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Stromelysin 3: An Independent Prognostic Factor for Relapse-Free Survival in Node-Positive Breast Cancer and Demonstration of Novel Breast Carcinoma Cell Expression

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Stromelysin 3 (ST3) is a matrix metalloproteinase implicated in mammary carcinoma progression. To date, localization of ST3 expression in breast cancer by *in situ* hybridization and immunocytochemistry has shown that the expression of the enzyme is limited to only the stromal fibroblasts surrounding the cancer cells. We have immunostained a large group of ductal carcinoma *in situ* and invasive breast carcinomas using a monoclonal antibody (5ST-4A9) raised against the hemopexin-like domain of human ST3. We show that invasive lobular carcinomas express significantly less ST3 than invasive ductal carcinomas (IDCs) ($P = 0.002$). We also show, for the first time, that certain breast carcinoma cells that have undergone a degree of epithelial-to-mesenchymal transition, the so-called metaplastic carcinomas, can express ST3 mRNA and protein, which may in part explain the increased metastatic propensity seen in a number of these tumors. In addition, patients with IDC who had moderate to strong ST3 levels had significantly shorter disease-free survival than those with negative or weak ST3 levels ($P = 0.02$). Furthermore, in node-positive IDC patients, multivariate analysis revealed that ST3 level was a strong, independent prognostic parameter for disease-free survival ($P = 0.005$). *(Am J Pathol 1998, 152:721–728)*

Dissolution of the extracellular matrix (comprising the interstitial stroma and the vascular basement membrane) is an essential prerequisite for cancer cell metastasis.[1,2] The extracellular-matrix-degrading enzyme family of matrix metalloproteinases has been implicated strongly in tumor invasion and metastasis.[3] Stromelysin 3 (ST3), a member of this family, was identified initially by subtractive hybridization using breast carcinoma and fibroadenoma cDNA libraries and was found to be specifically overexpressed in breast carcinoma.[4] Subsequently, ST3 mRNA/protein expression has been found in most invasive breast cancers studied so far,[5] and this expression is limited to only stromal fibroblast-like cells surrounding the cancer cells, rather than by the neoplastic cells themselves.[5,6] Furthermore, using *in situ* hybridization, Wolf et al[6] showed that significant ST3 mRNA expression in preinvasive breast lesions, such as ductal carcinoma *in situ* (DCIS), is confined mainly to the most aggressive or poorly differentiated (comedo type) DCIS, whereas ST3 expression is rarely found in benign breast tissue. These data suggest that ST3 may play an important part in promotion of breast cancer invasion and are supported by the recent demonstration that ST3 expression by stably transfected cells promotes tumor take in nude mice.[7]

In this study we have examined a large group of invasive and *in situ* mammary carcinomas both to extend previous prognostic correlates and to study the relationship of the pattern of ST3 expression to the tumor morphology. In particular, we have included in our series a group of aggressive primary mammary carcinomas, the so-called metaplastic carcinomas, in which at least part of the carcinoma has acquired mesenchymal attributes with spindle-shaped/fusiform neoplastic cells or more differentiated cell types such as chondrocytic cells.[8–10] In such carcinomas, the spindle-shaped cancer cells often exhibit both epithelial and fibroblastic characteristics, such as the co-expression of the intermediate filament proteins vimentin and keratin.[11] This co-expression pattern in human breast cancer has been shown to be associated with the capacity for invasion and metastasis.[11]

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Table 1. Histological Subtypes of Breast Cancers Studied

Histological subtype	Grade	Number of cases
IDC	Grade I	30
	Grade II	31
	Grade III	30
Invasive lobular carcinoma		28
DCIS	Well differentiated (low grade)	8
	Moderately differentiated	9
	Poorly differentiated (high grade/comedo)	11
Metaplastic carcinomas		14

Materials and Methods

Immunohistochemistry

Several 3-μm, paraffin-embedded, formalin-fixed breast carcinoma sections (obtained from mastectomy or excision biopsy samples) from patients presenting to the Imperial Cancer Research Fund Clinical Oncology Unit, Guy's Hospital, between 1990 and 1994 were studied. The main prognostic group was chosen to represent consecutive tumors of each of the grades of ductal carcinoma and lobular carcinoma. Table 1 shows the number and histological subtype distribution of the tumors studied. The 14 metaplastic carcinomas were also selected in this way but include 10 referral cases from other units. In addition, 20 cases of benign (fibroadenoma, fibrocystic disease, sclerosing adenosis, and inflammatory disease) or normal breast tissue have been analyzed.

