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# Fibrotic Focus in Invasive Ductal Carcinoma: An Indicator of High Tumor Aggressiveness

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**Histological examination of invasive ductal carcinoma of the breast often demonstrates the presence of an extensive central fibrotic focus (FF). The clinicopathological significance of the FF, or scar, in primary invasive ductal carcinoma is still ambiguous. One hundred and fifty-three cases of invasive ductal carcinoma (IDC) were classified into two groups, those with and those without FF. The differences in frequency of immunohistochemically detected overexpression of c-*erb*B-2 protein and nuclear accumulation of p53 protein, and the labeling index of proliferating cell nuclear antigen (PCNA), as well as histopathological parameters were compared between these two groups. IDCs smaller than 50 mm with FF showed a higher frequency of high-grade tumors, a higher frequency of lymph node metastasis, and a significantly higher frequency of c-*erb*B-2 protein overexpression than those without FF. In tumors of 20 mm or less, the incidence of nuclear accumulation of p53 protein was significantly higher in tumors with than those without FF. Tumors with FF showed a significantly higher PCNA labeling index than those without FF, regardless of tumor size. The present results indicate that the presence of FF is an important clinicopathological parameter associated with a higher degree of malignancy in IDCs, especially those smaller than 50 mm. Therefore, dividing IDCs into those with and those without FF appears to be meaningful clinicopathologically.**

Key words: Invasive ductal carcinoma — Fibrotic focus — c-*erb*B-2 protein — p53 protein — Proliferating cell nuclear antigen

When the primary site of surgically resected breast cancer is examined histopathologically, we often encounter tumors containing an extensive central fibrotic focus and with actively proliferating neoplastic cells in the periphery. The clinicopathological significance of such fibrotic focus (FF), or scar, in primary breast cancer is still ambiguous. This type of breast cancer was previously reported as cancer with sclerotic foci by Linell *et al.*,[1] and as scar cancer by Fisher *et al.*[2] Although scar cancer was shown to have a better prognosis and to be richer in steroid hormone receptors than non-scar cancers,[3,4] these studies did not divide such cancers into histological subtypes and included not only invasive ductal carcinoma (IDC), but also other histological types, e.g., lobular, mucinous, medullary, or squamous cell carcinomas, and non-invasive ductal carcinoma. In order to clarify the clinicopathological significance of FF or scars in primary breast cancer more accurately, it is necessary to study tumors of the same histological type. Therefore, we investigated the differences in histopathological features, nodal status, hormone receptor status, c-*erb*B-2 and p53 expression, and proliferative activity between IDCs with and those without FF.

The c-*erb*B-2 protooncogene product is a glycoprotein of the tyrosine kinase family with a molecular mass of 185 kDa. Overexpression of c-*erb*B-2 protein has been shown to be associated with breast cancers that have a poorer prognosis.[5–7] p53 is a 53-kDa nuclear phosphoprotein and the wild-type form is known to suppress the growth of transformed cells in culture.[8] Mutations of the *p53* gene are frequently observed in various human tumors,[9,10] and it is widely accepted that such mutations play an important role in tumor development and progression. Immunohistochemically detectable nuclear accumulation of p53 protein occurs frequently in breast cancer and is an indicator of poor prognosis.[11–13] Proliferating cell nuclear antigen (PCNA) is a 36-kDa acidic nuclear protein[14,15] present in the nuclei of cells in late G1 and S phase, thus serving as a marker of proliferating cells. Proliferative activity defined by PCNA labeling index has been correlated with the prognosis of breast cancer.[16,17] Thus, it was expected that the results of immunohistochemical staining for c-*erb*B-2, p53, and PCNA would provide objective information on the usefulness of FF for predicting the aggressiveness of breast cancer.

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## MATERIALS AND METHODS

**Cases** One hundred and fifty-three cases of invasive ductal carcinoma of the breast constituted the basis for this study; these cases had been consecutively treated by surgery between July, 1992 and June, 1994 at the National Cancer Center Hospital, East. Clinical information was obtained from the patients' medical records. All the patients were Japanese women ranging in age from 28 to 87 years (average, 53 years), and all had solitary lesions. Sixty-eight patients were pre-menopausal and 64 post-menopausal. Menopausal status was unknown in five patients. Standard radical mastectomy was performed on 38 patients, modified radical mastectomy on 107, extended radical mastectomy on four, quadrantectomy on two, and glandectomy on five. Axillary lymph node dissection was done in 151 patients. None of the patients received radiotherapy or chemotherapy before surgery. Estrogen receptor (ER) assay was performed on 116 of the 153 tumors. ER positivity was observed in 83 tumors and the remaining 33 were negative.

For pathological examination, surgically resected tissue specimens were fixed in 10% formalin overnight at room temperature and the entire tumor was cut into slices at intervals of about 0.5 to 0.7 cm. The size and gross appearance of the cancer were recorded, and the former was validated by comparison with the tumor size on histologic slides. Multiple histological sections were taken from each tumor in order to measure the maximum tumor diameter and area. The sections were processed routinely and embedded in paraffin.

