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PRECLINICAL STUDY

Clinicopathological analysis of homologous recombination-deficient breast cancers with special reference to response to neoadjuvant paclitaxel followed by FEC

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Abstract

Purpose This study aimed to elucidate the clinicopathological characteristics of breast tumors with homologous recombination deficiency (HRD) and the sensitivity to neoadjuvant paclitaxel followed by fluorouracil, epirubicin, and cyclophosphamide (P-FEC).

Methods Tumor biopsy samples obtained before P-FEC from 141 patients with stages II–III breast cancer including the luminal ($n=76$), luminal-HER2 ($n=13$), HER2 ($n=17$), and triple-negative (TNBC, $n=35$) subtypes were subjected to assay for HRD score using the OncoScan CNV FFPE Assay Kit. HRD score was a simple sum of NtAI, LOH, and LST (cutoff, 42). TNBCs were also subjected to the gene expression assay using the Affymetrix microarray (U133 plus 2.0) and to the *BRCA1* promoter methylation assay using the methylation-specific real-time PCR.

Results Of the 141 breast tumors, 45 samples (32%) had high HRD scores and were associated with high histological grade ($P=0.001$), negative progesterone receptor ($P=0.018$), high Ki67 index ($P=0.032$), and *BRCA1* promoter methylation ($P=3.6e{-}07$). The proportion of tumors with high HRD scores was significantly higher in the TNBC subtype than the others ($P=0.006$). In the TNBC subtype, but not the others, high HRD scores were significantly ($P=0.001$) associated with a low pathological complete response rate to P-FEC. Among the molecular TNBC subtypes, a majority of tumors belonging to the basal-like 1, immunomodulatory, mesenchymal, mesenchymal stem-like, but not luminal androgen receptor (LAR), subtypes had high HRD scores.

Conclusions Approximately one-third of sporadic breast tumors show a high HRD score, indicating the presence of homologous recombination dysfunction, and they are characterized by biologically aggressive phenotypes, most commonly in the TNBC subtype, and less sensitive to P-FEC.

Keywords Breast cancer · Chemosensitivity · HRD score · OncoScan

Abbreviations

ER	Estrogen receptor
PR	Progesterone receptor
HER2	Human epidermal growth factor receptor 2
TNBC	Triple-negative breast cancer
TILs	Tumor-infiltrating lymphocytes
HG	Histological grade
IHC	Immunohistochemistry
pCR	Pathologic complete response

Introduction

The breast cancer susceptibility genes *BRCA1* and *BRCA2* are involved in homologous recombination (HR) and play a pivotal role in the repair of DNA double-strand breaks [1]. *BRCA1*- or *BRCA2*-associated breast cancers (*BRCA1/2* breast cancer), which lack the HR function due to the inactivation of these genes, are known to be sensitive to platinum and poly (adenosine diphosphate-ribose) polymerase (PARP) inhibitors, which induce DNA double-strand breaks [2]. Since HR deficiency (HRD) can be induced by the

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inactivation of genes involved in the HR pathway other than *BRCA1* and *BRCA2*, it is expected that breast cancers with HRD, even though they do not harbor a *BRCA1* or *BRCA2* mutation, are sensitive to platinum and PARP inhibitors. Hence, the HRD score was developed to precisely reflect the HR status of cancers with an expectation that it would be a clinically useful predictor for response to platinum and PARP inhibitors.

The HRD score consists of three components, the number of subchromosomal regions with allelic imbalance extending to the telomere (NtAI), large-scale transition (LST), and HRD-loss of heterozygosity (LOH) [3], where NtAI is reportedly correlated with the sensitivity of ovarian cancers to platinum-containing regimens [4], LST was developed as an index to distinguish the *BRCA1* or *BRCA2* mutant from wild-type breast cancer cell lines [5], and HRD-LOH is reported to not only distinguish *BRCA1* or *BRCA2* from wild-type ovarian cancer but also is a prognostic marker of ovarian cancer [6]. The HRD score, as a combination of these three factors, is high (≥ 42) in almost all *BRCA1/2* breast cancers [6] and is reportedly correlated with high sensitivity to platinum-containing regimens in neoadjuvant settings, irrespective of the presence or absence of the *BRCA1* or *BRCA2* mutation [3, 7, 8], while it has also been reported that addition of carboplatin led to higher pCR rates in both HR-deficient and non-deficient tumors in the neoadjuvant settings [9] and that HRD status was not significantly associated with response to carboplatin in the metastatic settings [10].

