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Predictive value of BRCA1/2 mRNA expression for response to neoadjuvant chemotherapy in BRCA-negative breast cancers

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It is well known that *BRCA1* and *BRCA2* play a central role in DNA repair, but the relationship between BRCA1 and BRCA2 mRNA expression and response to neoadjuvant chemotherapy in sporadic breast cancer patients has not been well established. Here, we investigate the association between BRCA1 or BRCA2 mRNA expression levels and pathological response in 674 BRCA1/2 mutation-negative breast cancer patients who received neoadjuvant chemotherapy. BRCA1 and BRCA2 mRNA expression were assessed using quantitative real-time polymerase chain reaction in core biopsy breast cancer tissue obtained prior to the initiation of neoadjuvant chemotherapy. A total 129 patients (19.1%) achieved pathological complete response (pCR) after neoadjuvant chemotherapy. Among patients treated with anthracycline-based chemotherapy (n = 531), BRCA1 mRNA low expression patients had a significantly higher pCR rate than intermediate or high BRCA1 mRNA expression groups (24.6% vs 16.8% or 14.0%, $P = .031$) and retained borderline significance (OR = 1.54, 95% CI = 0.93-2.56, $P = .094$) in multivariate analysis. Among the 129 patients who received a taxane-based regimen, pCR rate showed no differences in BRCA1 low, intermediate, and high mRNA level subgroups (19.6%, 26.8% and 21.4%, respectively; $P = .71$). BRCA2 mRNA level was not associated with pCR rate in the anthracyline-based treated subgroup ($P = .60$) or the taxane-based regimen subgroup ($P = .82$). Taken together, our findings suggested that BRCA1 mRNA expression could be used as a predictive marker in BRCA1/2 mutation-negative breast cancer patients who received neoadjuvant anthracycline-based treatment.

KEYWORDS
BRCA1 gene, *BRCA2* gene, breast cancer, mRNA expression, neoadjuvant chemotherapy

1 | INTRODUCTION

BRCA1 and *BRCA2* genes play important roles in DNA repair, cell cycle checkpoint control, transcriptional regulation, and

Abbreviations: CI, confidence interval; DRFS, distant recurrence-free survival; ER, estrogen receptor; HER2, human epidermal growth factor receptor-2; OR, odds ratio; pCR, pathological complete response; PR, progesterone receptor; qPCR, quantitative polymerase chain reaction.

ubiquitination.[1-3] Thus, deficiencies in these genes can impact the response of cancer cells to chemotherapy drugs. Several studies have demonstrated that BRCA1 or BRCA2 germline mutation carriers were more sensitive than non-carriers to chemotherapy drugs.[4-9] However, BRCA1/2 germline mutations are rarely observed in sporadic breast cancer, comprising only approximately 2% of breast cancers overall[10,11] and approximately 1.1% of breast cancer cases in China.[12]

In addition to BRCA1/2 germline mutations, other BRCA defects, including methylation of the BRCA1 promoter, low expression, and copy number deletions in some sporadic cancers share similar phenotypic characteristics with tumors that carry BRCA1/2 mutations.[13] Decreased BRCA1 mRNA expression in breast cancer cell lines increased sensitivity to etoposide and cisplatin,[14,15] whereas overexpression of BRCA1 in murine ovarian cancer cells increased resistance to cisplatin, etoposide, and doxorubicin.[16,17] Consistent with these in vitro findings, we previously demonstrated that methylation of the BRCA1 promoter is significantly associated with sensitivity to adjuvant chemotherapy in triple-negative breast cancer patients and with good outcome.[18] However, several studies also showed that inhibition of BRCA1 expression resulted in resistance to paclitaxel and vincristine.[14,19] Moreover, relatively few studies to date have reported the relationship of BRCA2 mRNA expression and response to chemotherapy in breast cancers. Thus, the role of BRCA1 and BRCA2 as predictors of differential response to neoadjuvant chemotherapy in breast cancer has not been well established. In particular, few studies have excluded the effect of BRCA1/2 germline mutations to determine whether BRCA1 or BRCA2 mRNA expression levels contribute to response to chemotherapy agents.