Morphological Assessment

Grading of ductal carcinomas was performed in accordance with the recommendations of Elston and Ellis.[12] DCIS was classified as either well, moderately, or poorly (comedo-type) differentiated.[13] Other factors examined for included pathological tumor size, vascular invasion, estrogen/progesterone receptor levels in tumor cytosols,[14] and axillary nodal status.

The criteria for metaplastic carcinoma used in this study included 1) co-existent conventional mammary carcinoma, 2) spindle-shaped malignant cells/malignant stromal fibroblast-like cells merging with areas of conventional adenocarcinoma, and 3) cytokeratin positivity, often with vimentin positivity, in at least some of the malignant spindle cell population.

Immunostaining

The 3-μm sections were mounted onto Vectabond-coated slides (Vector Laboratory, Peterborough, UK) and stored at room temperature until required. Before staining, sections were heated overnight at 56°C and then dewaxed in xylene and alcohol. Endogenous peroxidase was blocked using a methanol/hydrogen peroxide solution, followed by washing (tap water) and immersion in 0.01 mmol/L, pH 6.0 citrate buffer and pressure cooker heat-mediated antigen retrieval. After washes in water and 0.01 mmol/L Tris-buffered saline (TBS), pH 7.6, incubation in 20% normal rabbit serum in TBS (Dako, High Wycombe, UK) for 10 minutes was performed. Subsequently, sections were incubated overnight in the primary antibody (MAb 5ST-4A9) raised against the hemopexin-like domain of human ST3[15] diluted 1:2000 in TBS. After washing, sections were incubated in Fab'2 biotinylated rabbit anti-mouse (Dako), diluted 1:200 in 3% human serum (Dako), and then peroxidase-conjugated streptavidin diluted 1:500, for 30 minutes each. The reaction was completed by addition of 3,3'-diaminobenzidine tetrachlorohydrate (DAB) activated with 0.3% hydrogen peroxide. Nuclei were counterstained with Mayer's hematoxylin.

CAM 5.2 and vimentin staining were carried out according to standard protocols.

Assessment

Analysis of ST3 immunostaining was performed by simultaneous assessment of sections by two authors (A. Hanby and E. Dublin). Fibroblastic or cancer cell cytoplasmic ST3 staining was graded with the strongest staining area of each section designated as absent (0), weak (1), moderate (2), or strong (3) for ST3 expression.

In Situ *Hybridization*

Specific localization of the mRNA for ST3 was accomplished by *in situ* hybridization using a ^{35}S-labeled antisense RNA probe (^{35}S at ~800 Ci/mmol; Amersham, Little Chalfont, UK). The methods applied for pretreatment, hybridization, washing, and dipping of slides in Ilford K5 for autoradiography essentially were as described by Senior et al[16] for formalin-fixed, paraffin-embedded tissue.

The template for riboprobe synthesis was an *Xba*I-linearized pBluescript II SK+ plasmid that contained the ST3 cDNA insert subcloned in the *Eco*RI site. The riboprobe contained 467 bp of vector sequence complementary to nucleotides 1127 to 1594.[17] The region of sequence used to produce the riboprobe did not show significant homology to any other known sequences in the database.

