values: 0 for none; 0. 25 for minute and less than 10%; 0.50 for minute and 10–50%; 0.75 for minute and more than 50% of tumour cells; 1 for weak and less than 10%; 2 for moderate and less than 10%; 3 for strong and less than 10%; 4 for weak and 10–50%; 5 for moderate and 10–50%; 6 for strong and 10–50%; 7 for weak and more than 50%; 8 for moderate and more than 50%; and 9 for strong and more than 50% of tumour cells expressing TF

garding TF expression, macrophages or histiocytes showing the respective staining were extremely rare. The appearance of TF in macrophages and histiocytes was neither associated with particular histological carcinoma types, nor with the front of tumour invasion.

The immunohistochemically elaborated numerical TF score corresponded well to the PCA in 31 benign and malignant tumours processed for both methods (Fig. 6). The correlation was statistically significant ($P<0.001$).

Discussion

In the normal resting breast TF staining was restricted to myoepithelial cells. In the pregnancy-stimulated mammary gland, however, TF appeared in a certain number of the acini. Fibrocystic disease was characterized by the expression of TF in luminal layer cells and myoepithelial cells of ductal structures. In fibroadenomas, TF was detectable in epithelial and myoepithelial components, but not in mesenchymal structures. The tendency for TF to be stronger and more frequently expressed in cells of the proliferating breast tissue is remarkable. Since myoepithelial cells are the only type weakly positive for TF in the resting mammary gland, one might assume that myoepithelial cell clones would account for proliferation accompanied by differentiation. It seems possible that the normally occurring clones of myoepithelial cells have the intrinsic potential to express TF in the breast gland. The biological importance of TF expression in these tissues is unknown, and it is possibly a natural mechanism of "self-protection" in terms of haemostasis. Drake et al. (1989) have shown the cellular expression of TF in many organs, especially in the kidney, the epidermis, cerebral cortex and adventitia of vessels. Interestingly, the presence of TF is preferentially detectable in the biological boundary layers ("envelope concept" by Drake et al. 1989).

In breast cancer it seems possible that the transcriptional regulation of the TF gene undergoes certain changes during malignant transformation, as there are marked differences between normal breast, benign tumours and breast cancers. About 80% (93/115) of the carcinomas showed reaction with monoclonal antibodies to TF. In most breast carcinomas, there was only a weak TF staining in certain areas of the tumour cell nests. In some tumours a positive reaction was found in more than 50% of the neoplastic cells. Interestingly, the tubular structures of invasive ductal carcinomas were positive. However, if this pattern was absent (in less differentiated tumours), the TF staining was less intense or completely absent. The reason for the heterogeneous TF expression within a carcinoma is not clear, but may be due to the genotypic and phenotypic instability of malignant tumours (Novell 1986) reflecting general heterogeneity of tumour cell populations. Unexpectedly, stronger TF expression in invasive ductal carcinomas was associated less frequently with axillary lymph node metastases when compared with those expressing low or no detectable TF. We may speculate that the expression of TF is a marker of tumour cell clones bearing low metastatic potential; alternatively, it might reflect a higher degree of differentiation in general. If TF expression directly influences the metastatic potential of cancer cells, the mechanism is obscure. We should look to see, for example, whether tumour cells with TF activity will trigger the coagulation cascade within the lymph fluid leading to an immobilization of these cells in the lymph channels. Among the most important variables of prognostic value are morphological type, histological grading and axillary lymph node status (Bloom and Ri-

chardson 1957). Our data did not reveal a significant correlation between the histological tumour types and TF expression, but the number of cases is low in each of the defined histological groups of this study. From the methodological point of view, it seems remarkable that the immunohistochemically evaluated TF score and the tumour-cell-derived PCA showed a significant correlation.

In summary, we can conclude that in normal human resting mammary gland, TF was detected only in myoepithelial cells. In pregnancy, acinar epithelia start to express TF. Benign proliferation was accompanied by a pronounced TF expression in epithelial and myoepithelial cells. In contrast, TF was demonstrated with various intensity and distribution patterns in breast carcinoma possibly reflecting cellular differentiation events. We found a statistically significant relationship between TF expression in invasive ductal carcinoma and the extent of axillary lymph node involvement. This suggests that the expression of TF in tumour cells could be a marker of favourable prognosis.

