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PRECLINICAL STUDY

PAM50 for prediction of response to neoadjuvant chemotherapy for ER-positive breast cancer

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Abstract

Purpose There is an urgent need for the development of a predictor of response to chemotherapy for ER-positive breast cancer which is less chemosensitive than for ER-negative breast cancer in order to avoid unnecessary chemotherapy. In the present study, intrinsic subtyping by PAM50 was evaluated for its ability to predict a response to chemotherapy.

Patients and Methods For this study, 124 patients with ER-positive breast cancer treated with neoadjuvant sequential paclitaxel and FEC (NAC) were evaluated. Tumor biopsy specimens obtained before NAC were subjected to intrinsic subtyping (IS) by gene expression (GE) using PAM50 (PAM50-IS) or immunohistochemistry (IHC-IS).

Results Of the PAM50-ISs (Luminal A, Luminal B, HER2-enriched, and Basal-like), GE-Luminal A showed the lowest pCR rate (1.9%), and multivariate analysis revealed that GE-Luminal A was a significant ($P = 0.031$) predictor of non-pCR independently of other clinicopathological parameters, including Ki67, and tumor-infiltrating lymphocytes. Of the IHC-ISs, on the other hand, IHC-Luminal A was not significantly associated with pCR. We also found that breast tumors with low ER levels (1–9%), like ER-negative tumors, were mostly GE-HER2-enriched and GE-Basal-like, and more sensitive to NAC than those with high ER levels (≥ 10%).

Conclusions GE-Luminal A intrinsically subtyped by PAM50 was the least sensitive to NAC and very unlikely to attain pCR. IHC-Luminal A identified by IHC, on the other hand, was not significantly predictive of pCR. In addition, PAM50 revealed that tumors with low ER (1–9%) were more like ER-negative tumors than ER-positive tumors, and most such cases should therefore would better be treated with chemotherapy.

Keywords Breast cancer · ER positive · Neoadjuvant chemotherapy · PAM50 · Intrinsic subtype

Abbreviations

ER	Estrogen receptor
PR	Progesterone receptor
HER2	Human epidermal growth factor receptor 2
DMFS	Distant metastasis-free survival
pCR	Pathological complete response
IHC	Immunohistochemistry
FISH	Fluorescence in situ hybridization
HG	Histological grade
NAC	Neoadjuvant chemotherapy
GE	Gene expression
IS	Intrinsic subtype

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Introduction

ER-positive breast cancer is known to be less sensitive to chemotherapy than ER-negative breast cancer. It is reported that the pCR rate for neoadjuvant chemotherapy (NAC) is 1–15% for ER-positive breast cancer and 10–50% for ER-negative breast cancer [1, 2]. The pCR rate for the former is so low that seems to indicate that chemotherapy is not useful for most ER-positive breast cancers and thus, in order to avoid unnecessary chemotherapy, it is of vital importance to develop an accurate predictor of response to chemotherapy for ER-positive breast cancer.

Intrinsic subtype classification of breast cancer using DNA microarray-based gene expression analysis was initially reported in 2000 by Perou et al. [3]. In their study, the intrinsic gene set comprising 496 genes was used for the unsupervised cluster analysis which could classify breast cancers into five subtypes, i.e., Luminal A, Luminal B, HER2-enriched, Basal-like, and Normal-like. In 2009, PAM50 was developed which could be performed by RT-PCR or DNA microarray using 50 representative genes from the original 496 [4, 5], and intrinsic subtyping by PAM50 is currently widely used in breast cancer research [6]. It was introduced into clinical practice as Prosigna^{R} (Nano-String Co., Ltd, Seattle, WA) and could predict the prognosis for ER-positive breast cancer patients with a higher accuracy than could conventional histology [7].

In addition, a study by Prat et al. reported on correlation of intrinsic subtypes by PAM50 with sensitivity to chemotherapy. They found that intrinsic subtyping at diagnosis provides useful prognostic and predictive information for neoadjuvant chemotherapy-treated patients [8]. However, correlation of PAM50 with pCR for ER-positive breast cancer has not been studied satisfactorily even though prediction of chemo-sensitivity of ER-positive breast cancer is clinically important as explained above. In the present study, we first therefore examined the correlation of PAM50 intrinsic subtypes with pCR for ER-positive breast cancer treated with sequential taxane and anthracycline-based therapy. We also compared the PAM50 intrinsic subtyping by PAM50 (PAM50-IS) with that by the immunohistochemistry-based subtyping method (ER, PR, HER2, and Ki67) (IHC-IS) [9] for their ability to predict response to chemotherapy.

