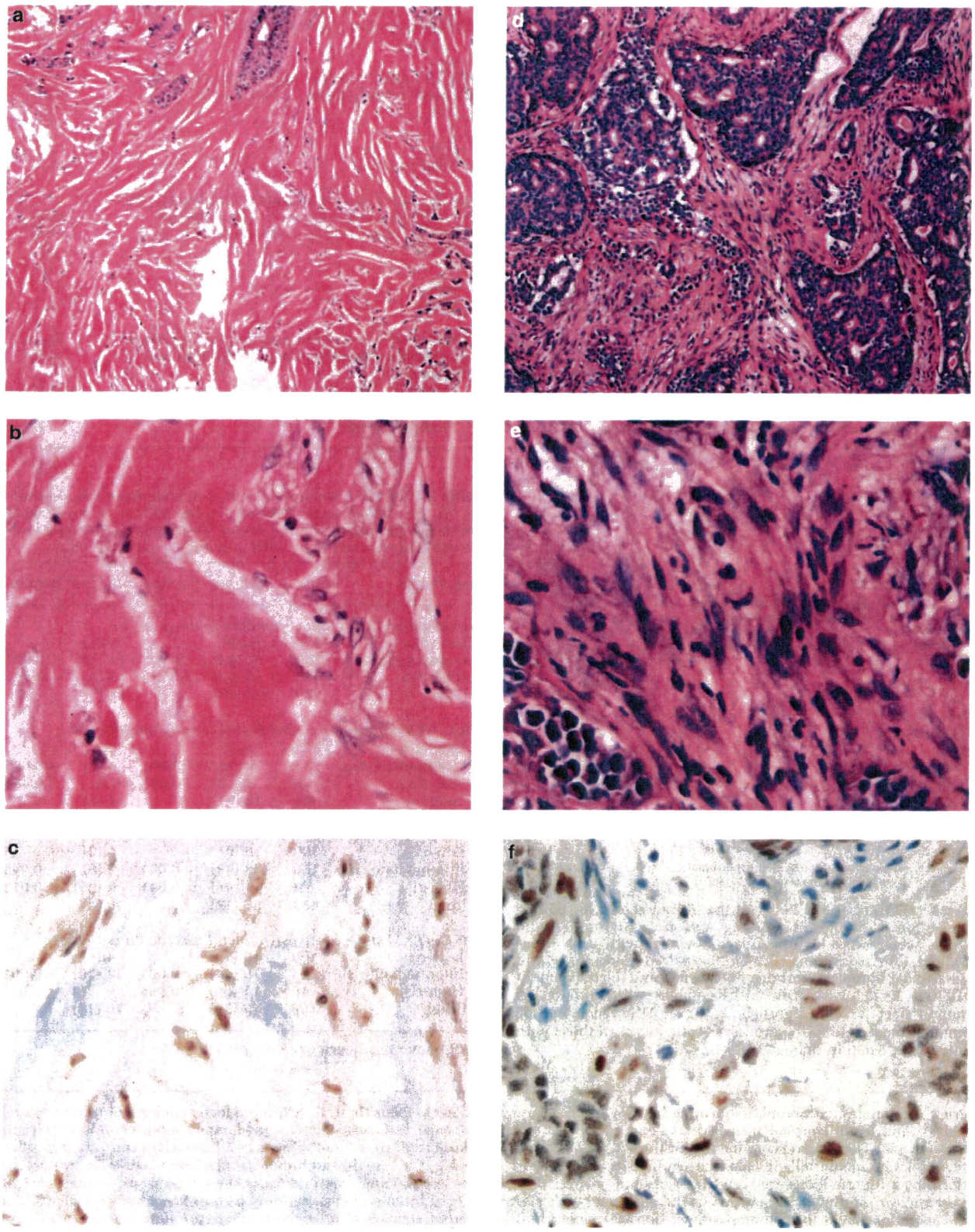
diameter >8 mm, the number of IDCs with Allred scores of 4–8 for p53 in tumor-stromal fibroblasts forming fibrotic foci was larger than that of IDCs with Allred scores of 0, 2, or 3 for p53 in tumor-stromal fibroblasts forming fibrotic foci (Figure 4b).



Allred Score Risk Classification for p53 in Tumor-Stromal Fibroblasts Forming and not Forming Fibrotic Foci in Patients with Invasive Ductal Carcinoma with and without Fibrotic Foci

We devised an Allred score risk classification for p53 in tumor-stromal fibroblasts in IDCs based on the combined Allred scores for p53 in tumor-stromal fibroblasts forming and not forming fibrotic foci (Table 1). This classification was successfully used to classify IDC patients with or without fibrotic foci into three risk classes (low risk, intermediate risk, and high risk) according to the ratios for tumor recurrence and tumor-related death (Table 2; Figure 5). Among the UICC pTNM stage I IDC patients, the patients in the intermediate- and high-risk classes showed a significantly higher tumor recurrence rate than the patients in the low-risk class (Table 2). Among the UICC pTNM stage II IDC

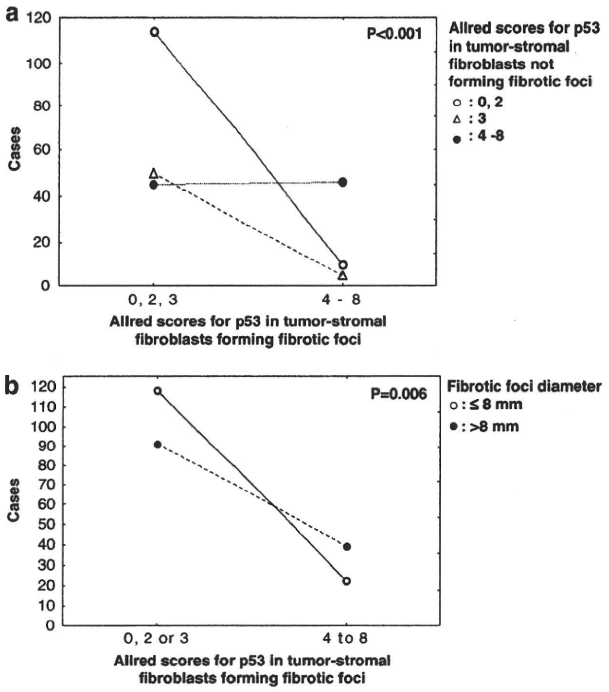


Figure 4 (**a**) Associations between the Allred scores for p53 in tumor -stromal fibroblasts forming and not forming fibrotic foci; the scores were significantly associated with each other ($P<0.001$). (**b**) Associations between the Allred scores for p53 in tumor-stromal fibroblasts forming fibrotic foci and the diameter of the fibrotic foci. Invasive ductal carcinomas with fibrotic foci >8 mm in diameter had a significantly higher Allred score for p53 in tumor-stromal fibroblasts forming fibrotic foci than those with fibrotic foci ≤8 mm in diameter ($P=0.006$).

Table 1 Overall Allred score classification of p53 in tumor-stromal fibroblasts forming and not forming a fibrotic focus

Invasive ductal carcinoma with a fibrotic focus	
A) The Allred scores of p53 in tumor-stromal fibroblasts forming a fibrotic focus	Score class
0, 2, or 3	0
4–8	2
B) The Allred scores of p53 in tumor-stromal fibroblasts not forming a fibrotic focus	Score class
0 or 2	0
3	1
4–8	2
Total (A+B)	0–4
Invasive ductal carcinoma without a fibrotic focus	
The Allred scores of p53 in tumor-stromal fibroblasts not forming a fibrotic focus	Score class
0 or 2	0
3	1
4–8	2
Total	0–2
The Allred score risk classes for p53 in tumor-stromal fibroblasts forming and not forming fibrotic foci	Score class
Low-risk class	0 and 1
Intermediate-risk class	2 and 3
High-risk class	4

Table 2 Tumor recurrence and tumor-related death rates according to the Allred score risk classes for p53 in tumor-stromal fibroblasts forming and not forming fibrotic foci in patients with invasive ductal carcinoma with or without a fibrotic focus

Risk classes	*Cases*	*TRR (%)*	*P-value*	*MR (%)*	*P-value*
Invasive ductal carcinoma patients as a whole					
Low-risk	648	36 (6)		9 (1)	
Intermediate-risk	232	52 (22)	<0.001	24 (10)	<0.001
High-risk	46	24 (52)	<0.001	15 (33)	0.001
Total	926	112 (12)		48 (5)	
UICC pTNM stage I invasive ductal carcinoma patients					
Low-risk	239	5 (2)		0	
Intermediate-risk	69	10 (15)	<0.001	4 (6)	<0.001
High-risk	6	2 (33)	0.295	0	0.454
Total	314	17 (5)		4 (1)	
UICC pTNM stage II invasive ductal carcinoma patients					
Low-risk	309	18 (6)		5 (2)	
Intermediate-risk	120	23 (19)	<0.001	7 (6)	0.045
High-risk	24	11 (46)	0.041	6 (25)	0.012
Total	453	52 (12)		18 (4)	
UICC pTNM stage III invasive ductal carcinoma patients					
Low-risk	100	13 (13)		4 (4)	
Intermediate-risk	43	19 (44)	<0.001	13 (30)	<0.001
High-risk	16	11 (69)	0.054	9 (56)	0.042
Total	159	43 (27)		26 (16)	

TRR, tumor recurrence rate; MR, mortality rate.

Figure 3 Tumor-stromal fibroblasts forming (**a, c, e**) and not forming a fibrotic focus (**b, d, f**). A fibrotic focus consists of tumor-stromal fibroblasts and hyalinized collagen fibers (**a** and **c**) and many tumor-stromal fibroblasts show a moderately intense nuclear staining pattern for p53. The Allred score for p53 in these tumor-stromal fibroblasts forming a fibrotic focus is 7 (intensity score, 2; proportion score, 5) (**e**). Carcinoma cells invade in irregular-shaped nests with a tubular structure (**b**) and tumor-stromal fibroblasts with oval nuclei not forming a fibrotic focus are seen (**d**). Many tumor-stromal fibroblasts not forming a fibrotic focus show a faint, moderate or strong intense nuclear staining pattern for p53, whereas tumor cells showing a faint intense nuclear staining pattern for p53 are visible (**f**). The Allred score for p53 in these tumor-stromal fibroblasts not forming a fibrotic focus is 8 (intensity score, 3; proportion score, 5).

