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ORIGINAL ARTICLE

Expression of DNA Damage Response Proteins and Associations with Clinicopathologic Characteristics in Chinese Familial Breast Cancer Patients with *BRCA1/2* Mutations

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Purpose: The characteristic expression of DNA damage response proteins in familial breast cancers with *BRCA1*, *BRCA2*, or non-*BRCA1/2* mutations has not been analyzed in Chinese patients. Our study aimed to assess the differential expression of microcephalin 1 (BRIT1), ATM serine/threonine kinase (ATM), checkpoint kinase 2 (CHEK2), BRCA1, RAD51 recombinase (RAD51), and poly (ADP-ribose) polymerase 1 (PARP-1) and establish the profile of Chinese familial breast cancers with different mutation status. **Methods:** We constructed five tissue microarrays from 183 familial breast cancer patients (31 with *BRCA1* mutations; 14 with *BRCA2* mutations, and 138 with non-*BRCA1/2* mutations). The DNA response and repair markers used for immunohistochemistry analysis included BRIT1, ATM, CHEK2, BRCA1, RAD51, and PARP-1. The expressions of these proteins were analyzed in *BRCA1/2* mutated tumors. The association between pathologic characteristics with *BRCA1/2* mutation status was also analyzed. **Results:** In familial breast cancer patients, *BRCA1* mutated tumors were more frequent with high nuclear grade, estrogen receptor/progesterone receptor/human epidermal growth factor receptor 2 negative, low Ki-67, and positive CK5/6. *BRCA1* mutated tumors had lower CHEK2 and higher cytoplasmic BRIT1 expression than *BRCA2* and non-*BRCA1/2* mutation tumors. *BRCA2*-associated tumors showed higher CHEK2 and cytoplasmic RAD51 expression than those in other groups. Nuclear PARP-1 expression in *BRCA1/2*-associated tumors was significantly higher than in non-*BRCA1/2* mutation tumors. Moreover, we found quite a few of negative PARP-1 expression cases in *BRCA1/2* mutated groups. **Conclusion:** The clinicopathologic findings of *BRCA1*-associated Chinese familial breast cancers were similar to the results of other studies. Chinese familial breast cancer patients with *BRCA1/2* mutations might have distinctive expression of different DNA damage response proteins. The reduced expression of PARP-1 in Chinese *BRCA1/2* mutated breast cancer patients could influence the therapeutic outcome of PARP-1 inhibitors.

Key Words: *BRCA1 genes, BRCA2 genes, Breast neoplasms, DNA repair, Poly (ADP-ribose) polymerase-1*

INTRODUCTION

Women with mutations of two high penetrance susceptibility genes, *BRCA1* and *BRCA2*, have an elevated risk for breast cancer and ovarian cancer [1]. In addition, the mutation frequency of *BRCA1/2* genes in breast cancer patients with a familial breast cancer history is approximately 20% [2]. A previous study by our group also demonstrated a similar result in a Chinese population [3]. Some studies concentrated on different biomarkers in the pathway of DNA damage response and repair [4,5]. However, there no similar study for Chinese familial breast cancer with *BRCA1/2* mutations has been reported. We investigated several proteins in DNA damage response and repair pathway to explore different expression patterns in a Chinese population.

Microcephalin 1 (BRIT1) expression is an early DNA repair mediator that regulates the recruitment of DNA repair proteins including BRCA1 and BRCA2, initiates the signaling pathways of ATM serine/threonine kinase or ATR serine/threonine kinase (ATM/ATR) after DNA damage. Reduced BRIT1 expression can lead to reduced BRCA1 expression [6].

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ATM kinase is a key protein responds to DNA double-strand break and coordinates the cell cycle and cell death pathways [7]. ATM can phosphorylate a range of downstream substrates, including human Cds1 kinase (CHEK2), p53, mouse double minute 2 homolog, BRCA1, and others. ATM protein expression is reduced more frequently in *BRCA1/2* tumors than non-*BRCA1/2* tumors [8]. After being phosphorylated by protein kinase ATM, CHEK2 regulates the release of BRCA1 after DNA damage by phosphorylating BRCA1 [9]. The genetic mutation of *CHEK2* has been associated with hereditary breast cancer and the downregulation of CHEK2 protein expression is also observed in these patients [10].

