FIGURE 3. Disease-free survival curves and overall survival curves of invasive ductal carcinoma patients with the UICC pTNM stage I (A and B) and luminal-A subtype invasive ductal carcinoma patients (C–H). A and B, Patients with invasive ductal carcinoma with fibrotic foci dimension >8 mm have shorter disease-free and overall survival times than patients without fibrotic foci and those with fibrotic foci <8 mm. IDC, invasive ductal carcinoma; FF, fibrotic foci. C and D, The disease-free and overall survival curves decreased significantly according to the grade of lymph vessel tumor embolus. Grade, lymph vessel tumor embolus grade. E and F, The disease-free and overall survival curves decreased significantly according to the number of mitotic figures in metastatic carcinoma to the lymph nodes. Node negative, no nodal metastasis; node positive, nodal metastases; mitotic figures, number of mitotic figures in metastatic carcinoma to the lymph nodes. G and H, Patients with types 2, 3, and 4 invasive ductal carcinoma have shorter disease-free and overall survival times than patients with type 1 invasive ductal carcinoma.

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TABLE 4. Multivariate Analyses for Tumor Recurrence and Tumor-Related Death in Patients With Invasive Ductal Carcinoma According to Biological Subtype

	Cases	Tumor Recurrence			Tumor-Related Death		
		Cases (%)	HR 95% CI	*P*	Cases (%)	HR 95% CI	*P*
Luminal A-subtype Patients (n = 658)							
Blood vessel invasion							
Absent	552	62 (11)	Referent		25 (5)	Referent	
Present	104	27 (26)	2.2 1.4-3.6	0.002	16 (15)	2.3 1.2-4.4	0.015
Grading system for lymph vessel tumor emboli							
Grade 0	425	36 (9)	Referent		17 (4)	Referent	
Grade 1	169	25 (15)	1.5 0.9-2.5	0.141	9 (5)	1.1 0.5-2.5	0.885
Grade 2	52	18 (35)	3.4 1.9-6.0	< 0.001	7 (14)	0.9 0.3-3.3	0.898
Grade 3	12	10 (83)	6.5 3.0-14.0	< 0.001	8 (66)	4.7 2.0-11.4	< 0.001
No. mitotic figures in metastatic carcinoma to lymph nodes							
n0	383	24 (6)	Referent		6 (2)	Referent	
≤5	200	27 (14)	Referent		8 (4)	Referent	
> 5	75	38 (51)	3.5 2.1-5.8	< 0.001	27 (36)	6.6 3.1-14.2	< 0.001
Types of invasive ductal carcinoma							
Type 1	409	38 (9)	Referent		16 (4)	Referent	
Type 2	22	9 (41)	1.9 0.8-4.3	0.152	5 (23)	1.6 0.4-5.7	0.470
Type 3	213	34 (16)	0.9 0.5-1.7	0.679	14 (7)	0.8 0.3-2.1	0.583
Type 4	14	8 (57)	2.4 1.0-5.6	0.045	6 (43)	3.9 1.5-9.9	0.005
UICC pN category							
pN0	383	24 (6)	Referent		6 (2)	Referent	
pN1	199	38 (19)	1.7 0.9-3.1	0.104	21 (11)	2.6 1.2-5.8	0.019
pN2	50	11 (22)	1.1 0.4-2.7	0.864	4 (8)	1.1 0.2-5.3	0.879
pN3	26	16 (62)	2.3 1.2-4.2	0.008	10 (39)	3.9 1.4-10.5	0.008
Luminal B-subtype Patients (n = 88)							
Types of invasive ductal carcinoma							
Type 1	51	8 (16)	Referent		3 (6)	Referent	
Type 2	6	4 (67)	8.6 2.4-30.7	0.001	3 (50)	21.2 2.5-184.7	0.006
Type 3	28	13 (46)	4.3 1.7-11.0	0.002	7 (25)	1.4 0.1-15.4	0.780
Type 4	3	2 (67)	13.2 2.5-68.2	0.002	2 (67)	150.9 1.1-2048.8	0.046
Equivocal HER2-subtype Patients (n = 182)							
Grading system for lymph vessel tumor emboli							
Grade 0	109	10 (9)	Referent		2 (2)	Referent	
Grade 1	49	10 (20)	1.7 0.6-4.5	0.299	5 (10)	7.7 1.4-42.9	0.020
Grade 2	18	10 (56)	6.8 2.1-22.6	0.002	5 (28)	14.3 2.6-77.9	0.002
Grade 3	6	2 (33)	3.7 0.9-16.1	0.079	2 (33)	16.8 2.2-127.6	0.006
Invasive tumor size (mm)							
≤20	84	6 (7)	Referent		2 (2)	Referent	
> 20-< 50	93	24 (26)	4.5 0.7-28.4	0.114	10 (11)	1.8 0.3-9.7	0.516
> 50	5	2 (40)	3.2 1.1-9.5	0.036	2 (40)	4.8 1.2-36.8	0.029
Types of invasive ductal carcinoma							
Type 1	95	13 (14)	Referent		6 (6)	Referent	
Type 2	6	2 (33)	2.7 0.5-15.7	0.265	0	Referent	
Type 3	75	13 (17)	0.8 0.4-2.0	0.697	6 (7)	1.3 0.4-4.5	0.692

TABLE 4. (*continued*)

	Cases	Tumor Recurrence			Tumor-Related Death		
		Cases (%)	HR 95% CI	*P*	Cases (%)	HR 95% CI	*P*
Type 4	6	4 (67)	11.9 3.1-44.8	< 0.001	2 (33)	7.6 1.5-38.0	0.013
Triple-negative Patients (n = 75)							
Grading system for lymph vessel tumor emboli							
Grade 0	51	6 (12)	Referent		2 (4)	Referent	
Grade 1	8	1 (13)	0.8 0.1-6.7	0.830	0	Referent	
Grade 2	11	10 (90)	19.1 3.6-101.2	< 0.001	7 (64)	24.1 4.7-122.8	< 0.001
Grade 3	5	4 (80)	65.3 5.9-722.6	< 0.001	3 (60)	32.6 5.2-209.0	< 0.001