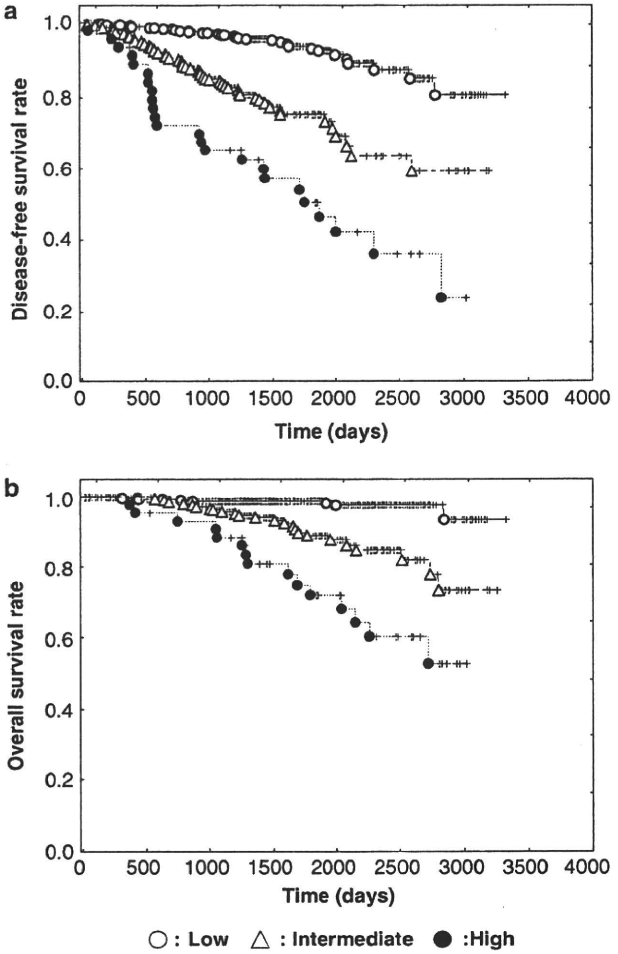


Figure 5 Disease-free survival curves and overall survival curves of invasive ductal carcinoma (IDC) patients overall (**a** and **b**) according to the Allred score risk classification for p53 in tumor-stromal fibroblasts forming and not forming a fibrotic focus (FF). The disease-free survival time (**a**) and the overall survival time (**b**) of the IDC patients significantly decrease with the risk class of the Allred score risk classification for p53 in tumor-stromal fibroblasts forming and not forming FF.

patients, the tumor recurrence rate and the mortality rate for each risk class were significantly increased according to the risk classes of the classification (Table 2). Among the UICC pTNM stage III IDC patients, the patients in the intermediate-risk class showed a significantly higher tumor recurrence rate and mortality rate than the patients in the low-risk class, and the patients in the high-risk class showed a marginally significantly higher tumor recurrence rate and a significantly higher mortality rate than the patients in the intermediate-risk class (Table 2).

Overall, the Allred score risk classification for p53 in tumor-stromal fibroblasts forming and not forming fibrotic foci (trend hazard rate, 2.9; trend 95% confidence interval, 1.6–5.2; *P*-value, <0.001) was superior to the Allred scores for p53 in tumor-stromal fibroblasts not forming fibrotic foci alone (trend hazard rate, 1.5; trend 95% confidence interval, 0.8–2.6; *P*-value, 0.172) for accurately predicting tumor-related death among patients with IDC, as shown in a multivariate analysis.

Factors Significantly Associated with Tumor Recurrence and Tumor-Related Death

Among the patients with UICC pTNM stage I IDC, an intermediate-risk class (hazard rate, 6.2; 95% confidence interval, 2.1–18.5; *P*-value, 0.001) and a high-risk class (hazard rate, 11.6; 95% confidence interval, 2.1–63.8; *P*-value, 0.005) for p53 in tumor-stromal fibroblasts forming and not forming fibrotic foci and a histological grade of 3 (hazard rate, 2.9; 95% confidence interval, 1.1–7.6; *P*-value, 0.034) significantly increased the hazard rates for tumor recurrence in a multivariate analysis.

Among the patients with UICC pTNM stage II IDC, an intermediate-risk class and a high-risk class for p53 in tumor-stromal fibroblasts forming and not forming fibrotic foci significantly increased the hazard rates for tumor recurrence and tumor-related death in the multivariate analyses (Table 3). Grades 2 and 3 lymph vessel tumor emboli and the presence of blood vessel invasion significantly increased the hazard rates for tumor recurrence in the multivariate analysis (Table 3). A UICC pN1 category and a fibrotic focus diameter >8 mm significantly increased the hazard rates for tumor-related death and an Allred score of 7 or 8 for the progesterone receptors in the tumor cells significantly decreased the hazard rate for tumor-related death in the multivariate analyses (Table 3).

Among the patients with a UICC pTNM stage III IDC, an intermediate-risk class and a high-risk class for p53 in tumor-stromal fibroblasts forming and not forming fibrotic foci, grade 3 lymph vessel tumor emboli and a UICC pN3 category significantly increased the hazard rates for tumor recurrence and tumor-related death in the multivariate analysis (Table 4). A fibrotic focus diameter >8 mm significantly increased the hazard rate for tumor recurrence and an Allred score of 7 or 8 for estrogen receptor in the tumor cells significantly decreased the hazard rate for tumor-related death in the multivariate analysis (Table 4).

Discussion

This study clearly showed that the values of the Allred scores for p53 in tumor-stromal fibroblasts not forming fibrotic foci were significantly higher than those in tumor-stromal fibroblasts forming fibrotic foci. Fibrotic foci are fibrotic scar-like lesions that mainly consist of tumor-stromal fibroblasts admixed with various numbers of tumor cells; some fibrotic foci do not contain any tumor cells.[1,2] In contrast, tumor-stromal fibroblasts not forming fibrotic foci commonly admix with many tumor cells that show stromal invasion. This difference

Table 3 Multivariate analyses for tumor recurrence and tumor-related death in UICC pTNM stage II invasive ductal carcinoma patients ($n=453$)

Factors	*Tumor recurrence*		*Tumor-related death*	
	HR (95% CI)	*P-value*	*HR (95% CI)*	*P-value*
p53 Allred score risk classes of tumor-stromal fibroblasts forming and not forming a fibrotic focus				
Low-risk	Referent		Referent	
Intermediate-risk	3.5 (1.4–4.4)	0.003	3.3 (1.0–10.5)	0.043
High-risk	5.2 (1.8–6.5)	<0.001	4.7 (1.3–17.3)	0.021
Grading system for lymph vessel tumor emboli				
Grade 0	Referent		Referent	
Grade 1	1.5 (0.8–3.0)	0.226	0.5 (0.1–2.5)	0.421
Grades 2 and 3	2.5 (1.4–4.4)	0.003	2.0 (0.6–6.3)	0.275
Blood vessel invasion				
Absent	Referent		Referent	
Present	2.1 (1.1–3.8)	0.017	1.1 (0.3–3.8)	0.914
The Allred scores for progesterone receptors in tumor cells				
0 or 2	Referent		Referent	
3–6	—		0.8 (0.2–3.0)	0.729
7 or 8	—		0.2 (0.07–0.7)	0.009
UICC pN category				
pN0	Referent		Referent	
pN1	—		14.7 (1.9–113.1)	0.010
Fibrotic focus, diameter				
Absent	Referent		Referent	
≤8 mm	—		1.3 (0.2–8.5)	0.763
>8 mm	—		3.4 (1.2–9.8)	0.025

HR, hazard rate; CI, confidence interval; —, not significance in univariate analysis.
The multivariate analysis for tumor recurrence was performed using the p53 Allred score risk classes in tumor-stromal fibroblasts forming and not forming a fibrotic focus, grading system for lymph vessel tumor emboli, blood vessel invasion, histological grade, and age.
The multivariate analysis for tumor-related death was performed using the p53 Allred score risk classes in tumor-stromal fibroblasts forming and not forming a fibrotic focus, grading system for lymph vessel tumor emboli, blood vessel invasion, the Allred scores for progesterone receptors in tumor cells, UICC pN category, fibrotic focus diameter, and age.

strongly suggests that the tumor cell–stromal cell interaction occurs more frequently in the outer area of a fibrotic focus than in the inner area of a fibrotic focus within IDCs,[10,11] probably resulting in the higher Allred scores for p53 in tumor-stromal fibroblasts not forming fibrotic foci. However, the Allred scores for p53 in tumor-stromal fibroblasts forming fibrotic foci were significantly associated with those for p53 in tumor-stromal fibroblasts not forming fibrotic foci. Thus, the tumor cell–stromal cell interaction probably occurs more frequently in IDCs with fibrotic foci than in IDCs without fibrotic foci.

We and others have already reported that the fibrotic focus diameter is a significant outcome predictor among patients with IDC who have fibrotic foci,[1–5] and our previous study showed that a fibrotic focus diameter of greater than 8 mm, similar to the Allred score for p53 in tumor-stromal fibroblasts not forming a fibrotic focus, was a significant outcome predictor for patients with IDC independent of the UICC pTNM stage.[19] In this study, a fibrotic focus diameter was also a significant outcome predictor for IDC patients of UICC pTNM stage II and IDC patients of UICC pTNM stage III, and IDCs with fibrotic foci greater than 8 mm in diameter showed a significantly higher Allred score for p53 in tumor-stromal fibroblasts forming fibrotic foci than IDCs with fibrotic foci of 8 mm or less in diameter. Thus, one can conclude that p53-expressing tumor-stromal fibroblasts located in both the inner and outer regions of fibrotic foci heighten the malignant potential of IDCs, probably accounting for the prognostic value of the fibrotic focus diameter. In addition, the grading system for lymph vessel tumor emboli significantly increased the hazard rates for tumor recurrence or tumor-related death in multivariate analyses performed for IDC patients with UICC pTNM stage II and UICC stage III. Therefore, the fibrotic focus diameter and the grading system for lymph vessel tumor emboli are likely to be very important histological outcome predictors for patients with IDC.