Both BRCA1 and BRCA2 proteins play critical roles in DNA repair and recombination, especially in homologous recombination. BRCA1 is activated by ATM or CHEK2 kinase phosphorylation and works as a signal processor for DNA damage response. BRCA2, which has a more specific role than BRCA1, binds directly with RAD51 recombinase and carries it to the site of DNA double strand break for homologous repair [11]. With the aid of mediator proteins such as BRCA2 and the RAD51 paralogs (RAD51B, RAD51C, RAD51D, X-ray repair cross complementing 2 [XRCC2], and XRCC3), RAD51 localizes to the foci of DNA damage and promotes the recombinational repair of DNA double strand breaks [12].

Besides proteins associated with homologous recombination, we also focused on the expression of poly (ADP-ribose) polymerase 1 (PARP-1), which is critical for the base excision repair pathway. The inhibition of PARP-1 is a therapeutic strategy for *BRCA1/2*-associated breast cancer [13].

The present study included six DNA damage response and repair proteins (BRIT1, ATM, CHEK2, BRCA1, RAD51, and PARP-1). Their expression in familial breast cancer patients with *BRCA1* or *BRCA2* gene mutations was compared to that in patients with non-*BRCA1/2* mutation. We also determined the clinical pathologic characteristics of these familial breast cancer patients to explore the association between the *BRCA1/2* mutation status and the clinicopathologic characteristics. The aim of these analyses was to discover specific protein expression patterns in different populations and the tumor biology of *BRCA1/2*-associated breast cancer. We hope that our findings will have therapeutic significance for Chinese familial breast cancer patients.

METHODS

Patients

Familial breast cancers (n = 185) from 183 breast cancer patients who were diagnosed and underwent curative surgery at the Fudan University Shanghai Cancer Center from June 2011 to July 2017. The breast cancer patients were required to meet the following inclusion criteria: (1) age up to 35 years with at least one other blood relative suffering from any type of cancer; (2) age 35 to 50 years with two blood relatives in the same lineage suffering from any type of cancer; or (3) older than 50 years of age with three blood relatives in the same lineage suffering from any type of cancer. Informed consent was obtained from each participant for collection of their blood and tissue specimens for scientific research. The genetic testing of *BRCA1/2* mutation was performed using targeted capture and massively parallel sequencing technology. The results were validated by conventional Sanger sequencing, as previously described [3]. A documented informed consent form was obtained from each patient for future use of her/his samples for breast cancer-related genetic studies and this study was approved by the Scientific and Ethical Committee of the Shanghai Cancer Center (IRB number: 1412142-11).

Tumor pathology

Relevant clinicopathologic characteristics were collected from the department of pathology, Fudan University Shanghai Cancer Center. The characteristics included the age of diagnosis, tumor type (ductal carcinoma *in situ*, invasive ductal or lobular carcinoma, and other types of malignant tumor), nuclear grade, pathological size of the tumor, estrogen receptor (ER) status, progesterone receptor (PR) status, human epidermal growth factor receptor 2 (HER2) expression in primary tumor, Ki-67, CK5/6 expression, and the number of positive lymph nodes.

Tissue microarray construction and immunohistochemistry

The formalin-fixed, paraffin-embedded specimens were obtained after curative surgery of the breast cancer patients. Two representative areas of each tumor were selected from hematoxylin and eosin stained sections and marked on the corresponding paraffin specimens. Two tissue cores (0.5 mm in diameter) were obtained from each block. We also included one normal breast tissue core as an internal control from each adjacent nontumorous breast tissues. The tissue cores were arrayed onto five independent new paraffin blocks using a tissue microarray technology. Multiple sections (5 μm thick) were used for immunohistochemistry. In brief, paraffin-embedded tissue microarray sections were deparaffinized and washed with a 1.5% hydrogen peroxide-methanol solution to block endogenous peroxidase activity for 30 minutes. For BRCA1 and PARP-1, antigen retrieval was carried out in 0.01 mol/L sodium citrate (pH 6.0) for 15 minutes and for BRIT1, CHEK2, ATM, and Rad51, antigen was retrieved in 0.05 mol/L

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Table 1. Antibodies used in the immunohistochemical staining

