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
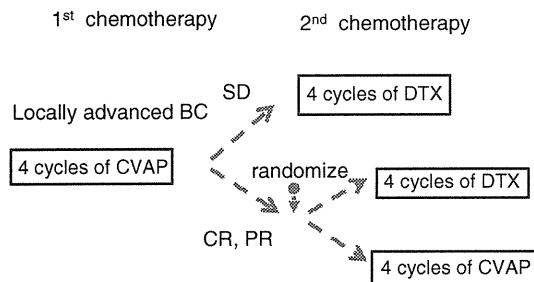

Fig. 1 Aberdeen breast group study. In the Aberdeen study, 162 patients with large and locally advanced breast cancer underwent four cycles of CVAP (cyclophosphamide/vincristine/doxorubicin/prednisone) primary chemotherapy. Patients with a complete or partial response were then randomized to either four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

Takahiro Hasebe MD, PhD [a],*, Motoki Iwasaki MD, PhD [b],
Sadako Akashi-Tanaka MD, PhD [c], Takashi Hojo MD, PhD [c], Chikako Shimizu MD, PhD [d],
Masashi Andoh MD, PhD [d], Yasuhiro Fujiwara MD, PhD [d], Tatsuhiro Shibata MD, PhD [e],
Yuko Sasajima MD, PhD [f], Takayuki Kinoshita MD, PhD [c], Hitoshi Tsuda MD, PhD [f]

[a]*Clinical Trials and Practice Support Division, Pathology Consultation Service, Center for Cancer Control and Information Services, National Cancer Center, Tokyo, 104-0045, Japan*
[b]*Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo 104-0045, Japan*
[c]*Department of Breast Surgery, National Cancer Center Hospital, Tokyo 104-0045, Japan*
[d]*Division of Breast and Medical Oncology, National Cancer Center Hospital, Tokyo 104-0045, Japan*
[e]*Cancer Genomics Project, National Cancer Center Research Institute, Tokyo 104-0045, Japan*
[f]*Clinical Laboratory Division, National Cancer Center Hospital, Tokyo 104-0045, Japan*

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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* Corresponding author.
  E-mail address: thasebe@ncc.go.jp (T. Hasebe).

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 ≤ 20, >20-≤ 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
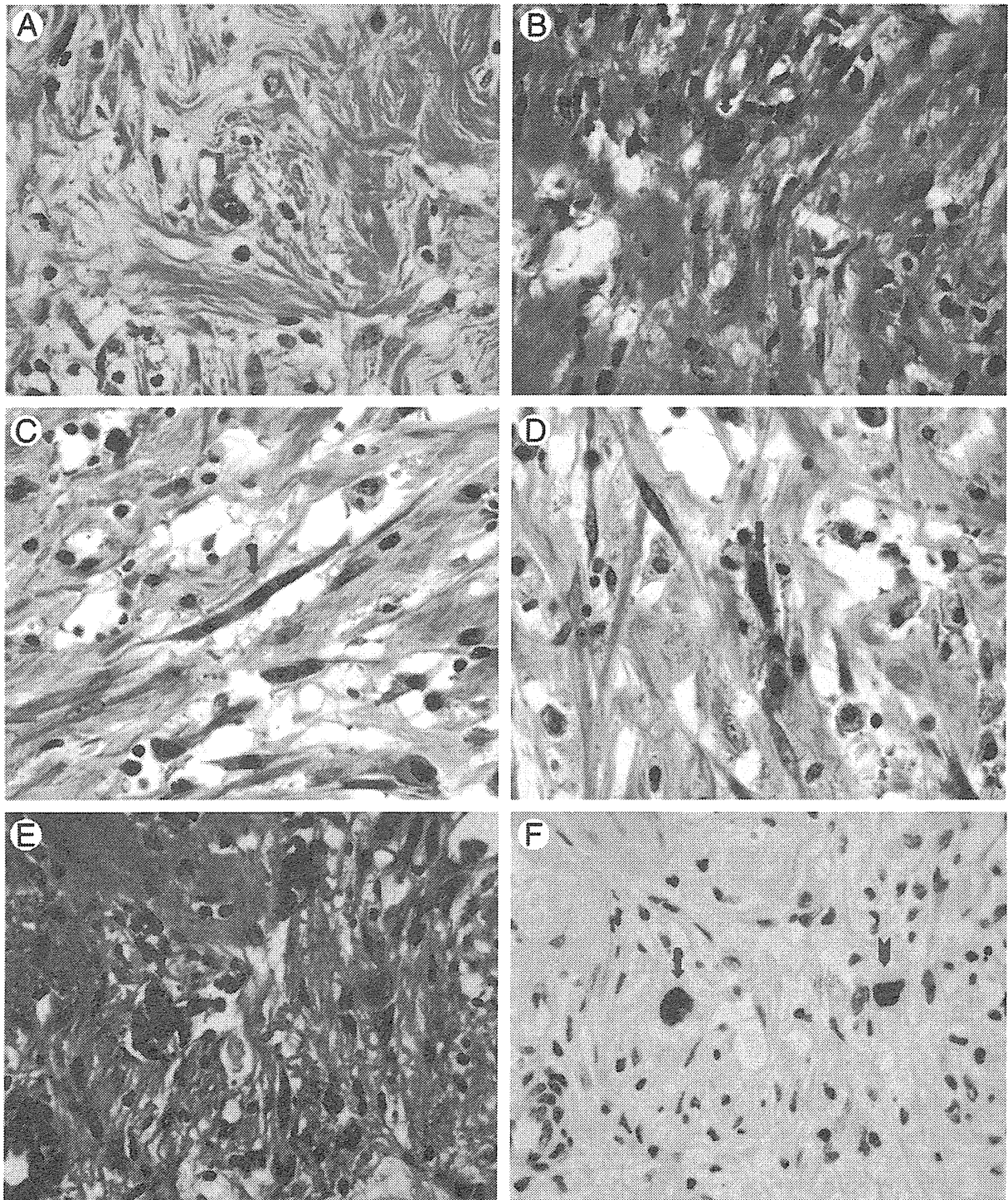
Fig. 1 Histologic features of atypical tumor-stromal fibroblasts in the tumor stroma (A-F). A, One atypical tumor-stromal fibroblast with bizarre and convoluted large nucleus containing 2 large-eosinophilic nucleoli is visible (arrows). B, One atypical tumor-stromal fibroblast containing a bizarre and 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).

