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# Elastosis in Breast - Correlation with Epithelial Proliferation in Benign Disease and Carcinomatous Growth

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## ABSTRACT

Elastosis in the breast is an unusual phenomenon and its morphogenesis has not yet been fully ascertained. The degree of elastosis in the breast associated with benign diseases, including fibroadenoma and fibrocystic disease as well as with breast carcinoma, was examined with special reference to the correlation between the degree of epithelial proliferation and elastosis. Using the immunohistochemical method, the presence of elastase (EL) and $\alpha$ 1-antichymotrypsin (ACT), one of the protease inhibitors, in these epithelial cells was also investigated to elucidate the role of an imbalance in these enzymes in the morphogenesis of the elastosis. Consequently, it was shown that there is a tendency in fibroadenoma and fibrocystic disease, for epithelial proliferation to be related to the degree of elastosis, and that the lack of EL in proliferated epithelial cells might play a role in the occurrence of elastosis, although ACT has no significant correlation. On the contrary, in our study noninvasive carcinoma showed marked periductal elastosis but no stromal elastosis, while invasive carcinoma showed various degrees of periductal and stromal elastosis. In invasive carcinoma, especially scirrhous carcinoma, the degree of ACT in cancer cells correlated well with stromal elastosis, although there was no correlation with EL. These findings suggest that an imbalance of the protease-antiprotease system, produced by epithelial cells of the breast, contribute to the morphogenesis of elastosis, although the physiological event, aging, is only marginally related to elastosis. Further investigation of the cells producing elastin and regulatory factors may be necessary.

***Key words:*** *Elastosis, Breast, Carcinoma*

Elastosis in the breast, that is, the presence of clumps of elastic fibers which appear as eosinophilic hyaline acellular areas in section stained with hematoxylin-eosin, occurs at three anatomical sites, periductal, stromal and perivascular, and has been a focus of interest. In breast carcinoma, a number of studies have been made to ascertain the relationship between the degree of elastosis and prognosis based on patients' outcome, but there is some controversy[6]. Furthermore, it is now known that elastosis accompanies benign breast disease. With regard to benign breast disease, a hypothesis that the degree of elastosis increases progressively in fibrocystic disease with the severity of epitheliosis has been proposed[30].

However, it remains unclear whether elastosis is to be regarded as a nonspecific stromal reaction to the malignant or benign disease or constitutes a more active component of an associated disease process. Also, it has been suggested that changes in the elastic fibers of the breast are associated with age, parity and reproductive status[10]. Thus, elastosis in the breast remains a mysterious phenomenon since its morphogenesis has not been ascertained. Recently, a hypothesis was put forward regarding the morphogenesis of elastosis which proposed that the presence of protease inhibitors might influence the metabolism of elastic fibers, facilitating elastic fiber proliferation by the inhibition of elastinolytic enzymes[9].

The present study is directed towards showing the degree of elastosis in the breast associated with benign disease, including fibroadenoma and fibrocystic disease, as well as with breast carcinoma, with special reference to the correlation between the degree of epithelial proliferation and elastosis. It is speculated that the epithelial cells produce protease inhibitors and an attempt is made by the use of immunohistochemical method, to show the presence of elastase and $\alpha$ 1-antichymotrypsin,

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one of the protease inhibitors, in these epithelial cells in order to examine the role of these enzymes in the morphogenesis of elastosis in the breast.

## MATERIALS AND METHODS

1. *Materials*: One hundred and twenty-one mastectomised female breasts with primary carcinoma were obtained from surgical files of Hiroshima University Hospital. The patients' ages ranged from 31 to 82. The mastectomised breasts were fixed with formalin and 1 or 2 specimens from the main tumor in each case were used for the present study. Among the cases with carcinoma, the breasts of 69 cases were cut by stepwise method with 5mm intervals to examine non-neoplastic areas microscopically, according to the method which we have previously reported[19]. Among 4412 specimens made from non-neoplastic areas, 32 lesions of radial scar measuring more than 3mm were detected. Radial scar was determined as a lesion with a central fibroelastotic core surrounded by stellate proliferation of the duct system[18,33,40].

Fifty-six cases of fibroadenoma, most of which were obtained by excisional biopsy, and 23 biopsy cases diagnosed as fibrocystic disease only, were selected from the same surgical files. The age of patients with fibroadenoma or fibrocystic disease ranged from 16 to 72 and 26 to 73, respectively. In these cases of benign breast disease, representative sections were selected for microscopic examination.

2. *Staining*: All sections were stained with hematoxylin-eosin (H&E), and in order to ascertain elastic fibers, elastica-van-Gieson (EVG) staining was performed in all sections.

3. *Grading of elastosis*: Elastosis in the breast was noted as three modes, that is: periductal, stromal and perivascular. The present study focused on the former two modes of elastosis because perivascular elastosis was thought to be influenced by factors other than epithelial cell proliferation. The elastosis was graded as follows: total lack of clumps of elastic fibers, negative (–); mild to moderate increase of elastic fibers in the periductal region or the presence of newly formed-elastic fibers in the stroma, mild to moderate (+); prominent increase of elastic fibers in the periductal region and in the stroma, marked (++).

4. *Immunohistochemistry*: Among cases with breast carcinoma, 116 cases with periductal elastosis and 102 cases with stromal elastosis were examined immunohistochemically, because in 5 cases with solid-tubular carcinoma, the ductal component was absent, and in 19 cases with noninvasive ductal carcinoma, no stromal component was observed. All of the cases with fibroadenoma and fibrocystic disease were examined immunohistochemically.

Serial sections of the microscopically examined section were prepared, and by means of the avidin-biotiń-peroxidase complex (ABC) method, localization of elastase (EL) and $\alpha$ 1-antichymotrypsin (ACT) was examined. Polyclonal antibodies against EL and ACT were purchased from Ventrex Laboratories (Portland, USA) or Dako Co. Ltd. (Glostrup, Denmark), respectively. The antibodies against EL and ACT were used at a dilution of 1: 200 and 1: 1500, respectively. As an internal control, the immunoreactivity of EL was observed at neutrophils infiltrated to the breast, and that of ACT at histiocytes.

The immunohistochemical staining was evaluated as follows: total lack of staining in any of the epithelial cells, negative (–); less than 49% of the epithelial cells were positive or weakly positive, positive (+); more than 50% of the epithelial cells were positive and strongly positive, strongly positive (++).

## RESULTS

1. Incidence and degree of elastosis

(1) Elastosis in fibroadenoma

Cases with fibroadenoma were divided into three groups according to the patients' age, that is, below 29, third decade and above 40 (Table 1). Elastosis in fibroadenoma appeared to increase in incidence progressively with age, although no statistically significant difference was noted. Elastosis in fibroadenoma was mostly of the stromal type.

Epithelial proliferation was occasionally associated in the ducts or tubules of fibroadenoma (Fig. 1-a). Marked stromal elastosis is seen in fibroadenoma (Fig. 1-b). Cases were divided into three groups, according to the presence and degree of this change. The degree of elastosis in the special area showing the epithelial proliferation is shown in Table 2. Cases with more epithelial proliferation appeared to show a higher incidence of elastosis, although this tendency was not statistically significant.

