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Fibrotic Focus in Invasive Ductal Carcinoma of the Breast: A Histopathological Prognostic Parameter for Tumor Recurrence and Tumor Death within Three Years after the Initial Operation

Takahiro Hasebe,[1,4] Hitoshi Tsuda,[2] Yoshitaka Tsubono,[2] Shigeru Imoto[3] and Kiyoshi Mukai[1]

[1]Pathology Division, [2]Epidemiology and Biostatistics Division, National Cancer Center Research Institute, East, [3]Department of Surgery, National Cancer Center Hospital East, 6-5-1 Kashiwanoha, Kashiwa 277 and [4]Pathology Division, National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104

We investigated whether the presence of a fibrotic focus (FF) in the primary lesion and in lymph node metastasis is a good predictor of early tumor recurrence or death in patients with invasive ductal carcinoma (IDC). Multivariate relative risk (RR) of tumor recurrence and death according to the presence of FF in the primary tumor was estimated using the Cox proportional hazards regression model with adjustment for other prognostic factors (histologic grade, T classification, nodal status, tumor necrosis, DNA ploidy, c-*erb*B-2 protein expression, *p53* protein expression, and labeling index of proliferating cell nuclear antigen). For the evaluation of the metastatic status in the axillary lymph nodes, RR of multivariate analysis was adjusted for the presence of FF in the metastatic tumor and the number of lymph nodes involved (1–3 and >3). The presence of FF increased the RR of tumor recurrence significantly for the cases in all stages, and especially for those in stages I and II (RR=6.9, $P<0.05$ and RR=25.0, $P<0.005$, respectively). All cases that died of disease had FF. Among IDCs with FF, 24 cases had FF in lymph node metastasis. Significantly higher RRs of tumor recurrence and death were observed in cases with FF in lymph node metastasis than in those without it (RR=2.0, $P<0.001$ and RR=5.9, $P<0.05$, respectively). It was suggested that the presence of FF is an important predictor of early tumor recurrence or death in patients with IDCs. The presence of FF in lymph node metastatic lesions is also a significant prognostic parameter.

Key words: Invasive ductal carcinoma — Breast cancer — Fibrotic focus — Lymph node metastasis — Prognosis

Some invasive ductal carcinomas (IDCs) have a central fibrotic focus (FF) containing proliferating tumor cells within or at the periphery. We have already reported that IDCs with FFs are associated with higher histologic grade, higher frequency of lymph node metastasis and c-*erb*B-2 protein expression, and higher proliferative activity than those without FFs.[1)]

The present study was conducted to determine whether the presence of FFs in IDCs can be used as a predictor of early tumor recurrence or death within three years after the initial operation. In addition, we examined whether the presence of FF in lymph node metastasis correlates with tumor recurrence or tumor death.

MATERIALS AND METHODS

Cases One hundred and fifty-two consecutive patients with IDC of the breast surgically treated between July 1992 and June 1994 at the National Cancer Center Hospital East constituted the basis of this study. Clinical information was obtained from the patients' medical records. All of the patients were Japanese women ranging in age from 28 to 87 years (average, 53 years), and all had a solitary lesion. Seventy-five patients were premenopausal and 71 post-menopausal. The menopausal status of six patients was unknown. Standard radical mastectomy was performed in 38 patients, modified radical mastectomy in 103, extended radical mastectomy in four, quadrantectomy in two, and glandectomy in five. Axillary lymph node dissection was carried out in 150 patients. None of the patients received radiotherapy or chemotherapy before surgery. Estrogen receptor assay was performed on 113 of the 152 tumors, and 82 tumors were positive. After the operation, 84 patients were treated with single agent 5-fluorouracil (5-FU) chemotherapy ($n=28$), or combination chemotherapy consisting of combinations of three agents; 1) cyclophosphamide (CPA)+methotrexate+5-FU ($n=20$), 2) CPA+adriamycin+5-FU ($n=36$). Tamoxifen hormone therapy was given to 96 patients. In four cases it was unknown whether chemotherapy or hormone therapy had been given. Only nine patients were treated with radiation. All lesions were classified according to the pathological TNM (pTNM) classification.[2)]

[4] To whom correspondence should be addressed.