Autoradiography was for 10 and 13 days at 4°C (two exposures per section) before developing in Kodak D19 and counterstaining by Giemsa's method. Sections were examined under conventional or reflected light dark-field conditions that allowed individual autoradiographic silver

Table 2. Patient Presentation Characteristics (n = 119)

Characteristic	n
Pathological tumor size	
<2 cm	66
>2 cm and <5 cm	49
>5 cm	2
Unknown	2
Menopausal status	
Pre	40
Peri	8
Post	70
Uncertain	1
Histological type and grade	
Ductal grade I	30
Ductal grade II	31
Ductal grade III	30
Lobular	28
Pathological nodal involvement	
Negative	45
1 to 3 positive	46
>4 positive	28
Estrogen receptor status	
<10 fmol/mg	16
>10 fmol/mg	79
Unknown	24
Progesterone receptor status	
<10 fmol/mg	23
>10 fmol/mg	72
Unknown	24

Median patient age was 55 (range, 28 to 84) years. Median tumor size was 2.00 (range, 0 to 7.00) cm.

grains to be seen as bright objects on a dark background.

The presence of mRNA in all compartments of the tissues studied was established by hybridizing additional sections to antisense β-actin mRNA generated with SP6 RNA polymerase and *Dra*I-linearized phβA-10, prepared by subcloning a ~450-bp region of a human β-actin cDNA into pSP73.[18]

Statistical Methods

Differences between groups were determined using the χ^2 test or Fisher's exact test as indicated. Disease-free survival was calculated from the time of diagnosis to first relapse. Patients who were alive without evidence of relapse were evaluated at the date they were last known to be alive. Patients who died without evidence of recurrent breast cancer were evaluated at their date of death. Survival curves were produced by the method of Kaplan and Meier.[19] Differences between curves were determined using the logrank test.[20] Multivariate analysis was performed using Cox's proportional hazards model.[21]

Results

Characteristics of Patients

The clinical and pathological characteristics of the 119 patients with invasive ductal and lobular carcinoma are shown in Table 2. Median follow-up for these cases is 60 months (range, 12.5 to 72.6 months). Both tumor grade ($\chi^2 = 8.17$; $P = 0.02$) and axillary nodal status ($\chi^2 = 4.37$; $P = 0.04$) were important predictors of relapse-free survival in this group of patients. The characteristics of the metaplastic carcinomas are discussed separately; as the majority of these cases were obtained from other centers, follow-up is not available.

Immunohistochemistry of ST3 in Benign Breast Tissue

ST3 expression was not observed in nonmalignant breast tissue (20 of 20 cases being negative; Table 3).

Immunohistochemistry of ST3 in Conventional in Situ and Invasive Carcinoma

In all of the *in situ* and invasive carcinomas studied only stromal fibroblast-like cells surrounding neoplastic cells were shown to express ST3 as revealed by diffuse brown cytoplasmic immunostaining. Neoplastic cell staining was never seen. Figure 1 shows the typical staining pattern observed in an invasive ductal carcinoma, with the fibroblasts in immediate juxtaposition to the tumor cells displaying strongest ST3 expression.

Fibroblasts surrounding 6 of 28 cases (21%) of DCIS expressed ST3. Although equal numbers of poorly (comedo-type), intermediate, and well differentiated DCIS were included in the study, there was no trend observed between ST3 expression and grade of DCIS. Indeed, of the two strongest staining cases of DCIS, one was poorly and the other well differentiated (Table 3).

In contrast to preinvasive disease, stromal fibroblasts in 72 of 91 cases (80%) of invasive ductal carcinoma expressed ST3 ($P < 0.0001$). However, there was no

Table 3. Expression of ST3 in Normal/Benign Breast Tissue, Preinvasive Breast Disease (CDIS), and Invasive Ductal (Grades I, II, and III) and Lobular Breast Carcinoma

	Number of cases					
ST3 expression	Normal/Benign	DCIS	Lobular	Ductal (GI)	Ductal (GII)	Ductal (GIII)
0	20	22	13	8	5	6
1		4	13	12	11	9
2		2*	2	8	8	6
3		0	0	2	7	9

Fibroblastic cytoplasmic ST3 staining was graded with the strongest staining area of each section designated as absent (0), weak (1), moderate (2), or strong (3) for ST3 expression.

*One case of poorly differentiated (comedo-type) DCIS and one case of well differentiated DCIS.

