長期安定性（破損，緩み，再発，局所根治性）よりも，機能回復が早い手術侵襲の少ない安価な再建手術が望ましい。合併症の頻度は，骨盤周辺で15～20%，腫瘍用巨大人工関節置換術で約５%の創治癒不全，感染症が発生し，脊椎後方固定で２%，前方固定で10数%の感染，完全麻痺の発生は３%程度である。

ビスホスホネートの意義

消化器癌骨転移に対しての，ビスホスホネートの臨床的有用性を証明した研究は少なく，基礎的研究では，胃癌[18]，膵臓癌[19]の培養細胞に抗癌剤との併用で，抗腫瘍効果を認めるとの研究や，末期骨転移の疼痛軽減を認めたとの症例報告がされているものの，第２，３相臨床試験によるビスホスホネートの消化器癌骨転移に対する治療効果については明らかではない。化学療法により，生命予後が，２倍から３倍と改善していること，化学療法が実施され，その有用性が高まれば高まるほど，骨転移発症率が高くなる事実から，ビスホスホネートを積極的に併用することは意義が高い。今後の研究の結果が待たれる。

そのほかの緩和治療

低悪性度の消化器癌の骨転移は，低感受性，放射線治療抵抗性，とくに直腸癌は，その傾向が強い。IVRや画像支援技術の進歩により，セメント注入充塡，椎体形成術，RF消却術，術中IVR併用治療，塞栓，セメント充塡，手術＋RF波併用治療など，さまざまな治療が試みられているが，消化器癌の骨転移に対する有用性は確定していない。

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Original contribution

p53 expression in tumor-stromal fibroblasts is closely associated with the nodal metastasis and outcome of patients with invasive ductal carcinoma who received neoadjuvant therapy☆

Takahiro Hasebe MD, PhD[a,*], Nobuko Tamura MD[b], Nao Okada MD[b], Takashi Hojo MD[b], Sadako Akashi-Tanaka MD, PhD[b], Chikako Shimizu MD[c], Histoshi Tsuda MD, PhD[d], Tatsuhiro Shibata MD, PhD[e], Yuko Sasajima MD, PhD[d], Motoki Iwasaki MD, PhD[f], Takayuki Kinoshita MD, PhD[b]

[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]*Department of Breast Surgery, National Cancer Center Hospital, Tokyo 104-0045, Japan*
[c]*Division of Breast and Medical Oncology, National Cancer Center Hospital, Tokyo 104-0045, Japan*
[d]*Clinical Laboratory Division, National Cancer Center Hospital, Tokyo 104-0045, Japan*
[e]*Cancer Genomics Project, National Cancer Center Research Institute, Tokyo 104-0045, Japan*
[f]*Epidemiology and Prevention Division, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo 104-0045, Japan*

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Summary The purpose of this study was to determine whether p53 immunoreactivity in tumor-stromal fibroblasts assessed by the Allred scoring system in biopsy specimens obtained before neoadjuvant therapy and assessed in surgical specimens obtained after neoadjuvant therapy is significantly associated with nodal metastasis by invasive ductal carcinoma and with the outcome of 318 patients with invasive ductal carcinoma who received neoadjuvant therapy, according to UICC pathologic TNM stage, in multivariate analyses with well-known clinicopathologic factors. The Allred scores for p53 in tumor-stromal fibroblasts in the surgical specimens were significantly associated with the presence of nodal metastasis. The Allred scores for p53 in the tumor-stromal fibroblasts of biopsy and surgical specimens were a very important outcome predictive factor for patients who received neoadjuvant therapy, independent of UICC pathologic TNM status, but the outcome predictive power of the Allred scores for p53 in tumor-stromal fibroblasts assessed in the surgical specimens was superior to that of the Allred scores for p53 in tumor-stromal fibroblasts in the biopsy specimens. The results indicated a close association between p53 protein expression in tumor-stromal fibroblasts, especially in surgical

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

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specimens, and both the presence of nodal metastasis and the outcome of invasive ductal carcinoma patients who received neoadjuvant therapy.

1. Introduction

It has recently been reported that the gene expression profile and protein expression profile of the tumor stroma play a very important role in tumor progression in carcinoma [1-3] and that the interaction between tumor cells and stromal cells also plays a very important role in tumor progression by carcinoma [4,5]. We have already reported that the proliferative activity of tumor-stromal fibroblasts plays a very important role in nodal metastasis and distant organ metastasis by invasive ductal carcinoma (IDC) of the breast [6,7]. Recently, a high frequency of p53 mutations in tumor cells and the surrounding stroma has also been reported [8], and p53 mutations in breast cancer stromal cells have been reported to be closely associated with nodal metastasis [9]. These findings strongly suggest a significant role of the tumor stroma in tumor progression by IDC, and the p53 status of tumor-stromal fibroblasts may play a very important role in tumor progression by IDC.

The purpose of the present study was to determine whether p53 protein expression in tumor-stromal fibroblasts assessed in biopsy specimens obtained before neoadjuvant therapy and surgical specimens obtained after neoadjuvant therapy is significantly associated with the presence of nodal metastasis by IDC, and significantly associated with the outcome of IDC patients who received neoadjuvant therapy, according to the UICC (International Union Against Cancer) pathologic TNM (pTNM) stage. The results indicated that p53 protein expressions in tumor-stromal fibroblasts in both the biopsy specimens and the surgical specimens were closely associated with the presence of nodal metastasis and the outcome of IDC patients who received neoadjuvant therapy.

2. Materials and methods

2.1. Cases

The subjects of this study were 318 consecutive patients with IDC of the breast and who received neoadjuvant therapy before surgery at the National Cancer Center Hospital between January 2000 and December 2005. The IDCs were diagnosed preoperatively by needle biopsy, aspiration cytology, mammography, or ultrasonography. Clinical information was obtained from the patients' medical records after complete histologic examination of all IDCs. All patients were Japanese women, and they ranged in age from 26 to 75 years (median, 54 years). All had a solitary lesion; 127 patients were premenopausal and 191 were postmenopausal. Partial mastectomy had been performed in 152 and modified radical mastectomy in 166. Level I and level II axillary lymph node dissection had been performed in all patients, and level III axillary lymph node dissection had been performed in some of patients with IDC.

Of the 318 patients, 37 (12%) achieved a pathologic complete response (34, no residual tumor; 3, only residual ductal carcinoma in situ; they have no nodal metastasis) to neoadjuvant therapy.

The neoadjuvant therapy consisted of chemotherapy in 235 patients, endocrine therapy in 43 patients, and chemoendocrine therapy in 3 patients; and 214 of 281 patients received adjuvant therapy, which consisted of chemotherapy in 47 patients, endocrine therapy in 116 patients, and chemoendocrine therapy in 51 patients. The chemotherapy regimens used were anthracycline-based with or without taxane and non–anthracycline-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. There were no cases of inflammatory breast cancer in this series. All tumors were classified according to the UICC pTNM classification.

For the pathologic 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 tumor size of the surgically resected specimens was confirmed by comparison with the tumor size on histologic slides; if more than one invasive focus was present, the size of the largest invasive focus was recorded as the residual invasive tumor size in this study.

2.2. Histologic examination

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 examined histologically to confirm the diagnosis, and another section was subjected to immunohistochemistry. The following 9 histologic features of the primary invasive tumors were evaluated in the biopsy specimens obtained before neoadjuvant therapy and the surgical specimens obtained after neoadjuvant therapy: (1) residual tumor size (no residual tumor or residual ductal

carcinoma in situ; residual tumor ≤20 mm, >20 to ≤50 mm, >50 mm), (2) histologic grade (1, 2, 3) [10], (3) tumor necrosis (absent, present) [11], (4) fibrotic focus (FF) (biopsy specimen: absent, present; surgical specimen: absent; FF diameter ≤8 mm, FF diameter >8 mm) [12,13], (5) lymph vessel invasion (absent, present), (6) blood vessel invasion (absent, present), (7) adipose tissue invasion (absent, present), (8) skin invasion (absent, present), and (9) muscle invasion (absent, present). We also evaluated the outcome predictive power of Fisher's neoadjuvant-therapy-effect classification for surgical specimens obtained after neoadjuvant therapy [14,15].

2.3. Immunohistochemistry

Immunohistochemical staining for estrogen receptors (ERs), progesterone receptors (PRs), p53, and HER2 products was performed with autoimmunostainer (Optimax Plus; BioGenex, San Ramon, CA). Antigen retrieval device for Optimax Plus was autoclave and each specimen was immersed in citrate buffer and incubated at 121°C for 10 minutes. Immunoperoxidase staining was performed by using a labeled streptavidin biotin staining kit (BioGenex) according to the manufacturer's instructions. The antibodies used were mouse anti-ER monoclonal antibody (mAb), ER88 (BioGenex), mouse anti-PR mAb, PR88 (BioGenex), and mouse anti-HER2 mAb, CB11 (BioGnex) and mouse p53 mAb, DO7 (Dako, Glostrup, Denmark). ER88, PR88, and CB11 were already diluted and DO7 was applied at 1:100 dilution. After immunostaining, the sections were counterstained with hematoxylin. Sections of IDCs positive for ER, PR, HER2, and p53 were used each time as positive control. As a negative control, the primary antibody was replaced with normal mouse immunoglobulin.

