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PARP-1 expression in breast cancer including *BRCAl*-associated, triple negative and basal-like tumors: Possible implications for PARP-1 inhibitor therapy

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Abstract

Purpose. Despite ongoing trials of PARP inhibitors in the treatment of breast cancer (BC), the extent of PARP-1 protein expression in BCs, which may influence treatment results, is not known. The purpose of this report is to assess expression of PARP-1 in BC including *BRCA1*-associated, triple negative (TN) and basal-like tumors. **Methods.** Immunohistochemistry with a PARP-1 antibody on tissue microarrays from 130 *BRCA1*-associated and 594 *BRCA1*-non-related BCs was used. **Results.** The vast majority of breast carcinomas expressed high level of nuclear PARP-1 protein and a small percentage of tumors exhibited both nuclear and cytoplasmic PARP-1 expression. There was a significant difference between the mean nuclear PARP-1 quickscore in *BRCA1*-associated versus *BRCA1*-non-associated carcinomas in all tumors ($p < 0.0001$), in the basal-like group ($p = 0.0086$), TN ($p = 0.0015$) and non-basal-like groups ($p = 0.016$) but not in the non-TN group. Among *BRCA1*-associated BCs, low PARP-1 expression was found in 18.5% of all cases, 18.9% of basal-like and 21% of TN cancers. Among *BRCA1*-non-related tumors, low PARP-1 expression was found in 8.8% of all cases, 3.1% of basal-like, and 2.7% of TN cancers. **Conclusions.** PARP-1 expression is significantly associated with *BRCA1* status in basal-like and TN BCs. ~~Due to low PARP-1 expression, roughly 1 in 5 patients with *BRCA1*-associated cancers (and TN *BRCA1*-associated tumors) treated with PARP-1 inhibitors may either not benefit from this type of therapy or may benefit very little.~~ The assessment of PARP-1 expression in tumor samples may improve the selection of BC patients for PARP inhibitor therapy.

Keywords: Breast cancer, PARP-1, BRCA1, Basal-like, Triple negative

Introduction

Poly(ADP-ribose)polymerase-1 (PARP-1) is a nuclear enzyme that participates in the repair of DNA single-strand breaks via the base excision repair pathway [1]. In *BRCA*-associated breast cancers this repair pathway can be made nonfunctional by the use of PARP inhibitors. Because unrepaired single-strand breaks are converted into double-strand breaks and *BRCA*-associated tumors cannot repair them by homologous recombination, the result is tumor cell death. Initial studies showing that PARP-1 inhibition results in synthetic lethality in *BRCA1* and *BRCA2*-deficient cell lines [2, 3] and the preclinical studies that followed [4-6] paved the way for clinical studies. There are now several PARP inhibitors undergoing clinical trials in *BRCA*-associated and triple negative (TN) breast cancers [7]. However, it is not known which patients may be best suited for PARP inhibitor therapy. Clinical studies with PARP inhibitors suggest that the extent of PARP enzyme inhibition may be important because the results are dose-dependent [8]. Nuclear expression of PARP-1 has been reported in colorectal carcinomas [9] and melanomas [10] however, the extent of PARP protein expression in clinical specimens of breast cancer is not known. The purpose of this report is to assess the expression of PARP-1 protein in breast cancer including *BRCA1*-associated, TN and basal-like tumors.

Materials and Methods

Patients

This study included two groups of women: 140 with *BRCA1*-associated and 625 with *BRCA1*-non-related invasive breast cancers. The first group consisted of 140 consecutive patients with *BRCA1* mutations (mean age 46.2) diagnosed in Szczecin (from 1996 to 2008) and Olsztyn (from 1998 to 2007). The second group consisted of 625 consecutive *BRCA1*-non-related patients (mean age 58.5) diagnosed at the West Pomeranian Oncological Center in Szczecin from 2006 to 2008. Patients did not receive endocrine therapy, chemotherapy, or radiotherapy before surgery. The study was approved by the Ethics Committee of the Pomeranian Medical University in Szczecin, Poland.

