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Predictors of response to exemestane as primary endocrine therapy in estrogen receptor–positive breast cancer

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Endocrine therapy is the most important treatment of choice for estrogen receptor (ER)-positive breast cancer. Potential mechanisms for resistance to endocrine therapy involve ER-coregulatory proteins and cross-talk between ER and other growth factor–signaling networks. However, the factors and pathways responsible for endocrine therapy resistance, particularly resistance to aromatase inhibitors, have not been clearly established. Sixteen postmenopausal patients with ERα-positive primary breast cancer were treated daily with 25 mg of exemestane (an aromatase inhibitor) for 6 months. Expressions of ERα, ERβ, progesterone receptor (PgR), androgen receptor (AR), amplified in breast cancer 1 (AIB1), aromatase, epidermal growth factor receptor, human epidermal growth factor receptor type 2, Ki67, cyclin D1, p53, Bcl2, signal transducer and activator of transcription 5 (Stat5), and insulin-like growth factor binding protein 5 (IGFBP5), and phosphorylations of ERα serine (Ser) 118, ERα Ser167, Akt Ser473, and p44/42 MAPK threonine (Thr) 202/tyrosine (Tyr) 204, were examined by immunohistochemistry on pretreatment tumor biopsies and post-treatment surgical specimens. Analyses were made to test for correlations with response to exemestane. Of the 16 patients, seven responded and nine retained stable disease. High-level expression of AIB1 and phosphorylation of Akt Ser473 were significantly associated with a better response to exemestane, suggesting that these factors could be considered as predictors of exemestane response. Expressions of ERα, ERβ, PgR, aromatase, Ki67, cyclin D1, and p53, and phosphorylations of ERα Ser118, ERα Ser167, and p44/42 MAPK Thr202/Tyr204, were decreased, whereas expressions of Stat5 and IGFBP5 were increased in post-treatment specimens compared to the values in pretreatment biopsies. Thus, the analysis of factors involved in the estrogen-dependent growth-signaling pathways may be useful in identifying patients responsive to exemestane. (*Cancer Sci* 2009; 100: 2028–2033)

Endocrine therapy has become the most important treatment option for women with ER-positive breast cancer. Local aromatization of androgens to estrogens is the primary source of estradiol in the breasts of postmenopausal women. Large-scale adjuvant clinical trials of nonsteroidal aromatase inhibitors, such as anastrozole and letrozole, and the steroidal aromatase inhibitor exemestane, have shown improved disease-free survival for patients with early stage breast cancer randomized to 5 years of aromatase inhibitor treatment as compared to 5 years of tamoxifen treatment.[1,2] When exemestane was administered following 2–3 years of tamoxifen, this improvement in disease-free survival was shown to translate into an overall survival benefit above that obtained with 5 years of tamoxifen treatment.[3] Therefore, aromatase inhibitors are now considered to be the gold standard endocrine therapy for hormone-receptor positive breast cancer in postmenopausal women. However, many breast cancer patients with tumors expressing high levels of ER are unresponsive to aromatase inhibitors, and all patients with advanced disease eventually develop resistance to the therapy. Identification of response predictors for aromatase inhibitors is critical to help reveal such patients prior to treatment.

Recent neoadjuvant aromatase inhibitor studies demonstrated that the expression of the proliferation marker Ki67 after short-term presurgical endocrine therapy showed a significant correlation with both response to the therapy and recurrence-free survival.[4,5]

We previously investigated prognostic and predictive factors for endocrine therapy in ER-positive breast cancer, and found various molecular predictors, such as phosphorylation of ERα Ser118 and ERα Ser167,[6,7] and expression of aromatase,[8] AIB1,[7] p53,[9,10] Stat5,[11] and IGFBP5,[12] as well as expression of HER2 and Ki67.[7,9,10] However, the endocrine therapies in those studies were mostly tamoxifen. In this study, we examined expressions and phosphorylations of molecular markers, including those factors examined by immunohistochemistry on pretreatment biopsies and post-treatment surgical specimens in postmenopausal patients with ER-positive primary breast cancer who were treated daily with 25 mg of exemestane for 6 months. Correlations between response to exemestane and expressions and phosphorylations of molecular markers were analyzed to identify predictors for the treatment.