CI indicates confidence interval; HR, hazard ratio; n0, no nodal metastasis; pN, pathologic regional lymph node; pN0, no nodal metastasis; pN1, 1 to 3 nodal metastases; pN2, 4 to 9 nodal metastases; pN3, 10 or more nodal metastases.

for tumor recurrence, and the UICC pN2 category ($P = 0.019$), a fibrotic focus diameter 8 mm ($P < 0.001$), and an invasive tumor size > 50 mm ($P = 0.047$) had significantly higher hazard ratio for tumor-related death in a multivariate analysis.

Among patients with HER2-subtype invasive ductal carcinoma (n = 39), the presence of blood vessel invasion ($P = 0.009$) and the UICC pN3 category ($P = 0.007$) had significantly higher hazard ratio for tumor recurrence in a multivariate analysis. As only 7 patients died as a result of their disease, a multivariate analysis for tumor-related death could not be performed in this patient series.

Among the patients with equivocal HER2 invasive ductal carcinoma, lymph vessel tumor embolus grade 2, invasive tumor size > 50 mm, and type 4 invasive ductal carcinoma had significantly higher hazard ratios for tumor recurrence and tumor-related death in multivariate analyses (Table 4). Lymph vessel embolus grades 1 and 3 (Table 4) and histologic grade 3 ($P = 0.010$) had significantly higher hazard ratio for tumor-related death in a multivariate analysis. Among the patients with triple-negative invasive ductal carcinoma, lymph vessel tumor embolus grades 2 and 3 had significantly higher hazard ratios for tumor recurrence and tumor-related death in multivariate analyses (Table 4).

DISCUSSION

The histologic factors that significantly increased the hazard ratios for tumor recurrence or tumor-related death are shown in Table 5. Histologic factors that significantly increased hazard ratios for both tumor recurrence and tumor-related death were evaluated as A rank predictors, those that significantly increased the hazard ratio only for tumor-related death were evaluated as B rank predictors, those that significantly increased the hazard ratio only for tumor recurrence were evaluated as C rank predictors, and those that failed to significantly increase the hazard ratio for either tumor recurrence or tumor-related death were evaluated as D rank predictors.

The 20 histologic factors were then ranked in decreasing order of their contribution to the accurate prediction of tumor recurrence or tumor-related death according to various tumor statuses (Table 5). Among them, type 4 invasive ductal carcinoma was evaluated as A rank predictors in 7 of the tumor status classifications (A7), followed by lymph vessel tumor embolus grades 3 and 2 (A6), blood vessel invasion (A4), > 5 mitotic figures in metastatic carcinoma to the lymph nodes (A4), a fibrotic focus diameter > 8 mm (A3), histologic grade 3 (A2), UICC pN3 category (A2), invasive tumor size > 50 mm (A1), type 2 invasive ductal carcinoma (A1), HER2 category 3 (A1), and an Allred score of 7 or 8 for progesterone receptors in tumor cells (A1). These 12 histologic factors were also evaluated as B or C rank predictors in other tumor status classifications in which they were not identified as A rank predictors. Thus, these 12 histologic factors are likely to be very important histologic outcome predictors for patients with invasive ductal carcinoma of the breast.

Of note, only C rank predictors (blood vessel invasion and UICC pN3 category) were identified among these 12 histologic factors for patients with HER2-subtype invasive ductal carcinoma. This result may reflect the relatively small number of patients with HER2-subtype invasive ductal carcinoma in this study (39 patients, 4%), although this study included 1042 patients with invasive ductal carcinoma of the breast. Thus, a multi-institutional case study may be needed to clarify the optimal histologic predictors of outcome for patients with HER2-subtype invasive ductal carcinoma.

Although an absolute histologic predictor of outcome applicable to all patients with invasive ductal carcinoma was not identified in this study, appropriate histologic predictors of outcome were identified for various tumor statuses. We listed only the A rank predictors for each tumor status in Table 6. These factors are the core histologic predictors of outcome for patients with specific invasive ductal carcinoma tumor statuses

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TABLE 5. Ranking of 18 Histologic Factors According to Outcome Predictive Power

	Rank	Overall (n = 1042)	N0 (n = 591)	N+ (n = 451)	Stage I (n = 363)	Stage II (n = 487)	Stage III (n = 192)	Luminal A (n = 658)	Luminal B (n = 88)	HER2 (n = 39)	eHER2 (n = 182)	TN (n = 75)
Type 4	A7; B0 C0; D4	A	A	A	D	A	D	A	A	D	A	D
Ly grade 3	A6; B1 C3; D1	A	C	A	C	A	A	A	C	D	B	A
Ly grade 2	A6; B0 C4; D1	A	C	A	C	A	A	C	C	D	A	A
BV invasion	A4; B0 C1; D6	A	D	A	D	A	D	A	D	C	D	D
> 5 MF, met ca to LN	A4; B0 C0; D6	A	NA	A	D	A	D	A	D	D	D	D
FF diameter > 8 mm	A3; B1 C2; D5	A	C	D	A	D	A	C	B	D	D	D
Histologic grade 3	A2; B1 C0; D7	A	C	D	A	D	D	D	D	D	B	D
pN3	A2; B0 C2; D4	D	NA	D	NA	NA	A	A	C	C	D	D
Tumor size > 50 mm	A1; B1 C0; D8	D	D	D	NA	D	D	D	B	D	A	D
Type 2	A1; B0 C4; D6	C	C	C	D	C	D	D	A	D	D	D
HER2 category 3	A1; B0 C2; D3	C	C	D	D	D	A	NA	NA	NA	NA	NA
PR Allred 7 or 8	A1; B0 C0; D6	A	D	D	D	D	D	NA	NA	NA	D	NA
Ly grade 1	A0; B2 C0; D9	D	D	D	D	B	D	D	D	D	B	D
MAI of > 20 in PIT	A0; B2 C0; D9	D	B	D	D	D	B	D	D	D	D	D
Histologic grade 2	A0; B1 C2; D8	B	C	D	C	D	D	D	D	D	D	D
pN2	A0; B1 C0; D7	D	NA	D	NA	NA	D	D	B	D	D	D
Skin invasion	A0; B1 C0; D8	B	D	D	NA	NA	D	D	D	D	D	D
pN1	A0; B1 C0; D9	D	NA	D	D	D	D	B	D	D	D	D
ER Allred 7 or 8	A0; B0 C1; D6	D	D	D	C	D	D	NA	NA	NA	D	NA
Type 3	A0; B0 C1; D10	D	D	D	D	D	D	D	C	D	D	D

