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Original Article

Immunohistochemical Evaluation of Hormone Receptor Status for Predicting Response to Endocrine Therapy in Metastatic Breast Cancer

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***Background*:** The importance of establishing hormone receptor status of tumors for the treatment of women with hormone receptor-positive breast cancer has been emphasized, however, there is no general agreement as to how immunohistochemical assays should be evaluated. It is critical to evaluate hormone receptor status when considering response to endocrine therapy.

***Methods*:** Estrogen receptor (ER) and progesterone receptor (PgR) expression was examined by immunohistochemistry using Allred's score for primary breast tumors from 75 metastatic breast cancer patients who received first-line treatment with endocrine therapy (56 patients received tamoxifen, 11 patients received aromatase inhibitors, and 8 patients received LH-RH agonist or other endocrine reagents) on relapse. Correlation between hormone receptor status and response to endocrine therapy as well as post-relapse survival was analyzed.

***Results*:** The most significant correlation between positive ER expression and response to any endocrine therapy ($p = 0.011$) or tamoxifen only ($p = 0.030$) occurred when the cutoff score was set at 10%. When the evaluation was based on Allred's score (TS), a cutoff point of TS $\geqq 4$ showed a more significant association between positive ER expression and response to all kinds of endocrine therapy ($p = 0.020$) or tamoxifen only ($p = 0.047$). When evaluated at a cutoff point of 1% positive cells, there were fifteen patients with both ER- and PgR-negative tumors, and three patients (20.0%) responded to the therapy. Patients with 1% or more ER or PgR positive cells had better survival after relapse ($p = 0.0005$ and $p = 0.0008$, respectively).

***Conclusions*:** The proportion score alone might be enough to predict hormone responsiveness and post-relapse survival in metastatic breast cancer. The cutoff might be set low, for example 1%, especially for metastatic disease.

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Key words: Breast cancer, Estrogen receptor, Progesterone receptor, Immunohistochemistry, Endocrine therapy

Estrogen receptors (ER) have been measured in human breast cancer tissues for more than 30 years, and ER status is used as a predictive and prognostic factor. The presence of ER is related to a favorable response to endocrine therapy and improves overall survival. Recently antibodies for hormone receptors, such as ER and progesterone receptor (PgR) were introduced that can be used on fixed, paraffin-embedded sections after heat-mediated antigen retrieval, and hormone receptor status can be analyzed by immunohistochemistry. However, there is no general agreement as to how the immunohistochemical assays should be evaluated, despite the widely recognized importance of determining hormonal receptor status, which is essential for deciding whether endocrine therapy is indicated in a given patient. Patients with ER- or PgR-positive tumors are candidate for endocrine therapy, and the choice among endocrine treatments should be made on the basis of hormone

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Abbreviations:
ER, Estrogen receptor; PgR, Progesterone receptor; IHC, Immunohistochemistry; TS, Total score; PS, Proportion score

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receptor status. There are two main features of the appearance of the stained tumor cells, the proportion of cells stained and the intensity of the staining. An additional feature is the uniformity of staining within the positive cells. Several different methods of evaluating hormone receptor status based on the proportion and/or intensity of positive cells have been reported, such as quick score[1], Histo (H) score[2], Allred's score[3], and proportion of positive cells.

There is much debate about the correct cutoff point to distinguish ER-positive from ER-negative tumors. One of the problems is achieving a balance between sensitivity and specificity. If 'any staining' is considered to be positive then the sensitivity will be very high and very few responders will be included in the negative group. However, some non-responders will be included in the positive group, thus reducing the specificity. Conversely, if only tumors with staining in almost all of the cells are called positive, the specificity will be high at the expense of sensitivity and if this is used to select women for endocrine therapy it will not identify all of the patients who could benefit[4]. The San Antonio group has found a significant benefit in women with tumors containing only 1% positive cells[5], and the St. Gallen recommendations on the primary therapy of early breast cancer in 2003 stated that even a low number of cells staining positive (as low as 1% of tumor cells) identified a cohort of tumors having some responsiveness to endocrine therapies[6].