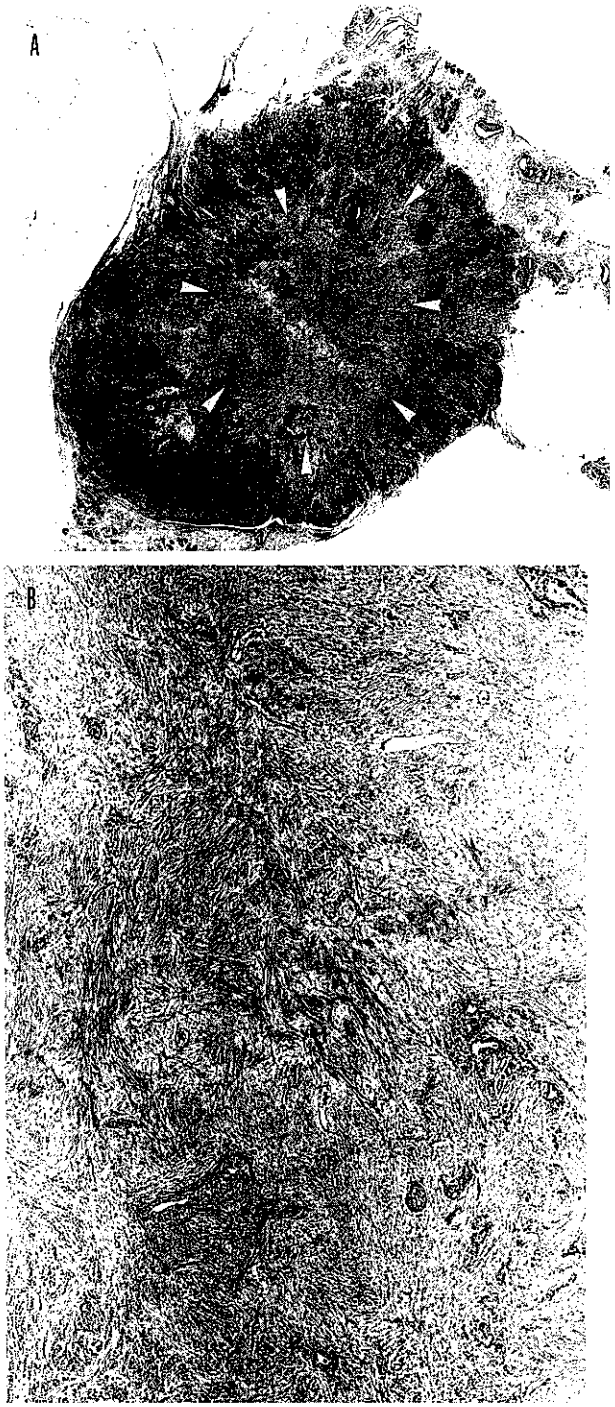

Fig. 1. IDC with FF. A, FF measuring 14×10 mm in size is observed within the tumor (arrowheads). The FF is irregular in shape and contains residual tumor islands of various sizes. B, The fibroblasts and collagen fibers composing the FF are arranged in irregular or storiform-like patterns. Tumor cells growing in solid nests or strands are seen within the FF. A: HE, panoramic view, B: HE, ×2.

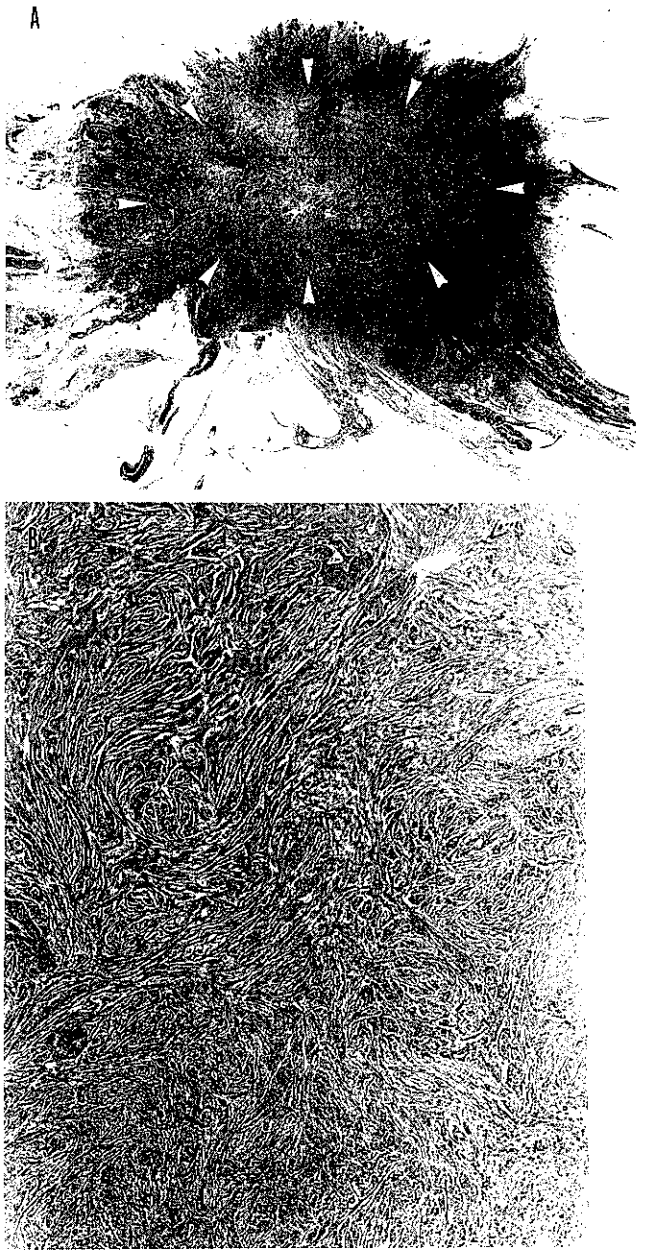

Fig. 2. IDC with FF. A, FF measuring 14×9 mm in size is seen within the tumor (arrowheads). The FF is rich in fibroblasts and collagen fibers. Tumor cell nests are present mainly in the periphery of the FF. B, The FF is composed mainly of collagen fibers arranged in irregular or storiform-like patterns and contains a small number of tumor cell nests growing in scirrhous fashion. A: HE, panoramic view, B: HE, ×2.

For pathological examination, the surgically resected specimens were fixed in 10% formalin overnight at room temperature and the entire tumor was cut into slices at