A rank of A was given for factors significantly associated with tumor recurrence and tumor-related death in multivariate analyses; a rank of B was given for factors that were significantly associated with tumor-related death in a multivariate analysis; a rank of C was given for factors that were significantly associated with tumor recurrence in a multivariate analysis; a rank of D was given for factors that were not associated with either tumor recurrence or tumor-related death in multivariate analyses or in univariate analyses; Overall, all the patients in this study; N0, patients without nodal metastasis; N+, patients with nodal metastasis; Stages I, II, and III, UICC pTNM stages I, II and III, respectively; Luminal A, luminal-A subtype invasive ductal carcinoma patients; Luminal B, luminal-B subtype invasive ductal carcinoma patients; HER2, HER2-subtype invasive ductal carcinoma patients; eHER2, equivocal HER2 subtype; TN, triple-negative invasive ductal carcinoma patients; Type 4, type 4 invasive ductal carcinoma; Ly grade, grading system for lymph vessel tumor emboli; Ly grade 3, grade 3 lymph vessel tumor emboli; Ly grade 2, grade 2 lymph vessel tumor emboli; BV, blood vessel; MF, mitotic figures; met, metastatic; ca, carcinoma; LN, lymph nodes; FF, fibrotic focus; pN3, UICC pN3 category; Tumor size, primary invasive tumor size; Type 2, type 2 invasive ductal carcinoma; PR, progesterone receptor; Allred, Allred score; Ly grade 1, grade 1 lymph vessel tumor emboli; MAI, mitotic activity index; PIT, primary invasive tumor; pN2, UICC pN2 category; pN1, UICC pN1 category; ER, estrogen receptor; Type 3, type 3 invasive ductal carcinoma; NA, not available.

and may enable pathologists or clinicians to predict the outcomes of many patients with invasive ductal carcinoma accurately. Among these factors, a fibrotic focus diameter > 8 mm, lymph vessel tumor embolus grades 2 and 3, types 2 and 4 invasive ductal carcinoma, and > 5 mitotic figures in metastatic carcinoma to the lymph nodes were histologic factors that we proposed.[12–16,18–20] Thus, many readers may believe that the reliabilities of these factors as outcome predictors are inferior to those of well-known histologic factors, such as the presence of blood vessel invasion, histologic grade 3, HER2 category 3, and UICC pN3 category. However, outcome predictive power of a fibrotic focus among patients with invasive ductal carcinoma without nodal metastasis or patients with early invasive ductal carcinoma has also been confirmed by other investigators.[3,6,23] We have also confirmed the outcome predictive powers of the grading system for lymph vessel tumor emboli and the presence of > 5 mitotic figures in metastatic carcinoma to the lymph nodes in different invasive ductal carcinoma patient groups.[15,16,18,20] Thus, the proposed histologic factors seem to be very useful as predictors of outcome

TABLE 6. Best histologic Factors for Predicting Outcome Among Patients With Invasive Ductal Carcinoma According to Tumor status

Lymph Node Status	
Lymph node-negative invasive ductal carcinoma	
Type 4 invasive ductal carcinoma	
Lymph node-positive invasive ductal carcinoma	
Blood vessel invasion	Lymph vessel tumor embolus grades 2 and 3
> 5 mitotic figures in metastatic carcinoma to lymph nodes	Type 4 invasive ductal carcinoma

UICC pTNM stage	
UICC pTNM stage I invasive ductal carcinoma	
Fibrotic foci diameter > 8 mm	Histologic grade 3
UICC pTNM stage II invasive ductal carcinoma	
Blood vessel invasion	Lymph vessel tumor embolus grades 2 and 3
> 5 mitotic figures in metastatic carcinoma to lymph nodes	Type 4 invasive ductal carcinoma
UICC pTNM stage III invasive ductal carcinoma	
Fibrotic foci diameter > 8 mm	HER2 category 3
Lymph vessel tumor embolus grades 2 and 3 and UICC pN3	Type 4 invasive ductal carcinoma

Carcinoma subtype	
Luminal A invasive ductal carcinoma	
Blood vessel invasion	Lymph vessel tumor embolus grade 3
> 5 mitotic figures in metastatic carcinoma to lymph nodes and UICC pN3	Type 4 invasive ductal carcinoma
Luminal B invasive ductal carcinoma	
Types 2 and 4 invasive ductal carcinoma	
Equivocal HER2 invasive ductal carcinoma	
Invasive tumor size > 50 mm	Lymph vessel tumor embolus grade 2
Type 4 invasive ductal carcinoma	
Triple-negative invasive ductal carcinoma	
Lymph vessel tumor embolus grades 2 and 3	

among patients with invasive ductal carcinoma of the breast.