In the present study, we examined hormone receptor status by immunohistochemistry using Allred's score in primary breast tumor specimens from 75 metastatic breast cancer patients who received first-line treatment with endocrine therapy on relapse, and analyzed the correlation between hormone receptor status and response to endocrine therapy as well as post-relapse survival.

Table 1. Clinicopathological Characteristics of Patients, Primary Breast Tumors, and Treatment

	Number of patients (%)
Total number of patients	75
Age at diagnosis (years)	
≦ 50	35 (46.7)
> 50	40 (53.3)
Range	29 to 77
Tumor size (cm)	
< 2.0	20 (26.7)
≧ 2.0	55 (73.3)
Number of positive lymph nodes	
0	21 (28.0)
1-3	21 (28.0)
> 3	33 (44.0)
Histological grade	
1	12 (16.0)
2	43 (57.3)
3	20 (26.7)
HER2	
Negative	63 (84.0)
Positive	12 (16.0)
Adjuvant therapy	
None	5 (6.7)
Endocrine therapy	32 (42.7)
Chemotherapy	2 (2.6)
Combined	36 (48.0)
First-line endocrine therapy for metastatic breast cancer	
Tamoxifen	56 (74.7)
Aromatase inhibitors	11 (14.7)
LH-RH agonist	3 (4.0)
LH-RH agonist + tamoxifen	4 (5.3)
Fulvestrant	1 (1.3)

SD, standard deviation; LH-RH agonist, luteinizing hormone-releasing hormone agonist

Materials and Methods

Patients and Breast Cancer Tissues

Breast tumor specimens from 75 female metastatic breast cancer patients, who were treated at Nagoya City University Hospital between 1982 and 2002, were included in this study (Table 1). The study protocol was approved by the institutional review board and conformed with the guidelines of the 1975 Declaration of Helsinki. All patients had undergone surgical treatment for primary breast cancer (either mastectomy or lumpectomy). After surgery, five patients (6.7%) received no additional therapy. Of the remaining 70 patients, 32 (42.7%) received systemic adjuvant therapy with endocrine therapy (tamoxifen) alone, two (2.7%) received chemotherapy alone, and 36 (48%) received combined endocrine therapy and chemotherapy. Patients who were positive for axillary lymph node metastases received either oral 5-fluorouracil derivatives for 2 years or a combination of cyclophosphamide, methotrexate, and fluorouracil (CMF). Patients were observed for disease recurrence at least once every six months for the first 5 years after the surgery and thereafter once every year. The median disease-free interval was 38 months (mean ± SD, 39.9 ± 26.4 months; range,

1 to 123 months).

First-Line Endocrine Therapy for Metastatic Breast Cancer and Response Criteria

When the patients relapsed and were diagnosed with metastatic breast cancer, they started endocrine therapy (Table 1). Patients were assessed monthly for clinical response, which was defined according to World Health Organization criteria as complete response, partial response, no change, or progressive disease. The presence of progressive disease indicated treatment failure; all other clinical responses were considered to show efficacy of treatment.

Immunohistochemical Analysis

One 4-μm section of each submitted paraffin block was stained first with hematoxylin and eosin to verify that a sufficient number of invasive carcinoma cells were present and that the fixation quality was adequate for immunohistochemical (IHC) analysis. Serial sections (4 μm) were prepared from selected blocks and float-mounted on adhesive-coated glass slides, for ER and PgR staining as described previously[7]. Primary antibodies included monoclonal mouse anti-human ERα antibody (1D5, DAKO, Glostrup, Denmark) at 1:100 dilution for ER, and monoclonal mouse anti-human PgR antibody (636, DAKO) at 1:100 dilution for PgR. The DAKO Envision system (DAKO EnVision labelled polymer, peroxidase) was used for detection as described previously[7].

Immunohistochemical Scoring

Immunostained slides were scored after the entire slide was evaluated by light microscopy. The immunostaining of ER and PgR was assessed by two independent investigators (H. Y. and Z. Z.), and discordant results were resolved by consultation with a third investigator (H. I.). Hormone receptor expression was scored by assigning proportion and intensity scores, according to Allred's procedure[3]. In brief, a proportion score (PS) represented the estimated proportion of tumor cells staining positive, as follows: 0 (none); 1 ($<$1/100); 2 (1/100 to 1/10); 3 (1/10 to 1/3); 4 (1/3 to 2/3); and 5 ($>$2/3). Any brown nuclear staining in invasive breast epithelium counted towards the proportion score. An intensity score (IS) represented the average intensity of the positive cells, as follows: 0 (none); 1 (weak); 2 (intermediate); and 3 (strong). The proportion and intensity scores were then added to obtain a total score (TS), which could range from 0 to 8.

