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Original contribution

Evaluation of the BRCAness phenotype and its correlations with clinicopathological features in triple-negative breast cancers☆,☆☆

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Summary Some sporadic triple-negative breast cancers (TNBCs) share similar clinicopathological and molecular characteristics to *BRCA1/2*-mutant breast cancers, a phenotype described as "BRCAness." Identifying BRCAness in TNBCs could expand the target group for platinum salts and poly(adenosine diphosphate-ribose) polymerase inhibitors. The aims of our study were to assess the clinical validity of *BRCA1/2* promoter methylation and BRCA1-like genomic profile to identify BRCAness and to evaluate its correlations with clinicopathological features in TNBCs. Formalin-fixed, paraffin-embedded tissues and fresh tissues of 151 primary invasive TNBCs were collected. *BRCA1/2* promoter methylation and BRCA1-like genomic profile were detected using methylation-specific multiplex ligation-dependent probe amplification and multiplex ligation-dependent probe amplification assay, respectively. BRCA1/2 messenger RNA expression was evaluated by quantitative reverse-transcription polymerase chain reaction. Of the 151 patients, 38 (25.2%) showed *BRCA1* promoter methylation. Of the 124, 52 (41.9%) had a BRCA1-like multiplex ligation-dependent probe amplification profile. The frequency of BRCAness phenotype was 54.8% (68/124). *BRCA1* germline mutation and *BRCA1* promoter methylation were mutually exclusive ($P = .002$). The BRCAness phenotype was significantly associated with large tumor size (>2 cm, $P = .009$), positive lymph nodes ($P = .008$), grade 3 tumor ($P = .0001$), high Ki-67 levels ($P = .001$), and basal-like breast cancers ($P = .0001$). Combined detections of *BRCA1* promoter methylation and BRCA1-like genomic profile could identify more BRCAness cases in TNBCs. *BRCA1* promoter methylation might rule out *BRCA1* germline mutation in TNBCs. BRCAness was associated with aggressive clinicopathological features. These findings might have clinical values in hereditary breast cancer screening and target treatment of TNBCs.

1. Introduction

Triple-negative breast cancer (TNBC), which accounts for 15% to 20% of all breast cancers, is defined by lacking of expression of estrogen receptor (ER), progesterone receptor (PR), and HER2 [1]. Most TNBCs have a higher rate of distant recurrence and a poorer prognosis compared with other breast

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cancer subtypes. However, there are no targeted therapies for this subset of breast cancer [2,3]. Therefore, new therapeutic strategies are highly needed to improve the outcomes in TNBCs.

Almost 5% to 10% of breast cancers carry *BRCA1/2* deleterious germline mutations [4-6]. *BRCA1/2*-mutant breast cancers are particularly sensitive to poly(adenosine diphosphate-ribose) polymerase (PARP) inhibitors and platinum salts, owning to their deficiency in DNA repair via homologous recombination repair (HRR) and nucleotide excision repair [7,8]. Not so many TNBC patients could take advantage of these therapies because only 15% to 20% of TNBCs carry *BRCA1/2* germline mutations [9-11]. Some sporadic TNBCs share similar clinicopathological and molecular characteristics to *BRCA1/2*-mutant breast cancers, a phenotype recently described as "BRCAness" [12,13]. Identifying the phenotype of BRCAness in TNBCs may expand the target group for platinum salts and PARP inhibitors.

Because of genomic instability caused by HRR dysfunction, *BRCA1/2*-mutant breast cancers and other sporadic breast cancers with an HRR defect often display a very characteristic pattern of gains and losses of genomic DNA, which is called BRCA1/2-like genomic profile [14,15]. Because BRCA2-like genomic profile presents in ER+ breast cancers more frequently [16], BRCA1-like genomic profile is assumed to be a measure of BRCAness in TNBCs. Recently, multiplex ligation-dependent probe amplification (MLPA) assay for the BRCA1ness classification of breast tumors was developed as an alternative for the rather complex array comparative genomic hybridization method [17,18]. According to the low frequency of *BRCA1/2* mutations in sporadic breast cancers, *BRCA1/2* promoter methylation may be the major cause of *BRCA1/2* somatic inactivation, which could also be a sign of BRCAness [19,20].