8 factors: the Allred scores for ER, PR, and p53 in the tumor cells; the Allred scores for p53 in the tumor-stromal fibroblasts; the HER2 category of the tumor cells; the patient age (<39 years, >39 years); the type of neoadjuvant therapy (endocrine therapy, chemotherapy, and chemoendocrine therapy); the response to neoadjuvant therapy (pathological complete response or no pathological complete response); and adjuvant therapy (no, yes). Factors that were significantly associated with outcome in the univariate analyses using the Cox proportional hazard regression model were then entered together into the multivariate analyses according to nodal status or hormone receptor status. Because immunohistochemical examinations for ER, PR, p53, and HER2 were performed using both biopsy specimens obtained before neoadjuvant therapy and surgical specimens obtained after neoadjuvant chemotherapy, to accurately assess the prognostic value of each of these factors in multivariate analyses, their mutual influence on outcome was avoided by conducting separate analyses of the prognostic predictive powers of the findings in the biopsy specimens obtained before neoadjuvant therapy and the findings in the surgical specimens obtained after neoadjuvant therapy (model 1, immunohistochemical findings assessed based on biopsy specimens obtained before neoadjuvant therapy; model 2, immunohistochemical findings assessed based on surgical specimens obtained after neoadjuvant therapy). The case-wise and step-down method was applied until all of the remaining factors were significant at a P value less than .05. All analyses were performed using Statistica/Windows software (StatSoft, Tulsa, OK).

3. Results

3.1. Factors significantly associated with the presence of atypical tumor-stromal fibroblasts

The association between the presence of atypical tumor-stromal fibroblasts assessed using biopsy materials and the Allred scores for p53 was marginally significant for tumor-stromal fibroblasts assessed using biopsy materials (Table 1, Fig. 1F) and significantly for tumor cells assessed using surgical materials (P = .032). Other factors, such as the Allred scores for p53 in the tumor cells assessed using biopsy

Table 1 Association between the presence or absence of atypical tumor-stromal fibroblasts and Allred scores for p53 in tumor-stromal fibroblasts

	Cases	Absent (%)	Present (%)	P
Atypical tumor-stromal fibroblasts assessed using biopsy materials				
Allred scores for p53 in tumor-stromal fibroblasts assessed using biopsy materials				
0 or 2	86	86 (32)	0	.059
3	61	58 (21)	3 (43)	
4-8	132	128 (47)	4 (57)	
Total	279	272	7	
Atypical tumor-stromal fibroblasts assessed using surgical materials				
Allred scores for p53 in tumor-stromal fibroblasts assessed using surgical materials				
pCR	34	33 (11)	1 (2)	<.001
0 or 2	142	137 (49)	5 (23)	
3	39	38 (14)	1 (2)	
4 - 8	88	72 (26)	16 (73)	
Total	304	282	22	

Abbreviation: pCR, pathological complete response.

O : Invasive ductal carcinomas without atypical tumor-stromal fibroblast
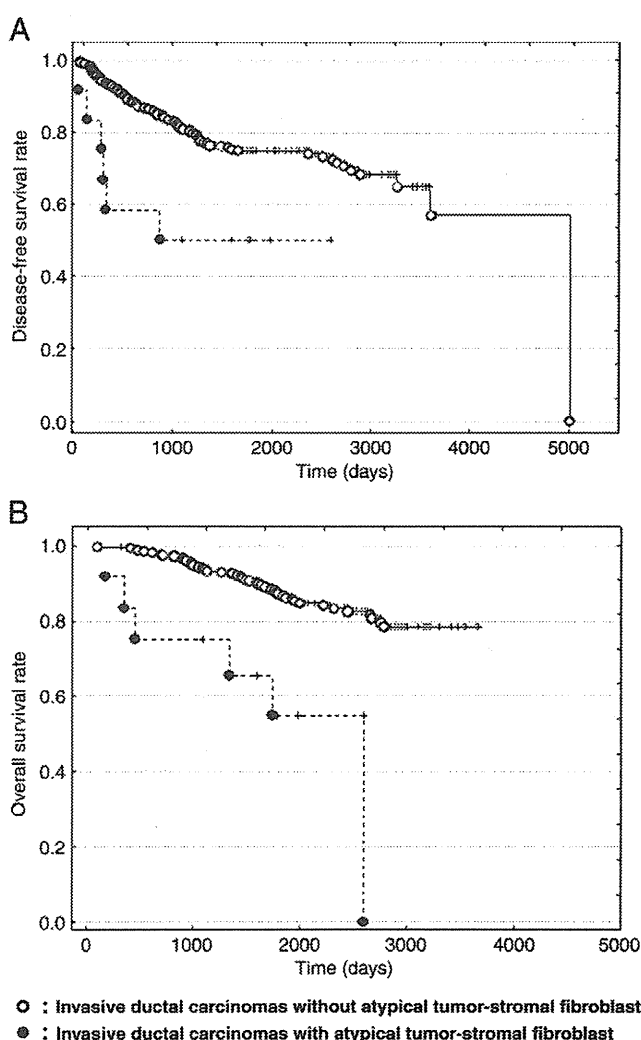● : Invasive ductal carcinomas with atypical tumor-stromal fibroblast