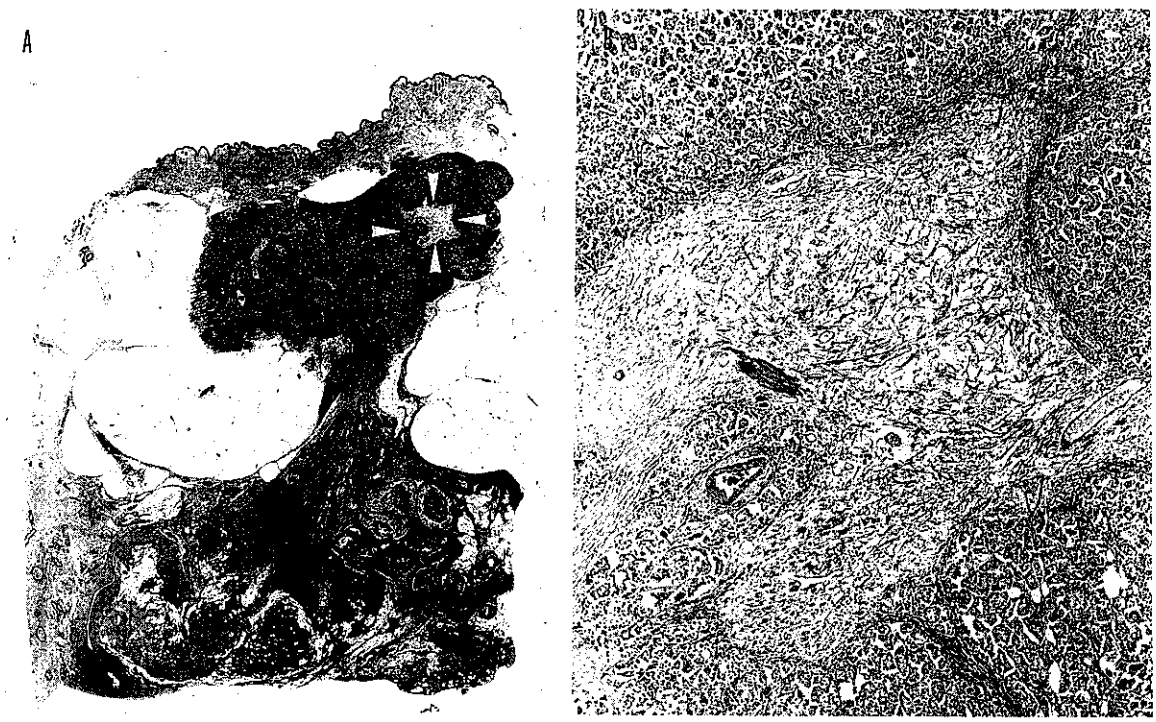

Fig. 3. IDC with FF. A, The tumor invading the dermis contains a 2×2 mm FF. B, The FF consists mainly of fibroblasts arranged in irregular fashion with mild inflammatory cell infiltration. A: HE, panoramic view, B: HE, ×40.

intervals of 0.5 to 0.7 cm. The size and gross appearance of the cancer were recorded, and the former was validated by comparison with 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 and examined pathologically to confirm the diagnosis. The remaining sections were used for immunohistochemistry and DNA analysis. All tumors were classified according to the guidelines of the World Health Organization,[3] and their histologic grade was evaluated by applying the classification of Elston,[4] which is a modification of the Bloom and Richardson classification.[5]

Histological appearance of fibrotic focus FF was defined according to the following criteria[1]: 1) FF consisting of an increased number of fibroblasts and/or collagen fibers is located within the tumor and occupying various percentages of the tumor area (Figs. 1A, 2A, and 3A). 2) When FF is 3 mm or smaller in size, tumor cells are infrequently seen within it (Fig. 3B). However, tumor cells growing in a scirrhous fashion or in solid nests are seen within FF of larger size (Figs. 1B and 2B). 3) Fibroblasts or collagen fibers in FF are arranged in irregular or storiform-like patterns with increased fibroblast cellularity and/or collagenization. Elastic tissue may be abundant (Fig. 3B). The arrangement of fibroblasts or collagen fibers forming FF appears different from normal breast tissue stroma or the surrounding tumor stroma, which is more orderly, 4) FF with necrosis is defined as FF containing tumor cells that have undergone coagulation necrosis and are surrounded by fibroblasts or collagen fibers (Fig. 4, A and B). The area of tumor cell coagulation necrosis within FF is smaller than the area occupied by fibroblasts or collagen fibers. Coagulation necrosis within the tumor without accompanying proliferation of fibroblasts or collagen fibers is not considered to be FF. FF with one or only a few necrotic tumor cells is not counted as "FF with tumor necrosis."

FF was divided into three grades according to the degree of fibrosis,[1] 1) grade 1, FF with a large number of fibroblasts but a small amount of collagen fibers (Fig. 5A), 2) grade 3, FF consisting for the most part of hyalinized collagen fibers with few fibroblasts (Fig. 5C), 3) grade 2, FF intermediate between grades 1 and 3, with fibroblasts and collagen fibers intermixed in various ratios (Fig. 5B). When several FFs of various sizes and degrees of fibrosis were present within one tumor, the largest FF was taken as the deciding one. We also divided

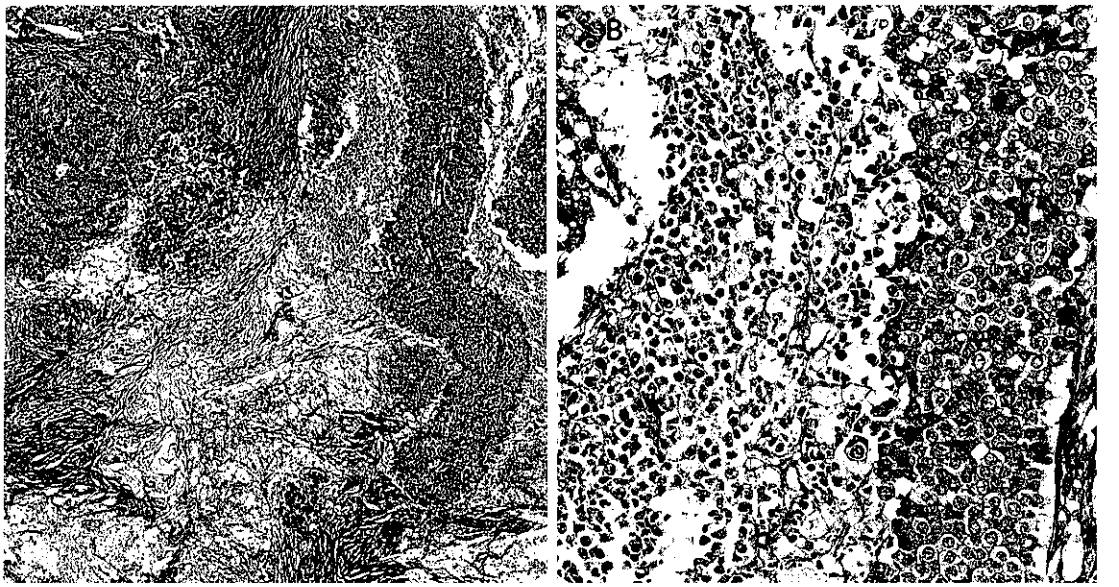

Fig. 4. IDC with FF. A, Tumor cell necrosis is observed in the periphery of the FF. B, Higher magnification of tumor necrosis. A: HE, ×4, B: HE, ×200.