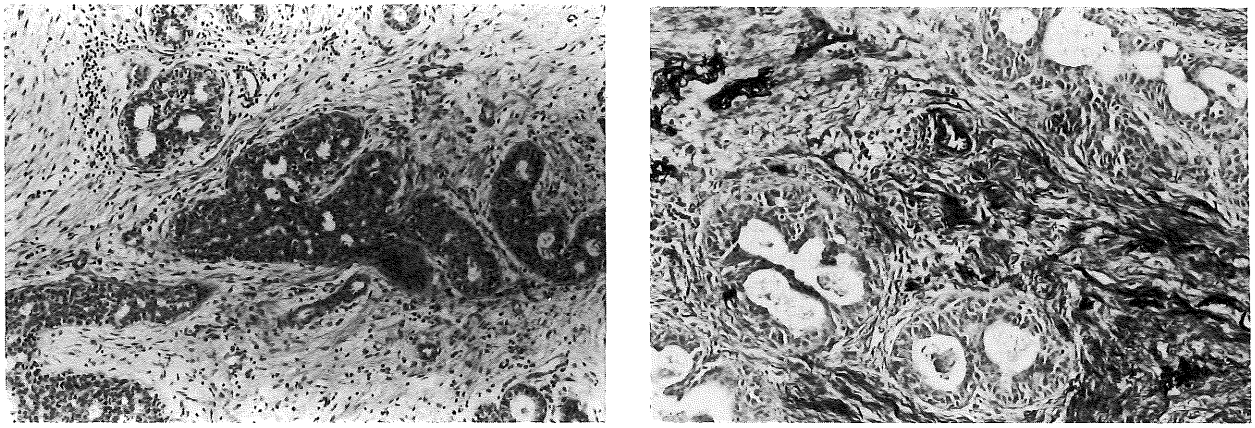
(2) Elastosis in fibrocystic disease (epitheliosis)

Elastosis in cases with fibrocystic disease was seen more frequently than in cases with fibroadenoma (57% versus 34%). The type of elastosis was mostly periductal (Fig. 2-a). Marked periductal elastosis is seen in fibrocystic disease (Fig. 2-b). Twenty-three cases with fibrocystic disease were

**Table 1.** Relationship between Degree of Stromal Elastosis in Fibroadenoma and Age of the Patients

| Age | Total no. of cases | No. of cases (%) with stromal elastosis at degrees of: – | + | ++ |
|---|---|---|---|---|
| ≦29 | 19 | 15 (79) | 4 (21) | 0 |
| 30–39 | 15 | 10 (67) | 4 (27) | 1 (7) |
| 40≦ | 22 | 12 (55) | 8 (36) | 2 (9) |
| Total | 56 | 37 (66) | 16 (29) | 3 (5) |

(N.S.)



(a) (b)

**Fig. 1.** Fibroadenoma with stromal elastosis and epithelial proliferation. (a: H&E stain, b: EVG stain, ×33)

**Table 2.** Relationship between Degree of Stromal Elastosis and Degree of Epithelial Proliferation in Fibroadenoma

| Degree of epithelial proliferation | Total no. of cases | No. of cases (%) with stromal elastosis at degrees of: | | |
|---|---|---|---|---|
| | | − | + | ++ |
| + | 20 | 15 (75) | 3 (15) | 2 (10) |
| ++ | 23 | 16 (70) | 6 (26) | 1 (4) |
| +++ | 13 | 6 (46) | 7 (54) | 0 |

(N.S.)

**Table 3.** Relationship between Degree of Periductal Elastosis in Fibrocystic Disease and Age of Patients

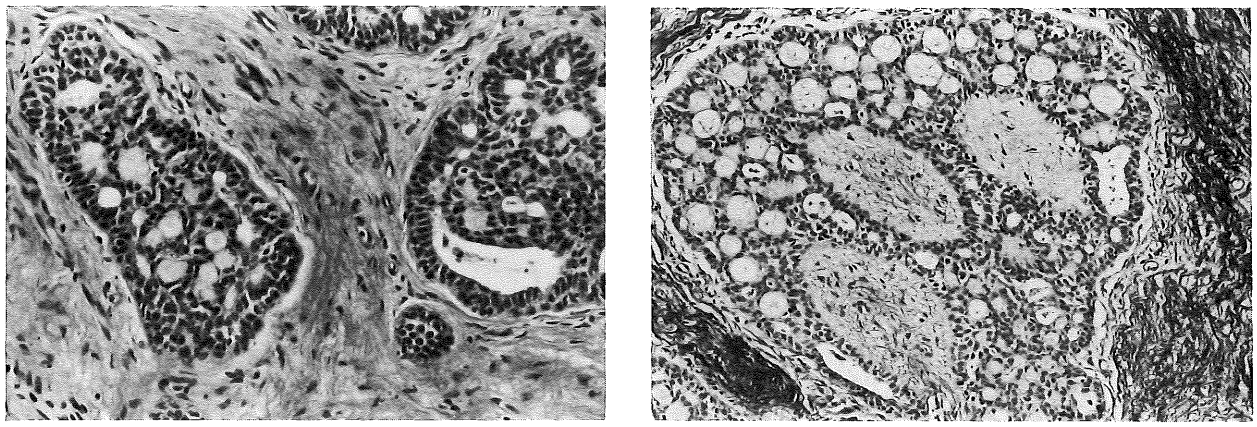
| Age | Total no. of cases | No. of cases (%) with periductal elastosis at degrees of: | | |
|---|---|---|---|---|
| | | − | + | ++ |
| ≦39 | 10 | 4 (40) | 4 (40) | 2 (20) |
| ≧40 | 13 | 6 (46) | 6 (46) | 1 (8) |
| Total | 23 | 10 (43) | 10 (43) | 3 (13) |

(N.S.)

divided into two groups according to the patients' age: below 39 and above 40. There was no correlation between the patients' age and elastosis (Table 3). Cases were divided into three groups according to the degree of ductal epithelial hyperplasia (epitheliosis). It appeared that the degree of elastosis was positively related to the degree of epitheliosis, although the number of cases was small and there was no statistical difference (Table 4).

(3) Elastosis in radial scar

Radial scar was almost always associated with elastosis. The type was periductal (Fig. 3-a). Marked periductal elastosis is noted in radial scar (Fig. 3-b). The relation to patients' age was not significant. Table 5 shows the correlation between the degree of elastosis and the degree of epithelial



(a) (b)

**Fig. 2.** Fibrocystic disease with periductal elastosis and epithelial proliferation. (a: H&E stain, b: EVG stain, ×33)

proliferation, which was not statistically significant.

(4) Elastosis in breast carcinoma

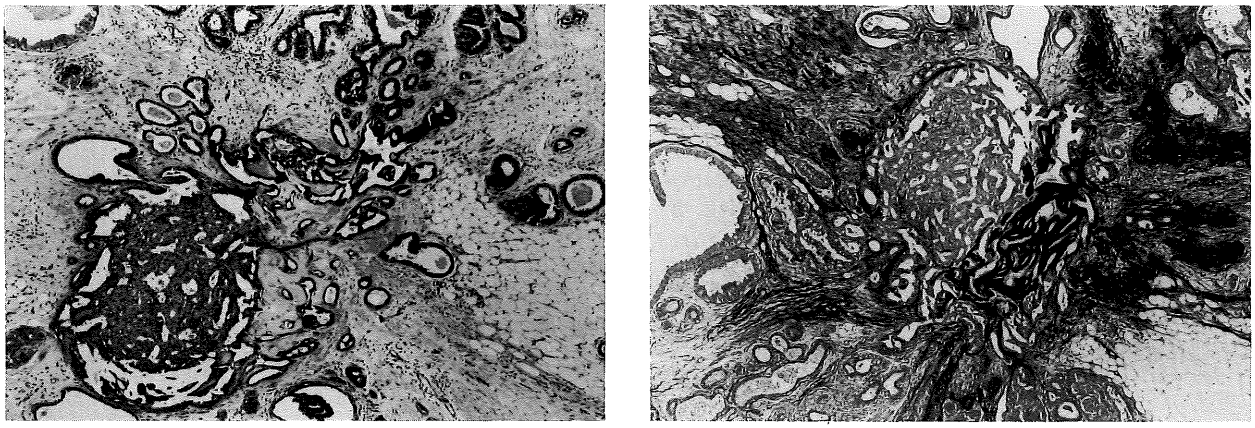
Breast carcinoma cases were divided into two groups according to the patients' age: below 49 and above 50. Cases above 50 years showed a higher incidence of elastosis than those with aged below 49 ($p<0.05$) (Table 6).