2.4. Assessment of ER, PR, p53, and HER2 expression

Sections of biopsy specimens and surgical specimens immunostained for ER, PR, and p53 in tumor cells were scored by the Allred system as described previously [16-19]. In brief, each entire slide was evaluated by light microscopy as follows. First, one of the following proportion scores was assigned according to the estimated proportion of tumor cells that stained positive: 0, 0/100 (0%); 1, <1/100 (<1%); 2, 1/100 to 1/10 (1% to 10%); 3, >1/10 to 1/3 (>10% to 33%); 4, >1/3 to 2/3 (>33% to 67%); 5, >2/3 (>67%). Next, one of the following intensity scores was assigned according to the average intensity of staining by the positive tumor cells: 0, no staining; 1, weak; 2, intermediate; 3, strong. The proportion score and intensity score were then added to obtain a total score, with possible total scores ranging from 0 and 2 to 8. However, the number of tumor-stromal fibroblasts that express p53 in tumors is relatively small, and examination of the distribution of tumor-stromal fibroblasts expressing p53 shows that they are scattered even in IDCs with tumor-stromal fibroblasts having Allred scores of 4 to 8. We therefore modified the Allred scoring system to assess nuclear expression of p53 in tumor-stromal fibroblasts by identifying one field with the highest of both proportion score and intensity score for p53 nuclear expression in the whole tumor area by scanning the tumor section stained for p53 at medium-power field (×20 objective and ×10 ocular). The highest intensity score, not the average intensity score, for nuclear expression of p53 was assigned to the tumor-stromal fibroblast staining, and the highest p53 nuclear expression proportion score and intensity score were then evaluated in one high power field (×40 objective and ×10 ocular) (Fig. 1). 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 [20]. Immunohistochemistry was used to score 290 of the 318 IDCs for ER, PR, HER2, and p53 expression in biopsy specimens. In 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.

One author (T. H.) assessed all of the immunohistochemical parameters, and 1 of 3 other authors (H. T., T. S., or Y. S.) identified the immunohistochemical parameters to confirm the IDC immunohistochemical characteristics recorded by TH. Discordant results were reevaluated jointly to reach a consensus. The histologic examination and immunohistochemical examination were performed without knowledge of the patient's outcome.

2.5. Patient outcome and statistical analysis

Survival was evaluated by follow-up for a median period of 52 months (range, 18-102 months) until June 2008. At that time, 199 of the 281 patients were alive and well, 82 had developed tumor recurrence, and 24 had died of their disease. The measurements of tumor recurrence–free survival and overall survival started at the time of surgery. Tumor relapse was considered to have occurred whenever there was evidence of metastasis.

The correlation analyses were performed using Pearson correlation coefficients. The univariate and multivariate analyses for pathologic complete response were performed by using the logistic regression model for all patients. We analyzed the outcome predictive power for tumor recurrence and tumor-related death by the univariate and multivariate analyses using the Cox proportional hazard regression model. The factors analyzed were the mentioned 9 factors, age (≤39, >39 years), type of neoadjuvant therapy (endocrine therapy, chemotherapy, and chemoendocrine therapy), adjuvant therapy (no, yes), and the factors that were significantly associated with outcome in the univariate analyses were then entered together into the multivariate analyses according to UICC pTNM stage. Because the 9 factors were examined using both biopsy specimens obtained before neoadjuvant therapy and surgical specimens obtained

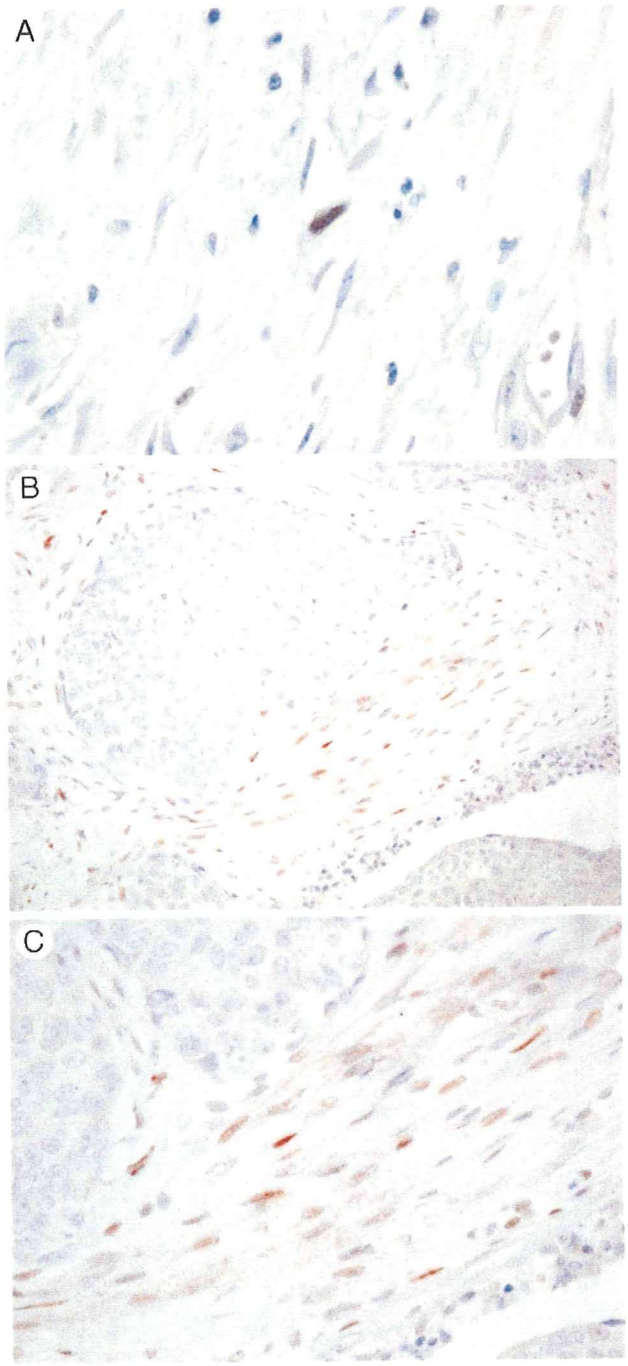

Fig. 1 p53 expression in tumor-stromal fibroblasts of IDCs. (A) Allred score of 3 for p53 in tumor-stromal fibroblasts. One tumor-stromal fibroblast shows moderately intense nuclear staining for p53 in the high-power field (magnification ×40). (B and C) Allred score of 7 for p53 in tumor-stromal fibroblasts. Several tumor-stromal fibroblasts show moderately intense nuclear staining for p53 at the medium-power field (B) and in the high-power field (C). None of the nuclei of the tumor cell have stained positive for p53 (B, magnification ×20; C, magnification ×40).

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 power of the findings in the biopsy specimens obtained before neoadjuvant therapy and the surgical specimens obtained after neoadjuvant therapy (model 1, factors examined based on biopsy specimens obtained before neoadjuvant therapy; model 2, factors examined 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. Because there were fewer than 10 tumor deaths among the patients with UICC pTNM stage 0 and I disease and the patients with UICC pTNM stage II disease, we were unable to perform multivariate analyses for tumor death in these groups. Survival curves were drawn by the Kaplan-Meier method. All analyses were performed with Statistica/Windows software (StatSoft, Tulsa, OK).

3. Results

3.1. Correlations between Allred scores for ER, PR, and p53, and HER2 category assessed in the biopsy specimens and assessed in the surgical specimens

The Allred scores for ER, PR, and p53 in tumor cells and HER2 category in the biopsy specimens were significantly correlated with the Allred scores for ER, PR, and p53 in tumor cells and HER2 category in tumor cells in the surgical specimens (ER: $r = 0.730$, $P < .001$; PR: $r = 0.407$, $P < .001$; p53: $r = 0.576$, $P < .001$; HER2, $r = 0.550$, $P < .001$). There were marginally significant correlations between the Allred scores for p53 in tumor-stromal fibroblasts assessed in the biopsy specimens and the Allred scores for p53 in tumor-stromal fibroblasts assessed in the surgical specimens ($r = 0.109$, $P = .088$) (Table 1, Fig. 1).

3.2. Analysis for nodal metastasis

Although the Allred scores for p53 in tumor-stromal fibroblasts in the biopsy specimens were not significantly associated with the presence of nodal metastasis, the Allred scores for p53 in tumor-stromal fibroblasts in the surgical specimens were significantly associated with the presence of nodal metastasis (Table 1).

3.3. Factors significantly associated with pathologic complete response

In the multivariate analysis, UICC pTNM-pathologic node (pN) category significantly decreased the trend values for the relative risk for pathologic complete response, and

Table 1 Nodal metastasis, tumor recurrence, and tumor-related deaths according to Allred scores for p53 in tumor-stromal fibroblasts in all patients with IDC who received neoadjuvant therapy

	Cases (%)	Number of patients (%)					
		Nodal metastasis		Tumor recurrence		Tumor-related death	
		Present	Absent	Present	Absent	Yes	No
Model 1			0.680		<0.001		0.035
Allred score	290	166 (57)	124 (43)	75 (26)	215 (74)	21 (7)	269 (93)
0	63 (22)	33 (52)	30 (48)	3 (5)	60 (95)	0	63 (100)
2	24 (8)	14 (58)	10 (42)	4 (17)	20 (83)	0	24 (100)
3	65 (22)	38 (58)	27 (42)	17 (26)	48 (74)	7 (11)	58 (89)
4	64 (22)	40 (63)	24 (37)	23 (36)	41 (64)	8 (13)	56 (87)
5	29 (10)	18 (63)	11 (37)	8 (28)	21 (72)	1 (3)	28 (97)
6	27 (9)	12 (44)	15 (56)	12 (44)	15 (56)	5 (19)	22 (81)
7	16 (6)	10 (63)	6 (37)	7 (44)	9 (56)	0	16 (100)
8	2 (1)	1 (50)	1 (50)	1 (50)	1 (50)	0	2 (100)
Model 2			<0.001		<0.001		<0.001
Allred score	273	176 (65)	97 (35)	80 (29)	193 (71)	24 (9)	249 (91)
0	142 (52)	77 (54)	65 (46)	19 (14)	123 (86)	4 (3)	138 (97)
2	4 (1)	3 (75)	1 (25)	0	4 (100)	0	4 (100)
3	39 (14)	27 (69)	12 (31)	13 (33)	26 (67)	1 (3)	38 (97)
4	44 (16)	37 (84)	7 (16)	23 (52)	21 (48)	7 (16)	37 (84)
5	33 (12)	26 (79)	7 (21)	19 (58)	14 (42)	9 (27)	24 (73)
6	9 (3)	5 (56)	4 (44)	5 (56)	4 (44)	2 (22)	7 (78)
7	1 (1)	0	1 (100)	1 (100)	0	1 (100)	0
8	1 (1)	1 (100)	0	0	1 (100)	0	1 (100)

NOTE. Model 1: Allred scores for p53 in tumor-stromal fibroblasts based on biopsy specimens obtained before neoadjuvant therapy. Model 2: Allred scores for p53 in tumor-stromal fibroblasts based on surgical specimens obtained after neoadjuvant therapy.

