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

## METHODS

### Cases

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

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

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

### Histologic Examination

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

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

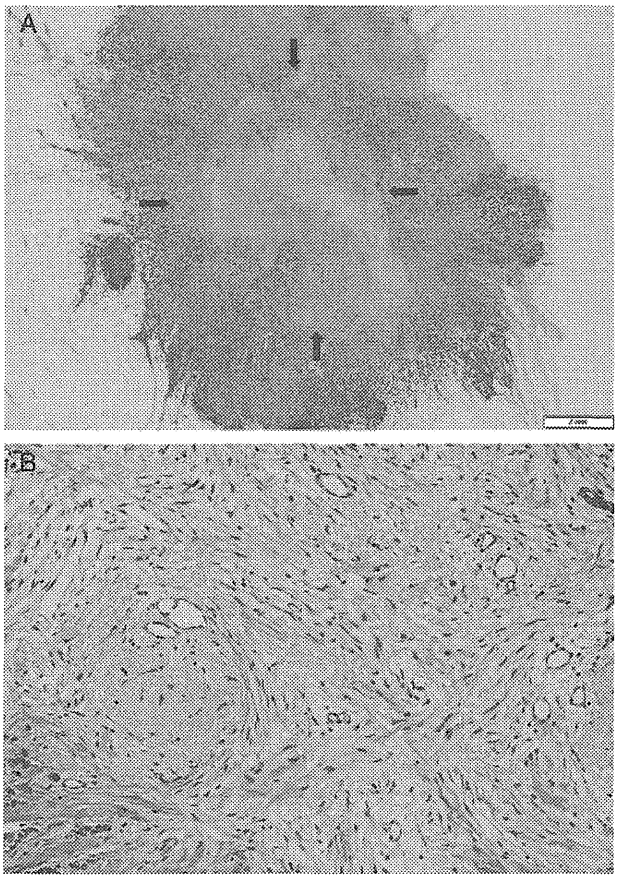


**FIGURE 1.** Invasive ductal carcinomas with fibrotic foci (A, B). A, A fibrotic focus measuring 7.8 × 5.6 mm is visible within the tumor (panoramic view, arrows). The fibrotic focus shows a scar-like feature, and is surrounded by invasive ductal carcinoma cells. B, The fibrotic focus area consists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