Statistical Analysis

The chi-square test, the Mann-Whitney U test, and the unpaired *t*-test were used to compare the IHC scores of hormone receptors with response to endocrine therapy. Both the Mann-Whitney U test and the unpaired *t* test were used to analyze correlation between IHC scores and response to endocrine therapy. Although the non-parametric Mann-Whitney U test might be better, parametric characteristics (mean ± SD) were obtained by the unpaired *t* test. Estimation of post-relapse survival was performed using the Kaplan-Meier method, and differences between survival curves were assessed with the log-rank test.

Results

Immunohistochemical Evaluation of ER in Primary Breast Tumors and Response to Endocrine Therapy in Metastatic Breast Cancer

All patients received endocrine therapy as first-line treatment for metastatic breast cancer at relapse; thirty-five (46.7%) patients responded. We first analyzed whether expression levels of ER in the primary breast tumors affected response to endocrine therapy. We focused on Allred's score (TS) and the proportion score (PS) for immunohistochemical evaluation of hormone receptors because these two methods, but not the intensity score (IS), are the most widely used in the world. Patients who responded to endocrine therapy had significantly higher expression of ER as estimated by Allred's score (TS) than did non-responders (Table 2, $p = 0.0045$ and $p = 0.0046$ by Mann-Whitney and the unpaired *t*-tests, respectively). When ER expression was analyzed only by the proportion score (PS), a significant association was also observed between higher ER expression and response to endocrine therapy (Table 2, $p = 0.019$ and $p = 0.011$).

Immunohistochemical Evaluation of PgR in Primary Breast Tumors and Response to Endocrine Therapy in Metastatic Breast Cancer

We next analyzed whether expression levels of PgR affected response to endocrine therapy. Patients who responded to endocrine therapy had

Table 2. Correlation Between IHC Scores of ER and PgR and Response to Endocrine Therapy

	Responders (n = 35) (Mean ± SD)	Non-responders (n = 40) (Mean ± SD)	p [a]	p [b]
ER score				
Allred's score (TS)	5.8 ± 2.3	4.1 ± 2.9	0.0045*	0.0046*
Proportion score (PS)	3.9 ± 1.5	2.8 ± 2.0	0.019*	0.011*
PgR score				
Allred's score (TS)	5.5 ± 2.5	3.6 ± 2.5	0.0008*	0.0014*
Proportion score (PS)	3.6 ± 1.7	2.4 ± 1.9	0.0033*	0.005*

[a] Mann-Whitney U test
[b] Unpaired *t*-test
* $p < 0.05$ is considered significant.

Table 3. Correlation Between ER and PgR IHC Scores and Response to Tamoxifen

	Responders (n = 20) (Mean ± SD)	Non-responders (n = 36) (Mean ± SD)	p [a]	p [b]
ER score				
Allred's score (TS)	5.7 ± 2.3	3.9 ± 2.3	0.038*	0.030*
Proportion score (PS)	3.8 ± 1.5	2.7 ± 2.0	0.098	0.047*
PgR score				
Allred's score (TS)	5.9 ± 2.4	3.4 ± 2.5	0.0004*	0.0007*
Proportion score (PS)	3.9 ± 1.6	2.3 ± 1.8	0.0018*	0.0025*

[a] Mann-Whitney U test
[b] Unpaired *t*-test
* $p < 0.05$ is considered significant.

significantly higher expression of PgR as estimated by Allred's score (TS) than did non-responders (Table 2, $p = 0.0008$ and $p = 0.0014$). When PgR expression was analyzed only by the proportion score (PS), a significant association was also observed between higher PgR expression and response to endocrine therapy (Table 2, $p = 0.0033$ and $p = 0.005$).

