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# Stromelysin 3 belongs to a subgroup of proteinases expressed in breast carcinoma fibroblastic cells and possibly implicated in tumor progression

(breast cancer/metalloproteinases/stroma/invasion/cancer progression)

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ABSTRACT **The expression of the stromelysin 3 (ST3) gene, which encodes a putative matrix metalloproteinase, was studied during breast cancer progression. The ST3 gene is expressed in all invasive breast carcinomas, in a number of their metastases, and in some *in situ* carcinomas where the probability of detecting ST3 transcripts correlates with the known risk of these carcinomas to become invasive. ST3 RNA and protein were specifically detected in fibroblastic cells immediately surrounding the neoplastic cells in both primary and metastatic tumors. This expression pattern distinguishes the ST3 gene from other matrix metalloproteinase genes, most notably from the 72-kDa type IV collagenase gene, which can be expressed in fibroblastic cells distributed throughout the stroma of primary breast carcinomas. Furthermore, high levels of 72-kDa type IV collagenase, but not of ST3 transcripts, are detected in benign breast fibroadenomas. Interestingly, the urokinase and ST3 genes exhibit very similar patterns of expression in breast carcinomas, which suggests that their products may cooperate during cancer progression.**

Breast cancer, whose incidence is increasing worldwide, is a leading cause of cancer deaths among women in the industrialized world. While it is clear that more specific treatments are necessary, early detection based on mammography screening and a better administration of present systemic therapies should help to stabilize this unfavorable evolution (1). In this respect, there is a need for new prognostic and predictive factors to better define the patients who are the most likely to benefit from adjuvant therapy (1, 2).

In this context, we have designed a strategy based on the differential screening of breast cancer cDNA libraries to identify genes whose expression is modified in breast carcinomas and that may play a role in breast cancer progression. We have previously identified the stromelysin 3 (ST3) gene, which encodes a putative matrix metalloproteinase (MMP) and represents a potential marker of tumor invasion (3, 4). MMPs are believed to play a role in cancer progression (5, 6), and it has been proposed that they could cooperate with other types of secreted proteinases, including urokinase (7). In the present study, we demonstrate that ST3 gene expression is modulated during breast cancer progression, in ways suggesting that ST3 may be implicated from the earliest stages of tumor invasion.

## MATERIALS AND METHODS

**Tissue Collection and ST3 RNA Analyses.** The surgical specimens were cut in sections and immediately frozen in liquid nitrogen for RNA analysis by Northern blot (3). Adjacent sections, fixed in 10% buffered formalin, were taken for histological examination and *in situ* RNA hybridization (3). Sections for immunohistochemical detection of ST3 protein were frozen in 2-methylbutane at −180°C.

**ST3 Immunohistochemical Analysis.** The polyclonal antibody 349 was obtained by rabbit immunization with a peptide corresponding to the 25 C-terminal amino acid residues of human ST3 (3) and used at 1:1000 dilution for immunohistochemical analysis. The monoclonal antibody 5ST-4A9-3 (IgG1, λ) was obtained by immunization of BALB/c mice (8) with recombinant human ST3 that was extracted from inclusion bodies of *Escherichia coli* (BL21 strain) cells expressing ST3 cDNA inserted in a pET-3 vector (9). Ascites fluid diluted 1:500 and a peroxidase-antiperoxidase system (DAKO, Carpinteria, CA) were used for ST3 immunostaining, which was scored (1+ to 3+) according to the intensity of cytoplasmic staining, the surface of ST3-expressing tumor areas, and the percentage of ST3-expressing fibroblastic cells in the positive tumor areas.

## RESULTS

**ST3 Gene Expression in Primary Breast Carcinomas.** ST3 RNA has been detected by Northern blotting in all invasive ductal and lobular breast carcinomas so far analyzed, but not in normal breast samples (Fig. 1; ref. 3; and data not shown). By using *in situ* RNA hybridization, 10 (83%) of 12 lobular and 89 (97%) of 92 ductal invasive carcinomas were found to express the ST3 gene (Table 1). In contrast, only 1 (from a pregnant patient) out of 21 breast fibroadenomas expressed significant levels of ST3 RNA.