Materials and methods

Patients

For this study, 156 patients with ER-positive ($n = 124$) or ER-negative ($n = 32$) breast cancer who had been treated with NAC and subsequent surgery (mastectomy or breast conserving surgery) between 2004 and 2013 at Osaka University Hospital were retrospectively recruited. Patient characteristics of those with ER-positive breast cancer are shown in Table 1 and Supplementary Table 1. NAC consisted of paclitaxel 80 mg/m2 weekly for 12 cycles followed by a combination of 5-FU [500 mg/m^2], epirubicin [75 mg/m^2], and cyclophosphamide [500 mg/m^2] every 3 weeks for 4 cycles (P-FEC). Before NAC administration, all patients underwent a tumor biopsy with a vacuum-assisted core-biopsy instrument (Mammotome 8G HH; Ethicon Endosurgery Inc., Cincinnati, OH) under ultrasonographic guidance. Some tumor biopsy samples of each patient were fixed in 10% buffered formaldehyde for histological examination, and the others were snap frozen in liquid nitrogen and stored at − 80 °C until use for RNA extraction for gene expression analysis using a DNA microarray. Prior to the tumor biopsy, informed consent regarding this study was obtained from every patient, and this study was approved by the ethics committee at our hospital.

For postoperative adjuvant therapy, hormonal therapy (tamoxifen ± LH-RH agonist, aromatase inhibitor, or others) was given to all the patients with ER-positive breast cancer, and trastuzumab was given to 16 of 31 HER2-positive breast cancer patients essentially in accordance with the Japanese Breast Cancer Guideline [http://jbcs.gr.jp/guidline/]. The median follow-up time for these patients was 49 months with a range of 1 to 97 months.

RNA extraction and DNA microarray analysis

TRIzol (Invitrogen, Carlsbad, CA) or Qiagen RNeasy mini kit (QIAGEN Sciences, Germantown, MD) was used to extract RNA from the tumor biopsy samples, and 50 ng of the extracted RNA was subjected to gene expression analysis with a DNA microarray (Human Genome U133 Plus 2.0 Array; Affymetrix, Santa Clara, CA) using a previously described method [10, 11].

Histological evaluation of response to NAC

The pathological response to NAC was evaluated by using surgical specimens obtained during surgery. The specimens were cut into 5-mm slices, and hematoxylin and eosin-stained sections were prepared to determine the presence or absence of tumor cells. A complete absence of invasive tumor cells in the breast and lymph nodes was defined as pCR irrespective of the presence or absence of non-invasive components. Residual cancer burden (RCB) was determined by means of the Residual Cancer Burden Calculator (MD Anderson Cancer Center, Houston, TX) [12].

Immunohistologic examination

ER, PR, and Ki67 levels in tumor biopsy samples were determined immunohistochemically in accordance with previously described methods [13, 14]. The cut-off values were 1% for ER, 20% for PR, and 20% for Ki67. HER2 amplification was determined by means of fluorescence in situ hybridization (FISH) using the PathVysion HER-2 DNA Probe Kit (Vysis/Abbott Molecular Inc., Chicago, IL). A tumor was classified as HER2-amplified if the FISH ratio was ≥ 2.0. Tumor-infiltrating lymphocytes (TILs) were assessed with the procedure based on recommendations by the International TILs Working Group 2014 [15]. Intrinsic subtyping by biomarkers, determined by immunohistochemistry (IHC-IS) and performed in accordance with a previously described

Table 1 Clinicopathological characteristics of ER-positive breast cancers according to intrinsic subtypes by PAM50

	PAM50 intrinsic subtypes				
	Luminal A ($n=52$)	Luminal B ($n=32$)	HER2-enriched ($n=24$)	Basal-like ($n=16$)	P value
Age					
Median (range)	50 (30–73)	53 (27–74)	53 (31–72)	49 (24–68)	
Menstrual status					
Premenopausal	28	15	10	10	0.556
Postmenopausal	24	17	14	6	
T category					
1	4	2	1	0	0.547
2	39	20	20	12	
3	7	5	2	2	
4	2	5	1	2	
N category					
0	16	12	8	5	0.934
1–3	36	20	16	11	
Histological grade					
1	14	5	3	3	0.019
2	31	25	15	5	
3	7	2	6	7	
Unknown	0	0	0	1	
TILs					
< 15%	44	24	17	5	< 0.001
≥ 15%	8	8	7	11	
Ki67					
< 20%	39	13	9	4	< 0.001
≥ 20%	13	19	15	12	
PR					
< 20%	14	12	17	10	< 0.001
≥ 20%	38	20	7	6	
HER2 ratio					
< 2	46	25	7	15	< 0.001
≥ 2	6	7	17	1	

method [9], yielded three subtypes, i.e., IHC-Luminal A (ER-positive, PR-positive, HER2-negative, Ki67-negative), IHC-Luminal B (ER-positive and HER2-negative and PR-negative or Ki67-positive) and IHC-Luminal HER2 (ER-positive and HER2-positive and any PR and any Ki67).