This study clearly demonstrated that the outcome predictive power of the invasive tumor size is inferior to that of a fibrotic focus, blood vessel invasion, the grading system for lymph vessel tumor emboli, histologic grade, or the type of invasive ductal carcinoma. The outcome predictive power of the number of nodal metastases was also inferior to the number of mitotic figures in metastatic carcinoma to the lymph nodes in this study. The number of nodal metastases and the invasive tumor size reflect the quantity of invasive ductal carcinoma cells, whereas the presence of a fibrotic focus, blood vessel invasion, grading system for lymph vessel tumor emboli, histologic grade, type of invasive ductal carcinoma, and the number of mitotic figures in metastatic carcinoma to the lymph nodes reflect the tumor characteristics of invasive ductal carcinomas. Furthermore, we observed that 1 UICC stage I patient with 1 micrometastasis with > 5 mitotic figures died of her diseases in this study (data not shown). Thus, histologic factors reflecting tumor characteristics are most likely superior to histologic factors reflecting tumor quantity as outcome predictors.

In conclusion, this study clearly demonstrated that our proposed histologic factors, such as type 4 invasive ductal carcinoma, lymph vessel tumor embolus grades 2 and 3, presence of > 5 mitotic figures in metastatic carcinoma to the lymph nodes, and a fibrotic focus diameter > 8 mm, are very important histologic factors for accurately predicting the outcomes of patients with invasive ductal carcinoma. Combined pathologic examinations based on these histologic factors and A-ranked, well-known histologic factors (blood vessel invasion, histologic grade, HER2 category, and UICC pN category) would most likely enable pathologists to assess the true malignant potential of invasive ductal carcinomas of the breast accurately.

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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

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

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 fibro-
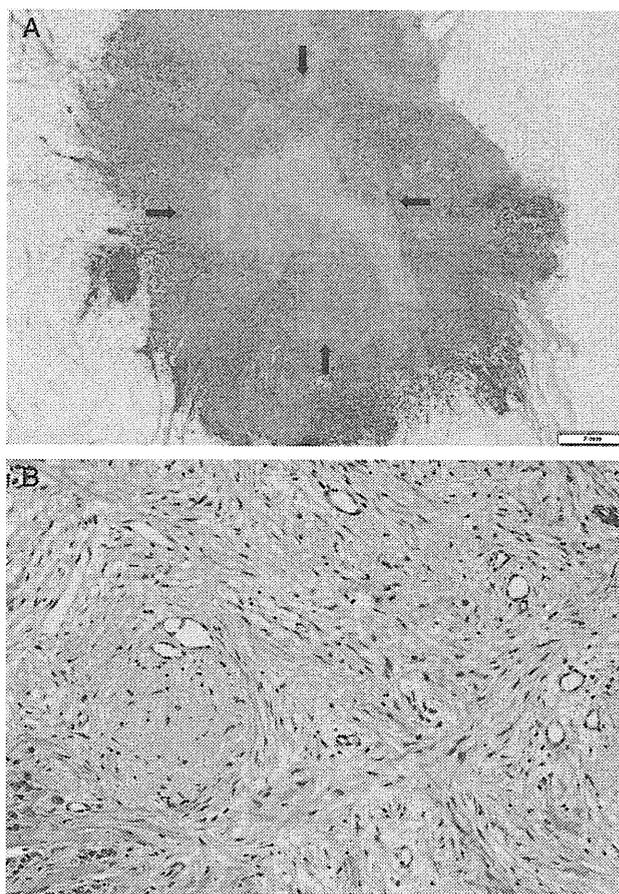

FIGURE 1. Invasive ductal carcinomas with fibrotic foci (A, B). A, A fibrotic focus measuring 7.8 × 5.6 mm is visible within the tumor (panoramic view, arrows). The fibrotic focus shows a scar-like feature, and is surrounded by invasive ductal carcinoma cells. B, The fibrotic focus area consists mainly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

blasts 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
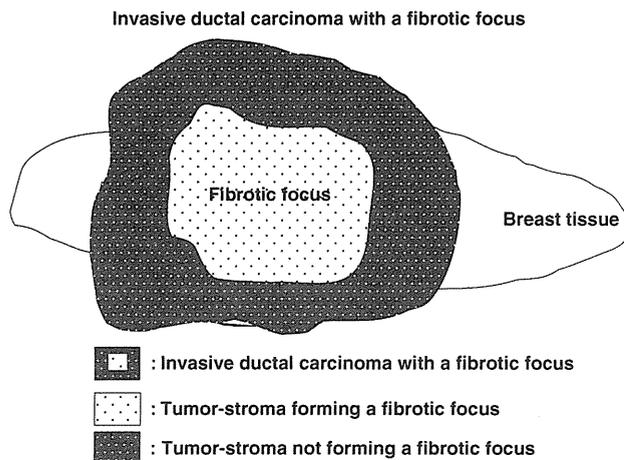
Invasive ductal carcinoma with a fibrotic focus