HER2 category in tumor cells significantly increased the trend values for relative risk for pathologic complete response (Table 2).

3.4. Factors significantly associated with tumor recurrence and tumor death

The univariate analyses of all of the cases as a whole showed that the Allred scores for p53 in tumor-stromal fibroblasts in the biopsy specimens and the surgical specimens were significantly associated with tumor recurrence and tumor-related death (Table 1, Fig. 2). In the multivariate analyses using model 1, UICC pTNM-pN category and the presence of lymph vessel invasion significantly increased the trend values for the hazard rates (HRs) for tumor recurrence and tumor-related death (data not shown). The Allred scores for p53 in tumor-stromal fibroblasts and the presence of an FF significantly increased the trend values for the HRs for tumor recurrence, and the Allred scores for ER in tumor cells significantly increased the trend value for the HR for tumor-related death in the multivariate analyses (data not shown). When model 2 was used, the Allred scores for p53 in tumor-stromal fibroblasts, the Allred scores for ER in tumor cells, UICC pTNM-pN category, and histologic grade significantly increased the trend values for the HRs for tumor recurrence and tumor-related death, and the Allred scores for p53 in tumor cells and residual tumor size significantly increased the trend values for the HRs for tumor-related death in the multivariate analyses (data not shown).

Table 2 Multivariate analysis for pathologic complete response in all patients with IDC who received neoadjuvant therapy

	Pathologic complete response	
	Trend RR (trend 95% CI)	*P* for trend
UICC pTNM-pN category (N0, N1, N2, N3)	0.07 (0.02-0.27)	<.001
HER2 category in tumor cells (0, 1, 2, 3)	1.81 (1.19-2.76)	.005

Abbreviations: RR, relative risk; N0, no nodal metastasis; N1, 1 to 3 nodal metastases; N2, 4 to 9 nodal metastases; N3, 10 or more nodal metastases.
NOTE. Pathologic complete responses were adjusted for UICC pTNM-pN category assessed in surgical specimens obtained after neoadjuvant therapy, and HER2 category in tumor cells, Allred scores for ERs, Allred scores for PRs, histologic grade, and tumor necrosis assessed in biopsy specimens obtained before neoadjuvant therapy.

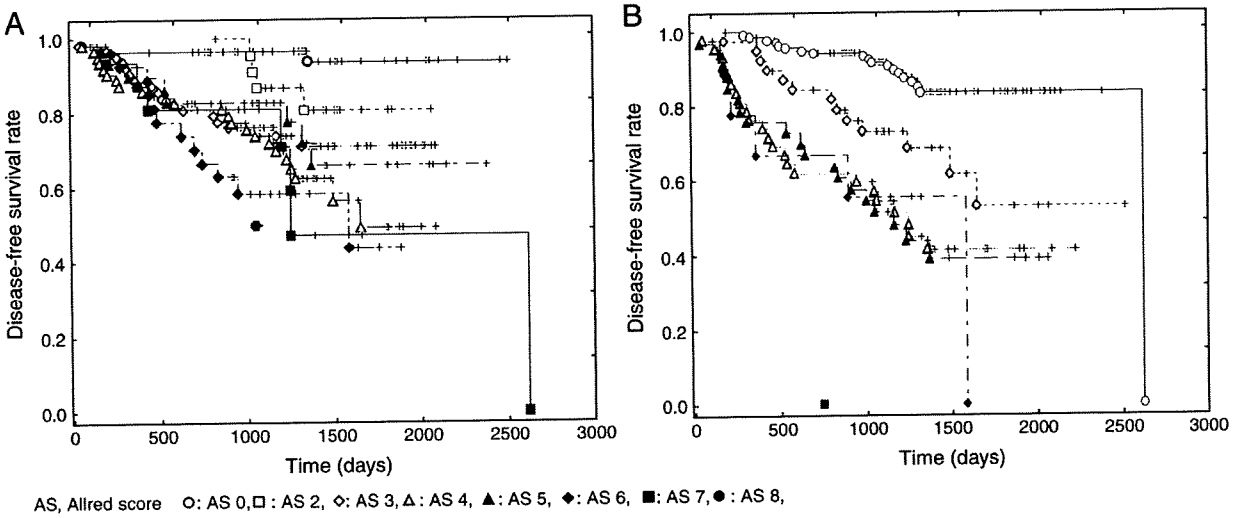

Fig. 2 Disease-free survival curves of patients with IDC according to the Allred scores for p53 in their tumor-stromal fibroblasts assessed in biopsy specimens obtained before neoadjuvant therapy (A) and in surgical specimens obtained after neoadjuvant therapy (B). Disease-free survival of patients with IDC classified by the Allred scores for p53 in tumor-stromal fibroblasts in biopsy and surgical specimens is significantly shortened as the scores increase (A, B: $P < .001$).

In the UICC pTNM stage 0 and I group of patients with IDC who received neoadjuvant therapy, age, lymph vessel invasion, and the Allred scores for p53 in tumor-stromal fibroblasts in the biopsy specimens obtained before neoadjuvant therapy significantly increased the trend values for the HRs for tumor recurrence in the multivariate analyses (Table 3, model 1). Among the factors in the surgical specimens obtained after neoadjuvant therapy, only the Allred scores for p53 in tumor stromal fibroblasts significantly increased the trend values for the HRs for tumor recurrence in the univariate analysis (data not shown).

In the group of UICC pTNM stage II IDC patients who received neoadjuvant therapy, the Allred scores for p53 in tumor-stromal fibroblasts, the Allred scores for PRs in tumor cells, and HER2 category in tumor cells in the biopsy specimens obtained before neoadjuvant therapy significantly increased the trend values for the HRs for tumor recurrence in the multivariate analyses (Table 4, model 1), and the Allred scores for ERs in tumor cells and the Allred scores for p53 in tumor-stromal fibroblasts in the surgical specimens obtained after neoadjuvant therapy significantly increased the trend values for the HR for tumor recurrence in the multivariate analysis (Table 4, model 2).

Table 3 Multivariate analysis for tumor recurrence in UICC pTNM stage 0 and I patients with IDC who received neoadjuvant therapy

	Tumor recurrence	
	Trend HR (trend 95% CI)	*P* for trend
Model 1 (n = 92)		
Age (≤39, >39 y)	0.21 (0.06-0.80)	.020
Lymph vessel invasion (absent, present)	4.49 (1.09-18.38)	.036
AS for p53 in tumor-stromal fibroblasts (0, 2-8)	1.45 (1.01-2.21)	.048

Abbreviation: AS, Allred score.
NOTE. Model 1: tumor recurrence was adjusted for age, lymph vessel invasion, and Allred scores for p53 in tumor-stromal fibroblasts assessed in biopsy specimens obtained before neoadjuvant therapy.

Table 4 Multivariate analyses for tumor recurrence in UICC pTNM stage II patients with IDC who received neoadjuvant therapy

	Tumor recurrence	
	Trend HR (Trend 95% CI)	*P* for trend
Model 1 (n = 86)		
AS for p53 in tumor-stromal fibroblasts (0, 2-8)	1.56 (1.15-2.51)	.007
AS for PRs in tumor cells (0, 2-8)	0.83 (0.71-0.98)	.026
HER2 category in tumor cells (0, 1, 2, 3)	1.61 (1.01-2.59)	.047
Model 2 (n = 89)		
AS for ERs in tumor cells (0, 2-8)	0.87 (0.76-0.99)	.035
AS for p53 in tumor-stromal fibroblasts (0, 2-8)	1.22 (1.00-1.49)	.044

NOTE. Model 1: tumor recurrence was adjusted for the Allred scores for p53 in tumor-stromal fibroblasts, the Allred scores for PRs in tumor cells, HER2 category in tumor cells, and Allred scores for ERs in tumor cells and lymph vessel invasion in the biopsy specimens obtained before neoadjuvant therapy. Model 2: tumor recurrence was adjusted for the Allred scores for ERs in tumor cells and Allred score for p53 in tumor-stromal fibroblasts in surgical specimens obtained after neoadjuvant therapy.

Table 5 Multivariate analyses for tumor recurrence and tumor death in UICC pTNM stage III patients with IDC who received neoadjuvant therapy

	Tumor recurrence		Tumor-related death	
	Trend HR (trend 95% CI)	*P* for trend	Trend HR (trend 95% CI)	*P* for trend
Model 1 (n = 112)				
Histologic grade (1, 2, 3)	2.60 (1.51-4.52)	<.001	3.87 (1.25-12.14)	.019
AS for p53 in tumor-stromal fibroblasts (0, 2-8)	1.21 (1.04-1.37)	.010	0.93 (0.70-1.26)	.651
AS for ERs in tumor cells (0, 2-8)	0.95 (0.85-1.07)	.397	0.77 (0.63-0.94)	.014
Adjuvant therapy (no, yes)	–		0.29 (0.09-0.97)	.043
HER 2 category in tumor cells (0, 1, 2, 3)	–		1.71 (1.01-2.91)	.048
Model 2 (n = 120)				
AS for ERs in tumor cells (0, 2-8)	0.87 (0.80-0.94)	<.001	0.77 (0.66-0.90)	<.001
AS for p53 in tumor-stromal fibroblasts (0, 2-8)	1.36 (1.14-1.63)	<.001	1.44 (1.12-1.87)	.005
Histologic grade (1, 2, 3)	1.84 (1.15-2.91)	.013	6.00 (1.96-18.31)	.002
AS for p53 in tumor cells (0, 2-8)	1.14 (1.01-1.28)	.040	1.09 (0.85-1.41)	.492
Residual invasive tumor size (≤20, >20 to ≤50, >50 mm)	2.21 (1.30-3.71)	.003	–	

NOTE. –, not significant in univariate analysis. Model 1: tumor recurrence was adjusted for histologic grade, the Allred scores for p53 in tumor-stromal fibroblasts, the Allred scores for ERs in tumor cells, the Allred scores for p53 in tumor cells, and Allred scores for PRs in tumor cells assessed in biopsy specimens obtained before neoadjuvant therapy, and adjusted for type of neoadjuvant therapy.