To address these problems, we measured mRNA expression levels of BRCA1 and BRCA2 in a cohort of 674 Chinese breast cancer patients without BRCA1/2 germline mutations who received neoadjuvant chemotherapy. We also evaluated the effect of BRCA1 and BRCA2 mRNA expression levels on the prediction of various neoadjuvant chemotherapy regimens.

2 | MATERIALS AND METHODS

2.1 | Patients

This study included 774 operable primary breast cancer patients (stage I-III) who received neoadjuvant chemotherapy at the Breast Center, Peking University Cancer Hospital from May 2004 to January 2011. Breast cancer was diagnosed by a core-needle biopsy using a 14-gauge needle at the Breast Center. Fresh core-needle breast tumor samples were available for all patients. Of these 774 patients, 73 patients were excluded because tumor tissue was unavailable or insufficient for BRCA1 and BRCA2 assessment, poor quality of the RNA samples, or bad repeat results of real-time quantitative PCR. Of these remaining 701 patients, 27 patients were excluded because they carried BRCA1 or BRCA2 germline mutations. Thus, a total 674 breast cancer patients who were assessed and confirmed as negative for BRCA1/2 germline mutations were analyzed in this study.

Patient age at diagnosis ranged from 25 to 73 years, with a median of 49 years. Tumors were graded according to the modified Bloom-Richardson system. Tumor stage was classified according to TNM classification of the Union International Cancer Control. Tumor size was defined as the maximum tumor diameter measured by a mammogram and/or ultrasound at the time of diagnosis. Treatments were obtained from review of medical records. This study was conducted in accordance with the ethics principles of the Declaration of Helsinki and approved by the Research and Ethics Committee of Peking University Cancer Hospital. All patients provided written informed consent.

2.2 | Neoadjuvant chemotherapy regimens

Among the total 674 BRCA1/2 mutation-negative patients, 94% received four to eight cycles of neoadjuvant chemotherapy. Treatments were categorized into three subgroups.

1. A total of 531 patients received an anthracycline-based regimen. Of these, 236 patients received anthracycline regimens; the common regimens were CTF (5-fluorouracil, pirarubicin, and cyclophosphamide) or FEC (5-fluorouracil, epirubicin, and cyclophosphamide) regimens, which were described previously;[20] 118 patients received two cycles of anthracycline regimens followed by four cycles of paclitaxel alone (80 mg/m^2 i.v. once a week for 12 weeks) or docetaxel plus cyclophosphamide (docetaxel 75 mg/m^2 i.v. on day 1, cyclophosphamide 600 mg/m^2 i.v. on day 1, every 3 weeks); and 177 patients received two cycles of anthracycline regimens followed by paclitaxel plus carboplatin (paclitaxel 175 mg/m^2 i.v. on day 1, or paclitaxel 60 mg/m^2 i.v. on day 1, day 8, and day 15, and carboplatin AUC6, i.v. on day 1, every 3 weeks).
2. A total of 129 patients received a taxane-based regimen without anthracyclines. Of these, 94 patients received four cycles of paclitaxel alone; 20 patients received paclitaxel plus carboplatin. The remaining 15 patients received docetaxel plus cyclophosphamide.
3. The remaining 14 patients received other regimens.

After completion of neoadjuvant chemotherapy, patients were treated with mastectomy (n = 398) or breast-conserving surgery (n = 276) depending on tumor size, presence of multiple lesions or patient preference. pCR was defined as no invasive breast cancer cells in the breast after completion of neoadjuvant chemotherapy.[21]

2.3 | Real-time quantitative PCR

Breast tumor RNA was extracted from all core-needle biopsy samples obtained before the start of neoadjuvant chemotherapy using Trizol reagent (Life Technologies Inc., Gaithersburg, MD, USA) according to the manufacturer's instructions. RNA (500 ng) was

transcribed to cDNA in a total 20 μL RT reaction solution containing 4.0 μL 5 × first strand buffer, 2.0 μM DTT, 20 U RNase inhibitor, 1 mM dNTP, 1 μM random primer and 200 U superscript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA). Real-time quantitative PCR reactions of the *BRCA1* and *BRCA2* genes were carried out using the TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA) in the Applied Biosystems StepOne-plus Real-time PCR System, following the manufacturer's instructions. We used commercially available primers and probes for PCR analyses (TaqMan Gene Expression Assays, Assay ID: Hs01556193_m1 for BRCA1, Hs00609073_m1 for BRCA2, and Hs99999903_m1 for β-actin as an endogenous control; Applied Biosystems). PCR conditions were as follows: 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds and 60°C for 1 minute. Each sample was assayed in triplicate with RNase-free water as negative control.