ST3 RNA was also detected in some *in situ* breast carcinomas. Eight (61%) of 13 ductal *in situ* carcinomas of the comedo type but only 2 (8%) of 25 lobular *in situ* carcinomas expressed ST3 RNA, and an intermediate value of 31% (7 out of 22 cases) was obtained for ductal *in situ* carcinomas of the noncomedo type. Furthermore, both the number of ducts associated with ST3 RNA and the transcript levels were higher in *in situ* carcinomas of the comedo type than in those of the other types (data not shown). Thus, the frequency of ST3 gene expression in each *in situ* carcinoma subgroup was well correlated with the known risk of these carcinomas to become invasive (1, 12).

As previously observed for invasive cancers (3), ST3 transcripts present in *in situ* carcinomas were not detected in the neoplastic cells, but in fibroblastic cells of the tumoral stroma (Fig. 2 *a–l*). However, ST3 gene expression in *in situ*

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Abbreviations: ST1, ST2, and ST3, stromelysins 1, 2, and 3; IV COL(72), 72-kDa type IV collagenase; MMP, matrix metalloproteinase.

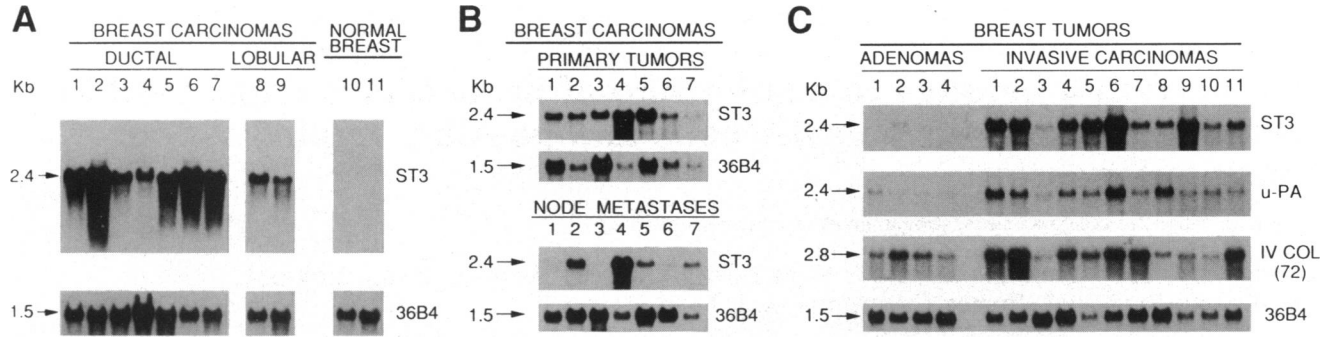


FIG. 1. Northern blot analysis of ST3 RNA in normal and tumoral breast tissue and in breast cancer metastatic lymph nodes. Eight micrograms of total RNA was loaded in each lane. Primary tumors (invasive ductal carcinomas) and metastatic lymph nodes in each lane of *B* were obtained from the same patient. In *C Right*, where lanes 1–10 correspond to ductal carcinomas and lane 11 corresponds to a lobular carcinoma, the blot was successively hybridized with ST3, urokinase (u-PA), and IV COL(72) $^{32}$P-labeled cDNA probes. Hybridization with 36B4 cDNA was performed to check for RNA loading and transfer in each lane. Probes are as in refs. 3, 4, 10, and 11. Autoradiography was for 2 days (ST3), 4 days (urokinase), and 1 day [IV COL(72)].

carcinomas was often limited to part of the stroma immediately surrounding the cancer ducts (Fig. 2 *a* and *b*, *d* and *e*, *g* and *h*), whereas ST3 transcripts were generally found everywhere at the epithelial–stromal interface in invasive carcinomas (3). Interestingly, both ST3 expressing and non-expressing ducts containing *in situ* carcinoma could be observed in the same tumoral area (Fig. 2 *i* and *j*, *k* and *l*). Altogether, these observations suggest that ST3 gene expression is induced by neoplastic cells in discrete areas of tumoral stroma during breast cancer progression, possibly by "pre-invasive" neoplastic cells. In support of this possibility, we note that ST3 transcripts were often detected in fibroblastic cells of tumoral stroma, when the integrity of the adjacent basement membrane could be questioned and/or when the adjacent stroma exhibited signs of remodeling (see Fig. 2 *a–f* and its legend; and data not shown), although in a few cases ST3 expression was also observed in the absence of obvious stroma remodeling (Fig. 2 *g–l*).