The histological distribution of breast carcinoma according to the classification proposed by the Japanese Breast Cancer Society is shown in Table 7. Elastosis was evaluated separately in the periductal region or in the stroma. Two-thirds of

**Table 4.** Relationship between Degree of Periductal Elastosis and Degree of Epitheliosis in Fibrocystic Disease

| Degree of epitheliosis | Total no. of cases | No. of cases (%) with periductal elastosis at degrees of: | | |
|---|---|---|---|---|
| | | − | + | ++ |
| + | 13 | 7 (54) | 4 (31) | 2 (15) |
| ++ | 6 | 2 (33) | 3 (50) | 1 (17) |
| +++ | 4 | 1 (25) | 3 (75) | 0 |

(N.S.)



(a) (b)

**Fig. 3.** Radial scar with periductal elastosis. (a: H&E stain, b: EVG stain, ×25)

**Table 5.** Relationship between Degree of Elastosis and Degree of Epithelial Proliferation in Radial Scar

| Degree of epithelial proliferation | Total no. of lesions | No. of lesions (%) with elastosis at degrees of: | | |
|---|---|---|---|---|
| | | + | ++ | +++ |
| − | 12 | 1 (8) | 3 (25) | 8 (67) |
| + | 13 | 1 (8) | 4 (31) | 8 (62) |
| ++ | 7 | 1 (14) | 1 (14) | 5 (71) |
| Total | 32 | 3 (9) | 8 (25) | 21 (66) |

(N.S.)

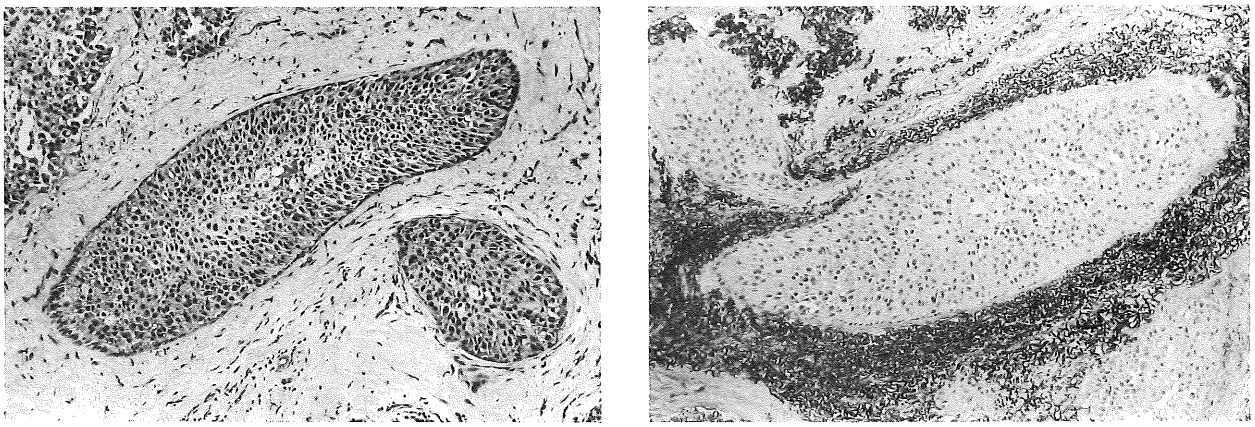
**Table 6.** Relationship between Degree of All Types of Elastosis in Breast Carcinoma and Age of Patients

| Age | Total no. of cases | No. of cases (%) with elastosis at degrees of: | |
|---|---|---|---|
| | | − | + |
| ≦49 | 62 | 19 (31) | 43 (69) |
| 50≦ | 59 | 8 (14) | 51 (86) |
| Total | 121 | 27 (22) | 94 (78) |

($p<0.05$)

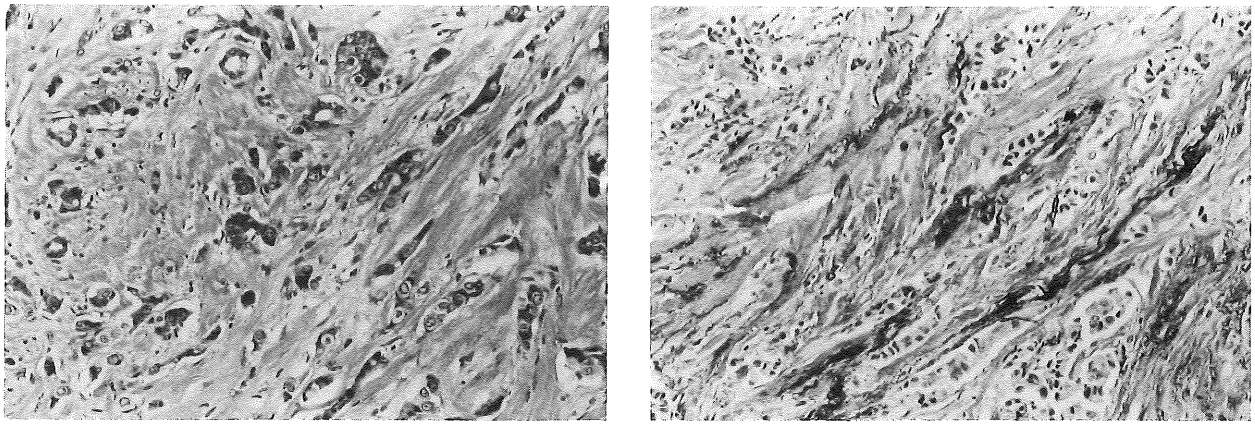
**Table 7.** Relationship between Histological type of Breast Carcinoma and Degree of Periductal and Stromal Elastosis

| Histological type of carcinoma | Total no. of cases | No. of cases (%) with: | | | | | |
|---|---|---|---|---|---|---|---|
| | | Periductal elastosis at degrees of: | | | Stromal elastosis at degrees of: | | |
| | | − | + | ++ | − | + | ++ |
| Noninvasive ductal carcinoma | 19 | 7 (37) | 10 (53) | 2 (11) | − | − | − |
| Invasive ductal carcinoma | | | | | | | |
| Papillotubular carcinoma | 16 | 9 (56) | 5 (31) | 2 (13) | 13 (81) | 2 (13) | 1 (6) |
| Solid-tubular carcinoma | 20 | 6 (40) | 7 (47) | 2 (13) | 9 (45) | 11 (55) | 0 |
| Scirrhous carcinoma | 57 | 3 (5) | 14 (25) | 40 (70) | 8 (14) | 18 (32) | 31 (54) |
| Medullary carcinoma | 2 | 2 (100) | 0 | 0 | 2 (100) | 0 | 0 |
| Lobular carcinoma | 3 | 2 (67) | 1 (33) | 0 | 2 (67) | 1 (33) | 0 |
| Tubular carcinoma | 3 | 1 (33) | 1 (33) | 1 (33) | 0 | 2 (67) | 1 (33) |
| Mucinous carcinoma | 1 | 1 (100) | 0 | 0 | 0 | 1 (100) | 0 |