Relative gene expression quantifications were calculated according to the comparative Ct method using β-actin as an endogenous control and normal peripheral blood mononuclear cell RNA control as calibrators in each plate. Final results were determined by the formula $2^{-\Delta\Delta Ct22}$ and were analyzed with DataAssist™ software. Results obtained were the mean of three independent experiments.

2.4 | BRCA1 and BRCA2 germline mutation analysis by sequencing

Genomic DNA was isolated from peripheral mononuclear blood cells using the phenol-chloroform assay. *BRCA1* and *BRCA2* genes were screened by PCR sequencing assay as described elsewhere.[23] We screened the entire coding regions and exon-intron boundaries of BRCA1 and BRCA2 for all 701 breast cancer patients. All fragments were sequenced using the BigDye Terminator Cycle Sequencing Kit and ABI 3730 automated sequencer (Applied Biosystems). Each mutation was confirmed in duplicate. Only the mutations that lead to a truncated protein or that have been previously reported as disease-associated (according to Breast Cancer Information Core database) were considered to be deleterious.

2.5 | ER, PR, and HER2 status

ER, PR, and HER2 status were determined by immunostaining in the core-needle biopsy breast cancer tissue obtained before the start of neoadjuvant chemotherapy. ER or PR immunostaining was considered positive when ≥1% of tumor cells showed positive nuclear staining. HER2 positivity was defined as a score of 3 + (immunohistochemistry) or *HER2* gene amplification (FISH) in core-biopsy breast cancer tissue.[20]

2.6 | Statistical analysis

Median values and ranges were calculated for mRNA expression. To provide an easily interpretable evaluation of the effect of BRCA1 and BRCA2 mRNA expression, gene expression values were divided into tertiles. Associations between BRCA1 or BRCA2 mRNA levels, clinicopathological characteristics, and pathological response to neoadjuvant chemotherapy were determined by Pearson's chi-squared test. A logistic regression model was applied to determine whether a factor was an independent predictor of pCR in multivariate analysis. DRFS was defined as the time from the date of diagnosis to first distant recurrence (not including second primary malignancies) or death from breast cancer without a recorded relapse. Survival curves were derived from Kaplan-Meier estimates and compared using log-rank tests. All statistical tests were two-sided, and *P*-values <.05 were considered statistically significant. Statistical analyses were carried out using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA).

3 | RESULTS

3.1 | Patient characteristics and BRCA1 and BRCA2 mRNA expression levels

We studied 674 patients who were previously tested and found to be negative for BRCA1/2 germline mutations.[24,25] We carried out quantitative real-time PCR of BRCA1 and BRCA2 mRNA expressions in the 674 total samples negative for BRCA1/2 germline mutations, using the reference gene of β-actin for normalization. Relative median mRNA expression levels of BRCA1 and BRCA2 were 1.71 (range 0.03-34.72) and 1.33 (range 0.03-43.87), respectively. Patients were subdivided into three groups based on low (0.03-1.08; mean, 0.57), intermediate (1.10-2.83; mean, 1.82), and high (2.85-34.7; mean, 6.73) levels of BRCA1 mRNA relative expression. Patients were also divided into three subgroups based on BRCA2 mRNA relative expression: low (0.03-0.88; mean, 0.52), intermediate (0.91-1.89; mean, 1.33), and high (1.92-68.29; mean, 5.17) levels.

Table 1 shows the clinicopathological characteristics for all patients (n = 674). Patients with low BRCA1 mRNA level were more likely to have ER-negative ($P = .002$), PR-negative ($P = .006$), and HER2-positive expression ($P = .025$), whereas patients with low BRCA2 mRNA level were more likely to be ER-positive ($P = .032$), PR-positive ($P = .01$), HER2-negative ($P = .011$), and lymph node positive ($P = .027$). Patients with low BRCA1 mRNA expression level were also more likely to be triple-negative ($P = .014$). Neither BRCA1 nor BRCA2 mRNA expression was significantly associated with age at diagnosis or tumor size (Table 1).

