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.
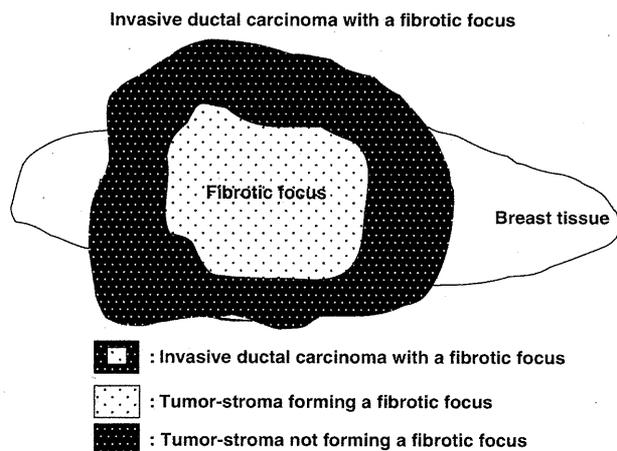
Histologic Examination

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 (≤20 mm, >20 to ≤50 mm, >50 mm), (2) histologic grade (1, 2, 3),[4] (3) tumor necrosis (absent, present),[6] (4) fibrotic focus (absent, fibrotic focus diameter ≤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-
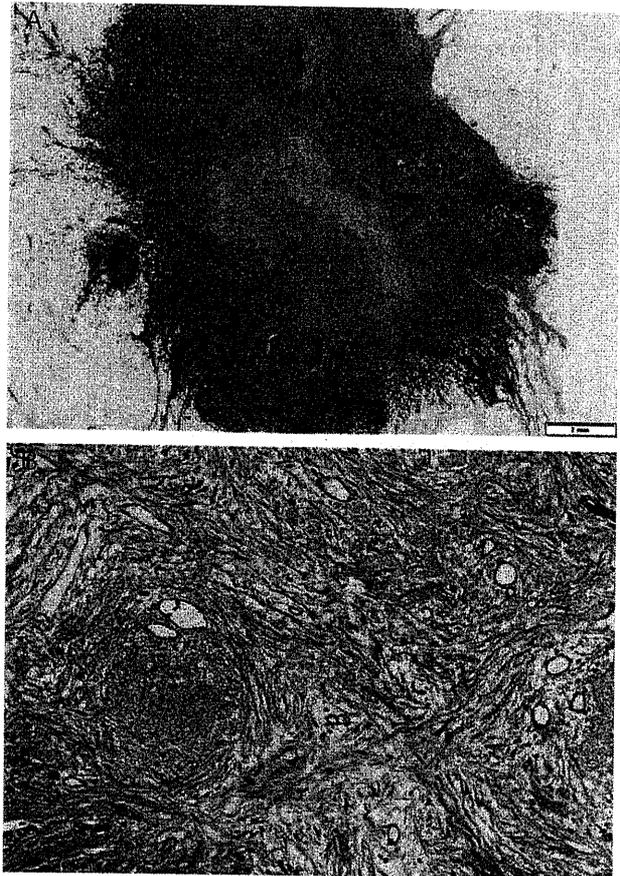
FIGURE 1. Invasive ductal carcinomas with fibrotic foci (A, B). A, A fibrotic focus measuring 7.8 × 5.6 mm is visible within the tumor (panoramic view, arrows). The fibrotic focus shows a scar-like feature, and is surrounded by invasive ductal carcinoma cells. B, The fibrotic focus area consists mainly of fibroblasts arranged in a storiform pattern. full color online