**Histological examination** Serial sections of each tumor were cut from the paraffin blocks. One section was stained with hematoxylin and eosin (HE) and examined pathologically to confirm the diagnosis. Another section was stained with elastica stain to assess the degree of elastosis in FF. Tumor area was calculated by multiplying two maximum diameters at right angles on histological sections. All the tumors were classified according to the guidelines of the World Health Organization,[18] and their histologic grade was evaluated by using the classification of Elston,[19] which is a modification of the Bloom and Richardson classification.[20] The tumors were divided into size classes of $\leq$20 mm, 21 to 49 mm, and $\geq$50 mm, and also into two groups according to their growth pattern: 1) tumor cells growing in solid nests, 2) tumor cells growing in strands. In addition, the presence or absence of coagulation necrosis of tumor cells within FF was also recorded.

**Histological examination of fibrotic focus** FF was defined according to the following criteria: 1) FF was composed of proliferated fibroblasts and/or collagen fibers, with or without tumor cells. 2) FF was located within the tumor and surrounded by tumor cells (Figs. 1 and 2). The area of FF was calculated by multiplying the two maximum diameters at right angles. Then, the percentage of the area of FF with respect to that of the whole tumor was obtained. The degree of fibrosis in FF was divided into three grades according to the following criteria: 1) grade 1 FF consisted of a large number of fibroblasts with a small amount of collagen fibers. 2) grade 3 FF consisted mainly of collagen fibers, most of which were hyalinized. 3) grade 2 FF was intermediate between grades 1 and 3, and fibroblasts and collagen

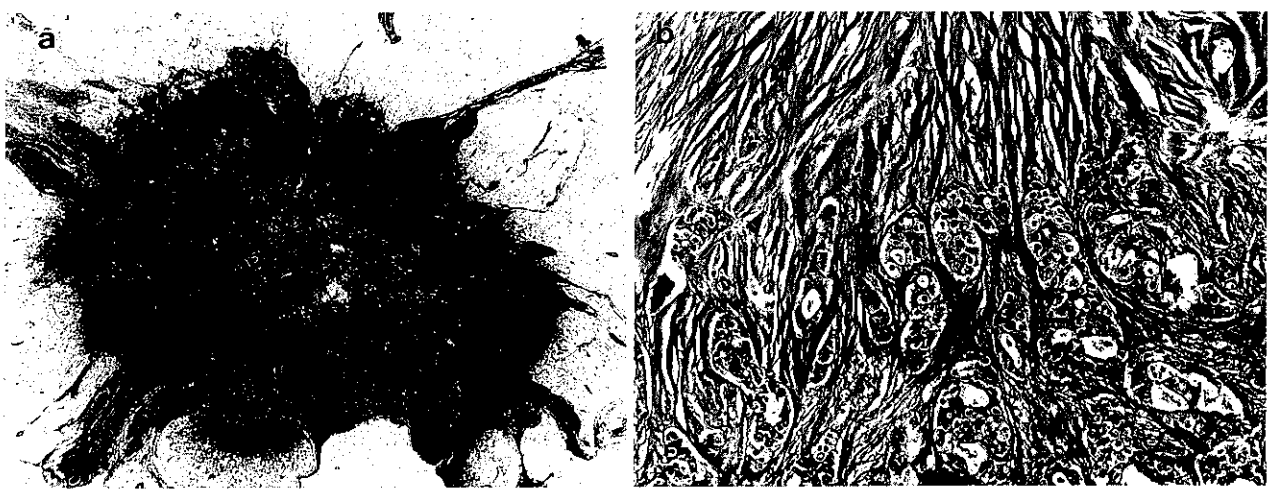


Fig. 1. Histology of invasive ductal carcinoma with a large fibrotic focus. a: Panoramic view. The fibrotic focus measures 8 mm in maximum diameter. b: Fibrotic focus consisting of hyalinized collagen fibers (FF of grade 3); carcinoma cells infiltrate as solid nests and small tubular structures (HE, original magnification $\times$100).