The results of this study clearly show that the Allred score risk classification for p53 in tumor-stromal fibroblasts forming and not forming fibrotic foci had a greater outcome predictive power than the Allred scores for p53 in tumor-stromal fibroblasts not forming fibrotic foci alone. Furthermore, the Allred score risk classification for p53 in tumor-stromal fibroblasts forming and not forming fibrotic foci is a very important outcome predictor for patients with IDC

Table 4 Multivariate analyses for tumor recurrence and tumor-related death in UICC pTNM stage III invasive ductal carcinoma patients

Factors	*Tumor recurrence*		*Tumor-related death*	
	HR (95% CI)	*P-value*	*HR (95% CI)*	*P-value*
p53 Allred score risk classes of tumor-stromal fibroblasts forming and not forming a fibrotic focus				
Low-risk	Referent		Referent	
Intermediate-risk	2.9 (1.3–6.3)	0.009	5.2 (1.6–17.2)	0.007
High-risk	6.0 (2.6–13.9)	<0.001	20.1 (5.8–69.0)	<0.001
Grading system for lymph vessel tumor emboli				
Grade 0	Referent		Referent	
Grade 1	0.6 (0.2–1.8)	0.340	0.5 (0.1–3.1)	0.480
Grade 2	0.6 (0.2–1.6)	0.281	1.7 (0.5–5.8)	0.426
Grade 3	6.5 (2.9–14.4)	<0.001	2.6 (1.0–6.7)	0.045
UICC pN category				
pN0	Referent		Referent	
pN1	6.3 (0.5–81.3)	0.166	8.8 (0.4–203.7)	0.171
pN2	6.9 (0.6–70.2)	0.108	5.0 (0.3–80.1)	0.256
pN3	2.8 (1.5–5.3)	0.001	3.3 (1.4–7.8)	0.005
Fibrotic focus, diameter				
Absent	Referent		Referent	
≤8 mm	1.6 (0.6–4.3)	0.383	1.3 (0.2–8.6)	0.777
>8 mm	2.8 (1.3–6.2)	0.009	2.1 (0.5–9.5)	0.337
The Allred scores for estrogen receptor in tumor cells				
0 or 2	Referent		Referent	
3–6	0.7 (0.3–1.9)	0.488	1.2 (0.3–5.0)	0.836
7 or 8	0.6 (0.2–1.5)	0.257	0.4 (0.2–0.9)	0.033

HR, hazard rate; CI, confidence interval; pN, pathological regional lymph node; N0, no nodal metastasis; N1, 1–3 nodal metastases; N2, 4–9 nodal metastases; N3, 10 or more nodal metastases.
The multivariate analysis for tumor recurrence was performed using the p53 Allred score risk classes in tumor-stromal fibroblasts forming and not forming a fibrotic focus, grading system for lymph vessel tumor emboli, UICC pN category, fibrotic focus diameter, the Allred scores for estrogen receptors in tumor cells, the Allred scores for progesterone receptors in tumor cells, the Allred scores for p53 in tumor cells, invasive tumor size, tumor necrosis, and histological grade.
The multivariate analysis for tumor death was performed using the p53 Allred score risk classes in tumor-stromal fibroblasts forming and not forming a fibrotic focus, grading system for lymph vessel tumor emboli, UICC pN category, fibrotic focus diameter, the Allred scores for estrogen receptors in tumor cells, the Allred scores for p53 in tumor cells, HER2 category in tumor cells, age, invasive tumor size, and histological grade.

and an intermediate-risk or high-risk classification significantly increased the hazard rates for tumor recurrence and tumor-related death independent of the UICC pTNM stage in multivariate analyses that included well-known prognostic factors. Thus, we can conclude that the Allred score risk classification based on the Allred score for p53 in tumor-stromal fibroblasts forming and not forming fibrotic foci appears to be an excellent histological predictor of outcome among patients with IDC with or without fibrotic foci. However, as we could not analyze the outcome predictive power of the Allred score risk classification for p53 in tumor-stromal fibroblasts forming and not forming fibrotic foci among patients with IDC according to the types of adjuvant therapy (chemotherapy, endocrine therapy, and chemoendocrine therapy) in detail, the predictive power of the Allred score risk classification for p53 in tumor-stromal fibroblasts forming and not forming fibrotic foci should be analyzed separately among IDC patients treated with chemotherapy, endocrine therapy, and chemoendocrine therapy in the future.

In this study, we did not investigate the associations of the Allred scores for p53 with the presence of *p53* gene abnormalities in tumor-stromal fibroblasts. Although *p53* mutations in tumor-stromal fibroblasts are relatively common among primary breast cancers and other cancers and have been reported to exert a positive effect on cancer growth,[12–15] some studies have not shown any p53 mutations in the tumor-stroma of breast cancer.[16–18] We have already reported that fibroblasts forming fibrotic foci show significantly higher proliferative activities than those not forming fibrotic foci and found that no significant association exists between the proliferative activity of fibroblasts forming fibrotic foci and the fibrotic foci diameter.[7] In contrast, the Allred scores for p53 in tumor-stromal fibroblasts forming fibrotic foci were significantly lower than the Allred scores for p53 in tumor-stromal fibroblasts not forming fibrotic foci, and a significant association between the increase in the Allred scores for p53 in tumor-stromal fibroblasts forming fibrotic foci and the fibrotic foci diameter was observed in this study. Thus, although the mechanism that increases the malignant potential of IDCs through the expression of p53 in tumor-stromal fibroblasts should be investigated from the viewpoint of

p53 gene abnormalities, p53 immunoreactivity in tumor-stromal fibroblasts produced by tumor cell–stromal cell interactions inside and outside fibrotic foci might in fact reflect specific reactive changes other than the proliferative activity of fibroblasts forming fibrotic foci within the stroma that might be correlated with the prognosis.

In conclusion, this is the first study to show clearly that p53 expression in tumor-stromal fibroblasts forming and not forming fibrotic foci is strongly associated with the outcome of IDC patients. Because p53 expression in tumor-stromal fibroblasts forming and not forming fibrotic foci might be important in tumor progression in IDCs, p53 expression could be a very important target for tumor gene therapy for IDCs, suppressing tumor cell–stromal cell interactions arising from *p53* gene abnormalities or p53-related tumor microenvironment reactions.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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Atypical Tumor-stromal Fibroblasts in Invasive Ductal Carcinoma of the Breast

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Abstract: Tumor-stromal fibroblasts have recently been reported to play important roles in the tumor progression of cancer in various organs. The purpose of this study was to investigate whether any characteristic histologic features of tumor-stromal fibroblasts could accurately predict the outcome of 1042 patients with invasive ductal carcinoma of the breast. We observed a small number of tumor-stromal fibroblasts with characteristic nuclear features existing inside and outside of fibrotic foci and named them atypical tumor-stromal fibroblasts. We then classified invasive ductal carcinomas into 4 types (1, 2, 3, and 4) according to the absence or presence of fibrotic foci and the absence or presence of atypical tumor-stromal fibroblasts. We then analyzed the outcome predictive powers of these types of invasive ductal carcinomas using multivariate analyses that included well-known clinicopathologic factors. The multivariate analyses showed that type 4 invasive ductal carcinomas with fibrotic foci and atypical tumor-stromal fibroblasts had significantly higher hazard ratios for tumor recurrence and tumor-related death, independent of the nodal status and histologic grade, and the type 2 invasive ductal carcinomas without fibrotic foci but with atypical tumor-stromal fibroblasts had a significant higher hazard ratio for tumor recurrence among patients with invasive ductal carcinoma with nodal metastasis and those with histologic grade 3 disease. The results of this study clearly indicated that the presence of atypical tumor-stromal fibroblasts, especially in fibrotic foci, is significantly associated with tumor recurrence and tumor-related death of patients with invasive ductal carcinoma of the breast.

Key Words: fibroblast, fibrotic focus, p53, tumor cell-stromal cell interaction, breast

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Tumor-stromal fibroblasts, or the so-called cancer-associated fibroblasts, have recently been reported to play important roles in the tumor progression of cancer in various organs.[9,10,21,23,25,28,30] Among tumor-stromal fibroblasts, tumor-stromal fibroblasts that form fibrotic foci have a more significant power for the accurate prediction of the outcome of patients with invasive ductal carcinoma than tumor-stromal fibroblasts that do not form fibrotic foci.[10] A fibrotic focus is a characteristic histologic feature of tumor stroma with scar-like features or a radiating fibrosclerotic core that is surrounded by invasive ductal carcinoma cells.[8,10,11] A fibrotic focus is composed of a mixture of fibroblasts and various amounts of collagen fibers, with the fibroblasts and collagen fibers composing the fibrotic focus exhibiting a storiform arrangement. We and other researchers have already reported that a fibrotic focus is a very useful histologic tumor-stromal indicator for accurately predicting the outcome of patients with invasive ductal carcinoma.[3,8,10,11,26] In addition, we recently showed that p53 expression in tumor-stromal fibroblasts was a very important outcome predictor for patients with invasive ductal carcinoma who had or had not received neoadjuvant therapy.[14,17] Among tumor-stromal fibroblasts expressing p53, the tumor-stromal fibroblasts that also formed fibrotic foci apparently played a very important role in tumor progression in invasive ductal carcinoma of the breast.[15]

The purpose of this study was to investigate whether characteristic histologic features of tumor-stromal fibroblasts could accurately predict the outcome of patients with invasive ductal carcinoma. This is because no other studies earlier have investigated the histologic features of tumor-stromal fibroblasts and their association with the outcome of patients with invasive ductal carcinoma of the breast. The results of this study clearly indicated that characteristic histologic features of the nuclei in

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tumor-stromal fibroblasts are significantly associated with tumor recurrence and the tumor-related death of patients with invasive ductal carcinoma of the breast. We named such tumor-stromal fibroblasts as atypical tumor-stromal fibroblasts.

METHODS

Cases

The participants of this study were 1042 consecutive patients with invasive ductal carcinoma of the breast who did not receive neoadjuvant therapy and were surgically treated at the National Cancer Center Hospital between January 2000 and December 2005 (almost the same case series as that used in our earlier study).[15] The invasive ductal carcinomas were diagnosed preoperatively using needle biopsy, aspiration cytology, a mammography, or ultrasonography. All the patients were Japanese women, ranging in age from 23 to 72 years (median, 55 y). All the patients had a solitary lesion; 498 patients were premenopausal and 544 patients were postmenopausal. A partial mastectomy had been performed in 458 patients, and a modified radical mastectomy had been performed in 584 patients. Level I and level II axillary lymph node dissection had been performed in all the patients, and a level III axillary lymph node dissection had been performed in some of the patients with invasive ductal carcinoma.

Of the 1042 patients, 873 received adjuvant therapy, consisting of chemotherapy in 218 patients, endocrine therapy in 281 patients, and chemoendocrine therapy in 374 patients. The chemotherapy regimens used were anthracycline based with or without taxane and nonanthracycline based, and the endocrine therapy regimens consisted of tamoxifen with or without a gonadotropin-releasing hormone agonist, tamoxifen, with or without an aromatase inhibitor, an aromatase inhibitor alone, or a gonadotropin-releasing hormone agonist alone. No cases of inflammatory breast cancer were included in this series. All the tumors were classified according to the pathologic UICC-TNM (pTNM) classification.[24] The protocol of this study (20–112) was reviewed by the Institutional Review Board of the National Cancer Center.

For the pathologic examination, the surgically resected specimens were fixed in 10% formalin, and the size and gross appearance of the tumors were recorded. The tumor size was confirmed by comparison with the tumor size on the histologic slides.

Histologic Examination

Serial sections of each tumor area were cut from paraffin blocks. One section from each tumor was stained with hematoxylin and eosin and was examined histologically to confirm diagnosis, and another section was subjected to immunohistochemistry. The following 9 histologic factors were evaluated: (1) invasive tumor size ($\leq$20 mm, $>$20 to $\leq$50 mm, $>$50 mm), (2) histologic grade (1, 2, 3),[4] (3) tumor necrosis (absent, present),[6] (4) fibrotic focus (absent, fibrotic focus diameter $\leq$8 mm, fibrotic focus diameter $>$8 mm) (Fig. 1),[8,10,11] (5) grading system for lymph vessel tumor emboli,[13,16] (6) blood vessel invasion (absent, present), (7) adipose tissue invasion (absent, present), (8) skin invasion (absent, present), and (9) muscle invasion (absent, present).