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).
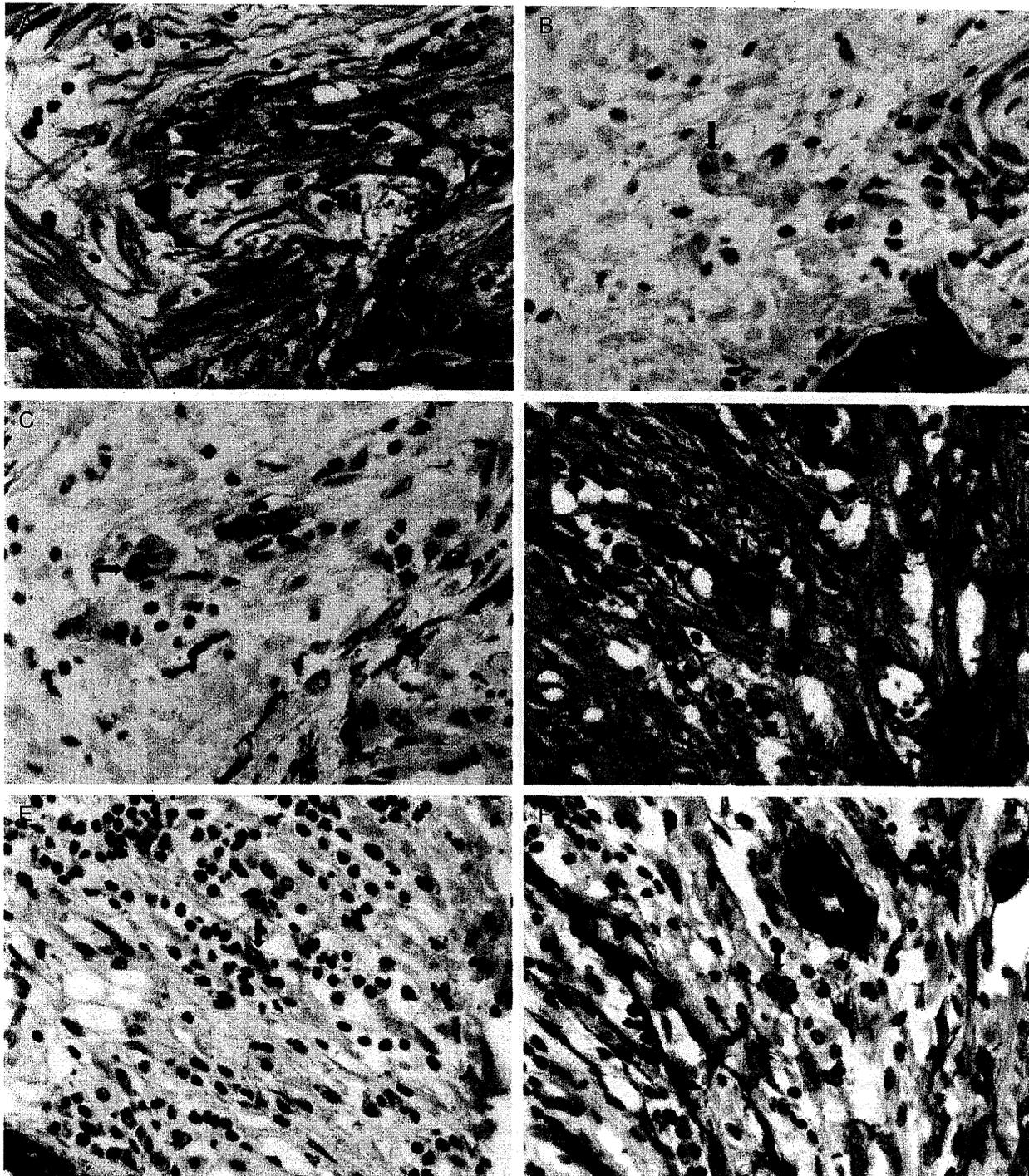
FIGURE 3. Immunohistochemical characteristics of atypical tumor-stromal fibroblasts inside fibrotic foci (A to F). A, One atypical tumor-stromal fibroblast with 1 bizarre and convoluted large nucleus with obvious nucleoli is visible (arrow). The fibroblast shows negative staining for keratins (B), but 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).
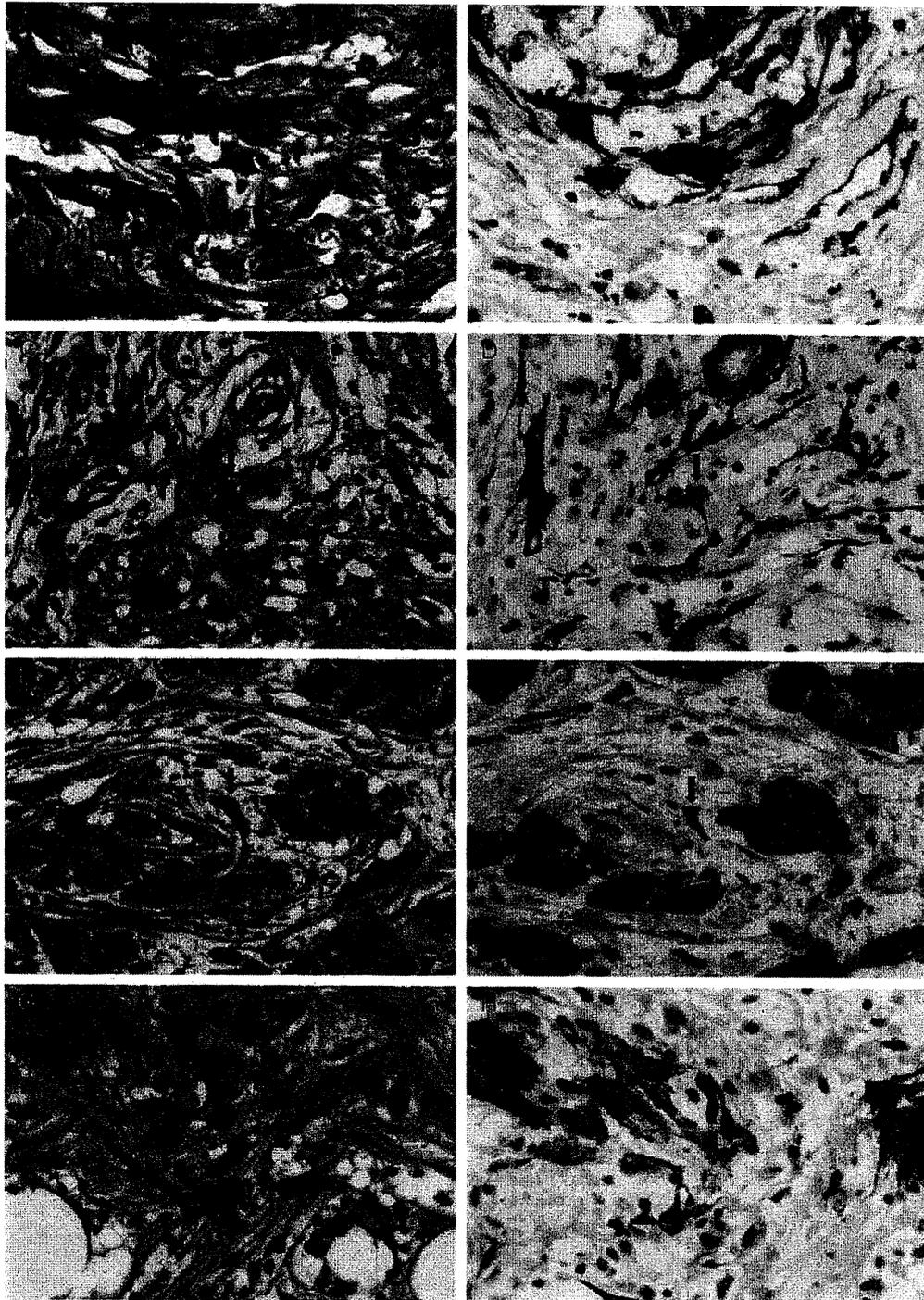
FIGURE 4. Histologic features of atypical tumor-stromal fibroblasts (A to H). A and B, One atypical tumor-stromal fibroblast with convoluted large nuclei