Immunohistochemical Evaluation of ER in Primary Breast Tumors and Response to Tamoxifen in Metastatic Breast Cancer

The preceding analyses included all types of endocrine treatment. We then narrowed the focus to examine the correlation between response to tamoxifen treatment and hormone receptor expression, starting with ER, because it is critical to analyze response to each endocrine reagent individually. Fifty-six patients were treated with tamoxifen in this study, and twenty (35.7%) patients responded. Responders had significantly higher ER expression, as estimated with the Allred's score (TS), than non-responders (Table 3, $p = 0.038$ and $p = 0.030$). The average Allred's score (TS) in responders to tamoxifen was 5.7 (Table 3), which was almost the same as that in responders to endocrine therapies of all kinds (TS = 5.8, Table 2). When ER expression was analyzed only with the proportion score (PS), a significant association was also observed between higher ER expression and response to tamoxifen (Table 3, $p = 0.047$ by unpaired *t*-test). The average proportion score (PS) in responders to tamoxifen was 3.8 (Table 3), which was similar to that in responders to any kind of endocrine therapy (PS = 3.9, Table 2). Collectively, these results suggested that expression levels of ER, as evaluated by either Allred's score (TS) or the proportion score (PS), affect response to tamoxifen.

Immunohistochemical Evaluation of PgR in Primary Breast Tumors and Response to Tamoxifen in Metastatic Breast Cancer

We then examined correlation between PgR expression levels and tamoxifen response. Patients who responded to tamoxifen therapy had sig-

Table 4. Correlation Between ER IHC Status and Response to Endocrine Therapy

	Allred's score (TS ≧ 4)		Proportion of positive cells (≧ 10%)		Proportion of positive cells (≧ 1%)	
	positive/total (%)	*p*	positive/total (%)	*p*	positive/total (%)	*p*
Response to endocrine therapy (n = 75)						
total	56/75 (74.7)		55/75 (73.3)		57/75 (76.0)	
responders	31/35 (88.6)	0.020*	31/35 (88.6)	0.011*	31/35 (88.6)	0.034*
non-responders	25/40 (62.5)		24/40 (60.0)		26/40 (65.0)	
Response to tamoxifen (n = 56)						
total	40/56 (71.4)		39/56 (69.6)		41/56 (73.2)	
responders	18/20 (90.0)	0.047*	18/20 (90.0)	0.030*	18/20 (90.0)	0.072
non-responders	22/36 (61.1)		21/36 (58.3)		23/36 (63.9)	
Response to aromatase inhibitors (n = 11)						
total	8/11 (72.7)		8/11 (72.7)		8/11 (72.7)	
responders	7/9 (77.8)		7/9 (77.8)		7/9 (77.8)	
non-responders	1/2 (50.0)		1/2 (50.0)		1/2 (50.0)	

p value was assessed with the chi-square test. *$p < 0.05$ is considered significant.

nificantly higher expression of PgR as estimated by Allred's score (TS) than did non-responders (Table 3, $p = 0.0004$ and $p = 0.0007$). The average Allred's score (TS) in responders to tamoxifen was 5.9 (Table 3), which was higher than that in responders to endocrine therapies of all kinds (TS = 5.5, Table 2). When PgR expression was analyzed only with the proportion score (PS), a significant association was also observed between higher PgR levels and response to tamoxifen (Table 3, $p = 0.0018$ and $p = 0.0025$). The average proportion score (PS) in responders to tamoxifen was 3.9 (Table 3), which was higher than that in responders to endocrine therapies of all kinds (PS = 3.6, Table 2). Thus, expression levels of PgR, as well as ER, as evaluated by either the Allred's score (TS) or the proportion score (PS), affect response to tamoxifen. Furthermore, patients with higher levels of PgR expression responded better to tamoxifen.