■ : Invasive ductal carcinoma with a fibrotic focus

▦ : Tumor-stroma forming a fibrotic focus

▓ : Tumor-stroma not forming a fibrotic focus

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 (≤ 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).
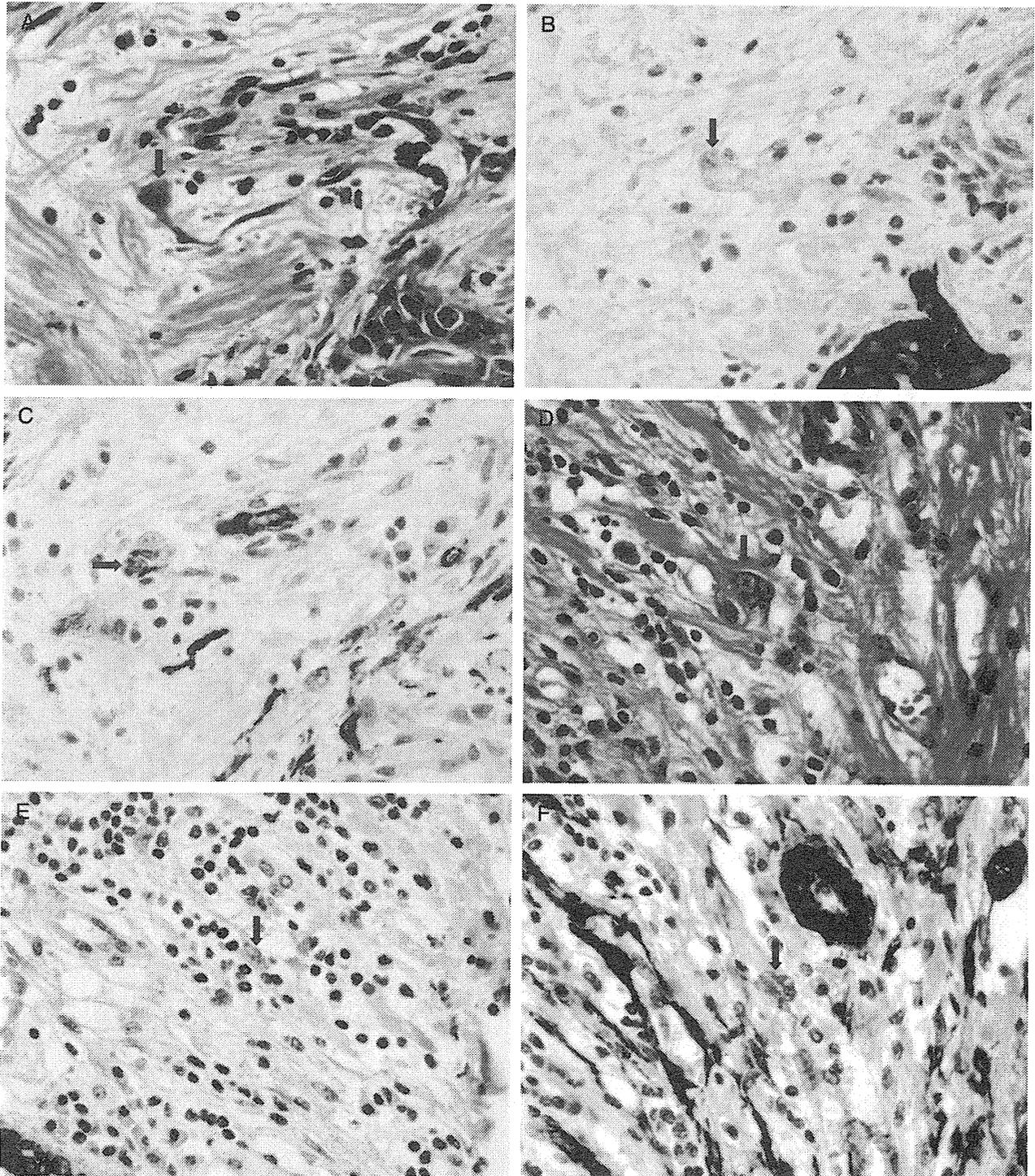
FIGURE 3. Immunohistochemical characteristics of atypical tumor-stromal fibroblasts inside fibrotic foci (A to F). A, One atypical tumor-stromal fibroblast with 1 bizarre and convoluted large nucleus with obvious nucleoli is visible (arrow). The fibroblast shows negative staining for keratins (B), but 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).
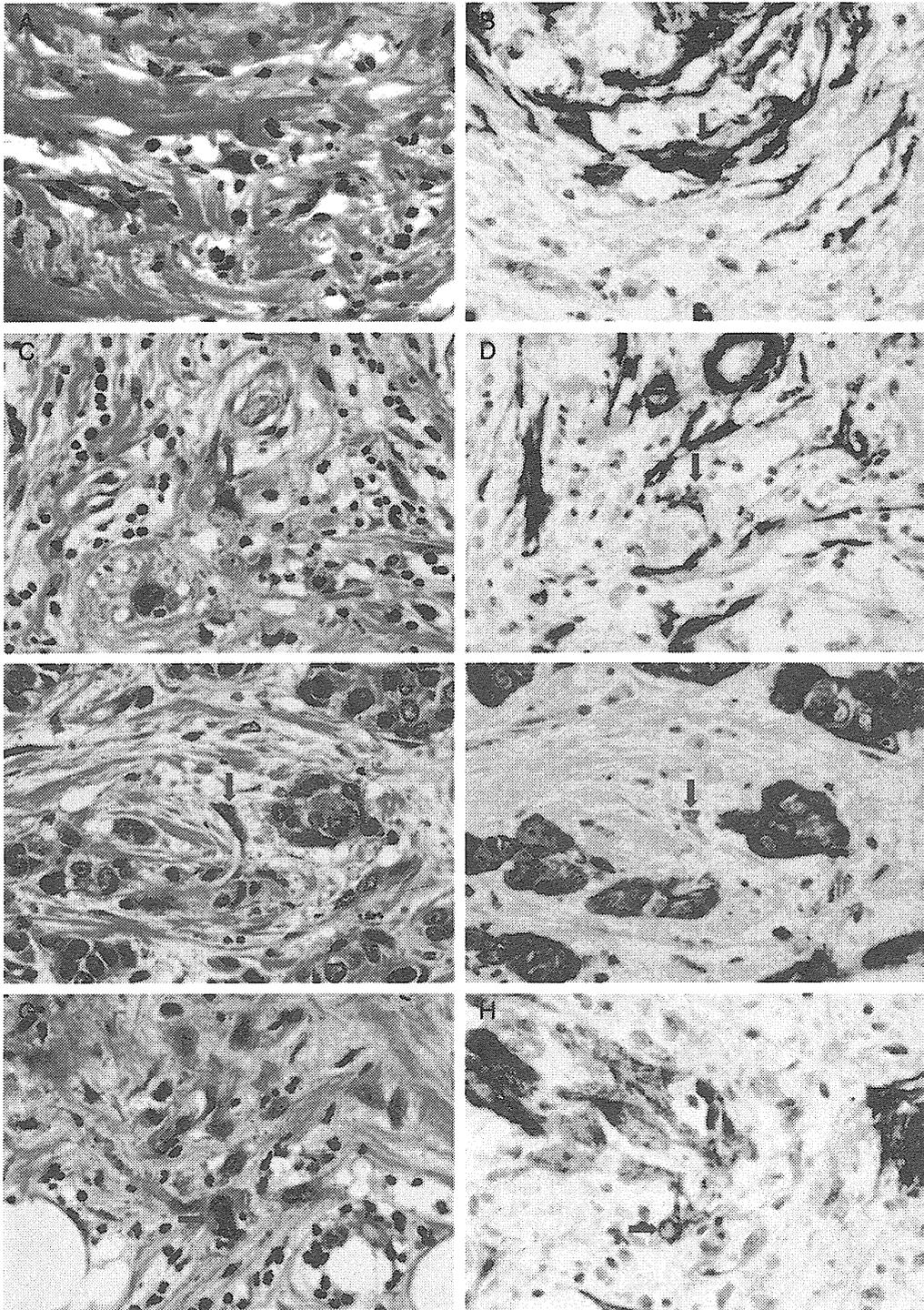
FIGURE 4. Histologic features of atypical tumor-stromal fibroblasts (A to H). A and B, One atypical tumor-stromal fibroblast with convoluted large nuclei with small nucleoli is visible (arrow) and shows positive staining for α-smooth muscle actin (arrow). C and D, One atypical tumor-stromal fibroblast 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.