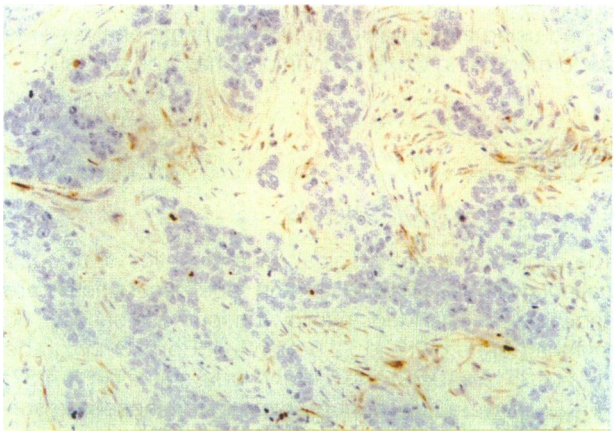

Figure 1. Immunostaining of a grade III invasive ductal carcinoma with monoclonal antibody 5ST-4A9 as described in Materials and Methods. The photograph shows high levels of ST3 expression in the form of a brown diaminobenzidine reaction product in stromal fibroblasts surrounding the breast carcinoma cells. Magnification, ×20.

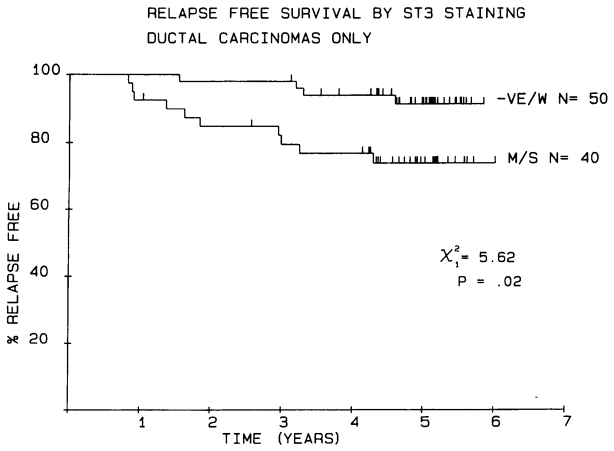

Figure 2. Relapse-free survival of ductal carcinomas according to ST3 staining. VE/W, cases that had negative or weak staining; M/S, moderate or strongly staining cases.

significant association between histological grade, axillary nodal status, tumor size, estrogen/progesterone receptor status, or the presence of vascular invasion and level of ST3 expression in the ductal carcinomas studied.

Immunostaining of 28 lobular breast carcinomas revealed significantly less stromal cell ST3 staining in these than in invasive ductal tumors ($P = 0.002$), with 13 of 28 lobular carcinomas not expressing ST3. Of the remaining 15 (53%) ST3-positive cases, 13 showed very weak staining (Table 3). There was a suggestion of a trend between the presence of vascular invasion in the lobular carcinomas and positive ST3 expression that just failed to reach statistical significance ($P = 0.08$), with 12 of 13 ST3-negative sections showing no vascular invasion in contradistinction to 8 of the 14 ST3-positive cases that did.

Prognostic Significance of ST3 Expression

As cases of invasive ductal and lobular carcinoma clearly have very different levels of ST3 expression, the prognostic significance of ST3 expression in these two groups was analyzed separately. Patients with invasive ductal carcinoma who had moderate or strong levels of fibroblastic ST3 expression (n = 40) had significantly shorter disease-free survival than those patients with negative or weak ST3 levels (n = 50; $\chi^2 = 5.62$; $P = 0.02$; Figure 2). The prognostic significance of ST3 levels in comparison with nodal status, histological grade, tumor size, and age was then evaluated in these patients using univariate analysis (Table 4). As can be seen, ST3 level is a strong prognostic factor in predicting disease-free survival than is nodal status in this study group, and is of similar prognostic value to tumor grade. When patients were subdivided according to nodal status, ST3 level was not predictive for outcome in the node-negative subset, but was a strong predictive factor for relapse-free survival in the 53 patients with node-positive breast cancer ($\chi^2 = 7.01$; $P = 0.0081$; Figure 3). Furthermore, multivariate analysis that included other parameters previously identified as prognostic factors in breast cancer revealed that, in node-positive patients, ST3 level was a strong, independent prognostic parameter for disease-free survival ($P = 0.005$; Table 5). ST3 level was of no prognostic significance in patients with lobular carcinoma.