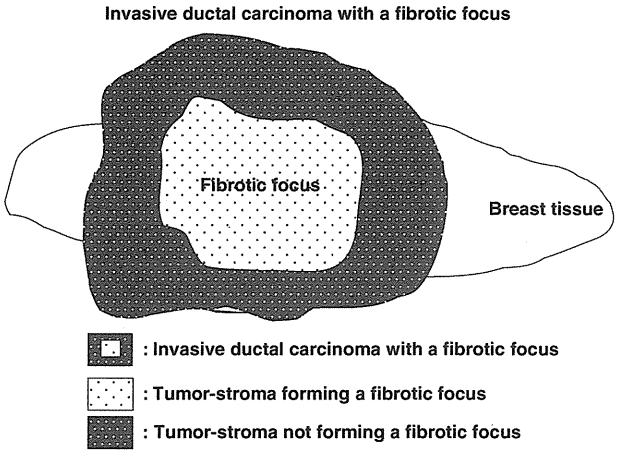


**FIGURE 2.** Schematic illustration of an invasive ductal carcinoma with a fibrotic focus.

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

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

## Patient Outcome and Statistical Analysis

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

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

# RESULTS

## Factors Significantly Associated With the Types of Invasive Ductal Carcinoma

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

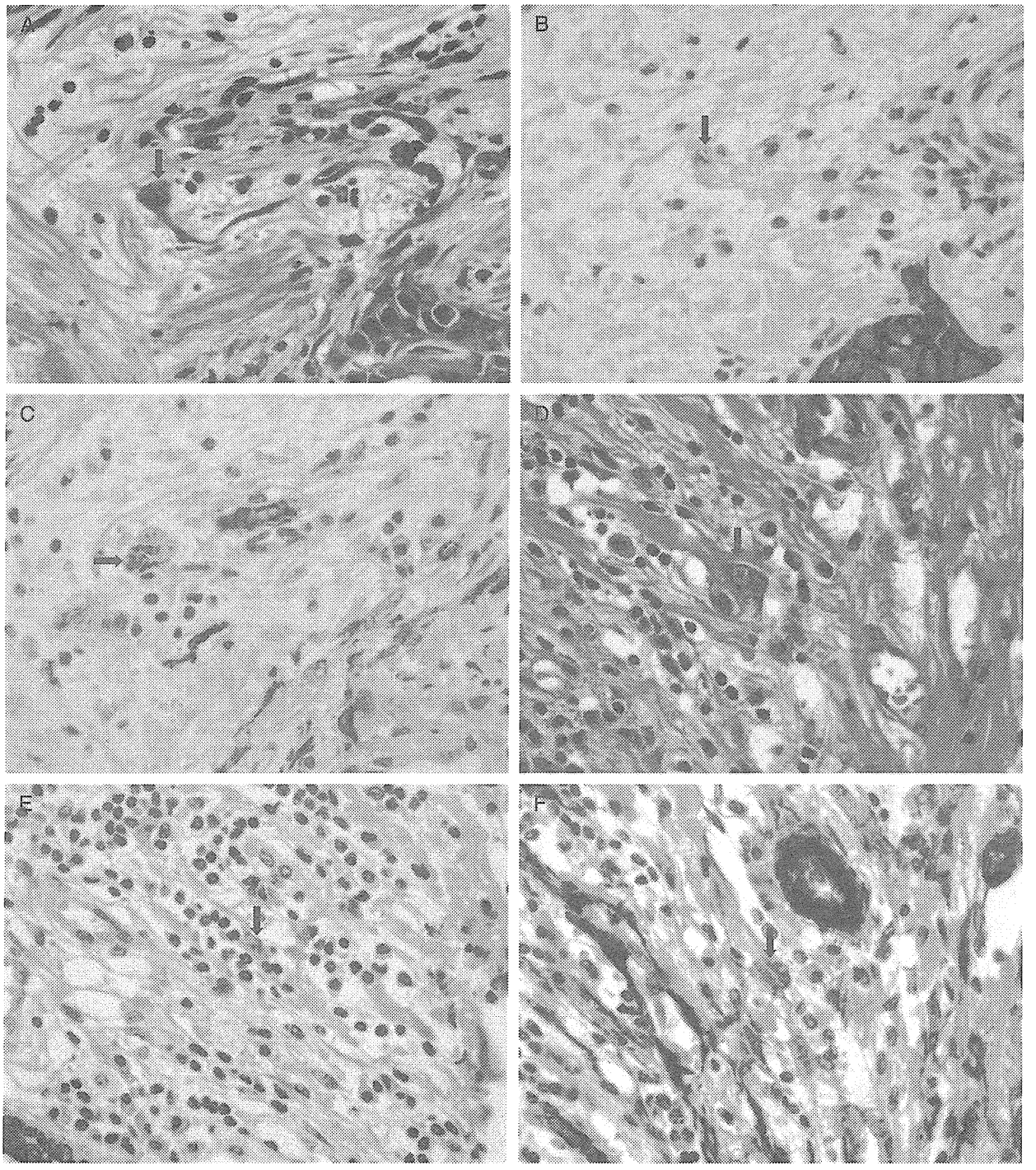


**FIGURE 3.** Immunohistochemical characteristics of atypical tumor-stromal fibroblasts inside fibrotic foci (A to F). A, One atypical tumor-stromal fibroblast with 1 bizarre and convoluted large nucleus with obvious nucleoli is visible (arrow). The fibroblast shows negative staining for keratins (B),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