As we have already reported that the characteristic cytoplasmic features or nuclear features of tumor-stromal fibroblasts in extrahepatic bile duct carcinomas are closely associated with the outcome of patients with extrahepatic bile duct carcinoma,[12] we examined whether tumor-stromal fibroblasts with characteristic cytoplasmic features or nuclear features could also be identified inside or outside of fibrotic foci in invasive ductal carcinomas (Fig. 2). We observed a small number of tumor-stromal fibroblasts with characteristic nuclear features existing inside and outside of fibrotic foci (Figs. 3, 4) and named them atypical tumor-stromal fibroblasts. The characteristic nuclear histologic features of atypical tumor-stromal fibroblasts are listed in Table 1. We then examined the presence or absence of atypical tumor-stromal fibroblasts in the tumor stroma inside and outside of fibrotic foci in invasive ductal carcinoma (Fig. 2). We classified the invasive ductal carcinomas into 4 types according to the presence or absence of fibrotic foci and the presence or absence of atypical tumor-stromal fibroblasts (Table 1). The presence of atypical tumor-stromal fibroblasts was defined based on the presence of 1 or more atypical tumor-stromal fibroblasts in the tumor stroma inside and outside of the fibrotic foci in invasive ductal carcinoma. We avoided a decision regarding the presence or absence of atypical tumor-stromal fibroblasts in the following situations while examining the presence or absence of atypical tumor-stromal fibroblasts in the tumor stroma: (1) the presence of atypical tumor-stromal fibroblast-like cells that were difficult to differentiate from the surrounding invasive tumor cells are

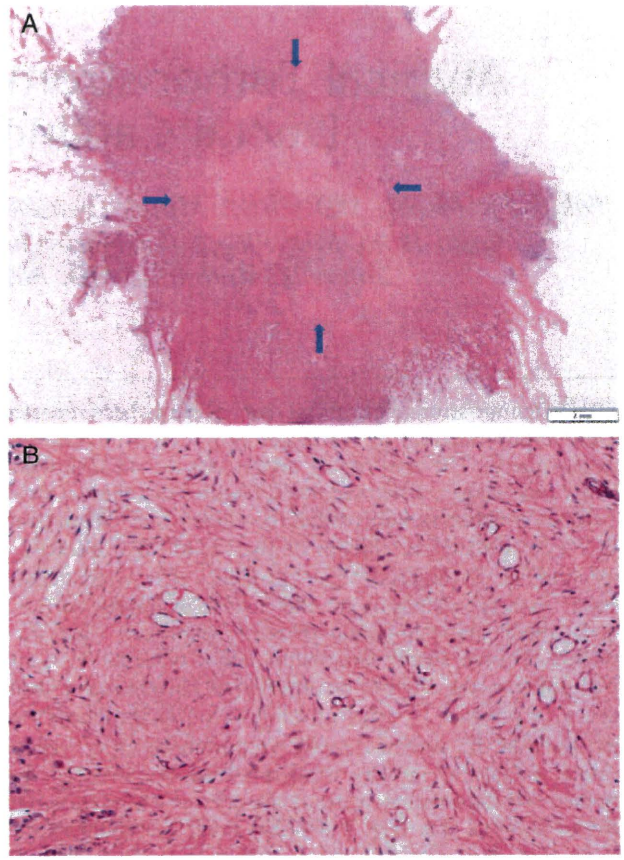

FIGURE 1. Invasive ductal carcinomas with fibrotic foci (A, B). A, A fibrotic focus measuring 7.8 × 5.6 mm is visible within the tumor (panoramic view, arrows). The fibrotic focus shows a scar-like feature, and is surrounded by invasive ductal carcinoma cells. B, The fibrotic focus area consists mainly of fibroblasts arranged in a storiform pattern. full color

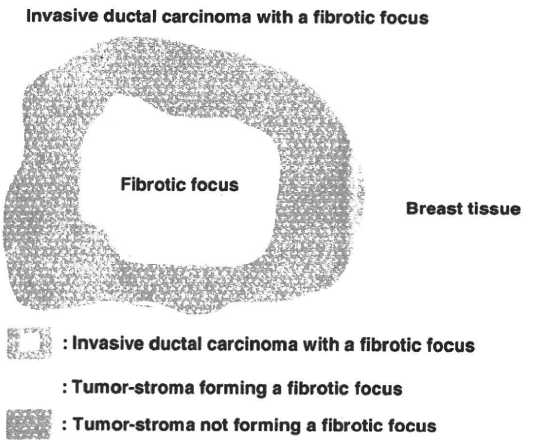

FIGURE 2. Schematic illustration of an invasive ductal carcinoma with a fibrotic focus.

present among invasive tumor cells; (2) the presence of atypical tumor-stromal fibroblast-like cells with gland-like structures that could possibly represent endothelial cells; and (3) the presence of atypical tumor-stromal fibroblast-like cells within an area of severe inflammatory cell infiltration that could possibly represent macrophages. Although atypical tumor-stromal fibroblasts were occasionally distributed at random locations in the tumor stroma inside and outside of fibrotic foci, they tended to exist within the cellular area of the tumor-stromal fibroblasts.

Immunohistochemical staining for estrogen receptors, progesterone receptors, p53, and HER2 products was performed using an autoimmunostainer (Optimax Plus; BioGenex, San Ramon, CA). The antigen retrieval device for Optimax Plus was an autoclave, and each specimen was immersed in citrate buffer and incubated at 121°C for 10 minutes. Immunoperoxidase staining was performed using a labeled streptavidin-biotin staining kit (BioGenex) according to the instructions of the manufacturer. The antibodies used were the antiestrogen receptor mouse monoclonal antibody ER88 (BioGenex), the antiprogesterone receptor mouse monoclonal antibody PR88 (BioGenex), the anti-HER2 mouse monoclonal antibody CB11 (BioGenex), and the p53 mouse monoclonal antibody DO7 (Dako, Glostrup, Denmark). ER88, PR88, and CB11 were previously diluted, and DO7 was applied at a dilution of 1:100. After immunostaining, the sections were counterstained with hematoxylin. Sections of the invasive ductal carcinomas that were positive for estrogen receptor, progesterone receptor, HER2, and p53 were used each time as a positive control. As a negative control, the primary antibody was replaced with normal mouse immunoglobulin. Slides of the tumor cells immunostained for estrogen receptor, progesterone receptor, and p53 were scored using the Allred scoring system, as described earlier,[2,7,20] and the Allred scores for estrogen receptor, progesterone receptor, and p53 expression in the tumor cells were classified into the following 3 categories[14,15]: (1) Allred score for estrogen receptor in tumor cells (0 or 2, 3 to 6, and 7 or 8); (2) Allred score for progesterone receptor in tumor cells (0 or 2, 3 to 6, and 7 or 8); and (3) Allred scores for p53 in tumor cells (0 or 2 or 3, 4 to 6, and 7 or 8). The Allred score risk classification for p53 in tumor-stromal fibroblasts forming and not forming fibrotic foci was described in our earlier study.[16] The HER2 status of the tumor cells was semiquantitatively scored on a scale of 0 to 3 according to the level of HER2 protein expression,[29] and was classified into 3 categories: 0 or 1, 2, and 3. In addition, all types 2 and 4 invasive ductal carcinomas were immunohistochemically studied using monoclonal antibodies to keratins (AE1/3) to confirm that the atypical tumor-stromal fibroblasts were not modified invasive tumor cells, and fibroblasts that were negative for keratins were considered to be atypical tumor-stromal fibroblasts (Figs. 3, 4). We also performed immunohistochemical staining for α-smooth muscle actin for types 2 and 4 invasive ductal carcinomas to investigate whether atypical tumor-stromal fibroblasts are myofibroblasts (Figs. 3, 4), and the presence of atypical tumor-stromal fibroblasts stained positive for α-smooth muscle actin was observed in 60 (87%) of 69 types 2 and 4 invasive ductal carcinomas (type 2: 35 of 40 cases, 88% and type 4: 25 of 29 cases, 86%).

Patient Outcome and Statistical Analysis

Survival was evaluated using a median follow-up period of 78 months (range, 32 to 116 mo) until April 2010. Of the 1042 invasive ductal carcinoma patients, 868 patients were alive and well, 174 had developed tumor recurrences, and 81 had died of their disease. The tumor recurrence-free survival and overall survival periods were calculated using the time of surgery as the starting point. Tumor relapse was considered to have occurred whenever evidence of metastasis was found. The correlation analyses were performed using Fisher exact test.

We analyzed the outcome predictive power of the types of invasive ductal carcinomas, the 9 histologic factors, the Allred scores for estrogen receptor in tumor cells, the Allred scores for progesterone receptor in tumor cells, the Allred scores for p53 in tumor cells, the category of HER2 expression in tumor cells, the Allred score risk classification for p53 in tumor-stromal fibroblasts forming and not forming fibrotic foci,[15] adjuvant therapy (yes or no), age ($\leq$39 y and $>$39 y) and the UICC pathologic nodal status[24] for tumor recurrence, and tumor-related death in univariate analyses using the Cox proportional hazard regression model. The factors significantly associated with outcome in the univariate analyses were then entered together into the multivariate analyses using the Cox proportional hazards regression model according to nodal status. The case-wise and step-down method was applied until all the remaining factors were significant at a P value < 0.05. All the analyses were carried out using Statistica/Windows software (StatSoft, Tulsa, OK).

RESULTS

Factors Significantly Associated With the Types of Invasive Ductal Carcinoma

The types of invasive ductal carcinoma were significantly associated with the use of adjuvant therapy ($P = 0.002$), invasive tumor size ($P < 0.001$), histologic grade ($P < 0.001$), grading system for lymph vessel tumor emboli ($P = 0.004$), the presence of blood vessel invasion ($P < 0.001$), the UICC pathologic nodal status ($P < 0.001$), and the Allred score risk classification for p53 in tumor-stromal fibroblasts forming and not forming fibrotic foci (Table 2, Figs. 5A–D). Other factors, for example the Allred scores for estrogen receptor in tumor cells and the Allred scores for p53 expression in tumor cells, were not significantly associated with the types of invasive ductal carcinoma (data not shown).