In current practice, the sequential use of an anthracycline-containing regimen (A) and taxane (T) is the most frequently used chemotherapeutic regimen in neoadjuvant and adjuvant settings. Thus, we thought that it would be interesting to determine whether there is a correlation between the HRD score and response to anthracycline/taxane (A/T) since such a study might provide evidence for better selection of a chemotherapeutic regimen based on the HRD score. It is reported that *BRCA1/2* breast cancers have a higher pCR rate to A/T than non-*BRCA1/2* breast cancers [11–17]. Among sporadic breast cancers, triple-negative breast cancer (TNBC) with HRD, which is determined by multiplex ligation-dependent probe amplification or array comparative genomic hybridisation, is reportedly associated with resistance to A/T [18] but not the response to A/T [19] or A [20] in the neoadjuvant settings. Moreover, it was very recently reported that breast tumors with high HRD scores are more sensitive to A/T in the neoadjuvant setting [8, 21, 22].

Thus, the aims of the present study were, first, to elucidate the clinicopathological characteristics of breast cancers with high HRD scores and, second, to elucidate the correlation between the HRD score and the response to neoadjuvant A/T. Although the correlation between high HRD scores and chemosensitivity has been mostly studied in TNBC, the present study included all breast cancer subtypes since a significant proportion of the subtypes, other than TNBC, has high HRD scores [23, 24].

Materials and methods

Patients and samples

The medical records of 167 patients with stages II and III primary breast cancer who received neoadjuvant chemotherapy (NAC) and underwent subsequent surgery (mastectomy or breast-conserving surgery) between 2004 and 2017 at Osaka University Hospital were retrospectively reviewed. NAC consisted of weekly paclitaxel at 80 mg/m^2 for 12 cycles followed by a combination of 5-fluorouracil (500 mg/m^2), epirubicin (75 mg/m^2), and cyclophosphamide (500 mg/m^2) (P-FEC) every 3 weeks for four cycles. Sixteen patients had at least one first-degree relative with breast and/or ovarian cancer. Before NAC, all patients underwent vacuum-assisted core biopsy (Mammotome 8G HH; Ethicon Endo-Surgery, Inc., Blue Ash, OH, USA) under ultrasonographic guidance. In each patient, some tumor biopsy samples 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.

Copy number variation analysis with a DNA microarray

DNA was extracted from the tumor biopsy samples (frozen tissues, $n = 164$; FFPE tissues, $n = 3$) using the DNeasy Blood and Tissue kit or the QIAamp DNA FFPE Tissue Kit (Qiagen Sciences, Inc., Germantown, MD, USA) and then subjected to copy number variation (CNV) analysis using the OncoScan CNV FFPE Assay Kit (Affymetrix - Thermo Fisher Scientific, Inc., Santa Clara, CA, USA), in accordance with the manufacturers' instructions.

Gene expression analysis with a DNA microarray

RNA was extracted from the TNBC biopsy samples ($n = 35$) using TRIzol® Reagent (Thermo Fisher Scientific, Waltham, MA, USA) or the RNeasy Mini Kit (Qiagen, Hilden, Germany) and then subjected to gene expression analysis using a DNA microarray (Human Genome U133 plus 2.0 Array; Affymetrix, Thermo Fisher Scientific, Inc.), according to a previously described method [25]. The gene expression analysis could be done successfully in all TNBCs, and subtyping of TNBCs into five categories was carried out according to the method described by Bareche et al. [26].

Determination of HRD score

The three components of the HRD score (i.e., NtAI, LST, and HRD-LOH) were calculated using R code, as reported by Marquard et al. [23]. The HRD score was determined as a simple sum of these three factors with a cutoff value of 42 [3]. Probe data were extracted using OncoScan Console 1.3 Software (Affymetrix, Thermo Fisher Scientific, Inc.), while ploidy and normal cell contamination were determined using allele-specific copy number analysis of tumors (ASCAT) [27]. Of the 167 tumor biopsy samples, 157 passed the quality check of the ASCAT algorithm, but 16 were excluded due to a tumor cell content of 0%, thus the HRD score was determined for the remaining 141 samples. Moreover, from the Cancer Genome Atlas (TCGA) data [dbGAP project ID:13643], HRD scores were calculated using Affymetrix SNP6.0 CEL files of 853 breast cancers and 23 *BRCA1*- or *BRCA2*-associated breast cancers (*BRCA1/2* breast cancer). Samples with an aberrant cell fraction of <0.36 were excluded from the analysis, in accordance with the method described by Marquard et al. [23].