Materials and Methods

Patients and breast cancer tissues. Sixteen postmenopausal patients over age 70 with primary ERα-positive breast cancer were treated daily with 25 mg of exemestane for 6 months between 2003 and 2006 (Table 1). Clinical measurement of tumor size and nodal status was performed monthly, and the final clinical sonographic measurements were performed 6 months after the start of treatment prior to the planned surgical excision of the tumor. Clinical response was defined as complete response (CR), partial response (PR), or stable disease (SD) according to the Response Evaluation Criteria in Solid Tumors (RECIST, 2000). Pretreatment specimens were taken by core needle biopsies. Post-treatment specimens were obtained at surgical treatment. The pathological response was assessed as grades 1 to 3 according to the following criteria: 0 (no response), 1 (mild to moderate response), 2 (marked response), 3 (complete response). The study protocol was approved by the institutional

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Table 1. Clinicopathological characteristics of patients and primary breast tumors

		Number of patients
Total number of patients		16
Age at diagnosis (years)	Mean ± SD	79.1 ± 6.3
	Range	70 to 88
Tumor size (cm)	<3.0	12
	≥3.0	4
Lymph node status (N)	Negative	16
	Positive	0
Nuclear grade	1	12
	2	3
	3	1
HER2	0	5
	1+	10
	2+	1
	3+	0
EGFR	0	16
Type of surgery	Breast-conserving surgery	15
	Mastectomy	1
Adjuvant therapy	Exemestane	16
Follow up (months)	Mean ± SD	46.0 ± 17.0
	Range	18 to 67

EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor type 2.

Table 2. List of antibodies used for the immunohistochemical markers

Markers	Antibodies	References
ERα	1D5; Dako, Carpinteria, CA, USA	13
pERα Ser118	No. 2515; Cell Signaling, Baverly, MA, USA	7
pERα Ser167	No. 2514; Cell Signaling	7
ERβ1	Generated rabbit polyclonal antibodies	14
ERβcx/β2	Generated rabbit polyclonal antibodies	14
PgR	636; Dako	13
AR	441, sc-7305; Santa Cruz Biotechnology, Santa Cruz, CA, USA	ND
AIB1	Clone 34; BD Biosciences, San Jose, CA, USA	7
Aromatase	Kindly provided by Nobuhiro Harada	15
HER2	c-erbB-2; Dako	9
EGFR	EGFR; Dako	ND
pAkt	No. 9277; Cell Signaling	7
pMAPK	No. 9101; Cell Signaling	7
Ki67	MIB-1; Dako	9
Cyclin D1	bcl-1 Ab-4; Neo Markers, Fremont, CA, USA	16
p53	PAb1801; Novocastra, Newcastle, UK	9
Bcl2	Clone 124; Dako	16
Stat5	Stat5b (G-2), sc-1656; Santa Cruz Biotechnology	11
IGFBP5	H-100, sc-13093; Santa Cruz Biotechnology	12

AIB1, amplified in breast cancer 1; AR, androgen receptor; EGFR, epidermal growth factor receptor; ER, estrogen receptor; HER2, human epidermal growth factor receptor type 2; IGFBP5, insulin-like growth factor binding protein 5; pAkt, phosphorylated Akt; pER, phosphorylated ER; PgR, progesterone receptor; pMAPK, phosphorylated MAPK, Ser, serine; Stat5, signal transducer and activator of transcription 5.

review boards and conformed with the guidelines of the 1996 Declaration of Helsinki.

Immunohistochemical (IHC) analysis. One 4-μm section of each submitted paraffin block was stained first with hematoxylin–eosin to verify that an adequate number of invasive carcinoma cells were present and that the fixation quality was adequate for IHC analysis. Serial sections (4 μm) were prepared from selected blocks and float-mounted on adhesive-coated glass slides for staining of expression of ERα,[13] ERβ1,[14] ERβcx/β2,[14] PgR,[13] AR, AIB1,[7] aromatase,[15] EGFR, HER2,[9] Ki67,[9] cyclin D1,[16] p53,[9] Bcl2,[16] Stat5,[11] and IGFBP5,[12] and phosphorylation of ERα Ser118,[7] ERα Ser167,[7] Akt Ser473,[7] and p44/42 MAPK Thr202/Tyr204,[7] as described previously. Primary antibodies used in this study are listed in Table 2.

Immunostained slides were scored after the entire slide was evaluated by light microscopy. The expression of ERα, ERβ1, ERβcx/β2, PgR, and AR, and the phosphorylation of ERα Ser118 and ERα Ser167, were scored by assigning proportion and intensity scores, according to Allred's procedure.[17] In brief, a proportion score 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 breast epithelium counted towards the proportion score. An intensity score 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 which could range from 0 to 8. Tumors with scores ≥3 for ERα were included in this study. Expression of AIB1, aromatase, Ki67, cyclin D1, p53, Bcl2, Stat5, and IGFBP5, and phosphorylation of Akt and p44/42 MAPK, were scored by assigning proportion scores 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). Expression of Stat5 and IGFBP5 was assessed by cytoplasmic and nuclear staining, respectively. Expression of AIB1, Ki67, cyclin D1, p53, and Bcl2, and phosphorylation of Akt and p44/42 MAPK, were assessed by nuclear staining only. Expression of aromatase was assessed by cytoplasmic staining in cancer cells and stromal cells, respectively. Immunostaining of EGFR and HER2 was evaluated using the same method as is employed by the HercepTest (Dako, Glostrup, Denmark). To determine the score of EGFR and HER2 expression, the membrane staining pattern was estimated and scored on a scale of 0 to 3.