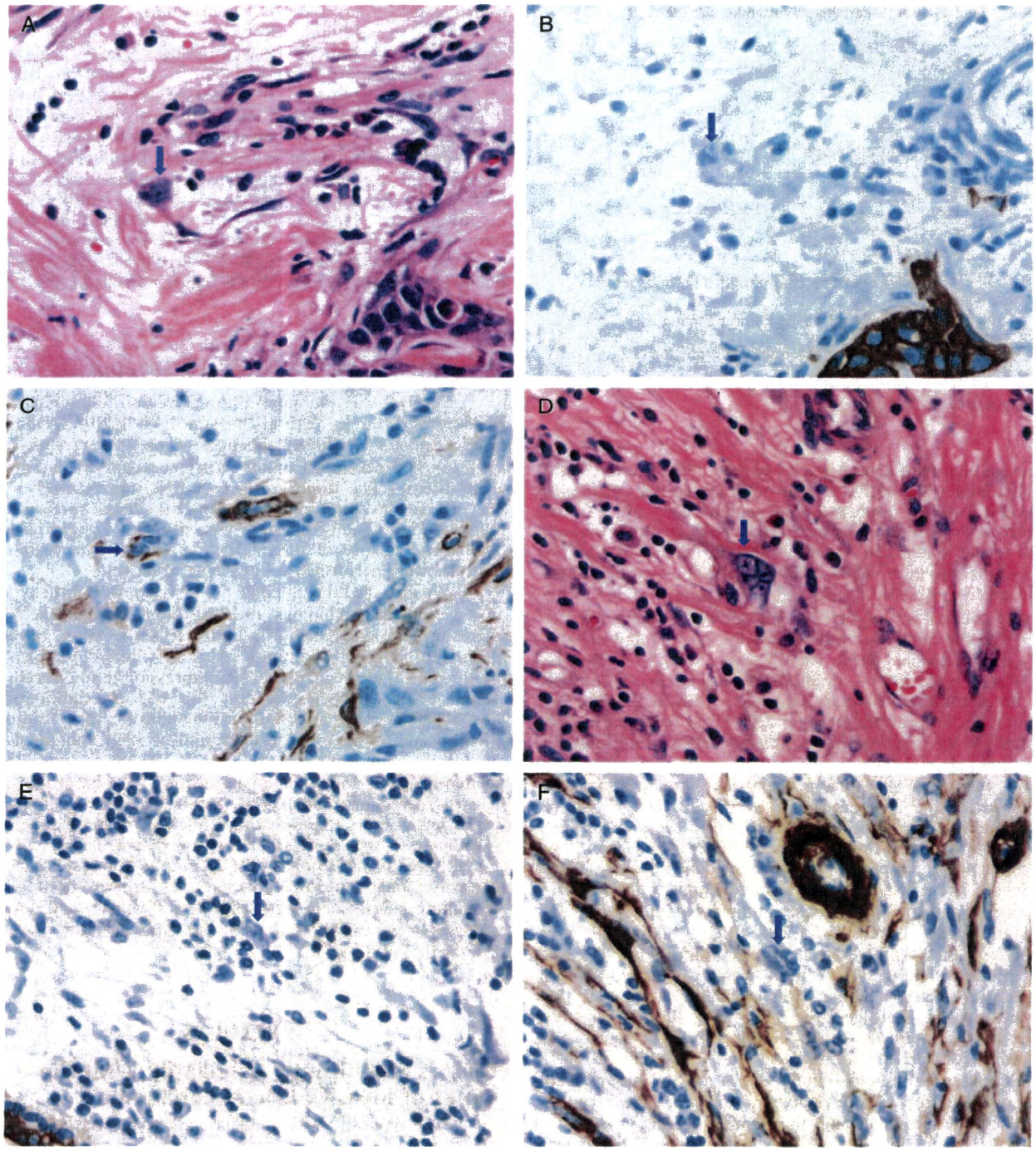

FIGURE 3. Immunohistochemical characteristics of atypical tumor-stromal fibroblasts inside fibrotic foci (A to F). A, One atypical tumor-stromal fibroblast with 1 bizarre and convoluted large nucleus with obvious nucleoli is visible (arrow). The fibroblast shows negative staining for keratins (B), but shows positive cytoplasmic staining for α-smooth muscle actin (C) (arrow). One tumor nest stained for keratins is also visible (right –lower corner) (B). D, One atypical tumor-stromal fibroblast containing 1 bizarre mulberry-like large nucleus with obvious nucleoli is visible (arrow) and shows negative staining both for keratins (E) and α-smooth muscle actin (F) (arrows). Duct epithelial cells showing positive staining for keratins are observed (left lower corner) (E).

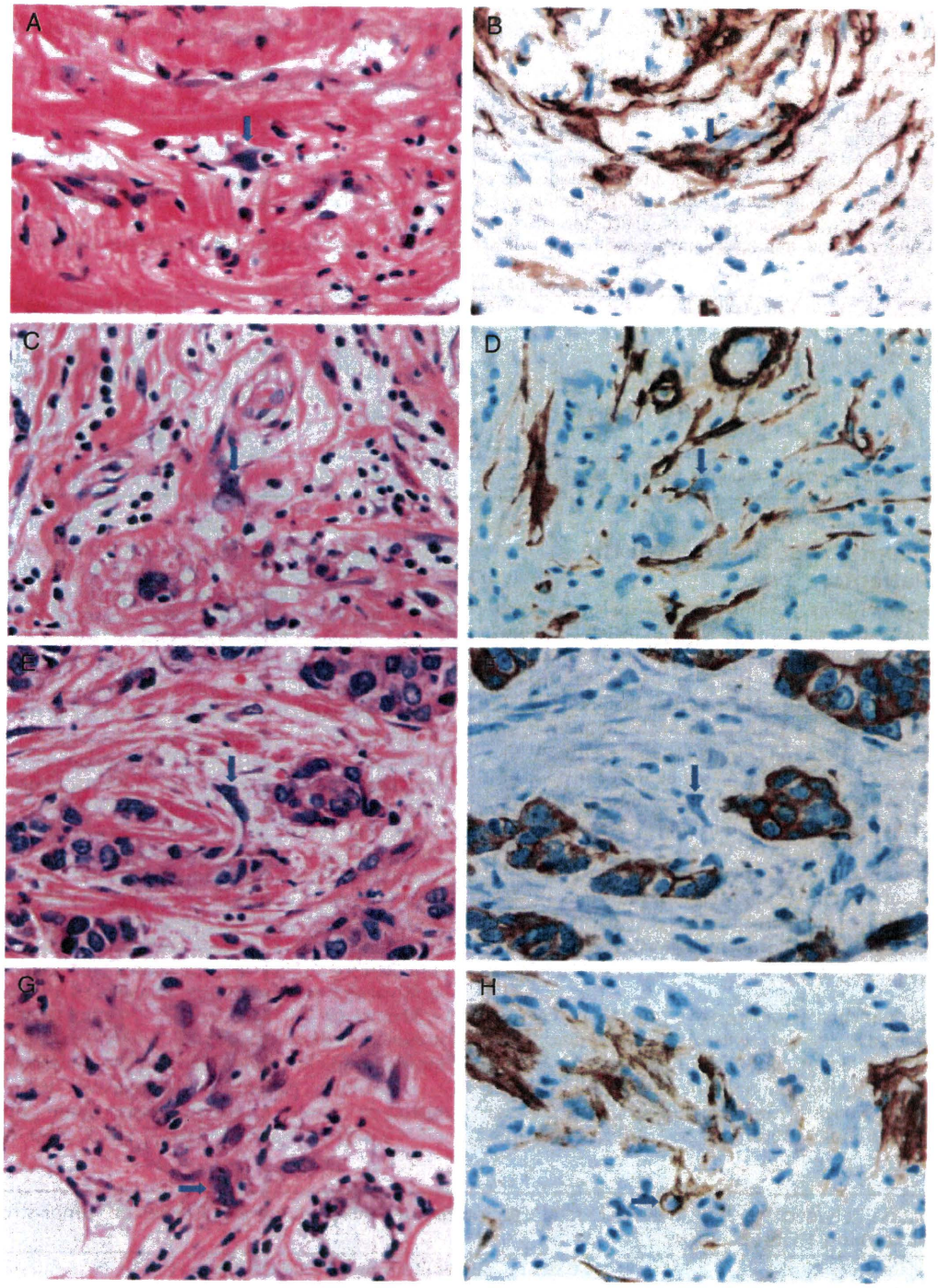

FIGURE 4. Histologic features of atypical tumor-stromal fibroblasts (A to H). A and B, One atypical tumor-stromal fibroblast with convoluted large nuclei with small nucleoli is visible (arrow) and shows positive staining for α-smooth muscle actin (arrow). C and D, One atypical tumor-stromal fibroblast with bizarre nucleus with obvious small nucleoli is visible (arrow) and shows positive staining for α-smooth muscle actin (arrow). E and F, One atypical tumor-stromal fibroblast with a large bizarre nucleus with obvious large nucleoli and coarsely granulated nuclear chromatins is visible among the tumor cells (arrow); the fibroblast exhibits negative staining for keratin (arrow), but tumor cells surrounding the fibroblast are positive for keratin. G and H, One atypical tumor-stromal fibroblast with 1 large bizarre nucleus and obvious small nucleoli is visible (arrow), and tumor-stromal fibroblasts with large nuclei are also visible in a scattered manner in the area surrounding the fibroblast (G). The fibroblast (arrow) and tumor-stromal fibroblasts are positive for α-smooth muscle actin (H).

TABLE 1. Characteristic Histologic Features of Atypical Tumor-stromal Fibroblasts and Types of Invasive Ductal Carcinomas of the Breast

Characteristic Histologic Features of Atypical Tumor-stromal Fibroblasts

The nucleus or nuclei of atypical tumor-stromal fibroblasts exhibit several characteristic histologic features as follows:
(1) The number of nuclei in an atypical tumor-stromal fibroblast is 1 or more
(2) The nuclear size of an atypical tumor-stromal fibroblast is 2 or more times larger than that of an ordinary tumor-stromal fibroblast
(3) The nuclear features of atypical tumor-stromal fibroblast include an irregular or convoluted shape, and also include various bizarre shapes
(4) Small-to-large-sized obvious nucleolus or nucleoli are seen in the nucleus or nucleoli of atypical tumor-stromal fibroblasts, and some atypical tumor-stromal fibroblasts show a coarsely granulated nuclear chromatin pattern
(5) Some atypical tumor-stromal fibroblasts may fuse with each other to produce atypical tumor-stromal fibroblasts with multiple nuclei

Criteria for Types of Invasive Ductal Carcinomas

Type	Fibrotic focus	Atypical tumor-stromal fibroblast not forming a fibrotic focus	Atypical tumor-stromal fibroblast forming a fibrotic focus
1	Absent	Absent	Not applicable
2	Absent	Present	Not applicable
3	Present	Not assessed	Absent
4	Present	Not assessed	Present

Present, 1 or more atypical tumor-stromal fibroblasts are present.

Factors Significantly Associated With the Outcome of Patients

Among the patients as a whole, type 4 invasive ductal carcinoma (Figs. 6A, B), a fibrotic focus diameter > 8 mm, lymph vessel tumor embolus grades 2 and 3, an intermediate-risk class and a high-risk class for p53 in tumor-stromal fibroblasts forming and not forming fibrotic foci, and UICC pN1, pN2, and pN3 categories had significantly higher hazard ratios for tumor recurrence and tumor-related death in the multivariate analyses (Table 3). An Allred score of 7 or 8 for progesterone receptors in the tumor cells had significantly lower hazard ratios for tumor recurrence and tumor-related death in multivariate analyses (Table 3). Type 3 invasive ductal carcinoma (Table 3, Fig. 6A), histologic grade 3 ($P = 0.032$), and the presence of blood vessel invasion ($P = 0.022$) had significantly higher hazard ratios for tumor recurrence, whereas the presence of skin invasion had a significantly higher hazard ratio for tumor-related death in the multivariate analyses ($P = 0.003$).