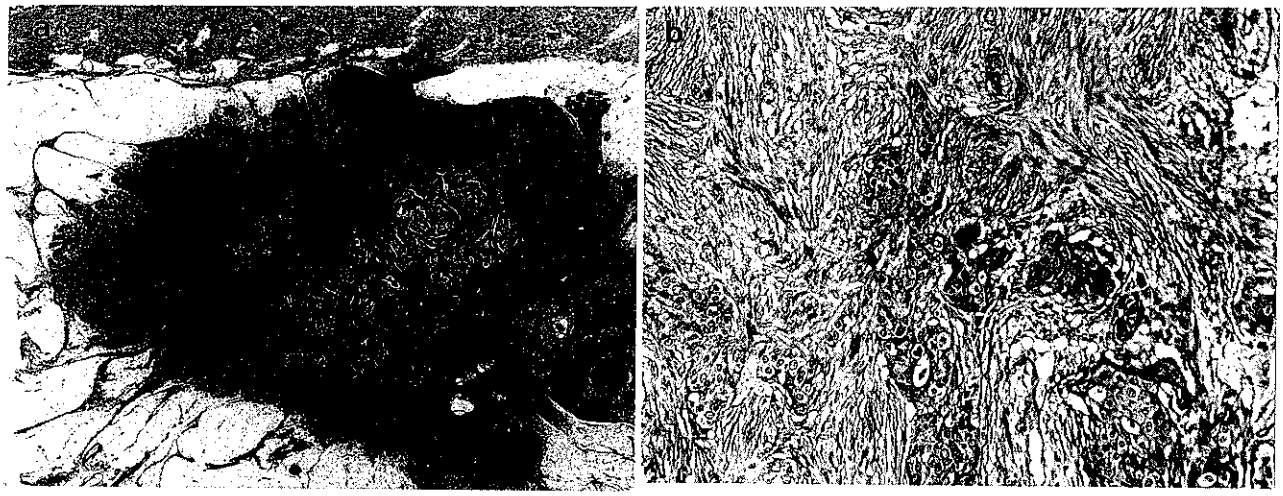

Fig. 2. Invasive ductal carcinoma with a small fibrotic focus. a: Panoramic view. The fibrotic focus is 2 mm in diameter (arrows). b: Fibrotic focus contains a large number of fibroblasts (FF of grade 1), and carcinoma cells grow in solid nests (HE, original magnification ×100).

fibers were intermixed in various ratios. If several FFs with varying sizes and grades were present within one tumor, the size and grade of the largest FF was used for the study. As well as grade, the presence of elastosis in FF was examined in each case.

**Immunohistochemistry** Immunohistochemical staining for c-*erb*B-2, p53, and PCNA products was performed by the avidin-biotin-peroxidase complex (ABC) technique.[21] The primary antibodies employed were an affinity-purified polyclonal antibody specific for 185-kDa c-*erb*B-2 protein (Nichirei, Tokyo), used at 1:200 dilution, a polyclonal antibody against p53, RSP53 (Nichirei), applied at 1:20,000 dilution, and a mouse monoclonal antibody against PCNA (PC10, Novocastra, UK), used at 1:100 dilution. RSP53 recognizes a linear epitope in human p53 located between amino acids 54 and 69, and was shown beforehand to clearly detect p53 protein in immunoblot analysis (data not shown). Microwave treatment was performed before ABC immunohistochemical staining for p53.[22] After immunostaining, the sections were counterstained with hematoxylin. Sections of invasive ductal carcinoma positive for c-*erb*B-2 protein, p53 protein, and PCNA were used each time as a positive control. As a negative control, the primary antibody was replaced with normal rabbit serum or normal mouse immunoglobin. Brown to dark-brown staining of tumor cell nuclei was judged to be positive for PCNA and p53, and faintly stained nuclei were considered negative. When a few tumor cells showed positive staining for p53 protein or c-*erb*B-2 protein, it was very difficult to judge its significance; therefore, nuclear staining for p53 in more than 10% of the tumor cells in the whole tumor area was judged to be positive. For c-*erb*B-2 protein, cell membrane staining in more than 10% of the tumor cells throughout the tumor was judged to be positive.

**Assessment of PCNA** PCNA index was calculated as the percentage of cells with positively stained nuclei among all tumor cells counted. The fields for cell counting were selected randomly in the tumor area. All tumor cells in each high-power field (×400) were examined, and at least 1,000 tumor cells in each tumor were counted without prior knowledge of nodal status or c-*erb*B-2/p53 expression.

**Statistical analysis** The chi-squared test or Fisher's exact probability test was used for statistical analysis of correlations between the presence or absence of FF in a tumor and clinicopathological findings, overexpression of c-*erb*B-2 protein, abnormal nuclear accumulation of p53 or nodal status. The Wald-Wolfowitz runs test was used to analyze whether there were significant differences in PCNA index between IDCs with and without FF in tumors ≤20 mm, 21 to 49 mm, and ≥50 mm in diameter. All the statistical analyses were carried out using Statistica/DOS software (StatSoft, Tulsa, OK).