Statistical analysis. The Mann–Whitney *U*-test and the unpaired *t*-test were used to compare the IHC scores of molecular markers with response to exemestane. To examine the change of expression and phosphorylation status between pretreatment and post-treatment tumors, the one-sample Wilcoxon signed rank test was used.

Results

Clinical and pathological responses. The patients' characteristics are summarized in Table 1. All patients completed the 6-month treatment. Of the 16 patients, seven responded and nine retained SD according to the clinical evaluation (Table 3). A pathological response (grades I to III) was obtained in eight patients. One patient presented both a clinical CR and a pathological CR. No patient showed progressive disease. A significant correlation was seen between clinical and pathological responses ($P = 0.035$) (Table 3).

High expression of AIB1 and high-level phosphorylation of Akt Ser473 are significantly associated with better responses to exemestane. We first analyzed the potential correlation between IHC scores of molecular markers in tumors before treatment and response to exemestane. ERα Allred expression scores were 5 or greater in all tumors before treatment. All tumors were negative for EGFR. As shown in Table 4, patients with primary tumors that had higher expressions of AIB1 and high-level phosphorylations of Akt Ser473 responded significantly to the treatment according to the clinical evaluation (Mann–Whitney *U*-test, $P = 0.037$ and $P = 0.0006$, respectively). Although IHC scores of HER2 were higher in responders compared to nonresponders

(scores 1 or 2 *vs* 0 or 1), none of them were overexpressed (Fig. 1). Similarly, the expression levels of p53 were below 10% in all tumors, although IHC scores of p53 were higher in responders than in nonresponders (scores 0 to 2 *vs* 0 or 1) (Fig. 2). The other molecular markers, including phosphorylation of ERα and expression of PgR (Fig. 3a), did not affect the clinical response to exemestane. When the response was evaluated pathologically, phosphorylation of Akt Ser473 was the only factor that affected the response (Mann–Whitney *U*-test, $P = 0.020$) (Table 5). We concluded that higher expression of AIB1 and high-level phosphorylation of Akt Ser473 are predictors for an exemestane response.

Comparison of expression and phosphorylation levels of molecular markers in tumors before and after exemestane treatment. We next examined potential correlations between IHC scores of molecular markers in tumors after treatment and response to exemestane. Only 15 paired tumors were analyzed

Table 3. Correlation between clinical and pathological responses

Clinical response	Pathological response (grade)				
	0	1	2	3	Total
SD	6 (2)†	3	0	0	9
PR	2 (0)†	2	2	0	6
CR	0	0	0	1	1
total	8	5	2	1	16

†Number of cases that no pathological response was observed.
CR, complete response; PR, partial response; SD, stable disease.

Table 4. Correlation between immunohistochemical scores of biological markers in tumors before treatment and response to exemestane

	PR and CR ($n = 7$) Mean ± SD (median; range)	SD ($n = 9$) Mean ± SD (median; range)	*P*-values†	*P*-values‡
ERα	7.6 ± 1.1	7.7 ± 0.7	0.82	0.84
pERα Ser118	7.1 ± 1.1	7.4 ± 0.8	0.58	0.55
pERα Ser167	7.7 ± 0.8	7.2 ± 0.8	0.16	0.24
ERβ1	6.9 ± 1.5	6.6 ± 0.7	0.28	0.60
ERβcx/β2	4.9 ± 2.4	3.7 ± 2.8	0.41	0.38
PgR	4.9 ± 3.6	5.1 ± 2.6	0.79	0.87
AR	3.0 ± 1.3	2.7 ± 0.7	0.77	0.52
AIB1	3.0 ± 2.1	0.6 ± 1.7	0.037*	0.020*
Aromatase (cancer)	4.6 ± 0.8	4.8 ± 0.7	0.44	0.58
Aromatase (stroma)	3.6 ± 1.0	4.0 ± 1.0	0.44	0.40
HER2	1.1 ± 0.4	0.4 ± 0.5	0.016*	0.010*
pAkt	4.0 ± 1.5	0.3 ± 1.0	0.0006*	<0.0001*
pMAPK	3.7 ± 1.8	2.3 ± 1.9	0.12	0.17
Ki67	2.4 ± 0.5	2.6 ± 0.7	0.81	0.70
Cyclin D1	4.9 ± 0.4	4.8 ± 0.4	0.70	0.71
p53	1.0 ± 1.0	0.1 ± 0.3	0.038*	0.025*
Bcl2	3.1 ± 0.8	3.0 ± 1.1	0.96	0.78
Stat5 (cytoplasm)	2.1 ± 2.7	2.8 ± 2.6	0.63	0.64
Stat5 (nuclei)	4.9 ± 0.4	3.7 ± 2.1	0.17	0.17
IGFBP5 (cytoplasm)	3.1 ± 2.3	2.0 ± 2.5	0.39	0.36
IGFBP5 (nuclei)	4.6 ± 0.8	3.2 ± 2.1	0.18	0.13