Among the patients with invasive ductal carcinoma without nodal metastasis, type 4 invasive ductal carcinoma, a fibrotic foci diameter > 8 mm, and a high-risk class for p53 in tumor-stromal fibroblasts forming and not forming fibrotic foci had significantly higher hazard ratios for tumor recurrence and tumor-related death in multivariate analyses (Table 4). Lymph vessel tumor embolus grades 2 ($P < 0.001$) and 3 ($P < 0.001$), histologic grades 2 ($P = 0.033$) and 3 ($P = 0.009$), and HER2 category 3 ($P = 0.044$) had significantly higher hazard ratios for tumor recurrence, and an Allred score of 7 or 8 for estrogen receptor in the tumor cells had a significantly lower hazard ratio for tumor-related death in multivariate analyses ($P = 0.008$).

Among patients with invasive ductal carcinoma with nodal metastases, type 4 invasive ductal carcinoma, the presence of blood vessel invasion, lymph vessel tumor embolus grade 3, UICC pN3 category, and an intermediate-risk class and a high-risk class for p53 in tumor-stromal fibroblasts forming and not forming fibrotic foci had significantly higher hazard ratios for tumor recurrence and tumor-related death in multivariate analyses (Table 5). An Allred score of 7 or 8 for progesterone receptors in tumor cells had significantly lower hazard ratios for tumor recurrence and tumor-related death in the multivariate analyses (Table 5). Type 2 invasive ductal carcinoma (Table 5) and lymph vessel tumor embolus grade 2 (Table 5) had significantly higher hazard ratios for tumor recurrence in multivariate analyses, and invasive tumor sizes of > 20 to ≤ 50 mm ($P = 0.003$) and > 50 mm ($P = 0.008$) and the presence of skin invasion ($P = 0.014$) had significantly higher hazard ratios for tumor death in the multivariate analyses.

Among patients with invasive ductal carcinoma of histologic grade 1, lymph vessel tumor embolus grades 1 ($P = 0.019$) and 2 ($P = 0.048$), UICC pN1 ($P = 0.018$), pN2

TABLE 2. Association Between Types of Invasive Ductal Carcinomas and Allred Score Risk Classes of Tumor-stromal Fibroblasts Forming and Not Forming a Fibrotic Focus

	Types of Invasive Ductal Carcinomas (%)				
	1	2	3	4	*P*
p53 Allred score risk classes of tumor-stromal fibroblasts forming and not forming a fibrotic focus					
Low risk	464 (75)	20 (54)	224 (66)	9 (31)	< 0.001
Intermediate risk	156 (25)	17 (46)	82 (24)	8 (28)	
High risk	0	0	34 (10)	12 (41)	
Total	620	37	340	29	

Type 1, invasive ductal carcinomas not having fibrotic foci and atypical tumor-stromal fibroblasts; type 2, invasive ductal carcinomas not having fibrotic foci, but having atypical tumor-stromal fibroblasts; type 3, invasive ductal carcinomas having fibrotic foci but not having atypical tumor-stromal fibroblasts within the fibrotic foci; type 4, invasive ductal carcinomas having fibrotic foci and atypical tumor-stromal fibroblasts within the fibrotic foci.

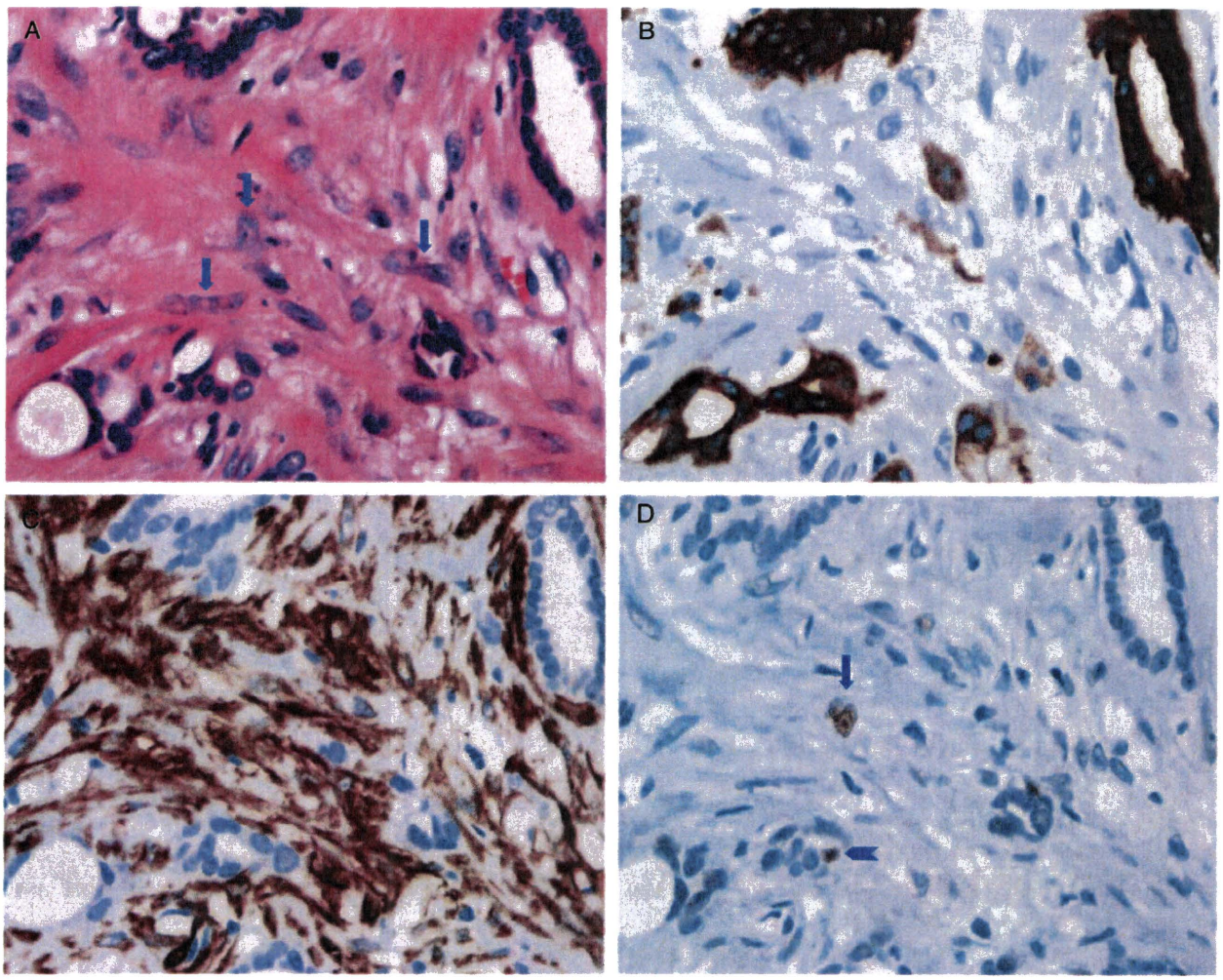

FIGURE 5. Nuclear staining for p53 in atypical tumor-stromal fibroblasts. A, Three atypical tumor-stromal fibroblasts with a large convoluted nucleus or bizarre nucleus (arrows) and tumor-stromal fibroblasts with large oval nuclei are visible within the tumor stroma. These atypical tumor-stromal fibroblasts are negative for keratin (B), but they are positive for smooth muscle actin (C). One of atypical tumor-stromal fibroblast shows a positive nuclear staining for p53 (arrow) and 1 tumor cell is also positive for p53 (arrowhead) (D).

($P = 0.004$), and pN3 categories ($P = 0.004$), and a high-risk class for p53 in tumor-stromal fibroblasts forming and not forming fibrotic foci had significantly higher hazard ratios for tumor recurrence in the multivariate analyses. Types 2 and 4 invasive ductal carcinomas had a marginally significant higher hazard ratio for tumor recurrence in the multivariate analysis ($P = 0.063$). As only 1 patient with invasive ductal carcinoma of histologic grade 1 died, we could not carry out a multivariate analysis for tumor-related death.

Among patients with invasive ductal carcinoma of histologic grade 2, type 4 invasive ductal carcinoma (Figs. 6C, D) and an intermediate-risk class and a high-risk class for p53 in tumor-stromal fibroblasts forming and not forming fibrotic foci had significantly higher hazard ratios for tumor recurrence and tumor-related death in the multivariate analyses (Table 6). Allred scores of 3 to 6 and an Allred score of 7 or 8 for estrogen receptors in tumor cells had significantly lower hazard ratios for tumor recurrence and tumor-related death in the multivariate analyses (Table 6). Fibrotic focus diameter > 8 mm ($P = 0.004$) and lymph vessel tumor embolus grades 2 ($P < 0.001$) and grades 3 ($P = 0.004$) had significantly higher hazard ratios for tumor recurrence in multivariate analyses, and the UICC pN1 ($P = 0.008$), pN2 ($P = 0.004$), and pN3 ($P < 0.001$) categories had significantly higher hazard ratios for tumor death in the multivariate analyses.