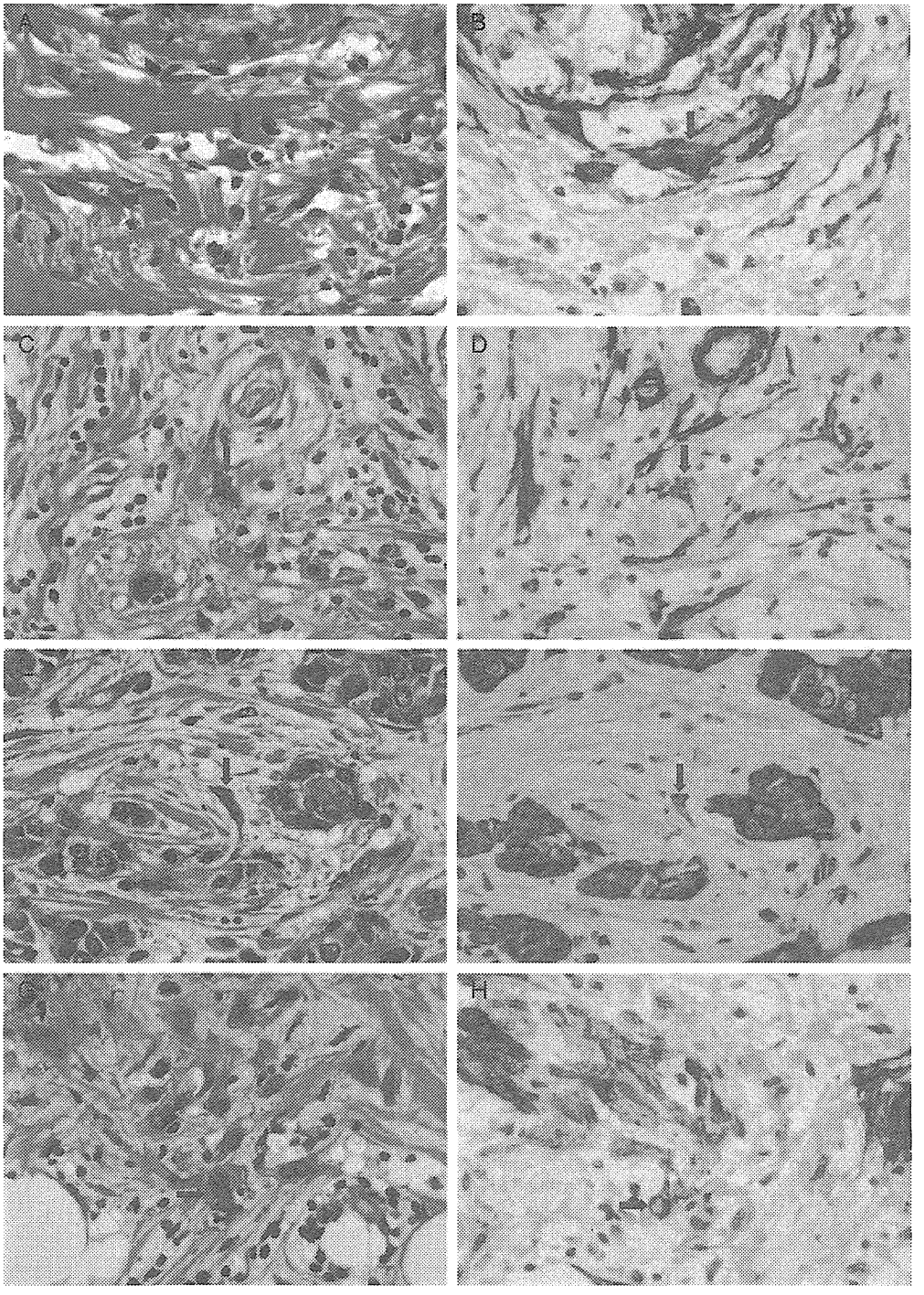


**FIGURE 4.** Histologic features of atypical tumor-stromal fibroblasts (A to H). A and B, One atypical tumor-stromal fibroblast with convoluted large nuclei with small nucleoli is visible (arrow) and shows positive staining for α-smooth muscle actin (arrow). C and D, One atypical tumor-stromal fibroblast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