Genotyping

In 1996, a national program began in Poland to evaluate pathologic and clinical characteristics of hereditary breast cancer. Patients were invited to participate during hospital stays or through a mailed invitations. During the interview, the goals of the study were explained, informed consent was obtained, genetic counseling was given and a blood sample was taken for DNA analysis.

BRCA1 genetic testing was conducted at the Department of Genetics and Pathology, Pomeranian Medical University, Szczecin. Genomic DNA was prepared from 5-10 ml of peripheral blood. Mutation analysis for the common Polish mutations was performed as described previously [11]. There are three common founder mutations in *BRCA1* in Poland. The 4153delA and 5382insC mutations were detected using a multiplex-specific polymerase chain reaction (PCR) assay. The third mutation (C61G) generates a novel restriction enzyme site in exon 5. This mutation is detected after digesting amplified DNA with *AvaII*. To visualize the different *BRCA1* alleles, the PCR products were

subjected to electrophoresis in a 1.5% agarose gel and, stained with ethidium bromide. To avoid false results in all reactions, positive and negative controls (without DNA) were used. DNA testing results indicating the occurrence of mutations were confirmed by sequencing of material from a second blood sample obtained on a different day.

Tumor Pathology

Pathology review was conducted at the Department of Pathology, Pomeranian Medical University in Szczecin by two pathologists (PD, TH) associated with the study. In cases where there was disagreement, consensus was reached by consultation with a third reviewer (WD). Representative histological slides organized according to an assigned random number were evaluated to confirm the diagnosis of breast cancer type and classified according to the Elston-Ellis histological grade [12]. The Nottingham Prognostic Index was also established [13]. After review, 135 *BRCA1*-associated and 598 *BRCA1*-non-related cases had sufficient tumor available in the blocks for arraying.

Tissue microarray construction

Two different regions of tumors in the area of the outer invasive margin of cancer were identified and marked on hematoxylin and eosin-stained sections. Sections were matched to their corresponding wax blocks (the donor blocks), and two 0.6 mm diameter cores of the tumor were removed from these donor blocks and inserted into the recipient master block using a Tissue Microarrayer (Beecher Instruments, Silver Spring, MD). The recipient block was cut and sections were transferred to coated slides.

Immunohistochemistry and fluorescent in situ hybridization

Slides were deparaffinized, rehydrated and immersed in pH 6.0 buffer (PARP-1) or pH 9.0 buffer (ER, PR, CK5/6). Heat-induced antigen retrieval was performed in a water bath at 98°C for 20 min (PARP-1), or a pressure cooker at 120°C for 3 min (ER, PR, CK 5/6). The following monoclonal antibodies were used: anti-PARP-1 (F-2, dilution 1:500; Santa Cruz Biotechnology, Santa Cruz, CA), anti-Estrogen Receptor (clone 1D5, dilution 1:50; Dako, Glostrup, Denmark), anti-Progesterone Receptor (clone PgR 636, dilution 1:50; Dako), anti-CK5/6 (clone D5/16 B4, dilution 1:50; Dako). Slides were incubated with the primary antibodies for 30 min and immunostained using the Dako EnVision™+ kit according to the manufacturer's instructions. The reaction was developed with a diaminobenzidine substrate-chromogen solution, and slides were counterstained with hematoxylin. EGFR staining was performed using the EGFR pharmDx kit (Dako) with incubation with proteinase K for 5 min for enzymatic antigen retrieval. Expression of HER-2 was tested using the HercepTest kit (Dako) according to the manufacturer's instructions. Appropriate positive and negative controls were included. Cases with HER-2 staining of 2+ were further evaluated by fluorescent in situ hybridization for HER-2 gene amplification. In this assay, slides were hybridized with probes to LSI HER-2/neu and CEP17 with the PathVysion HER-2 DNA Probe Kit (Abbott Laboratories, Abbott Park, IL) according to the manufacturer's instructions.