The aim of this study was to assess the validity of *BRCA1/2* promoter methylation and BRCA1-like genomic profile to identify BRCAness in TNBCs. We also investigated the relationships between *BRCA1/2* promoter methylation, BRCA1-like genomic profile, and *BRCA1/2* germline mutation. Moreover, we explored the associations between BRCAness phenotype and clinicopathological parameters in TNBCs.

2. Materials and methods

2.1. Patients and samples

One hundred fifty-one cases of primary invasive TNBCs were derived from the pathology archives at Fudan University Shanghai Cancer Center. All patients were diagnosed and treated by surgery without neoadjuvant therapy in 2015 at the center. All patients were informed consent. All patients were younger than 55 years. The status of *BRCA1/2* germline mutation of the 151 TNBCs had been tested by next-generation sequencing and had been confirmed through mutation analysis. In 151 TNBCs, 20 cases carried *BRCA1* pathogenic mutation and 9 cases carried *BRCA2* pathogenic mutation.

Formalin-fixed, paraffin-embedded tissues of 151 cases were collected, 4-μm-thick slices of representative tumor blocks were stained with hematoxylin and eosin. All cases were reviewed by 2 experienced breast pathologists to confirm the histologic type and grade, according to 2012 World Health Organization *Classification of Tumours of the Breast* [21]. Tumors were defined as triple negative as follows: less than 1% of ER and PR immunoreactivity and absence of HER2 protein overexpression or gene amplification. The "IHC signature" of Nielsen et al [22] was used as a surrogate of gene microarray expression to identify the basal-like breast cancer (BLBC). This signature includes lack of expression of ER, PR, and HER2, and overexpression of cytokeratin 5/6 and/or epidermal growth factor receptor.

Fresh-frozen specimens were available in 78 of 151 TNBCs and were collected from the Tissue Bank at Fudan University Shanghai Cancer Center.

2.2. DNA isolation

We selected tumor blocks that contained a region with a minimum of 60% tumor cells. Breast cancer tissues were harvested from 4-μm-thick tissue sections. Areas with necrosis, preinvasive lesions, and extensive inflammation were avoided. DNA extraction was performed using the QIAamp DNA Mini Kit according to the manufacturer's protocol (Qiagen, Hilden, Germany). DNA concentration was measured using Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA).

2.3. *BRCA1/2* promoter methylation

BRCA1/2 promoter methylation was detected using methylation-specific MLPA (MS-MLPA) analysis. We used ME053-BRCA1-BRCA2 methylation kit (MRC-Holland, Amsterdam, the Netherlands), which contained 3 and 4 probes to detect *BRCA1* and *BRCA2* promoter methylation, respectively. These probes targeted 3 CpG sites in the *BRCA1* promoter region and 5 CpG sites in the *BRCA2* promoter region. A total quantity of 50 to 250 ng of DNA in a 5-μL volume was used for MS-MLPA analysis. Three normal breast formalin-fixed, paraffin-embedded tissues were used as reference samples. The MS-MLPA principle and protocol had been described in previous literatures [23,24].

Coffalyser.Net software (MRC-Holland) was used for methylation data analysis. Quality control showed that the results of the control probes and control samples were adequate. A CpG site was considered to be methylated when the methylation dosage ratio between digested and undigested sample was superior to the cutoff threshold of 20%, as previously reported [25-27]. It was interpreted as *BRCA1* or *BRCA2* promoter methylation when 3 *BRCA1*-methylation probes and 4 *BRCA2* methylation probes showed methylation.

Table 1 Clinical characteristics of 151 TNBCs

Characteristics	n (%)
Age (y)	
<50	108 (71.5)
≥50	43 (28.5)
Tumor size (cm)	
pT1 (≤2.0)	78 (51.7)
pT2 (2.1-5.0)	67 (44.4)
pT3 (>5.0)	6 (3.9)
Nodal status	
pN0 (0)	113 (74.8)
pN1 (1-3)	24 (15.9)
pN2/N3 (4+)	14 (9.3)
Histologic grade	
2	36 (23.8)
3	115 (76.2)
Histologic type	
Invasive carcinoma of no special type	134 (88.7)
Special type	17 (11.3)
Lymphovascular invasion	
Yes	54 (35.8)
No	97 (64.2)
Basal-like phenotype	
Yes	101 (66.9)
No	50 (33.1)