As expected from the above data, the ST3 protein could also be detected in primary breast carcinomas. By using frozen sections, it was present in 80 (78%) of 103 invasive ductal carcinomas, but only in 1 of 6 invasive lobular carcinomas and 3 of 12 *in situ* ductal carcinomas (Table 2), which suggests that protein detection may be less sensitive than RNA to evaluate ST3 gene expression. However, the frequency of ST3-positive tumors was similar to that found by *in situ* RNA hybridization when the immunohistological analysis was performed on microwave-treated paraffin-embedded tissue sections (N.R. and J.-P.B., unpublished observation). The ST3 protein was present in the cytoplasm of elongated, fibroblast-like cells immediately surrounding islands of neoplastic cells, using either polyclonal antibody 349 (Fig. 2*m*; and data not shown) or monoclonal antibody 5ST-4A9-3 (Fig. 2 *n* and *o*; and data not shown). However, ST3 was not detected either in neoplastic cells or in normal breast tissue located at a distance from the cancer cells (data not shown). Taken together, these observations indicate that ST3 RNA and protein expression patterns are superimposable.

Table 1. Detection of ST3 RNA by *in situ* hybridization in breast tumors

| Diagnosis | Number of cases: Total | Number of cases: ST3-positive (%) |
|---|---|---|
| Fibroadenoma | 21 | 1 (5) |
| *In situ* carcinoma | | |
| Lobular | 25 | 2 (8) |
| Noncomedo ductal | 22 | 7 (31) |
| Comedo ductal | 13 | 8 (61) |
| Invasive carcinoma | | |
| Lobular | 12 | 10 (83) |
| Ductal | 92 | 89 (97) |
| Metastasis | | |
| Lymph nodes | 13 | 11 (85) |
| Bone | 6 | 4 (67) |
| Other organs | 8 | 4 (50) |

A single tissue section of each tumor was analyzed.

**ST3 RNA Expression in Metastatic Breast Carcinomas.** ST3 RNA was detected by *in situ* hybridization in 19 (70%) of 27 breast cancer metastases (Table 1). As in the case of primary tumors, ST3 gene expression was selectively found in fibroblastic cells and was not associated with any particular tissular localizations, since it occurred in all types of breast cancer metastases tested, including those in lymph nodes, bone, skin, pleura, and liver (Fig. 3; and data not shown). There was no strict correlation between ST3 RNA levels in the primary tumor and metastatic lymph nodes from the same patient (Fig. 1*B*), and low levels of ST3 RNA in metastatic nodes were not necessarily correlated with a weak stromal reaction in the nodes (data not shown).

**The Expression Pattern of the ST3 Gene in Breast Carcinoma Is Similar to That of the Urokinase Gene, but Different from That of Other MMP Genes.** Thirteen breast tumors, including 2 fibroadenomas and 11 invasive ductal carcinomas, were analyzed for the presence of transcripts of urokinase and seven MMP genes (Table 3). ST1 RNA was not detected in any of the tumors analyzed. ST2 RNA was detected in a single carcinoma, which exhibited malpighian differentiation and in which ST2 transcripts were specifically detected in neoplastic cells distributed at the periphery of neoplastic islands (data not shown), as previously observed in head and neck squamous cell carcinomas (10). In 3 carcinomas, type I collagenase RNA was specifically detected in fibroblastic cells surrounding islands of neoplastic cells, but, in any given tumor, more tumoral areas expressed the ST3 gene than the collagenase gene (data not shown). ST2 and interstitial type I collagenase RNAs were not detected in the 2 fibroadenomas tested or in the normal breast structures also present in the tissue sections (Table 3; and data not shown).

The other MMP genes and the urokinase gene were expressed in most of the tumors analyzed (Table 3). Putative metalloproteinase 1 RNA was detected in both nonneoplastic and neoplastic epithelial cells, whereas 92-kDa type IV collagenase RNA was found in inflammatory cells infiltrating the malignant tumors and in few neoplastic cells (Table 3; and data not shown). In contrast, 72-kDa type IV collagenase [IV COL(72)], ST3, and urokinase RNAs were exclusively, or almost exclusively, found in fibroblastic cells (Table 3 and