Immunohistochemistry scoring

Tumor cores were independently assessed by two observers (PD and WD) who were blinded to mutation status and clinico-pathological data. In cases of disagreement, the result was reached by consensus. Tumors with lost cores, or insufficient tumor in the cores, were excluded from the analysis, leaving 130 *BRCA1*-associated and 594 *BRCA1*-non-related cases. Various scoring systems have been used in the literature to assess the immunohistochemical expression of proteins. Of the three most frequently applied (intensity score, pattern score or both combined), we used the multiplicative quickscore method (QS), because it seems to be the most reliable and proved to be useful and reproducible [14]. This system accounts for both the intensity and the extent of cell staining. Briefly, the proportion of positive cells was estimated and given a score on a scale from 1 to 6 (1=1% to 4%; 2=5% to 19%; 3=20% to 39%; 4=40% to 59%; 5=60% to 79%; and 6=80% to 100%). The average intensity of the positively staining cells was given a score from 0 to 3 (0=no staining; 1=weak, 2=intermediate, and 3=strong staining). A QS was then calculated by multiplying the percentage score by the intensity score, to yield a minimum value of 0 and a maximum value of 18. Based on the QS, nuclear PARP-1 expression was graded as low (0-9) or high (10-18). For cytoplasmic PARP-1 expression unequivocal staining in $\geq 1\%$ cells was graded as positive.

Tumors were considered as HER-2 positive if scored as 3+ according to standard criteria [15]. Fluorescent in situ hybridization with amplification ratio >2.2 was used to segregate cases with 2+ results. ER and PR were considered positive if staining was detected in $\geq 1\%$ nuclei [16]. EGFR and CK5/6 stains were considered positive if membranous or cytoplasmic/membranous immunostaining, respectively, was detected in $\geq 1\%$ tumor cells.

Immunophenotypic classification of breast cancer

ER-negative, PR-negative, HER-2-negative, CK5/6 and/or EGFR-positive tumors were diagnosed as basal-like carcinomas [17, 18]. Tumors that were negative for ER, PR and HER-2 were considered as triple negative cancers.

Statistics

Because the distribution of the PARP-1 quickscore was significantly different from the normal distribution (Shapiro-Wilk test), nonparametric tests were used for the analysis. Analysis of differences in distributions of PARP-1 expression between the two groups of patients was performed using the Mann-Whitney U test and between more than two groups were evaluated by the Kruskal-Wallis test. Fisher's exact test or Chi-square test when appropriate was used when PARP-1 expression was categorized as "low" or "high" according to the aforementioned criteria. All reported p values were two sided. For all statistical analyses, a p value <0.05 was considered as significant. Statistical analyses were performed using Graphpad Prism 5.0 software.

Results

Immunohistochemical staining with the PARP-1 antibody revealed a strong nuclear reaction in tumor cells (Fig. 1a,b,d) in the majority of breast carcinomas however, there were also PARP-1 negative

cases (Fig. 1a,c,e) or tumors with low PARP-1 expression. In a small number of cancers (18/130 = 13.8% of *BRCA1*-associated and 37/594 = 6.2% of *BRCA1*-non-associated, $p=0.006$) both cytoplasmic and nuclear expression was seen. We noticed that many tumor cells in mitosis exhibited cytoplasmic PARP-1 expression. The distribution of nuclear and cytoplasmic PARP-1 quickscores among 130 *BRCA1*-associated and 594 *BRCA1*-non related breast carcinomas is shown in Fig. 2.

Nuclear PARP-1 expression

BRCA1-associated breast carcinomas. High PARP-1 immunoreactivity (QS=10-18) was found in 81.5% (106/130) of all cases, in 81% (73/90) of basal-like cancers and in 79% (83/105) of triple negative tumors. Conversely 18.5% (24/130) of all tumors, 18.9% (17/90) of basal-like cancers and 21% (22/105) of triple negative tumors showed low PARP-1 expression (QS=0-9) (Table 1). Nine (6.9%) *BRCA1*-associated tumors, 7 (7.7%) basal-like cancers and 8 (7.6%) TN carcinomas were PARP-1 negative (QS=0). Within the group of *BRCA1*-associated cancers there were no statistically significant associations between mean PARP-1 scores in basal-like or TN categories *versus* non-basal-like and non-TN. However, the mean PARP-1 score was higher in ductal *versus* non-ductal cancers ($p=0.017$) (Table 2).