Tumor-related death was adjusted for histologic grade, the Allred scores for p53 in tumor-stromal fibroblasts, the Allred scores for ERs in tumor cells, HER 2 category in tumor cells, the Allred scores for PRs in tumor cells assessed in biopsy specimens obtained before neoadjuvant therapy, and adjusted for adjuvant therapy.

Model 2: tumor recurrence was adjusted for the Allred scores for ERs in tumor cells, the Allred scores for p53 in tumor-stromal fibroblasts and tumor cells, histologic grade, residual invasive tumor size, the Allred scores for PRs in tumor cells, tumor necrosis, lymph vessel invasion in the surgical specimens obtained after neoadjuvant therapy, and adjusted for type of neoadjuvant therapy.

Tumor-related death was adjusted for the Allred scores for ERs in tumor cells, the Allred scores for p53 in tumor-stromal fibroblasts and tumor cells, histologic grade, the Allred scores for PRs in tumor cells, HER 2 category in tumor cells, and tumor necrosis in the surgical materials obtained after neoadjuvant therapy, and adjusted for adjuvant therapy.

In the group of UICC pTNM stage III IDC patients who received neoadjuvant therapy, in model 1, histologic grade significantly increased the trend values for the HRs for tumor recurrence and tumor-related death in the multivariate analyses (Table 5). The Allred scores for p53 in tumor-stromal fibroblasts significantly increased the trend values for the HR for tumor recurrence, and the Allred scores for ERs in tumor cells, HER2 category in tumor cells, and adjuvant therapy status significantly increased the trend values for the HR for tumor-related death in the multivariate analysis (Table 5). In model 2, the Allred scores for ERs in tumor cells, the Allred scores for p53 in tumor-stromal fibroblasts, and histologic grade significantly increased the trend values for the HRs for tumor recurrence and tumor-related death in the multivariate analyses (Table 5). The Allred scores for p53 in tumor cells and residual invasive tumor size significantly increased the trend values for tumor recurrence in the multivariate analysis (Table 5).

4. Discussion

This study clearly demonstrated significant correlations between the Allred scores for ER, PR, and p53 in tumor cells and HER2 category in tumor cells assessed in the biopsy specimens obtained before neoadjuvant therapy and in the surgical specimens obtained after neoadjuvant therapy, and these findings also confirmed the results of other studies [21-23]. It can therefore be concluded that expression of ER, PR, p53, and HER2 in tumor cells is not modified by neoadjuvant therapy. By contrast, although a marginally significant correlation was observed between the Allred scores for p53 in tumor-stromal fibroblasts in the biopsy specimens and in the surgical specimens, the Allred scores for p53 in tumor-stromal fibroblasts tended to be lower in the surgical specimens than in the biopsy specimens. The following 2 explanations for this finding appear to be possible: (1) neoadjuvant therapy down-regulates the status of p53 expression in tumor-stromal fibroblasts and (2) the fixation time interval by 10% formalin suppresses p53 immunoreactivity that reflects some reactive changes within tumor-stromal fibroblasts in biopsy specimens in surgical specimens because the time interval for tissue fixation by 10% formalin is usually shorter in biopsy specimens than in surgical specimens. Because there was a significant correlation between p53 expression in tumor cells in the biopsy specimens before neoadjuvant therapy and in the surgical specimens after neoadjuvant therapy in this study, p53 immunoreactivity in tumor-stromal fibroblasts in biopsy specimens that reflect reactive changes unrelated to nodal metastasis may be

suppressed in tumor-stromal fibroblasts in surgical specimens for the possible reasons mentioned above, thereby resulting in Allred scores for p53 in tumor-stromal fibroblasts in the surgical specimens being probably significantly associated with the presence of nodal metastasis of IDC in this study.

Negative lymph node status, tumor cells being negative for hormone receptor immunoreactivity, and tumor cells being positive for HER2 immunoreactivity have been found to be significantly associated with a pathologic complete response of patients with breast cancer in other studies [24-26], and all these factors were also significantly associated with a pathologic complete response in the univariate analyses or the multivariate analysis in this study. Although tumor fibroblasts play a significant role in regulating tumor sensitivity to a variety of chemotherapeutic agents [27], and p53 activation in tumor-stromal fibroblasts sensitizes tumors to chemotherapy [28,29], no significant association between either p53 expression in tumor-stromal fibroblasts or in tumor cells and pathologic complete response was observed in this study.

This results of this study also clearly demonstrated that Allred scores for p53 in tumor-stromal fibroblasts in biopsy or surgical specimens are a very important outcome predictive factor for IDC patients who have received neoadjuvant therapy independent of UICC pTNM status, but the outcome predictive power of the Allred scores for p53 in tumor-stromal fibroblasts assessed in the surgical specimens obtained after neoadjuvant therapy was superior to that of the Allred scores for p53 in tumor-stromal fibroblasts assessed in the biopsy specimens obtained before neoadjuvant therapy. Thus, we can conclude that the outcome predictive power of the Allred scores for p53 in tumor-stromal fibroblasts should be evaluated in surgical specimens obtained after neoadjuvant therapy.

This study did not investigate Allred scores for p53 for associations with the presence of p53 gene abnormalities in tumor-stromal fibroblasts. Although p53 mutations in tumor-stromal fibroblasts are a common lesion in primary breast cancer and other cancers and have a positive effect on cancer growth [8,30-32], some studies have shown an absence of p53 mutations in the tumor-stroma of breast cancer [33,34], and the possibility of technical problems, for example, polymerase chain reaction artifacts mimicking p53 gene abnormalities, has been pointed out by Campbell et al [35]. Thus, although the mechanism responsible for increasing the malignant potential of IDCs that is related to the expression of p53 in tumor-stromal fibroblasts should be investigated from the standpoint of p53 gene abnormalities, the p53 immunoreactivity in tumor-stromal fibroblasts may in fact reflect specific reactive changes within tumor-stromal fibroblasts that are related to the outcome of patients with IDC.

In previous studies, we and others have reported that an FF, a characteristic histologic feature of the tumor stroma in primary invasive tumors, is a very useful and accurate prognostic histologic tumor-stromal indicator for the outcome of patients with IDC who have not received neoadjuvant therapy [12,13,36,37]. The present study clearly demonstrated that the presence of an FF (in the biopsy specimens obtained before neoadjuvant therapy, but not in the surgical specimens obtained after neoadjuvant therapy) was a factor that was significantly associated with tumor recurrence. This strongly suggests that neoadjuvant therapy produced FF-like stromal changes in the IDCs of the patients who received neoadjuvant therapy and that the true FFs in the IDCs could not be differentiated from the FF-like stromal changes. Thus, the outcome predictive power of FFs should be assessed in biopsy specimens obtained before neoadjuvant therapy.

In conclusion, this is the first study to clearly demonstrate that p53 expression by tumor-stromal fibroblasts, especially in surgical specimens obtained after neoadjuvant therapy, is strongly associated with the presence of nodal metastasis and the outcome of IDC patients who received neoadjuvant therapy. The modified Allred scoring system is very suitable for accurately assessing p53-expressing tumor-stromal fibroblasts in IDCs independent of the UICC pTNM stage of the IDC. p53 expression in tumor-stromal fibroblasts will probably become a very important target for tumor-gene therapy of IDCs of the breast in patients treated with neoadjuvant therapy.

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SURGERY TODAY

Original Articles

Clinical and Pathological Features of Intracystic Papillary Carcinoma of the Breast

TOMONORI AKAGI[1], TAKAYUKI KINOSHITA[1], TADAHIKO SHIEN[1], TAKASHI HOJO[1], SADAKO AKASHI-TANAKA[1], and YUSUKE MURATA[2]

[1]Division of Breast Surgery and [2]Pathological Division, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan

Abstract

Purpose. To evaluate the clinicopathological features of intracystic papillary carcinoma (ICPC), which have not been established given its rarity and lack of standard diagnostic criteria.

Methods. We reviewed the clinicopathological findings and treatment outcomes of 14 patients with ICPC diagnosed between 2002 and 2006.

Results. Intracystic papillary carcinoma was diagnosed by fine-needle aspiration biopsy in three patients and by core-needle biopsy in six patients. A preoperative diagnosis was not made in five patients. Three patients underwent magnetic resonance imaging preoperatively, which helped to differentiate benign tumors and maintain free surgical margins. The final pathological diagnosis was invasive carcinoma in 2 (14.2%) of the 14 patients. The patients were followed up for 1–72 months, during which time only one died, of a cancer-unrelated cause.

Conclusion. Our results show that ICPC is more difficult to diagnose than common breast cancer preoperatively. Excisional biopsy was necessary when fine-needle aspiration and core-needle biopsy could not provide a diagnosis. Magnetic resonance imaging is helpful to differentiate a benign tumor from invasive disease.