with small nucleoli is visible (arrow) and shows positive staining for α-smooth muscle actin (arrow). C and D, One atypical tumor-stromal fibroblast 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.
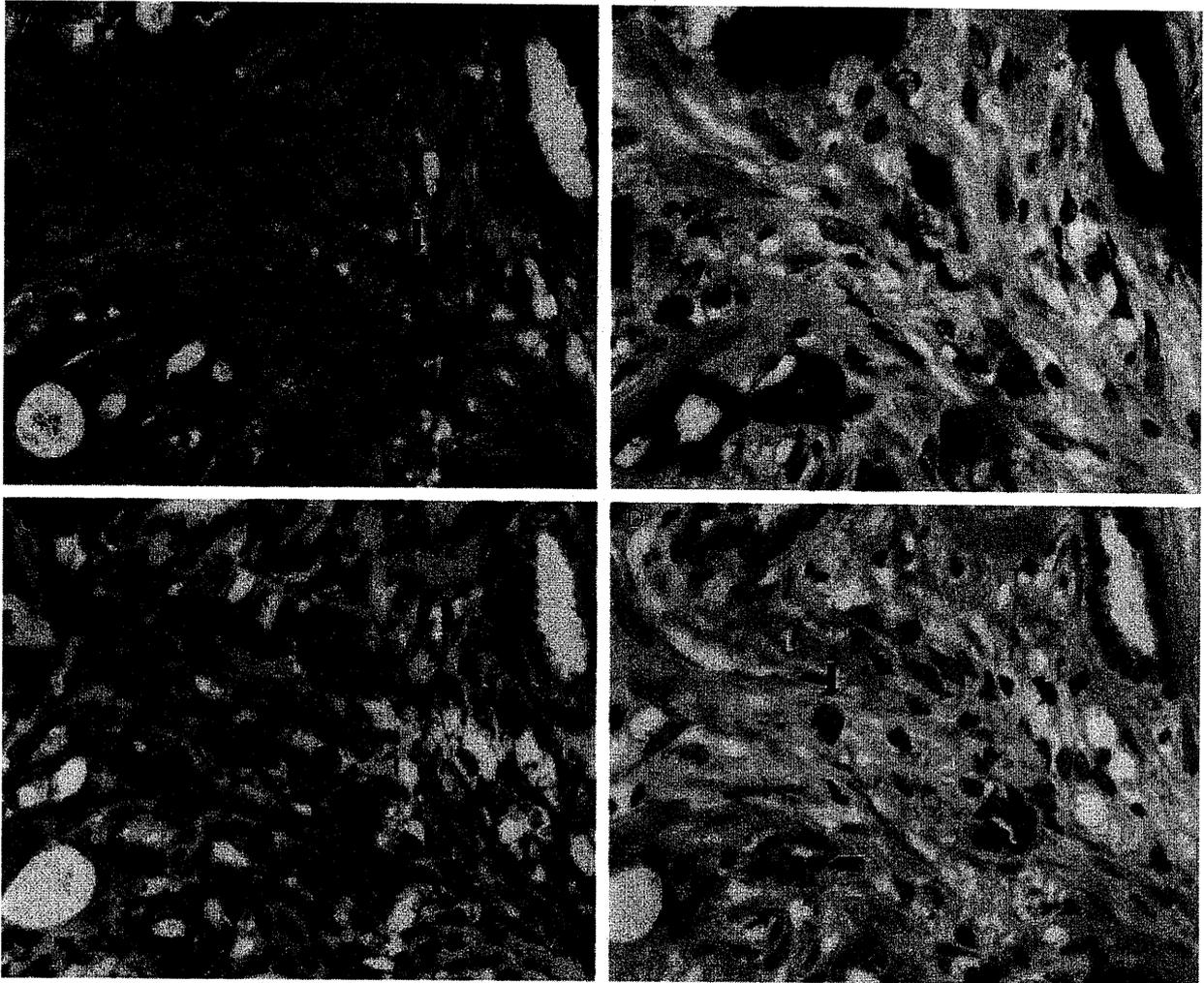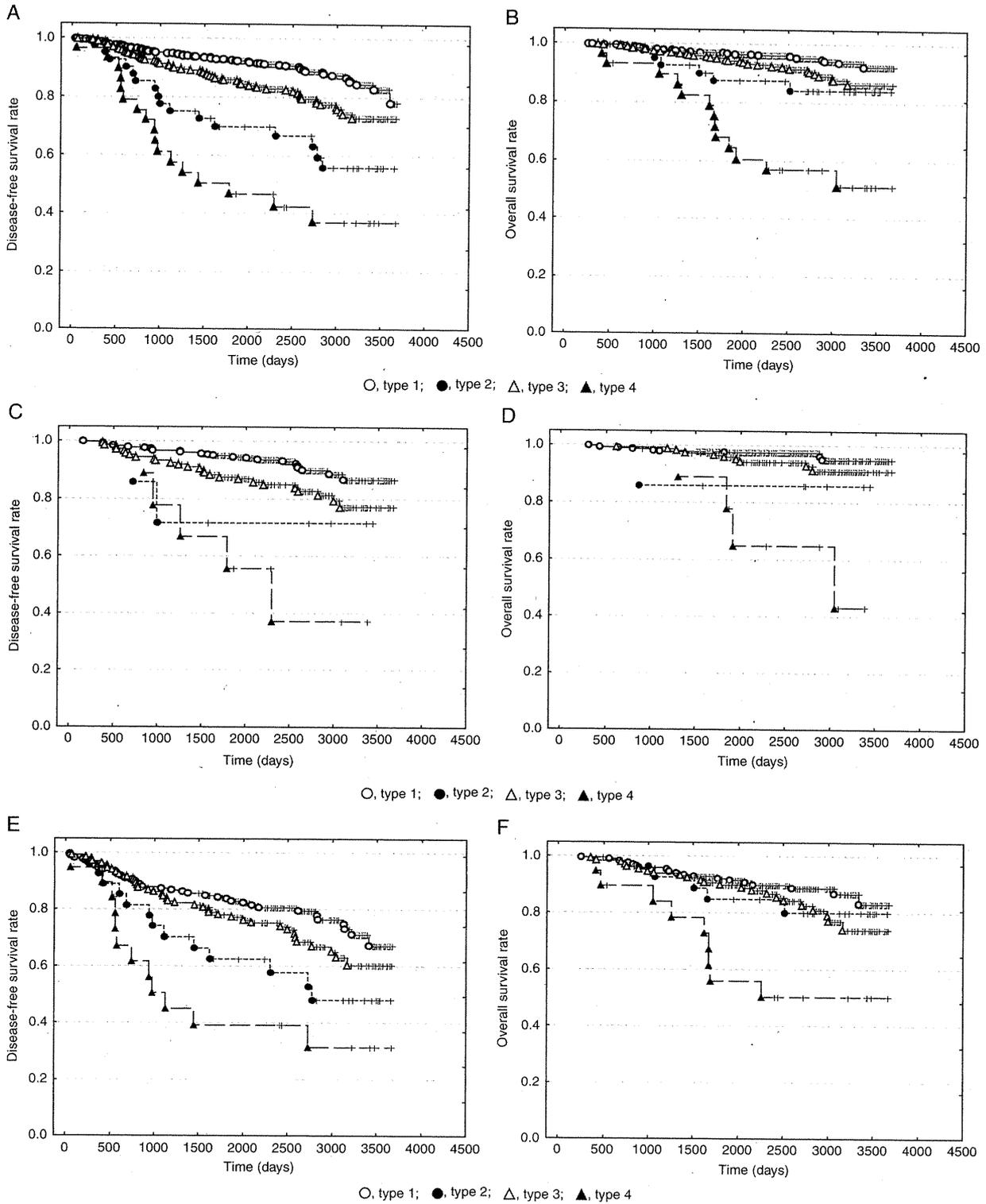
FIGURE 5. Nuclear staining for p53 in atypical tumor-stromal fibroblasts. A, Three atypical tumor-stromal fibroblasts with a large convoluted nucleus or bizarre nucleus (arrows) and tumor-stromal fibroblasts with large oval nuclei are visible within the tumor stroma. These atypical tumor-stromal fibroblasts 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