BRCA1-non-related breast carcinomas. High PARP-1 immunoreactivity was observed in 91.2% (542/594) of all tumors, in 96.9% (63/65) of basal-like cancers and 97.3% (72/74) of TN breast carcinomas. Low PARP-1 expression was found in 8.8% (52/594) of all tumors, in 3.1% (2/65) of basal-like carcinomas, and in 2.7% (2/74) of TN breast cancers (Table 1). Eighteen tumors (3%) in this group were PARP-1 negative, and almost all of them belonged to non-basal-like and non-TN cancers. Only one basal-like and one TN carcinoma were PARP-1 negative among 594 *BRCA1*-non-related cancers. Within the *BRCA1*-non-related group, TN cancers were more likely to have high PARP-1 expression than were non-TN tumors although the difference was almost statistically insignificant ($p=0.048$) (Table 2).

Comparison of nuclear PARP-1 expression between BRCA1-associated and BRCA1-non-related tumors.

There was a statistically significant difference between the mean nuclear PARP-1 score in *BRCA1*-associated tumors *versus* *BRCA1*-non-related carcinomas in all tumors ($p<0.0001$), in the basal-like group of cancers ($p=0.0086$) and in the TN ($p=0.0015$) and non-basal-like groups ($p=0.016$), but not in the non-TN group ($p=0.48$) (Table 1). *BRCA1*-associated tumors were more likely to exhibit low PARP-1 expression than were *BRCA1*-non-related carcinomas ($p=0.0023$). *BRCA1*-associated basal-like carcinomas and TN cancers were also more likely to exhibit low PARP-1 expression than were *BRCA1*-non-related basal-like and TN cancers ($p=0.0026$, and $p=0.0003$ respectively) (Table 1).

Similar percentages of basal-like (18.9%) and TN (21%) tumors showed low PARP-1 expression within the *BRCA1*-associated group of cancers. Within the *BRCA1*-non-related category, this percentage was much lower but also similar between basal-like (3.1%) and TN (2.7%) carcinomas (Table 2).

Cytoplasmic PARP-1 expression

There was a statistically significant difference between the mean cytoplasmic PARP-1 score in *BRCA1*-associated tumors versus *BRCA1*-non-related carcinomas in all tumors (0.90 ± 2.86 vs. 0.37 ± 1.93 , $p=0.003$), as well as in non-basal-like category (0.68 ± 1.87 vs. 0.32 ± 1.86 , $p=0.008$) but not in the TN, non-TN and basal-like group of cancers. *BRCA1*-associated cancers more frequently expressed PARP-1 in the cytoplasm than did *BRCA1*-non-related tumors (18/130 vs. 37/594, $p=0.006$). In the non-basal-like group, cytoplasmic expression of PARP-1 was about three times more frequent in *BRCA1*-associated than in *BRCA1*-non-related tumors (6/40 vs. 26/529, $p=0.019$).

Within the *BRCA1*-non-related group, the mean PARP-1 score was higher in basal-like, TN, grade III and high NPI cancers as compared to non-basal-like, non-TN, grade I-II and low NPI tumors, respectively ($p=0.0002$, $p=0.0002$, $p<0.0001$, and $p=0.039$, respectively) (Table 3). Cytoplasmic expression of PARP-1 was more frequent in basal-like, TN, grade III and high NPI cancers as compared to non-basal-like, non-TN, grade I-II and low NPI tumors, respectively ($p=0.001$, $p=0.0008$, $p<0.0001$, and $p=0.035$, respectively). Within the *BRCA1*-associated group of tumors, statistical analysis could not be performed due to the small numbers in the subgroups.