## RESULTS

**Fibrotic focus and clinicopathological parameters in invasive ductal carcinoma** Correlations of the presence or absence of FF with menopausal status, ER level, tumor size, and tumor histologic grade were examined. FF was observed in 23 (41%) of 56 tumors measuring ≤20 mm, 46 (64%) of 72 tumors measuring 21 to 49 mm, and 9

Table I. Differences in Frequency of Histologic Grades[a] between Invasive Ductal Carcinoma with FF and That without

| | No. of patients (%) | | | *P*-value |
|---|---|---|---|---|
| | Total | IDC | | |
| | | $FF^+$ | $FF^-$ | |
| | 152 | 80 | 72 | |
| HG 1 | 21 | 2 (10) | 19 (90) | |
| HG 2 and 3 | 131 | 78 (60) | 53 (40) | $P<0.001$ |
| Tumor size (mm) | | | | |
| ≤20 | 56 | 23 | 33 | |
| HG 1 | 12 | 1 (9) | 11 (91) | |
| HG 2 and 3 | 44 | 22 (50) | 22 (50) | $P<0.009$ |
| 21 to 49 | 74 | 47 | 27 | |
| HG 1 | 6 | 0 (0) | 6 (100) | |
| HG 2 and 3 | 68 | 47 (69) | 21 (31) | $P<0.002$ |
| ≥50 | 22 | 3 | 19 | |
| HG 1 | 3 | 1 (33) | 2 (67) | |
| HG 2 and 3 | 19 | 9 (47) | 10 (53) | NS |

IDC, Invasive ductal carcinoma; FF, fibrotic focus; $FF^+$, fibrotic focus present; $FF^-$, fibrotic focus absent; HG, histologic grade; NS, not significant.
*a*) Histologic grade was not examined in one case.

Table II. Differences in Frequency of Lymph Node Metastasis[a] between Invasive Ductal Carcinoma with FF and That without

| | No. of patients (%) | | | *P*-value |
|---|---|---|---|---|
| | Total | IDC | | |
| | | $FF^+$ | $FF^-$ | |
| | 151 | 78 | 73 | |
| $LN^-$ | 77 | 29 (38) | 48 (62) | |
| $LN^+$ | 74 | 49 (66) | 25 (34) | $P<0.001$ |
| Tumor size (mm) | | | | |
| ≤20 | 56 | 23 | 33 | |
| $LN^-$ | 40 | 13 (32) | 27 (68) | |
| $LN^+$ | 16 | 10 (63) | 6 (37) | $P<0.040$ |
| 21 to 49 | 73 | 46 | 27 | |
| $LN^-$ | 34 | 15 (44) | 19 (56) | |
| $LN^+$ | 39 | 31 (80) | 8 (20) | $P<0.002$ |
| ≥50 | 22 | 9 | 13 | |
| $LN^-$ | 3 | 1 (33) | 2 (67) | |
| $LN^+$ | 19 | 8 (42) | 11 (58) | NS |

IDC, Invasive ductal carcinoma; FF, fibrotic focus; $FF^+$, Fibrotic focus present; $FF^-$, fibrotic focus absent; LN, lymph node; $LN^-$, lymph node metastasis absent; $LN^+$, lymph node metastasis present; NS, not significant.
*a*) Nodal status was unknown in two cases.

(39%) of 23 tumors measuring ≥50 mm. FF was present in two (10%) of 21 grade 1 tumors, 36 (58%) of 62 grade 2 tumors, and 42 (61%) of 69 grade 3 tumors.

The incidence of tumors with histologic grades 2 and 3 was significantly higher in IDCs with FF than in those without FF in tumors measuring ≤20 mm ($P<0.009$) and measuring 21 to 49 mm ($P<0.002$) (Table I). However, in tumors measuring ≥50 mm, there was no significant difference in the incidence of histologic grade 2 and 3 tumors between IDCs with and without FF. The incidence of axillary lymph node metastasis was significantly higher in IDCs with FF than in those without FF in tumors measuring ≤20 mm ($P<0.04$) and 21 to 49 mm ($P<0.002$) (Table II), whereas in tumors of ≥50 mm

Table III. Association of Fibrotic Focus with c-*erb*B-2 Expression[a] in Invasive Ductal Carcinoma

| | No. of patients (%) | | | *P*-value |
|---|---|---|---|---|
| | Total | IDC | | |
| | | $FF^+$ | $FF^-$ | |
| | 152 | 80 | 72 | |
| c-*erb*B-$2^+$ | 66 | 45 (68) | 21 (32) | |
| c-*erb*B-$2^-$ | 86 | 35 (41) | 51 (59) | $P<0.0009$ |
| Tumor size (mm) | | | | |
| ≤20 | 55 | 23 | 32 | |
| c-*erb*B-$2^+$ | 18 | 11 (61) | 7 (39) | |
| c-*erb*B-$2^-$ | 37 | 12 (32) | 25 (68) | $P<0.05$ |
| 21 to 49 | 74 | 47 | 27 | |
| c-*erb*B-$2^+$ | 36 | 28 (78) | 8 (22) | |
| c-*erb*B-$2^-$ | 38 | 19 (50) | 19 (50) | $P<0.02$ |
| ≥50 | 23 | 10 | 13 | |
| c-*erb*B-$2^+$ | 12 | 6 (50) | 6 (50) | |
| c-*erb*B-$2^-$ | 11 | 4 (36) | 7 (64) | NS |

IDC, Invasive ductal carcinoma; FF, fibrotic focus; $FF^+$, fibrotic focus present; $FF^-$, fibrotic focus absent; c-*erb*B-$2^+$, c-*erb*B-2 positive; c-*erb*B-$2^-$, c-*erb*B-2 negative; NS, not significant.
*a*) c-*erb*B-2 expression was not examined in one case.