3.2 | Response to neoadjuvant chemotherapy

Among the 674 BRCA1/2 germline mutation negative patients, 129 patients (19.1%, 129/674) achieved pCR. Patients with low BRCA1 mRNA level had a higher pCR rate than those with intermediate or high BRCA1 mRNA level (23.6% vs 18.7% or 15.2%, respectively; $P = .077$), but the difference did not reach statistical significance. Low, intermediate or high BRCA2 mRNA expression level was not significantly associated with pCR rate (18.7%, 18.2% or 20.4%, respectively; $P = .82$). Additional factors associated with pCR included tumor size ($P = .003$), lymph node status ($P < .001$), tumor

TABLE 1 Association of patient and tumor characteristics with BRCA1 or BRCA2 mRNA expression status

Characteristic	N	BRCA1 Low N (%)	BRCA1 Intermediate N (%)	BRCA1 High N (%)	P	BRCA2 Low N (%)	BRCA2 Intermediate N (%)	BRCA2 High N (%)	P
Age, years	674	225	225	224	.89	224	225	225	.23
≤50	363	119 (52.9)	124 (55.1)	120 (53.6)		128 (57.1)	124 (55.1)	111 (49.3)	
>50	311	106 (47.1)	101 (44.9)	104 (46.4)		96 (42.9)	101 (44.9)	114 (50.7)	
Tumor size,					.55				.16
≤2 cm	224	81 (36.0)	71 (31.6)	72 (32.1)		78 (34.8)	64 (28.4)	82 (36.4)	
>2 cm	450	144 (64.0)	154 (68.4)	152 (67.9)		146 (65.2)	161 (71.6)	143 (63.6)	
Lymph node status					.69				.027
Negative	355	123 (54.7)	119 (53.1)	113 (50.7)		102 (45.7)	130 (58.0)	123 (54.7)	
Positive	317	102 (45.3)	105 (46.9)	110 (49.3)		121 (54.3)	94 (42.0)	102 (45.3)	
Unknown	2								
Tumor grade					.17				.06
I	46	17 (7.8)	12 (5.5)	17 (7.8)		17 (7.9)	14 (6.4)	15 (6.8)	
II	501	155 (71.1)	176 (80.0)	170 (78.0)		175 (81.0)	167 (76.6)	159 (71.6)	
III	109	46 (21.1)	32 (14.5)	31 (14.2)		24 (11.1)	37 (17.0)	48 (21.6)	
Unknown	18								
ER status					.002				.032
Negative	230	97 (43.1)	64 (28.7)	69 (30.8)		65 (29.0)	74 (33.0)	91 (40.6)	
Positive	442	128 (56.9)	159 (71.3)	155 (69.2)		159 (71.0)	150 (67.0)	133 (59.4)	
Unknown	2								
PR status					.006				.01
Negative	299	119 (53.6)	88 (39.6)	92 (41.8)		82 (37.4)	102 (45.7)	115 (51.8)	
Positive	365	103 (46.4)	134 (60.4)	128 (58.2)		137 (62.6)	121 (54.3)	107 (48.2)	
Unknown	10								
HER2 status					.025				.011
Negative	465	149 (66.5)	146 (64.9)	170 (75.9)		171 (76.3)	151 (67.4)	143 (63.6)	
Positive	208	75 (33.5)	79 (35.1)	54 (24.1)		53 (23.7)	73 (32.6)	82 (36.4)	
Unknown	1								
Triple-negative					.014				0.17
Yes	123	53 (23.6)	29 (12.9)	41 (18.3)		36 (16.1)	37 (16.4)	50 (22.2)	
No	551	172 (76.4)	196 (87.1)	183 (81.7)		188 (83.9)	188 (83.6)	175 (77.8)	
Surgery type					.14				.19
BCS	276	91 (40.4)	103 (45.8)	82 (36.6)		91 (40.6)	83 (36.9)	102 (45.3)	
Mastectomy	398	134 (59.6)	122 (54.2)	142 (63.4)		133 (59.4)	142 (63.1)	123 (54.7)	
Chemotherapy					.92				.35
Anthracycline- based	531	175 (77.8)	178 (79.1)	178 (79.5)		177 (79.0)	171 (76.0)	183 (81.3)	
Taxane	129	46 (20.4)	41 (18.2)	42 (18.8)		45 (20.1)	47 (20.9)	37 (16.4)	
Others	14	4 (1.8)	6 (2.7)	4 (1.8)		2 (0.9)	7 (3.1)	5 (2.2)	