2.4. BRCA1-like genomic profile analysis

Classification of BRCA1-like genomic profile was performed using MLPA with P376-B3 BRCA1ness probemix (MRC-Holland) as previously reported [17,18]. This probemix contains 34 target probes covering the chromosomal regions that have been found to be gained in 3q22-29,6p21-22, 10p14, 12p13, and 13q31-34, and lost in 3p21, 5q12-23, 10q23, 12q21-23, 14q22-24, and 15q15-21 translocation, which were the most important genomic regions of the BRCA1-like classifier described in previous studies [17]. The assay was performed according to the standard MLPA protocol from the manufacturer's instructions.

Coffalyser.Net software (MRC-Holland) was used for MLPA data analysis. BRCA1-like class prediction was performed using prediction analysis for microarrays and R statistics. The training set generated by MRC-Holland with P376-B3 was used for the prediction analysis for microarray. For the MLPA classifier, the cutoff value to classify a sample as "BRCA1-like" was set at 0.5 or greater. Below this score, a sample was classified as "non-BRCA1-like" [17].

2.5. RNA isolation and quantitative reverse-transcription polymerase chain reaction

BRCA1 messenger RNA (mRNA) expression levels were evaluated using quantitative reverse-transcription polymerase chain reaction. Total RNA was extracted from fresh tumor tissue samples using TRIzol (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. The reverse-transcription reactions were carried out using a PrimeScript RT reagent Kit (Takara, Dalian, China); the quantitative polymerase chain reaction reactions were then conducted using SYBR Premix Ex TaqTM (Takara). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was set as the endogenous control to normalize the data. The primer sequences are listed in Supplementary Table S1.

2.6. Statistical analysis

Associations between *BRCA1* promoter methylation, BRCA1-like genomic profile, and *BRCA1/2* mutation status, and associations between BRCAness phenotype and clinicopathological parameters were assessed using the Pearson χ^2 test, Fisher exact test, or Mann-Whitney test. Data analysis was performed using IBM SPSS Statistics version 20.0 (IBM, Chicago, IL) and R software version 3.2.3 (www.R-project.org).

3. Results

3.1. Clinicopathological characteristics

The clinicopathological characteristics of 151 TNBC patients are listed in Table 1. The mean patients' age was 45 years (range, 24-55 years). One hundred thirty-four (88.7%) TNBCs were diagnosed as invasive carcinoma of no special type, and 17 (11.3%) were invasive breast carcinoma of special subtypes (metaplastic carcinoma in 7 cases, carcinoma with apocrine differentiation in 3 cases, carcinoma with central necrosis in 2 cases, pleomorphic invasive lobular carcinoma in 3 cases, invasive micropapillary carcinoma in 1 case, and adenoid cystic carcinoma in 1 case; Fig. 1). One hundred one (66.9%) cases were BLBCs defined by immunohistochemistry (IHC). The mean Ki-67 index was 59% (range, 10%-95%).

3.2. BRCAness phenotype in TNBCs

MS-MLPA analysis showed that 38 (25.2%) of 151 TNBCs exhibited *BRCA1* promoter methylation, and no case exhibited *BRCA2* promoter methylation. One hundred twenty-four of 151 TNBCs had adequate quality to assess the BRCA1-like profile by MLPA. Of the 124 TNBCs, 52 (41.9%) had a BRCA1-like profile (Fig. 2). In these BRCA1-like TNBCs, 42.3% (22/52) tumors had a *BRCA1* promoter methylation, whereas 21.2% (11/52) had a *BRCA1* germline mutation and 5.8% (3/52) had a *BRCA2* germline mutation. For the 124 TNBCs, the frequency of BRCAness phenotype (defined as *BRCA1/2* germline mutation and/or *BRCA1* promoter methylation and/or BRCA1-like genomic profile) was 54.8% (68/124; Fig. 3).