Key words Intracystic papillary carcinoma · Magnetic resonance imaging

Introduction

Intracystic papillary carcinoma (ICPC) of the breast is a rare malignant tumor, accounting for fewer than 2% of breast cancers.[1] According to the Japanese Society for Breast Cancer, ICPC includes ductal carcinoma in situ (DCIS). Several reports have described invasive ICPC with synchronous liver metastases.[2–6] Intracystic papillary carcinoma is more difficult to diagnose than common breast cancer. Because of the lack of standard criteria for diagnosis and treatment, the clinicopathological features and treatments of this type of breast cancer have not been defined. We reviewed the clinical and pathological features of 14 patients who underwent surgery for ICPC between 2002 and 2006.

Patients and Methods

Between 2000 and 2006, 2700 cases of primary breast cancer were diagnosed at the National Cancer Center Hospital, 14 of which were diagnosed as ICPC based on clinicopathological analysis. We reviewed the clinical features, pathological findings, and treatments of these 14 patients. Immunohistochemical evaluation was performed according to the DAKO criteria, with the ABC staining method. Immunohistochemical examinations for ER and PgR were defined as positive when there was positive nuclear reactivity. Positivity was scored as follows: 0, 0% positive cells; 1+, less than 10% positive cells; 2+, 10%–50% positive cells; and 3+, more than 50% positive cells. Immunohistochemical examinations for p53 were also defined as positive when there was positive nuclear reactivity. Positivity was scored as follows: 0, 0% of positive cells; +/–, less than 10%; 1+, 10 to 50%; and 2+, more than 50%. HER2 was defined as positive depending on the cytoplasmic membrane reactivity. The grading system for HER2 was also scored from 0 to 3+ by the immunohistological method reported by Bilous et al.[7]

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Table 1. Clinicopathological features of the 14 patients with intracystic papillary carcinoma

No.	Age (years)/sex	Duration from detecting the tumor to operation (months)	Location	US cystic size (mm)	US solid size (mm)	US shape of solid component	MMG shape of mass	MMG calcification	MRI	FNA	CNB	Preoperative diagnosis
1	84/F	2	A	22	5	Irregular	Irregular	None	—	—	–	Not given
2	83/F	2	D	11	6	Regular	Regular	None	—	Class 5	–	DC
3	75/F	3	A	22	7	Irregular	Irregular	A	—	Class 3	+	Not given
4	60/F	4	B	36	10	Regular	Regular	None	—	Class 2	+	Not given
5	43/F	3	A	15	3	Regular	Regular	None	—	—	+	Not given
6	36/F	9	C	34	17	Irregular	No mass	None	—	—	+	ICPC
7	57/F	4	E	10	4	Regular	Regular	None	—	Class 5	–	DC
8	70/M	6	E	50	15	Irregular	Irregular	None	—	—	+	ICPC
9	75/F	2	A	28	20	Regular	Regular	A	—	Class 5	–	DC
10	48/F	3	A	23	5	Regular	Regular	P	—	Class 2	+	Not given
11	74/F	8	A	14	14	Regular	—	—	—	—	+	ICPC
12	82/F	24	C	200	30	Regular	—	—	BCP	Class 2	+	ICPC
13	81/F	2	A	170	52	Irregular	Irregular	None	BCP	Class 2	+	ICPC
14	71/F	2	E	60	21	Irregular	Regular	None	BCP	—	+	ICPC

US, ultrasonography; MMG, mammography; MRI, magnetic resonance imaging; FNA, fine-needle aspiration; CNB, core-needle biopsy; A, amorphous; P, pleomorphic; BCP, breast cancer type in MRI; DC, ductal carcinoma

Results

The clinical data are summarized in Table 1. The patients consisted of one man and 13 women and their ages ranged from 36 to 82 years (median 72.5 years). The initial manifestation was a breast lump in all patients, 13 of whom noticed the breast lump, whereas it was detected by breast cancer screening in 1 patient. The time from tumor detection to treatment ranged from 2 to 24 months (median, 5.2 months). The size of the cystic component ranged from 1 to 20 cm (mean, 4 cm), and the size of the solid component ranged from 3 to 52 mm (median, 12 mm). The tumor was located in areas A, B, C, D, and E in seven, one, two, one, and three patients, respectively.

Ultrasonography showed a multicystic lesion in one patient, and a unicystic lesion in 13 patients. All patients had solid components with intracystic growth. The cystic component ranged from 11 to 220 mm (median, 22.5 mm), and the solid component ranged from 3 to 52 mm (median, 12 mm). The solid components were variable, regular, or irregular in shape.

Twelve patients underwent mammography, which showed a smooth mass in seven, an irregular mass in four, and no mass in one. Four patients had amorphous or pleomorphic calcifications. Magnetic resonance imaging (MRI) showed a breast cancer pattern in all three patients who underwent this examination. It also showed invasion of the cystic wall in one patient.

Fine-needle aspiration was done in 8 of the 14 patients and the tumor was designated as class 5 in 3 (37%) patients, class 3 in 1, and class 2 in 4. Core-needle biopsy was done of five of the tumors designated as class 3 or class 2. Five other patients underwent core-needle biopsy without fine-needle aspiration. A diagnosis of ICPC was made in six (60%) of these ten patients. A diagnosis was not able to be made by core-needle biopsy in four patients, who required excisional biopsy for a definite diagnosis. One patient did not undergo fine-needle aspiration or core-needle biopsy preoperatively.

The pathological features are summarized in Table 2. Thirteen patients underwent mastectomy or partial mastectomy; with axillary lymph node dissection in five, without axillary lymph node dissection in four, and with sentinel lymph node dissection in four. The intracystic fluid was either serous or bloody. Pathological findings revealed invasive ICPC in 2 (14.2%) patients and DCIS was detected around the ICPC in 3 (21.4%) patients. Axillary lymph node metastasis was found in one patient. Estrogen receptor, progesterone receptor, HER2, and p53 were positive in 14 (100%), 13 (92.8%), 3 (21.4%) and 2 (14.2%) patients, respectively.

Thirteen patients were treated with tamoxifen postoperatively, and three of the eight who underwent

Table 2. Pathological findings of intracystic papillary carcinoma (ICPC)

No.	Proposed operation	Invasion of cystic wall	DCIS around ICPC	Lymph node metastasis	ER PgR HER2	p53	Histologic grade	Nuclear grade
1	Bp	–	–	No dissection	ER2 PgR2 HER2 1	–	1	1
2	Bp	–	–	No dissection	ER2 PgR0 HER2 0	–	1	1
3	Bt+sampling	–	–	0/2	ER2 PgR2 HER2 0	+	2	2
4	Bq	–	–	No dissection	ER2 PgR1 HER2 0	–	2	2
5	Bp+Ax	–	–	0/11	ER2 PgR2 HER2 0	–	2	2
6	Bp+Ax	–	+	0/22	ER2 PgR2 HER2 0	–	1	1
7	Bt+Ax	–	–	0/20	ER2 PgR2 HER2 1	–	2	2
8	Bp	–	–	No dissection	ER2 PgR2 HER2 2	2+	2	2
9	Bt+Ax	–	–	0/18	ER1 PgR1 HER2 0	–	2	3
10	Bq+SLN	–	–	0/4	ER1 PgR2 HER2 0	–	1	1
11	Bp	+	–	No dissection	ER2 PgR2 HER2 0	–	1	1
12	Bt+SLN	–	–	1/5	ER3 PgR3 HER2 0	–	1	1
13	Bt+SLN	–	+	0/5	ER3 PgR3 HER2 0	–	1	1
14	Bt+SLN	+	+	0/3	ER3 PgR2 HER2 0	–	1	1

DCIS, ductal carcinoma in situ

partial mastectomy were also treated with radiation therapy. All 14 patients were followed up for 1–72 months. At the time of writing, 13 patients were alive without evidence of recurrence and one had died of a cause unrelated to cancer.

Discussion

Intracystic papillary carcinoma is a rare type of breast cancer characterized by papillary growth within a macroscopic cyst. It accounts for fewer than 2% of all breast cancers.[1] Generally, ICPC shows no invasive growth outside of the cyst and is treated similarly to DCIS. However, there are reports of invasive ICPC with synchronous liver metastases.[2–6] Yet, because of its rarity and the lack of diagnostic criteria, the clinicopathological features and treatments of ICPC have not been established.

The average age of onset is higher than that for the more common types of breast cancer, at about 65 years old (range, 34–92 years).[8–10] The average age of onset in this series was 36–82 years old (median, 72.5). Some studies have reported a longer period from tumor detection to treatment for ICPC than for common breast cancer.[8,9] In our study, it ranged from 1 to 24 months (median, 5 months), which suggests that ICPC grows more slowly than common breast cancer, and that it has a lower pathological grade and a tendency not to form ulcerations.

Intracystic papillomas are difficult to diagnose. Previous studies reported that the average age of onset was 40.7–47 years, and that 81% of intracystic papillomas in patients older than 60 years old were carcinoma.[8–10] Intracystic papillary carcinoma tumors tend to be larger than intracystic papillomas, but this does not necessarily help differentiate malignancy from benign growth.[11]

Ultrasonography was thought to be a useful modality to differentiate malignant from benign tumors, but as seen in this series, the shapes of the solid components can be variable, regular, or irregular in malignant and benign tumors. Thus, several studies have found that ultrasonography is not useful for identifying benign tumors.[11,12] Although ultrasonography can differentiate malignancy from benign tumors relatively easily when there is invasion, ICPC without invasion is difficult to diagnose with ultrasonography.

Magnetic resonance imaging is one of the most useful diagnostic techniques for common breast cancer, as it shows the patterns of the time–intensity curves of the lesion, allowing us to differentiate cancerous from benign tumors. Naoshige et al. reported that dynamic MRI imaging is very useful in the differential diagnosis of ICPC.[6] Kusuma et al. also reported that the MRI findings correlated with the pathological findings.[13] Only three of our patients underwent MRI, which showed malignant patterns in the time–intensity curve in all three. Moreover, in one patient it showed invasive growth outside of the cyst, corresponding to the pathological findings (Fig. 1). This finding demonstrates the strong potential of MRI to differentiate benign tumors from invasive disease.