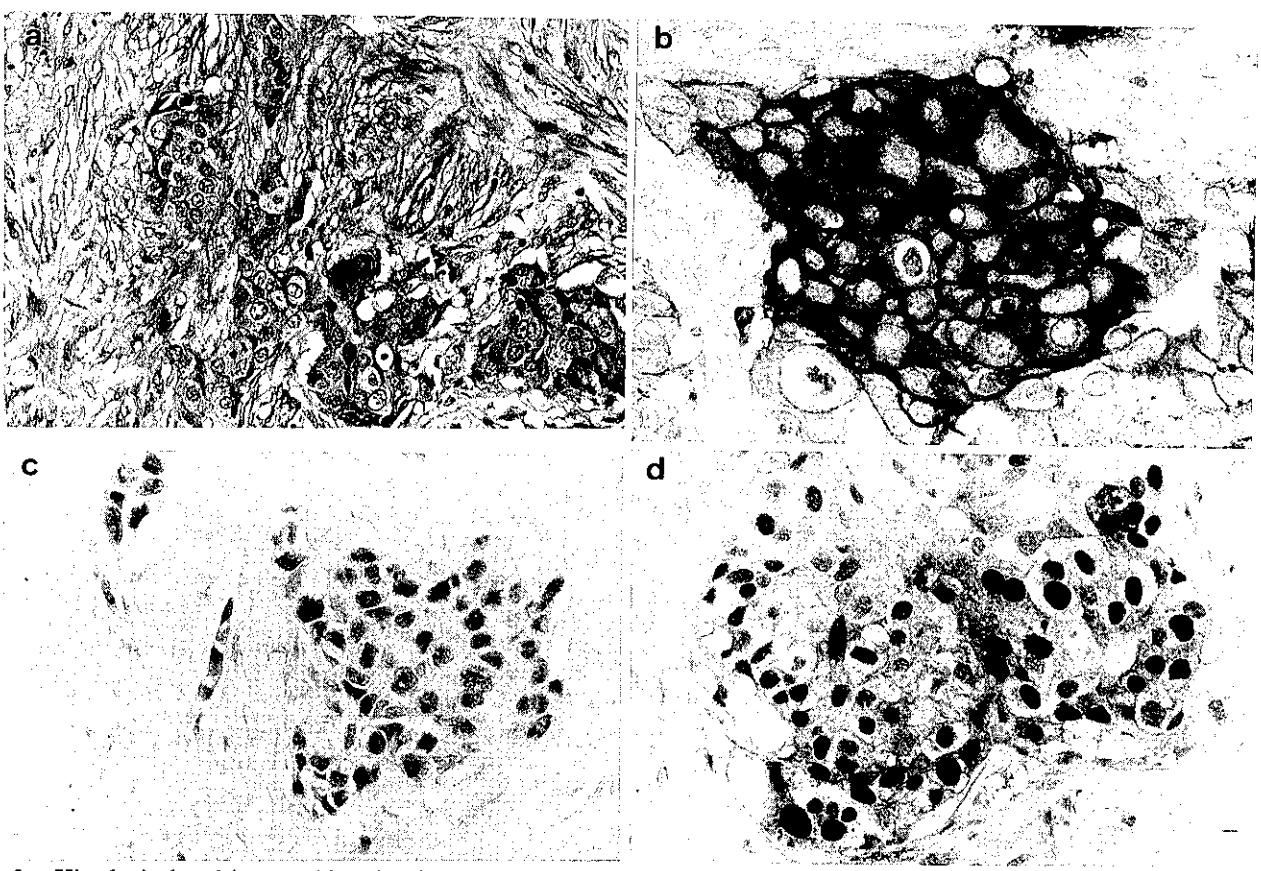


Fig. 3. Histological and immunohistochemical features of ductal carcinoma cells invading a fibrotic focus. a: Ductal carcinoma cells invading within a fibrotic focus (HE, original magnification ×200). b: Ductal carcinoma cells show intense cell membrane staining for c-*erb*B-2 protein (immunostain for c-*erb*B-2, original magnification ×400). c: Ductal carcinoma cells show nuclear accumulation of p53 protein (immunostain for p53, original magnification ×400). d: Ductal carcinoma cells show positive staining for PCNA in nuclei (immunostain for PCNA, original magnification ×400).

there was no significant difference in the rate of lymph node metastasis between IDCs with and without FF. Although there was no significant correlation between the presence or absence of FF and menopausal status or ER level, ER-negative IDCs had FF more frequently than ER-positive IDCs ($P=0.07$) (data not shown).