Among patients with invasive ductal carcinoma of histologic grade 3, type 4 invasive ductal carcinoma (Figs. 6E, F), lymph vessel tumor embolus grade 3, an intermediate-risk class and a high-risk class for p53 in tumor-stromal fibroblasts forming and not forming fibrotic foci, and UICC pN3 category had significantly higher hazard ratios for tumor recurrence and tumor-related death in the multivariate analyses (Table 7). Type 2 invasive ductal carcinoma (Table 7, Fig. 6E), lymph vessel tumor embolus grade 2 (Table 7), and the presence of blood vessel tumor embolus ($P = 0.002$) had significantly higher hazard ratios for tumor recurrence, and UICC pN1 and pN2

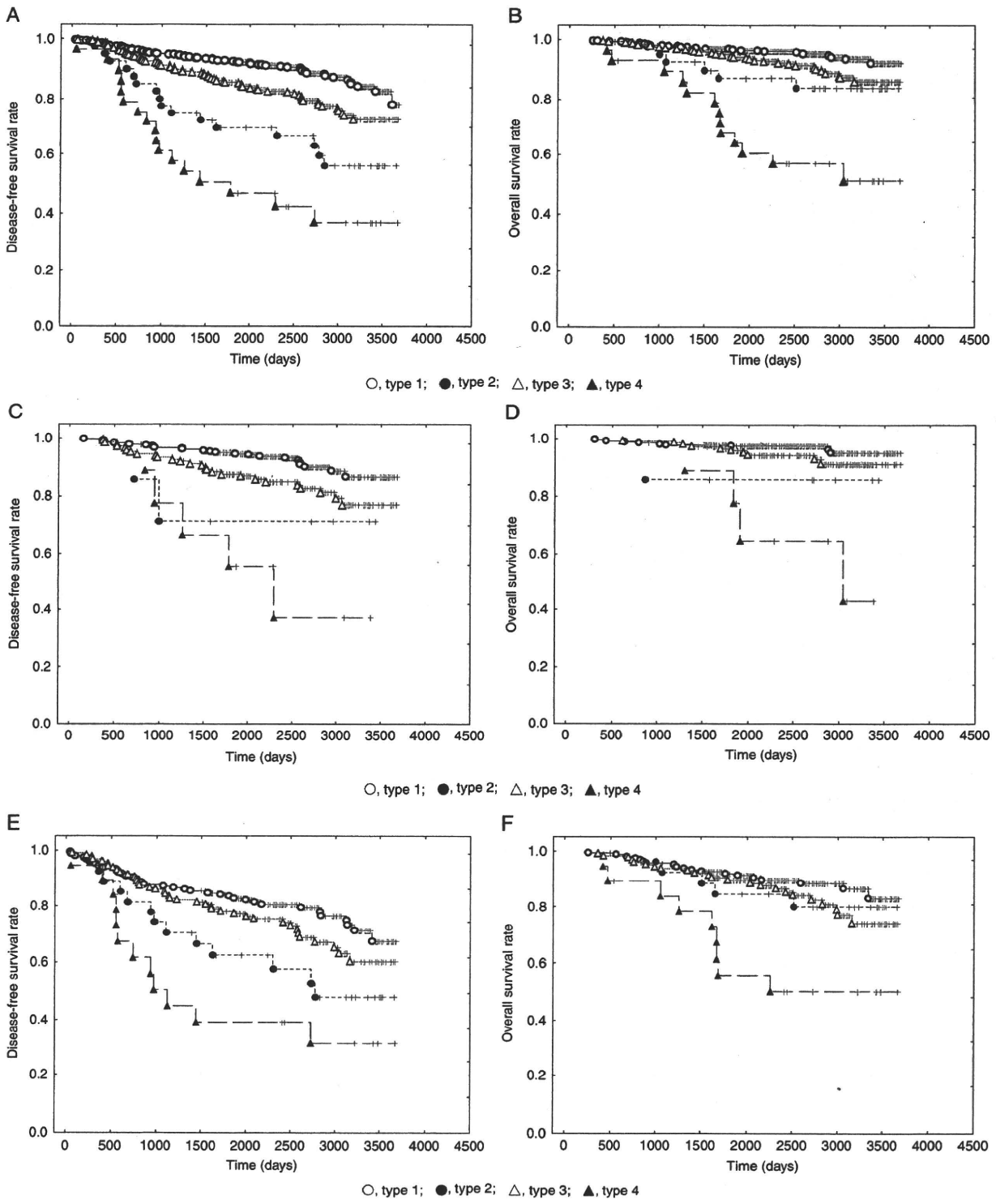

FIGURE 6. Disease-free survival curves and overall survival curves of invasive ductal carcinoma of patients overall (A, B), those of invasive ductal carcinoma patients of histologic grade 2 (C, D) and those of patients with invasive ductal carcinoma of histologic grade 3 (E, F) according to the type of invasive ductal carcinoma. Patients with types 2, 3, and 4 invasive ductal carcinoma have a shorter disease-free survival time and overall survival time than patients with type 1 invasive ductal carcinoma among invasive ductal carcinoma patients overall (A, B), patients with invasive ductal carcinoma of histologic grade 2 (C, D), and patients with invasive ductal carcinoma of histologic grade 3 (E, F).

TABLE 3. Multivariate Analyses for Tumor Recurrence and Tumor-related Death in Invasive Ductal Carcinoma patients as a Whole (n = 1042)

Types	Cases	TRR (%)	HR (95% CI)	*P*	MR (%)	HR (95% CI)	*P*
Types of invasive ductal carcinomas							
1	627	69 (11)	Referent		29 (5)	Referent	
2	40	16 (40)	2.4 (1.3-4.4)	0.005	6 (15)	1.5 (0.5-4.5)	0.481
3	346	72 (21)	1.4 (0.8-2.3)	0.219	33 (10)	1.4 (0.6-3.1)	0.432
4	29	17 (59)	2.8 (1.5-5.8)	0.007	13 (45)	3.1 (1.5-6.5)	0.002
Allred scores for progesterone receptors in tumor cells							
0 or 2	183	45 (25)	Referent		23 (13)	Referent	
3 to 6	303	59 (20)	0.7 (0.4-1.1)	0.090	35 (12)	0.8 (0.4-1.6)	0.585
7 or 8	556	70 (13)	0.5 (0.3-0.9)	0.009	23 (4)	0.3 (0.2-0.6)	< 0.001
Fibrotic focus, diameter							
Absent	667	85 (13)	Referent		35 (5)	Referent	
≤ 8 mm	221	37 (17)	Referent		14 (6)	Referent	
> 8 mm	154	52 (33)	1.8 (1.2-2.7)	0.003	32 (21)	1.8 (1.0-3.2)	0.038
Grading system for lymph vessel tumor emboli							
Grade 0	666	71 (11)	Referent		28 (4)	Referent	
Grade 1	250	39 (16)	1.2 (0.8-1.8)	0.341	15 (6)	1.2 (0.6-2.4)	0.600
Grade 2	97	43 (44)	2.4 (1.6-3.8)	< 0.001	22 (23)	1.8 (1.0-3.2)	0.048
Grade 3	29	21 (72)	4.2 (2.2-6.3)	< 0.001	16 (55)	2.5 (1.2-5.2)	0.018
p53 Allred score risk classes of tumor-stromal fibroblasts forming and not forming a fibrotic focus							
Low risk	559	52 (9)	Referent		16 (3)	Referent	
Intermediate risk	173	21 (12)	2.3 (1.6-3.4)	< 0.001	5 (3)	4.8 (2.6-8.7)	< 0.001
High risk	294	97 (33)	3.4 (1.9-5.9)	< 0.001	57 (19)	6.5 (3.0-13.9)	< 0.001
UICC pN category							
pN0	591	52 (9)	Referent		13 (2)	Referent	
pN1	318	68 (21)	1.9 (1.2-2.8)	0.003	33 (10)	4.7 (2.3-9.7)	< 0.001
pN2	85	28 (33)	2.3 (1.3-4.1)	0.004	15 (18)	5.9 (2.6-13.8)	< 0.001
pN3	48	26 (15)	3.7 (2.1-6.6)	< 0.001	20 (25)	8.0 (3.4-18.8)	< 0.001

CI indicates confidence interval; HR, hazard rate; MR, mortality rate; TRR, tumor recurrence rate; type 1, invasive ductal carcinomas not having fibrotic foci and atypical tumor-stromal fibroblasts; type 2, invasive ductal carcinomas not having fibrotic foci but having atypical tumor-stromal fibroblasts; type 3, invasive ductal carcinomas having fibrotic foci but not having atypical tumor-stromal fibroblasts within the fibrotic foci; type 4, invasive ductal carcinomas having fibrotic foci and atypical tumor-stromal fibroblasts within the fibrotic foci.

categories had significantly higher hazard ratios for tumor-related death in the multivariate analyses (Table 7).

DISCUSSION

Although we have reported earlier that the biological characteristics of tumor-stromal fibroblasts are closely associated with the nodal metastasis or distant organ metastasis of invasive ductal carcinoma,[9,10] the specific histologic features of tumor-stromal fibroblasts associated with the outcome of patients with invasive ductal carcinoma have not been described. This study clearly showed that type 4 invasive ductal carcinoma had the highest biological malignant potential among the various classification of invasive ductal carcinomas. Furthermore, type 2

TABLE 4. Multivariate Analyses for Tumor Recurrence and Tumor-related Death in Invasive Ductal Carcinoma Patients Without Nodal Metastasis (n = 591)

	Tumor Recurrence		Tumor-related Death	
Factors	HR (95% CI)	*P*	HR (95% CI)	*P*
Types of invasive ductal carcinomas				
Type 1	Referent	—	Referent	—
Type 2	2.9 (0.9-8.8)	0.056	2.0 (0.2-17.5)	0.540
Type 3	0.9 (0.3-2.7)	0.945	0.7 (0.1-7.5)	0.789
Type 4	5.3 (2.1-13.7)	< 0.001	9.5 (1.8-51.2)	0.009
Fibrotic focus, diameter				
Absent	Referent	—	Referent	—
≤ 8 mm	Referent	—	Referent	—
> 8 mm	2.2 (1.1-4.4)	0.023	4.2 (1.2-14.1)	0.020
p53 Allred score risk classes of tumor-stromal fibroblasts forming and not forming a fibrotic focus				
Low risk	Referent	—	Referent	—
Intermediate risk	1.9 (0.6-6.2)	0.305	2.8 (0.1-55.7)	0.492
High risk	2.6 (1.5-4.6)	0.001	15.3 (2.9-79.6)	0.001

— indicates no significance in univariate analysis; CI, confidence interval; HR, hazard rate; type 1, invasive ductal carcinomas not having fibrotic foci and atypical tumor-stromal fibroblasts; type 2, invasive ductal carcinomas not having fibrotic foci but having atypical tumor-stromal fibroblasts; type 3, invasive ductal carcinomas having fibrotic foci but not having atypical tumor-stromal fibroblasts within the fibrotic foci; type 4, invasive ductal carcinomas having fibrotic foci and atypical tumor-stromal fibroblasts within the fibrotic foci.