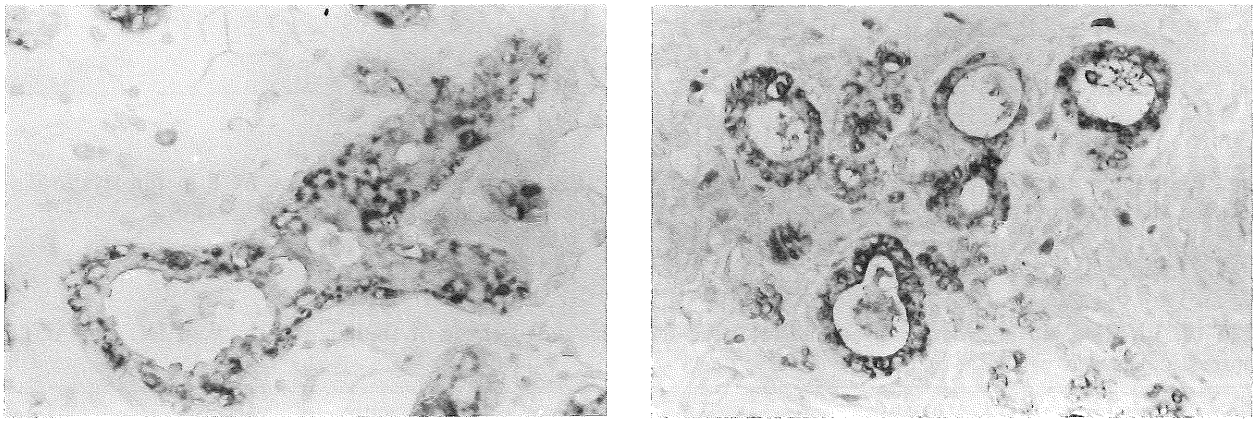
(a) (b)

**Fig. 4.** Noninvasive carcinoma with periductal elastosis. (a: H&E stain, b: EVG stain, ×33)



(a) (b)

**Fig. 5.** Scirrhous carcinoma with stromal elastosis. (a: H&E stain, b: EVG stain, ×33)



(a) (b)

**Fig. 6.** Immunoreactivity of EL(a) and ACT(b) in normal ductal cells were noted. (ABC method, ×33)

cases with non-invasive ductal carcinoma showed periductal elastosis (Fig. 4-a). Periductal elastosis is observed markedly in Fig. 4-b. It is evident that stromal elastosis is related to the invasive growth of the carcinoma and to the degree of differentiation among the invasive carcinoma (Fig. 5-a). Marked stromal elastosis is observed in scirrhous carcinoma (Fig. 5-b). Periductal elastosis was also

**Table 8.** Relationship between Degree of Stromal Elastosis and Degree of Immunoreactivity of EL and ACT in Fibroadenoma

| Degree of stromal elastosis | Total no. of cases | No. of cases (%) with: EL in epithelial cells at degrees of: − | + | ++ | ACT in epithelial cells at degrees of: − | + | ++ |
|---|---|---|---|---|---|---|---|
| − | 37 | 20 (54) | 13 (35) | 4 (11) | 1 (3) | 14 (38) | 22 (59) |
| + | 16 | 9 (56) | 5 (31) | 2 (13) | 0 | 8 (50) | 8 (50) |
| ++ | 3 | 3 (100) | 0 | 0 | 0 | 2 (66) | 1 (33) |
| Total | 56 | 32 (57) | 18 (32) | 6 (11) | 1 (2) | 24 (43) | 31 (55) |
| | | | | (N.S.) | | | (N.S.) |

**Table 9.** Relationship between Degree of Epithelial Proliferation and Degree of Immunoreactivity of EL and ACT in Fibroadenoma

| Degree of epithelial proliferation | Total no. of cases | No. of cases (%) with: EL in epithelial cells at degrees of: − | + | ++ | ACT in epithelial cells at degrees of: − | + | ++ |
|---|---|---|---|---|---|---|---|
| + | 20 | 14 (70) | 5 (25) | 1 (5) | 1 (5) | 11 (55) | 8 (40) |
| ++ | 23 | 15 (65) | 4 (17) | 4 (17) | 0 | 11 (48) | 12 (52) |
| +++ | 13 | 3 (23) | 9 (69) | 1 (8) | 0 | 2 (15) | 11 (85) |
| | | | | ($p<0.05$) | | | (N.S.) |

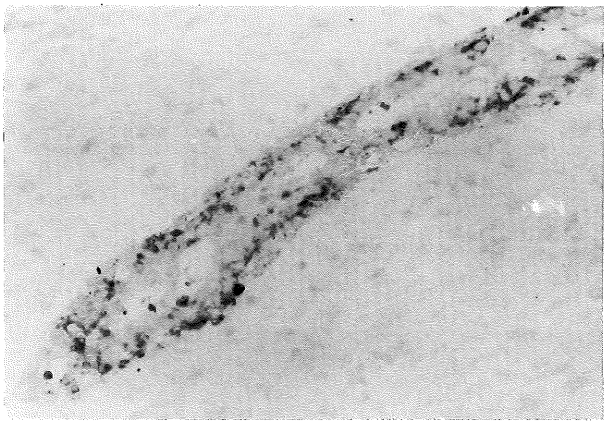


**Fig. 7.** Immunoreactivity of EL in fibroadenoma was noted. (ABC method, ×33)

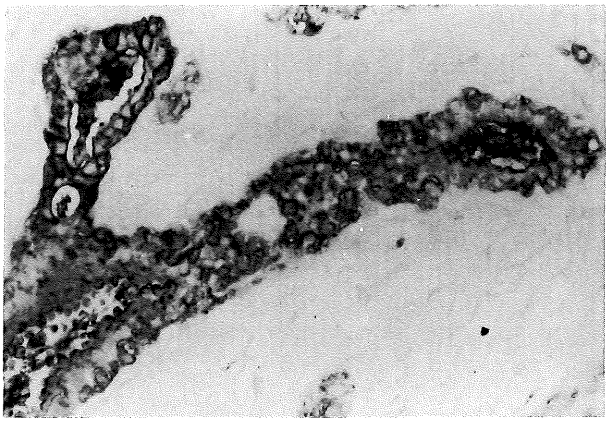


**Fig. 8.** Immunoreactivity of ACT in fibrocystic disease was noted. (ABC method, ×50)

**Table 10.** Relationship between Degree of Periductal Elastosis and Degree of Immunoreactivity of EL and ACT in Fibrocystic Disease

| Degree of periductal elastosis | Total no. of cases | No. of cases (%) with: EL in epithelial cells at degrees of: − | + | ++ | ACT in epithelial cells at degrees of: − | + | ++ |
|---|---|---|---|---|---|---|---|
| − | 10 | 7 (70) | 3 (30) | 0 | 0 | 7 (70) | 3 (30) |
| + | 10 | 8 (80) | 2 (20) | 0 | 1 (10) | 4 (40) | 5 (50) |
| ++ | 3 | 3 (100) | 0 | 0 | 1 (33) | 2 (66) | 0 |
| Total | 23 | 18 (78) | 5 (22) | 0 | 2 (9) | 13 (57) | 8 (35) |
| | | | | (N.S.) | | | (N.S.) |

related to invasive growth, and thus scirrhous carcinoma showed the highest incidence.