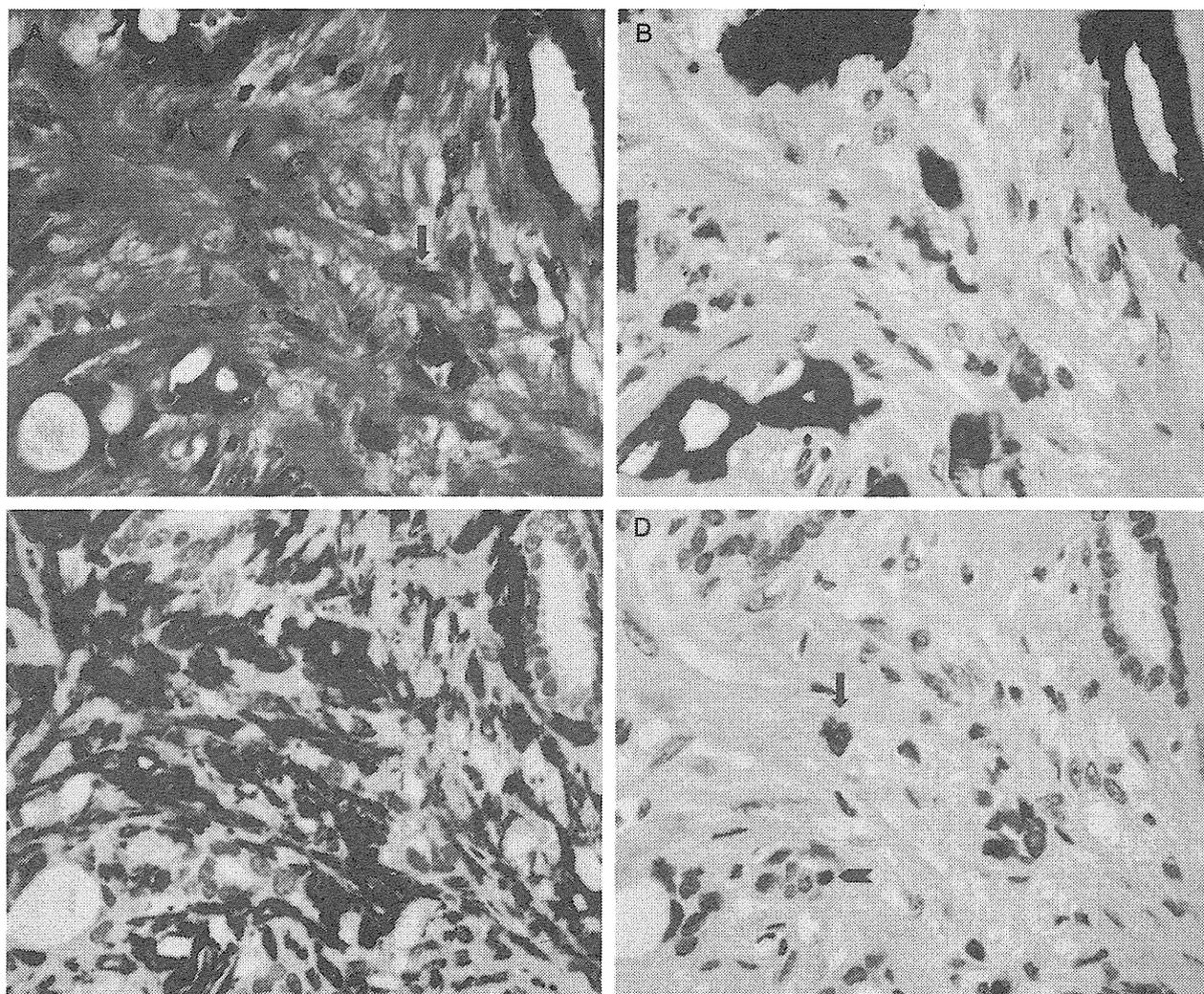
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FIGURE 5. Nuclear staining for p53 in atypical tumor-stromal fibroblasts. A, Three atypical tumor-stromal fibroblasts with a large convoluted nucleus or bizarre nucleus (arrows) and tumor-stromal fibroblasts with large oval nuclei are visible within 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
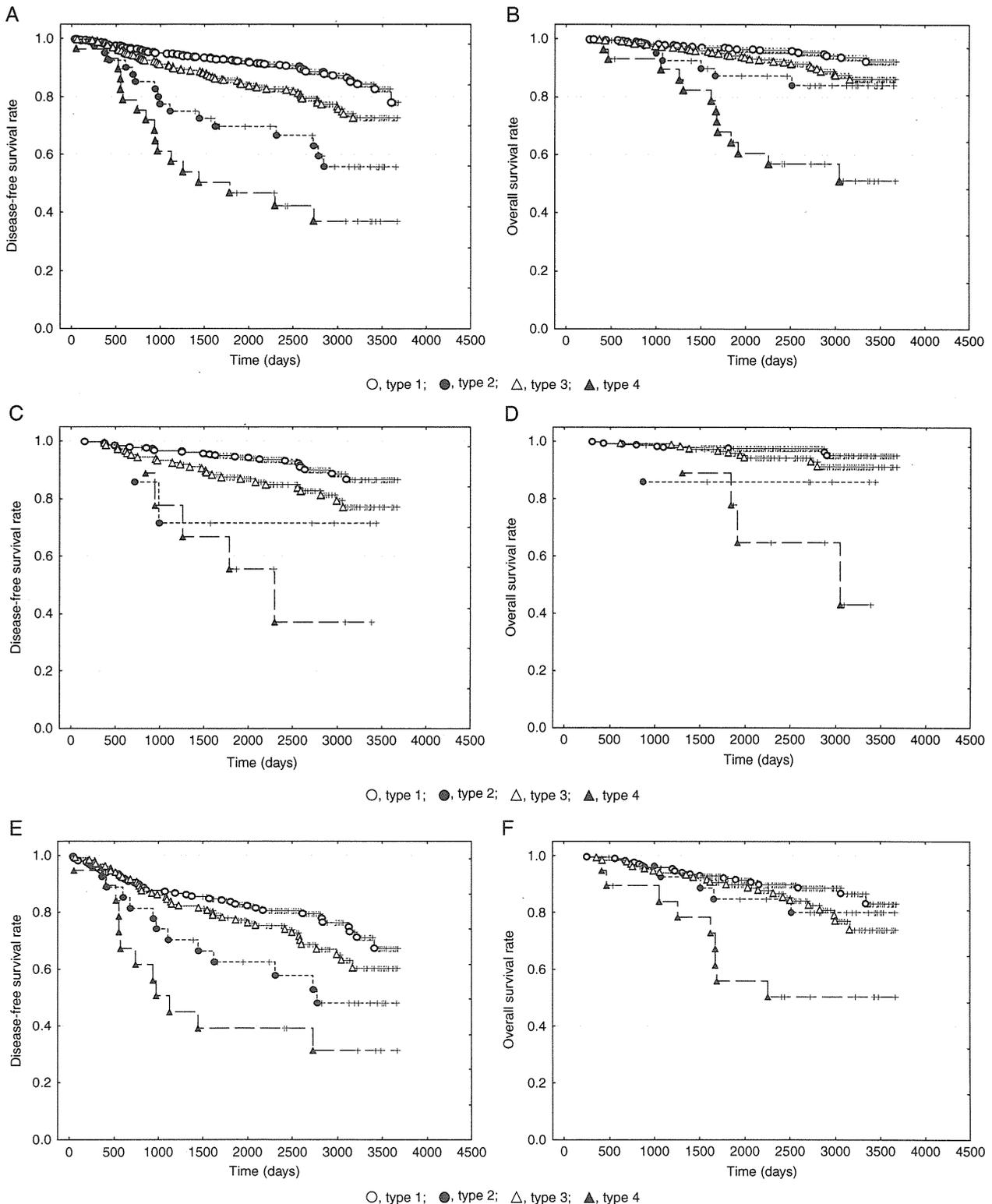
FIGURE 6. Disease-free survival curves and overall survival curves of invasive ductal carcinoma of patients overall (A, B), those of invasive ductal carcinoma patients of histologic grade 2 (C, D) and those of patients with invasive ductal carcinoma of histologic grade 3 (E, F) according to the type 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).