A

B

O, type 1; ●, type 2; △, type 3; ▲, type 4

C

D

O, type 1; ●, type 2; △, type 3; ▲, type 4

E

F

O, type 1; ●, type 2; △, type 3; ▲, type 4

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)

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.

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.

ACKNOWLEDGMENTS

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Preoperative therapy: recent findings

Takayuki Kinoshita

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	814	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

examining pCR, disease-free survival (DFS) and overall survival (OS) rates showed no improvement with the combination of the two agents in overall survival time until now, although some improvement has been found in disease-free survival time (Table 2) [4]. Indeed, the pCR rate is improved by the sequential administration of anthracycline and taxane agents, but the requirement of postoperative treatments for patients with non-pCRs remains an
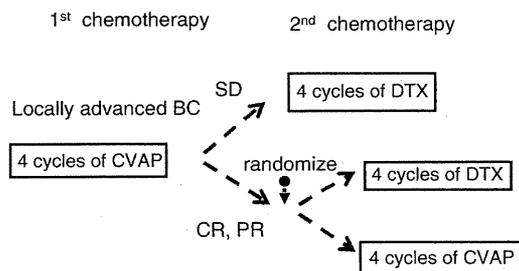
Fig. 1 Aberdeen breast group study. In the Aberdeen study, 162 patients with large and locally advanced breast cancer underwent four cycles of CVAP (cyclophosphamide/vincristine/doxorubicin/prednisone) primary chemotherapy. Patients with a complete or partial response were then randomized to either four further cycles of CVAP or four cycles of docetaxel (100 mg/m^2). It was shown that the addition of sequential docetaxel (100 mg/m^2) to CVAP neoadjuvant chemotherapy resulted in a significantly enhanced clinical response rate (94 vs. 64%) and a substantially increased complete histopathological response rate (34 vs. 16%) when compared to patients receiving CVAP alone. Furthermore, patients receiving docetaxel had an increased breast conservation rate (67 vs. 48%) and an increased survival at a median follow-up of 3 years

issue. Tailoring of therapy according to the outcome of neoadjuvant chemotherapy has become increasingly important in postoperative treatment. In the Aberdeen trial, patients who responded to the primary treatment with anthracycline agents were evaluated to determine if the same therapy should be continued or a new regimen including taxane agents should be applied (Fig. 1) [5]. The Gepartrio trial tailored therapy in non-responders, whereas the M.D. Anderson group randomly assigned patients to receive postoperative therapy depending on residual tumor size (Table 1) [4]. The Aberdeen trial and M.D. Anderson trial demonstrated that survival rates were improved by altering therapy before and after operation. However, it is not yet clear what benefit the preoperative responders or nonresponders can get from changing therapy.