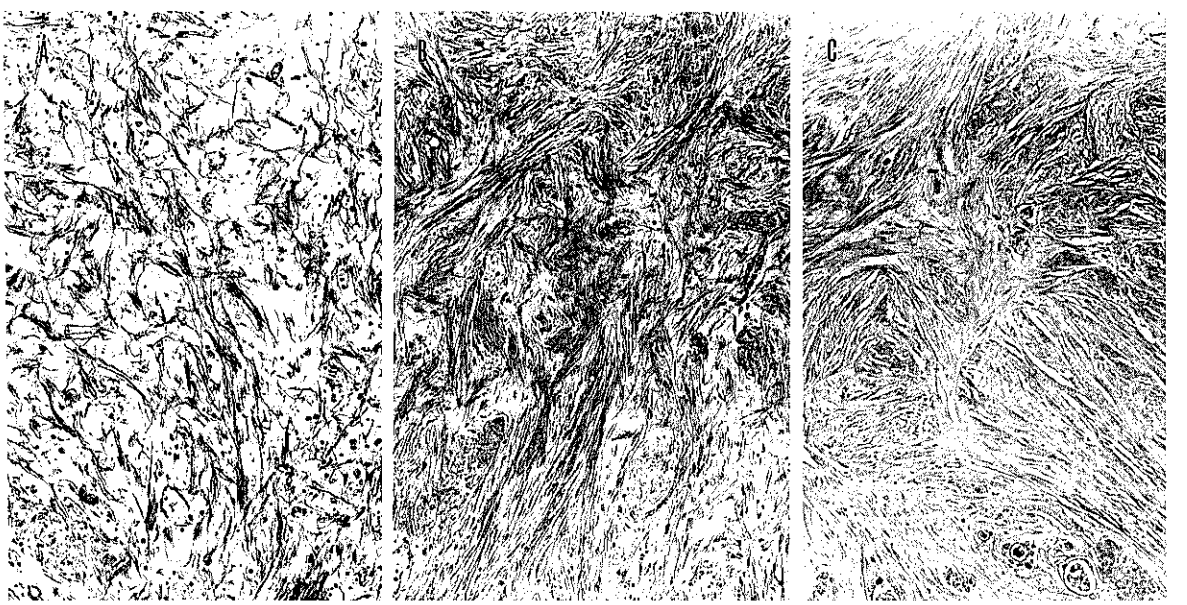

Fig. 5. Fibrosis grades of FF. A, Fibrosis grade 1 FF. The FF is composed of fibroblasts and has an edematous appearance. B, Fibrosis grade 2 FF. The FF is composed of fibroblasts mixed with collagen fibers arranged in an irregular fashion. C, Fibrosis grade 3 FF. The FF is mainly composed of hyalinized collagen fibers arranged in a storiform-like pattern. A, B, C: HE, ×100.

the growth pattern of the tumor cells in or around FF into solid and scirrhous (Fig. 6, A and B). We defined FF in lymph node metastasis in the same manner as FF in the primary tumor (Fig. 7).

Immunohistochemistry Immunohistochemical staining for c-*erb*B-2, *p53*, and PCNA products was performed by the avidin-biotin-peroxidase complex technique.[6] The primary antibodies employed were an affinity-purified

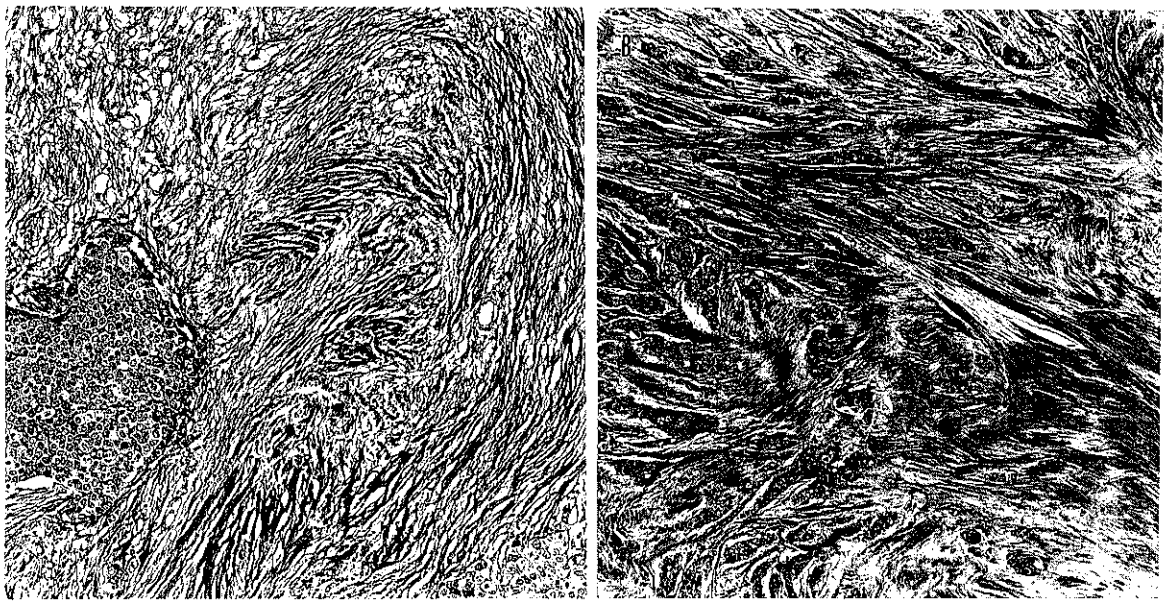

Fig. 6. Growth patterns of tumor cells within an FF. A, Tumor cells growing in a solid nest are seen within the FF. B, Tumor cells are growing in a scirrhous fashion within the FF. A, B: HE, ×100.