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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)

Factors	Tumor Recurrence		Tumor-related Death	
	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)

Factors	Tumor Recurrence		Tumor-related Death	
	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)

Factors	Tumor Recurrence		Tumor-related Death	
	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.

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

Factors	Tumor Recurrence		Tumor-related Death	
	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.

ACKNOWLEDGMENTS

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Preoperative therapy: recent findings

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Abstract Preoperative systemic therapy (PST) is the standard treatment for locally advanced breast cancer and a standard option for primary operable breast cancer. PST for breast cancer is as effective as postoperative adjuvant therapy, which permits more lumpectomies and can be used to study breast cancer biology. For locally advanced breast cancer patients, the primary aim of PST is to improve surgical option. For operable breast cancer patients, the primary aim of PST is to obtain freedom from disease. Because of recent advances in treatment and our understanding of the disease, we summarized the current consensus on the adoption and benefits of PST, especially for operable breast cancer patients.

Keywords Breast cancer · Preoperative therapy · Preoperative chemotherapy · Regimen

Introduction

Surgery followed by drug therapy (chemotherapy, hormone therapy) and/or radiotherapy, according to prognosticators or outcome predictors, has been standard therapy in patients with operable primary breast cancer. Previously, preoperative therapy was administered in patients with inoperable, locally advanced cancer. However, preoperative chemotherapy, a new therapeutic regimen where patients receive chemotherapy prior to surgery, has gained recognition since

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Fisher et al. reported the results of the National Surgical Adjuvant Breast and Bowel Project (NSABP) B-18 in 1997 [1]. Initially, a regimen of combination therapy with anthracycline and taxane was adopted. However, preoperative systemic therapy administering molecular targeted therapies, such as trastuzumab (Herceptin), and new hormone blockers, such as aromatase inhibitors, have been added to the regimen for the past 10 years. In this report, current consensus on the adoption and benefits of preoperative therapy is summarized, and the related issues and future challenges are reviewed.