Fig. 2 Disease-free survival curves and overall survival curves of patients with IDC who received neoadjuvant therapy as a whole determined according to the absence or presence of atypical tumor-stromal fibroblasts assessed using biopsy materials (A and B). Patients with IDC with atypical tumor-stromal fibroblasts have a significantly shorter disease-free survival time (A) and overall survival time (B) than do patients with IDC without atypical tumor-stromal fibroblasts.

materials, were not significantly associated with the presence of atypical tumor-stromal fibroblasts assessed using biopsy materials (data not shown).

The presence of atypical tumor-stromal fibroblasts assessed using surgical materials was significantly associated with the residual invasive tumor size ($P < .001$), the presence of skin invasion ($P = .015$), the histologic grade ($P < .001$), the grading system for lymph vessel tumor emboli ($P = .015$), the UICC pN category ($P = .045$), the Allred scores for ER in tumor cells assessed using biopsy materials ($P = .003$) and surgical materials ($P < .001$), the Allred scores for PR in tumor cells assessed using biopsy materials ($P < .001$) and surgical materials ($P < .001$), and the Allred scores for p53 in tumor-stromal fibroblasts assessed using surgical materials (Table 1, $P < .001$). No significant associations were observed between the presence of atypical tumor-stromal fibroblasts assessed using surgical materials and other factors (data not shown).

3.2. Multivariate analyses for outcome predictive power of the presence of atypical tumor-stromal fibroblasts assessed using biopsy materials

The presence of atypical tumor-stromal fibroblasts significantly increased the hazard ratios (HRs) for tumor recurrence and tumor-related death in models 1 and 2 of the multivariate analyses in the patients with IDC overall (Fig. 2), in the patients with IDC with nodal metastasis, and in the patients with IDC positive for both ER and PR or positive for either ER or PR (Table 2).

Among the patients with IDC negative for both ER and PR, because the Allred scores for ER and PR in the tumor cells, the Allred scores for p53 in the tumor cells, and the HER2 category assessed using biopsy materials showed no significant increase in the HRs for tumor recurrence and tumor-related death in the univariate analyses (data not shown), only the model 2

Table 2 Multivariate analyses for tumor recurrence and tumor-related death rates according to the absence or presence of atypical tumor-stromal fibroblasts assessed using biopsy materials

Atypical fibroblast IDC patients as a whole	Cases	TRR (%)	HR (95% CI)	P	MR (%)	HR (95% CI)	P
Model 1							
Absent	273	78 (29)	Referent	.008	45 (17)	Referent	.006
Present	12	6 (50)	3.5 (1.4-9.0)		6 (50)	4.3 (1.5-12.0)	
Model 2							
Absent	273	78 (29)	Referent	.028	45 (17)	Referent	.002
Present	12	6 (50)	3.5 (1.1-11.1)		6 (50)	5.3 (1.8-15.2)	
Total	285	84 (30)			51 (18)		
IDC patients with nodal metastasis							
Model 1							
Absent	148	63 (43)	Referent	.010	36 (24)	Referent	.025
Present	8	6 (75)	5.1 (1.5-17.5)		6 (75)	5.2 (1.2-22.4)	
Model 2							
Absent	148	63 (43)	Referent	.008	36 (24)	Referent	.006
Present	8	6 (75)	8.3 (1.8-38.8)		6 (75)	8.1 (1.9-35.1)	
Total	156	69 (44)			42 (27)		
IDC patients positive for both ERs and PRs or positive for either ERs or PRs							
Model 1							
Absent	190	52 (27)	Referent	.020	26 (14)	Referent	.037
Present	8	3 (38)	4.4 (1.3-15.6)		3 (38)	6.4 (1.1-36.8)	
Model 2							
Absent	190	52 (27)	Referent	.021	26 (14)	Referent	.005
Present	8	3 (38)	5.8 (1.3-26.4)		3 (38)	14.4 (2.3-90.2)	
Total	198	55 (28)			29 (15)		
IDC patients negative for both ER and PR							
Model 2							
Absent	83	26 (31)	Referent	.035	19 (23)	Referent	.004
Present	4	3 (75)	10.1 (1.2-86.1)		3 (75)	32.2 (3.1-331.0)	
Total	87	29 (33)			22 (25)		

NOTE. Model 1: immunohistochemical findings were assessed based on biopsy specimens obtained before neoadjuvant therapy. Model 2: immunohistochemical findings were assessed based on surgical specimens obtained after neoadjuvant therapy.
Abbreviations: Atypical fibroblast, atypical tumor-stromal fibroblast; TRR, tumor recurrence rate; CI, confidence interval; MR, mortality rate.