Antibody	Dilution	Clone*	Staining localization	Cutoff (range)
BRIT1	1:200	Polyclonal rabbit	Cytoplasmic	≥12 (0–18)
BRCA1	1:200	MS110	Nuclear	≥12 (0–18)
CHEK2	1:100	Polyclonal rabbit	Nuclear	≥6 (0–18)
RAD51	1:300	Polyclonal mouse	Cytoplasmic	≥6 (0–12)
PARP-1	1:100	E102	Nuclear	≥9 (0–18)
ATM	1:100	Y170	Nuclear	≥12 (0–18)

BRIT1 = microcephalin 1; CHEK2 = checkpoint kinase 2; RAD51 = RAD51 recombinase; PARP-1 = poly (ADP-ribose) polymerase 1; ATM = ATM serine/threonine kinase.
*Supplier: Abcam, Cambridge, UK.

Tris-ethylenediaminetetraacetic acid for 12 minutes. After incubating with primary antibodies at 37°C for 60 minutes, the slides were placed in moist chamber at 4°C overnight. The antibodies and dilutions used are listed in Table 1. The dilutions for immunohistochemistry that were used were specified by the manufacturer. On the second day, the REAL EnVision Detection System (Dako, Carpinteria, USA) consisting of horseradish peroxidase-labeled anti-rabbit or anti-mouse secondary antibody according to the manufacturer's instructions. After washing three times with phosphate buffered saline, the products of the antigen–antibody reactions were visualized by incubating the sections in 3,3′-diaminobenzidine (Dako). The length of incubation was determined by the microscopy examination of the samples. Cell nuclei were stained with hematoxylin (Bio-Optica, Milan, Italy). The MS110 antibody against BRCA1 protein used for nuclear staining reacted with the N-terminal portion of the BRCA1 protein.

Immunohistochemistry assessment

The immunohistochemical score was independently evaluated by three experienced pathologists who were blinded to genetic mutation information, clinicopathological data, and prognosis status. Results were reached by consensus in cases of disagreement. Many scoring systems have been used in previous studies to evaluate the immunohistochemical expression of proteins. We invited the pathologists to choose the proper method to interpret the expression of proteins. They decided on the quickscore (QS) method to score the immunoactivity of BRIT1, ATM, CHEK2, BRCA1, RAD51, and PARP-1. It achieved better consistency in the results of the three observers than the other methods, supporting the reported reliability and reproducibility of the QS method for immunohistochemistry assessment [14-16]. This system accounted for both the extent of cell staining and the staining intensity. The proportion of positive cells was estimated and given a score on a scale from 1 to 6, score 1 (≤4%); score 2 (≤19%); score 3 (≤39%); score 4 (≤59%); score 5 (≤79%); score 6 (≤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). QS was calculated by multiplying the percentage score by the intensity score. Two cores from each tumor were evaluated individually and the mean value of the two scores was calculated. If one core was lost or contained no tumor tissues, we scored the remaining core as the final score. For nuclear BRCA1, CHEK2, PARP-1, and ATM expression, and cytoplasmic BRIT1 and RAD51 expression, the median scores calculated on the all cases of familial breast cancers were considered as the cutoff. According to the median score, the expression of protein was classified as positive if the final score of one breast cancer case was the same or greater than the median score. Table 1 summarizes the range of scores and the median scores for each protein. The QS of RAD51 ranged from 0 to 12, and the expression was graded as negative (0–5) or positive (6–12). We considered the tumor cell as negative if the score of normal tissue was higher, even the score of tumor cell was higher than the cutoff score.

Statistical analyses

The chi-square test was applied to analyze the difference of clinicopathological characteristics and protein expression between groups. Univariate and multivariate analyses were performed by logistic analysis. SPSS version 22.0 statistical software (IBM Corp., Armonk, USA) was used to perform the statistical analyses. All *p*-values were two-sided. All statistical differences were considered significant if $p < 0.05$.