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

**Characteristic Histologic Features of Atypical Tumor-stromal Fibroblasts**

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

**Criteria for Types of Invasive Ductal Carcinomas**

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

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

## Factors Significantly Associated With the Outcome of Patients

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

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

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

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

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

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

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

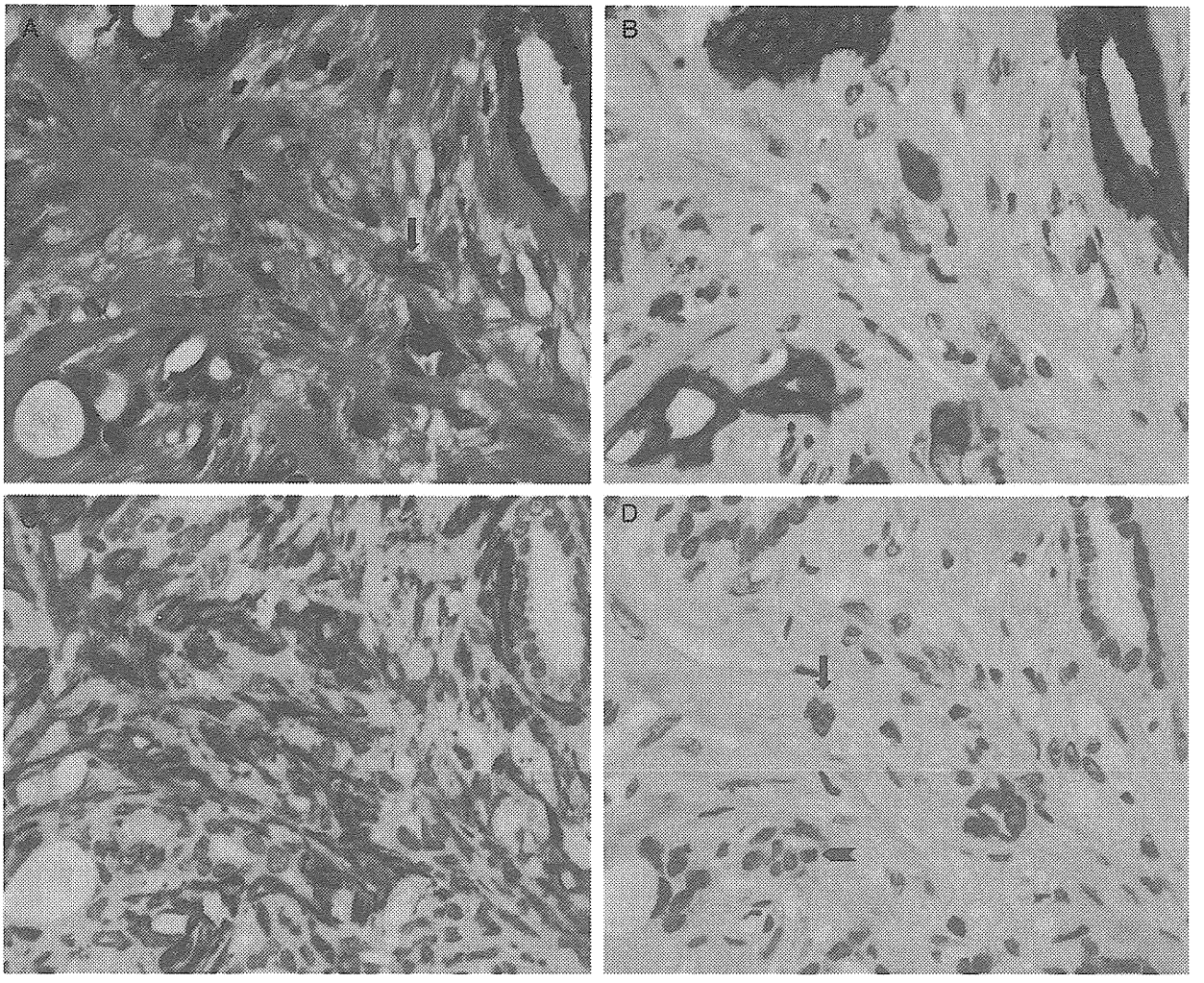


**FIGURE 5.** Nuclear staining for p53 in atypical tumor-stromal fibroblasts. A, Three atypical tumor-stromal fibroblasts with a large convoluted nucleus or bizarre nucleus (arrows) and tumor-stromal fibroblasts with large oval nuclei are visible within the tumor stroma. These atypical tumor-stromal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