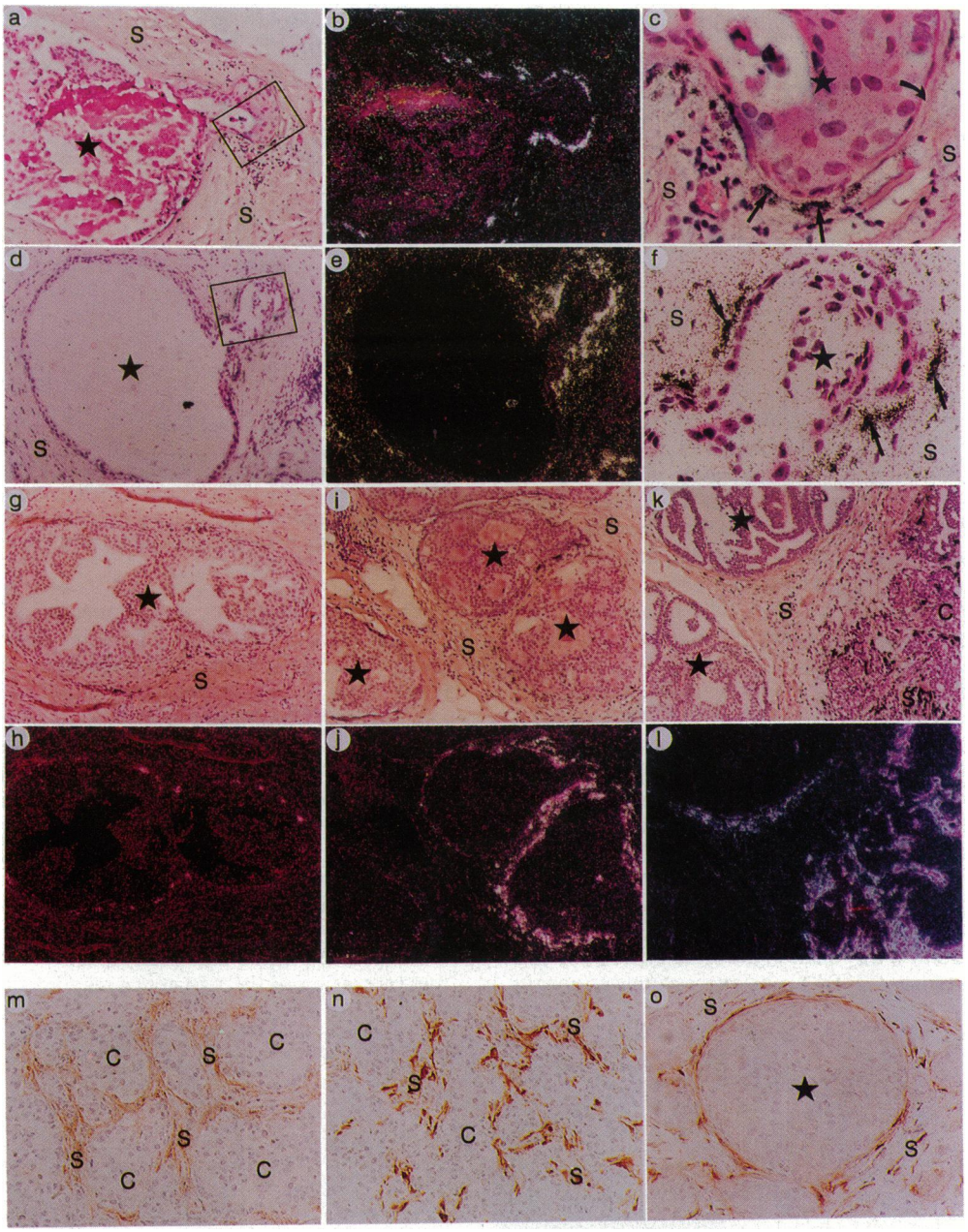


FIG. 2. Fibroblastic expression of ST3 RNA and protein in primary breast carcinoma. (*a–l*) *In situ* RNA hybridization. Bright-field (*a*, *c*, *d*, *f*, *g*, *i*, and *k*) and dark-field (*b*, *e*, *h*, *j*, and *l*) photomicrographs of paraffin-embedded tissue sections stained with hematoxylin, after *in situ* hybridization with $^{35}$S-labeled ST3 antisense RNA and autoradiography for 4 weeks are shown. S, stroma; ★, *in situ* carcinoma; C, invasive cancer cells. Comedo (*a* and *b*) and mucinous (*d* and *e*) ductal *in situ* carcinomas with ST3 gene expression in stromal cells surrounding the cancer-containing duct are shown. Note that expression is maximal where the tumoral stroma exhibits remodeling (boxed). (*c* and *f*) Higher power views of *a* and *d*, respectively, where straight arrows indicate fibroblastic cells expressing ST3 transcripts. Note that the ST3-expressing cells are found where the basement membrane integrity is questionable, but not where it appears intact (curved arrow). (*g* and *h*) Micropapillary ductal carcinoma *in situ* with few ST3-expressing stromal cells immediately surrounding the cancer-containing duct. Cribriform (*i* and *j*) and micropapillary (*k* and *l*) ductal *in situ* carcinomas are shown. Note that *in situ* carcinoma-containing ducts can be surrounded by stromal cells expressing or not expressing the ST3 gene, in a given tumoral area; in *k* and *l*, note the high levels of ST3 transcripts in the stromal cells surrounding the invasive cancer cells associated with the *in situ* carcinoma. (*m–o*) Indirect immunoperoxidase staining of ST3 protein. Photomicrographs of frozen tissue sections of invasive (*m* and *n*) and *in situ* (comedo type; *o*) ductal carcinomas, after immunohistochemical analysis with polyclonal antibody 349 (*m*) and monoclonal antibody 5ST-4A9-3 (*n* and *o*) are shown. ST3 is exclusively detected in elongated fibroblast-like cells of tumoral stroma surrounding neoplastic cells. (Original magnifications: *a*, *b*, *d*, *e*, and *g–l*, ×100; *c* and *f*, ×400; *m–o*, ×200.)