Table 3   Multivariate analyses for tumor recurrence and tumor-related death rates according to the absence or presence of atypical tumor-stromal fibroblasts assessed using surgical materials

Atypical fibroblast IDC patients as a whole	Cases	TRR (%)	HR (95% CI)	P	MR (%)	HR (95%CI)	P
Model 1							
pCR	34	3 (9)	Referent	.002	3 (9)	Referent	.712
Absent	258	77 (30)	Referent		48 (19)	Referent	
Present	22	16 (73)	2.9 (1.5-5.8)		11 (50)	0.8 (0.3-2.2)	
Model 2							
pCR	34	3 (9)	Referent	.055	3 (9)	Referent	.772
Absent	258	77 (30)	Referent		48 (19)	Referent	
Present	22	16 (73)	2.0 (0.9-3.9)		11 (50)	0.1 (0.3-2.2)	
Total	314	96 (31)			62 (20)		
IDC patients without nodal metastasis							
Model 1							
pCR	34	3 (9)	Referent	.002	3 (9)	Referent	.007
Absent	98	12 (12)	Referent		5 (5)	Referent	
Present	4	3 (75)	7.7 (2.2-30.0)		3 (75)	7.9 (1.7-35.8)	
Model 2							
pCR	34	3 (9)	Referent	.092	3 (9)	Referent	.168
Absent	98	12 (12)	Referent		5 (5)	Referent	
Present	4	3 (75)	3.6 (0.8-15.5)		3 (75)	3.4 (0.6-20.0)	
Total	136	18 (13)			11 (8)		
IDC patients with nodal metastasis							
Model 1							
Absent	160	65 (41)	Referent	.009	43 (27)	Referent	.409
Present	18	13 (72)	3.0 (1.3-6.8)		8 (44)	0.6 (0.2-2.2)	
Model 2							
Absent	160	65 (41)	Referent		43 (27)	Referent	.198
Present	18	13 (72)	1.5 (0.7-3.0)	.291	8 (44)	0.4 (0.1-1.5)	
Total	178	69 (44)			42 (27)		
IDC patients positive for both ERs and PRs or positive for either ERs or PRs							
Model 1							
pCR	15	1 (7)	Referent	.143	1 (7)	Referent	.777
Absent	194	55 (28)	Referent		30 (15)	Referent	
Present	8	6 (75)	2.6 (0.7-9.0)		4 (50)	0.8 (0.1-4.4)	
Model 2							
pCR	15	1 (7)	Referent	.437	1 (7)	Referent	.815
Absent	194	55 (28)	Referent		30 (15)	Referent	
Present	8	6 (75)	1.7 (0.4-6.7)		4 (50)	0.8 (0.1-4.5)	
Total	217	62 (29)			35 (16)		
IDC patients negative for both ER and PR							
Model 2							
pCR	19	2 (11)	Referent	.869	2 (11)	Referent	.744
Absent	64	22 (34)	Referent		18 (28)	Referent	
Present	14	10 (71)	1.1 (0.2-5.4)		7 (50)	1.3 (0.3-6.8)	
Total	97	34 (35)			27 (28)		

NOTE. Model 1: immunohistochemical findings were assessed based on biopsy specimens obtained before neoadjuvant therapy. Model 2: immunohistochemical findings were assessed based on surgical specimens obtained after neoadjuvant therapy.

Abbreviations: Atypical fibroblast, atypical tumor-stromal fibroblast; TRR, tumor recurrence rate; CI, confidence interval; MR, mortality rate; pCR, pathological complete response.

multivariate analysis was performed. The presence of atypical tumor-stromal fibroblasts significantly increased the HRs for tumor recurrence and tumor-related death in the multivariate analyses (Table 2).

In IDC patients without nodal metastasis, the presence of atypical tumor-stromal fibroblasts was noticed in 4 patients; none of these patients exhibited tumor recurrence.

3.3. Multivariate analyses for outcome predictive power of the presence of atypical tumor-stromal fibroblasts assessed using surgical materials

In the IDC patients overall, although the presence of atypical tumor-stromal fibroblasts significantly increased the HR for tumor recurrence in model 1 and marginally significantly increased the HR for tumor recurrence in model 2, the presence of atypical tumor-stromal fibroblasts failed to significantly increase the HR for tumor-related death in models 1 and 2 of the multivariate analyses (Table 3).

In the IDC patients without nodal metastasis, the presence of atypical tumor-stromal fibroblasts significantly increased the HRs for tumor recurrence and tumor-related death in model 1 but failed to significantly increase the HRs for tumor recurrence and tumor-related death in model 2 of the multivariate analyses (Table 3).

In the IDC patients with nodal metastasis, the presence of atypical tumor-stromal fibroblasts significantly increased the HR for tumor recurrence but failed to significantly increase the HR for tumor-related death in model 1 of the multivariate analyses (Table 3). In model 2, the presence of atypical tumor-stromal fibroblasts failed to significantly increase the HRs for tumor recurrence and tumor-related death in the multivariate analyses (Table 3).

In the IDC patients who were positive for both ER and PR or positive for either ER or PR and in the IDC patients who were negative for both ER and PR, the presence of atypical tumor-stromal fibroblasts failed to significantly increase the HRs for tumor recurrence or tumor-related death in models 1 and 2 of the multivariate analyses (Table 3).

4. Discussion

This study clearly demonstrated that the presence of atypical tumor-stromal fibroblasts within the tumor stroma is a definitely useful histologic feature for accurately predicting the degree of malignant potential of IDCs treated with neoadjuvant therapy, and the presence of atypical tumor-stromal fibroblasts assessed using biopsy materials is the most important outcome predictive histologic feature of patients with IDC who received neoadjuvant therapy. On the other hand, the presence of atypical tumor-stromal fibroblasts assessed using surgical materials could not maintain a significant increase in the HRs for tumor recurrence and tumor-related death in the

multivariate analyses in this study. The presence of atypical tumor-stromal fibroblasts assessed using surgical materials was significantly associated with many factors, such as the lymph vessel tumor grade and the Allred scores for ER and PR assessed using both biopsy and surgical materials, whereas the presence of atypical tumor-stromal fibroblasts assessed using biopsy materials was only significantly associated with the Allred scores for p53 in tumor cells assessed using surgical materials. Thus, significant mutual relationships between the presence of tumor-stromal fibroblasts assessed using surgical materials and other factors may weaken the outcome predictive power of the presence of atypical tumor-stromal fibroblasts assessed using surgical materials in the multivariate analyses in this study. It can therefore be concluded that pathologists should assess the presence or absence of atypical tumor-stromal fibroblasts in the tumor stroma using biopsy materials to accurately predict the outcome of patients with IDC who received neoadjuvant therapy.