Statistics

The CEL file of gene expression (GE) data obtained by DNA microarray was used to perform intrinsic subtyping by PAM50 (PAM50-IS) into GE-Luminal A, GE-Luminal B, GE-HER2-enriched, GE-Basal-like, and GE-Normal-like in accordance with a previously described method [5]. Since GE-Normal-like subtypes are thought to contain a very small amount of tumor cells mostly contaminated by normal tissue and thus unlikely to represent the tumor cell phenotype [3], they were excluded from the analysis. Distant metastasis-free survival (DMFS) was calculated with the Kaplan–Meier method and evaluated with a log-rank test. Correlation of the intrinsic subtypes with the various clinicopathological parameters was assessed by means of Fisher's exact test. All statistical analyses were two-sided and $P < 0.05$ was considered to be significant.

Results

Correlation of PAM50-based intrinsic subtype (PAM50-IS) with clinicopathological parameters

Total of 124 ER-positive breast cancers were analyzed by PAM50, and classified into 52 GE-Luminal A, 32

GE-Luminal B, 24 GE-HER2-enriched, and 16 GE- Basal-like subtypes. Associations of PAM50-IS with the various clinicopathological parameters are shown in Table 1 and Supplementary Table 1. Menstrual status, tumor size, and lymph node status were not significantly different among the PAM50-IS determined subtypes but histological grade, Ki67, PR, HER2, and TILs were significantly different. Tumors with a high histological grade occurred more frequently in GE-Basal-like and GE-HER2-enriched subtypes than in GE-Luminal A and GE-Luminal B, and tumors with a low histological grade were most frequent in GE-Luminal A. Ki67 high tumors were more frequent in GE-Luminal B, GE-HER2-enriched, and GE-Basal-like subtypes than in GE-Luminal A, while PR positivity was higher in GE-Luminal A and GE-Luminal B than in GE-HER2-enriched and GE-Basal-like. HER2 positivity was highest in GE-HER2-enriched, followed by GE-Luminal B, GE-Luminal A, and GE-Basal-like, in that order.

Correlation of IHC-based intrinsic subtype (IHC-IS) with PAM50-IS

Classification by IHC of 124 ER-positive breast cancers yielded 3 IHC-ISs [9], 37 IHC-Luminal A, 56 IHC-Luminal B, and 31 IHC-Luminal-HER2 subtypes (Fig. 1). IHC-Luminal A tumors mostly comprised GE-Luminal A tumors (73.0%) followed by GE-Luminal B (16.2%), GE-Basal-like (8.1%), and GE-HER2-enriched (2.7%) tumors. IHC-Luminal B tumors mostly consisted of GE-Luminal B (33.9%) and GE-Luminal A (33.9%) tumors of similar frequency followed by GE-Basal-like (21.4%) and GE-HER2-enriched (10.7%) tumors. Finally, IHC-Luminal-HER2 tumors mostly consisted of GE-HER2-enriched (54.8%) tumors followed by GE-Luminal B (22.6%), GE-Luminal A (19.4%), and GE-Basal-like (3.2%) tumors.

Correlation of PAM50-IS and IHC-IS with pCR

Of the PAM50 ISs, GE-Luminal A showed the lowest pCR rate (1.9%) compared with the other subtypes (Fig. 2a), and the pCR rate of GE-Luminal A was significantly ($P=0.014$) lower than that of a combination of the other three subtypes (15.3%, Fig. 2b). Of the IHC-ISs, IHC-Luminal A showed the lowest pCR rate (5.4%, Fig. 2a) but the pCR rate of IHC-Luminal A was not significantly different from that of a combination of the other two subtypes ($P=0.507$,11.5%).

Univariate analysis of the correlations of the various clinicopathological parameters with pCR revealed that the correlation with pCR was significant only for PAM50-IS ($P=0.007$) (Table 2). Menstrual status ($P=0.055$) and TILs ($P=0.080$) tended to be associated with pCR although this tendency was statistically not significant. Multivariate analysis of these three parameters with a P value less than 0.1 showed that only PAM50-IS was significantly associated with pCR ($P=0.031$).

Correlation of PAM50-IS with prognosis

Univariate analysis of the correlation of various clinicopathological parameters with distant metastasis-free survival (DMFS) revealed that RCB and tumor size, but not PAM50, were significantly correlated with DMFS ($P<0.001$ and $P=0.040$, respectively), while multivariate analysis showed that only RCB was significantly correlated with DMFS ($P<0.001$) (Table 3).