The relations between 3 BRCAness abnormalities (*BRCA1/2* germline mutation, *BRCA1* promoter methylation,

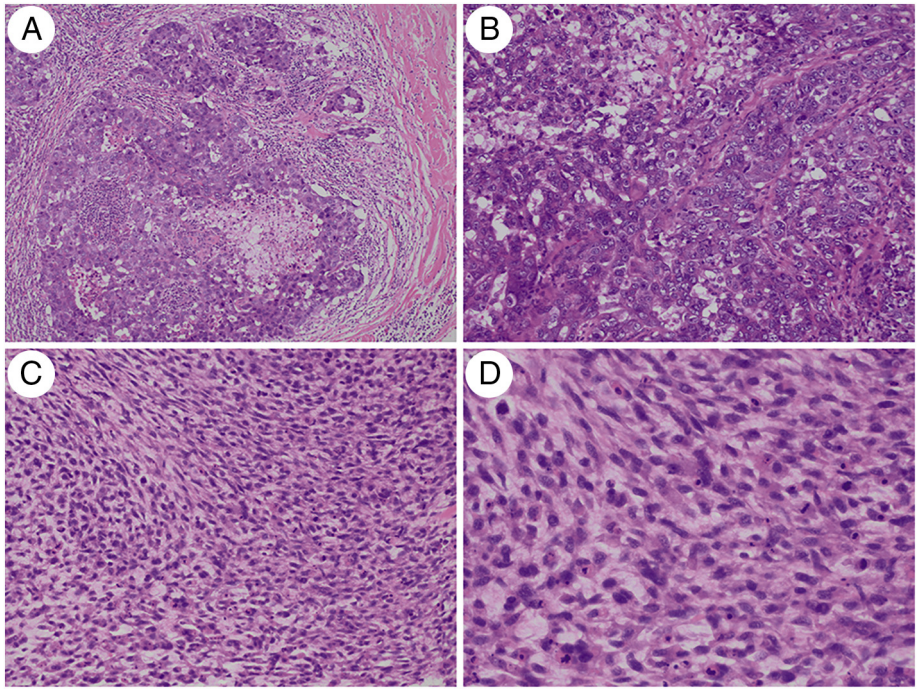

Fig. 1 TNBCs with BRCAness phenotype. A and B, Invasive carcinoma of no special type with *BRCA1* germline mutation and BRCA1-like genomic profile. C and D, Spindle cell metaplastic carcinoma with *BRCA1* promoter methylation and BRCA1-like genomic profile. Original magnifications ×10 (A and C) and ×20 (B and D).

and BRCA1-like genomic profile) were analyzed. No case was found with a concurrent *BRCA1* germline mutation and somatic *BRCA1* promoter methylation. *BRCA1* promoter methylation and *BRCA1* germline mutation were mutually exclusive (P = .002, Fisher exact test; Table 2). If *BRCA1* promoter methylation analysis would be conducted to exclude a germline *BRCA1* mutation, then the specificity would be 100% (20/20). Positive predictive value would be 100% (38/38) as well. Sensitivity and negative predictive values would be 29.0% (38/131) and 17.7% (20/113), respectively.

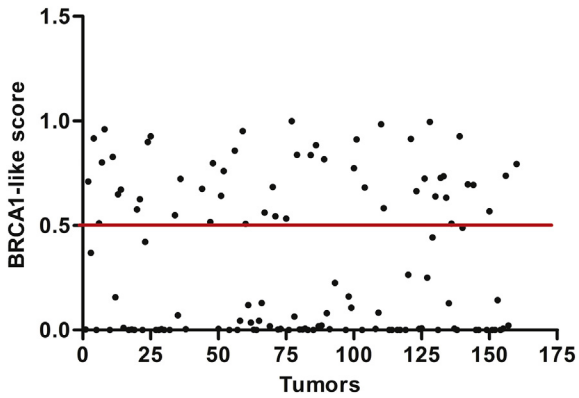

Fig. 2 BRCA1-like MLPA profile analyses in TNBCs. *y*-Axis represents the BRCA1-like score. Each dot represents a tumor. The red horizontal line indicates the 0.5 cutoff value. Dots above the red line represent TNBCs with BRCA1-like genomic profile (52/124), and dots below the red line show TNBCs with non-BRCA1-like genomic profile (72/124).