Patients whose Primary Breast Tumors Contain 10% or More ER-Positive Cells Efficiently Respond to Endocrine Therapy

We evaluated cutoff points in the assessment of ER as a predictor of response to endocrine therapy. In terms of Allred's score (TS), the most significant correlation between positive ER expression and response to endocrine therapy, whether all kinds ($p = 0.020$) or tamoxifen only ($p = 0.047$) was obtained when the cutoff point was set at TS ≧4, (Table 4). By this criterion, fifty-six tumors (74.7%) were positive for ER. When the evaluation was based on the proportion of cells positive for ER, a cutoff value of 10% gave a more significant association between positive ER expression and response to endocrine therapy than a cutoff of 1% (Table 4, $p = 0.011$ and $p = 0.034$, respectively). There was no correlation between response to endocrine therapy and ER expression when the cutoff point was set at PS ≧4. Using any of these three criteria (TS ≧4, PS ≧10%, or PS ≧1%), there were thirty-one (88.6%) patients whose tumors were ER-positive in thirty-five responders. Response to tamoxifen was significantly correlated with positive ER expression when the PS cutoff was set at 10% (Table 4, $p = 0.030$), but not at 1% (Table 4, $p = 0.072$). Using PS ≧10% as the criterion of ER positivity also produced stronger associations (lower *p* values) in terms of both response to endocrine therapy and response to tamoxifen than were obtained using Allred's score with a cutoff of TS ≧4. Eleven patients were treated with aromatase inhibitors, and nine (81.8%) responded. Seven (77.8%) of these responders had ER-positive tumors by any method of evaluation (Table 4). From these analyses, we concluded that the best cutoff point for ER was 10% of the proportion of positive cells with regard to the response to endocrine therapy, and that the cutoff point could be set at 1%, especially for metastatic breast cancer.

Table 5. Correlation Between PgR IHC Status and Response to Endocrine Therapy

	Allred's score (TS ≧ 5)		Proportion of positive cells (≧ 10%)		Proportion of positive cells (≧ 1%)	
	positive/total (%)	*p*	positive/total (%)	*p*	positive/total (%)	*p*
Response to endocrine therapy (n = 75)						
total	43/75 (57.3)		47/75 (62.7)		55/75 (73.3)	
responders	25/35 (71.4)	0.038*	26/35 (74.3)	0.087	30/35 (85.7)	0.044*
non-responders	18/40 (45.0)		21/40 (52.5)		25/40 (62.5)	
Response to tamoxifen (n = 56)						
total	41/56 (73.2)		35/56 (62.5)		40/56 (71.4)	
responders	16/20 (90.0)	0.013*	17/20 (85.0)	0.021*	18/20 (90.0)	0.047*
non-responders	15/36 (41.7)		18/36 (50.0)		22/36 (61.1)	
Response to aromatase inhibitors (n = 11)						
total	4/11 (36.3)		4/11 (36.3)		7/11 (63.6)	
responders	3/9 (33.3)		3/9 (33.3)		6/9 (66.7)	
non-responders	1/2 (50.0)		1/2 (50.0)		1/2 (50.0)	

p value was assessed with the chi-square test. $^{*}p < 0.05$ is considered significant.

Patients with Primary Breast Tumors with an Allred's Score of 5 or More for PgR Efficiently Respond to Endocrine Therapy

We next evaluated cutoff points for the assessment of PgR as a predictor of response to endocrine therapy. In terms of Allred's score (TS), the most significant correlation between positive PgR expression and response to endocrine therapy of any kind ($p = 0.038$) or tamoxifen only ($p = 0.013$) was obtained when the cutoff point of TS was set at 5 (Table 5). When the evaluation was based on the proportion of cells positive for PgR, a significant association between positive PgR expression and response to endocrine therapy was observed at a cutoff point of 1%, but not at 10% (Table 5, $p = 0.044$ and $p = 0.087$, respectively). However, response to tamoxifen was more strongly correlated with positive PgR expression using a cutoff value of 10% rather than 1% (Table 5, $p = 0.021$ and $p = 0.047$, respectively). When PgR expression was evaluated by Allred's score using a cutoff of TS ≧ 5, a stronger association (lower *p* values) with both response to endocrine therapy and response to tamoxifen was observed than when using a proportion score with a cutoff 10% or 1%. In contrast, of nine responders to aromatase inhibitors, only three (33.3%) had PgR-positive tumors as defined by an Allread's score ≧5 or a proportion score ≧ 10% (Table 5). From these analyses, we concluded that the best cutoff point for PgR with regard to response to tamoxifen was an Allred's score of 5.