TABLE 5. Multivariate Analyses for Tumor Recurrence and Tumor-related Death in Invasive Ductal Carcinoma Patients with Nodal Metastases (n = 451)

	Tumor Recurrence		Tumor-related Death	
Factors	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Types of invasive ductal carcinomas				
Type 1	Referent	—	Referent	—
Type 2	2.2 (1.1-4.5)	0.021	1.9 (0.6-6.1)	0.274
Type 3	1.1 (0.7-1.9)	0.716	0.8 (0.4-1.8)	0.589
Type 4	2.8 (1.4-5.5)	0.003	4.3 (2.1-9.0)	< 0.001
Allred scores for progesterone receptors in tumor cells				
0 or 2	Referent	—	Referent	—
3 to 6	0.6 (0.4-1.0)	0.050	0.7 (0.3-1.3)	0.243
7 or 8	0.5 (0.3-0.8)	0.009	0.4 (0.2-0.7)	< 0.001
Blood vessel invasion				
Absent	Referent	—	Referent	—
Present	1.6 (1.1-2.4)	0.024	2.0 (1.0-3.7)	0.045
Grading system for lymph vessel tumor emboli				
Grade 0	Referent	—	Referent	—
Grade 1	1.5 (0.9-2.4)	0.152	1.5 (1.7-3.3)	0.333
Grade 2	2.1 (1.4-3.2)	< 0.001	1.7 (0.8-3.5)	0.146
Grade 3	4.0 (2.3-7.1)	< 0.001	2.6 (1.1-6.3)	0.035
p53 Allred score risk classes of tumor-stromal fibroblasts forming and not forming a fibrotic focus				
Low risk	Referent	—	Referent	—
Intermediate risk	2.1 (1.4-3.3)	< 0.001	5.0 (2.8-9.1)	< 0.001
High risk	4.3 (2.4-7.5)	< 0.001	10.4 (5.0-22.0)	< 0.001
UICC pN category				
pN1	Referent	—	Referent	—
pN2	1.3 (0.8-2.1)	0.339	1.4 (0.7-2.8)	0.345
pN3	1.8 (1.1-2.9)	0.016	2.3 (1.3-4.1)	0.006

— indicates no significance in univariate analysis; CI, confidence interval; HR, hazard rate; pN, pathologic regional lymph node; pN1, 1 to 3 nodal metastases; pN2, 4 to 9 nodal metastases; pN3, 10 or more nodal metastases; type 1, invasive ductal carcinomas not having fibrotic foci and atypical tumor-stromal fibroblasts; type 2, invasive ductal carcinomas not having fibrotic foci but having atypical tumor-stromal fibroblasts; type 3, invasive ductal carcinomas having fibrotic foci but not having atypical tumor-stromal fibroblasts within the fibrotic foci; type 4, invasive ductal carcinomas having fibrotic foci and atypical tumor-stromal fibroblasts within the fibrotic foci.

invasive ductal carcinoma had a higher biological malignant potential than types 1 and 3 invasive ductal carcinomas. Types 4 and 2 invasive ductal carcinomas exhibited atypical tumor-stromal fibroblasts in the tumor stroma inside and outside of the fibrotic foci, respectively. Thus, the presence of atypical tumor-stromal fibroblasts within the tumor stroma of fibrotic foci and nonfibrotic foci is definitely a useful histologic feature for accurately predicting the degree of the malignant potential of invasive ductal carcinomas. Thus, a detailed histologic examination of the nuclei of tumor-stromal fibroblasts is likely to be useful for accurately predicting the degree of the malignant potential of invasive ductal carcinomas of the breast.

The numbers of patients with type 4 invasive ductal carcinoma and type 2 invasive ductal carcinoma were 29 (2.8%)

TABLE 6. Multivariate Analyses for Tumor Recurrence and Tumor-related Death in Histologic Grade 2 Invasive Ductal Carcinoma Patients (n = 439)

	Tumor Recurrence		Tumor-related Death	
Factors	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Types of invasive ductal carcinomas				
Type 1	Referent	—	Referent	—
Type 2	4.0 (0.7-22.0)	0.112	12.0 (0.6-237.3)	0.101
Type 3	1.3 (0.6-2.7)	0.451	1.1 (0.3-3.8)	0.937
Type 4	3.2 (1.1-9.2)	0.031	6.7 (1.1-40.2)	0.039
Allred scores for estrogen receptors in tumor cells				
0 or 2	Referent	—	Referent	—
3 to 6	0.2 (0.07-0.7)	0.007	0.1 (0.02-0.9)	0.049
7 or 8	0.3 (0.2-0.6)	0.001	0.1 (0.03-0.4)	0.001
p53 Allred score risk classes of tumor-stromal fibroblasts forming and not forming a fibrotic focus				
Low risk	Referent	—	Referent	—
Intermediate risk	2.6 (1.5-4.6)	< 0.001	8.4 (2.6-27.3)	< 0.001
High risk	3.4 (1.2-9.9)	0.021	8.7 (1.5-51.1)	0.016

— indicates no significance in univariate analysis; CI, confidence interval; HR, hazard rate; type 1, invasive ductal carcinomas not having fibrotic foci and atypical tumor-stromal fibroblasts; type 2, invasive ductal carcinomas not having fibrotic foci but having atypical tumor-stromal fibroblasts; type 3, invasive ductal carcinomas having fibrotic foci but not having atypical tumor-stromal fibroblasts within the fibrotic foci; type 4, invasive ductal carcinomas having fibrotic foci and atypical tumor-stromal fibroblasts within the fibrotic foci.

TABLE 7. Multivariate Analyses for Tumor Recurrence and Tumor-related Death in Histologic Grade 3 Invasive Ductal Carcinoma Patients (n = 341)

	Tumor Recurrence		Tumor-related Death	
Factors	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Types of invasive ductal carcinomas				
Type 1	Referent	—	Referent	—
Type 2	2.0 (1.0-4.1)	0.049	1.0 (0.3-3.7)	0.985
Type 3	1.1 (0.6-1.9)	0.877	1.1 (0.4-2.5)	0.911
Type 4	2.2 (1.1-4.4)	0.023	3.1 (1.4-7.1)	0.007
Grading system for lymph vessel tumor emboli				
Grade 0	Referent	—	Referent	—
Grade 1	0.8 (0.4-1.5)	0.532	0.9 (0.4-2.2)	0.896
Grade 2	2.8 (1.7-4.4)	< 0.001	1.3 (0.6-3.1)	0.500
Grade 3	5.6 (3.0-10.3)	< 0.001	2.8 (1.4-5.7)	0.004
p53 Allred score risk classes of tumor-stromal fibroblasts forming and not forming a fibrotic focus				
Low risk	Referent	—	Referent	—
Intermediate risk	1.9 (1.2-3.0)	0.006	4.4 (2.0-9.4)	< 0.001
High risk	2.8 (1.5-5.3)	0.001	7.7 (3.2-18.5)	< 0.001
UICC pN category				
pN0	Referent	—	Referent	—
pN1	1.5 (0.9-3.6)	0.125	4.0 (1.7-9.2)	0.001
pN2	1.7 (0.8-3.7)	0.174	5.0 (1.9-13.3)	0.001
pN3	2.4 (1.3-4.2)	0.003	10.8 (4.4-27.1)	< 0.001

— indicates no significance in univariate analysis; CI, confidence interval; HR, hazard rate; type 1, invasive ductal carcinomas not having fibrotic foci and atypical tumor-stromal fibroblasts; type 2, invasive ductal carcinomas not having fibrotic foci but having atypical tumor-stromal fibroblasts; type 3, invasive ductal carcinomas having fibrotic foci but not having atypical tumor-stromal fibroblasts within the fibrotic foci; type 4, invasive ductal carcinomas having fibrotic foci and atypical tumor-stromal fibroblasts within the fibrotic foci.

and 40 (3.8%), respectively. As these 2 types of invasive ductal carcinoma accounted for a very small proportion of the invasive ductal carcinomas, the type of invasive ductal carcinomas may be of limited usefulness as a prognostic histologic feature of invasive ductal carcinomas. However, this observation also suggests that many patients with type 1 or type 3 invasive ductal carcinoma had a better prognosis than those with type 4 or type 2. Thus, the type of invasive ductal carcinoma may actually be very useful for the histologic classification of patients with invasive ductal carcinoma. This study is the first to report the prognostic significance of atypical tumor-stromal fibroblasts in invasive ductal carcinomas in an analysis of a large number of patients with invasive ductal carcinoma of the breast.

A significant association between the presence of atypical tumor-stromal fibroblasts within the tumor stroma inside and outside of fibrotic foci and the p53 Allred score risk classes of tumor-stromal fibroblasts forming and not forming a fibrotic focus was observed in this study. This finding clearly indicates that the presence of atypical nuclear features is closely associated with p53 expression in tumor-stromal fibroblasts. p53 mutations in tumor-stromal fibroblasts are relatively common among primary breast cancers and have been reported to exert a positive effect on cancer growth.[19,22] Nevertheless, some studies have not reported any p53 mutations in the tumor-stroma of breast cancers.[1,5] Although the presence or absence of p53 gene abnormalities in tumor-stromal fibroblasts remains controversial, p53 gene abnormalities or specific reactive changes in p53 immunoreactivity in tumor-stromal fibroblasts produced by tumor cell-stromal cell interactions inside and outside of fibrotic foci probably lead to the expression of p53 in tumor-stromal fibroblasts. Consequently, some tumor-stromal fibroblasts expressing p53 inside and outside of fibrotic foci probably transform into atypical tumor-stromal fibroblasts. Furthermore, as many atypical tumor-stromal fibroblasts were also stained for smooth muscle actin in this study, one can conclude that many of the atypical tumor-stromal fibroblasts have biological characteristics of myofibroblasts.[18,27] Thus, these atypical tumor-stromal fibroblasts likely play important roles in the tumor progression of invasive ductal carcinomas of the breast.

In conclusion, this is the first study to clearly show definite histologic features of tumor-stromal fibroblasts that are closely associated with the outcome of patients with invasive ductal carcinoma of the breast. Indeed, while routine pathologic examinations of atypical tumor-stromal fibroblasts within the tumor stroma would require a careful examination, the resulting information would enable pathologists or clinicians to evaluate the malignant potential of invasive ductal carcinomas of the breast more precisely. In addition, the presence of atypical tumor-stromal fibroblasts may also be a useful outcome predictor for patients with invasive ductal carcinoma who have been classified according to phenotypic classifications, that is, luminal A or luminal B, based on the hormone receptor status or HER2 status of the tumors.

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Original contribution

Prognostic significance of mitotic figures in metastatic mammary ductal carcinoma to the lymph nodes☆,☆☆

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Summary We previously reported that the number of mitotic figures in metastatic mammary carcinoma to the lymph nodes accurately predicted the outcome of patients with invasive ductal carcinoma with nodal metastasis. To confirm these previous findings, the present study investigated the number of mitotic figures and other histologic characteristics in metastatic mammary carcinoma to the lymph nodes and their associations with patient outcome according to nodal status and the histologic grade of primary invasive ductal carcinomas in a different series of 1039 patients with invasive ductal carcinoma. Multivariate analyses examining well-known clinicopathologic factors, the number of mitotic figures in the primary invasive ductal carcinomas, the grading system for lymph vessel tumor emboli, the p53 Allred score risk classes of tumor-stromal fibroblasts forming and not forming a fibrotic focus, and 9 histologic features of metastatic mammary carcinoma to the lymph nodes were performed. The presence of 6 or more mitotic figures in metastatic mammary carcinoma to the lymph nodes significantly increased the hazard ratios for tumor recurrence and tumor-related death among patients with invasive ductal carcinoma as a whole, those with nodal metastasis, and those with a histologic grade of 2 or 3. The presence of 6 or more mitotic figures in metastatic mammary carcinoma to the lymph nodes also significantly increased the hazard ratio for tumor recurrence among patients with histologic grade 1 invasive ductal carcinoma. In conclusion, this study clearly confirmed the excellent outcome predictive power of the number of mitotic figures in metastatic mammary carcinoma to the lymph nodes.

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