Outcome predictors of preoperative chemotherapy

In order to tailor therapy based on outcome, it is important to clarify the outcome predictors of preoperative chemotherapy. Several studies including ETOC have evaluated the relationship between numerous factors (age, tumor size, malignancy, status of hormonal receptors, etc.) and pCR rates (Table 3). The common finding between these studies is that pCR rates in patients with hormone receptor-negative tumors were between 22 and 42%, which were significantly higher than in patients with receptor-positive tumors. At our facility, we analyzed clinical outcomes in

Table 3 Comparison of pCR rates and hormone receptor status for PST

Study	Subjective No. of patients	Treatment	pCR (%) Hormone receptor status	
GEPARDUO study	783	AC-DOC and ADOC	Negative	22.8
			Positive	6.2 ($p = 0.0001$)
GEPARTRIO pilot study	285	TAC (2 cycles)	Negative	26.7
			Positive	2.6 ($p = 0.003$)
ECTO (The European Cooperative Trial in Operable Breast Cancer)	451	AT-CMF	Negative	42
			Positive	12 ($p < 0.001$)
Marco Colleoni et al.	399	ECF or AT or ET or Navelbine containing	Negative	33.3
			Positive	7.6 ($p < 0.0001$)

Table 4 Comparison of pathological response and results of immunohistochemical staining

	Triple negative (%)	Endocrine: (+) HER2: over exp (%)	Endocrine: (+) HER2: (−) (%)	HER2 over expression (%)
3	12 (13.1)	1 (2.2)	5 (2.7)	12 (16.7)
2	25 (27.5)	14 (31.1)	16 (8.6)	29 (40.3)
1b	9 (9.9)	8 (17.8)	34 (18.3)	14 (19.4)
1a	37 (40.7)	16 (35.6)	97 (52.2)	14 (19.4)
0	5 (5.5)	2 (4.4)	15 (8.1)	1 (1.4)

Table 5 Clinical trials using trastuzumab in PST

Author/trial	No. of patients	Regimen	cRR (%)	pCR (%)
Burstein et al. 2003	40	PH	75	18[a]
Coudert et al. 2004	33	DH	73/97	47/54[b]
Harris et al. 2003	28	NH	93	NA[b]
Hurley et al. 2002	36	DCaH	NA	26
Buzdar et al. 2005	42	CT/H	NA	26/65[c]
Bines et al. 2003	33	DH w	70	12
Molucon et al. 2003	18	DH	95	28[b]
Limentani et al. 2003	17	DNH dd	89	24[d]
Steger et al. 2002	9	EDH	100	22[b]

PST preoperative chemotherapy, *cRR* clinical remission rate, *pCR* pathologic complete remission, *PH* paklitaxel and trastuzumub, *DH* docetaxel and trastuzumab, *NH* vinorelbine and trastuzumab, *DCaH* docetaxel, carboplatin and trastuzumab, *CT/H* chemotherapy with trastuzumab, *DNH* docetaxel, vinorelbine and trastuzumab, *dd* dose dense, *EDH* epirubicin, docetaxel and trastuzumab, *w* weekly, *NA* not available

[a] ypT0 ypTis, ypN0

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

[c] Definition NA

[d] Breast only: ypT0 regardless of nodal status

400 cases where preoperative chemotherapy was administered by adding the status of HER2 to that of hormone receptors (Table 4) [4]. We confirmed that the number of grade 3 cases in which cancer cells were completely eliminated was significantly smaller for the hormone receptor-positive group, and better clinical outcomes were obtained in the HER2-positive group.

Preoperative chemotherapy in patients with overexpressed HER2 breast cancer

According to clinical studies in patients with HER2 overexpressing breast cancer, preoperative chemotherapy administering a molecular targeted therapy, trastuzumab (Herceptin), resulted in 18–65% pCR rates (Table 5) [4].

Recent studies have indicated that the combination of trastuzumab with taxane agents is likely to be effective and the combination with anthracycline agents also showed high pCR rates. However, more clinical research is required to clarify these effects.

Preoperative hormone therapy: preoperative therapy for hormone-sensitive breast cancer

For hormone-sensitive breast cancer, higher efficacy and lower side effects can be a key factor in the choice of treatment. Table 6 summarizes the results of clinical trials on the preoperative hormone therapy available to date. In many of the clinical studies comparing aromatase inhibitors and tamoxifen, aromatase inhibitors showed higher response rates and breast conservation rates than tamoxifen. Table 7 shows the results of tamoxifen and anastrozole administered as preoperative chemotherapy conducted at our facility. Semiglazov et al. [6] reported a comparative study between preoperative hormone therapy and chemotherapy in postmenopausal patients with ER-positive breast cancer. The primary endpoint of that study was response rate, and median ages were 69 and 67 for hormone therapy and chemotherapy, respectively. There was no observed difference in response rate or breast conservation rate between hormone therapy and chemotherapy. This result indicates that preoperative hormone therapy can be an effective treatment option in elderly patients with ER-positive breast cancer. Some of the future areas of investigation in preoperative hormone therapy include the determination of endpoints, best administration period, and criteria for histological effects.