Fine-needle aspiration or core-needle biopsy is important if the preoperative image indicates a potential malignancy. Fine-needle aspiration should be done initially, followed by core-needle biopsy, unless the fine-needle biopsy reveals class 5. In this series excisional biopsy was necessary when fine-needle aspiration or core-needle biopsy could not provide a diagnosis. It is more difficult to diagnose ICPC than common breast

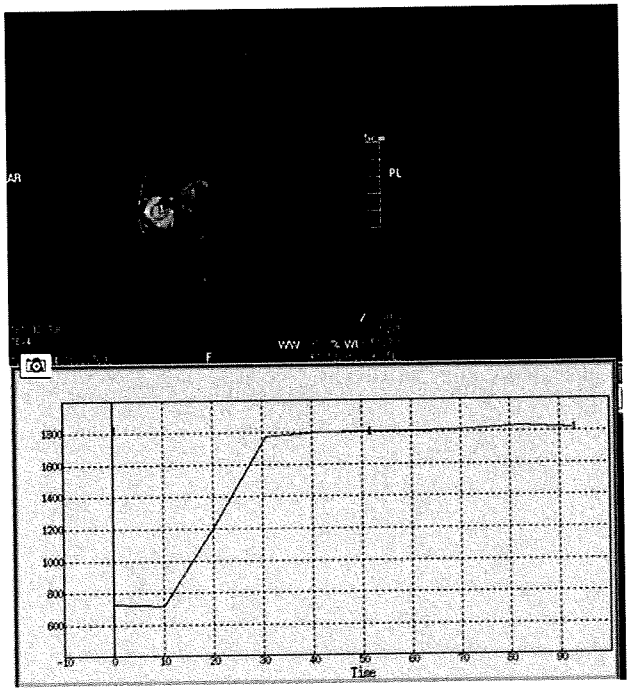

Fig. 1. Dynamic magnetic resonance imaging showed malignant patterns in the time–intensity curve of the lesion

cancer because nuclear atypicality of ICPC is not prominent. Therefore, a correct diagnosis is dependent on an adequate preoperative biopsy specimen.

The treatments for ICPC and DCIS are generally the same, although cases of invasive ICPC with synchronous liver metastases have been reported.[2–6] According to Yamashita et al., invasive ICPC is no longer rare and intraductal spread beyond 2 cm from the cystic wall is possible.[10] In this series, two patients had invasive ICPC and another patient had axillary lymph node metastases despite no evidence of invasion in any pathologic section. It is likely that this patient had invasive disease that was missed on the available pathologic sections. Thus, it is important to obtain negative pathological surgical margins. Intracystic papillary carcinoma has the potential to be invasive, which can be evaluated by MRI. Standard neoadjuvant and adjuvant treatments have not been established and surgical resection remains the first line of treatment. The frequency of lymph node metastasis of ICPC has been reported as 0%–36%, which is lower than that of common breast cancer.[9,14] Four patients in this series were treated with sentinel biopsy, which we have been performing in our department since 2004. It is now reasonable not to perform axillary lymph node dissection, as sentinel biopsy is an accepted indicator of ICPC.

All of the tumors in this series were positive for estrogen and progesterone receptors, and the patients were given tamoxifen as adjuvant therapy. Eight patients who underwent breast-conserving treatment received radiation. Although no definitive conclusions about adjuvant treatments have been made, ICPC should g enerally be treated like DCIS.

Based on our experience and review of the literature, we conclude that it is critical to evaluate the malignant potential of ICPC and to decide on the most appropriate adjuvant treatment for each individual patient.

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CLINICAL TRIAL

Comparison among different classification systems regarding the pathological response of preoperative chemotherapy in relation to the long-term outcome

Tadahiko Shien · Chikako Shimizu · Kunihiko Seki · Taro Shibata · Takashi Hojo · Masashi Ando · Tsutomu Kohno · Noriyuki Katsumata · Sadako Akashi-Tanaka · Takayuki Kinoshita · Yasuhiro Fujiwara

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Abstract Neoadjuvant chemotherapy (NAC) is increasingly used for operable disease. However there are several pathological response classification systems and the correlation between the pathological response to NAC according to each system and the patient outcome is still under debate. From 1998 to 2006, 370 primary breast cancer patients underwent curative surgical treatment after NAC containing both anthracycline and taxane at the National Cancer Center Hospital. We retrospectively evaluated the clinical and pathological response using the cTMN, Fisher's, Chevailler's, and the Japanese Breast Cancer Society classification systems (JBCS) respectively, and analyzed the correlation between each pathological response and disease free survival (DFS). Ninety-five (26%) patients had tumor recurrence. The five-year DFS according to Fisher's system was pCR, 80% and pINV, 63%. The five-year DFS according to Chevallier's system was Grade 1, 83%, Grade 2, 85%, Grade 3, 62%, and Grade 4, 65%. The five-year DFS according to the JBSC system was Grade 3, 77%, Grade 2, 68%, Grade 1a, 68%, Grade 1b, 58%, and Grade 0, 52%. None of the pathological response systems reached a statistically significant difference. In the classification by the post-treatment number of metastatic axillary lymph nodes, the 5-year DFS was $n = 0$, 86%; $n = 1$–3, 64%; $n = 4$–9, 44%; and $n > 10$ positive: 25% ($P < .0001$). In pathologically node negative patients, there were no significant differences in the DFS among all the classification systems. All three classifications analyzed were considered inadequate as the prognostic marker of the long-term outcome after NAC and further studies are warranted to optimize the prediction.

Keywords Breast cancer · Neoadjuvant · Chemotherapy · Response · Predictor

T. Shien · T. Hojo · S. Akashi-Tanaka · T. Kinoshita
Department of Breast Surgery, National Cancer Center Hospital, Tokyo, Japan

T. Shien · C. Shimizu (✉) · M. Ando · T. Kohno · N. Katsumata · Y. Fujiwara
Breast and Medical Oncology Division, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan
e-mail: cshimizu@ncc.go.jp

K. Seki
Department of Pathology, National Cancer Center Hospital, Tokyo, Japan

T. Shibata
Statistics and Cancer Control Division, National Cancer Center, Tokyo, Japan

Introduction

Breast cancer has recently become the most common malignancy among Japanese women. Approximately 40,000 women are annually affected and breast cancer mortality has been increasing. National efforts to establish an early detection system by screening mammography has begun, but many of the primary cases still present with a palpable mass in the breast.

Neoadjuvant chemotherapy (NAC) has been accepted as one of the standards of care not only for locally advanced breast cancer but also for primary operable breast cancer. The disease free survival (DFS) and overall survival (OS) of patients treated with NAC is at least equivalent to those treated with post-operative adjuvant chemotherapy and the chance of breast conservation increases in patients with larger tumors [1, 2]. Although the benefit of the addition of taxane to anthracycline in the preoperative setting in terms of long-term outcome remains controversial, regimens that

combine both anthracycline and taxane, either sequentially or concomitantly, are widely used.

Prognostic factors after primary chemotherapy include the clinical and pathological response to primary chemotherapy, the cTNM stage, and axillary lymph-node status after chemotherapy. "Pathological complete response (pCR)" correlates with an improved DFS and OS and has often been used as the surrogate primary endpoint for NAC. However, the classification systems for pathological response vary among studies and the system that best reflects the long-term outcome remains unidentified. Thus in this study, we applied various pathological response systems in the published literature to the same patient cohort treated with NAC including anthracycline and taxane to compare their usefulness in the prediction of the long-term outcome after NAC.

Patients and methods

Patients and treatments

All breast cancer patients treated with NAC containing both anthracycline and taxane between May 1998 and October 2006 at the National Cancer Center Hospital were extracted from the surgical database to be included in this retrospective study. NAC was indicated in patients with clinical stage II or III primary breast cancer with tumors larger than 3 cm. Core needle biopsy was performed before NAC to obtain a pathological diagnosis. The NAC regimens included (1) four cycles of doxorubicin (DOX, 50 mg/m^2) and docetaxel (DOC, 60 mg/m^2) (AT) followed by additional adjuvant treatment with two cycles of AT or four cycles of iv CMF (cyclophosphamide, methotrexate and 5FU), (2) four cycles of fluorouracil (500 mg/m^2)/epirubicin (100 mg/m^2)/cyclophosphamide (500 mg/m^2) plus 12 weekly cycles of paclitaxel (80 mg/m^2) (FECT), (3) four cycles of doxorubicin (60 mg/m^2)/cyclophosphamide (600 mg/m^2) plus 12 weekly cycles of paclitaxel (80 mg/m^2) (ACT). After November 2002, in patients with HER2-overexpression tumors, trastsuzumab (initially 4 mg/kg and 2 mg/kg weekly) was administered with paclitaxel for 12 weeks in the ACT and FECT treated populations (ACTH and FECTH, respectively). Five years of endocrine therapy was scheduled when either the pretreatment biopsy specimen or surgical specimen post-chemotherapy were positive for the estrogen or progesterone receptor.

Evaluation of pathological factors

Pretreatment diagnosis was established by pathologists from a core needle biopsy specimen. Surgical specimens were sectioned at about 7–10 mm and the pathological response was evaluated by pathologists. The expression levels of ER (1D5, Dako Cytomation), PgR (1A6, Novocastra) and HER2 (HercepTest®, Dako Cytomation) were examined with immunohistological staining. ER and PgR were classed as positive when more than 10% of cancer cell nuclei were stained, regardless of the intensity of the staining. HER2 was scored as follows: (0): negative for cells, (1+): slightly positive in more than 10% of cancer cells, (2+): moderately positive in more than 10% of cancer cells, (3+): markedly positive in more than 10% of cancer cells. Additional fluorescent in situ hybridization (FISH) for HER2 amplification (Pathvision, Vysis) was performed and when IHC (3+) or FISH-positive (HER2/CEP17 signal ratio $\geq$ 2.0) were defined as HER2-positive.