Immunostaining and in Situ *Hybridization of Metaplastic Breast Carcinoma*

The ST3 expression levels in each of the 14 cases of metaplastic carcinoma are shown in Table 6. As can be seen, in 11 of 14 cases, ST3 expression was observed in stromal fibroblasts, and in 9 of these, it was also observed in carcinoma cells. ST3 expression in neoplastic cells was limited only to the spindle-shaped cancer cells (**arrows** in Figure 4) and never seen in co-existing adenocarcinoma cells of conventional morphology. These pleomorphic, spindle-shaped neoplastic cells often stained positively for both fibroblastic (vimentin; Figure 5) and epithelial (cytokeratin-CAM 5.2; Figure 6) markers.

In situ hybridization using an antisense ST3 RNA probe was then performed in eight of the above cases of metaplastic carcinoma. The pattern of expression of ST3 mRNA in these cases closely resembled that seen immunohistochemically, with stromal fibroblasts labeling vari-

Table 4. Evaluation of Prognostic Significance of ST3 Levels in Comparison with Tumor Size, Grade, Nodal Status, and Age in Patients with Invasive Ductal Carcinoma

	Univariate			
Variable	χ^2	*P* value	RR	95% CI
ST3 level*	6.00	0.01	1.96	1.12–3.44
Age*	2.95	0.09	1.04	0.99–1.09
Tumor size*	2.56	0.11	1.34	0.95–1.89
Grade†	6.72	0.01	2.52	1.18–5.39
Nodal status‡	3.28	0.07	1.89	0.94–3.77

RR, relative risk; CI, confidence interval on relative risk.
*Variables treated as continuous.
†Grade I *versus* grade II *versus* grade III.
‡Node negative *versus* 1 to 3 nodes positive *versus* ≤4 nodes positive.

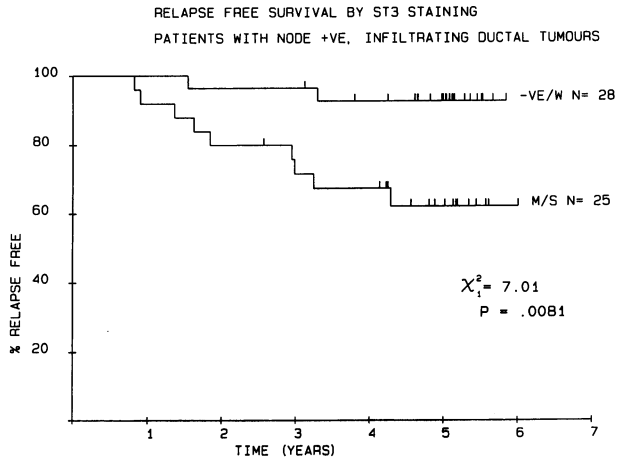

Figure 3. Relapse-free survival of node-positive ductal carcinomas according to ST3 staining. VE/W, cases that had negative or weak staining; M/S, moderate or strongly staining cases.

ably strongly and with cytologically malignant spindle cells that were consistent with metaplastic cells also showing unequivocal labeling. A representative light-field micrograph is shown in Figure 7 where these large spindle-shaped cells with pleomorphic nuclei are seen to be overlain by clusters of granules at the sites of the hybridized probe.

Discussion

In this study we report two major novel findings. We have shown, first, the ability of certain breast (metaplastic) carcinoma cells to express ST3 mRNA and protein and, second, that in node-positive breast cancer ST3 may represent a new, independent prognostic factor in predicting relapse-free survival.