Adoption of preoperative chemotherapy

Preoperative therapy has been adopted since the 1970s to improve surgical outcomes for permanent cure of inoperable locally advanced breast cancer and inflammatory breast cancer. Preoperative therapy remains the standard for patients with these breast cancers, although there is inadequate scientific evidence for the efficacy of this therapeutic strategy. In two large randomized trials, NSABP B-18 [1, 2] and EORTC1902 [3], higher breast conservation rates were found in patients who received neoadjuvant chemotherapy than adjuvant chemotherapy, although no differences were found in either survival rates or disease-free survival rates between the two therapies. Consequently, preoperative chemotherapy has become widely accepted as the treatment of choice for patients with operable breast cancer (stages II–IIIA), for improved breast conservation rates as well as for a new predictor: pathological complete response (pCR). In general preoperative therapy has been increasingly adopted as a principal treatment option for breast cancer patients.

The best regimen of preoperative chemotherapy

This section discusses the best regimen of preoperative chemotherapy. Several multicenter prospective randomized studies have been conducted to extensively review anthracycline and taxane agents, which generally produce good outcome for breast cancer patients with a single agent administration, by administering either of them alone or in combination, in patients with operable breast cancer. Anthracycline agents, if combined with taxane agents, showed a higher pCR rate of 15 to 20% compared to using each of the agents alone (Table 1) [4]. However, studies

Table 1 Results of preoperative systemic therapy using taxanes

Author/trial	No. of Patients	Regimen 1	Regimen 2	pCR (%)
Bear et al. 2003 (NSABP-B27)	2,411	AC	AC-D	9.6 vs. 18.9[a]
				13.7 vs. 26.1[b]
von Minckwiz et al. 2005 (Gepaduo)	913	AD	AC-D	7 vs. 14.3[c]
				7.4 vs. 15.9[d]
				11 vs. 22.3[a]
Moliterni et al. 2004	811	AT	EV	4 vs. 8
Untch et al. 2002	475	ET	ET	10 vs. 18[d]
Evans et al. 2005	363	AC × 6	AD × 6	16 vs. 12[c]
von Minckwiz et al. 2004 (Gepartrio)	296	TAC × 6	TAC-NX	NR: 4.3 vs. 3.1
		TAC × 6		R: 23
von Minckwiz et al. 2001 (Gepartrio)	248	AD	AD + Tam	10.3 vs. 9.1[a]
Dieras et al. 2004	200	AC × 4	AT × 4	10 vs. 16[c]
Steger et al. 2004	292	3 × ED every 21 days	6 × ED every 21 days	7.7 vs. 18.6[d]
Green et al. 2005	258	Paclitaxel every 21 days	Paclitaxel w	13.7 vs. 28[d]
Buzdar et al. 1999	174	FAC × 4	Paclitaxel × 4	16.4 vs. 8.1[d]
				23 vs. 14[b]
Smith et al. 2002 (Aberdeen)	104	CVAP	CVAP-D	15.4 vs. 30.8[b]

AC doxorubicin and cyclophosphamide, *AC-D* AC and docetaxel, *AD* doxorubicin and docetaxel, *AT* doxorubicin and paclitaxel, *EV* epirubicin and vincristine, *ET* epirubicin and paclitaxel, *TAC* paclitaxel, doxorubicin, and cyclophosphamide, *NX* vinorelbine and capecitabine, *Tam* tamoxifene, *FAC* fluoruracil, doxorubicin and cyclophosphamide, *CAVP-D* cyclophosphamide, doxorubicin, vincristine, predonisone and docetaxel, *w* weekly, *NR* noneresponder, *R* responder after two cycles of CMF, cyclophosphmide, methotrexate and fluorouracil

[a] Breast only: ypT0 regardless of nodal status

[b] Breast only: ypT0 ypTis regardless of nodal status

[c] ypT0, ypN0 only

[d] ypT0 ypTis, ypN0

Table 2 Comparison of pCR rates and survival data for PST

Author/trial	Regimen 1	Regimen 2	pCR (%)	DFS (%)	OS (%)
Fisher et al. Wolmark et al. (NASBP-B27)	AC × 4		9.4[a]	53 (9 years)	70 (9 years)
Bear et al. (NSABP-B27)	AC	AC-D	9.6 vs. 18.9[a]	69 vs. 74	81 vs. 82
Therassa et al.	CEF	EC	14 vs. 10[b]	34 vs. 33.7	53 vs. 51
Smith et al. (Aberdeen)	CAVP-D	CAVP	30.8 vs. 15.4[c]	90 vs. 72 (5 years)	NA
Diaras et al.	AT × 4	AC × 4	16 vs. 10[d]	87 vs. 79 (3 year)	NA

PST preoperative chemotherapy, *pCR* pathologic complete remission, *DFS* disease-free survival, *OS* overall survival, *AC* doxorubicin and cyclophosphamide, *AC-D* AC and docetaxel, *CEF* cyclophosphamide, epirubicin and fluorouracil, *EC* epirubicin and cyclophosphamide; *CAVP-D* cyclophosphamide, doxorubicin, vincristine, prednisone and docetaxel, *AT* doxorubicin and paclitaxel, *NA* not available

[a] Breast only: ypT0 regardless of nodal status

[b] Definition NA

[c] Breast only: ypT0 ypTis regardless of nodal status

[d] ypT0, ypN0 only