Conclusion

In the future, adoption of preoperative therapy is likely to increase the rates of tailored therapy, which best suites the

Table 6 Studies for neoadjuvant endocrine therapy

Author or trial name	No. of patients	Design	Treatment period (month)	Clinical ORR
V. Semiglazov	239	Chem vs. ANA vs. EXE	3	63 vs. 62 vs. 67%
IMPACT	330	ANA vs. TAM vs. ANA + TAM	3	37 vs. 36 vs. 39%
PROACT	451	ANA vs. TAM	3	49.7 vs. 39.7%
PO24 Trial	337	LET vs. TAM	4	55 vs. 36%
Russian study	151	EXE vs. TAM	3	76.3 vs. 40%
GENARI trial	27	EXE	4	37.00%
French study	38	EXE	4–5	70.60%
Gil Gil (spain)	55	EXE	6	50%
Mustacchi	44	EXE	6	66%

Table 7 Results of neoadjuvant endocrine therapy

	Tamoxifen ($n = 32$)	Anastrozole ($n = 47$)
Age/median (range)	60.9 (51–77)	64.3 (51–87)
Clinical ORR[a] (%)	45.50	57.4
US ORR[a] (%)	21.20	23.4
Pathologic response rate[b] (%)	17.60	22.2

[a] cCR and cPR

[b] Pathologic response; Grade 1b, 2 and 3

need of each patient, based on clinical evidence. Introduction of personalized medicine and new molecular targeted therapies are expected to provide higher pCR rates in preoperative chemotherapy. In addition, more treatments focused on improving quality of life are expected to be available, especially for elderly patients on preoperative hormone therapy.

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original article

FcγR2A and 3A polymorphisms predict clinical outcome of trastuzumab in both neoadjuvant and metastatic settings in patients with HER2-positive breast cancer

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Background: Antibody-dependent-mediated cytotoxicity (ADCC) is one of the modes of action for trastuzumab. Recent data have suggested that fragment C γ receptor (FcγR) polymorphisms have an effect on ADCC. This prospective phase II trial aimed to evaluate whether these polymorphisms are associated with clinical efficacies in patients who received trastuzumab.

Patients and methods: Patients in a neoadjuvant (N) setting received Adriamycin and cyclophosphamide followed by weekly paclitaxel/trastuzumab. Patients in a metastatic (M) setting received single trastuzumab until progression. In total, 384 distinct single nucleotide polymorphisms of different FcγR, HER2, and fucosyltransferase loci were assessed.

Results: Fifteen operable and 35 metastatic HER2-positive breast cancer patients were enrolled in each of the N and M settings, respectively. The FcγR2A-131 H/H genotype was significantly correlated with the pathologically documented response (pathological response) (P = 0.015) and the objective response (P = 0.043). The FcγR3A-158 V/V genotype was not correlated with the pathological response, but exhibited a tendency to be correlated with the objective response. Patients with the FcγR2A-131 H/H genotype had significantly longer progression-free survival in the M setting (P = 0.034).

Conclusion: The FcγR2A-131 H/H polymorphism predicted the pathological response to trastuzumab-based neoadjuvant chemotherapy in early-stage breast cancer, and the objective response to trastuzumab in metastatic breast cancer.

Key words: ADCC, FcγR, trastuzumab

introduction

The humanized HER-2/*neu* immunoglobulin G (IgG) 1 monoclonal antibody (mAb) trastuzumab is an effective treatment of HER-2/*neu*-positive breast cancer. However, large differences in clinical outcome remain among patients treated with trastuzumab. Identifying molecular markers that can select patients who are to benefit from trastuzumab treatment is crucial for avoiding chemotherapy toxicity and reducing treatment costs.

Antibody-dependent cytotoxicity (ADCC) mediated by fragment C γ receptor (FcγR) on immune cells such as macrophages and natural killer cells plays an important role in the antitumor effect of IgG1 antibodies [1]. Genetic polymorphisms have been identified in genes encoding the

activating receptors FcγR2A and 3A. A histidine (H)/arginine (R) polymorphism at position 131 for FcγR2A and a valine (V)/phenylalanine (F) polymorphism at position 158 for FcγR3A are two polymorphisms that affect the affinity of the receptors to human IgG [2–4]. Clinical studies have shown that FcγR2A-131 H/H and FcγR3A-158 V/V genotypes are associated with better clinical outcomes following the administration of rituximab as a first-line treatment of follicular lyoma [5, 6] and diffuse large lyoma [7] and cetuximab as a first-line treatment of metastatic colorectal cancer [8].