2. Immunohistochemistry of elastase (EL) and α 1-antichymotrypsin (ACT)

(1) Localization of EL and ACT in normal breast

The constituent cells in the normal lobule were positive for EL and ACT. The relation to patients' age was not significant. It is uncertain whether one cell simultaneously had a positive finding of both EL and ACT. The myoepithelial cells were positive for both EL and ACT. The constituent cells in the normal duct were positive for EL and ACT (Fig. 6). No correlation to the patients' age was noted, as with the lobule.

(2) EL and ACT in fibroadenoma

The proportion of fibroadenoma with positive EL and ACT in the epithelial cells was 43% and 98%, respectively. Table 8 shows the correlation between stromal elastosis and the positivity of EL and ACT in the epithelial cells. EL was not detected in the cases with marked elastosis, but there was no correlation between the degree of elastosis and the positivity of ACT in the epithelial cells. As shown in Table 9, the fibroadenoma with marked epithelial proliferation had a higher frequency of positive EL ($p<0.05$) (Fig. 7) and a greater positivity of ACT. The positivity of EL was not related to that of ACT in any of the epithelial cells.

(3) EL and ACT in fibrocystic disease (epitheliosis)

The proportion of fibrocystic disease (epitheliosis) with positive EL and ACT in epithelial cells was 22% and 91%, respectively (Table 10). As with fibroadenoma, the number of cases with positive ACT (Fig. 8) was larger than those with positive EL.

EL was not detected in cases with marked elastosis, as with fibroadenoma. There was no correlation between the degree of elastosis and the

**Table 11.** Relationship between Degree of Epitheliosis and Degree of Immunoreactivity of EL and ACT in Fibrocystic disease

| Degree of epitheliosis | Total no. of cases | No. of cases (%) with: EL in epithelial cells at degrees of: − | + | ++ | ACT in epithelial cells at degrees of: − | + | ++ |
|---|---|---|---|---|---|---|---|
| + | 13 | 10 (77) | 3 (23) | 0 | 1 (8) | 9 (69) | 3 (23) |
| ++ | 6 | 5 (83) | 1 (17) | 0 | 0 | 3 (50) | 3 (50) |
| +++ | 4 | 3 (75) | 1 (25) | 0 | 1 (25) | 1 (25) | 2 (50) |
| | | | | (N.S.) | | | (N.S.) |

**Table 12.** Relationship between Degree of Periductal Elastosis and Degree of Immunoreactivity of EL and ACT in Radial Scar

| Degree of periductal elastosis | Total no. of lesions | No. of lesions (%) with: EL in epithelial cells at degrees of: − | + | ++ | ACT in epithelial cells at degrees of: − | + | ++ |
|---|---|---|---|---|---|---|---|
| + | 3 | 2 (67) | 1 (33) | 0 | 0 | 1 (33) | 2 (67) |
| ++ | 8 | 5 (63) | 2 (25) | 1 (13) | 2 (25) | 4 (50) | 2 (25) |
| +++ | 21 | 11 (52) | 8 (38) | 2 (10) | 7 (33) | 8 (38) | 6 (29) |
| Total | 32 | 18 (56) | 11 (34) | 3 (9) | 9 (28) | 13 (41) | 10 (31) |
| | | | | (N.S.) | | | (N.S.) |

**Table 13.** Relationship between Degree of Epithelial Proliferation and Degree of Immunoreactivity of EL and ACT in Radial Scar

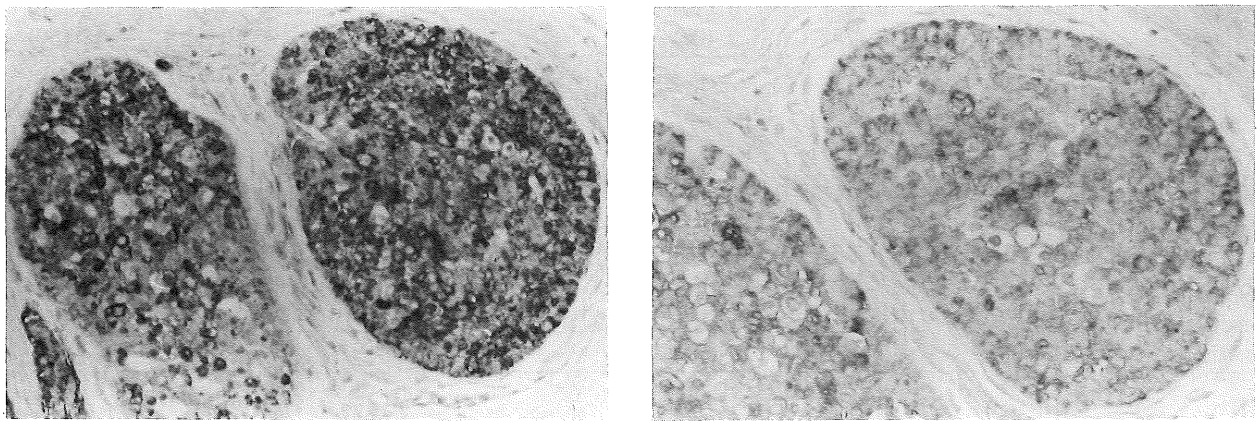
| Degree of epithelial proliferation | Total no. of cases | No. of cases (%) with: EL in epithelial cells at degrees of: + | ++ | +++ | ACT in epithelial cells at degrees of: + | ++ | +++ |
|---|---|---|---|---|---|---|---|
| − | 12 | 6 (50) | 5 (42) | 1 (8) | 6 (50) | 3 (25) | 3 (25) |
| + | 13 | 8 (62) | 3 (23) | 2 (15) | 2 (15) | 7 (54) | 4 (31) |
| ++ | 7 | 4 (57) | 3 (43) | 0 | 1 (14) | 3 (43) | 3 (43) |
| | | | | (N.S.) | | | (N.S.) |

**Table 14.** Relationship between Degree of Periductal Elastosis and Degree of Immunoreactivity of EL and ACT in Cancer Cells

| Degree of periductal elastosis | Total no. of cases | No. of cases (%) with: EL in cancer cells at degrees of: | | | ACT in cancer cells at degrees of: | | |
|---|---|---|---|---|---|---|---|
| | | − | + | ++ | − | + | ++ |
| − | 30 | 17 (57) | 7 (23) | 6 (20) | 14 (47) | 4 (13) | 12 (40) |
| + | 38 | 4 (11) | 17 (45) | 17 (45) | 9 (24) | 5 (13) | 24 (63) |
| ++ | 48 | 16 (33) | 13 (27) | 19 (40) | 10 (21) | 9 (19) | 29 (60) |
| Total | 116 | 37 (32) | 37 (32) | 42 (36) | 33 (28) | 18 (16) | 65 (56) |
| | | | | (N.S.) | | | (N.S.) |

**Table 15.** Relationship between Degree of Stromal Elastosis and Degree of Immunoreactivity of EL and ACT in Cancer Cells

| Degree of stromal elastosis | Total no. of cases | No. of cases (%) with: EL in cancer cells at degrees of: | | | ACT in cancer cells at degrees of: | | |
|---|---|---|---|---|---|---|---|
| | | − | + | ++ | − | + | ++ |
| − | 34 | 17 (50) | 10 (29) | 7 (21) | 17 (50) | 2 (6) | 15 (44) |
| + | 35 | 12 (34) | 10 (29) | 13 (37) | 12 (34) | 8 (23) | 15 (43) |
| ++ | 33 | 8 (24) | 12 (36) | 13 (39) | 5 (15) | 2 (6) | 26 (79) |
| Total | 102 | 37 (36) | 32 (31) | 33 (32) | 34 (33) | 12 (12) | 56 (55) |
| | | | | (N.S.) | | | ($p<0.01$) |



(a) (b)

**Fig. 9.** Immunoreactivities of EL(a) and ACT(b) in noninvasive carcinoma. EL was markedly positive and ACT was moderately positive. (ABC method, ×33)

positivity of ACT in the epithelial cells (Table 10). Table 11 shows the correlation between the degree of epitheliosis and the positivity of EL and ACT. There was no significant correlation between the two. The positivity of EL was not related to that of ACT in any of the epithelial cells.