Fig. 4; and data not shown). However, the levels of IV COL(72) RNA were similar in benign and malignant tumors, whereas the ST3 and urokinase genes were expressed at significant levels in only the malignant tumors (Fig. 1*C*). Furthermore, IV COL(72) (Fig. 4*c*), but not ST3 (Fig. 4*b*) or urokinase (data not shown), transcripts were detected in fibroblastic cells located at a distance from the neoplastic cells (see Fig. 4*a*). Interestingly, although the levels of ST3 RNA in breast carcinomas were generally higher than those of urokinase RNA (Fig. 1*C* and its legend), the expression patterns of both genes were very similar (Fig. 4 *d–i*).

## DISCUSSION

We have shown here that expression of the ST3 gene can be detected in all invasive breast carcinomas and in a number of their metastases, but only in a restricted number of *in situ*

Table 2. Immunodetection of ST3 in breast carcinomas

| Diagnosis | Number of cases | | | | |
|---|---|---|---|---|---|
| | | ST3-positive* | | | |
| | Total | 1+ | 2+ | 3+ | Total |
| *In situ* carcinoma | | | | | |
| Lobular | 2 | — | — | — | — |
| Ductal | 12 | 1 | 1 | 1 | 3 |
| Invasive carcinoma | | | | | |
| Lobular | 6 | — | 1 | — | 1 |
| Ductal | 103 | 20 | 30 | 30 | 80 |

*Using monoclonal antibody 5ST-4A9-3 on frozen tissue sections. Immunoscoring was performed as described in *Materials and Methods*.

carcinomas. Interestingly, most of the *in situ* carcinomas expressing ST3 were associated with obvious remodeling of the surrounding stroma and/or questionable integrity of the basement membrane. Moreover, ST3 RNA was detected in almost two-thirds of *in situ* carcinomas of the comedo type, which are known to often become invasive (1), but only in few lobular *in situ* carcinomas, which more rarely and more slowly evolve toward invasive tumors (12). Altogether, these observations suggest that ST3 may be implicated in early stages of cancer cell invasion. In this respect, it is noteworthy that angiogenesis and HER-2 overexpression, which both may represent critical steps in the triggering of tumor invasion (13, 14), are also observed in a number of *in situ* breast carcinomas (15, 16). Although its *in vivo* substrate is presently unknown, ST3 is likely to be a proteinase, since it exhibits proteolytic activities after protein purification (G. Murphy, P.C., and P.B., unpublished results). Thus, it is probable that ST3 belongs to the group of proteinases that are expressed in stromal fibroblast-like cells of carcinoma and may be involved in tumor progression (3, 4, 10, 11, 17–21).