Several studies have reported that tumor-stromal fibroblasts, so-called cancer-associated fibroblasts, play important roles in the tumor progression of various types of carcinomas [1-3,19,20]. We previously reported that the biological characteristics of tumor-stromal fibroblasts are closely associated with the nodal metastasis or distant-organ metastasis of IDC [4,5] and also recently reported that p53 expression in tumor-stromal fibroblasts is closely associated with the outcome of IDC patients who had or who had not received neoadjuvant therapy [6,7]. However, we and other authors have not previously described a specific histologic feature of tumor-stromal fibroblasts that was significantly associated with the outcome of patients with IDC of the breast. Thus, pathologists could not assess the degree of malignant potential of IDC from the viewpoint of the histologic features of tumor-stromal fibroblasts during routine pathological examinations for patients with IDC. Therefore, this study clearly demonstrated that a detailed histologic examination of the nuclei of tumor-stromal fibroblasts enables accurate predictions of the degree of the malignant potential of IDCs of the breast that were treated with neoadjuvant therapy.

A significant association between the presence of atypical tumor-stromal fibroblasts assessed using surgical materials and the Allred scores for p53 in tumor-stromal fibroblasts assessed using surgical materials was observed, and a marginally significant association between the presence of atypical tumor-stromal fibroblasts assessed using biopsy materials and the Allred scores of p53 in tumor-stromal fibroblasts assessed using biopsy materials was also observed in this study. These findings clearly indicate that the presence of atypical nuclear features in tumor-stromal fibroblasts 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 [21,22]. However, some studies have not

reported any p53 mutations in the tumor stroma of breast cancers [23,24]. Although the presence or absence of p53 gene abnormalities in tumor-stromal fibroblasts is a matter of debate, p53 gene abnormalities or the specific reactive changes of p53 immunoreactivity in tumor-stromal fibroblasts produced by tumor cell–stromal cell interactions probably produce tumor-stromal fibroblasts expressing p53 and some tumor-stromal fibroblasts expressing p53 probably transform into atypical tumor-stromal fibroblasts and play important roles in the tumor progression of IDCs treated with neoadjuvant therapy.

In conclusion, this is the first study to clearly demonstrate definite histologic features of tumor-stromal fibroblasts that are closely associated with the outcome of patients with IDC of the breast who received neoadjuvant therapy. The presence of atypical tumor-stromal fibroblasts may provide pathologists or clinicians with more precise information regarding the malignant potential of IDCs of the breast treated with neoadjuvant therapy.

Acknowledgments

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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

K. Tamura[1]*, C. Shimizu[1], T. Hojo[2], S. Akashi-Tanaka[2], T. Kinoshita[2], K. Yonemori[1], T. Kouno[1], N. Katsumata[1], M. Ando[1], K. Aogi[3], F. Koizumi[4], K. Nishio[5] & Y. Fujiwara[1]

[1]Departments of Breast and Medical Oncology and [2]Breast Surgery, National Cancer Center Hospital, Tokyo; [3]Department of Breast Oncology, National Hospital Organization Shikoku Cancer Center, Ehime; [4]Shien Lab, National Cancer Center Hospital, Tokyo; [5]Department of Genome Biology, Kinki University School of Medicine, Osaka, Japan

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]

*Correspondence to: Dr K. Tamura, Department of Breast and Medical Oncology, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan. Tel: +81-3-3542-2511; Fax: +81-3-3542-3815; E-mail: ketamura@ncc.go.jp

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 $\geq$ 20 years, Eastern Cooperative Oncology Group performance status of 0–2, and adequate organ function (white blood cell count $\geq$ 4000/μl, platelet count $\geq$ 100 000/μl, hemoglobin concentration $\geq$ 9.0 g/dl, serum bilirubin $\leq$ 2.0 mg/dl, aspartate aminotransferase and alanine aminotransferase $\leq$ 100 IU/l, serum creatinine $\leq$ institutional upper limit of normal range, PaO$_2$ $\geq$ 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.

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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		M setting	
	No.	%	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.

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Table 3. FcγR polymorphisms and pathological responses to trastuzumab in an N setting

Polymorphism	Patients	Pathological response (grade)							
		1a		1b		2		3 (pCR)	
		No.	%	No.	%	No.	%	No.	%
FcγR2A									
H/H	7	0	0	0	0	2	29	5	71
H/R	6	1	17	3	50	2	33	0	0
R/R	2	1	50	0	0	1	50	0	0
Fisher's exact test: P		0.015							
Chi-square test (pCR versus others): P^a		0.007							
Linear correlation test: P		0.0076							
ANOVA test: P		0.0088							
FcγR3A									
V/V	7	0	0	1	14	2	29	4	57
F/V	6	1	17	2	33	2	33	1	17
F/F	2	1	50	0	0	1	50	0	0
Fisher's exact test: P		0.45							
Chi-square test (pCR versus others): P^a		0.12							
Linear correlation test: P		0.069							
ANOVA test: P		0.16							

[a]Comparison of H/H versus R carrier (H/R + R/R) or V/V versus F carrier (F/V + F/F).

N, neoadjuvant; FcγR, fragment C γ receptor; pCR, pathological complete response; ANOVA, analysis of variance.