Next, using the public databases (GSE4922, GSE6532, GSE9195, GSE17705, GSE26971) which comprise 339 stage II/III breast cancer patients with ER-positive tumors treated with adjuvant hormonal therapy alone, DMFS of GE-Luminal A was compared with that of the other subtypes. This comparison demonstrated that patients with GE-Luminal A tumors showed a significantly better prognosis

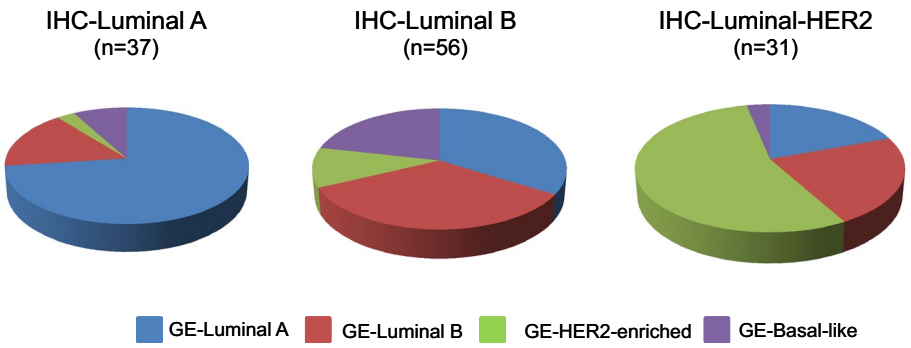

Fig. 1 Comparison of PAM50-IS and immunohistochemistry-based intrinsic subtype (IHC-IS) in ER-positive breast cancers. ER-positive breast cancers ($n=124$) were classified by IHC into three subtypes (IHC-IS; IHC-Luminal A, IHC-Luminal B, and IHC-Luminal-HER2) and classified by PAM50 into four subtypes (PAM50-IS; GE-Luminal A, GE-Luminal B, GE-HER2-enriched, GE-Basal-like). Correlation between IHC-IS and PAM50-IS is also shown

A

	pCR rate	
PAM50-IS*		
Luminal A	1.9% (1/52)	
Luminal B	9.4% (3/32)	Others
HER2-enriched	25.0% (6/24)	
Basal-like	12.5% (2/16)	
IHC-IS		
Luminal A	5.4% (2/37)	
Luminal B	8.9% (5/56)	Others
Luminal-HER2	16.1% (5/31)	

*IS, intrinsic subtype

B

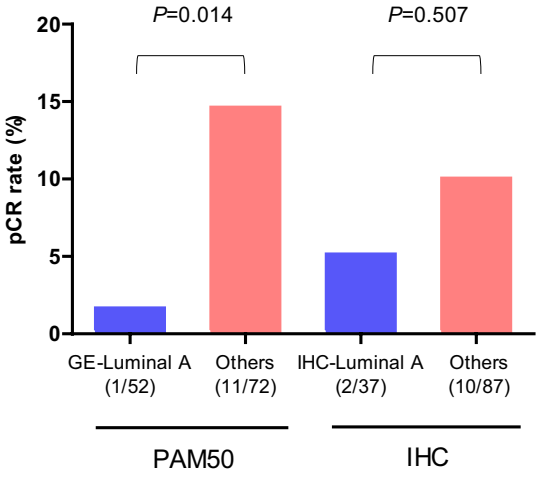

Fig. 2 **a** pCR rates for PAM50-IS and IHC-intrinsic subtypes (IHC-IS). **b** Comparison of pCR rates between GE-Luminal A and other subtypes for PAM50-IS and IHC-IS. pCR rates were compared for GE-Luminal A and other ISs determined by PAM50, and for IHC-Luminal A and other ISs determined by IHC

Table 2 Univariate and multivariate analysis of various clinicopathological parameters in their correlation with pCR to neoadjuvant chemotherapy

Parameters[a]	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P* value	HR	95% CI	*P* value
Menstrual status[b]						
Pre vs Post	3.462	0.974–16.217	0.055	3.735	0.932–19.49	0.064
T category						
T1 + 2 vs T3 + 4	0.218	0.048–1.383	0.125			
N category						
Negative vs Positive	1.508	0.421–5.050	0.512			
Histological grade						
1 + 2 vs 3	0.400	0.109–1.219	0.990			
PR						
< 10% vs ≥ 10%	0.324	0.082–1.091	0.126			
HER2						
Negative vs Positive	2.363	0.652–8.036	0.182			
Ki67						
< 20% vs ≥ 20%	2.392	0.711–9.385	0.161			
TILs						
< 15% vs ≥ 15%	3.000	0.874–10.333	0.080	2.555	0.653–10.26	0.176
PAM50						
Luminal A vs others	9.197	1.702–170.9	0.007	6.982	1.168–133.97	0.031

[a]All the clinical and pathological parameters were obtained before neoadjuvant chemotherapy from clinical staging and pathological/microarray analysis of tumor biopsy samples