BCS, breast-conserving surgery; ER, estrogen receptor; HER2, human epidermal growth factor receptor-2; PR, progesterone receptor.

grade ($P < .001$), ER status ($P < .001$), PR status ($P < .001$), and HER2 status ($P = .01$) (Table 2).

Patients were also divided into three treatment subgroups according to treatment regimen. No significant differences in BRCA1 and BRCA2 mRNA expression were found among the anthracycline-based treatment group, taxane-treated group, and other regimen groups ($P = .92$ and $P = .35$, respectively) (Table 1). pCR rates were also not significantly different in the three treatment groups (18.5% vs 22.5% vs 14.3%, $P = .52$) (Table 2).

Among the patients who received anthracycline with or without taxane-based neoadjuvant chemotherapy regimen (n = 531), patients with low BRCA1 mRNA expression had significantly higher pCR rate

TABLE 2 Pathological complete response rates by clinical characteristics and BRCA1 or BRCA2 mRNA expression level

Characteristic	N	Non-pCR N (%)	pCR N (%)	*P*-value
Age, years				.28
≤50	363	299 (82.4)	64 (17.6)	
>50	311	246 (79.1)	65 (20.9)	
Tumor size, cm				.003
≤2	224	167 (74.6)	57 (25.4)	
>2	450	378 (84.0)	72 (16.0)	
Lymph node status				<.001
Negative	355	262 (73.8)	93 (26.2)	
Positive	317	281 (88.6)	36 (11.4)	
Unknown	2			
Tumor grade				<.001
I	46	41 (89.1)	5 (10.9)	
II	501	416 (83.0)	85 (17.0)	
III	109	70 (64.2)	39 (35.8)	
Unknown	18			
ER status				<.001
Negative	230	149 (64.8)	81 (35.2)	
Positive	442	394 (89.1)	48 (10.9)	
PR status				<.001
Negative	299	210 (39.1)	89 (70.1)	
Positive	365	327 (60.9)	38 (29.9)	
HER2 status				.01
Negative	465	388 (83.4)	77 (16.6)	
Positive	208	156 (75.0)	52 (25.0)	
Chemotherapy type				.52
Anthracycline based	531	433 (81.5)	98 (18.5)	
Taxane	129	100 (77.5)	29 (22.5)	
Others	14	12 (85.7)	2 (14.3)	
BRCA1				.077
Low	225	172 (76.4)	53 (23.6)	
Intermediate	225	183 (81.3)	42 (18.7)	
High	224	190 (84.8)	34 (15.2)	
BRCA2				.82
Low	224	182 (81.3)	42 (18.7)	
Intermediate	225	184 (81.8)	41 (18.2)	
High	225	179 (79.6)	46 (20.4)	

BCS, breast-conserving surgery; ER, estrogen receptor; HER2, human epidermal growth factor receptor-2; pCR, pathological complete response; PR, progesterone receptor.

than intermediate or high BRCA1 mRNA level patients (24.6% vs 16.9% or 14.0%, respectively; $P = .031$) (Table 3). In multivariate analysis, low BRCA1 mRNA expression showed a borderline significance (OR = 1.54; 95% CI, 0.93-2.56; $P = .094$) for correlation with pCR to anthracycline-based chemotherapy after adjusting for age, tumor size, tumor grade, ER and PR status, and HER2 status

TABLE 3 pCR rate based on BRCA1 or BRCA2 mRNA expression status according to neoadjuvant chemotherapy regimens