**Association of fibrotic focus with growth type of invasive ductal carcinoma** FF was observed in 43 of 88 tumors growing in solid nests, and 37 of 65 tumors growing in strands. FF with tumor coagulation necrosis, suggesting that FF had developed through necrosis of tumor cells, was noted in 12 of 43 (28%) cases in the former category, but in only two of 37 (5%) cases in the latter. The difference was statistically significant ($P<0.008$).

**Relationship of fibrotic focus with c-*erb*B-2 and p53 proteins** The tumors with FF showed a significantly higher frequency of overexpression of c-*erb*B-2 protein than those without FF when tumors of $\leq 20$ mm ($P<0.05$) and those of 21 to 49 mm ($P<0.02$) were analyzed (Table III) (Fig. 3). In tumors measuring $\geq 50$ mm, the

Table IV. Association of Fibrotic Focus with p53 Expression[a] in Invasive Ductal Carcinoma

| | No. of patients (%) | | | *P*-value |
|---|---|---|---|---|
| | Total | IDC | | |
| | 152 | $FF^+$ 80 | $FF^-$ 72 | |
| $p53^+$ | 32 | 21 (66) | 11 (34) | |
| $p53^-$ | 120 | 59 (49) | 61 (51) | NS |
| Tumor size (mm) | | | | |
| $\leq 20$ | 55 | 23 | 32 | |
| $p53^+$ | 13 | 10 (71) | 3 (29) | |
| $p53^-$ | 42 | 13 (31) | 29 (69) | $P<0.004$ |
| 21 to 49 | 74 | 47 | 27 | |
| $p53^+$ | 15 | 9 (60) | 6 (40) | |
| $p53^-$ | 59 | 38 (64) | 21 (36) | NS |
| $\geq 50$ | 23 | 10 | 13 | |
| $p53^+$ | 4 | 2 (50) | 2 (50) | |
| $p53^-$ | 19 | 8 (42) | 11 (58) | NS |

IDC, Invasive ductal carcinoma; FF, fibrotic focus; $FF^+$, fibrotic focus present; $FF^-$, fibrotic focus absent; $p53^+$, p53 positive; $p53^-$, p53 negative; NS, not significant.
*a*) p53 expression was not examined in one case.

Table V. Correlation between Overexpression of c-*erb*B-2 and Histological Features of Fibrotic Focus in Tumors Less than 20 mm in Diameter

| Fibrotic focus | No. of patients (%) | | | *P*-value |
|---|---|---|---|---|
| | Total | c-*erb*B-2 | | |
| | | + | − | |
| Diameter (mm) | 23 | 11 | 12 | |
| $\leq 6$ | 17 | 6 (35) | 11 (65) | |
| $>6$ | 6 | 5 (83) | 1 (17) | $P<0.05$ |
| Area (%) | 23 | 11 | 12 | |
| $\leq 13$ | 17 | 6 (35) | 11 (65) | |
| $>13$ | 6 | 5 (83) | 1 (17) | $P<0.05$ |
| Fibrosis grade | 23 | 11 | 12 | |
| 1 | 8 | 1 (13) | 7 (87) | |
| 2 | 8 | 5 (63) | 3 (37) | |
| 3 | 7 | 5 (71) | 2 (29) | $P<0.02$[a] |
| Elastosis | 23 | 11 | 12 | |
| Present | 13 | 7 (54) | 6 (46) | |
| Absent | 10 | 4 (40) | 6 (60) | NS |

c-*erb*B-$2^+$, c-*erb*B-2 positive; c-*erb*B-$2^-$, c-*erb*B-2 negative; NS, not significant.
*a*) 1 vs. 2/3.

incidence of c-*erb*B-2 overexpression did not differ between those tumors with FF and those without. As for abnormal nuclear accumulation of p53 protein, tumors with FF showed a significantly higher incidence than those without FF only in the ≤20 mm class ($P<0.004$) (Table IV) (Fig. 3). In tumors larger than 20 mm, the incidence of nuclear p53 protein accumulation did not differ significantly between those with and those without FF. There was no statistically significant difference in the frequency of overexpression of c-*erb*B-2 protein or p53 protein accumulation between tumors growing in solid nests and those growing in strands (data not shown).

**Correlation between overexpression of c-*erb*B-2 protein and histological characteristics of FF in tumors measuring ≤20 mm** Since a significant association of FF with overexpression of c-*erb*B-2 or nuclear accumulation of p53 proteins was noted in tumors smaller than 50 mm, more detailed analysis was performed to examine whether there was a correlation between expression of c-*erb*B-2/p53 proteins and the size, area, fibrosis grade, or elastosis of FF.