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

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

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

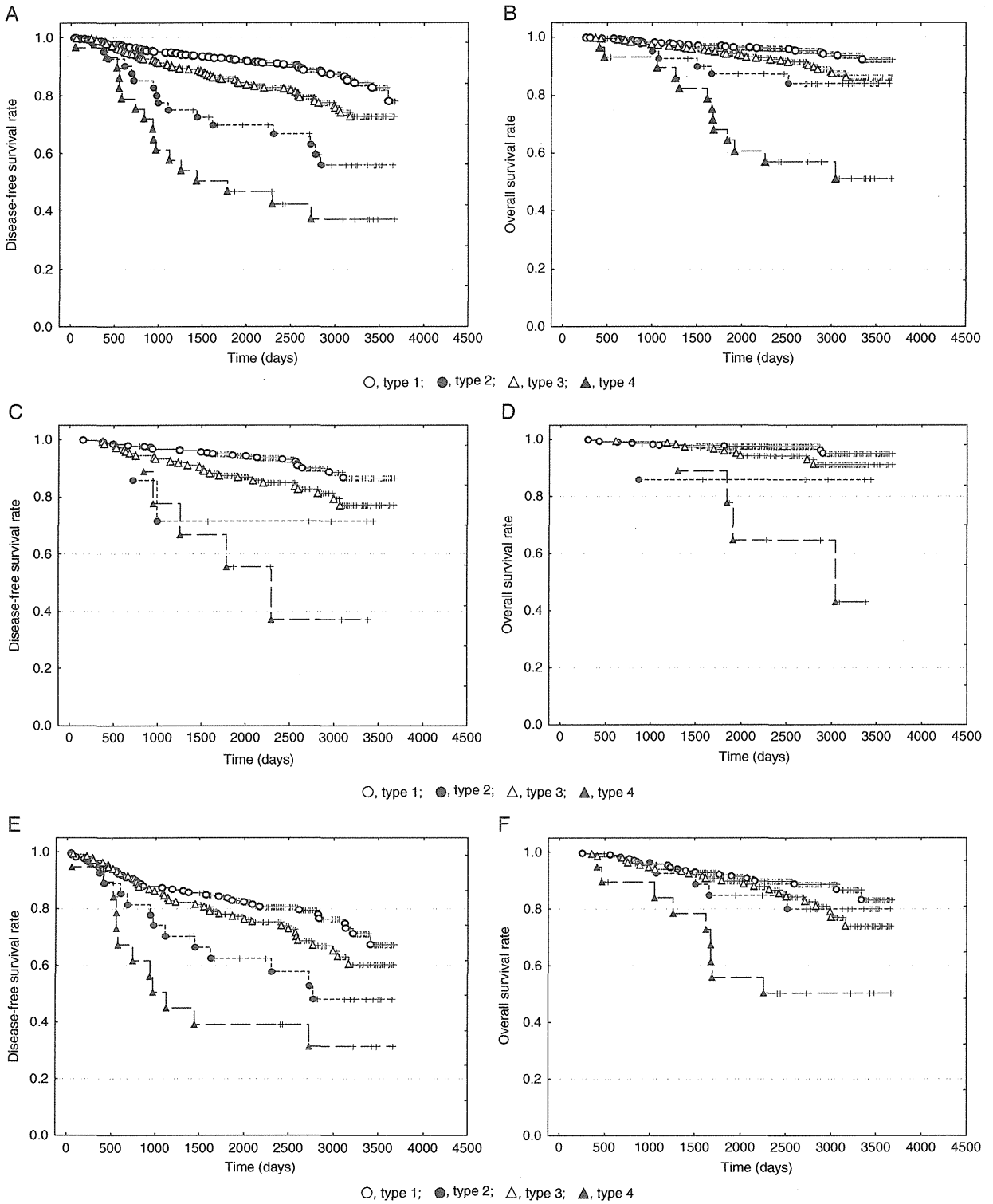


**FIGURE 6.** Disease-free survival curves and overall survival curves of invasive ductal carcinoma of patients overall (A, B), those of invasive ductal carcinoma patients of histologic grade 2 (C, D) and those of patients with invasive ductal carcinoma of histologic grade 3 (E, F) according to the type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

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

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

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

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

## DISCUSSION

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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# 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 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.

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## 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. | AC × 4 | | 9.4[a] | 53 (9 years) | 70 (9 years) |
| Wolmark et al. (NASBP-B27) | | | | | |
| 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 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.

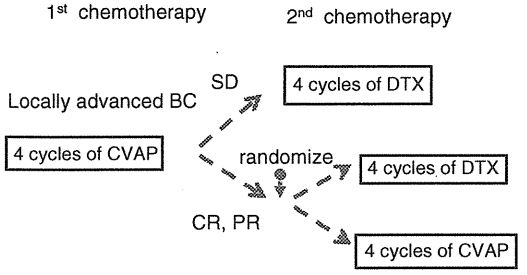


**Fig. 1** Aberdeen breast group study. In the Aberdeen study, 162 patients with large and locally advanced breast cancer underwent four cycles of CVAP (cyclophosphamide/vincristine/doxorubicin/prednisone) primary chemotherapy. Patients with a complete or partial response were then randomized to either 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

## 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 | Treatment | pCR (%) | |
|---|---|---|---|---|
| | No. of patients | | 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 contribution

# Atypical tumor-stromal fibroblasts in invasive ductal carcinomas of the breast treated with neoadjuvant therapy

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**Summary** Tumor-stromal fibroblasts have recently been reported to play important roles in the tumor progression of cancer in various organs. The purpose of the present study was to investigate whether any characteristic histologic features of tumor-stromal fibroblasts could accurately predict the outcome of 318 patients with invasive ductal carcinoma of the breast who had received neoadjuvant therapy. We observed a small number of tumor-stromal fibroblasts with characteristic nuclear features existing in the tumor stroma and named these cells "atypical tumor-stromal fibroblasts." We then assessed the absence or presence of atypical tumor-stromal fibroblasts in biopsy (taken before neoadjuvant therapy) and surgical (taken after neoadjuvant therapy) materials and analyzed the outcome predictive powers of the presence of atypical tumor-stromal fibroblasts in biopsy and surgical materials using multivariate analyses that included well-known clinicopathological factors. The multivariate analyses demonstrated that the presence of atypical tumor-stromal fibroblasts assessed using biopsy materials had significantly higher hazard ratios for tumor recurrence and tumor-related death in patients with nodal metastasis and also significantly higher hazard ratios for tumor recurrence and tumor-related death independent of the hormone receptor status of the tumors. The results of this study clearly indicated that the presence of atypical tumor-stromal fibroblasts, especially in biopsy materials, is significantly associated with tumor recurrence and the tumor-related death of patients with invasive ductal carcinoma of the breast who have received neoadjuvant therapy.