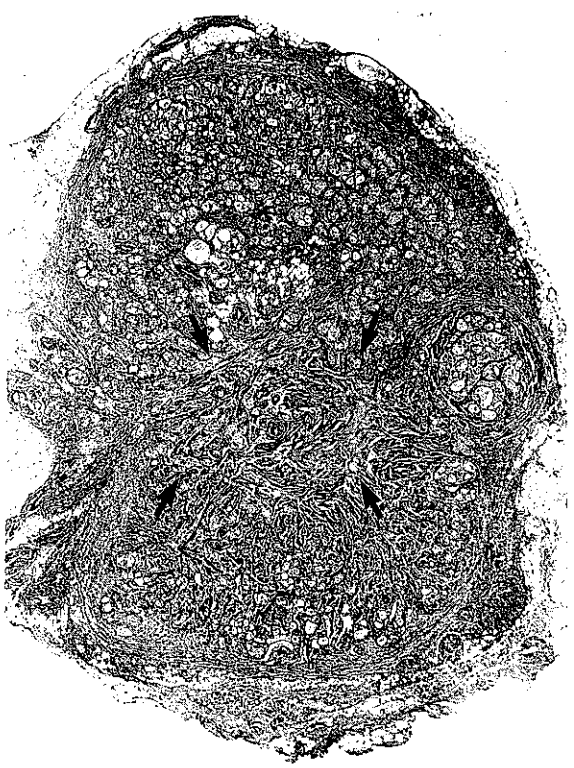

Fig. 7. FF in a lymph node metastasis. The 6×5 mm metastatic tumor in the lymph node contains a 1.5×1.5 mm FF (arrows). The FF consists of hyalinized collagen fibers (fibrosis grade 3), and contains small tumor cell nests. HE, ×2.

polyclonal antibody specific for 185-kDa c-erbB-2 protein (Nichirei, Tokyo) used in a 1 : 200 dilution, a polyclonal antibody against p53, RSP53 (Nichirei), applied in a 1 : 20,000 dilution, and a mouse monoclonal antibody against PCNA (PC10, Novocastra, UK), used in a 1 : 100 dilution. RSP53 recognizes a linear epitope in human p53 located between amino acids 54 and 69, and was shown beforehand to detect clearly the p53 protein in immunoblot analysis (data not shown). Microwave treatment was performed before immunohistochemical staining for p53.[7] After immunostaining, the sections were counterstained with hematoxylin. Sections of IDC positive for c-erbB-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 immunoglobulin.

Assessment of immunohistochemical results Tumor cell nuclei that stained brown to dark brown were considered positive for PCNA and p53, and faintly stained nuclei were considered negative. When only a few tumor cells stained positive for p53 protein or c-erbB-2 protein, it was very difficult to judge the significance of the staining. Therefore, nuclear staining for p53 was only considered positive when more than 10% of the tumor cells in the entire tumor area was judged to be positive. Tumors were judged to be positive for c-erbB-2 protein when the cell membrane of more than 10% of the tumor cells throughout the tumor stained positive. The PCNA labeling index (LI) is the percentage of tumor cells with positively

stained nuclei among the total number of 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.

Nuclear DNA content analysis Five-micron sections cut from formalin-fixed, paraffin-embedded tissue blocks were stained by the Feulgen method for DNA analysis with a CAS DNA staining kit (Cell Analysis Systems, Inc., Elmhurst, IL). DNA ploidy analysis was performed with a CAS 200 Image Analysis System (Cell Analysis Systems, Inc.). One hundred and fifty to 200 tumor cells were analyzed on each slide, and 50 lymphocytes were evaluated as a normal diploid control in each slide. A DNA index of 0.9 to 1.1 was considered diploid, and DNA indices of less than 0.9 and greater than 1.2 were considered aneuploid.[8]

Outcome The survival of the patients was determined by follow-up to June 1994, a median period of 28 months. One hundred and thirty-one patients were alive and well, 11 had tumor recurrence, and 10 had died of their disease. Measurement of disease-free survival and overall survival was started at the time of surgery. Tumor relapse was defined as any evidence of metastasis or local recurrence. Only deaths due to breast cancer were considered for the purpose of this study.

Statistical analysis Tumor death and tumor recurrence were considered as the two endpoints for determining the relationship between FF and outcome. The Cox proportional hazards regression model was used to estimate the univariate and multivariate relative risks (RR) of tumor death and tumor recurrence (with their 95% confidence intervals (CI)).[9] The following variables were examined as potential prognostic factors: 1) FF (absent or present), 2) menopausal status (pre- or post-), 3) chemotherapy (not given or given), 4) tumor necrosis (absent or present), 5) nodal status (0, 1–3, or >3), 6) T classification (T1, T2, T3, or T4), 7) histologic grade (1, 2, or 3), 8) DNA ploidy (diploid or aneuploid), 9) c-*erb*B-2 protein expression (negative or positive), 10) *p53* protein expression (negative or positive), and 11) PCNA LI (≤50% or >50%). All of the variables considered were entered into the regression model in the multivariate analyses. In addition to the analysis of all cases ($n=152$), RRs were computed for subgroups of cases in early pathological stages (stages I, IIA, and IIB, $n=124$) and those without lymph node metastases ($n=80$). For the cases with FFs at all stages ($n=79$), we analyzed the relationship between outcome and the pathological features of FF, which included the following variables: 1) tumor necrosis (present and absent), 2) growth pattern of tumor cells (solid and scirrhous), 3) size (≤5, 6–10 mm, and >10 mm), and 4) fibrosis grade (1, 2, and 3), in a similar way. For the cases with FFs, the RRs of tumor recurrence and death in cases with and without FFs in lymph node metastases were calculated by means of the Cox proportional hazards regression model and adjusted for nodal status (1 to 3, or >3). All analyses were conducted with Statistica/Windows software (StatSoft, Tulsa, OK).