In tumors of ≤20 mm, the maximum, minimum, and average diameters of FF were 11 mm, 1 mm, and 4.8 mm, respectively. Maximum, minimum, and average proportions of FF relative to the whole tumor area were 61%, 0.6%, and 13%, respectively. Although a significant difference in the frequency of c-*erb*B-2 overexpression was not noted between tumors with smaller than average FF and those with average size or larger FF, c-*erb*B-2 protein overexpression was observed more frequently in tumors with FF 6 mm or larger than those with FF smaller than 6 mm ($P<0.05$) (Table V). Similarly, the incidence of c-*erb*B-2 protein overexpression was higher in tumors where the proportion of FF relative to the total tumor area was average or larger than in tumors where the proportion of FF was smaller than average ($P<0.05$). Tumors with fibrosis of grades 2 and 3 showed a significantly higher frequency of c-*erb*B-2 protein overexpression than those with grade 1 fibrosis ($P<0.02$). There was no significant difference in the frequency of c-*erb*B-2 protein overexpression between tumors with elastosis in FF, and those without. Although abnormal nuclear accumulation of p53 protein was not correlated with size, fibrosis grade, or elastosis of FF in tumors ≤20 mm in size, tumors in which the proportion of FF relative to total tumor area was larger than average showed abnormal nuclear p53 protein accumulation more frequently than those in which the proportion of FF was smaller than average ($P=0.18$) (data not shown).

In tumors measuring 21 to 49 mm, the maximum, minimum, and average diameters of FF were 25 mm, 1 mm, and 7.6 mm, respectively. Maximum, minimum, and average areas of FF in tumors of this category were 40%, 0.1%, and 6.5%, respectively. There was no significant difference in the frequency of c-*erb*B-2 protein overexpression between tumors with FF of average or larger

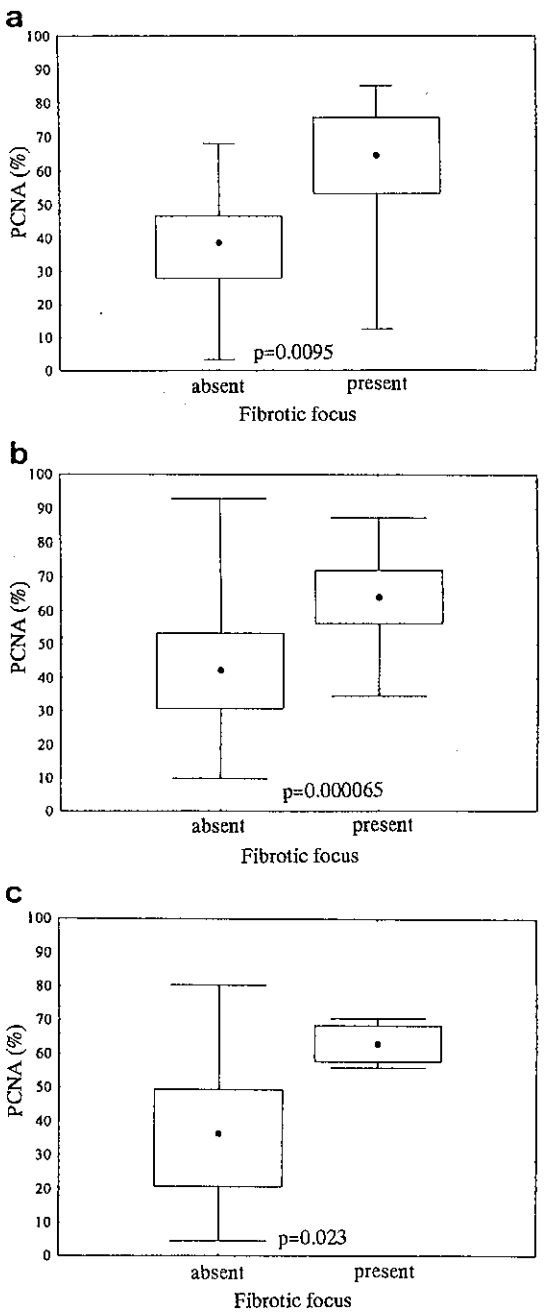


Fig. 4. Differences in proliferative activity of invasive ductal carcinomas with and without fibrotic focus. (a) Tumors ≤20 mm, (b) 21 to 49 mm, and (c) ≥50 mm with fibrotic focus show significantly higher proliferative activity than those without ($P=0.0095$, $P=0.000065$, and $P=0.023$). ⌶ min.–max., ▭ 25–75%, ● median.

size and those with FF smaller than average. The incidence of c-*erb*B-2 overexpression was not correlated with the proportion of FF relative to total tumor area, fibrosis grade, or between tumors with and without elastosis (data not shown).