*$P < 0.05$ is considered significant. †Mann–Whitney *U*-test. ‡Unpaired *t*-test. AIB, amplified in breast cancer 1; AR, androgen receptor; ER, estrogen receptor; HER2, human epidermal growth factor receptor type 2; IGFBP5, insulin-like growth factor binding protein 5; pAkt, phosphorylated Akt; pER, phosphorylated ER; PgR, progesterone receptor; pMAPK, phosphorylated MAPK, Ser, serine; Stat5, signal transducer and activator of transcription 5.

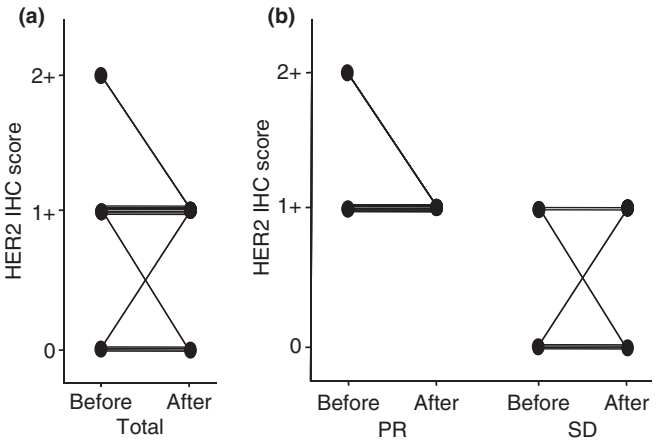

Fig. 1. Changes of immunohistochemical (IHC) expression scores for human epidermal growth factor receptor type 2 (HER2) in tumors in response to exemestane treatment. (a) All patients. (b) Responders (PR) and nonresponders (SD) were separately analyzed.

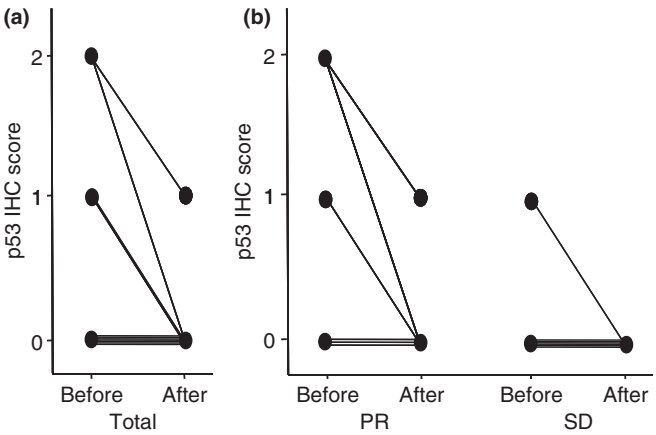

Fig. 2. Immunohistochemical (IHC) expression scores for p53 in tumors before and after exemestane treatment. (a) All patients. (b) Responders (PR) and nonresponders (SD) were separately analyzed.

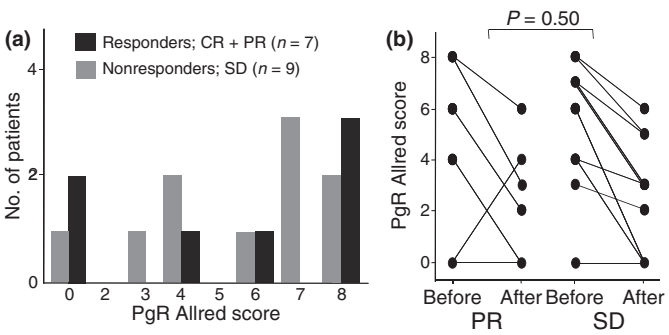

Fig. 3. (a) Progesterone receptor (PgR) Allred scores in tumors before exemestane treatment for responders and nonresponders. (b) Changes of immunohistochemical expression scores for PgR in tumors in response to exemestane treatment. Responders (PR) and nonresponders (SD) were separately analyzed.