The response criteria used in this study included Fisher's system [1], Chevalier's system [3] and the histological response criteria of the Japanese Breast Cancer Society (JBCS) [4, 5]. The key definitions of each response classification system are described in Table 1. To summarize, Fisher's system evaluated only the histological evidence of invasive disease in the primary tumor, Chevallier's system incorporated nodal status and the JBSC system measured morphological changes in of the tumor cells and the proportion of histological changes in the primary tumor. The histological effect in both the primary tumor and axillary lymph node should be separately evaluated in the JBCS system, but the standard of how to combine the effect is not mentioned. Therefore we used the pathological response in only the primary lesion in the present study.

In addition, we evaluated pretreatment clinical staging, the clinical response to preoperative chemotherapy and postoperative pathological lymph node status. The clinical response to preoperative chemotherapy was decided from the two greatest perpendicular diameters (before each chemotherapy treatment and before surgery) of tumors in the breast and axillary lymph nodes. No clinical evidence of palpable tumor in the breast and axillary lymph nodes was defined as a clinical complete response (cCR). A reduction in the total tumor size of 30% or greater was graded as clinical partial response (cPR). An increase in total tumor size of more than 20% or appearance of new suspicious ipsilateral axillary adenopathy was considered progressive disease (cPD). Tumors that did not meet the criteria for objective response and progression were considered as stable disease (cSD).

Statistical analysis

Disease free survival (DFS) was calculated from the date that NC was initiated to the date of the first relapse including loco-regional recurrence or the last visit without

Table 1 Pathological response classification systems

Classification system	Key definitions
Fisher et al. [1]	Classification based on microscopic evidence
	pCR = no histological evidence of invasive tumor cells (specimens with only noninvasive cells included)
	pINV = histological evidence of invasive disease of any extent
Chevallier et al. [3]	Classification using both microscopic and macroscopic evidence
	Grade 1 (pCR) = disappearance of all tumor on either macroscopic or microscopic assessment
	Grade 2 = presence of in situ carcinoma in the breast, no invasive tumor, no tumor in the ALNs
	Grade 3 = presence of invasive carcinoma with stromal alteration
	Grade 4 = no/few modifications of the tumor appearance
JBCS	Classification using both microscopic and macroscopic evidence in primary tumor
	Grade 0 = no therapeutic effect
	Grade 1 = <66% therapeutic effect, but >33% effect evident
	Grade 2 = subjectively >66% therapeutic effect, but <near total therapeutic effect
	Grade 3 = disappearance of all tumor on either macroscopic or microscopic assessment

pCR, pathological complete response; ALN, Axillary lymph node; pINV, pathological invasive disease; JBCS, Japanese breast cancer society

relapse. Kaplan–Meier plots and the log-rank test were used to assess the difference in survival. All comparisons were two-tailed. Cox-proportional hazards models were fitted for OS and DFS and included variables identified a priori as being associated with survival and the ALN status. Other variables not identified a priori were entered into the model one at a time and assessed for statistical significance. All pair-wise interactions were tested. The fit of the model and the proportional hazards assumption were assessed visually with residual plots. The statistical significance level (P) was taken as a measure of the strength of evidence against the null hypothesis, and $P < .05$ was considered statistically significant.

Results

Three hundred and seventy patients with operable breast cancer were included in this study. Table 2 lists the patient and tumor characteristics. The median age was 50 years (26–71) and 192 (52%) patients were over the age of 50. Clinical staging at diagnosis was IIA in 104 (28%), IIB in 114 (31%), IIIA in 75 (20%) and IIIB in 77 (21%). ER and PgR positive patients were respectively 148 (40%) and 152 (41%). 183 (49%) patients were treated with AT, 73 (20%) with ACT and 90 (24%) with FECT. Trastuzumab as administered to four patients among the ACT-treated patients (ACTH) and 20 among the FECT-treated patients. Ten percent of patients with HER2-positive breast cancer received trastuzumab in this study.

Ninety-six patients (26%) had tumor recurrence with a median follow-up of 45 months (range 4–104). Nine patients had only loco-regional recurrence without distant metastasis. Only 42 patients died within this period.

The clinical and pathological response results are shown in Table 3. The overall clinical response rate to NAC was 88% (cCR + cPR) and the cCR rate was 28%. According to Fisher's classification, pCR and pINV was 65 (18%) and 305 (82%). According to Chevallier's classification, 30 (8%) patients achieved a Grade 1 (disappearance of all

Table 2 Patient and tumor characteristics

Parameter	No. of patients (%)
Total	370
Age	
Age <50	179 (48)
Age >51	191 (52)
Pretreatment pathology	
Invasive ductal carcinoma	347 (94)
Invasive lobular carcinoma	13 (4)
Mucinous carcinoma	7 (2)
Others	3 (1)
Hormone receptors	
ER positive	148 (40)
PgR positive	152 (41)
HER2	
Positive (>2+)	132 (36)
Neoadjuvant chemotherapy	
AT	183 (49)
ACT	73 (20)
ACTH	4 (1)
FECT	90 (24)
FECTH	20 (5)
Surgery	
Partial mastectomy	136 (37)
Total mastectomy	234 (63)

Table 3 Response to neoadjuvant chemotherapy, Cox proportional hazards model for disease free survival

Parameter	No. of patients (%)	Hazard ratio (95% CI)
Fisher's classification		
pCR	65 (18)	1.00
pINV	305 (82)	1.07 (0.56–2.73)
Chevallier's classification		
Grade 1	30 (8)	1.00
Grade 2	21 (6)	1.03 (0.18–5.85)
Grade 3	172 (46)	1.00 (0.43–2.26)
Grade 4	147 (40)	1.31 (0.27–5.66)
JBCS classification		
Grade 3	34 (9)	1.00
Grade 2	102 (28)	1.39 (0.54–3.39)
Grade 1b	81 (22)	0.96 (0.36–2.32)
Grade 1a	141 (38)	0.61 (0.21–1.71)
Grade 0	12 (3)	0.50 (0.56–2.73)
Pathological lymph node status		
$n = 0$	174 (47)	1.00
$n = 1$–3	102 (28)	0.78 (0.54–1.10)
$n = 4$–9	57 (15)	1.57* (1.07–2.24)
$n > 10$	37 (10)	2.71* (1.83–3.95)
Clinical stage		
IIA	104 (28)	1.00
IIB	114 (31)	0.68 (0.45–1.01)
IIIA	75 (20)	1.23 (0.86–1.74)
IIIB	77 (21)	1.22 (0.85–1.74)
Clinical response		
CCR + cPR	324 (88)	1.00
CSD + cPD	46 (12)	1.44* (1.10–1.87)

CI, Confidence interval, * $P < .001$

tumors in either breast or lymph node) pathological response. According to the JBCS classification, there were 34 (9%) patients with Grade 3 pathological response (pathologically no residual tumor in the breast). Post-treatment pathological nodal status was negative in 174 (47%), 1–3 positive in 102 (28%), 4–9 positive in 57 (15%), and >10 positive in 37 (10%) patients, respectively. In the Cox proportional hazards model, the classification of pathological lymph node status and clinical response were identified as being independently significantly associated with patient outcomes (Table 3). Pretreatment hormone receptor status was not associated with pathological response or DFS. Inclusion of trastuzumab in NAC was associated with the pathological response in HER2-positive tumors ($P = 0.04$), but there was no statistical difference in the DFS (data not shown).

Figure 1 illustrates the Kaplan–Meier curves of the patient cohort of DFS according to each pathological response classification system (Fisher's, Chevallier's, JBCS). Among these classification systems, Fisher's tended to show a correlation with DFS, however, it did not reach a statistically significant difference ($P = .067$). The five-year DFS rates in Grade 3, Grade 2, Grade 1a, Grade 1b and Grade according to the JBCS system were 77%, 68%, 68%, 58%, and 52%, respectively ($P = .525$). According to Chevallier's system, the five-year DFS rates for Grade 1, Grade 2, Grade 3 and Grade 4 were 83%, 85%, 62% and 65%, respectively ($P = .16$).

The five-year DFS according to the number of post-treatment axillary node metastases was $n = 0$, 86%; $n = 1$–3, 64%; and $n = 4$–9, 25%. Figure 2 shows the DFS according to the pre-treatment cTMN classification, post-treatment pathological nodal status and clinical response to NAC. The pre-treatment clinical stage, clinical response to NAC and post-treatment pathological nodal status were strong predictors of DFS ($P < .0001$, $P = .0005$, $P < .0001$, respectively).

The pathological response results in post-treatment pathological node negative patients are shown in Fig. 3. Pathological node-negative patients accounted for 174 (47%) out of 370 patients. Since the number of Grade 0 patients according to the JBCS system was only two, they were excluded from the analysis. There were no significant relationships between the three pathological response classification systems and the DFS in pathologically node-negative patients. Neither clinical response ($P = .142$) nor pre-treatment clinical stage ($P = .231$) predicted DFS in node-negative patients.