Histological evaluation of response to NAC

The pathological response to NAC was evaluated using surgical specimens obtained after NAC. The surgical specimens were cut into 5-mm slices, which were stained with hematoxylin and eosin, and further prepared to determine the presence or absence of tumor cells. The complete absence of invasive tumor cells in the breast and axillary lymph nodes was defined as pCR irrespective of the presence or absence of non-invasive components.

Immunohistological and *BRCA1* promoter methylation analyses

The expression levels of estrogen receptor (ER), progesterone receptor (PR), and the Ki67 in the tumor biopsy samples were determined immunohistochemically, as described elsewhere [28]. The cutoff values of ER, PR, and Ki67 were 1%, 1%, and 20%, respectively. The amplification of human epidermal growth factor receptor 2 (HER2) was determined by fluorescence *in situ* hybridization (FISH) using the PathVysion HER-2 DNA Probe Kit (Vysis, Abbott Molecular Inc., Des Plaines, IL, USA). HER2 amplification was identified by a FISH ratio of ≥ 2.0. The presence of tumor-infiltrating lymphocytes (TILs) was assessed using the procedure recommended by the International TILs Working Group 2014 [29]. The methylation status of the *BRCA1* promoter was determined by a previously described method [30].

Statistical analysis

All statistical analyses were performed using R statistical software (version 3.5.1; http://www.r-project.org/). The Fisher's exact test was used to compare 2×2 groups and the Mann–Whitney *U* test was used to compare the HRD scores. Logistic regression model was used for the univariate and multivariate analysis in the prediction of pCR with the various parameters in TNBC. All statistical analyses were two-sided, and a probability (*P*) value of <0.05 was considered statistically significant.

Results

HRD scores of established TCGA data sets and those obtained from the present series

From the TCGA database, single-nucleotide polymorphism (SNP) microarray data including 853 breast cancers and 23 *BRCA1/2* breast cancers were downloaded, and the HRD scores were calculated for each cancer and then compared with those of the present series ($n=141$) (Fig. 1). The 853 breast cancers from the TCGA database and 141 obtained in the present series were unselected cases, and thus almost all were thought to be sporadic. HRD scores were significantly

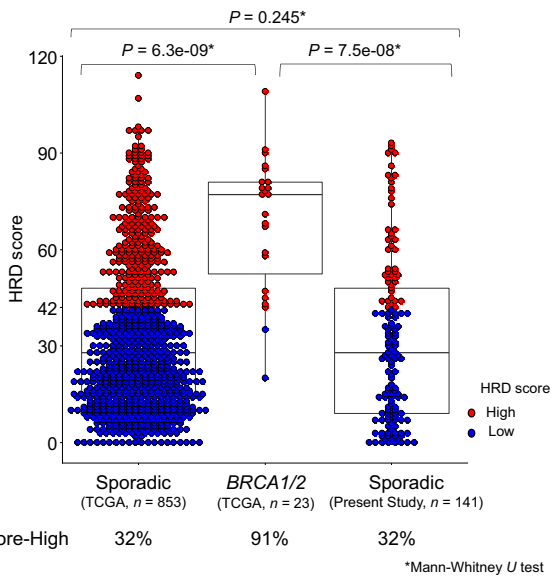

Fig. 1 HRD scores for breast cancers from the TCGA database and the present series. The distribution of HRD scores of 853 breast cancers (Sporadic) and 23 BRCA1/2 breast cancers from the TCGA database and 141 breast cancers (Sporadic) from the present series is illustrated in a boxplot diagram. Box outlines, 25th, and 75th percentiles; solid line in the middle, median; red dot, high HRD score (≥ 42); blue dot, low HRD score (<42); statistical analysis, Mann–Whitney *U* test

higher for *BRCA1/2* breast cancers than in the TCGA series ($P = 6.3e{-}09$) or the present series ($P = 7.5e{-}08$). Then, the tumors were classified as either HRD-high or HRD-low using a cutoff value of 42. A significantly greater proportion of *BRCA1/2* breast cancers was classified as HRD-high, compared with those in the TCGA series or the present series (91% [21 of 23] vs. 32% [277 of 853] and 32% [45 of 141], respectively).