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Table 5. Correlation between immunohistochemical scores of biological markers in tumors before treatment and pathological response

	Grade 1–3 (n = 8) Mean ± SD (median; range)	Grade 0 (n = 8) Mean ± SD (median; range)	P-values†	P-values‡
ERα	7.5 ± 1.1	7.8 ± 0.5	0.54	0.59
pERα Ser118	7.3 ± 1.1	7.4 ± 0.8	0.85	0.80
pERα Ser167	7.5 ± 0.6	7.4 ± 0.9	0.86	0.77
ERβ1	7.0 ± 0.6	6.4 ± 1.7	0.37	0.26
ERβcx/β2	3.4 ± 2.9	5.0 ± 2.1	0.21	0.23
PgR	5.5 ± 3.5	4.5 ± 2.5	0.29	0.52
AR	3.0 ± 0.9	2.6 ± 1.1	0.33	0.46
AIB1	2.1 ± 2.3	1.1 ± 2.1	0.39	0.38
Aromatase (cancer)	4.5 ± 0.9	4.9 ± 0.4	0.44	0.30
Aromatase (stroma)	4.3 ± 0.9	3.4 ± 0.9	0.077	0.073
HER2	0.9 ± 0.6	0.6 ± 0.5	0.42	0.41
pAkt	3.3 ± 2.2	0.5 ± 1.4	0.020*	0.013*
pMAPK	2.5 ± 2.3	3.4 ± 1.6	0.55	0.39
Ki67	2.8 ± 0.7	2.3 ± 0.5	0.12	0.12
Cyclin D1	4.8 ± 0.5	4.9 ± 0.4	0.54	0.55
p53	0.9 ± 1.0	0.1 ± 0.4	0.083	0.063
Bcl2	3.4 ± 0.9	2.8 ± 1.0	0.27	0.22
Stat5 (cytoplasm)	3.1 ± 2.6	1.9 ± 2.6	0.33	0.35
Stat5 (nuclei)	3.6 ± 2.3	4.8 ± 0.5	0.44	0.19
IGFBP5 (cytoplasm)	3.0 ± 2.5	2.0 ± 2.3	0.37	0.42
IGFBP5 (nuclei)	3.3 ± 2.3	4.4 ± 0.9	0.38	0.21

*P < 0.05 is considered significant. †Mann–Whitney U-test. ‡Unpaired t-test. AIB1, amplified in breast cancer 1; AR, androgen receptor; ER, estrogen receptor; HER2, human epidermal growth factor receptor type 2; IGFBP5, insulin-like growth factor binding protein 5; pAkt, phosphorylated Akt; pER, phosphorylated ER; PgR, progesterone receptor; pMAPK, phosphorylated MAPK; Ser, serine; Stat5, signal transducer and activator of transcription 5.

Table 6. Correlation between immunohistochemical scores of biological markers in tumors after treatment and response to exemestane

	PR (n = 6) Mean ± SD (median; range)	SD (n = 9) Mean ± SD (median; range)	P-values†	P-values‡
ERα	6.8 ± 1.9	6.9 ± 1.1	0.58	0.94
pERα Ser118	4.3 ± 1.8	4.8 ± 1.7	0.55	0.63
pERα Ser167	5.5 ± 1.8	5.6 ± 1.4	0.95	0.95
ERβ1	3.0 ± 3.0	3.9 ± 2.7	0.59	0.56
ERβcx/β2	1.7 ± 2.0	1.4 ± 2.2	0.79	0.84
PgR	2.5 ± 2.3	2.3 ± 2.0	0.90	0.88
AR	2.5 ± 0.5	2.9 ± 1.1	0.56	0.42
AIB1	2.3 ± 2.6	0.4 ± 1.3	0.08	0.08
Aromatase (cancer)	4.8 ± 0.4	4.4 ± 1.1	0.67	0.44
Aromatase (stroma)	2.3 ± 0.8	3.1 ± 1.5	0.27	0.26
HER2	1.0 ± 0.0	0.4 ± 0.5	0.031*	0.024*
pAkt	1.7 ± 2.6	1.0 ± 1.3	0.89	0.52
pMAPK	1.3 ± 1.4	1.4 ± 1.5	0.80	0.89
Ki67	1.5 ± 0.5	1.7 ± 0.7	0.69	0.63
Cyclin D1	3.8 ± 1.8	4.0 ± 1.4	0.95	0.85
p53	0.2 ± 0.4	0.0 ± 0.0	0.22	0.23
Bcl2	3.3 ± 1.6	1.9 ± 1.3	0.10	0.08
Stat5 (cytoplasm)	5.0 ± 0.0	3.2 ± 2.4	0.07	0.10
Stat5 (nuclei)	5.0 ± 0.0	4.6 ± 0.7	0.13	0.16
IGFBP5 (cytoplasm)	5.0 ± 0.0	3.3 ± 2.1	0.030*	0.7
IGFBP5 (nuclei)	5.0 ± 0.0	3.7 ± 1.6	0.020*	0.06