Discussion

Pathological and biological markers predicting "pCR" in NAC have been evaluated in several studies [6, 7], but there is no consensus on the definition of pathological response. It is particularly unclear whether the classification needs the measurement of the extent of therapeutic effect including the disappearance of tumor cells and decrease of tumor cellularity [1–3, 8, 9]. The frequency distribution of residual tumor size was altered markedly by the inclusion of tumor cellularity, and the accurate pathologic response information may be provided the product of pathologic size and tumor cellularity [10]. The results in our study showed that the evaluation of tumor cellularity and tumor size by both Chevallier's and he JBCS classification systems was not useful for predicting prognosis in both all patients and node-negative patients. This result was in contrast to another study, where the reduction of tumor cellularity significantly correlated with the overall and disease free survival [11]. The negative finding in our study may be due to the small sample size of the study and limited number of events in each category of the

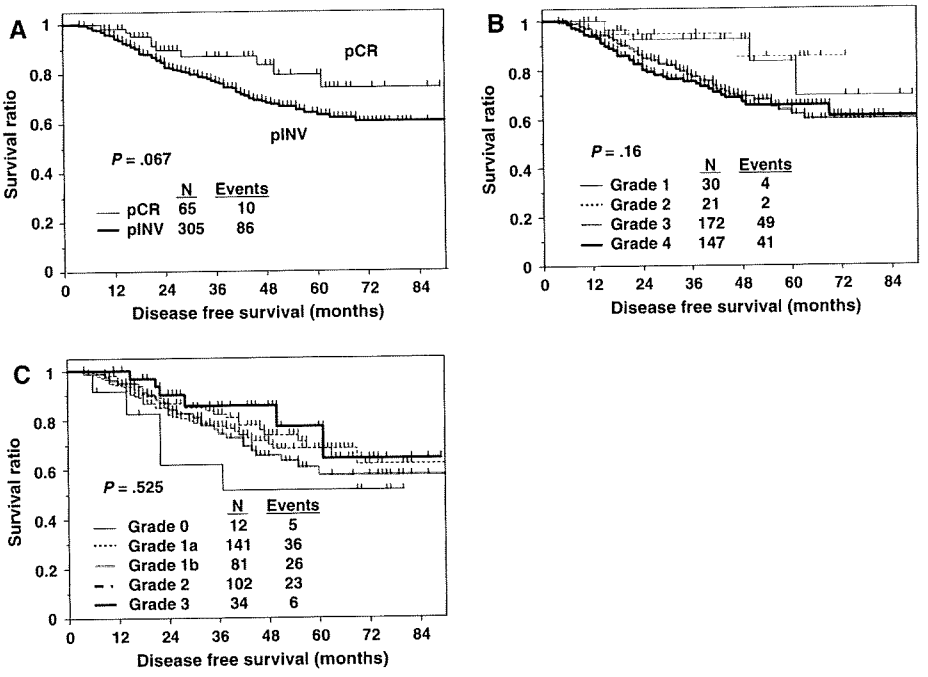

Fig. 1 Kaplan–Meier curves of disease free survival according to pathological response classification systems examined. (**a**) Fisher's classification; (**b**) Chevallier's classification; (**c**) JBCS classification

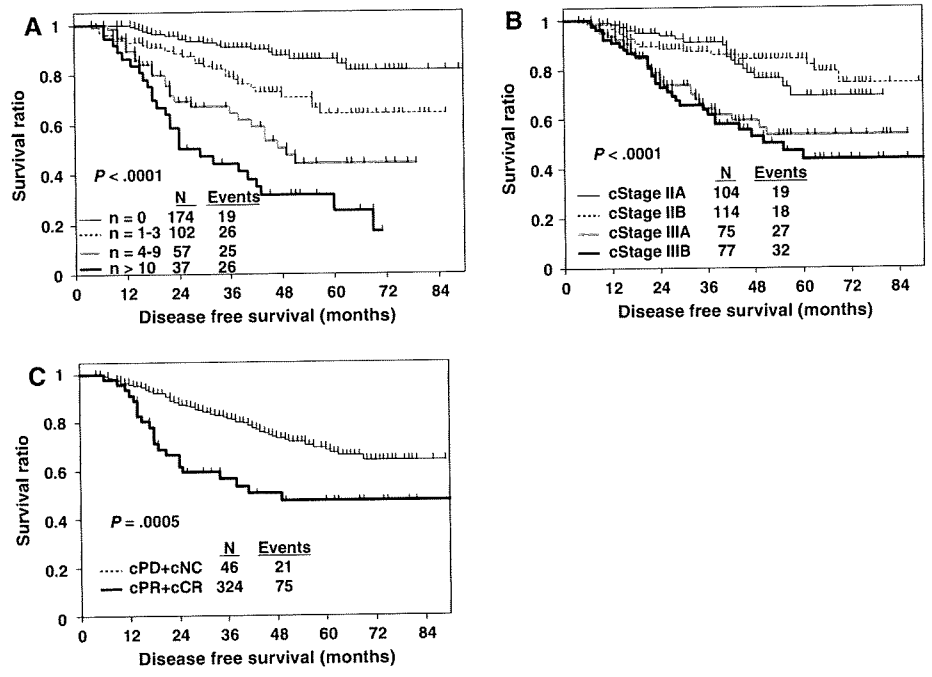

Fig. 2 Kaplan–Meier curves of disease free survival according to (**a**) Pathologic nodal status; (**b**) Clinical staging and (**c**) Clinical response

classification. Moreover the variety of chemotherapy regimens used as NAC may have affected the result. Particularly, trastuzumab was used only in the recent HER2-positive patient cohort.

However, studies including ours indicate the importance of incorporating the pathological nodal status in the prediction of prognosis for patients after NAC [12–15]. Fisher's classification is the most popular classification system using major clinical trials such as NSABP trials, but this classification system is diagnosed simply based on the disappearance of invasive tumor cells, regardless of non-invasive tumor cells, only in the primary tumor. Although Fisher's system is simple, objective and its usefulness as a predictive marker has been validated [1–3, 9, 14], incorporation of the therapeutic effect in axillary lymph nodes may be necessary for more precise outcome prediction.

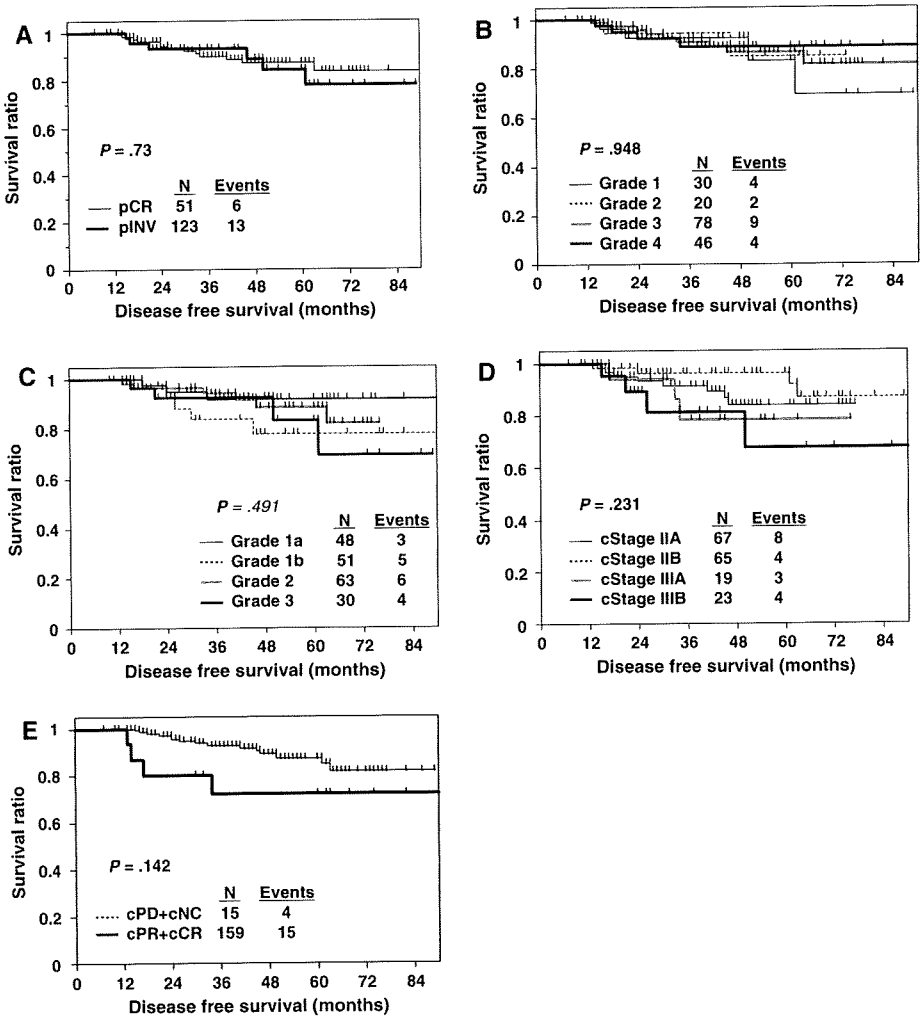

Fig. 3 Kaplan–Meier curves of disease free survival in node negative patients (**a**) Fisher's classification; (**b**) Chevallier classification; (**c**) JBCS classification; (**d**) Clinical staging; (**e**) Clinical response

On the other hand, clinical response was the significant predictor of the disease free survival in this study as reported in several other papers [13, 16–19]. Clinical response reflects the activity of chemotherapeutic agents. Clinical responders had a better prognosis compared with non-responders. The pretreatment clinical stage correlated with disease free survival, but there were good responders among the patients with advanced primary lesions and clinically positive axillary lymph nodes. Although pCR significantly correlated with the clinical response, the importance of the clinical response in outcome prediction may remain in patients with residual tumor or pathologically negative axillary lymph node after NAC.

In conclusion, we think that all three classifications analyzed in this study were not adequate as a prognostic marker of long-term outcome after NAC. The evaluation of the therapeutic effect in primary tumors warrants further study, especially in pathologically node-negative patients after NAC. Given the suggestion that the benefit of certain chemotherapy regimens might be different depending on the biological tumor characteristics (e.g. hormone responsive, HER2, triple negative), the validity of pCR as a prognostic marker might better be tested independently in each biological subset. Moreover, the validity of pCR with NAC including biologically targeted drugs such as trastuzumab should also be revisited.

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