Clinicopathological characteristics of HRD-high breast cancers

Of the 141 breast cancers in the present series, 45 were classified as HRD-high and 96 as HRD-low. HRD-high tumors were significantly associated with a high histological grade, PR-negativity, and a high Ki67 (Table 1). Then, the tumors were further classified into one of the four subtypes according to the status of ER, PR, and HER2; that is, luminal ($n = 76$, ER- and/or PR-positive and HER2-negative), luminal-HER2 ($n = 13$, ER- and/or PR-positive and HER2-positive), HER2 ($n = 17$, ER- and PR-negative and HER2-positive), and TNBC ($n = 35$, ER- and PR-negative and HER2-negative) (Table 2; Fig. 2). There was no significant difference in HRD scores among the luminal, luminal-HER2 and HER2 subtypes although the scores of the TNBC subtype were significantly higher than those of the luminal subtype and the HER2 subtype ($P = 0.0012$ and $P = 0.014$, respectively) (Fig. 2, left panel). *BRCA1* promoter methylation was observed in 8.5% (12 of 141) of breast cancers, and the HRD scores were significantly ($P = 5.6e{-}08$) higher in breast cancers with *BRCA1* promoter methylation than without (Fig. 2, right panel).

Correlations of the subtypes with *BRCA1* methylation status and HRD score are shown in Table 2. Positivity of *BRCA1* methylation and the proportion of HRD-high tumors were significantly ($P = 0.002$ and 0.006, respectively) higher for the TNBC subtype than the other subtypes.

Table 1 Clinicopathological parameters and HRD score

	HRD score < 42 ($n = 96$)	HRD score ≥ 42 ($n = 45$)	OR	95% CI	*P* value[a]
Menopause					
Premenopausal	42	21	1		
Postmenopausal	54	24	0.89	0.41–1.93	0.856
cT					
1 + 2	76	32	1		
3 + 4	20	13	1.54	0.62–3.72	0.295
HG					
1 + 2	72	21	1		
3	24	24	3.4	1.52–7.72	0.001
cN					
Negative	29	16	1		
Positive	67	29	0.79	0.35–1.80	0.564
ER					
Negative	32	23	1		
Positive	64	22	0.48	0.22–1.05	0.063
PR					
Negative	43	29	1		
Positive	53	16	0.45	0.20–0.98	0.032
HER2					
Negative	72	38	1		
Positive	24	7	0.55	0.18–1.48	0.276
Ki67					
< 20	46	10	1		
≥ 20	49	34	3.17	1.34–8.04	0.005
Unknown	1	1			
TILs					
< 20	70	31	1		
≥ 20	26	14	1.21	0.51–2.80	0.690

cT clinical tumor size, *cN* clinical nodal status, *OR* odds ratio, *CI* confidence interval

[a]Fisher's exact test. Unknown data were not included in the analysis

Correlation between HRD scores and pCR to neoadjuvant P-FEC

Patients ($n = 130$) who completed neoadjuvant P-FEC were including in this analysis. Postmenopausal status, negative ER, negative PR, high Ki 67 index, and high TILs, but not HRD scores, were significantly associated with pCR (Table 3). Then, the correlation of HRD scores with pCR was analyzed in each subtype (Table 4). Interestingly, HRD-high tumors showed a significantly ($P = 0.001$) lower pCR rate than HRD-low tumors in the TNBC subtype but not in the other three subtypes. Then, the association of the various parameters with pCR was investigated in TNBCs by univariate analysis which showed that high HRD scores and low TILs were significantly ($P = 0.002$ and $P = 0.047$, respectively) associated with non-pCR and that *BRCA1* methylation tended ($P = 0.065$) to show an association with non-pCR (Table 5). These three parameters were further evaluated by the multivariate analysis which showed that high HRD scores and low TILs were independently and significantly ($P = 0.012$ and $P = 0.047$, respectively) associated with non-pCR.

Then, TNBCs were classified into four groups according to the HRD score and TILs (Fig. 3a). The highest pCR rate (87.5%) was observed for HRD-low and TIL-high tumors, while the lowest (0%) was observed for HRD-high and TIL-low tumors, and the HRD-high and TIL-high tumors and the HRD-low and TIL-low tumors showed intermediate pCR rates (37.5% and 62.5%, respectively).