Discussion

The introduction of PARP inhibitors has become an exciting breakthrough in treatment of *BRCA*-related breast cancer. However, although there have been numerous ongoing clinical trials on PARP-1 inhibitors for the treatment of breast cancer [7] there are no published reports on the amount of PARP-1 protein expression in clinical specimens of breast cancer. PARP-1 was found to be less abundant in MCF-7 cells that are resistant to mitoxantrone and etoposide than in drug-susceptible MCF-7 cells [19], indicating that the level of PARP-1 protein expression may have therapeutic relevance. A dose dependent clinical response to PARP inhibitor therapy [8] also suggests that it may be worthwhile to consider the amount of PARP expression in tumor cells.

Here we show that: (1) The majority of breast cancers exhibit high expression of nuclear PARP-1 protein either in *BRCA1*-associated or *BRCA1*-non-related groups. (2) The level of PARP-1 nuclear expression is lower in the *BRCA1*-associated group as compared to the *BRCA1*-non-related group. (3) PARP-1 nuclear expression is significantly associated with *BRCA1* status in basal-like and TN breast carcinomas. (4) Cytoplasmic PARP-1 expression, although rare, is about twice as frequent in *BRCA1*-associated as compared to *BRCA1*-non-related cancers. There is a significant association of cytoplasmic PARP-1 expression with grade III and high NPI cancers, suggesting the prognostic significance of this parameter. (5) Approximately 7% of *BRCA1*-associated cancers (but only 3% of *BRCA1*-non-related tumors) do not exhibit expression of PARP-1 protein at all, and altogether, 18.5% of *BRCA1*-associated cancers (and 21% of TN *BRCA1*-associated tumors) exhibit low PARP-1 nuclear expression. Because it is possible that *BRCA1*-associated cancers with null or low PARP expression may be refractory to treatment with PARP inhibitors it is vital to identify such tumors because

different therapeutic strategies may then be applied to such patients e.g. with inhibitors of Tankyrase-1 [20]. Bieche et. al. have shown that levels of PARP mRNA in 35 primary breast cancer tumor samples vary from low in 15 tumors to high in 10 [21]. Although in such studies a mixture of mRNA from tumor cells and stromal benign cells (including lymphocytes) was assessed altogether, their results support our findings that breast cancers with low PARP expression do exist. ~~Perhaps different maximum tolerated dose may have to be required for patients with high and low PARP-1 expression.~~

It has been known that not all *BRCA1* or *BRCA2* mutation carriers respond to the inhibition of PARP [8]. Various explanations have been proposed, e.g. different sensitization to PARP inhibition depending on the type of mutations or preexisting genetic resistance due to secondary *BRCA2* mutations which may restore *BRCA* function. The results of our study suggest that low PARP protein expression may be another reason.

Knowledge of the level of PARP-1 protein expression may be useful not only for adequate treatment of *BRCA1*-associated breast cancers but also for those without constitutional *BRCA1* mutations. *BRCA1* dysfunction has been reported in basal-like “sporadic” cancers, which suggests that they may be sensitive to PARP inhibitors [22]. Certain (phenanthridine-derived) PARP inhibitors arrest proliferation and cause death of MCF-7 and MDA231 human breast cancer cell lines lacking mutations that impair DNA repair (without adverse effects on normal cells), and therefore, it seems that *BRCA1*-non-related tumors may also constitute a potential target for some PARP inhibitors [23].

In conclusion, we showed here for the first time that the vast majority of breast carcinomas expressed high level of nuclear PARP-1 protein but a small percentage of tumors exhibited both nuclear and cytoplasmic PARP-1 expression. Nuclear PARP-1 expression was significantly associated with *BRCA1* status in basal-like and TN breast carcinomas. Our findings suggest that due to low PARP-1 expression, a subset of patients with *BRCA1*-associated cancers (and TN *BRCA1*-associated tumors) treated with PARP-1 inhibitors may either not benefit from this type of therapy or may benefit very little ~~however, further research on the association of PARP-1 expression with clinical outcome of patients with *BRCA1*-associated, triple-negative and basal-like breast carcinomas is needed for its application in PARP inhibitor therapy strategy. Therefore, the assessment of PARP-1 expression in tumor samples by immunohistochemistry may improve the selection of breast cancer patients, including those with *BRCA1* associated and TN tumors, for PARP inhibitor therapy. This strategy could also apply to basal-like “sporadic” cancers.~~ Because a sizable percentage of *BRCA1*-associated cancers with low PARP expression is likely to influence the results of clinical trials of such therapy, especially in small groups of patients, we suggest that future clinical trials involving PARP inhibitors should take into account not only constitutional genetic background but also PARP protein expression in tumor cells.