ST3 mRNA and protein expression in breast cancer have been studied by a number of groups,[22–24] but these studies have been restricted to analysis of ST3 levels in either DCIS, invasive ductal, or lobular carcinomas. In all breast carcinomas studied to date, localization of ST3 expression is limited to the stromal fibroblasts surrounding the epithelial carcinoma cells (Figure 3), and ST3 expression has never been observed in the neoplastic

Table 5. Evaluation of Prognostic Significance of ST3 Levels in Comparison with Tumor Size, Grade, Nodal Status, and Age in Patients with Node-Positive Invasive Ductal Carcinoma

Variable	Multivariate			
	χ^2	*P* value	RR	95% CI
ST3 level*	7.87	0.005	2.44	1.24–4.83
Age*	4.40	0.04	1.06	1.00–1.12
Tumor size*	2.68	0.10	1.43	0.97–2.10
Grade†	1.50	0.22	1.88	0.67–7.22
Nodal status‡	1.03	0.31	1.97	0.54–7.22

RR, relative risk; CI, confidence interval on relative risk.
*Variables treated as continuous.
†Grade I *versus* grade II *versus* grade III.
‡Node negative *versus* 1 to 3 nodes positive *versus* ≤4 nodes positive.

Table 6. ST3 Immunostaining Scores of 14 Cases of Metaplastic Breast Carcinoma

Case	Stromal fibroblast score	Cancer cell
1	2	2
2	1	2
3	2	3
4	0	0
5	3	3
6	3	3
7	1	1
8	0	0
9	1	2
10	1	1
11	1	0
12	0	0
13	3	0
14	3	2

Fibroblastic or cancer cell cytoplasmic ST3 staining was graded with the strongest staining area of each section designated as absent (0), weak (1), moderate (2), or strong (3) for ST3 expression. (Separate scores are provided for ST3-positive stromal fibroblasts and cancer cells.)

cells themselves. Immunohistochemical analysis of our subset of metaplastic breast carcinomas demonstrates that, in addition to the stromal fibroblasts, breast carcinoma cells within these tumors also express ST3 protein. This novel demonstration of expression of ST3 is confirmed at the mRNA level using *in situ* hybridization. The nomenclature of such malignant epithelial tumors showing transition to a spindle cell morphology, so-called epithelial-to-mesenchymal transition, is varied, both between body sites and even within the breast where the term metaplastic carcinoma encompasses lesions with a wide range of appearances.[8,9,10,25] We do not seek here to address the complications of this terminology other than to provide evidence that alteration in neoplastic cellular morphology is accompanied by immunophenotypic, and potentially functional, changes. These spindle-shaped cells often co-express fibroblastic and epithelial cell markers,[8–10] which was confirmed in our study where many of the ST3-expressing metaplastic cancer cells stained positively for both cytokeratin and vimentin. In this regard, it is interesting to note that expression of vimentin in human breast cancer is associated with markers of disease aggression such as high grade, low estrogen receptor status, and high Ki-67 growth fraction.[26,27] In addition, human breast carcinoma cell lines that express vimentin are highly invasive *in vitro* and highly metastatic in nude mice in comparison with vimentin-negative cell lines.[28,29] However, neither vimentin-expressing nor vimentin-negative breast cancer cell lines from a small panel examined express detectable levels of ST3 mRNA *in vitro* (data not shown).

The lack of detectable ST3 expression in mammary carcinoma cells of conventional morphology in the sections studied suggests that transition from adenocarcinoma to spindle/metaplastic cell type may be accompanied by cellular genetic changes allowing the constitutive expression of the ST3 gene product. Such putative changes may alter signal transduction pathways involved in regulation of ST3 gene expression or may result in the cell developing a more mesenchymal pattern of gene