In breast carcinomas, as well as in the other types of human carcinomas so far analyzed, ST3 gene expression was always

Table 3. Comparative expression of MMP and urokinase RNAs in breast tumors

| RNA | Number of positive tumors* | | Expressing cell type |
|---|---|---|---|
| | Adenomas | Carcinomas | |
| ST1 | 0 | 0 | — |
| ST2 | 0 | 1 | Neoplastic cells |
| I COL(F) | 0 | 3 | Fibroblastic cells |
| Pump-1 | 2 | 10 | Normal and neoplastic epithelial cells |
| IV COL(92) | 0 | 11 | Inflammatory and few neoplastic cells |
| IV COL(72) | 2 | 11 | Fibroblastic cells |
| ST3 | 0 | 11 | Fibroblastic cells |
| Urokinase | 0 | 11 | Fibroblastic and few neoplastic cells |

I COL(F), interstitial type I collagenase; IV COL(92), 92-kDa type IV collagenase. Pump-1, putative metalloproteinase 1.
*Two fibroadenomas and 11 invasive ductal carcinomas were analyzed by *in situ* RNA hybridization; probes were as in refs. 3, 4, 10, and 11.

restricted to fibroblastic cells immediately surrounding the neoplastic cells (3, 4, 10, 11). This restricted expression pattern distinguishes the ST3 gene from all the other MMP genes, most notably from the IV COL(72) gene, which can be expressed in fibroblastic cells located at distance from the cancer cells. It also differs from that of the urokinase gene, which is expressed not only in fibroblastic cells of colon adenocarcinomas (18) but also in neoplastic cells of squamous cell carcinomas (ref. 22; C.W., unpublished results). Interestingly, our present data show that ST3 and urokinase gene expression are highly correlated in breast carcinomas and that, with the exception of a few neoplastic cells that express the urokinase gene only, ST3 and urokinase RNAs are expressed in the same fibroblastic compartment. Thus, the expression of the two genes may be similarly regulated,

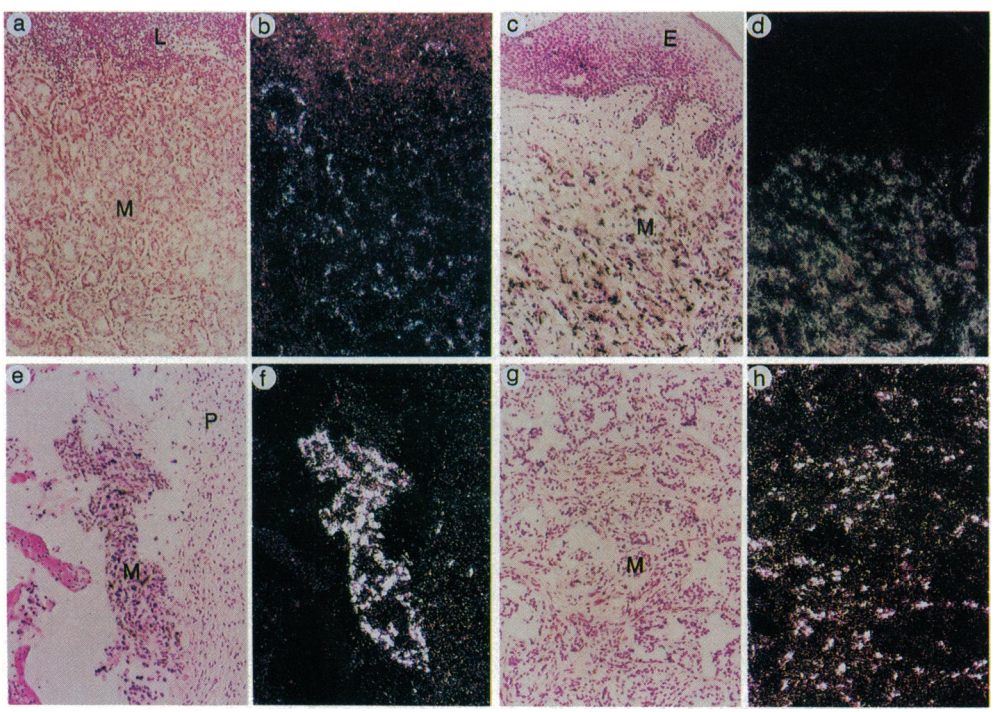


FIG. 3. Expression of ST3 RNA in metastases of ductal breast carcinomas. Bright-field (*a*, *c*, *e*, and *g*) and dark-field (*b*, *d*, *f*, and *h*) photomicrographs of paraffin-embedded tissue sections stained with hematoxylin, after *in situ* hybridization with $^{35}$S-labeled ST3 antisense RNA and autoradiography for 4 weeks are shown. (*a* and *b*) Lymph node metastasis. (*c* and *d*) Skin metastasis. (*e* and *f*) Pleura metastasis. (*g* and *h*) Bone metastasis. M, metastatic tumor; L, lymphocytes; E, epiderm; P, pleura. (Original magnification: ×100.)