FcγR-deficient mice show a significantly reduced antitumor effect after trastuzumab treatment, with wild-type mice [9]. HER-2/*neu*-positive breast cancer cell lines are susceptible to ADCC in the presence of trastuzumab [10–12]. The activity of trastuzumab *in vivo* has also been correlated with a significant increase in the numbers of peritumoral lyomonocytes and *in vitro* ADCC [13]. In a clinical trial, Musolino et al. [14]

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demonstrated that a better response to trastuzumab-based therapy in metastatic breast cancer (MBC) was associated with the above two genotypes. In contrast, a recent large prospective trial (BCIRG006) [15] showed that the two FcγR single nucleotide polymorphisms (SNPs) did not predict disease-free survival in early breast cancer or progression-free survival (PFS) in MBC after trastuzumab-based therapy. Most of the previous studies reporting that FcγR SNPs are correlated with outcome [5–8, 14] have been under-powered, with the exception of BCIRG006. However, this inconsistency might have been influenced by the different modalities of therapeutic use [i.e. adjuvant and metastatic (M) settings] or the combinations of cytotoxic agents.

The goal of our prospective study was to determine the predictive values of these two SNPs as biomarkers in predicting the objective response to the single use of trastuzumab and to these predictive values with other SNPs in FcγR, HER2 or fucosyltransferase in MBC patients. We also analyzed their potential as a predictive marker of pathological complete response (pCR) in a neoadjuvant (N) setting with trastuzumab-based chemotherapy.

materials and methods

eligibility criteria
Eligible patients had histologically confirmed breast cancer, operable stage II–IIIA disease (tumor size > 3 cm) in an N setting or stage IV disease in an M setting (recurrent disease after curative surgery was also eligible), HER2-positive (IHC 3+ or FISH positive), chemotherapy, measurable disease, age ≥ 20 years, Eastern Cooperative Oncology Group performance status of 0–2, and adequate organ function (white blood cell count ≥ 4000/μl, platelet count ≥ 100 000/μl, hemoglobin concentration ≥ 9.0 g/dl, serum bilirubin ≤ 2.0 mg/dl, aspartate aminotransferase and alanine aminotransferase ≤ 100 IU/l, serum creatinine ≤ institutional upper limit of normal range, PaO$_2$ ≥ 60 mmHg, baseline left ventricular ejection fraction >50%). The main exclusion criteria were active concomitant malignancy, congestive heart failure, uncontrolled angina pectoris, arrhythmia, symptomatic infectious disease, severe bleeding, pulmonary fibrosis, obstructive bowel disease or severe diarrhea, symptomatic peripheral or cardiac effusion, and symptomatic brain metastasis. This study was conducted according to a protocol approved by the institutional review board/ independent ethics committee, and informed consent was obtained from all patients for the use of blood samples and the analysis of clinical information.

analysis of FcγR, HER2, and fucosyltransferase polymorphisms
In a previous study [14], the authors focused only on the hot spot of SNPs at FcγR2A-131 and FcγR3A-158. However, other loci of FcγR, including 2B-232 I/T, have already been reported [16] as potential markers for predicting the response to trastuzumab. Additionally, FUT8 is known to transfer a fucose residue to N-linked oligosaccharides on glycoproteins [17], and we reported that FUT8 plays an important role in ADCC activity [18]. Goldgate Genotyping is a novel technique that can be used to determine 384 SNPs quickly and simultaneously. Based on these backgrounds, 384 SNPs harboring FcγR1, R2, R3, HER2, and fucosyltransferase (FUT8) loci were custom designed using Goldgate Genotyping [19] (Illumina Co., CA) in this study. Among them, 67 SNPs were designed in exons. Genomic DNA was purified from peripheral blood using QIAamp Micro kits (QIAGEN K.K., Tokyo, Japan). Genomic DNA was isolated from specimens using QIAamp Micro kits. Genomic DNA

(250 ng) was hybridized using a bead array and the Goldgate Genotyping Assay manual [20]. The presence of SNPs was analyzed using a bead array reader. Gene clustering was carried out automatically using a software algorithm (Beadstudio) several times, and all the spots were confirmed by visual inspection. When the separation of the clustering was poor or when some samples provided inconsistent data, the assay was repeated to confirm the results. We also combined standard DNA (HapMap) with the assay as a control. Molecular data were independently interpreted by two biologists (FK and KN) who were blinded to the clinical outcomes of the study participants.

treatment and assessment
Treatment in the N setting consisted of Adriamycin and cyclophosphamide (60/600 mg/m^2) × 4 i.v. every 3 weeks followed by paclitaxel (80 mg/m^2) with trastuzumab (4 mg/kg followed by 2 mg/kg) × 12 i.v. every week. Treatment of MBC consisted of trastuzumab (8 mg/kg, followed by 6 mg/kg) every 3 weeks until disease progression. Routine clinical and laboratory assessments were carried out every 3 weeks, and a CT or echo examination of the target lesion was carried out every 2 months. The pathologically documented response (pathological response) after N therapy was assessed using the histopathological criteria of the Japanese Breast Cancer Society [21]. The objective response was evaluated every month using the Response Evaluation Criteria in Solid Tumors guidelines [22]. All the adverse effects that occurred during treatment were reported, and the severity of each adverse effect was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, Version 3.0.

statistical analysis
The association of each polymorphism with either the pathological response for the N setting or the objective tumor response for the M setting was the primary end point of the analysis. The association of each polymorphism with the PFS for the M setting and with a linkage analysis between FcγR2A and 3A were the secondary end points. First, all genotypes (wild, hetero and homo) of the 384 SNPs were assessed as to whether or not a difference in the primary end points was present with a statistical power <0.1. Second, the pathological responses and objective tumor responses of the patients were according to the selected FcγR polymorphisms using a two-tailed Fisher's exact test [23], chi-square test [23], linear correlation test [24], and analysis of variance (ANOVA) test [24]. Linkage disequilibrium was determined using a Fisher's exact test, chi-square test, and linear correlation test. The PFS was calculated as the length of time between the first day of trastuzumab treatment and the first observation of disease progression or death from any cause. If a patient had not progressed or died, the PFS was censored at the time of the last follow-up examination. The association of each polymorphism with PFS was analyzed using Kaplan–Meier curves [25] and the log-rank test [26]. All tests of statistical significance were two-tailed. The analyses were carried out using the SAS statistical package, version 9.0 (SAS Institute Inc., Cary, NC).