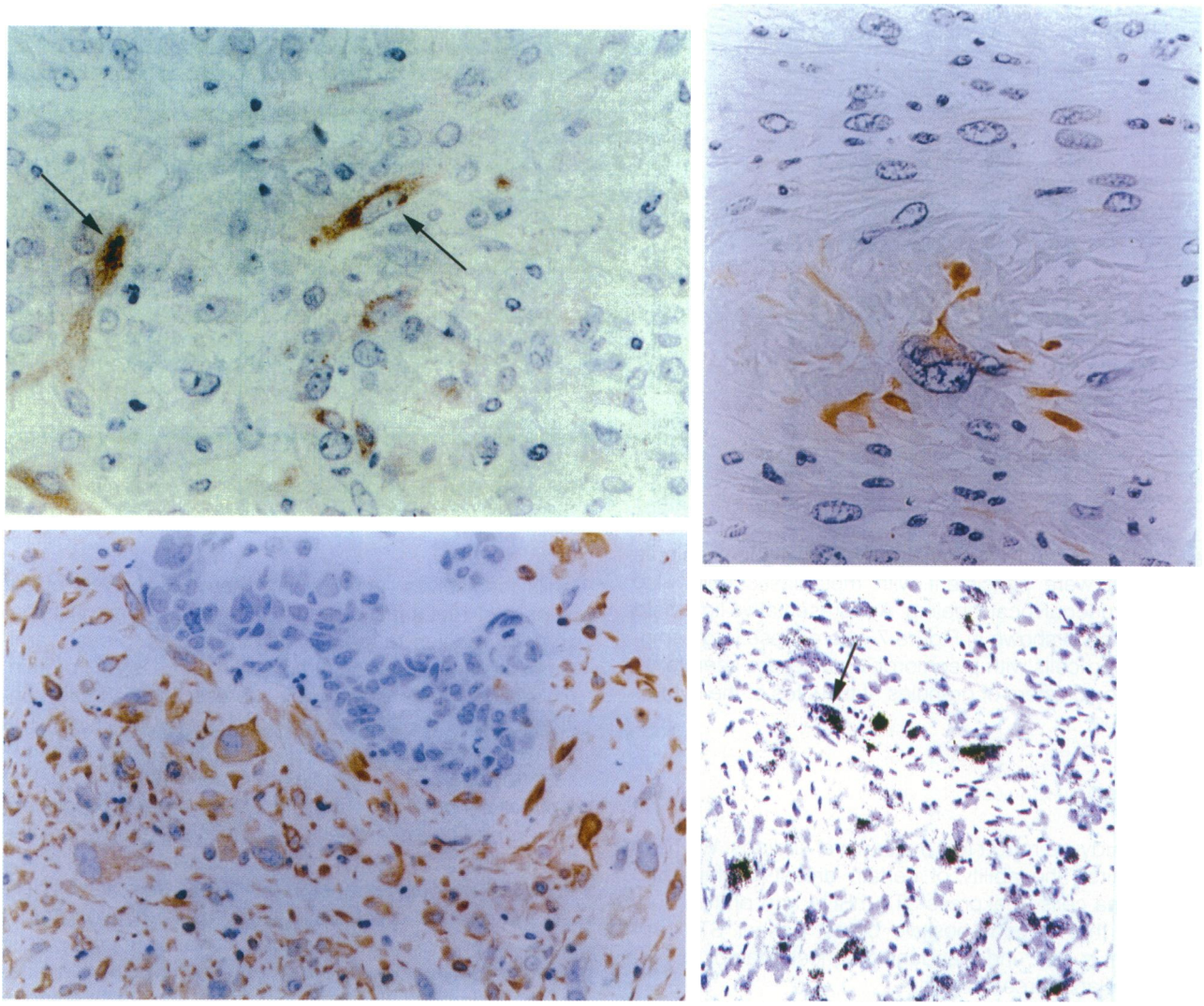

Figure 4. (top left) Immunostaining of metaplastic breast carcinoma using monoclonal antibody 5ST-4A9 as described in Materials and Methods. Spindle-shaped, metaplastic breast carcinoma cells can be seen to express ST3 (**arrows**). Magnification, ×40.

Figure 5. (top right) Representative photograph showing vimentin expression in spindle-shaped, metaplastic breast cancer cells (vimentin expression is not seen in adjacent mammary carcinoma of conventional morphology). Magnification, ×40.

Figure 6. (bottom left) Representative photograph showing cytokeratin (CAM 5.2) expression in a metaplastic breast carcinoma cell. Magnification, ×40.