Combined ER and PgR Status and Response to Endocrine Therapy

There were 44 (58.7%) patients whose tumors were both ER- and PgR- positive at a cutoff point of 10% positive cells (Table 6). The ratio of responders with both ER- and PgR-positive tumors was 56.8%. Analyzing response to tamoxifen, there were thirty-three (58.9%) patients whose tumors were both ER- and PgR- positive at a cutoff point of 10% positive cells and 17 (51.5%) were responsive, whereas only 1 in 6 (16.7%) patients with ER-positive, PgR-negative tumors responded to tamoxifen (Table 6). In contrast, all 5 patients with ER-positive, PgR-negative tumors at a cutoff point of 10% responded to aromatase inhibitors (Table 6). There were only 3 patients (4.0%) whose tumors were ER-negative and PgR-positive. Three of 17 patients (17.6%) with ER- and PgR-negative tumors at a cutoff point of 10% positive cells also responded to endocrine therapy. When evaluated at a cutoff point of 1% positive cells, there were 15 patients with both ER- and PgR-negative tumors, and 3 patients (20.0%) responded to the therapy. We concluded from these analyses that PgR positivity influenced the effects of tamoxifen and aromatase inhibitors in ER-positive tumors, and that the cutoff point could be set at 1% positive cells when treating metastatic breast cancer patients.

Table 6. Correlation Between Combined ER and PgR Status at a Cutoff Point of 10% Positive Cells and Response to Endocrine Therapy

	ER+ PgR+	ER+ PgR−	ER− PgR+	ER− PgR−
	No. of patients/total (%)	No. of patients/total (%)	No. of patients/total (%)	No. of patients/total (%)
Response to endocrine therapy (n = 75)				
total	44/75 (58.7)	11/75 (14.7)	3/75 (4.0)	17/75 (22.7)
responders	25/44 (56.8)	6/11 (54.5)	1/3 (33.3)	3/17 (17.6)
non-responders	19/44 (43.2)	5/11 (45.5)	2/3 (66.7)	14/17 (82.4)
Response to tamoxifen (n = 56)				
total	33/56 (58.9)	6/56 (10.7)	2/56 (3.6)	15/56 (26.8)
responders	17/33 (51.5)	1/6 (16.7)	0/2 (0)	2/15 (13.3)
non-responders	16/33 (48.5)	5/6 (83.3)	2/2 (100.0)	13/15 (86.7)
Response to aromatase inhibitors (n = 11)				
total	3/11 (27.3)	5/11 (45.5)	1/11 (9.1)	2/11 (18.2)
responders	2/3 (66.7)	5/5 (100.0)	1/1 (100.0)	1/2 (50.0)
non-responders	1/3 (33.3)	0/5 (0)	0/1 (0)	1/2 (50.0)

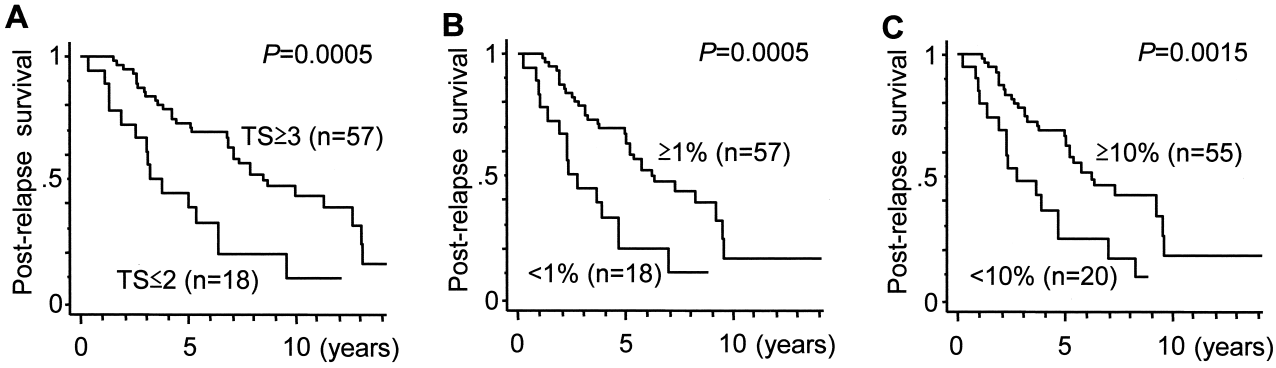

Fig 1. Post-relapse survival of patients according to expression levels of ER in primary breast tumors. Expression status was evaluated by Allred's score (A, cutoff point of TS is set at 3), and the proportion of positive cells (B, cutoff point of 1%; C, cutoff point of 10%). Higher expression levels of ER by any evaluation method were associated with better survival.