*P < 0.05 is considered significant. †Mann–Whitney U-test. ‡Unpaired t-test. AIB1, amplified in breast cancer 1; AR, androgen receptor; ER, estrogen receptor; HER2, human epidermal growth factor receptor type 2; IGFBP5, insulin-like growth factor binding protein 5; pAkt, phosphorylated Akt; pER, phosphorylated ER; PgR, progesterone receptor; pMAPK, phosphorylated MAPK; Ser, serine; Stat5, signal transducer and activator of transcription 5.

because one patient obtained a pathological CR. Patients who responded to the therapy had tumors that showed high expressions of HER2 or IGFBP5 in both cytoplasm and nuclei (Mann–Whitney U-test, $P = 0.031$, $P = 0.030$ and $P = 0.020$, respectively) (Table 6). When IHC scores of molecular markers were compared in tumors before and after exemestane treatment, expression levels of ERα, ERβ1, ERβcx/β2, PgR (Fig. 3b), aromatase in stromal cells, Ki67 (Fig. 4a), and cyclin D1, and phosphorylation levels of ERα Ser118, ERα Ser167, and p44/42 MAPK Thr202/Tyr204, were decreased, whereas expression levels of Stat5 and IGFBP5 in cytoplasm were increased in post-treatment tumors compared to the levels in pretreatment specimens regardless of the treatment response (Table 7). Although expression levels of p53 (Fig. 2a) also were decreased in post-treatment tumors compared to pretreatment specimens, these changes were not significant. On the other hand, expression levels of AR, AIB1, HER2 (Fig.1a), and Bcl2 did not change significantly during the treatment. Expression levels of Ki67 decreased more in post-treatment specimens of responders (grades 1 and 2) than in tumors of nonresponders (grade 0) according to the pathological evaluation ($P = 0.035$) (Fig. 4c). Interestingly, the phosphorylation levels of Akt Ser473 were decreased in the post-treatment specimens of responders (PR) but not of nonresponders (SD) according to the clinical evaluation ($P = 0.030$) (Fig. 5b). We suggest that these factors, especially those in the Akt signaling pathway, are involved in the estrogen-dependent growth.

Patients' outcomes. One patient developed bone metastasis during adjuvant exemestane treatment 36 months after surgery.

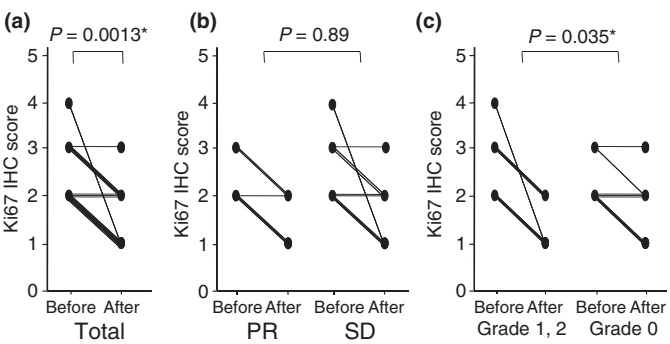

Fig. 4. Immunohistochemical (IHC) expression scores for Ki67 in tumors before and after exemestane treatment. (a) All patients. (b) Responders (PR) and nonresponders (SD) with clinical evaluation were separately analyzed. (c) Responders (grades 1 and 2) and nonresponders (grade 0) with pathological evaluation were separately analyzed.

The treatment was switched from exemestane to tamoxifen when the recurrence was diagnosed, and tamoxifen has been effective against her bone metastasis for more than 8 months. Another patient developed local recurrence in her breast during adjuvant exemestane treatment 45 months after surgery. Both patients showed stable disease with the primary exemestane treatment.

Table 7. Comparison of immunohistochemical scores of biological markers in tumors before and after exemestane treatment