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Figure Legends

Fig. 1 PARP-1 expression in breast carcinomas (immunohistochemical reaction with PARP-1 monoclonal antibody). **a** Fragment of a tissue microarray with four neighboring cores. Two cores from one cancer with strong nuclear PARP-1 expression and two cores from another tumor negative for PARP-1 (original magnification: x40). **b and c** Higher magnification of respective cores from Fig. 1a. **d and e** (original magnification: x100). High magnification of boxed area from Fig. 1b (strong nuclear PARP-1 expression) and Fig. 1c (PARP-1 negative tumor cells), respectively (original magnification: x400).

Fig. 2 Distribution of quickscores (QS) of nuclear and cytoplasmic PARP-1 expression in *BRCA1*-associated (a) and *BRCA1*-non-related carcinomas (b).

Table 1 Association between nuclear PARP-1 expression in *BRCA1*-associated and *BRCA1*-non-related tumors and immunophenotypic breast cancer types

	No. of cancers with PARP-1 expression				P	Mean QS ^a ± SD		
	<i>BRCA1</i> -associated		<i>BRCA1</i> -non-related			<i>BRCA1</i> -associated	<i>BRCA1</i> -non-related	P
	Low ^b	High ^c	Low ^b	High ^c				
All tumors	24/130	106/130	52/594	542/594	0.0023	14.62 ± 5.88	16.27 ± 4.40	< 0.0001
Basal-like	17/90	73/90	2/65	63/65	0.0026	14.61 ± 5.85	16.85 ± 3.41	0.0086
Triple negative	22/105	83/105	2/74	72/74	0.0003	14.24 ± 6.15	16.91 ± 3.27	0.0015
Non-basal-like	7/40	33/40	50/529	479/529	0.11	14.65 ± 6.02	16.20 ± 4.51	0.016
Non-triple negative	2/25	23/25	50/520	470/520	1.00	16.20 ± 4.33	16.18 ± 4.54	0.48

^a Quickscore; ^b Quickscore 0–9; ^c Quickscore 10–18

Table 2 Association between nuclear PARP-1 expression in *BRCA1*-associated and *BRCA1*-non-related tumors and immunophenotypic types and pathological factors of breast carcinomas