[b]Menstrual status; Premenopausal vs Postmenopausal

than that of the other subtypes ($P = 0.012$) (Fig. 3a). On the other hand, when prognosis of the patients with GE-Luminal A was compared with that of the other subtypes in our study and the public database (GSE25066) containing the 379 ER-positive tumors, where all the patients were treated with NAC and adjuvant hormonal therapy, no significant differences in DMFS were identified (Fig. 3b). Sixteen patients with HER2-positive breast cancer treated with post-operative trastuzumab were excluded from Fig. 3b since we wanted to establish the impact of chemotherapy on prognosis of the patients treated with hormonal therapy. The subset analysis according to pathological response to NAC showed

Table 3 Univariate and multivariate analysis of various clinicopathological parameters in their correlation with distant metastasis-free survival

Parameters[a]	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P* value	HR	95% CI	*P* value
Menstrual status[b]						
Pre. vs. Post	0.758	0.309–1.791	0.667			
T category						
T1 + 2 vs T3 + 4	2.661	1.049–6.339	0.040	0.645	0.253–1.736	0.373
N category						
Negative vs Positive	1.228	0.199–3.444	0.667			
Histological grade						
1 + 2 vs 3	1.012	0.341–4.329	0.985			
PR						
< 20% vs ≥ 20%	0.441	0.162–1.138	0.091			
HER2						
Negative vs Positive	1.097	0.391–2.702	0.850			
Ki67						
< 20% vs ≥ 20%	1.016	0.426–2.450	0.971			
TILs						
< 15% vs ≥ 15%	1.030	0.365–2.553	0.952			
PAM50						
Luminal A vs Others	1.190	0.50-3.007	0.680			
RCB [c]						
0 + 1 + 2 vs 3	8.991	3.560–22.39	< 0.001	0.152	0.060–0.398	< 0.001

[a]All the clinical and pathological parameters were obtained before neoadjuvant chemotherapy from clinical staging and pathological/microarray analysis of tumor biopsy samples

[b]Menstrual status, Premenopausal vs Postmenopausal

[c]RCB, residual cancer burden

that both GE-Luminal A tumors and the other subtypes had a similarly excellent prognosis in the pCR subset (Fig. 3c) but, in the non-pCR subset, GE-Luminal A tumors had a significantly ($P = 0.008$) better prognosis than the other subtypes (Fig. 3d).

Clinicopathological characteristics of breast tumors with low ER expression

ER-negative breast cancer patients treated with NAC ($n = 32$) during the same period as for the aforementioned 124 ER-positive breast cancer patients were combined, and classified into ER-negative (0%, $n = 32$), ER-low (1–9%, $n = 16$), and ER-high (≥ 10%, $n = 108$) groups (Table 4). There was a significant difference in histological grade ($P = 0.035$), TILs ($P = 0.019$), Ki67 ($P = 0.030$), PR ($P < 0.001$), and HER2 ($P = 0.004$) between ER-low and ER-high tumors, but there was no significant difference in any of the clinicopathological parameters between ER-low and ER-negative tumors (Table 4). Moreover, the pCR rate was significantly higher for ER-negative tumors (40.6%) than ER-high tumors (7.4%) but not than ER-low tumors (25.0%, $P = 0.296$). And the pCR rate was significantly higher ($P = 0.026$) for ER-low tumors (25.0%) than ER-high tumors (7.4%). DMFS was not significantly different between ER-negative, ER-low, and ER-high tumors (Supplementary Fig. 1).

Next, the ratios of PAM50-ISs were compared among ER-negative, ER-low, and ER-high tumors (Fig. 4). ER-negative tumors consisted mostly of GE-Basal-like and GE-HER2-enriched subtypes, while ER-high tumors comprised mostly GE-Luminal A and GE-Luminal B subtypes. ER-low tumors consisted mostly of GE-HER2-enriched and GE-Basal-like subtypes, while Luminal subtypes accounted for only 6.3%.

Discussion

For the present study, the correlations of PAM50-ISs with pCR for ER-positive breast cancer patients treated with NAC were investigated. To avoid unnecessary chemotherapy for a majority of ER-positive breast cancer patients, we believe it is very important to develop a predictor of response to chemotherapy by ER-positive breast tumors, which are less sensitive to chemotherapy than ER-negative tumors. Consistent with the findings of previous studies [11, 16], we found that GE-Luminal A was associated with the lowest pCR rate, and was the only significant predictor for resistance to NAC among the various clinicopathological parameters.