Figure 7. (bottom right) Light-field micrograph of *in situ* hybridization of a metaplastic breast carcinoma using a ^{35}S-labeled ST3 antisense RNA probe as described in Materials and Methods. Large spindle-shaped neoplastic cells with pleomorphic nuclei are seen to contain clusters of granules (example shown by **arrow**).

expression. Alternatively, they may result in the ability of these cancer cells to respond in an autocrine fashion to diffusible factors that they themselves elaborate and that can stimulate ST3 gene expression in neighboring fibroblasts.[30] A switch from stromal to tumour cell expression of another matrix metalloproteinase, stromelysin-1, has previously been observed in the transition from squamous to spindle cell carcinoma in a mouse skin tumor model.[31] The data presented here may help to explain why certain subtypes of metaplastic breast carcinoma are highly invasive and therefore associated with poor prognosis,[8–10] as ST3 is being expressed by both fibroblasts and cancer cells. Furthermore, although lymphatic dissemination of metaplastic carcinomas is recognized, vascular invasion and subsequent spread via the bloodstream is a far more common route of spread for this tumor type.[25] It is interesting to note that, as is the case with lobular carcinoma, which also has a predeliction for vascular dissemination, certain metaplastic or spindle carcinoma cells fail to express the homotypic cell adhesion molecule, E-cadherin.[32,33] Data presented here suggest that ST3 expression in lobular carcinomas may be associated with the likelihood of these tumors exhibiting vascular invasion ($P = 0.08$), although confirmation of these data requires larger patient numbers.

Evaluation of ST3 levels in invasive ductal carcinomas (IDCs) revealed higher ST3 levels in ductal rather than lobular carcinoma ($\chi^2 = 14.5$; $P = 0.002$), an observation in agreement with previous studies.[22,23] No association was found between ST3 expression level and histological

grade or axillary nodal status in IDC. Importantly, in our series, both tumor grade and extent of nodal involvement are important predictors of relapse-free survival, whereas in the recent paper from Chenard et al,[22] although ST3 protein level showed no significant relationship with tumor grade, the latter surprisingly was not a prognostic factor in their group of patients. The lack of correlation between ST3 expression level and grade may relate to the fact that histological grade is deduced by a composite score derived from mitotic activity, nuclear pleomorphism, and tubule formation. Examination of ST3 level clearly gives information that is not readily observable using these features, ie, regarding the ability to invade. Many other markers examined for prognostic effect are more directly related to cell cycle attributes and relate therefore to the mitotic component of grading and are by extension unlikely to achieve independence from it. We further show that tumor size, estrogen/progesterone receptor status, and presence of vascular invasion in IDC do not correlate with ST3 protein level. The absence of correlation between ST3 level and vascular invasion, or indeed axillary nodal status, may possibly be explained by the fact that ST3 may be promoting breast carcinoma progression not only by enhancing tumor invasion but also by contributing to the survival and implantation of tumor cells outside of their compartment of cellular origin.[7] Prognostic evaluation of ST3 level in the IDC patients studied reveals that moderate/strong ST3 staining predicts significantly shorter disease-free survival (Figure 2; Table 4). In node-positive patients, this effect on disease-free survival was more marked (Figure 3). In a multivariate analysis of the node-positive patients that included a number of established prognostic parameters, ST3 level was a highly significant independent prognostic parameter for disease-free survival ($P = 0.005$; $\chi^2 = 7.87$; relative risk = 2.4; 95% confidence interval = 1.2 to 4.8; Table 5). Previous reports have concluded that high levels of ST3 mRNA[23] and protein[22] are associated with poor prognosis. This study, on a different and larger group of patients and using a different technique to assess ST3 immunostaining from that used by Chenard et al,[22] is the first to report the independent prognostic effect of ST3 on disease-free survival in node-positive patients. In node-negative patients, ST3 was not a significant prognostic parameter. This may relate to the small number of events and short overall follow-up time in this group.

In conclusion, we have shown that ST3 expression in breast cancer can be seen in certain types of neoplastic as well as stromal cells; hence, in many of the metaplastic breast carcinomas studied we observed ST3 mRNA and protein in both stromal fibroblasts and cancer cells. This dual expression may contribute to the increased metastatic capability observed in a number of these tumor types. Our results in *in situ* and invasive carcinoma support and extend earlier data indicating that ST3 is implicated in breast carcinoma progression and provide compelling evidence that ST3 is an important, independent prognostic parameter in patients with node-positive breast cancer.

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