Regimens	No. patients	Non-pCR No.	Non-pCR %	pCR No.	pCR %	*P*-value
Anthracycline-based (n = 531)						
BRCA1						.031
Low	175	132	75.4	43	24.6	
Intermediate	178	148	83.1	30	16.9	
High	178	153	86.0	25	14.0	
BRCA2						.60
Low	177	147	83.1	30	16.9	
Intermediate	171	141	82.5	30	17.5	
High	183	145	79.2	38	20.8	
Taxane (n = 129)						
BRCA1						.71
Low	46	37	80.4	9	19.6	
Intermediate	41	30	73.2	11	26.8	
High	42	33	78.6	9	21.4	
BRCA2						.82
Low	45	34	75.6	11	24.4	
Intermediate	47	36	76.6	11	23.4	
High	37	30	81.1	7	18.9	
Others (n = 14)						
BRCA1						.57
Low	4	3	75.0	1	25.0	
Intermediate	6	5	83.3	1	16.7	
High	4	4	100.0	0	0	
BRCA2						.18
Low	2	1	50.0	1	50.0	
Intermediate	7	7	100.0	0	0	
High	5	4	80.0	1	20.0	

pCR, pathological complete response.

(Table 4). However, BRCA2 mRNA expression level was not associated with pCR rate in patients with anthracycline-based neoadjuvant chemotherapy (16.9%, 17.5% and 20.8% in low, intermediate and high BRCA2 mRNA groups, respectively; $P = .60$) (Table 3).

Among the patients who received taxane-based neoadjuvant chemotherapy (n = 129), pCR rate was not significantly associated with BRCA1 mRNA (19.6%, 26.8% and 21.4% in low, intermediate and high BRCA1 groups, respectively; $P = .71$) or BRCA2 mRNA level (24.4%, 23.4% and 18.9%; $P = .82$) (Table 3).

3.3 | Survival estimates

Follow-up data were available for all patients; median follow-up time was 66 months (range 5-113 months). A total of 116 (17.2%) patients experienced a distant recurrence or died of breast cancer during the follow-up period. The estimated 5-year DRFS for the entire study population was 83.3% (95% CI, 80.4%-86.2%). The

TABLE 4 Multivariate analysis of pathological complete response in anthracycline-based group

	Pathological complete response		
Variable	OR	95% CI	*P*-value
Age			
≤50 y vs >50 y	1.19	0.71-2.01	.50
ER status			
Negative vs Positive	3.01	1.43-6.33	.004
PR status			
Positive vs Negative	1.50	0.70-3.21	.30
HER2 status			
Negative vs Positive	1.43	0.85-2.42	.18
Tumor size			
≤2 cm vs >2 cm	2.28	1.36-3.82	.002
Tumor grade			
III vs I/II	2.41	1.32-4.40	.004
Lymph node status			
Negative vs Positive	4.93	2.47-9.84	<.001
BRCA1			
Low vs Intermediate/High	1.54	0.93-2.56	.094

CI, confidence interval; ER, estrogen receptor; HER2, human epidermal growth factor receptor-2; OR, odds ratio; PR, progesterone receptor.

estimated 5-year DRFS rates for anthracycline-based and taxane-based treated groups were 82.3% (78.9%-85.6%) and 87.4% (81.3%-93.5%), respectively. The sample size of patients who received other regimens was relatively small, so the survival curve was not analyzed in this subgroup. Patients who achieved a pCR had a significantly better 5-year DRFS rate than patients who did not, in the entire patient (92.8% vs 81.1%, $P = .001$) (Figure 1A) or in anthracycline-based groups (91.7% vs 80.2%, $P = .006$) (Figure 1B). Among taxane-based treated groups, patients who achieved pCR also had a better 5-year DRFS rate than patients who did not, but the difference did not reach statistical significance (96.4% vs 86.6%, $P = .12$) (Figure 1C).

Because BRCA1 mRNA level was associated with pCR in the 531 patients who received anthracycline-based neoadjuvant chemotherapy, we next analyzed the association between pCR and DRFS according to the BRCA1 mRNA level in this subgroup. There was no significant difference in 5-year DRFS between BRCA1 low and intermediate or high mRNA level (84.7% vs 81.5%, $P = .97$) (Figure 2A). Patients who achieved pCR had better 5-year DRFS compared with those who did not achieve pCR, in both BRCA1 low and intermediate or high mRNA groups ($P = .044$, Figure 2B).