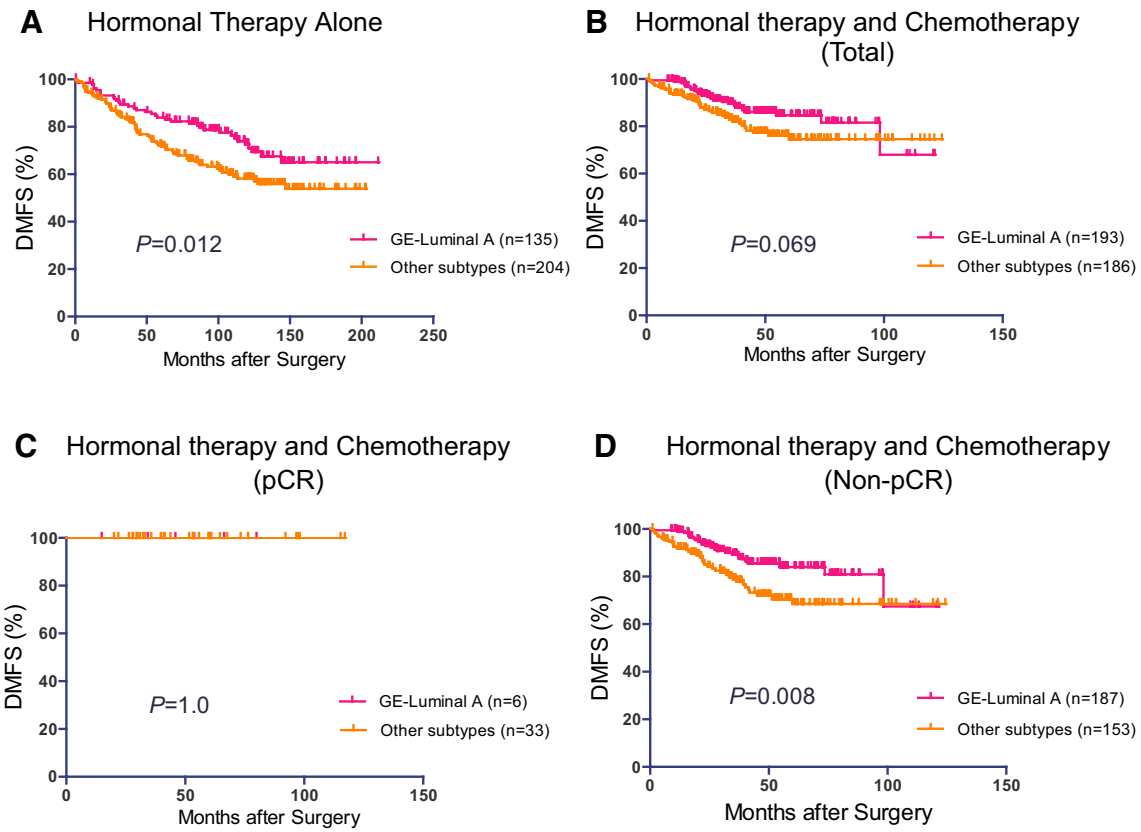

Fig. 3 **a** Comparison of distant metastasis-free survival (DMFS) between GE-Luminal A tumors and other subtypes treated with adjuvant hormonal therapy alone. Data on gene expression of primary tumors and prognosis for 339 ER-positive breast cancer patients treated with adjuvant hormonal therapy alone were extracted from public databases (GSE4922, GSE6532, GSE9195, GSE17705, and GSE26971). After classification into GE-Luminal A and the other subtypes (GE-Luminal B, GE-HER2-enriched, and GE-Basal-like) by PAM50, their DMFS was compared. **b** Comparison of DMFS between GE-Luminal A tumors and other subtypes treated with neoadjuvant chemotherapy and adjuvant hormonal therapy. Data on gene expression of primary tumors and prognosis for 379 ER-positive breast cancer patients treated with neoadjuvant chemotherapy and adjuvant hormonal therapy were extracted from our present series and public database (GSE25066). Patients with HER2-positive breast cancers and treated with adjuvant trastuzumab ($n=16$) were excluded from this analysis. After classification into GE-Luminal A and the other subtypes by PAM50, their DMFS was compared. **c** Comparison of DMFS between GE-Luminal A tumors and other subtypes in patients who achieved pCR. The patients ($n=39$) who achieved pCR were selected from those in Fig. 3b, and DMFS was compared between GE-Luminal A tumors and the other subtypes. **d** Comparison of DMFS between GE-Luminal A tumors and other subtypes in patients who did not achieve pCR. The patients ($n=340$) who did not achieve pCR were selected from those in Fig. 3b, and DMFS was compared between GE-Luminal A tumors and the other subtypes

Since subtyping by PAM50 is not easily available in clinical practice, subtyping by IHC for ER, PR, HER2, and Ki67 has often been used as a surrogate [17]. However, we found that IHC-Luminal A, unlike GE-Luminal A, was not significantly associated with a low pCR rate, while 27.0% of IHC-Luminal A and 66.1% of IHC-Luminal B consisted of subtypes other than GE-Luminal A and GE-Luminal B, respectively, indicating that results of subtyping by IHC are not identical to those by GE-IS even though the two procedures are significantly similar, as reported previously by Viale et al. [18]. These results seem to indicate that IHC-identified Luminal A cannot be substituted for GE-Luminal A identified by PAM50, at least for prediction of response to NAC.