RESULTS

Frequencies of variables The frequencies of the variables are listed in Table I. Among 152 cases of IDCs, nodal status, pTNM stage, DNA ploidy analysis, and PCNA LI were not examined in two cases.

Relationship between FF and tumor recurrence and tumor death FF was observed in 79 (52%) of 152 IDCs. A half of the cases in stages I and II, and 32 (40%) cases of 80 IDCs without lymph node metastasis had FF. In all cases, and in those in stages I and II, the presence of FF significantly increased the RR of tumor recurrence, and multivariate adjustment for other factors confirmed that the RR of tumor recurrence was significantly higher in cases with FF than in those without FF (Table II). In cases without lymph node metastasis, FF was associated with a significantly increased risk of recurrence in univariate analysis. Multivariate analysis was adjusted for the factors associated with high tumor recurrence rates.

Table I. The Frequencies of Each Variable

Variables	No. of patients (%)	Variables	No. of patients (%)
Histologic grade	152	Tumor necrosis	152
1	21 (14)	absent	101 (66)
2	62 (41)	present	51 (34)
3	68 (45)	DNA ploidy	150[a]
T classification	152	diploid	50 (33)
1	55 (36)	aneuploid	100 (67)
2	67 (44)	c-*erb*B-2 expression	152
3	17 (11)	negative	87 (57)
4	13 (9)	positive	65 (43)
Nodal status	150[a]	*p53* expression	152
negative	77 (51)	negative	121 (80)
1 to 3	39 (26)	positive	31 (20)
>3	34 (24)	PCNA LI (%)	150[a]
pTNM stage	150[a]	≤50	67 (45)
I	40 (27)	>50	83 (55)
IIA	46 (31)		
IIB	37 (25)		
IIIA	14 (9)		
IIIB	13 (8)		

LI, Labeling index.
a) Nodal status, pTNM stage, DNA ploidy analysis, and PCNA LI (%) were not examined in two cases.

Table II. Univariate and Multivariate Regression Analyses of Fibrotic Focus in Relation to Tumor Recurrence and Tumor Death in Patients with Invasive Ductal Carcinoma within Three Years after Surgery

Factor	No. of patients	Tumor recurrence					Tumor death		
		TRR (%)	Univariate		Multivariate		MR (%)	Univariate	
			RR	95% CI	RR	95% CI		RR	95% CI
All stages									
Fibrotic focus	152								
absent	73	4	1.0[a]		1.0[a]		0	1.0[a]	
present	79	23	6.5**	1.8–22.0	6.9*	1.2–40.6	13		NE
Stages I and II									
Fibrotic focus	124								
absent	62	2	1.0[a]		1.0[a]		0	1.0[a]	
present	62	20	13.5**	1.9–96.0	25.0*	1.3–438	8		NE
Cases without lymph node metastasis									
Fibrotic focus	80								
absent	48	2	1.0[a]		1.0[a]		0	1.0[a]	
present	32	16	7.6*	0.9–65.1	6.6	0.3–165	6		NE

TRR, tumor recurrence rate; MR, mortality rate; RR, relative risk; 95% CI, 95% confidence interval; Univariate, univariate analysis; Multivariate, multivariate analysis.
NE, not estimated; * P-value of <0.05; ** $P<0.001$. *a*) referent category.
All stages and stages I and II: RR of multivariate analysis adjusted for menopausal status, chemotherapy, tumor necrosis, nodal status, T classification, histologic grade, DNA ploidy, c-*erb*B-2 expression, *p53* expression, and PCNA LI using the Cox proportional hazards regression.
Cases without lymph node metastasis: RR of multivariate analysis adjusted for tumor necrosis, c-*erb*B-2 expression, DNA ploidy, and PCNA LI using the Cox proportional hazards regression.

These factors were tumor necrosis (presence vs. absence =15% vs. 4%), c-*erb*B-2 protein expression (positive vs. negative=12% vs. 6%), DNA ploidy (aneuploid vs. diploid=9% vs. 4%), and PCNA LI (>50% vs. ≤50% =14% vs. 2%). Although multivariate analysis failed to show a statistically significant increase in risk, the point estimate of RR was close to that estimated by univariate analysis (RR=6.6). Since all patients who died of their disease had FF, we could not perform a statistical analysis for tumor death.

Histological features of FF, and tumor recurrence and death The presence of tumor cell coagulation necrosis within FF significantly increased the RR of tumor recurrence in the univariate and multivariate analyses (Table III). Tumors having necrosis within FF were associated with a higher RR of tumor death than those without necrosis in the univariate and multivariate analyses, but the difference was not significant. Increase in size was significantly associated with tumor death in the univariate and multivariate analyses (P-trend; 0.030 and 0.019, respectively) (Table III). Neither the fibrosis grade of the FF nor the type of growth of tumor cells surrounding the FF significantly increased the RR of tumor recurrence or tumor death (data not shown).

When the analyses were performed for patients with stages I and II disease, those with tumor cell coagulation necrosis within FFs had a significantly higher RR of tumor recurrence than those without it in the univariate and multivariate analyses (Table III). Similarly, the presence of tumor cell coagulation necrosis within FF increased the RRs of tumor death (Table III). Although other factors, such as size, type of growth of tumor cells surrounding FF, and fibrosis grade of FF, were not significantly associated with tumor recurrence or death (data not shown), increasing size of FF increased the RR of tumor death (RR for per point increase=2.6, 95% CI =0.5–13.3, $P=0.26$). Since only 29 cases of IDCs without lymph node metastasis had FFs, we could not perform outcome analysis for this group.