4 | DISCUSSION

In the present study, we investigated the association between BRCA1 and BRCA2 mRNA levels and response to neoadjuvant chemotherapy in 674 Chinese breast cancer patients without BRCA1/2 germline mutations. We found that patients with low expression of BRCA1 mRNA were more likely to respond to anthracycline-based neoadjuvant chemotherapy than patients with intermediate or high BRCA1 mRNA levels.

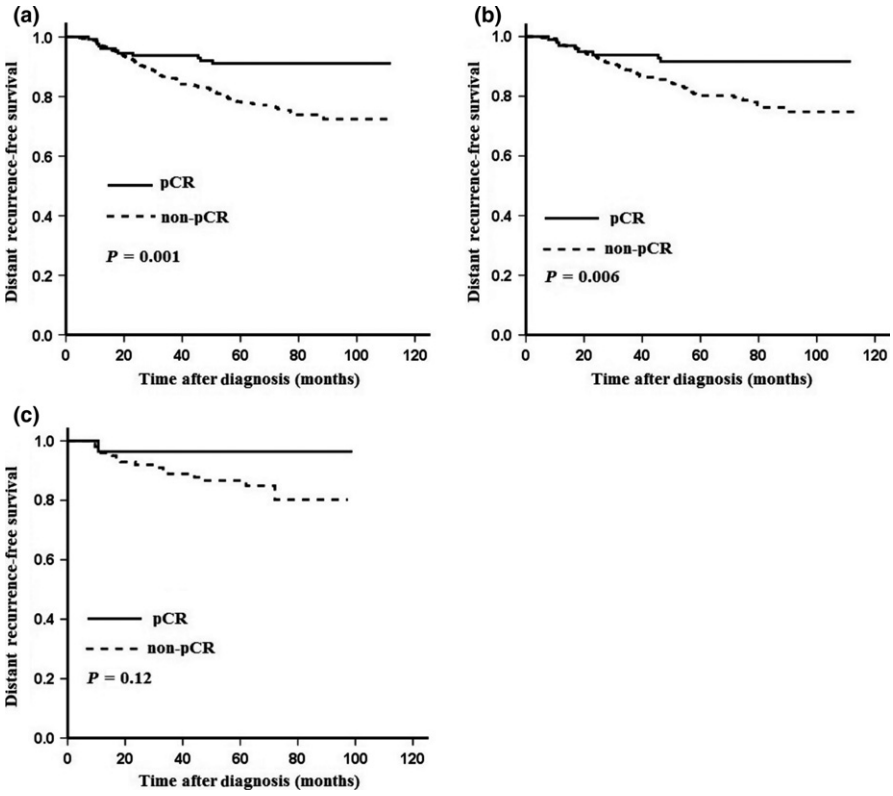

FIGURE 1 Kaplan-Meier estimates of distant recurrence-free survival by pathological complete response (pCR) status in (A) the entire 674 breast cancer patients who received neoadjuvant chemotherapy, (B) the 531 patients who received anthracycline-based neoadjuvant chemotherapy, and (C) the 129 patients who received taxane-based neoadjuvant chemotherapy

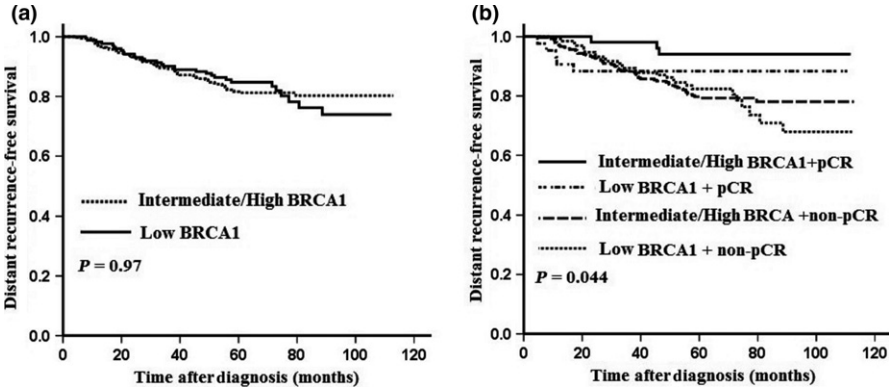

FIGURE 2 Kaplan-Meier estimates of distant recurrence-free survival (DRFS) by BRCA1 mRNA expression level and pathological complete response (pCR) status in 531 breast cancer patients who received anthracycline-based neoadjuvant chemotherapy. (A) DRFS by BRCA1 mRNA expression level. (B) DRFS by BRCA1 mRNA expression level and pCR status