Patients with GE-Luminal A were found to show a significantly better prognosis than those with other subtypes when they were treated with adjuvant hormonal therapy alone (Fig. 3a), consistent with previously reported findings [5, 19]. However, the prognosis of patients with GE-Luminal A and of those with the other subtypes were not significantly different when they were treated with neoadjuvant chemotherapy plus adjuvant hormonal therapy (Fig. 3b). These results seem to suggest that prognosis of the patients with the other subtypes, which are more sensitive to chemotherapy, was improved by chemotherapy, so that their prognosis was not significantly different from that of GE-Luminal A tumors.

Table 4 Clinicopathological characteristics of breast cancers according to ER status

Parameters[a]	ER (0%)	ER (1–9%)	*P* value[b]	ER (≥ 10%)	*P* value[c]
Age					
Median (range)	55 (28–70)	57 (43–68)		49 (24–74)	
Menstrual status					
Premenopausal	20	5	0.066	50	0.293
Postmenopausal	12	11		58	
T category					
1	1	1	0.460	6	0.454
2	24	14		77	
3	3	1		15	
4	4	0		10	
N category					
0	7	4	1.000	37	0.576
1–3	25	12		71	
Histological grade					
1	1	2	0.447	23	0.035
2	16	7		69	
3	15	7		15	
Unknown	0	0		1	
TILs					
< 15%	22	9		90	
≥ 15%	10	7	0.524	18	0.019
Ki67					
< 20%	5	4	0.457	61	0.030
≥ 20%	27	12		47	
PR					
< 20%	32	16		40	< 0.001
≥ 20%	0	0		68	
HER2 ratio					
Negative	20	7	0.237	86	0.004
Positive	12	9		22	
Pathological response					
pCR	13	4	0.296	8	0.026
Non-pCR	19	12		100	

[a]All the clinical and pathological parameters were obtained before neoadjuvant chemotherapy from clinical staging and pathological/microarray analysis of tumor biopsy samples

[b]*P* value for ER (0%) vs ER (1–9%)

[c]*P* value for ER (1–9%) vs ER (≥ 10%)

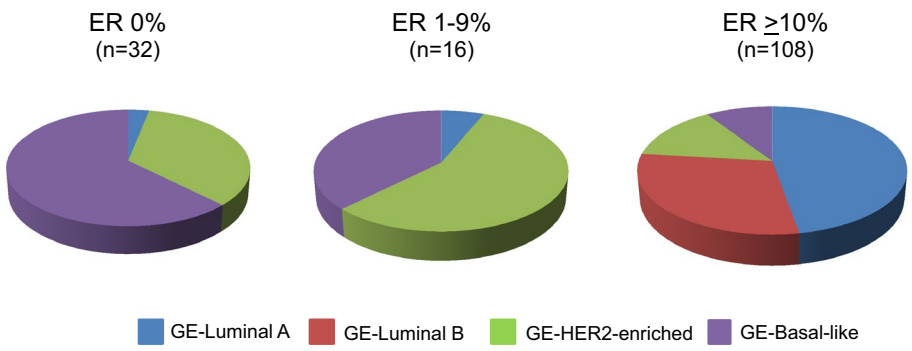

Fig. 4 Comparison of PAM50-IS and IHC-IS for ER-negative, ER-low, and ER-high breast cancers. A total of 156 breast cancers (32 ER-negative (0%), 16 ER-low (1–9%), and 108 ER-high (≥ 10%)) were classified by PAM50 into four subtypes and by IHC into three subtypes. Correlation between PAM50-IS and IHC-IS is also shown

Since GE-Luminal A tumors are more sensitive to hormonal therapy and less sensitive to chemotherapy than the other subtypes, neoadjuvant hormonal therapy can be a reasonable option for GE-Luminal A tumors. Recently, it has been reported that GE-Luminal A tumors without lymph node metastasis show such an excellent prognosis that the adjuvant chemotherapy can be avoided safely [20, 21] However, in the more advanced (stage II/III) tumors as shown in Fig. 3a, patients with GE-Luminal A tumors showed a better prognosis than those with the other subtypes but about 30% of patients with GE-Luminal A tumors developed distant recurrences at 10 years after surgery and thus those with stage II/III GE-Luminal A tumors are unlikely to be safely spared adjuvant chemotherapy. Therefore, in order to identify the subset of patients with stage II/III ER-positive tumors with an excellent prognosis who can forgo adjuvant chemotherapy, ALTERNATE trial which assesses a biomarker-driven strategy is ongoing [22]. Its results are eagerly awaited.