(4) EL and ACT in radial scar

In radial scar, the proportion of cases with positive EL and ACT in the epithelial cells was 14% and 72%, respectively, and there was no correlation between the degree of periductal elastosis and the positivity of EL and ACT in the epithelial cells (Table 12). Also, there was no correlation between the degree of epithelial proliferation and the positivity of EL and ACT in the epithelial cells (Table 13).

(5) EL and ACT in breast carcinoma

The proportion of carcinoma with positivity of EL or ACT was 68% and 72%, respectively. EL was detected more frequently compared to cases with benign disease, but ACT was detected less frequently in cases with carcinoma. As shown in Tables 14 and 15, the degree of periductal elastosis showed no correlation with the positivity of EL or

**Table 16.** Relationship between Histological Type of Breast Carcinoma and Degree of Immunoreactivity of EL and ACT in Cancer Cells

| Histological type of carcinoma | Total no. of cases | No. of cases (%) with: EL in cancer cells at degrees of: − | + | ++ | ACT in cancer cells at degrees of: − | + | ++ |
|---|---|---|---|---|---|---|---|
| Noninvasive ductal carcinoma | 19 | 3 (16) | 7 (37) | 9 (47) | 2 (11) | 7 (37) | 10 (53) |
| Invasive ductal carcinorna | | | | | | | |
| Papillotubular carcinoma | 16 | 4 (25) | 9 (56) | 3 (19) | 5 (31) | 1 (6) | 10 (63) |
| Solid-tubular carcinoma | 20 | 11 (55) | 6 (30) | 3 (15) | 9 (45) | 3 (15) | 8 (40) |
| Scirrhous carcinoma | 57 | 17 (30) | 15 (26) | 25 (44) | 16 (28) | 6 (11) | 35 (61) |
| Medullary carcinoma | 2 | 2 (100) | 0 | 0 | 2 (100) | 0 | 0 |
| Lobular carcinoma | 3 | 2 (67) | 1 (33) | 0 | 1 (33) | 0 | 2 (67) |
| Tubular carcinoma | 3 | 1 (33) | 1 (33) | 1 (33) | 0 | 2 (67) | 1 (33) |
| Mucinous carcinoma | 1 | 0 | 0 | 1 (100) | 1 (100) | 0 | 0 |

**Table 17.** Relationship between Degree of Periductal Elastosis and Degree of Immunoreactivity of EL and ACT in Cancer Cells of Noninvasive Carcinoma

| Degree of periductal elastosis | Total no. of cases | No. of cases (%) with: EL in cancer cells at degrees of: − | + | ++ | ACT in cancer cells at degrees of: − | + | ++ |
|---|---|---|---|---|---|---|---|
| − | 7 | 2 (29) | 2 (29) | 3 (43) | 1 (14) | 2 (29) | 4 (57) |
| + | 10 | 0 | 4 (40) | 6 (60) | 1 (10) | 4 (40) | 5 (50) |
| ++ | 2 | 1 (50) | 1 (50) | 0 | 0 | 1 (50) | 1 (50) |
| Total | 19 | 3 (16) | 7 (37) | 9 (47) | 2 (11) | 7 (37) | 10 (53) |
| | | | | (N.S.) | | | (N.S.) |

**Table 18.** Relationship between Degree of Stromal Elastosis and Degree of Immunoreactivity of EL and ACT in Cancer Cells of Scirrhous Carcinoma

| Degree of stromal elastosis | Total no. of cases | No. of cases (%) with: EL in cancer cells at degrees of: − | + | ++ | ACT in cancer cells at degrees of: − | + | ++ |
|---|---|---|---|---|---|---|---|
| − | 8 | 4 (50) | 1 (13) | 3 (38) | 5 (63) | 0 | 3 (38) |
| + | 18 | 5 (28) | 4 (22) | 9 (50) | 6 (33) | 4 (22) | 8 (44) |
| ++ | 31 | 8 (26) | 10 (32) | 13 (42) | 5 (16) | 2 (6) | 24 (77) |
| Total | 57 | 17 (30) | 15 (26) | 25 (44) | 16 (28) | 6 (11) | 35 (61) |
| | | | | (N.S.) | | | ($p<0.05$) |

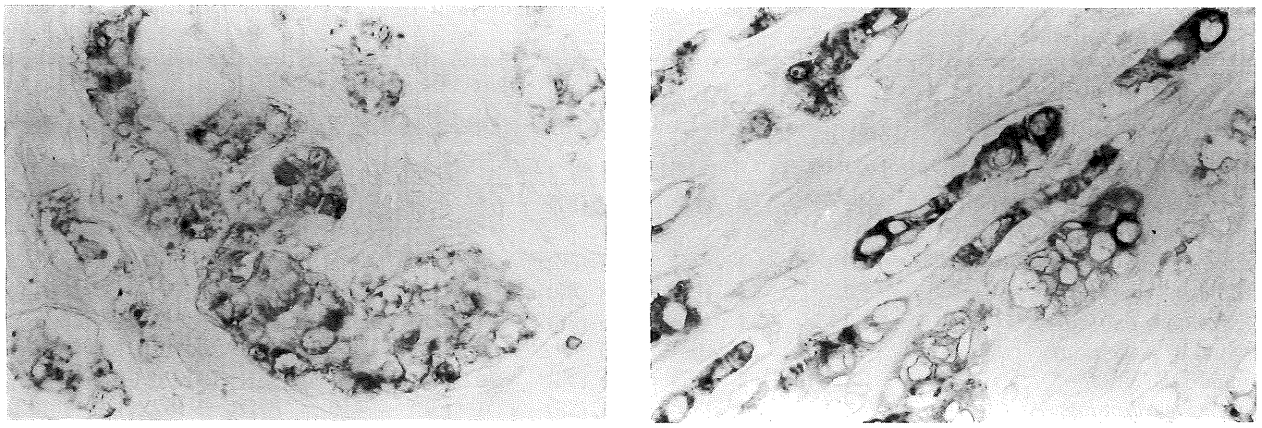
ACT in the cancer cells, but the degree of stromal elastosis clearly correlated with the positivity of ACT in the cancer cells ($p<0.01$).

On the basis of the histological type of breast carcinoma, noninvasive ductal carcinoma (Fig. 9) showed a higher frequency of positive EL and ACT in cancer cells than invasive carcinoma. In invasive carcinoma, there was no significant correlation between the histological type and the positivity of EL and ACT (Table 16). There was no correlation between the positivity of EL and ACT in any of the breast carcinoma.

In noninvasive carcinoma, there was no correlation between the degree of periductal elastosis and the positivity of EL and ACT in the cancer cells (Table 17). In scirrhous carcinoma (Fig. 10), there was no correlation between the degree of stromal elastosis and the positivity of EL in the cancer cells, but a significant correlation was noted between the degree of stromal elastosis and the positivity of ACT in the cancer cells ($p<0.05$), as shown in Table 18. There was no correlation between the positivity of EL and ACT in any of the scirrhous carcinoma.