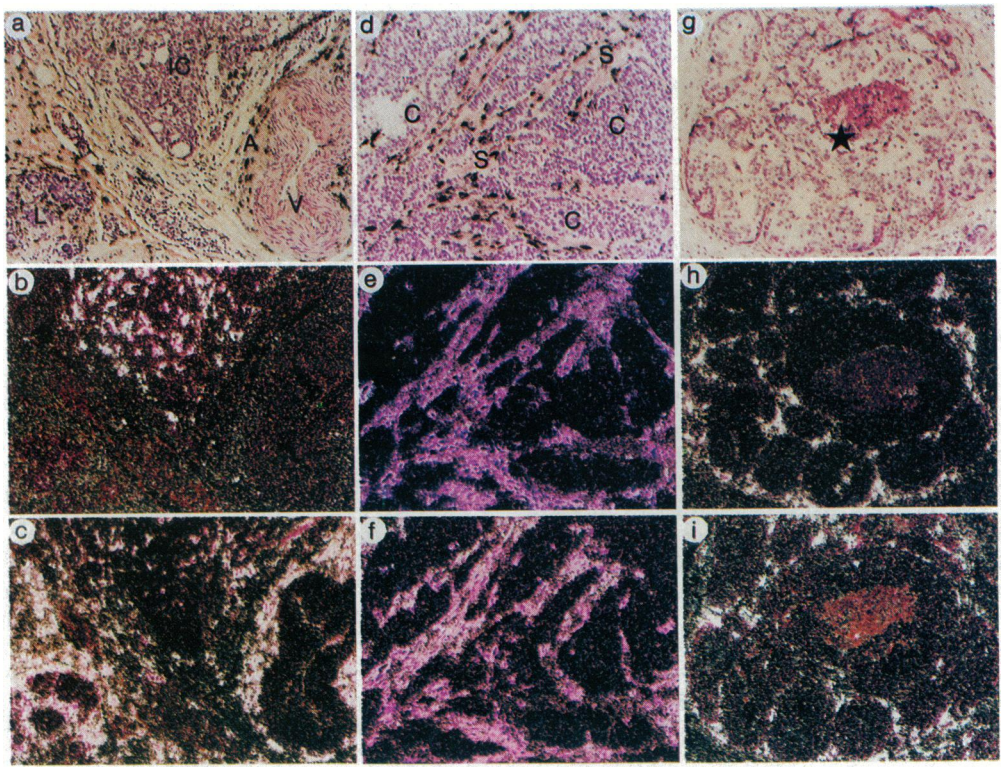


FIG. 4. Stromal expression of ST3, IV COL(72), and urokinase RNAs in breast carcinomas. Bright-field (*a*, *d*, and *g*) and dark-field (*b*, *c*, *e*, *f*, *h*, and *i*) photomicrographs of paraffin-embedded tissue sections stained with hematoxylin, after *in situ* hybridization with $^{35}$S-labeled ST3 (*b*, *e*, and *h*), 72-kDa type IV collagenase (*c*), and urokinase (*f* and *i*) antisense RNAs are shown. Probes are as in ref. 4. Exposure times were 2 weeks (*c*), 4 weeks (*b*, *e*, and *f*), and 6 weeks (*h* and *i*). *a–c*, *d–f*, and *g–i* correspond to serial sections of two invasive and one *in situ* ductal carcinomas, respectively. L, normal lobule; A, adventitia of a blood vessel (V); IC, area of invasive carcinoma, with intermixed cancer and stromal cells; S, stroma; ⋆, *in situ* carcinoma; C, invasive cancer cells. (*a–c*) ST3 transcripts were exclusively detected in stromal cells present in the area of invasive carcinoma (*b*), whereas IV COL(72) transcripts were also observed in connective tissue cells in the normal lobule and the blood vessel adventitia (*c*). ST3 (*e* and *h*) and urokinase (*f* and *i*) transcripts were detected in the same stromal cell compartment, in both invasive (*d–f*) and *in situ* (*g–i*) carcinomas. (Original magnification: ×100.)

and their products may cooperate during tumor progression, possibly through a proteinase cascade (7). In this respect, we note that high levels of urokinase have been reported to be associated with increased risk of relapse and death in breast cancer patients (23, 24). The possible implication of ST3 in tumor progression is also supported by the observation that in head and neck (10) and in skin basal cell (11) carcinomas, there was a good correlation between ST3 RNA levels and local invasiveness. Thus, ST3 gene expression appears well correlated with tumor invasion in both breast and other types of human carcinomas, which suggests that the presence of ST3 could be used as a prognostic marker to define subpopulations of aggressive tumors.