Table 2 Correlation of IHC subtype with *BRCA1* methylation status and HRD score

IHC subtype	*BRCA1*					HRD score				
	UM	M	OR	95% CI	*P* value[a]	Low	High	OR	95% CI	*P* value[a]
Luminal ($n=76$)	74	2	1			55	21	1		
Luminal-HER2 ($n=13$)	12	1	1			10	3	1		
HER2 ($n=17$)	16	1	1			14	3	1		
Triple negative ($n=35$)	27	8	7.41	1.82–36.23	0.002	17	18	3.07	1.29–7.38	0.006

M methylated, *UM* unmethylated, *High* HRD score ≥ 42, *Low* HRD score < 42, *OR* odds ratio, *CI* confidence interval

[a]Fisher's exact test for triple-negative vs other subtypes

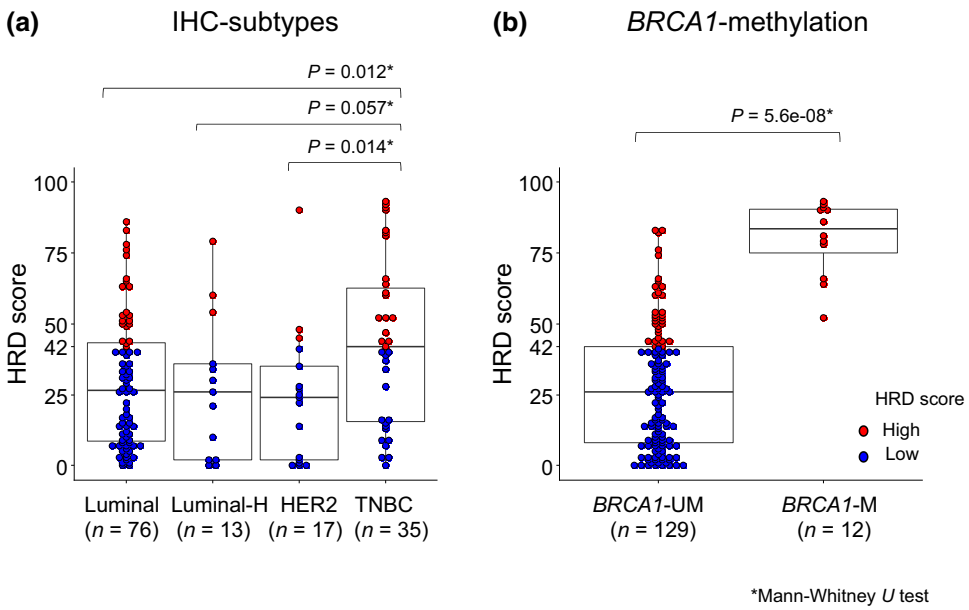

Fig. 2 HRD scores according to IHC subtypes and *BRCA1* methylation status. **a** HRD scores according to IHC subtypes. **b** HRD scores according to *BRCA1* methylation status. Box outlines, 25th, and 75th percentiles; solid line in the middle, median; red dot, high HRD score (≥ 42); blue dot, low HRD score (< 42); statistical analysis method, Mann–Whitney *U* test. *BRCA1*-M, *BRCA1*-methylated; *BRCA1*-UM, *BRCA1*-unmethylated; luminal-H, luminal-HER2; TNBC, triple-negative breast cancer

BRCA1 methylation status and HRD scores according to molecular subtypes of TNBC

TNBCs were classified into five molecular subtypes, basal-like 1 (BL1), mesenchymal (M), immunomodulatory (IM), mesenchymal stem-like (MSL), and luminal androgen receptor (LAR), according to the gene expression profile as determined by DNA microarray analysis [26]. *BRCA1* methylation was observed in the BL1, M, and IM subtypes but not in the MSL and LAR subtypes (Fig. 3b). A majority of tumors belonging to the BL1, M, IM, and MSL, but not LAR, subtypes were classified as HRD-high tumors (Fig. 3c).

Discussion

Although the proportion of HRD-high tumors was significantly lower in sporadic breast cancers than *BRCA1/2* breast cancers, still 32% of them were HRD-high tumors in the present study. The aim of this study was to investigate the clinicopathological characteristics of these HRD-high sporadic breast tumors with a special reference to the sensitivity to neoadjuvant P-FEC. First, the relationship between HRD scores and clinicopathological characteristics of breast tumors was investigated, which showed that HRD-high tumors were significantly more likely to have a high histological grade, be PR-negative and have a high Ki67 index. Thus, the phenotypes of HRD-high tumors are considered to be biologically aggressive, and the results of subtype analysis showed that HRD-high tumors were significantly more prevalent in the TNBC subtype than in the other three subtypes, consistent with the findings of previous reports [23, 24].