Table 4. FcγR polymorphisms and tumor responses to trastuzumab in an M setting

Polymorphism	Patients	Response					
		CR/PR		SD		PD	
		No.	%	No.	%	No.	%
FcγR2A							
H/H	15	6	40	7	47	2	13
H/R	18	2	12	8	44	8	44
R/R	2	0	0	0	0	2	100
Fisher's exact test: P		0.043					
Chi-square test (CR/PR versus SD/PD): P^a		0.051					
Linear correlation test: P		0.0077					
ANOVA test: P		0.029					
FcγR3A							
V/V	15	6	40	5	33	4	27
F/V	17	1	6	10	59	6	35
F/F	3	1	33	0	0	2	67
Fisher's exact test: P		0.053					
Chi-square test (CR/PR versus SD/PD): P^a		0.051					
Linear correlation test: P		0.12					
ANOVA test: P		0.16					

[a]Comparison between H/H versus R carrier (H/R + R/R) or V/V versus F carrier (F/V + F/F).

M, metastatic; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; FcγR, fragment C γ receptor; ANOVA, analysis of variance.

The median PFS time was 6.4 months (95% CI, 3.9–8.6 months). The PFS of patients with FcγR2A-131 H/H was significantly longer than that of patients with 131 H/R or R/R. (Figure 1A: 9.2 versus 3.5 months, P = 0.034). In contrast, no statistical difference in the PFS of patients with FcγR3A-158 V/V and that of patients with 158 F/V or F/F was observed (Figure 1B: 8.5 versus 5.3 months, P = 0.37). Linkage disequilibrium analyses were conducted among the two FcγR polymorphisms (Table 5). The incidence of the FcγR2A-131 genotype was associated with that of the FcγR3A-158 genotype according to a Fisher's exact test, a chi-square test, and a linear correlation test.

discussion

The overexpression of HER2 protein is observed in ~20–30% of patients with breast cancer and is correlated with a poor clinical outcome. Trastuzumab is an IgG1-type humanized HER2 mAb that has been shown to exhibit significant clinical efficacy as a treatment of MBC [27] and as an adjuvant treatment of operable breast cancer [28]. However, the clinical effectiveness of trastuzumab is somewhat limited: the response rate to single-agent trastuzumab as a first-line treatment is 20–30%; the pCR rate to neoadjuvant therapy including

trastuzumab is ~30%. A substantial numbers of HER2-positive tumors exhibit de novo resistance to trastuzumab; therefore, the development of biomarkers to select patients who might benefit from trastuzumab is warranted, as a way of decreasing toxicity and reducing unnecessary cost.

The principal mechanism of action of trastuzumab is HER2 blockade with the inactivation of the signal transduction pathway, leading to apoptosis. ADCC is another not insignificant and generally accepted mechanism of trastuzumab action. In ADCC, the cytotoxicity of mAbs that target tumor cells, is mediated by immune effector cells that express FcγR. Recently, two FcγR gene polymorphisms have been identified that affect the binding affinity of IgG, thus changing the effectiveness of ADCC and affecting tumor response. FcγR3A-158 V/V, either alone or in combination with the FcγR2A-131 H/H genotype, was significantly associated with a better response and PFS among patients with follicular lyoma [5, 6] and among MBC patients [14] treated with rituximab- or trastuzumab-based therapy, respectively. Inconsistent data have been reported in metastatic colorectal cancer patients who had not responded to previous irinotecan- or oxaliplatin-based therapy and were subsequently treated with single-agent cetuximab [29]. The
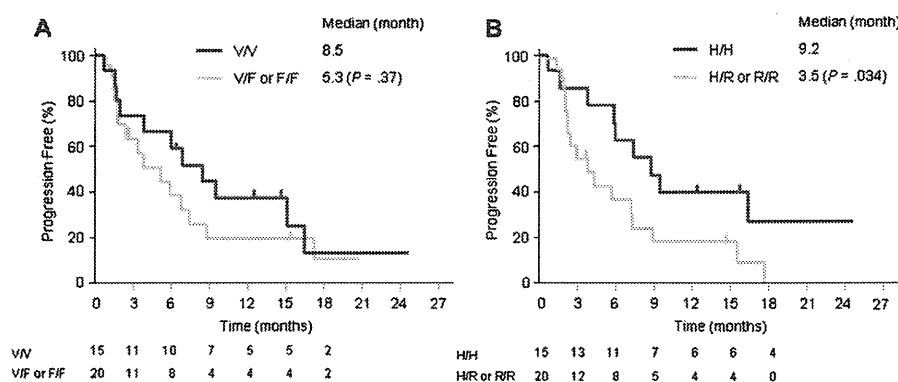
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Figure 1. Progression-free survival for patients with metastatic breast cancer receiving single-agent trastuzumab categorized according to fragment C γ receptor (FcγR) polymorphisms. (A) Progression-free survival (PFS) curves were plotted for FcγR2A-131 H/H and H/R or R/R carriers. (B) PFS curves were plotted for FcγR3A-158 V/V and F/V or F/F carriers. V, valine allele; F, phenylalanine allele; H, histidine allele; R, arginine allele.