Post-Relapse Survival Categorized by Expression Levels of ER in Primary Breast Tumors

Finally, we analyzed the survival after relapse categorized by ER positivity in primary breast tumors. The median follow-up period was 77 months (range, 4 to 234 months). Using Allred's score (TS), high expression of ER significantly increased post-relapse survival when the cutoff point of TS was set at 3 (Fig 1A, $p = 0.0005$). Similarly, a strong correlation was observed between ER expression and post-relapse survival when ER positivity was evaluated as 1% or more positive cells (Fig 1B, $p = 0.0005$). Although a significant association was detected between ER expression and post-relapse survival at a cutoff point of 10% (Fig 1C, $p = 0.0015$), the p value at 1% was less than that at 10%. These results indicate that patients with high ER expression levels had significantly longer post-relapse survival, and that the best cutoff points for ER were at an Allred's score of 3 and at 1% positive cells.

Post-Relapse Survival Categorized by Expression Levels of PgR in Primary Breast Tumors

We then analyzed the survival after relapse categorized by PgR positivity in primary breast tumors. Using an Allred's score (TS) of 3 as a cutoff, high expression of PR significantly increased post-relapse survival (Fig 2A, $p = 0.0008$). Similarly, a strong correlation was observed between PgR

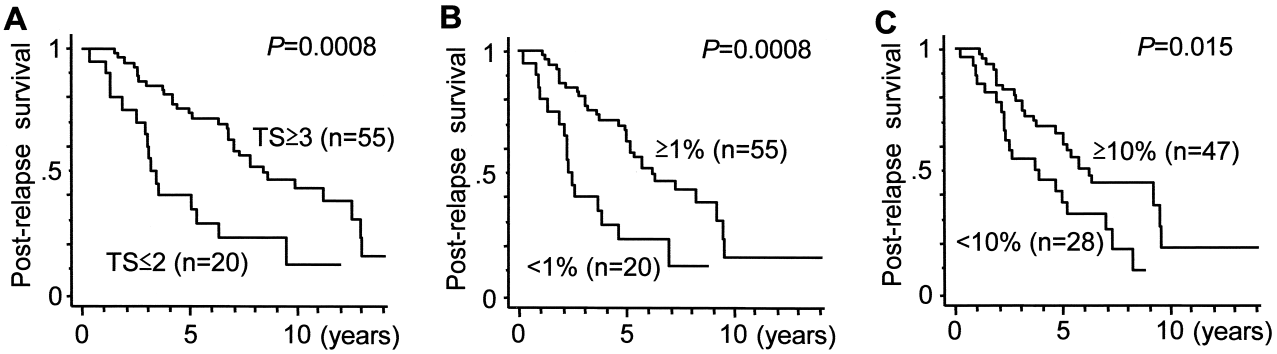

Fig 2. Post-relapse survival of patients according to expression levels of PgR in primary breast tumors. Expression status was evaluated by Allred's score (A, cutoff point of TS is set at 3), and the proportion of positive cells (B, cutoff point of 1%; C, cutoff point of 10%). Higher expression levels of PgR by any evaluation method were associated with better survival.

expression and post-relapse survival when PgR positivity was evaluated as 1% or more positive cells (Fig 2B, $p = 0.0008$). Although a significant association was also detected between post-relapse survival and PgR expression at a cutoff point of 10% (Fig 2C, $p = 0.015$), the p value at 1% was less than that at 10%. These results indicated that patients with high PgR expression levels, as well as ER, had significantly longer post-relapse survival, and that the best cutoff points for PgR were at an Allred's score of 3 and at 1% positive cells.

Discussion

We examined hormone receptor status by immunohistochemistry using Allred's score in primary breast tumors from 75 metastatic breast cancer patients who received first-line treatment with endocrine therapy on relapse, and analyzed the correlation between hormone receptor status and response to endocrine therapy as well as post-relapse survival.