Although the somatic *BRCA1* mutation is rare in sporadic breast cancers [31], *BRCA1* methylation has been reported in 7–31% of breast cancers [32]. In the present study, *BRCA1* methylation was found in 8.5% of all tumors and most frequently (22.9%) in the TNBC subtype. *BRCA1* methylation silences its mRNA expression, resulting in the loss of *BRCA1* function and manifestation of similar phenotypes

Table 3 Clinicopathological parameters and pCR

	Non-pCR ($n=104$)	pCR ($n=26$)	OR	95% CI	P value[a]
Menopause					
Premenopausal	56	5	1		
Postmenopausal	48	21	4.84	1.61–17.71	0.002
cT					
1 + 2	78	22	1		
3 + 4	26	4	0.55	0.13–1.83	0.436
cN					
Negative	31	9	1		
Positive	73	17	0.8	0.30–2.28	0.641
HG					
1 + 2	75	14	1		
3	29	12	2.2	0.82–5.84	0.098
ER					
Negative	27	21	1		
Positive	77	5	0.09	0.02–0.26	4.20E−07
PR					
Negative	41	22	1		
Positive	63	4	0.12	0.03–0.39	4.40E−05
HER2					
Negative	87	20	1		
Positive	17	6	1.53	0.44–4.76	0.403
Ki67					
< 20	49	3	1		
≥ 20	53	23	7.09	1.93–38.70	6.30E−04
Unknown	2	0			
TILs					
< 20	82	12	1		
≥ 20	22	14	4.29	1.59–11.83	0.002
BRCA1					
UM	93	25	1		
M	11	1	0.34	7.6E−03–2.55	0.310
HRD score					
Low	67	21			
High	37	5	0.43	0.12–1.31	0.159

cT clinical tumor size, *cN* clinical nodal status, *M* methylated, *UM* unmethylated, *High* HRD score ≥ 42, *Low* HRD score < 42, *OR* odds ratio, *CI* confidence interval

[a]Fisher's exact test. Unknown data were not included in the analysis

of *BRCA1* breast cancers, including HRD [3, 33]. We were also able to show that *BRCA1* methylated tumors had high HRD scores, as with *BRCA1* breast cancers. Thus, the reason why the proportion of HRD-high tumors was more prevalent in TNBCs can be explained, at least in part, by the fact that TNBCs are more likely to be positive for *BRCA1* methylation than the other three subtypes [30, 34].

Second, we examined the relationship between the HRD score and response to P-FEC. When all subtypes of tumors were considered, there was no significant association between the HRD score and pCR rate but, interestingly, when only TNBCs were considered, HRD-high tumors were significantly and independently associated with non-pCR. Since HRD-high tumors are speculated to be sensitive, although not yet proven, to platinum or PARP inhibitors, our results seem to suggest the possibility that the HRD score might be useful for the selection of a chemotherapeutic regimen for TNBCs, that is, platinum or PARP inhibitors for HRD-high TNBCs and P-FEC for HRD-low TNBCs. However, several conflicting results on the association between HRD and the response to A/T have been reported. Some reported an association of HRD

Table 4 IHC subtypes and pCR

IHC subtype	HRD score	Non-pCR	pCR	OR	95% CI	*P* value[a]
Luminal ($n=74$)	Low	50	3	1		
	High	19	2	1.74	0.14–16.46	0.618
Luminal-HER2 ($n=10$)	Low	8	0	1		
	High	2	0	NA	NA	NA
HER2 ($n=12$)	Low	5	6	1		
	High	1	0	0	0.00–39.00	1.000
Triple negative ($n=34$)	Low	4	12	1		
	High	15	3	0.07	8.7E−03–0.44	0.001

High HRD score ≥ 42, *Low* HRD score < 42, *OR* odds ratio, *CI* confidence interval

[a]Fisher's exact test

Table 5 Clinicopathological parameters and pCR in TNBC

	Non-pCR ($n=19$)	pCR ($n=15$)	Univariate			Multivariate		
			OR	95% CI	*P* value[a]	OR	95% CI	*P* value[a]
Menopause								
Premenopausal	6	2	1					
Postmenopausal	13	13	3	0.57–34.70	0.225			
cT								
1 + 2	13	12	1					
3 + 4	6	3	0.54	0.10–2.55	0.451			
cN								
Negative	5	6	1					
Positive	14	9	0.54	0.12–2.29	0.4			
HG								
1 + 2	6	3	1					
3	13	12	1.85	0.39–10.33	0.451			
Ki67								
<20	2	1	1					
≥20	17	14	1.65	0.14–37.65	0.696			
TILs								
<20	13	5	1			1		
≥20	6	10	4.33	1.07–19.89	0.047	9.95	1.03–95.95	0.047
BRCA1								
UM	12	14	1			1		0.802
M	7	1	0.12	6.1E−03–0.83	0.065	0.7	0.04–11.41	
HRD score								
Low	4	12	1			1		
High	15	3	0.07	0.01–0.32	0.002	0.04	3.3E−03–0.50	0.012