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## 1. Introduction

Tumor-stromal fibroblasts, or so-called cancer-associated fibroblasts, have recently been reported to play important roles in the tumor progression of cancer in various organs [1-3]. We have previously reported that highly proliferative fibroblasts in the tumor stroma of invasive ductal carcinoma (IDC) of the breast play a very important role in lymph node metastasis and distant-organ metastasis of IDC of the breast [4,5]. We also recently demonstrated that p53 expression in tumor-stromal fibroblasts was a very important outcome predictor for IDC patients who had or who had not received neoadjuvant therapy [6,7].

The purpose of the present study was to investigate whether characteristic histologic features of tumor-stromal fibroblasts could accurately predict the outcome of patients with IDC who received neoadjuvant therapy, because no other previous studies have investigated the histologic features of tumor-stromal fibroblasts and their association with the outcome of patients with IDC of the breast. The results of this study clearly indicated that characteristic histologic features of the nuclei in tumor-stromal fibroblasts assessed using biopsy materials are significantly associated with tumor recurrence and the tumor-related death of patients with IDC of the breast who received neoadjuvant therapy, and we named such tumor-stromal fibroblasts as "atypical tumor-stromal fibroblasts."

## 2. Materials and methods

### 2.1. Cases

The subjects of this study were 318 consecutive patients with IDC of the breast who had received neoadjuvant therapy and were surgically treated at the National Cancer Center Hospital between January 2000 and December 2005 (almost the same series of the patients as investigated in an earlier study [7]). The IDC diagnoses were made preoperatively based on the results of a needle biopsy, aspiration cytology, mammography, or ultrasonography. Clinical information was obtained from the patients' medical records after a complete histologic examination of all IDCs. All the patients were Japanese women ranging in age from 23 to 77 years (median, 55 years). All had a solitary lesion; 127 patients were premenopausal, and 191 were postmenopausal. A partial mastectomy had been performed in 152 patients, and a modified radical mastectomy had been performed in 166 patients. Level I and level II axillary lymph node dissections had been performed in all the patients, and a level III axillary lymph node dissection had been performed in some of the IDC patients.

Of the 318 subjects, 35 (11%) had exhibited a pathological complete response to neoadjuvant therapy [8] (32 with no residual tumor and no nodal metastasis, and 3 with residual ductal carcinoma in situ and no nodal metastasis). In addition, 2 patients with no residual tumor but with lymph node micrometastasis [9] were observed.

The neoadjuvant therapy consisted of chemotherapy in 235 patients, endocrine therapy in 43 patients, and chemoendocrine therapy in 3 patients; the chemotherapy regimens used were anthracycline based with or without taxane (132 patients) and nonanthracycline based (103 patients), and the endocrine therapy regimens consisted of tamoxifen with or without a gonadotropin-releasing-hormone agonist (18 patients), tamoxifen with or without an aromatase inhibitor (16 patients), an aromatase inhibitor alone, or a gonadotropin-releasing-hormone agonist alone (9 patients). Two hundred fourteen of the 281 patients who had received neoadjuvant therapy had also received adjuvant therapy, consisting of chemotherapy in 47 patients, endocrine therapy in 116 patients, and chemoendocrine therapy in 51 patients. No cases with inflammatory breast cancer were included in this series. All the tumors were classified according to the UICC pTNM classification [9]. The protocol of this study (20-112) was reviewed by the institutional review board of the National Cancer Center.

For the pathological examination, biopsy specimens obtained before neoadjuvant therapy and surgically resected specimens obtained after neoadjuvant therapy were fixed in 10% formalin and subsequently examined. The size and gross appearance of the surgically resected tumor specimens were recorded as the residual invasive tumor size. The residual tumor size of the surgically resected specimens was confirmed by comparison with the residual tumor size on histologic slides.

### 2.2. Histologic examination and immunohistochemistry

Serial sections of the biopsy specimens obtained before neoadjuvant chemotherapy and of the tumor area in the surgically resected specimens obtained after neoadjuvant therapy were cut from paraffin-wax blocks. One section of each biopsy specimen and surgical specimen was stained with hematoxylin and eosin and was examined histologically to confirm the diagnosis, whereas another section was subjected to immunohistochemistry. The following 8 histologic features of the primary-invasive tumors were evaluated in the surgical specimens obtained after neoadjuvant therapy: (1) residual invasive tumor size (no residual tumor or residual ductal carcinoma in situ, residual tumor $\leq$20, >20-$\leq$50, >50 mm), (2) histologic grade (1, 2, 3) [10], (3) tumor necrosis (absent, present) [11], (4) grading system for lymph vessel tumor emboli [12,13], (5) blood vessel invasion (absent, present), (6) adipose tissue invasion (absent, present), (7) skin invasion (absent, present), and (8) muscle invasion (absent, present). We also evaluated the outcome predictive power for a pathological complete response to

neoadjuvant therapy for surgical specimens obtained after neoadjuvant therapy [8].