We next evaluated whether all *BRCA1*-aberrent tumors showed BRCA1-like MLPA profile. There were 75.9% (22/29) tumors with *BRCA1* promoter methylation and 78.6%

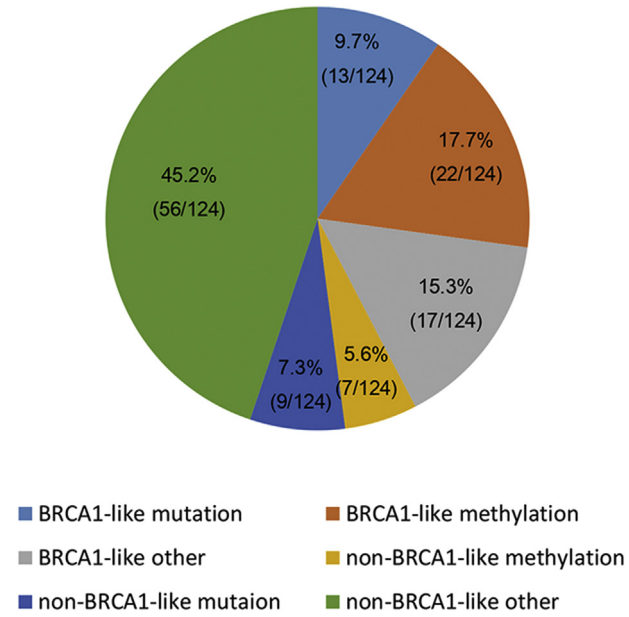

Fig. 3 Co-occurrence of BRCA1-like MLPA profile, *BRCA1/2* germline mutation, and *BRCA1* promoter methylation. This pie chart indicates the frequency of 3 BRCAness characteristics in 124 TNBCs. BRCA1-like profile included *BRCA1/2* germline mutation (BRCA1-like mutation), *BRCA1* promoter methylation (BRCA1-like methylation), and other tumors with no *BRCA* aberration (BRCA1-like other).

Table 2 Overlap between *BRCA1* germline mutation and *BRCA1* promoter methylation

	BRCA1 mutation	No *BRCA1* mutation	Total	*P* [a]
BRCA1 methylated	0	38	38	.002 *
BRCA1 unmethylated	20	93	113	
Total	20	131	151	

[a] Fisher exact test.
* *P* value was significant.

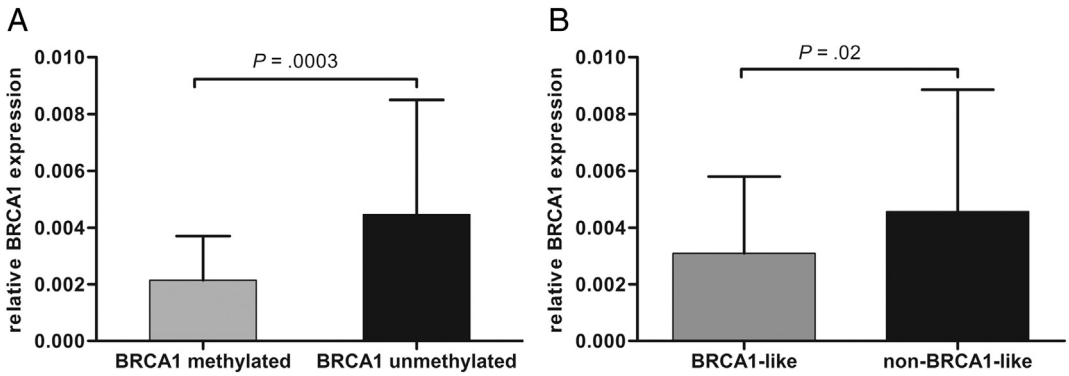

Fig. 4 Associations of *BRCA1* promoter methylation and BRCA1-like MLPA profile with BRCA1 mRNA expression. *y*-Axis shows the relative mRNA expression of BRCA1 (mean ± SD). A, *BRCA1* promoter methylation was associated with lower BRCA1 mRNA expression (*P* = .0003). B, BRCA1-like MLPA profile was associated with lower BRCA1 mRNA expression (*P* = .02).