## DISCUSSION

Elastosis around the ducts in the breast was described in scirrhous carcinoma by Billroth in 1860 followed by many pathological studies on this change. Early reports suggested that periductal elastosis resulted from the stagnation of secretion in carcinomatous lesions and that it represented a



(a) (b)

**Fig. 10.** Immunoreactivities of EL(a) and ACT(b) in scirrhous carcinoma. EL was mildly positive and ACT was markedly positive. (ABC method, ×33)

precancerous lesion[20]. Recently, with regard to breast carcinoma, attempts have been made to elucidate the correlation of elastosis with histopathological parameters, hormone receptor status, clinical course, response to endocrine therapy, prognosis and survival[3,6]. Shivas and Douglas[35] first found a clear correlation between the grade of elastosis and prognosis in patients with breast carcinoma. Their conclusion was supported by several other studies[26,42], in most of which focal periductal elastosis was measured and ascertained to correlate with estrogen receptor positive status. A totally contrary conclusion was reached in other studies, most of which measured stromal or total elastosis showing that marked elastosis correlated with estrogen receptor positive status, but no correlation shown between elastosis and prognosis[1,16,31,34]. More recently, Humeniuk et al[17] reported that focal elastosis was a disappointing indicator of prognosis, because the elastosis grade correlated with the presence of axillary metastasis[16], although it was more likely to be associated with carcinomas with differentiated characteristics[11]. A recent study in Japan, reported by Tamura & Enjoji[38], revealed that 105 cases with moderate to marked elastosis had a higher survival rate than 197 cases with no or only mild elastosis, and that this characteristic could serve as a prognostic indicator, but a correlation also existed between elastosis and the histologic grade of the carcinoma. This led to the consideration that well differentiated carcinomas progress slowly, and therefore contain more elastic tissue.

Consequently, the value of elastosis as a prognostic indicator in patients with breast carcinoma has remained unclear. However, it appears that periductal elastosis is influenced by the differentiation of breast carcinoma including the positive status of estrogen receptor, and therefore the prognosis of patients with breast carcinoma associated with periductal elastosis is better. On the basis of the above information, elastosis may not be directly related to the malignant behavior of breast carcinoma.

In the present study, the degree of elastosis in breast carcinoma cases was compared according to histological type. Periductal elastosis was frequently seen in noninvasive ductal carcinoma, but among invasive ductal carcinoma, poorly differentiated carcinoma (scirrhous carcinoma) showed a higher frequency. This observation did not necessarily support the previous view. The fact that stromal elastosis was frequently seen in scirrhous carcinoma suggests that the degree of elastosis should be evaluated separately according to the anatomical sites. It is possible that the mechanism of elastosis is different between the periductal and stromal sites.

In 1977, Martinez-Hernandes et al[25] stated that benign conditions, such as fibroadenoma and sclerosing adenosis, did not produce significant elastosis. In the same year, however, Tremblay et al[40] reported elastosis in 17 cases of benign sclerosing ductal proliferation of the female breast. Azzopardi[3] stated that some lesions of epitheliosis, sclerosing adenosis and duct ectasia are accompanied by elastosis. Recently, Parfrey et al[30] indicated that elastosis is a feature of fibrocystic disease and that the degrees of both stromal and periductal elastosis are related to the severity of epitheliosis. They confirmed that elastosis is not confined to carcinoma of the breast and that the degree of elastosis, especially stromal, increases progressively with the severity of breast disease, that is, from fibrocystic disease with increasingly severe epitheliosis, to noninvasive carcinoma, to invasive carcinoma. A peculiar hypothesis was proposed by them[30] that the epithelial cells of fibrocystic disease, as well as breast carcinoma cells, induce fibroblast to overproduce elastic fibers.

In the present study, elastosis associated with be-

nign breast disease including not only fibrocystic disease but also radial scar and fibroadenoma was examined. Consequently, the result that periductal elastosis in fibrocystic disease shows an increasing tendency with the degree of epithelial proliferation confirms the findings of Parfrey et al[30]. So far, no report is available on elastosis in fibroadenoma. It is interesting to note that in fibroadenoma a similar tendency to fibrocystic disease is seen. A common biological nature is suggested in epithelial proliferation of fibroadenoma and fibrocystic disease.

As mentioned above, elastosis has been a focus of interest, chiefly in relation to the presence of breast disease including carcinoma and fibrocystic disease. However, it has not been clear what degree of elastosis is observed in normal or healthy breast[10]. The amount of elastic fibers varies considerably according to the anatomical site, that is, periductal, stromal and perivascular[3,6]. There is a lack of agreement on the response of elastic tissue to fundamental physiological events, for example aging[10]. In some studies on breast with carcinoma, changes in elastic fibers with age, parity and reproductive status were an important concern, but the results in these breasts have been inconsistent. In general, it is accepted that elastosis is related to parity[8,10,26,32,38]. It makes sense to hypothesize that stimulus to the breast associated with lactation is reflected in an increase in bulk of elastic fibers, and that this could be recognized long after the event. However, this is not in agreement with some reports that no consistent diminution in elastic fibers occurs by decade[3,10,32]. The relationship to aging is not clear[8,25,32].

In the present study, normal or healthy breasts were not selected as materials and the correlation between elastosis and aging was considered in the lesions of fibroadenoma, fibrocystic disease and breast carcinoma. The fact that stromal elastosis in fibroadenoma and periductal or stromal elastosis in breast carcinoma increase in the older age group, suggests that aging in the breast can not be excluded as a factor in the morphogenesis of elastosis, even if the elastosis is within benign or malignant breast disease. However, it remains uncertain whether aging only implies the influence of parity or whether other complicated factors associated with aging play a role in the morphogenesis of elastosis.

It has been suggested that elastic fibers are resistant to degradation and, once deposited, elastic tissue will tend to remain because of this property. Therefore periductal elastic fibers show progressive deposition during the reproductive years and persist for life[10]. Consequently, it was proposed that elastosis is a monument to past events and not an active process, and so, this term should be reserved for use in premenopausal women in whom high grades of elastosis are uncommon[10].

On the contrary, in the report of elastosis in breast diseases examined by immunohistochemistry for elastin and electron microscopy[41], stromal elastosis was thought to be newly formed because it consisted of tannic acid-positive amorphous components and abundant microfibrils; periductal elastosis supposedly consisted of numerous fine amorphous components with plentiful microfibrils or condensed accumulation of irregularly arranged small amorphous components associated with only a few microfibrils, and were thought to be formed by excess production of elastic fibers and degradation of pre-existing and newly formed-elastic fibers. Similar changes of elastic fibers have been reported in experimental paraquat-treated lung[12,13] and solar elastosis in skin[7]. Fukuda et al[14] studied elastic fibers in patients with panacinar or centriacinar emphysema by electron microscopy and immunohistochemistry of elastin and found finely disrupted fibers presumed to have been damaged by elastase, small round amorphous components in elastic fibers, which might be formed from abnormal elastogenesis, and large confluent elastic masses, thought to be formed by aggregation in areas undergoing structural remodeling of the alveolar wall. Also, Fukuda et al[15] reported that the emphysema-like lesions of lymphangiomyomatosis were mediated by the degradation of elastic fibers, and that degraded elastic fibers were related to an imbalance of the elastase / $\alpha$ 1-antitrypsin system. On the basis of these facts, it seems that the elastic fibers present in the normal status of various organs including breast, are not resistant to degradation[22].