Table 5. Linkage analysis between FcγR2A and 3A alleles

	Patients	FcγR3A					
		V/V		F/V		F/F	
		No.	%	No.	%	No.	%
FcγR2A							
H/H	22	13	59	9	41	0	0
H/R	24	8	33	13	54	3	13
R/R	4	1	25	1	25	2	50
Total	50	22	44	23	46	5	10
Fisher's exact test: P		0.030					
Chi-square test (V/V versus F carrier) P^a		0.020					
Linear correlation test: P		0.0067					

[a]Comparison between H/H versus R carrier.
FcγR, fragment C γ receptor.

influence of cytotoxic agents in combination with antibody therapy or the retrospective natures of these analyses might explain the previous inconsistencies. In the present prospective study, the FcγR2A-131 H/H genotype was significantly associated with a stronger tumor response and a longer PFS, and the FcγR3A-158 V/V genotype tended to be correlated with the tumor response after single-agent trastuzumab therapy.

Metastatic cancer patients mostly have suppressed immune function. Thus, early-stage breast patients treated with trastuzumab might be more sensitive to ADCC activity. In the current study, we have demonstrated for the first time that the FCγR2A-131 H/H genotype was significantly correlated with the pathological response after neoadjuvant trastuzumab-based treatment. Our data suggest that this genotype was correlated with not only the pCR rate but also the gradation of the response based on a precise assessment of pathological responses using established histopathological criteria (grade 1a–3; Table 3). A recent large adjuvant trial with trastuzumab-based therapy [15] has raised questions regarding the usefulness of the two FcγR SNPs as predictive biomarkers for recurrence. The sample size of this trial was relatively large; thus, the results seemed to be

confirmatory. However, one possible explanation for this difference is that ADCC might be influenced by the existence of a target tumor volume. Another possible explanation is that the cytotoxic agents might influence outcome. Theoretically, the clinical efficacy of trastuzumab is based on both the direct blockade of signal transduction and its indirect effect, ADCC. On the other hand, the efficacy of cytotoxic agents is based on the direct DNA damaging effect and not on ADCC. Thus, the change in ADCC induced by different SNPs might be diluted in cases where trastuzumab and cytotoxic agents are combined, with cases in trastuzumab is used singly.

In this study, we examined 384 SNPs at FcγRI, RII, RIII, HER2 and FUT8 loci. Our findings demonstrated that only two SNP hot spots were correlated with the clinical efficacy of trastuzumab, indicating a high specificity. Our finding that the incidences of the two FCγR2A-131 H/H and FcγR3A-158 V/V genotypes were moderately linked with each other is inconsistent with a previous report [14]. One possible explanation for this discrepancy might be ethnical differences in SNP frequency. Zhang et al. [29] showed that the FCγR2A-131 H/H and FcγR3A-158 V/V genotypes were more frequent among Asian populations than among Western populations. Statistical approaches, including a linear correlation or ANOVA test, suggested that heterozygosity for the two SNPs might have a minimal effect on ADCC activity. Additional studies evaluating the relationship between ADCC activity and the SNP status are needed.

In conclusion, this study supports the hypothesis that FcγR polymorphisms play a role in trastuzumab-mediated ADCC and can predict the clinical outcome of patients with both early and MBC in Asian populations.

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original article

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disclosure

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Prognostic Factors in Young Japanese Women with Breast Cancer: Prognostic Value of Age at Diagnosis

Miwa Yoshida[1,2,*], Chikako Shimizu[3], Takashi Fukutomi[2], Hitoshi Tsuda[4], Takayuki Kinoshita[1], Sadako Akashi-Tanaka[1], Masashi Ando[3], Takashi Hojo[1] and Yasuhiro Fujiwara[3]

[1]Division of Breast Surgery, National Cancer Center Hospital, Tokyo, [2]Division of Breast and Endocrine Surgery, Aichi Medical University, Aichi, [3]Division of Breast and Medical Oncology, National Cancer Center Hospital and [4]Division of Diagnostic Pathology, National Cancer Center Hospital, Tokyo, Japan

*For reprints and all correspondence: Miwa Yoshida, Division of Breast and Endocrine Surgery, Aichi Medical University, 21 Nagakute-cho, Aichi-gun, Aichi 480-1195, Japan. E-mail: miwyoshi@aichi-med-u.ac.jp

Objective: The primary objective of this study was to verify whether breast cancer patients aged <35 at diagnosis have poorer prognoses than those aged 35–39, in other words, to identify the prognostic value of age in younger premenopausal patients under 40 years old. The secondary objective was to assess prognostic factors specific for younger premenopausal patients.

Methods: We identified 242 consecutive patients who were diagnosed with stage I–III breast cancer before the age of 40 and underwent surgery between 1990 and 2004. We compared disease-free survival and overall survival in patients aged <35 years and those aged 35–39 years, and evaluated clinicopathological factors associated with disease-free survival or overall survival in each age group and in all patients under the age of 40.

Results: Ninety-nine (41%) patients were younger than 35 years and 143 (59%) were between 35 and 39 years. No significant difference in disease-free survival or overall survival was found between the two groups. In our cohort of patients under the age of 40, the independent factors associated with poor disease-free survival and overall survival included positive axillary lymph nodes and triple-negative status, but not age at diagnosis. Adverse prognostic factors also did not differ considerably between the two age groups.

Conclusions: Age at diagnosis was not an independent prognostic factor in our study. Our findings suggest that other clinicopathological features rather than age should be used to determine individualized treatment courses for breast cancer patients younger than 40 years.

Key words: breast cancer — young — disease-free survival — overall survival

INTRODUCTION

Many studies have reported that younger women with primary breast cancer have poorer prognoses than older women. The St Gallen international expert consensus reports from 1998 to 2007 concluded the age of <35 years was a high-risk factor for relapse in node-negative breast cancer patients and recommended adjuvant chemotherapy for most young women with breast cancer (1–5). However, the decision regarding chemotherapy in young patients must be made after taking into consideration not only the risk of relapse but also the age-specific problems caused by chemotherapy such as infertility, bone loss and changes in sexual function and appearance.