(11/14) tumors with *BRCA1* mutation that showed a BRCA1-like genomic profile. There were 2.4% (3/124) tumors with *BRCA1* mutation and 5.6% (7/124) with *BRCA1* promoter methylation that did not show a BRCA1-like MLPA profile. Revision of these samples by a pathologist showed that the tumor cell percentage was less than 50%, and they all had too many normal cells in the biopsy material, which might lead to the false-negative result.

Moreover, *BRCA1* promoter methylation (*P* = .0003) and BRCA1-like genomic profile (*P* = .02) were significantly correlated with lower BRCA1 mRNA expression (Mann-Whitney test, Fig. 4).

3.3. Associations of BRCAness phenotype with clinicopathological parameters in TNBCs

The associations between clinicopathological variables and *BRCA1/2* mutation, *BRCA1* promoter methylation, and BRCA1-like MLPA status were evaluated, respectively. *BRCA1* promoter methylation was significantly associated with higher histologic grade (grade 3, *P* = .03, χ^2 test; Table 3), higher Ki-67 index (*P* = .02, Mann-Whitney test; Fig. 5A), and IHC-defined BLBC (*P* = .0001, χ^2 test; Table 3). BRCA1-like genomic profile was associated with higher histologic grade (grade 3, *P* = .001, χ^2 test) and IHC-defined

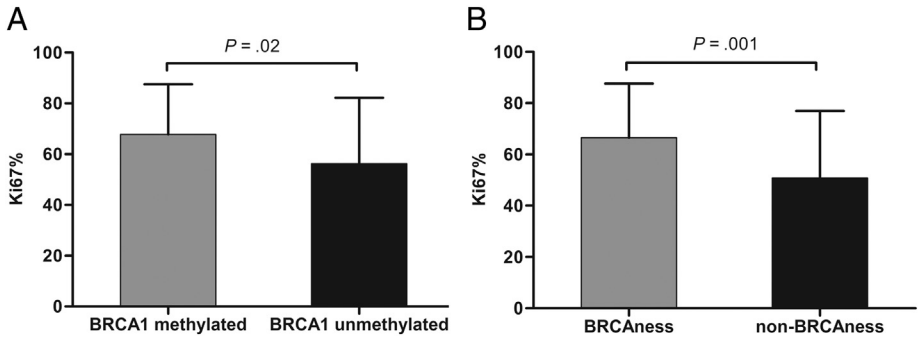

Fig. 5 Associations of *BRCA1* promoter methylation and BRCAness with Ki-67 index in TNBCs. *BRCA1* promoter methylation (A) and BRCAness phenotype (B) were significantly associated with higher Ki-67 index. *P* values are shown in the figure.

Table 3 Associations between BRCAness abnormalities and clinicopathological characteristics in TNBCs

Characteristics	TNBCs (n = 151)			TNBCs (n = 124)			TNBCs (n = 124)		
	BRCA1 methylated, n (%)	*BRCA1* unmethylated, n (%)	*P* [a]	MLPA BRCA1-like, n (%)	MLPA non-BRCA1-like, n (%)	*P* [a]	BRCAness, n (%)	Non-BRCAness, n (%)	*P* [a]
Tumor size (cm)									
pT1 (≤2.0)	16 (42.1)	62 (54.9)	.17	23 (44.2)	43 (59.7)	.09	29 (42.6)	37 (66.1)	.009 *
pT2 (2.1-5.0)	22 (57.9)	45 (39.8)		27 (52.0)	28 (38.9)		37 (54.4)	18 (32.1)	
pT3 (>5.0)	0 (0)	6 (5.3)		2 (3.8)	1 (1.4)		2 (3.0)	1 (1.8)	
Nodal status									
pN0 (0)	29 (76.3)	84 (74.3)	.81	36 (69.2)	56 (77.8)	.28	44 (64.7)	48 (85.8)	.008 *
pN1 (1-3)	7 (18.4)	17 (15.1)		9 (17.3)	10 (13.9)		15 (22.1)	4 (7.1)	
pN2/N3 (4+)	2 (5.3)	12 (10.6)		7 (13.5)	6 (8.3)		9 (13.2)	4 (7.1)	
Histologic grade									
2	4 (10.5)	32 (28.3)	.03 *	5 (9.6)	25 (34.7)	.001 *	7 (10.3)	23 (41.1)	.0001 *
3	34 (89.5)	81 (71.7)		47 (90.4)	47 (65.3)		61 (89.7)	33 (58.9)	
Histologic type									
Invasive carcinoma of no special type	36 (94.7)	98 (86.7)	.18	50 (96.2)	60 (83.3)	.07	64 (94.1)	46 (82.1)	.07
Special type	2 (5.3)	15 (13.3)		2 (3.8)	12 (16.7)		4 (5.9)	10 (17.9)	
Lymphovascular invasion									
Yes	12 (31.6)	42 (37.2)	.53	19 (36.5)	26 (36.1)	.96	27 (39.7)	18 (32.1)	.38
No	26 (68.4)	71 (62.8)		33 (63.5)	46 (63.9)		41 (60.3)	38 (67.9)	
Basal-like phenotype									
Yes	33 (86.8)	68 (60.2)	.003 *	44 (84.6)	39 (54.2)	.0001 *	55 (80.9)	28 (50.0)	.0001 *
No	5 (13.2)	45 (39.8)		8 (15.4)	33 (45.8)		13 (19.1)	28 (50.0)	
Ki-67 (%)	68	56	.02 *	64.6	55.8	.06	59	56	.001 *