From the viewpoint of an imbalance of elastinogenesis over elastinolysis, an evaluation of levels of elastinolytic enzymes in the lesion of elastosis is necessary. However, it is possible that much elastinolytic enzyme activity is attenuated by protease inhibitors such as $\alpha$ 1-antitrypsin, $\alpha$ 1-antichymotrypsin or $\alpha$ 2-macroglobulin, and that an excess of protease inhibitors relative to proteolytic enzymes may lead to overproduction of elastic fibers in the breast. Davies and Mera[9] reported that the immunoreactivities of protease inhibitors including $\alpha$ 1-antichymotrypsin were found in the fibers of elastosis. They suggested that extracellular regulation of enzymatic degradation occurs and that the plasma- or tissue-derived protease inhibitors are involved in the modulation of enzyme activity during morphogenesis of elastosis, also that the increased concentration of protease inhibitors, relative to elastinolytic enzymes, contributes to overproduction of elastic fibers in elastosis of the breast.

So far, elastatinal, Meo-Suc-(Ala)2-Pro-Val-CH2CL, SR26831, $\alpha$ 1-antitrypsin and $\alpha$ 1-antichymotrypsin and $\alpha$ 2-macroglobulin are known as elastase inhibitors and it is uncertain which is most effective as an elastase inhibitor.

Among them, the ones available to the paraffin-embedded material are α 1-antitrypsin, α 1-antichymotrypsin and α 2-macroglobulin. Therefore, the immunohistochemical staining using these three antibodies was performed in the present study and the results of α 1-antichymotrypsin were only evaluated, because the positive finding of α 1-antitrypsin and α 2-macroglobulin was limited.

In the report by Davies and Mera[9], the cellular origin of elastase and protease inhibitors was not described. The present study was attempted, therefore, to ascertain the source of elastase (EL) and a representative of protease inhibitor, α 1-antichymotrypsin (ACT). Immunohistochemical observation of epithelium in the normal lobule and duct of breast revealed immunoreactivities of EL and ACT. This fact suggests that production of EL and ACT is a normal function of breast epithelium. Furthermore, the observation that fibrocystic disease accompanies elastosis as well as breast carcinoma and that inflammatory cells including neutrophils or histiocytes are not prominent in most fibrocystic disease and breast carcinoma, indicates that ductal epithelial proliferation with preserved production of enzymes, either in the benign or malignant disease, plays a significant role in the occurrence of elastosis.

The present study has shown a different role for EL and ACT in benign and malignant breast disease. In fibroadenoma and fibrocystic disease, it is probable that marked epithelial proliferation lacks production of EL, and the ACT produced in epithelial cells ceases elastinolysis. On the other hand, the role of EL and ACT is variable among the histological types of carcinoma. In noninvasive ductal carcinoma, carcinoma cells preserve the productive activity of EL and ACT, but the degree of periductal elastosis is not related to the positivity of EL and ACT. Therefore, it seems that an imbalance of these enzymes is not related to the periductal elastosis. In invasive ductal carcinoma, however, the productive activity of ACT in carcinoma cells is related to stromal elastosis. In particular, scirrhous carcinoma shows a significant correlation between ACT in cancer cells and stromal elastosis.

The localization of EL in breast disease has not been reported so far, and the present study revealed a wide distribution of this enzyme and its significant role in the morphogenesis of elastosis. The immunohistochemical distribution of type IV collagenase in normal, benign and malignant breast has been recently reported[29], in which collagenase was frequently detected in the epithelial cells of the normal terminal ducts and hyperplastic lesion. Also, intraductal carcinoma and invasive carcinoma as well as metastasis in lymph nodes has shown positive immunoreactivity of this enzyme[29]. The result supported the role of type IV collagenase in the basement membrane remodeling of normal breast and in the invasion and metastasis of breast carcinoma. It has now become clear that the epithelial cells of the breast have the capacity to produce proteolytic enzyme including elastase and collagenase. Each of these enzymes appears to have a different role in the normal breast and in the pathological process and plays a role in the peculiar morphogenesis.

α 1-antichymotrypsin is an acute-phase plasma protease inhibitor and was first described in 1965. The physiological role of this enzyme has not yet been fully elucidated. Although a number of functions are recognized, it is certain that this enzyme is involved in the defense mechanism against protease and inhibits antibody-dependent cell-mediated cytotoxicity in vitro. So far the immunoreactivity of this enzyme has been reported in a large number of different types of tumors[24], and it was suggested that its presence is of little value in the differential diagnosis of either sarcomas or carcinomas. The immunoreactivity of ACT, in relation to various kinds of breast disease, has not been available in previous reports, but the present study has revealed that ACT plays a different role in the morphogenesis of elastosis among different types of breast disease.

On the other hand, the cellular origin of elastic fiber in the breast, either benign or with healthy-normal or malignant disease, remains uncertain. Two hypotheses have been suggested to explain the cellular origin of elastosis associated with breast carcinoma. Firstly, the carcinoma cells themselves may be responsible for the secretion of the material. In support of this hypothesis, it is reported that cultured breast carcinoma cells secrete collagen and elastin, although the amounts are small compared with cultured fibroblasts[21]. Recently, it was reported that the human breast cancer cell lines produce elastin in culture at rates equivalent to 1.6—15% of those of control fibroblastic cells, and northern blot analysis of total RNA showed the presence of a 3.5 kilobase elastin mRNA band in both the fibroblastic cells and the cancer cells lines[23]. Also, by use of in situ hybridization with an elastin complementary RNA probe, malignant cells of some breast carcinoma have been shown to be responsible for elastin synthesis[23]. Alternatively, it has been suggested that an inductive factor is secreted by carcinoma cells[4,39] and such a factor influences periductal fibroblasts, vascular smooth muscle cells or fibroblasts, causing myofibroblastic differentiation and the biosynthesis of elastic fibers.

Ultrastructural observation has revealed that myofibroblasts are not a component of normal breast stroma, but are a prominent feature in the stroma of invasive ductal carcinoma[36,37]. These findings suggest that myofibroblastic proliferation around invasive carcinoma occurs. Myofibroblasts are known to have well-developed synthetic and

contractile properties and the presence of myofibroblasts within the lesion with elastosis suggests that these cells are responsible for the morphogenesis of elastosis. Another report described the presence of myofibroblasts in radial scars and suggested that focal loss of basal lamina could contribute to the myofibroblastic reaction and consequently to the elastosis seen in the radial scar[5].

The study by use of in situ hybridization with an elastin complimentary RNA probe revealed that fibroblastic cells in the stroma and in the periductal region were responsible for elastin synthesis in most breast carcinoma[23]. Vascular endothelium also expressed the elastin gene in some breast carcinomas. From these results, it was speculated that the elastotic elastin might have different cellular origins in different portions of a single ductal carcinoma, and elastosis in breast carcinomas was likely to be a complex process with multifactorial regulatory mechanisms[23]. Other reports are in agreement with this view[32,38].

Recently the attention of many investigators has focused on the potential roles of soluble or secreted factors, the so-called growth factors, in modulating cell-cell communication in the normal and abnormal regulation of tissue growth and maturation[2]. Cultured human breast cancer cells have been shown to produce and respond to a number of growth factors, such as insulin-like growth factor II (IGF-II), epidermal growth factor (EGF), transforming growth factor-$\alpha$ (TGF-$\alpha$) and TGF-$\beta$. Most benign and malignant breast lesions involve abnormal proliferation and altered architecture of the stromal and/or epithelial elements. It is thus reasonable to hypothesize that altered cellular expression or response to growth factors in the breast play a role in the development, persistence or regression of such lesions[27]. With regard to morphogenesis of the elastosis in the breast, it might be important to consider some growth factors and inductive factors to elastin production. From this point of view, further investigations are necessary.

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