cT clinical tumor size, *cN* clinical nodal status, *M* methylated, *UM* unmethylated, *High* HRD score ≥ 42, *Low* HRD score < 42, *OR* odds ratio, *CI* confidence interval

[a]Logistic regression analysis

with resistance to A/T [18], while others found no such association [19, 20]. Furthermore, very recently, Telli et al. reported the opposite result that HRD-high TNBCs determined by next-generation sequencing were more sensitive to A/T than HRD-low TNBCs [21], and similar results have also been reported [8, 22]. The reason for this discrepancy is currently unknown but the difference in the methods for determination of HRD, that is, SNP array, multiplex ligation-dependent probe amplification, or next-generation sequencing, as well as the regimens for NAC (dose and schedule, etc.) might offers a partial explanation.

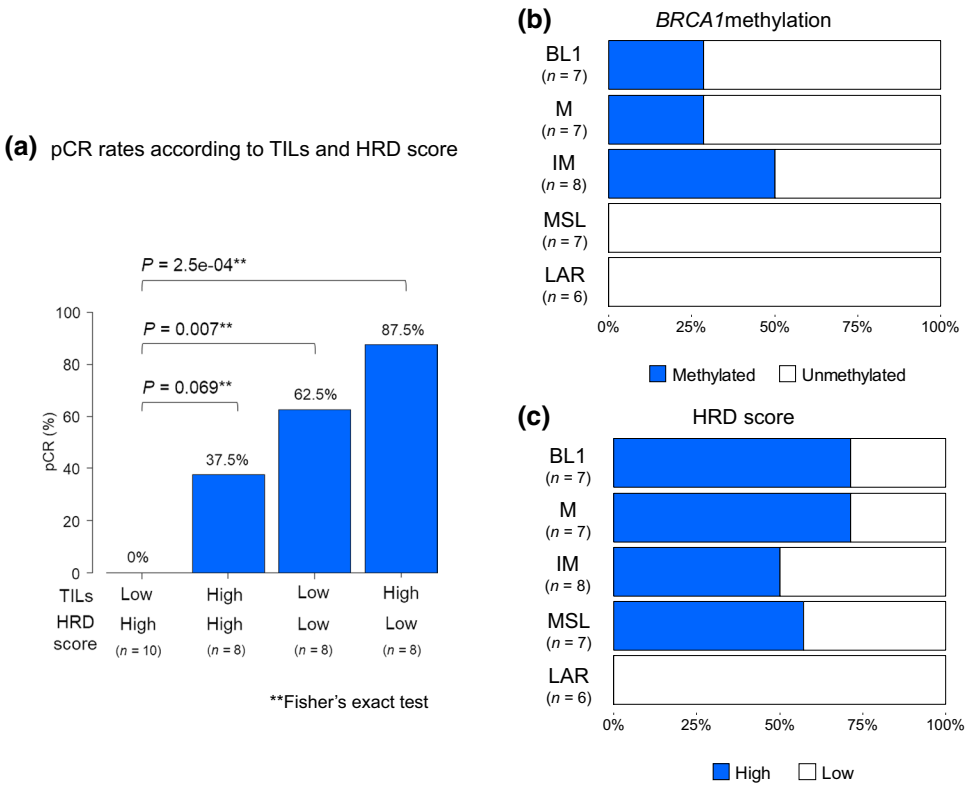

Fig. 3 pCR rates according to HRD score and TILs in TNBCs and correlation of molecular subtypes of TNBCs with *BRCA1* methylation status and HRD score. **a** pCR rates according to the combination of TILs and HRD score in TNBCs. **b** *BRCA1* methylation status according to molecular TNBC subtype. **c** HRD score according to the molecular TNBC subtype. *BL1* basal-like 1, *M* mesenchymal, *IM* immunomodulatory, *MSL* mesenchymal stem-like, *LAR* luminal androgen receptor