[a] χ^2 Test.
* *P* values were significant.

BLBC (*P* = .0001, χ^2 test; Table 3). However, there was no significant relationship between *BRCA1/2* germline mutation and clinicopathological characteristics and IHC-defined BLBC in this cohort (Supplementary Table S2).

In the 124 TNBCs with complete 3 types of data, BRCAness phenotype was associated with larger tumor size (>2 cm, *P* = .009), higher histopathologic grade (grade 3, *P* = .0001), positive lymph node involvement (*P* = .008, χ^2 test; Table 3), higher Ki-67 index (*P* = .001; Fig. 5B), and IHC-defined BLBCs (*P* = .0001, χ^2 test; Table 3).

In our study, the BRCAness phenotype was not associated with invasive carcinoma of special types (*P* = .07; Table 3). BRCAness phenotype was shown in 4 cases of special type invasive carcinoma. In the 7 metaplastic breast carcinoma cases, 1 case (14.3%) had *BRCA1* promoter methylation and BRCA1-like genomic profile. This case was diagnosed as spindle cell metaplastic carcinoma (Fig. 1C and D).

4. Discussion

BRCAness phenotype could be defined as *BRCA1/2* germline mutation and/or *BRCA1* promoter methylation and/or BRCA1-like genomic profile [12,13]. Identifying BRCAness in TNBCs could expand the target group for platinum salts and PARP inhibitors. Because the frequency of *BRCA1/2* germline mutation was relatively low in TNBCs, the aim of our study was to assess the clinical validity of *BRCA1/2* promoter methylation and BRCA1-like genomic profile to identify BRCAness. In our study, 38 (25.2%) of 151 TNBCs exhibited *BRCA1* promoter methylation. There were 41.9% (52/124) of patients who had a tumor with a BRCA1-like profile. *BRCA1/2* germline mutations had been confirmed in 19% (29/151) TNBCs in our previous work. In 124 TNBCs with complete 3 types of data, the frequency of BRCAness phenotype was 54.8% (68/124). Our study showed that combined detections of *BRCA1* promoter methylation and BRCA1-like genomic profile could identify more BRCAness cases in TNBCs.

In this study, we detected *BRCA1/2* promoter methylation using a new *BRCA* methylation MS-MLPA assay. Thirty-eight (25.2%) of 151 TNBCs exhibited *BRCA1* promoter methylation. *BRCA1* promoter methylation was correlated with decreased BRCA1 mRNA expression. In addition, *BRCA1* promoter methylation and *BRCA1* germline mutation were mutually exclusive. Increasing pieces of evidence have shown that *BRCA1* somatic mutation was rare in breast cancer, and the main mechanism of somatic *BRCA1* dysfunction was promoter methyaltion [19,20,28,29]. Brianese et al [20] recently