**Differences in proliferative activities between IDCs with and without FF** The average PCNA indices of tumors with FF and without FF were 61.1% (range: 12.3–85.2%) and 37.3% (range: 3.2–68.0%), respectively, in tumors measuring $\leq$20 mm; 63.7% (range: 34.7–87.4%) and 41.7% (range: 9.9–92.9%) in tumors measuring 21 to 49 mm; 62.9% (range: 56–70.7%) and 39% (range: 4.5–80.5%) in tumors measuring $\geq$50 mm (Fig. 2). In all groups, tumors with FF showed significantly higher proliferative activity than those without ($P=0.0095$, $P=0.000065$, and $P=0.023$, respectively) (Fig. 4).

## DISCUSSION

Fisher *et al.*[2] divided scar cancers into five subtypes according to the histological features of the sclerotic foci as follows: 1) type 1, composed of radiating relatively acellular hyaline containing isolated tubular carcinoma cells and resembling radial scars.[23] 2) type 2, having either edema or a dense acellular hyalinized core. In the latter instance, tumor cells are observed only rarely within the scar. 3) type 3, having a more evident radiating core than type 1 or 2, and with the tumor cells present within the scar as well as at its periphery. 4) type 4, with a fibrous stroma arranged in an interweaving pattern, isolating or compartmentalizing aggregates of tumor cells. A well-defined core or scar is not obvious within the tumor. 5) type 5, with a circumferential small core and obviously multifocal distribution. In this study, FF was defined as a sclerotic focus composed of proliferated fibroblasts and/or collagen fibers, with or without tumor cells, and located within the tumor and surrounded by tumor cells.[2] Therefore, it is considered that FF is similar to type 2 or type 3 of Fisher's classification. It was reported that scar cancers of types 1, 4, and 5 showed a better prognosis than non-scar cancers.[2] However, there were no significant differences in survival period between type 2 and 3 scar cancers and non-scar cancer.

Although scar cancer was shown to have a better prognosis,[2–4] these studies included not only IDC, but also other histological types with different aggressiveness. In order to clarify the clinicopathological significance of FF or scars in primary breast cancer more accurately, our study was concentrated on IDCs.

This study demonstrated that the presence of FF in primary IDC is associated with higher histologic grades and a higher frequency of lymph node metastasis. Such association was more obvious in tumors $\leq$20 mm and 21 to 49 mm in size, but not in tumors measuring $\geq$50 mm. Almost all IDCs with FF were tumors of histological grade 2 or 3. More than half of all tumors smaller than 50 mm with FF had metastasized to the axillary lymph nodes at the time of surgery. In other words, the presence of FF was associated with poor differentiation, and a high incidence of regional lymph node metastasis. The presence of FF was also seen significantly more frequently in tumors with c-*erb*B-2 oncoprotein overexpression, those with nuclear accumulation of p53 protein, and those with a higher than average PCNA labeling index. Because these immunohistochemical parameters have been associated with poor differentiation, high-grade nuclear atypia, and a high proliferation rate of breast cancer,[9–11, 15–17, 20, 21] our results confirmed the clinicopathological significance of the presence of FF in IDC. From these results, it appears that dividing IDC into two groups, i.e., IDC with FF and that without, may be meaningful. Although, in the present study, we did not examine whether the prognosis of patients having IDC with FF was poorer than that of patients having IDC without FF, our data clearly demonstrated the association of FF with higher malignant potential. Therefore, it seems necessary to do a more accurate analysis to see whether there is a significant difference in the patients' outcome between the two groups.

In tumors measuring $\geq$50 mm, since the number of cases showing high histologic grade, poor differentiation, or c-*erb*B-2 protein expression in IDCs without FF was increased and was similar to that of IDCs with FF, there was no significant association of the presence of FF with high histologic grade, poor differentiation, or high frequency of c-*erb*B-2 protein expression. These results indicate that there is a close association of tumor size with histologic grade, nodal status, or c-*erb*B-2 protein expression, and suggest that the biological characteristics of tumors $\geq$50 mm are different from those of tumors $<$50 mm.

In the present study, even among tumors measuring $\leq$20 mm, overexpression of c-*erb*B-2 was associated with a large size and large area of FF in the primary tumor. The aggressiveness of breast cancer is mostly determined at onset and highly aggressive breast cancer frequently shows c-*erb*B-2 overexpression and/or p53 accumulation even at an early stage. Solid tumors may proliferate so rapidly that a paucity of blood flow, or hypoxia may occur in the tumor, resulting in individual cell necrosis or coagulation necrosis of the tumor, which appears to be replaced by fibrosis or FF. In tumors growing in strands, since these tumor cells grow so rapidly, the desmoplastic reaction of the host to the tumor cells, or fibroblastic proliferation/fibrosis induced by the tumor cells may be accelerated more strongly in tumors with a large size and large area of FF than in those with a small size. Therefore, it is suggested that FF in the tumors growing in

strands originates from the interaction between tumor cells and tumor stroma.[24–26]

In conclusion, FF in IDC is associated with clinicopathological and phenotypic parameters of higher malignant potential. This suggests that the presence of FF in IDCs is a significant clinicopathological indicator of higher-grade malignancy in breast cancer.

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