	Before Mean ± SD	After Mean ± SD	*P*-values†	*P*-values‡
ERα	7.6 ± 0.9	6.9 ± 1.4	0.016*	0.010*
pERα Ser118	7.3 ± 0.9	4.6 ± 1.7	0.0014*	<0.0001*
pERα Ser167	7.4 ± 0.8	5.5 ± 1.5	0.0039*	0.0005*
ERβ1	6.7 ± 1.1	3.5 ± 2.7	0.0021*	0.0002*
ERβcx/β2	4.2 ± 2.6	1.5 ± 2.0	0.0071*	0.0012*
PgR	5.0 ± 3.0	2.4 ± 2.1	0.0089*	0.0025*
AR	2.8 ± 1.0	2.7 ± 0.9	>0.99	
AIB1	1.6 ± 2.2	1.2 ± 2.0	0.79	0.71
Aromatase (cancer)	4.7 ± 0.7	4.6 ± 0.9	0.83	0.84
Aromatase (stroma)	3.8 ± 1.0	2.8 ± 1.3	0.0065*	0.0021*
HER2	0.8 ± 0.6	0.7 ± 0.5	0.059	0.058
pAkt	1.9 ± 2.2	1.3 ± 1.9	0.29	0.43
pMAPK	2.9 ± 1.9	1.4 ± 1.4	0.020*	0.018*
Ki67	2.5 ± 0.6	1.6 ± 0.6	0.0013*	0.0005*
Cyclin D1	4.8 ± 0.4	3.9 ± 1.5	0.041*	0.037*
p53	0.5 ± 0.8	0.07 ± 0.3	0.059	0.055
Bcl2	3.1 ± 0.9	2.5 ± 1.6	0.30	0.26
Stat5 (cytoplasm)	2.5 ± 2.6	3.9 ± 2.0	0.035*	0.027*
Stat5 (nuclei)	4.1 ± 1.7	4.7 ± 0.6	0.38	0.23
IGFBP5 (cytoplasm)	2.5 ± 2.4	4.0 ± 1.8	0.033*	0.029*
IGFBP5 (nuclei)	3.8 ± 1.8	4.2 ± 1.4	0.36	0.44

*$P < 0.05$ is considered significant. †One-sample Wilcoxon signed rank test. ‡Paired *t*-test. AIB1, amplified in breast cancer 1; AR, androgen receptor; ER, estrogen receptor; HER2, human epidermal growth factor receptor type 2; IGFBP5, insulin-like growth factor binding protein 5; pAkt, phosphorylated Akt; pER, phosphorylated ER; PgR, progesterone receptor; pMAPK, phosphorylated MAPK; Ser, serine; Stat5, signal transducer and activator of transcription 5.

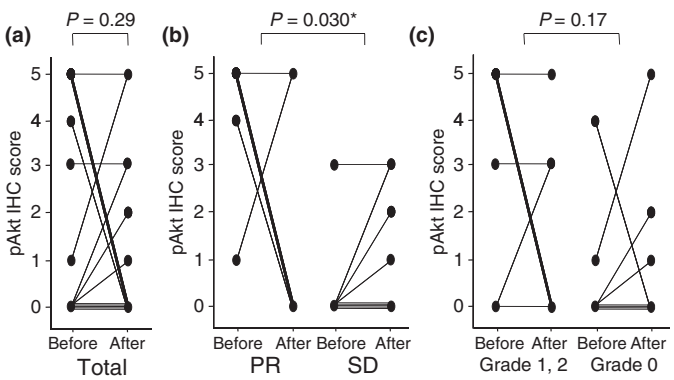

Fig. 5. Immunohistochemical (IHC) phosphorylation scores for Akt in tumors before and after exemestane treatment. (a) All patients. (b) Responders (PR) and nonresponders (SD) with clinical evaluation were separately analyzed. (c) Responders (grades 1 and 2) and nonresponders (grade 0) with pathological evaluation were separately analyzed.

Discussion

We investigated predictive factors for exemestane response using pretreatment tumor biopsies and post-treatment surgical specimens in ER-positive primary breast cancer. Although expression levels of ERα were 5 or greater by Allred score in all 16 pretreatment tumors, clinical and pathological responses to exemestane varied. Several clinical studies on exemestane as primary endocrine therapy in operable breast cancer have been reported.[18–20] The duration of neoadjuvant exemestane therapy was 4 months in two studies and 6 months in one study. The clinical response rates in these studies were 34–66%, which are comparable to the results in our present study.

Surprisingly, high expression of AIB1 and high-level phosphorylation of Akt Ser473 were significantly associated with better responses to the therapy. Moreover, phosphorylation of Akt Ser473 is a convincing predictive factor from the aspects of clinical and pathological responsiveness. Several studies have reported that phosphorylation of Akt predicts worse outcome and tamoxifen resistance in ER-positive breast cancer.[21–24] Our previous study did not indicate correlation between Akt phosphorylation and prognosis in ER-positive breast cancer, although phosphorylation of Akt was strongly and positively associated with phosphorylation of ERα Ser118, ERα Ser167, and MAPK.[7] It was reported that estradiol rapidly activates Akt via the HER2 signaling pathway.[25] Akt might be activated via growth factor signaling pathways, both estrogen-dependently and estrogen-independently in breast cancer. We previously reported that high expressions of AIB1 and HER2 were associated with significantly worsened disease-free survival in ER-positive breast cancer.[7] Moreover, p53 protein accumulation in primary breast tumors predicted resistance to endocrine therapy and decreased post-relapse survival in metastatic breast cancer.[10] The cut-off levels in our previous studies were 3 (overexpressed) for HER2 and more than 10% of cells positive for p53. All 16 tumors in this study were negative for HER2 and p53, although expression levels of HER2 and p53 were slightly higher in responders compared to nonresponders.