The cutoff value for classifying a patient as 'young' varies among studies and it is unclear whether the age of <35 years at diagnosis was an appropriate threshold to identify patients with primary breast cancer at high risk of relapse. It also remains to be determined whether Japanese patients aged <35 years at diagnosis have poorer prognoses since there have been few reports focusing on young Japanese women with breast cancer.

Prognostic factors in younger patients with primary breast cancer have been recently identified, but are not yet well

understood. A recent study showed that gene expression profile was a powerful predictor of disease outcome in young patients with breast cancer, but age was not an independent prognostic factor (6).

Gene expression profiling has identified intrinsic breast cancer subtypes that predict distinct clinical outcomes (7,8). In particular, triple-negative breast cancer, defined by the lack of expression of estrogen receptor (ER), progesterone receptor (PgR) and human epidermal growth factor receptor 2 (HER2), is known to be a subtype associated with poor clinical outcome. A high prevalence of triple-negative breast cancer has been reported to contribute to the poor prognosis of young African American women with breast cancer (9).

The primary objective of this study was to verify whether breast cancer patients aged <35 at diagnosis have poorer prognoses than those aged 35–39, in other words, to identify the prognostic value of age in younger premenopausal patients under 40 years old. The secondary objective was to assess the prognostic factors specific for younger premenopausal patients.

PATIENTS AND METHODS

PATIENTS AND TREATMENT

From the database of the National Cancer Center Hospital, Tokyo, Japan, we identified consecutive patients who were diagnosed with breast cancer before the age of 40 years and underwent surgery between January 1990 and December 2004. Only patients with stage I–III disease who underwent definitive surgery were included. Patients who had undergone preoperative adjuvant therapy or had excisional biopsy in a local clinic were also excluded because it is difficult to determine pathological factors influencing prognoses.

The complete medical records of patients enrolled in the study were reviewed. Information derived from the database and medical records included clinical and histological variables such as age; family history; pT (primary tumor) and pN (regional lymph node) status; histological type; histological grade; peritumoral vessel invasion (PVI) [including lymphatic vessel invasion (LVI) and blood vessel invasion (BVI)]; ER, PgR and HER2; tumor subtype stratified by hormone receptor (HR) and HER2 status; operative procedure; radiation therapy; adjuvant systemic therapy (chemotherapy and endocrine therapy).

Familial breast cancer (that does not fit hereditary breast cancer definition) was defined as breast cancer with a family history of one or more first- or second-degree relatives with breast cancer prior to or at the time of the patient's initial diagnosis (10,11). In all cases, pT and pN status were assessed according to the UICC TNM classification (6th edition) (12). Histological grade was evaluated according to Elston and Ellis (13). ER and PgR expression were determined by enzyme immunoassay or immunohistochemistry (IHC) (threshold for positivity: staining in more than 10% of tumor cells) (14). The definition of HER2 positive was a

score 3+ by IHC (uniform, intense membrane staining in more than 10% of invasive cancer cells) and/or a 2.0 or higher of HER2/CEP17 (centromere probe chromosome 17) ratio by fluorescence *in situ* hybridization (15). On the basis of the expression profile of HR and HER2, all tumors were categorized into one of the four subtypes: HR+HER2−, HR+HER2+, HR−HER2+, HR−HER2− (triple-negative). HR-positive status (HR+) was defined as ER and/or PgR positivity, and HR-negative status (HR−) was defined as ER and PgR negativity. PVI was determined by the presence of tumor emboli within peritumoral endothelial-lined spaces and was assessed on hematoxylin and eosin-stained slides by making a distinction between lymphatic and blood vessels. LVI was graded as absent, focal to moderate (one to five foci of tumor thrombi in all the tumor specimens examined) or extensive (more than five foci of tumor thrombi in all the tumor specimens examined) (16). BVI was classified as either absent or present.

All patients received clinically necessary local treatment (breast-conserving surgery or mastectomy) in addition to sentinel node biopsy or complete axillary dissection. Postoperative breast irradiation was indicated for all patients who underwent breast-conserving surgery. After 1999, patients with pT3 presentation who had undergone mastectomy received postoperative radiation to the chest wall. Patients with four or more metastatic axillary lymph nodes received postoperative radiation to the axillary and supraclavicular regions. Adjuvant chemotherapy was followed by radiotherapy for all indicated patients. The adjuvant chemotherapy regimen widely used prior to 1993 comprised doxorubicin, cyclophosphamide (AC), methotrexate and 5-fluorouracil. After 1993, patients generally received four cycles of intravenous doxorubicin and AC. After 1999, high-risk patients received AC followed by taxane (docetaxel or paclitaxel). For women with endocrine-responsive disease aged <40 years, adjuvant endocrine therapy was indicated, such as tamoxifen for 2–5 years or the combination of tamoxifen for 5 years plus gonadotropin-releasing hormone analogues for at least 2 years. Patients who received adjuvant chemotherapy for endocrine-responsive disease were treated with tamoxifen immediately after the completion of chemotherapy.

Patients were followed up every 3–6 months during the first 5 years and every 6–12 months from 5 to 10 years. In addition to physical examination, annual mammography with or without breast ultrasound was performed for 10 years. Blood tests including two tumor markers (carcinoembryonic antigen and cancer antigen 15-3), chest X-ray, abdominal ultrasonography and bone scintigraphy were performed when the patients complained of any symptoms and/ or tumor recurrence was suspected.

The study was conducted with support from the Health and Science Grants for Clinical Research in Cancer, as part of the investigations directed by the Ministry of Health, Labor and Welfare of Japan. The data on which the study was based were obtained in the course of daily clinical