Fibrotic foci in lymph node metastases on tumor recurrence and death FFs were present in 47 (65%) of the 72 IDCs with lymph node metastases. Twenty-four (51%) of the patients with FFs had FFs in their lymph node metastases, whereas six (24%) of the 25 patients without FFs in the primary tumor had FFs in their lymph node metastases, and the difference was statistically significant ($P=0.027$). In addition, the frequency of FFs in the lymph node metastases was significantly higher in patients with four or more lymph node metastases (21 [62%] of 34 cases) than in those with one to three lymph node metastases (9 [24%] of 38 cases) ($P=0.001$).

In order to investigate whether the presence of FF in the lymph node metastases is an important prognostic factor for tumor recurrence or death among patients

Table III. Univariate and Multivariate Regression Analyses of Histopathological Factors of Fibrotic Focus in Relation to Tumor Recurrence and Tumor Death in Patients with Invasive Ductal Carcinoma within Three Years after Surgery

Factor	No. of patients	Tumor recurrence					Tumor death				
		TRR (%)	Univariate		Multivariate		MR (%)	Univariate		Multivariate	
			RR	95% CI	RR	95% CI		RR	95% CI	RR	95%CI
All stages											
Tumor necrosis	79										
absent	65	18	1.0[a]		1.0[a]		9	1.0[a]		1.0[a]	
present	14	43	3.4*	1.3–8.7	3.7*	1.1–12.0	27	3.2	0.9–11.5	3.8	0.7–18.7
Size (mm)	79										
≤5	41	20	1.0[a]		1.0[a]		7	1.0[a]		1.0[a]	
6–10	20	20	1.2	0.3–3.8	0.8	0.3–4.5	11	2.1	0.4–10.7	2.5	0.5–13.4
>10	18	33	2.2	0.8–6.4	1.4	0.4–4.9	26	4.1	0.9–18.9	5.8	1.0–34.9
			P-trend : 0.212		P-trend : 0.514			P-trend : 0.030		P-trend : 0.019	
Stages I and II											
Tumor necrosis	62										
absent	51	14	1.0[a]		1.0[a]		6	1.0[a]		1.0[a]	
present	11	45	4.5*	1.4–14.3	4.6*	1.1–18.8	18	3.9	0.6–23.7	3.8	0.4–35.4

TRR, tumor recurrence rate; MR, mortality rate; RR, relative risk; 95% CI, 95% confidence interval; Univariate, univariate analysis; Multivariate, multivariate analysis; * P-value of <0.05.
a) referent category.
All stages: RR of multivariate analysis adjusted for growth type and fibrosis grade using the Cox proportional hazards regression.
Stages I and II: RR of multivariate analysis adjusted for growth type, fibrosis grade and dimension using the Cox proportional hazards regression.

Table IV. Effect of the Presence of Fibrotic Focus in the Primary Tumor and Lymph Node Metastases on Tumor Recurrence and Death

Factor	Primary tumor	Metastasis in LN	No. of patients	Tumor recurrence			Tumor death		
				TRR (%)	Multivariate		MR (%)	Multivariate	
					RR	95% CI		RR	95% CI
FF	present	absent	23	17	1.0[a]		4	1.0[a]	
FF	present	present	24	42	2.0**	1.2–3.4	29	5.9*	1.1–31.0

TRR, tumor recurrence rate; MR, mortality rate; FF, fibrotic focus; LN, lymph node; RR, relative risk; 95% CI, 95% confidence interval; Multivariate, multivariate analysis; * P-value of <0.05; ** $P<0.001$.
a) referent category.
RR of multivariate analysis adjusted for nodal status (1 to 3, and >3).

with FFs in the primary tumor, we conducted multivariate analysis adjusted for nodal status (1 to 3 and >3), excluding one case in which there were only a few metastatic tumor cells in one lymph node. Significantly higher RRs of tumor recurrence and death were observed in patients with FFs in lymph node metastases than in those without FFs (Table IV).

DISCUSSION

Most studies on prognostic parameters for breast cancer have focused on finding parameters that can predict 5-, 10-, or 15-year survival. Although some studies have touched on early tumor recurrence or death in breast cancer patients, the major interest has been long-term survival,[10–14] and thus, there has never been a study focused mainly on early tumor recurrence or death in breast cancer. IDCs associated with early tumor recurrence or death appear to have different biological characteristics from those associated with late tumor recurrence or death, indicating that the former tumors are more aggressive than the latter.[14] Since patients with highly aggressive IDCs have to be observed very carefully or treated with postoperative adjuvant chemotherapy or

radiotherapy in order to prevent early tumor recurrence or death, it is very important to identify the histopathological parameters associated with early tumor recurrence or death.

This study showed that the presence of FFs in IDCs is a good predictor of early tumor recurrence, both in all stages as a whole and in stages I and II alone. Although there was no significant difference between the RRs of early tumor recurrence in IDCs with and without FFs in the multivariate analysis of the cases without axillary lymph node metastasis, the former had an about 6 times higher RR than the latter. This suggests that the presence of FFs in IDCs is also a significant histological parameter for predicting early tumor recurrence even in cases without axillary lymph node metastasis. Although we could not analyze the significance of the presence of FFs in IDCs for early tumor death, all the patients who died of tumor had FFs. Thus, FFs appear to be a very important histopathological parameter for predicting tumor recurrence or death within three years after surgery. Furthermore, we clearly demonstrated that among the histological features of FF, the presence of tumor necrosis and a size of 11 mm or more were the most important histological factors associated with early tumor recurrence or death. In addition, FFs in lymph node metastases were shown to be an independently significant prognostic parameter for IDCs. These findings indicate that IDCs with FFs exhibiting tumor necrosis and with a size of 11 mm or more and those having lymph node metastatic tumors with FF pursue the worst clinical course.