results

patient characteristics
Between December 2005 and August 2008, 40 and 36 patients were prospectively screened for N and M settings, respectively. Out of the 40 patients in the N setting, 15 (37.5%) patients were diagnosed as being HER2-positive using tissue samples obtained during a core needle biopsy. One patient in an M setting was ineligible because of an incorrect diagnosis of breast cancer. The clinical and pathologic features of the patients are presented in Table 1.

genotypic frequencies of the polymorphisms

A total of 384 SNPs harboring FcγRI, RII, RIII, HER2 and FUT8 loci were custom designed and analyzed in all 50 patients (15 in an N setting and 35 in an M setting). After a study to establish the correlations between the genotypes of these SNPs and the clinical outcome, we found that only two hot spots, FCγR2A-131 H/H and FCγR3A-158 V/V, among the 384 loci were predictive markers of the response to trastuzumab-based therapy. Forty-four percent (22 of 50) of the patients were homozygous for the FCγR2A-131 H allele, 48% (24 of 50) were heterozygous (H/R), and 8% (4 of 50) were homozygous(R/R) for the 131R allele (Tables 3 and 4). Forty-four percent (22 of 50) of the patients were homozygous for the FCγR3A-158 F allele, 46% (23 of 50) were heterozygous carriers (F/V), and 10% (5 of 50) were homozygous for the 158V allele. The distribution of genotypes between the N and M settings was similar and was not significantly different from that would be expected if each group was in Hardy–Weinberg equilibrium.

Table 1. Patient characteristics

Characteristics	N setting No.	%	M setting No.	%
No. of patients	15	100	35	100
Median age, years	44		58	
Range	23–66		28–76	
Menopausal status				
Pre	9	60	14	40
Post	6	40	21	60
Eastern Cooperative Oncology Group performance status				
0	12	80	18	51
1	3	20	16	46
2	0	0	1	3
Stage				
II	11	73	0	0
III	4	27	0	0
IV (+recurrence)	0	0	35	100
Histological grade				
1	3	20	2	6
2	5	33	9	26
3	7	47	24	68
Estrogen receptor status				
Positive	6	40	12	34
Negative	9	60	23	66
Progesterone receptor status				
Positive	5	33	7	20
Negative	10	67	28	80
Number of axillary lymph node				
1	10	67	–	–
1–3	5	33	–	–
≧4	0	0	–	–
Number of metastatic sites				
1	–	–	16	46
2	–	–	12	34
≧3	–	–	7	20

N, neoadjuvant; M, metastatic.

clinical response to trastuzumab therapy and FcγR polymorphisms

The pCR rate for the N setting was 33% [95% confidence interval (CI), 11.6% to 61.6%] (Table 2). No significant difference in the pretreatment features was observed between the FCγR2A and R3A genotypes. The FcγR2A-131 H/H genotype was significantly correlated with the pathological response [71% (5/7) for H/H versus 0% (0/8) for H/R + R/R; $P = 0.015$, Fisher's exact test; $P = 0.007$, chi-square test; $P < 0.05$, both linear correlation test and ANOVA test; Table 3]. The FcγR3A-158 V/V genotype was not correlated with the pathological response.

The objective response rate for the M setting was 23% (95% CI, 10.4% to 40.1%), and the disease control rate was 66% (95% CI, 47.8% to 80.9%) (Table 2). The median duration time of stable disease ($n = 15$) was 9.5 (5.3–17.7) months. A significant difference in the objective response rate was observed between patients with FcγR2A-131 H/H and those with either the 131 H/R or the 131 R/R genotype ($P = 0.043$, Fisher's exact test; $P < 0.05$, both linear correlation test and ANOVA test; Table 4). Although this difference did not reach the level of statistical significance, patients with FcγR3A-158 V/V also showed an overall higher response rate than the other two FcγR3A-158 genotypes [40% (6/15) for V/V versus 10% (2/20) for F/V + F/F; $P = 0.053$, Fisher's exact test; $P = 0.051$, chi-square test].

PFS analysis according to FcγR polymorphisms

The median follow-up times for the N and M settings were 24.8 and 22.6 months, respectively. Six patients (four local, two distant) had already relapsed as of July 2010. The PFS was assessed at 1 year after the last patient's enrollment in the study.

Table 2. Responses of patients in M or N settings

Response	No.	%
Neoadjuvant setting ($n = 15$)		
Grade 3[a] (pathological CR)	5	33
Grade 2 (marked response)	5	33
Grade 1b (moderate response)	3	20
Grade 1a (mild response)	2	14
Grade 0 (no response)	0	0
Pathological CR rate	5	33
95% CI	11.6–61.6	
M setting ($n = 35$)		
Compete response	1	3
Partial response	7	20
Stable disease	15	43
Progressive disease	12	34
Objective response rate	8	23
95% CI	10.4–40.1	
Disease control rate	23	66
95% CI	47.8–80.9	

[a]Grade refers to the histopathological criteria for the assessment of therapeutic response [21].

N, neoadjuvant; M, metastatic; CR, complete response; CI, confidence interval.