Acknowledgements. The authors thank Mr. Thomas S. Edgington for the reference antibodies. We thank S. Thieme, S. Manthey, I. Peterson, and E. Müller for excellent technical assistance.

References

Bevilacqua MP, Pober JS, Majeau GR, Cotran RS, Gimbrone MA (1984) Interleukin 1 (IL-1) induces biosynthesis and cell surface expression of procoagulant activity in human vascular endothelial cells. J Exp Med 160:618–623

Bloom HJC, Richardson WW (1957) Histological grading and prognosis in breast cancer. Br J Cancer 2:359–377

Bonnard C, Papermaster DS, Kraehenbuhl JP (1984) The streptavidin-biotin bridge technique: application in light and electron microscope immunohistochemistry. In: Polak JM, Varndell JM (eds) Immunolabelling for electron microscopy. Elsevier, Amsterdam, pp 95–111

Campbell AM (1987) Monoclonal antibody technology. Laboratory techniques in biochemistry and molecular biology, vol 13. Elsevier, Amsterdam

Drake TA, Morrissey JM, Edgington TS (1989) Selective cellular expression of tissue factor in human tissues: implications for disorders of hemostasis and thrombosis. Am J Pathol 134:1087–1098

Edgington TS, Mackman N, Brand K, Ruf W (1991) The structural biology of expression and function of tissue factor. Thromb Haemost 66:67–79134

Edwards RL, Rickles FR, Bobrove AM (1979) Mononuclear cell tissue factor: cell of origin and requirements for activation. Blood 54:359–370

Fleck RA, Rao LVM, Rapaport SI, Varki N (1990) Localization of human tissue factor antigen by immunostaining with monospecific, polyclonal anti-human tissue factor antibody. Thromb Res 59:421–437

Gregory SA, Edgington TS (1985) Tissue factor induction in human monocytes: two distinct mechanisms displayed by different alloantigen responsive T cell clones. J Clin Invest 76:2440–2445

Hartzell S, Ryder K, Lanahan A, Lau LF, Nathans D (1989) A growth factor-responsive gene of murine balb/c 3T3 cells encodes a protein homologous to human tissue factor. Mol Cell Biol 9:2567–2573

Hsu SM, Raine L, Fanger H (1981) Use of avidin-biotin peroxidase complex (ABC) in immunoperoxidase technique: a comparison between ABC and an unlabeled antibody (PAP) procedure. J Histochem Cytochem 29:577–580

Hu T, Bach R, Konigsberg WH, Ezekowitz MD, Horton R, Todd M (1990) Tissue factor expression in human cancer cell lines. Proc Am Assoc Cancer Res 31:20

Levy GA, Leibowitz JL, Edgington TS (1981) Induction of monocyte procoagulant activity by murine hepatitis virus type 3 parallels disease suspectibility in mice. J Exp Med 151:1150–1163

Morrissey JM, Fair DS, Edgington TS (1988) Monoclonal antibody analysis of purified and cell-associated tissue factor. Thromb Res 52:247–261

Nawroth PP, Stern DM (1986) Modulation of endothelial cell hemostatic properties by tumor necrosis factor. J Exp Med 163:740–745

Nemerson Y (1988) Tissue factor and hemostasis. Blood 71:1–8

Nemerson Y, Bach R (1982) Tissue factor revisited. Prog Hemost Thromb 6:237–261

Novell PC (1986) Mechanism of tumor progression. Cancer Res 46:2203–2207

Rao LVM, Rapaport SI (1987) Affinity purification of human brain tissue factor utilizing factor VII bound to immobilized anti-factor VII. Anal Biochem 165:365–370

Sobin LH (1981) Histological typing of breast tumours. International histological classification of tumours. World Health Organization, Geneva

Weiss HJ, Lages B (1988) Evidence for tissue factor-dependent activation of classic extrinsic coagulation mechanism in blood obtained from bleeding tissue wounds. Blood 71:629–635

Wilcox JN, Smith KM, Schwartz SM, Gordon D (1989) Localization of tissue factor in normal vessel wall and in the atherosclerotic plaque. Proc Natl Acad Sci USA 86:2839–2843

Zotter S, Lossnitzer A, Kunze K-D, Müller M, Hilkens J, Hilgers J, Hagemann P (1985) Epithelial markers for paraffin-embedded human tissues. Virchows Arch [A] 406:237–251