reported that 1 (0.8%) of 131 TNBCs had a somatic pathogenic variant and 27 (20.6%) of 131 TNBCs showed *BRCA1* promoter methylation, especially in younger women. In line with our findings, their study also showed that no *BRCA1* promoter methylation was overlapped with *BRCA1* germline mutation. Lips et al [27] observed 20% to 30% TNBCs with *BRCA1* promoter methylation and also demonstrated that *BRCA1* germline mutation and *BRCA1* promoter methylation were mutually exclusive. Yamashita et al [30] observed that 11 (16%) of 69 TNBCs had *BRCA1* promoter methylation and *BRCA1* promoter methylation was associated with lower BRCA1 mRNA expression. One of the limitations in our study was that the number of cases was relatively low. Whether *BRCA1* promoter methylation analysis could be used as a quick and reliable method to exclude *BRCA1* germline mutation in breast cancers needed multicenter researches of larger samples.

In our study, BRCA1-like genomic profile was used as another measure to identify BRCAness in TNBCs. Fifty-two (41.9%) of 124 TNBCs had BRCA1-like genomic profile using MLPA assay. It showed that 75.9% tumors with *BRCA1* promoter methylation and 78.6% with *BRCA1* mutation were classified as BRCA1-like genomic profile in our cohort. Branham et al [31] observed that 10 (15.8%) of 63 breast cancers showed BRCA1-like genomic profile using MLPA assay, and BRCA1-like profile was associated with TNBCs. Lips et al [27] observed that nearly 70% TNBCs showed a BRCA1-like array comparative genomic hybridization pattern, and they observed that 4% tumors with *BRCA1* mutation and 4% with *BRCA1* methylation showed non–BRCA1-like genomic profile.

The associations between BRACness with clinicopathological features were analyzed in our study. The BRCAness phenotype was significantly associated with large tumor size, positive lymph nodes, grade 3 tumor, high Ki-67 levels, and BLBCs in our study. It showed that *BRCA1* promoter methylation was significantly associated with grade 3 tumor, high Ki-67 levels, and BLBCs in TNBCs. BRCA1-like MLPA profile was correlated with grade 3 tumor and basal-like phenotype. In line with our study, Yamashita et al [30] found that *BRCA1* promoter methylation was associated with younger patients, higher grade, and lymphovascular invasion. Lips et al [27] also observed that BRCA1-like genomic profile was associated with younger patients (<50) and high grade tumors. Zhu et al [32] found that *BRCA1* promoter methylation was correlated with the basal-like phenotype identified by IHC.

Our findings might have important implications for clinical practice. First, our study showed that combined detections of *BRCA1* promoter methylation and BRCA1-like genomic profile could identify more BRCAness cases in TNBCs, which might be significant to expand the target patients who might benefit from PARP inhibitors and platinum chemotherapy. Second, *BRCA1* promoter methylation analysis might be used as a cost-effective prescreening tool to exclude *BRCA1* germline mutations in TNBCs. To our knowledge, our present study was one of the few studies to comprehensively investigate *BRCA1/2* germline mutation, *BRCA1/2* promoter methylation, and BRCA1-like MLPA profile in TNBCs in a Chinese population.

5. Conclusions

In conclusion, our study showed that combined detections of *BRCA1* promoter methylation and BRCA1-like genomic profile could identify more BRCAness cases in TNBCs. *BRCA1* promoter methylation and *BRCA1* germline mutation were mutually exclusive. The BRCAness phenotype was associated with aggressive clinicopathological features. These findings may be helpful to identify patients who might benefit from targeted therapies such as PARP inhibitors and to the prescreening test for hereditary breast cancers.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humpath.2018.10.004.

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Availability of data and materials

The data sets generated and/or analyzed during the current study are not publicly available owing to privacy reasons for patients carrying *BRCA1* or *BRCA2* germline mutations, but they are available from the corresponding author on reasonable request.

Ethical approval

All procedures performed involving human participants were in accordance with the ethical standards of Ethics Institutional Review Board of Fudan University Shanghai Cancer Center and with the 1964 Helsinki declaration and its later amendments. Written informed consent was obtained from all patients of the study, who signed the informed consent form

allowing the use of their biological material, donated for our Biobank, for scientific projects, and for data publication.

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