Because 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 [14], we examined whether tumor-stromal fibroblasts with characteristic cytoplasmic features or nuclear features could also be identified in the tumor stroma of IDCs in biopsy and surgical specimens. We observed a small number of tumor-stromal fibroblasts with characteristic nuclear features existing in the tumor stroma in the biopsy specimens or in the surgical specimens (Fig. 1) and named these cells "atypical tumor-stromal fibroblasts." The presence of atypical tumor-stromal fibroblast was defined based on 1 or more atypical tumor-stromal fibroblasts in the tumor stroma, and the characteristic nuclear histologic features of atypical tumor-stromal fibroblasts are as follows: (1) atypical tumor-stromal fibroblast can have a single nucleus or may be multinucleated; (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, convoluted or bizarre shape; and (4) some atypical tumor-stromal fibroblasts may fuse with each other to produce atypical tumor-stromal fibroblasts with multiple nuclei. While examining the absence or presence of atypical tumor-stromal fibroblasts in the tumor stroma, we avoided a decision regarding the absence or presence of atypical tumor-stromal fibroblasts in the following situations: (1) the presence of atypical tumor-stromal fibroblast-like cells that were difficult to differentiate from surrounding 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, they tended to exist within the cellular area of the tumor-stromal fibroblasts. One author (T.H.) assessed the presence or absence of atypical tumor-stromal fibroblasts, and 1 of 2 other authors (T.S. or Y.S.) identified the presence or absence of atypical tumor-stromal fibroblasts to confirm the presence or absence of atypical tumor-stromal fibroblasts recorded by T.H. Discordant results were reevaluated jointly to reach a consensus.

Immunohistochemical staining for estrogen receptors (ERs), progesterone receptors (PRs), p53, and HER2 products was performed using an autoimmunostainer (Optimax Plus; BioGenex, San Ramon, CA). The antigen retrieval device for the 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 manufacturer's instructions. The antibodies used were the mouse anti-ER monoclonal antibody (mAb) ER88 (BioGenex), the mouse anti-PR mAb PR88 (BioGenex), and the mouse anti-HER2 mAb CB11 (BioGnex) and the mouse p53 mAb DO7 (Dako, Glostrup, Denmark). ER88, PR88, and CB11 were already diluted, and DO7 was applied at a 1:100 dilution. After immunostaining, the sections were counterstained with hematoxylin. Sections of the IDCs that were positive for ER, PR, HER2, and p53 were used each time as a positive control. As a negative control, the primary antibody was replaced with normal mouse immunoglobulin.

The sections of the biopsy and surgical specimens that were immunostained for ER, PR, and p53 and that contained tumor cells were scored using the Allred system as described previously [15-17], and the Allred scores for ER, PR, and p53 expression in the tumor cells were classified into the following 3 categories [6]: (1) Allred score for ER in tumor cells (0 or 2, 3 to 6, and 7 or 8); (2) Allred score for PR in tumor cells (0 or 2, 3 to 6, and 7 or 8); (3) Allred scores for p53 in tumor cells (0 or 2 or 3, 4 to 6, and 7 or 8); and (4) Allred scores for p53 in tumor-stromal fibroblasts (0 or 2, 3, and 4 to 8). We defined an Allred score of 0 or 2 for ER or PR as being negative for ER or PR and Allred scores of 3 or more for ER or PR as being positive for ER or PR. 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 [18] and was classified into 3 categories: 0 or 1, 2, and 3. Immunohistochemistry was used to score 290 of the 318 IDCs for ER, PR, HER2, and p53 expression in the biopsy specimens. In the surgical specimens, immunohistochemistry was used to score 273 of the 318 IDCs for ER, PR, and p53 expression and to score 271 of them for HER2 expression. The immunohistochemical examination was performed without knowledge of the patients' outcomes.

### 2.3. Patient outcome and statistical analysis

Survival was evaluated using a median follow-up period of 75 months (range, 50-117 months), ending in February 2010. As of the end of February 2010, 220 of the 381 patients were alive and well, 98 had developed tumor recurrence, and 63 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 the Fisher exact test.