	PARP-1 expression					
	n	Low ^a n (%)	High ^b n (%)	p	Mean QS ^c ± SD	p
<i>BRCA1</i>-associated						
Basal-like	90	17 (18.9)	73 (81.1)		14.61 ± 5.85	
Non-basal-like	40	7 (17.5)	33 (82.5)	1.00	14.65 ± 6.02	0.96
Triple negative	105	22 (21)	83 (79)		14.25 ± 6.15	
Non-triple negative	25	2 (8)	23 (92)	0.16	16.20 ± 4.33	0.24
Histological type						
Ductal	78	13 (16.7)	65 (83.3)		15.44 ± 5.20	
Non-ductal	52	11 (21.2)	41 (78.8)	0.65	13.40 ± 6.64	0.017
Grade						
I-II	9	2 (22.2)	7 (77.8)		13.89 ± 6.11	
III	121	22 (18.2)	99 (81.8)	0.67	14.68 ± 5.89	0.25
Nodal status						
N0	84	18 (21.4)	66 (78.6)		14.15 ± 6.11	
N1	42	6 (14.3)	36 (85.7)	0.47	15.52 ± 5.56	0.13
Tumor size (cm)						
≤ 2	55	12 (21.8)	43 (78.2)		13.98 ± 6.55	
> 2	66	11 (16.7)	55 (83.3)	0.49	14.97 ± 5.40	0.48
NPI ^d						
≤ 3.4	3	1 (33.3)	2 (66.7)		14.67 ± 5.77	
3.4-5.4	88	19 (21.6)	69 (78.4)		14.00 ± 6.29	
> 5.4	25	3 (12)	22 (88)	n.d. ^e	16.24 ± 5.01	0.11
<i>BRCA1</i>-non-related						
Basal-like	65	2 (3.1)	63 (96.9)		16.85 ± 3.42	
Non-basal-like	529	50 (9.5)	479 (90.5)	0.10	16.20 ± 4.51	0.47
Triple negative	74	2 (2.7)	72 (97.3)		16.91 ± 3.27	
Non-triple negative	520	50 (9.6)	470 (90.4)	0.048	16.18 ± 4.54	0.38
Histological type						
Ductal	431	36 (8.4)	395 (91.6)		16.41 ± 4.21	
Non-ductal	163	16 (9.8)	147 (90.2)	0.67	15.92 ± 4.87	0.20
Grade						
I-II	379	34 (9)	345 (91)		16.27 ± 4.38	
III	215	18 (8.4)	197 (91.6)	0.88	16.28 ± 4.45	0.91
Nodal status						
N0	372	39 (10.5)	333 (89.5)		16.00 ± 4.79	
N1	203	12 (5.9)	191 (94.1)	0.07	16.70 ± 3.71	0.13
Tumor size (cm)						
≤ 20	381	31 (8.1)	350 (91.9)		16.44 ± 4.22	
> 20	206	20 (9.7)	186 (90.3)	0.54	16.00 ± 4.70	0.21
NPI ^d						
≤ 3.4	214	24 (11.2)	190 (88.8)		15.94 ± 4.84	
3.4-5.4	269	20 (7.4)	249 (92.6)		16.46 ± 4.14	
> 5.4	85	6 (7.1)	79 (92.9)	0.29	16.42 ± 4.32	0.51

^a Quickscore 0–9; ^b Quickscore 10–18; ^c Quickscore; ^d Nottingham Prognostic Index; ^e Not determined due to small number of cases in NPI ≤ 3.4 group

Table 3 Association between cytoplasmic PARP-1 expression in *BRCA1*-non-related tumors and immunophenotypic types and pathological factors of breast carcinomas

	PARP-1 expression				Mean QS ^c ± SD	p
	n	Negative ^a n (%)	Positive ^b n (%)	p		
Basal-like	65	54 (83.1)	11 (16.9)		0.80 ± 2.41	
Non-basal-like	529	503 (95.1)	26 (4.9)	0.001	0.32 ± 1.86	0.0002
Triple negative	74	62 (83.8)	12 (16.2)		0.91 ± 2.81	
Non-triple negative	520	495 (95.2)	25 (4.8)	0.0008	0.30 ± 1.77	0.0002
Histological type						
Ductal	431	405 (94)	26 (6)		0.24 ± 1.27	
Non-ductal	163	152 (93.3)	11 (6.7)	0.71	0.72 ± 3.04	0.65
Grade						
I-II	379	368 (97.1)	11 (2.9)		0.25 ± 1.70	
III	215	189 (87.9)	26 (12.1)	0.0001	0.59 ± 2.28	< 0.0001
Nodal status						
N0	372	347 (93.3)	25 (6.7)		0.40 ± 1.94	
N1	203	192 (94.6)	11 (5.4)	0.59	0.35 ± 2.01	0.54
Tumor size (cm)						
≤ 20	381	361 (94.8)	20 (5.2)		0.33 ± 1.81	
> 20	206	190 (92.2)	16 (7.8)	0.28	0.45 ± 2.16	0.23
NPI ^d						
≤ 3.4	214	208 (97.2)	6 (2.8)		0.24 ± 1.54	
3.4-5.4	269	247 (91.8)	22 (8.2)		0.45 ± 2.13	
> 5.4	85	78 (91.8)	7 (8.2)	0.035	0.52 ± 2.36	0.039

^aQuickscore 0; ^bQuickscore 1–18; ^cQuickscore; ^dNottingham Prognostic Index