With regard to the mechanism of the formation of FFs in IDCs, we have already demonstrated that 1) FFs exhibiting tumor necrosis were observed more frequently in tumors growing in solid nests than in a scirrhous fashion ($P<0.008$). This suggests that FFs in tumors growing in solid nests developed as a result of tumor cell necrosis.[1] 2) Tumors growing in a scirrhous fashion with FFs displayed a significantly higher frequency of basic fibroblast growth factor (bFGF) protein expression than those growing in a scirrhous fashion without FFs or those growing in solid nests with FFs (unpublished data). bFGF is a potent stimulator of fibroblasts,[15] and excessive production of bFGF has been shown to contribute to fibroblastic proliferation and fibrosis in a number of conditions.[16–18] Thus, FFs in tumors growing in scirrhous fashion may be formed by interaction between the tumor cells and the stromal fibroblasts, whereas, in tumors growing in solid nests, the cohesive growth of tumor cells causes decreased blood flow or hypoxia within the tumor, resulting in coagulation necrosis of the tumor cells. The necrotic area is likely to be replaced by FF. In addition, it was demonstrated that the presence of FFs in lymph node metastases is significantly dependent upon the presence of FFs in the primary site, indicating that tumor cells are able to form FFs in different environments.

In conclusion, it was demonstrated that the presence of FFs in IDCs is an important histological parameter for predicting the outcome for patients within three years after surgery. In addition, the presence of FFs in lymph node metastases as well as in primary tumors can be regarded as a significant prognostic parameter for patients with IDCs.

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REFERENCES

1) Hasebe, T., Tsuda, H., Hirohashi, S., Shimosato, Y., Iwai, M., Imoto, S. and Mukai, K. Fibrotic focus in invasive ductal carcinoma: an indicator of high tumor aggressiveness. *Jpn. J. Cancer Res.*, **87**, 385–394 (1996).
2) Hermanek, P. and Sobin, L. H. "TNM Classification of Malignant Tumors," 4th Ed., p. 93 (1987). Springer-Verlag, Berlin.
3) WHO: Histological typing of breast tumors. *In* "International Histological Classification of Tumors," 2nd Ed., p. 806 (1981). World Health Organization, Geneva.
4) Elston, C. W. Grading of invasive carcinoma of the breast. *In* "Diagnostic Histopathology of the Breast," ed. D. L. Page and T. J. Anderson, pp. 300–311 (1987). Churchill Livingstone, New York.
5) Bloom, H. J. G. and Richardson, W. W. Histological grading and prognosis in breast cancer. *Br. J. Cancer*, **11**, 359–377 (1957).
6) Hsu, S. M., Raine, L. and Fanger, H. The use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase technique: a comparison between ABC and unlabelled antibody (PAP) procedures. *J. Histochem. Cytochem.*, **29**, 577–580 (1981).
7) Shi, S. R., Key, M. E. and Kalza, K. L. Antigen retrieval in formalin-fixed, paraffin-embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections. *J. Histochem. Cytochem.*, **39**, 741–746 (1991).
8) Shankey, T. V., Rabinovitch, P. S., Bagwell, B. and Bauer, K. D. Guidelines for implementation of clinical DNA cytometry. *Cytometry*, **14**, 472–477 (1993).
9) Cox, D. R. Regression models and life-tables. *J. R. Stat. Soc.*, **34**, 187–220 (1972).

10) Black, M. M., Opler, S. R. and Speer, F. D. Survival in breast cancer cases in relation to the structure of the primary tumor and regional lymph nodes. *Surg. Gynecol. Obstet.*, **100,** 543–551 (1955).
11) Fisher, B., Bauer, M., Wickerman, L., Redmond, C. K. and Fisher, E. R. Relation of number of positive axillary nodes to the prognosis of patients with primary breast cancer. *Cancer,* **52,** 1551–1557(1983).
12) Bryan, R. M., Mercer, R. J., Bennett, R. C. and Rennie, G. C. Prognostic factors in breast cancer and the development of a prognostic index. *Br. J. Surg.,* **73,** 267–271 (1986).
13) Le Doussal, V., Tubiana-Hulin, M., Friedman, S., Hacene, K., Spyratos, F. and Brunet, M. Prognostic value of histologic grade nuclear components of Scarff-Bloom-Richardson (SBR). An improved score modification based on a multivariate analysis of 1262 invasive ductal breast carcinomas. *Cancer,* **64,** 1914–1921 (1989).
14) Tahan, S. T., Neuberg, D. S., Dieffenbach, A. and Yacoub, L. Prediction of early relapse and shortened survival in patients with breast cancer by proliferating cell nuclear antigen score. *Cancer,* **71,** 3552–3559 (1993).
15) Rifkin, D.B. and Moscatelli, D. Recent developments in the cell biology of basic fibroblast growth factor. *J. Cell Biol.,* **109,** 1–6 (1989).
16) Henke, C., Marinelli, W., Jessurun, J., Fox, J., Harms, D., Peterson, M., Chiang, L. and Doran, P. Macrophage production of basic fibroblast growth factor in the fibroproliferative disorder of alveolar fibrosis after lung injury. *Am. J. Pathol.,* **143,** 1189–1199 (1993).
17) Friess, H., Yamanaka, Y., Büchler, M., Beger, H. G., Do, D. A., Kobrin, M. S. and Korc, M. Increased expression of acidic and basic fibroblast growth factors in chronic pancreatitis. *Am. J. Pathol.,* **144,** 117–128 (1994).
18) Tanimoto, H., Yoshida, K., Yokozaki, H., Yasui, W., Nakayama, H., Ito, H., Ohama, K. and Tahara, E. Expressions of basic fibroblast growth factor in human gastric carcinomas. *Virchows Arch. B,* **61,** 263–267 (1991).