Recently, the TAILORx trial has shown that ER-positive, HER2-negative, and node-negative patients with recurrence score of 11 to 25 by Oncotype DX derive no benefit from adding chemotherapy to adjuvant hormonal therapy [23]. In addition, the MINDACT trial has shown that the patients with clinically high-risk but genomically low-risk tumors by MammaPrint derive no significant benefit from adjuvant chemotherapy in distant disease-free survival and overall survival, indicating that such patients can be spared adjuvant chemotherapy [24]. In these trials, however, the prognosis of the patients treated with adjuvant hormonal therapy alone was so excellent that additional improvement by chemotherapy in prognosis, if any, seems to have been very difficult to be demonstrated. The benefit of adding adjuvant chemotherapy to hormonal therapy should be evaluated in the patients with a higher risk for relapse. Therefore, the RxPonder trial [25] which evaluates this benefit in ER-positive, HER2-negative, and 1–3 node-positive patients with recurrence score of 25 or less is ongoing.

We also investigated the clinicopathological characteristics of ER-low tumors (1–9%). We could demonstrate that these characteristics (histological grade, TILs, Ki67, PR, and HER2) were more similar to those of ER-negative tumors (0%) than of ER-high tumors (≥ 10%). These observations are also essentially consistent with those reported by other investigators [26]. Moreover, ER-low tumors in our study consisted mostly (93.7%) of GE-HER2-enriched and GE-Basal-like tumors, and only 6.3% of GE-Luminal A, while a majority of ER-high tumors consisted of GE-Luminal A and GE-Luminal B subtypes (Fig. 4). We also found that the pCR rate was significantly higher for ER-low than ER-high tumors. In this connection, it has also been reported that ER-low tumors are unlikely to benefit from adjuvant hormonal therapy [27–30]. Taken together, these findings suggest that ER-low tumors should be clinically regarded as ER-negative tumors because of their low sensitivity to hormonal therapy and high sensitivity to chemotherapy, even though adjuvant chemotherapy is thought to be administered in most cases with ER-low tumors(1–9%).

A limitation of our study is that HER2-positive breast cancer patients were treated with NAC alone but not with NAC plus trastuzumab, and thus the predictability of PAM50 for NAC plus trastuzumab could not be assessed. Since the main purpose of this study was to elucidate the sensitivity of various PAM50-ISs to chemotherapy alone, we did not include the patients treated with NAC plus trastuzumab. Although a majority of IHC-Luminal-HER2 were classified into GE-HER2-enriched (54.8%; 17/31) by PAM50, a significant proportion of these tumors were classified into other subtypes, i.e., GE-Luminal A (19.4%; 6/31), GE-Luminal B (22.6%; 7/31), and GE-Basal-like (1.3%; 1/31) tumors. Thus, it seems to be of interest to study the response to NAC plus trastuzumab by IHC-Luminal-HER2 tumors identified by PAM50-IS.

To conclude, we have shown that GE-Luminal A identified by PAM50 is the least sensitive to NAC and very unlikely to achieve pCR in ER-positive tumors. Although subtyping by IHC is often used in practice due to its easy availability, it is not equal to subtyping by PAM50, especially in its capability to predict pCR. It is thus not advisable to use subtyping by IHC in place of subtyping by PAM50. In addition, analysis of ER-low tumors (1–9%) by PAM50 revealed that they are more like ER-negative tumors (0%) than ER-high tumors (≥ 10%), and should be considered as the equivalent of ER-negative tumors, which need to be treated with chemotherapy in most cases. However, our observations presented here need to be verified by a future study including a larger number of patients.

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Compliance with ethical standards

Conflict of interest Dr. Shinzaburo Noguchi has been an advisor for Taiho, AstraZeneca and Novartis, and has received research funding for other studies from Sysmex, AstraZeneca, Novartis, Chugai, Daiichi-Sankyo, Kyowa-Kirin, Takeda, Pfizer, Ono, Taiho, and Eisai, and honoraria from AstraZeneca, Novartis, Pfizer, Chugai, Takeda, Sysmex, Nippon Kayaku, and Ono. Dr. Yasuto Naoi has received research funding from Sysmex and AstraZeneca. Dr. Naofumi Kagara has received honoraria from AstraZeneca and Novartis. Dr. Masafumi Shimoda has received research funding from Novartis and AstraZeneca, and honoraria from Chugai, Eisai, Novartis, and Takeda. Dr. Kenzo Shimazu has received honoraria from AstraZeneca, Chugai, and Sysmex. Dr. Seung Jin Kim received honoraria from AstraZeneca, Chugai, Eisai, Kyowa-Kirin, Novartis, Pfizer, Shimadzu, Taiho, and Takeda.

Ethical approal This study complies with the current relevant laws of and guidelines for Japan.

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