As for the correlation of HR deficiency with response to platinum-containing regimens, it has been reported that *BRCA1/2* breast cancers [35, 36] or HRD-high tumors [3, 8] are sensitive to platinum-containing regimens, suggesting a clinical utility of HRD score for the selection of breast tumors which are more likely to respond to platinum-containing regimen. Very recently, however, Telli et al. reported that addition of carboplatin resulted in the higher pCR rates in both HR-deficient and non-deficient tumors in the neoadjuvant settings [9]. In addition, Tutt et al. reported that the response rate to carboplatin was similar between HRD-high and HRD-low tumors in the metastatic settings (TNT trial) [7]. In this TNT trial, HRD scores were determined in the primary tumors, and treatment efficacy was evaluated in the metastatic tumors. Thus, it is possible that HRD scores in the primary tumors do not necessarily reflect those of the metastatic tumors. Therefore, further studies are needed to draw a final conclusion as to the clinical utility of HRD scores as a predictor for response to platinum-containing regimens.

Finally, we investigated the correlation between the HRD score and *BRCA1* methylation status among the molecular subtypes of TNBCs, as determined by gene expression profiling according to the method described by Bareche et al. [26], because TNBCs are very heterogeneous. The majority of BL1, M, IM, and MSL tumors were HRD-high tumors but *BRCA1* methylation was observed in less than 50% of BL1, M, and IM tumors and in none of MSL tumors, indicating that a significant proportion of TNBCs induces HRD through a mechanism, other than *BRCA1* methylation, which might involve the inactivation of other genes in the HR pathway. Interestingly, no LAR tumors were *BRCA1* methylated or HRD-high tumors. Although classified as TNBCs, LAR tumors are distinct from the other TNBC subtypes based on androgen receptor and luminal-like gene expression [26, 37].

This study has several limitations; firstly, this is a retrospective study on a limited number of patients so that a definitive conclusion as to the correlation of HRD score and pCR cannot be drawn. A further study including a larger number of TNBCs is required. Secondly, HRD score was determined by, not the Myriad Genetics (Salt Lake City, Utah, USA), but our laboratory. However, we believe that our assay, which was conducted according to the method described by Marquard et al. [23], was valid since almost all *BRCA1/2*-associated breast tumors and *BRCA1*-methylated breast tumors were classified as HRD-high tumors, and the phenotypes of HRD-high tumors were consistent with the previous reports using the HRD score determined by the Myriad Genetics [3]. Thirdly, *BRCA1/2* germline mutation was not assessed in the present study. Since about 10% of TNBCs are reported to have *BRCA1/2* mutation [12], the number of TNBCs with *BRCA1/2* germline mutation is estimated to be three or four in the present study. Such a small number of *BRCA1/2*-mutated tumors does not allow for a

meaningful analysis on the correlation between *BRCA1/2* mutation status and pCR. Lastly, it is possible that HRD plays a certain role in response to P-FEC in the other subtypes than TNBCs but this issue cannot be investigated in the present study due to the small number of tumors in each subtype.

In conclusion, the results of the present study showed that HRD-high tumors are associated with biologically aggressive phenotypes and *BRCA1* methylation and are most prevalent in TNBCs. It is also suggested that HRD-high tumors are resistant to chemotherapy (P-FEC) in TNBCs, indicating a possibility that the determination of the HRD score of TNBCs might be useful for the selection of a chemotherapeutic regimen. These observations should be confirmed in a future study with a larger number of TNBCs.

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Compliance with ethical standards

Conflict of interest Shinzaburo Noguchi has been an adviser for Taiho, AstraZeneca, and Novartis and has received research funding for this study from AstraZeneca and for other studies from Sysmex, 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. Yasuto Naoi has received research funding for this study from AstraZeneca and honoraria from Sysmex. Naofumi Kagara has received honoraria from AstraZeneca and Novartis. Masafumi Shimoda has received research funding for other studies from Novartis and AstraZeneca and honoraria from Chugai, Eisai, Novartis, and Takeda. Kenzo Shimazu has received honoraria from AstraZeneca, Chugai, and Sysmex. Seung Jim Kim has received honoraria from AstraZeneca, Chugai, Eisai, Kyowa-Kirin, Novartis, Pfizer, Shimadzu, Taiho, and Takeda. The other authors declare no conflicts of interest.

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

Informed consent The study protocol was approved by the Ethical Review Board of Osaka UniversityHospital, and informed consent was obtained from each patient before tumor biopsy.

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