The total 674 breast cancer patients included in this study received different chemotherapy regimens. Among the 531 patients treated with an anthracyline-based regimen, patients with low BRCA1 mRNA level had a significantly higher pCR rate than patients with intermediate or high BRCA1 mRNA level. However, among the 129 patients that received a taxane-based regimen, pCR rate was not different in BRCA1 low, intermediate, and high mRNA level subgroups ($P = .71$). BRCA2 mRNA level was not associated with pCR rate in either the anthracyline-based treated subgroup ($P = .60$) or the taxane-based regimen subgroup ($P = .82$).

Previous studies reported the association between BRCA1 and BRCA2 expression and response to chemotherapy; however, the results shown were less consistent.[26-28] Some clinical data suggested that tumors with low BRCA1 or BRCA2 mRNA expression responded better to DNA-damaging chemotherapy,[27-29] whereas some showed no relationship between BRCA1 or BRCA2 expression and chemotherapy sensitivity.[28,30] However, the sample size of previous studies was relatively small. Moreover, the previous studies did not take the BRCA1/2 germline mutations into consideration when investigating the relationship between mRNA expression level of BRCA1/2 and response to neoadjuvant chemotherapy, as BRCA1 germline mutations may affect the response to some chemotherapy drugs.[31] One advantage of the current study was that the germline mutations of *BRCA1* and *BRCA2* genes in this cohort had been analyzed through sequencing assay, and BRCA1/2 gene germline mutations status was clearly shown in our previous reports.[24,25] To the best of our knowledge, this is the first study to report that BRCA1 and BRCA2 mRNA expression level was associated with response to neoadjuvant chemotherapy in a relatively large sample size, excluding the effects of *BRCA1* and *BRCA2* gene mutations.

BRCA1 gene plays an important role in DNA damage repair, regulation of gene expression and cell cycle control. Tumors with low expression of BRCA1 mRNA are certainly different from typical tumors with BRCA1 germline mutation. However, there are some similarities between the morphology and molecular biology of breast cancer with low expression BRCA1 and BRCA1 mutated breast cancer that have led to the concept of "BRCAness". Both BRCA1 mutated breast cancers and reduced BRCA1 expression tend to be higher grade, hormone receptor-negative, and HER2-negative, or "triple negative" and frequently express a basal phenotype.[13,32-34] In vitro and in vivo studies have shown that cells deficient for BRCA1 could be more sensitive to chemotherapy agents that produce DNA damage through double-strand DNA breaks, such as anthracyclines and etoposide.[14,15,17,19] These preclinical observations are consistent with our results. Our findings indicate that patients with low BRCA1 mRNA level were more likely to respond to anthracycline-based neoadjuvant chemotherapy and gained more benefit from anthracycline-based therapy compared with patients with intermediate or high BRCA1 mRNA level, but this was not evident for BRCA2 mRNA expression. This may be because the relative roles of BRCA1 and BRCA2 in DNA repair of DNA double-strand breaks were not exactly the same. BRCA1 is a critical organizing molecule that has been linked to a range of cellular processes beyond DNA repair, such as transcriptional regulation and chromatin remodeling. Tumor cells with deficient BRCA1 may respond better to DNA-damaging chemotherapy. BRCA2 function in homologous recombination is primarily through regulation of RAD51 activity.[1,17,35]

The present study had several limitations. Neoadjuvant chemotherapy regimens were not assigned randomly, and a small number of patients received taxane-based neoadjuvant chemotherapy.

In summary, our study suggests that in the absence of BRCA1/2 germline mutations, breast cancer patients with low BRCA1 mRNA expression have a higher pCR rate to anthracycline-based neoadjuvant chemotherapy, but not to taxane regimens. Although BRCA2 plays an important role in DNA repair, BRCA2 mRNA expression may not predict response to anthracycline-based or taxane neoadjuvant chemotherapy. Nevertheless, future studies are warranted to confirm our current findings.

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CONFLICT OF INTEREST

Authors declare no conflicts of interest for this article.

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