Prognostic factors in ER-positive breast cancer, which we have demonstrated previously – such as phosphorylation of ERα Ser118 and ERα Ser167,[7] and expression of Stat5[11] – were not predictors for the response to exemestane as primary endocrine therapy for 6 months. We suggest that the discordance between predictors in this study and prognostic factors in our previous studies may be due to different types of endocrine therapy. Most patients were treated with tamoxifen in our previous studies of prognosis prediction, whereas exemestane (an aromatase inhibitor) was used in this study. The mechanisms of endocrine therapy are different for aromatase inhibitors, which block estrogen synthesis in peripheral adipose tissues, and selective ER modulators such as tamoxifen, which bind to ER and block the action of estrogen. The data from recent randomized clinical trials have shown that aromatase inhibitors substantially improve disease-free survival as compared to tamoxifen in postmenopausal women in the adjuvant setting. Viale and colleagues demonstrated that the magnitude of improved disease-free survival for letrozole *versus* tamoxifen was greater for patients with high tumor Ki67 expression than for those with low tumor Ki67 expression. Thus, predictors and/or prognostic factors may be different for different types of endocrine therapy. In addition, predictors for response to primary endocrine therapy evaluated in pretreatment biopsies may not be surrogate markers for prognosis in ER-positive breast cancer. Ellis and colleagues described that the aromatase inhibitor letrozole, but not tamoxifen, was effective in HER2-positive tumors as neoadjuvant endocrine therapy in ER-positive breast cancer.[26] On the other hand, a high HER2 expression is associated with a high risk of recurrence for 5 years after treatment with the aromatase inhibitors anastrozole and letrozole, as well as tamoxifen, in adjuvant endocrine therapy.[27,28] Therefore, response predictors for aromatase inhibitors assessed with a pretreatment tumor biopsy may not be prognostic markers for adjuvant endocrine therapy.

Our study indicated that expression levels of ERα, ERβ1, ERβcx/β2, PgR, aromatase in stromal cells, Ki67, and cyclin D1, and phosphorylation levels of ERα Ser118, ERα Ser167, and p44/42 MAPK Thr202/Tyr204, were decreased, whereas expression levels of Stat5 and IGFBP5 in cytoplasm were increased in post-treatment specimens compared to the levels in pretreatment tumors. It is suggested that inhibition of aromatase activity and estrogen production by exemestane affects molecules that are present downstream of ER signaling pathways regardless of the

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treatment response. However, expression of AIB1, a predictor for exemestane, was not affected during the treatment. In contrast, phosphorylation levels of Akt Ser473 and expression levels of Ki67 were more reduced in post-treatment specimens of responders than in those from the tumors of nonresponders. Dowsett and colleagues demonstrated that measurements of tumor Ki67 level after short-term endocrine treatment may improve the prediction of recurrence-free survival by integrating the prognostic value of Ki67 level at baseline with changes in Ki67 level that are associated with treatment benefits.[5] It is reasonable to assume that a response to neoadjuvant endocrine therapy affects prognosis when it is known that the same treatment has an effect when it is given as adjuvant therapy. On the other hand, it has been reported that Ki67 is a prognostic factor in postmenopausal women with ER-positive early breast cancer who were treated with letrozole or tamoxifen as adjuvant therapy, because high Ki67 expression levels in primary breast tumors were associated with worse disease-free survival.[29]

One patient developed bone metastasis during adjuvant exemestane treatment, and tamoxifen has been effective for the recurrent disease. It is not clear whether this phenomenon is intrinsic or whether it represents an acquired resistance to exemestane, although the patient showed SD with the neoadjuvant exemestane therapy. Because tumor biology changes during a long-term endocrine therapy, predictors for a short-term endocrine treatment response may not have prognostic value for adjuvant endocrine therapy.

In conclusion, the present data indicate that high expressions of AIB1 and high-level phosphorylation of Akt Ser473 are predictors for exemestane response, and that phosphorylation of Akt Ser473 is a convincing predictive factor from the aspects of clinical and pathological responsiveness. Our findings will be helpful when preoperative endocrine therapy is planned for women with ER-positive breast cancer. It might be necessary to consider predictors and prognostic factors for endocrine therapy separately in ER-positive breast cancer.

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