We analyzed the outcome predictive power for tumor recurrence and tumor-related death by the multivariate analyses using the Cox proportional hazard regression model. The factors analyzed were the above-mentioned

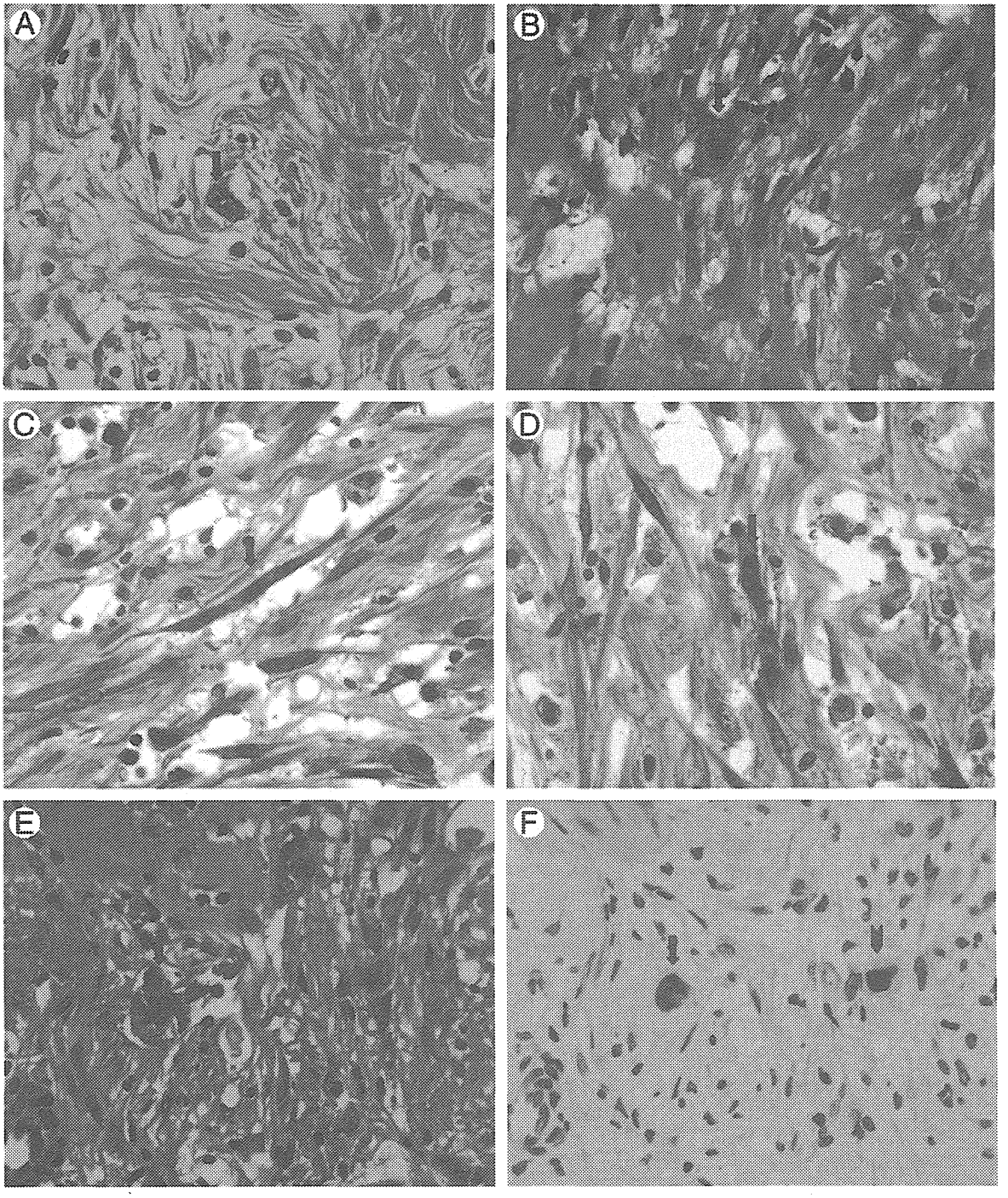


**Fig. 1** Histologic features of atypical tumor-stromal fibroblasts in the tumor stroma (A-F). A, One atypical tumor-stromal fibroblast with bizarre and convoluted large nucleus containing 2 large-eosinophilic nucleoli is visible (arrows). B, One atypical tumor-stromal fibroblast containing a bizarre and convoluted large nucleus is visible (arrows); the fibroblast has microcalcifications in its body. C, One atypical tumor-stromal fibroblast with 3 oval-shaped nuclei is visible in the stroma, suggesting that 3 tumor-stromal fibroblasts have fused with each other (arrows). D, One atypical tumor-stromal fibroblast with a large rosary-like nucleus is visible (arrow). E, One atypical tumor-stromal fibroblast with one nucleus of a dishcloth gourd-like feature and containing microcalcifications in its body is visible (arrow), and a tumor-stromal fibroblast with a large oval nucleus is also present (arrowhead). F, These cells exhibit moderate to strong positive nuclear staining for p53 (arrow and arrowhead).