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1. Harris, J. R., Lippman, M. E., Veronesi, U. & Willett, W. (1992) *N. Engl. J. Med.* **327,** 319–328, 390–398, 473–480.
2. Henderson, I. C. (1992) *N. Engl. J. Med.* **326,** 1774–1775.
3. Basset, P., Bellocq, J. P., Wolf, C., Stoll, I., Hutin, P., Limacher, J. M., Podhajcer, O. L., Chenard, M. P., Rio, M. C. & Chambon, P. (1990) *Nature (London)* **348,** 699–704.
4. Basset, P., Wolf, C. & Chambon, P. (1993) *Breast Cancer Res. Treat.*, in press.
5. Liotta, L. A., Steeg, P. S. & Stetler-Stevenson, W. G. (1991) *Cell* **64,** 327–336.
6. Matrisian, L. M. (1992) *BioEssays* **14,** 455–463.
7. Mignatti, P., Robbins, E. & Rifkin, D. B. (1986) *Cell* **47,** 487–498.
8. Rochette-Egly, C., Lutz, Y., Saunders, M., Scheuer, I., Gaub, M. P. & Chambon, P. (1991) *J. Cell Biol.* **115,** 535–545.
9. Studier, F. W., Rosemberg, A. H., Dunn, J. J. & Dubendorff, J. W. (1990) *Methods Enzymol.* **185,** 60–89.
10. Muller, D., Wolf, C., Abecassis, J., Millon, R., Engelmann, A., Bronner, G., Rouyer, N., Rio, M. C., Eber, M., Methlin, G., Chambon, P. & Basset, P. (1993) *Cancer Res.* **53,** 165–169.
11. Wolf, C., Chenard, M. P., Durand de Grossouvre, P., Bellocq, J. P., Chambon, P. & Basset, P. (1992) *J. Invest. Dermatol.* **99,** 870–872.
12. Page, D. L., Kidd, T. E., Dupont, W. D., Simpson, J. F. & Rogers, L. W. (1991) *Hum. Pathol.* **22,** 1232–1239.
13. Folkman, J. (1992) *Semin. Cancer Biol.* **3,** 65–71.
14. Slamon, D. J., Clark, G. M., Wong, S. G., Levin, W. J., Ullrich, A. & McGuire, W. L. (1987) *Science* **235,** 177–182.
15. Weidner, N., Semple, J. P., Welch, W. R. & Folkman, J. (1991) *N. Engl. J. Med.* **324,** 1–8.
16. Van de Vijver, M. J., Peterse, J. L., Mooi, W. J., Wisman, P., Lomans, J., Dalesio, O. & Nusse, R. (1988) *N. Engl. J. Med.* **319,** 1239–1245.
17. Goslen, J. B. & Bauer, E. A. (1986) *J. Dermatol. Surg. Oncol.* **12,** 812–817.
18. Grondhal-Hansen, J., Ralfkiaer, E., Kirkeby, L. T., Kristensen, P., Lund, L. R. & Dano, K. (1991) *Am. J. Pathol.* **138,** 111–117.
19. Pyke, C., Ralfkiaer, E., Huhtala, P., Hurskainen, T., Dano, K. & Tryggvason, K. (1992) *Cancer Res.* **52,** 1336–1341.
20. Poulsom, R., Pignatelli, M., Stetler-Stevenson, W. G., Liotta, L. A., Wright, P. A., Jeffery, R. E., Longcroft, J. M., Rogers, L. & Stamp, G. W. H. (1992) *Am. J. Pathol.* **141,** 388–396.
21. Polette, M., Clavel, C., Cockett, M., Girod de Bentzmann, S., Murphy, G. & Birembaut, P. (1993) *Invasion Metastasis*, in press.
22. Sappino, A. P., Belin, D., Huarte, J., Hirschel-Scholz, S., Saurat, J.-H. & Vassalli, J.-D. (1991) *J. Clin. Invest.* **88,** 1073–1079.
23. Duffy, M. J., Reilly, D., O'Sullivan, C., O'Higgins, N., Fennelly, J. J. & Andreasen, P. (1990) *Cancer Res.* **50,** 6827–6829.
24. Jänicke, F., Schmitt, M., Hafter, R., Hollrieder, A., Babic, R., Ulm, K., Gössner, W. & Graeff, H. (1990) *Fibrinolysis* **4,** 1–10.