Whatever the evaluation method used, there is no doubt that the more ER present in the tumor cells, the greater the likelihood of a favorable response to endocrine therapy for metastatic breast cancer. Our results showed that patients with higher expression of ER or PgR estimated by either Allred's score or the proportion score responded significantly to the therapy. The United Kingdom national external quality assessment scheme for immunohistochemistry (UK NEQAS-ICC) has been conducted to investigate interlaboratory variance in the sensitivity of detection and evaluation of scoring systems for hormone receptors since April 1994, and 200 laboratories in 26 countries have participated[8-10]. Based on the results, they proposed to use Allred's score because of its high reproducibility, good correlation with the ligand binding assay, and equally significant predictive and prognostic information, showing a 'working protocol' for immunohistochemical detection of steroid receptors in breast cancer[11]. We also used Allred's score for the evaluation of ER and PgR status, but our results indicated that the proportion score alone might be enough to predict hormone responsiveness and post-relapse survival in metastatic breast cancer.

Different cutoff values might be used depending on the clinical situation, whether in the adjuvant or metastatic setting. When the cutoff point is stringently set low and the assay is of high quality, patients with ER- and PgR-negative tumors will experience little benefit from endocrine therapy, especially as an adjuvant treatment. For metastatic disease, treating hormone receptor-negative tumors with endocrine therapy simply delays more appropriate treatment[12]. In our study, three of fifteen patients (20.0%) with ER- and PgR-negative tumors evaluated at a cutoff point of 1% responded to the therapy. Thus, the cutoff might be set low, such as 1%, especially for metastatic disease.

Since PgR is induced by ER, it has been studied as a surrogate marker for ER activity and has been used as an additional predictive factor for endocrine therapy. Reduced benefit with tamoxifen adjuvant therapy in ER-positive, PgR-negative tumors was reported in a very large data set in which the receptors were measured in a central reference laboratory[13]. It has recently been reported that growth factor signaling through IGF-IR or HER1/HER2 results in downregulation of transcription of the PgR gene[14]. This may be due to ER complexed with the transcription factors *fos*

and *jun* at an activator protein recognition site in the promoter of the PgR gene[15]. It has been reported that ER-positive, PgR-negative tumors more frequently express higher levels of HER2 than ER-positive, PgR-positive tumors[16]. Thus, estrogen deprivation therapy might be more beneficial than tamoxifen in ER-positive, PgR-negative tumors for reasons similar to those seen in HER2 positive tumors, in which the growth factor signaling cascade reduces the antagonist qualities of tamoxifen[17, 18]. Our results also indicated that tamoxifen was less effective in ER-positive, PgR-negative tumors, and that all patients with ER-positive, PgR-negative tumors responded to aromatase inhibitors. Recent studies indicated that HER1 and HER2 status affect resistance to endocrine therapy, particularly tamoxifen[19-21]. Ellis *et al*. reported that ER-positive, HER1- and/or HER2-positive primary breast cancer responded well to letrozole, but responses to tamoxifen were infrequent, suggesting that HER1 and HER2 signaling through ER is ligand-dependent and that the growth-promoting effects of these receptor tyrosine kinases on ER-positive breast cancer can be inhibited by potent estrogen deprivation[20].

Finally, our results demonstrated that patients with 1% or more ER or PgR positive cells had better survival after relapse. It was reported that using Allred's score, the best cutoff point was TS $\geqq$3, and ER status was a highly significant predictor for disease-free survival, indicating that tumors with as few as 1% to 10% weakly positive cells had a significantly improved response, compared with those with fewer positive cells[5]. There may be several explanations as to why such low scores predict better survival, including that low scores correspond to an ER-positive stem-cell population.

In conclusion, patients with primary breast tumors with 10% or more ER positive cells efficiently responded to endocrine therapy, and patients with 1% or more ER or PgR positive cells had better survival after relapse. Our results indicated that the proportion score alone might be enough to predict hormone responsiveness and post-relapse survival in metastatic breast cancer, and that the cutoff might be set low, such as 1%, especially for metastatic disease. It is important to consider individualized management for women with breast cancer, making treatment more effective and timely.

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