RESULTS

Clinicopathological characteristics between *BRCA1/2* and non-*BRCA1/2* breast tumors

Among the 183 familial breast cancer patients, we found 31 patients had *BRCA1* mutations (16.9%), 14 patients had

Table 2. Pathological characteristics of familial breast cancers

Characteristic	*BRCA1* mutation No. (%)	*p*-value*	*BRCA2* mutation No. (%)	*p*-value†	Non-*BRCA1/2* mutation No. (%)	*p*-value‡
Histology		0.061		0.413		0.091
DCIS	1 (3.2)		4 (28.6)		21 (15.0)	
IDC	30 (96.8)		10 (71.4)		105 (75.0)	
ILC	0		0		6 (4.3)	
Others	0		0		8 (5.7)	
T stage		0.024		0.400		0.232
Tis	1 (3.2)		4 (28.6)		21 (15.0)	
T1	17 (54.8)		8 (57.1)		69 (49.3)	
T2	11 (35.5)		2 (14.3)		46 (32.9)	
T3	2 (6.5)		0		4 (2.9)	
Nuclear grade		<0.001		0.898		<0.001
I	0		0		2 (2.0)	
II	4 (13.3)		6 (60.0)		62 (60.8)	
III	26 (86.7)		4 (40.0)		38 (37.3)	
LN metastasis		0.923		0.688		0.866
pN0	22 (71.0)		10 (71.4)		97 (69.3)	
pN1	6 (19.4)		3 (21.4)		22 (15.7)	
pN2	1 (3.2)		0		12 (8.6)	
pN3	2 (6.5)		1 (7.1)		9 (6.4)	
ER§		<0.001		0.253		<0.001
Positive	7 (22.6)		13 (92.9)		111 (80.4)	
Negative	24 (77.4)		1 (7.1)		27 (19.6)	
PR§		<0.001		0.479		<0.001
Positive	9 (29.0)		12 (85.7)		107 (77.5)	
Negative	22 (71.0)		2 (14.3)		31 (22.5)	
HER2‖		0.005		0.059		0.004
Positive	2 (6.5)		1 (7.1)		43 (31.2)	
Negative	29 (93.5)		13 (92.9)		95 (68.8)	
Ki-67 (%)		0.008		0.811		0.025
≤15	3 (10.3)		4 (40.0)		34 (36.2)	
>15	26 (89.7)		6 (60.0)		60 (63.8)	
CK5/6		<0.001		0.233		<0.001
Positive	15 (51.7)		0		12 (10.7)	
Negative	14 (48.3)		12 (100)		100 (89.3)	

DCIS = ductal carcinoma *in situ*; IDC = invasive ductal carcinoma; ILC = invasive lobular carcinoma; LN = lymph node; ER = estrogen receptor; PR = progesterone receptor; HER2 = human epidermal growth factor receptor 2.
*The *p*-value between *BRCA1* and non-*BRCA1/2* mutation; †The *p*-value between *BRCA2* and non-*BRCA1/2* mutation; ‡The *p*-value between *BRCA1* and *BRCA2* and *BRCA1/2* mutation; §ER and PR positive are at least 1% of tumor cells with nuclear immunoreactivity; ‖HER2 positive is at least 10% of tumor cells with continuous strong membranous reactivity or HER2 gene amplification.

BRCA2 mutations (7.7%), and 138 patients had non-*BRCA1/2* mutations (75.4%). The pathological characteristics of the familial breast cancers are presented in Table 2. Invasive ductal carcinoma (IDC) was the most common histological type in the three groups. Ductal carcinoma *in situ* (DCIS) and invasive lobular carcinoma were less frequently seen in *BRCA1* mutated breast cancers ($p = 0.061$). Although the differences were not statistically significant, there were more DCIS cases among patients with *BRCA2* mutated breast cancers (28.6%) than among those with *BRCA1* (3.2%) and non-*BRCA1/2* (15.0%) mutations. IDCs with *BRCA1* mutation showed higher nuclear grade than those with *BRCA2* or non-*BRCA1/2* mutations ($p < 0.001$). In addition, *BRCA1* tumors were more frequently ER negative, PR negative, HER2 negative, CK5/6 positive, and displayed a high proliferation index of Ki-67 compared with *BRCA2* and non-*BRCA1/2* tumors.

Expression of DNA repair proteins in *BRCA1/2* mutated breast cancer

Representative examples of immunohistochemistry staining cores are shown in Figure 1 and the staining localizations of each antibody are presented in Table 1. For RAD51 and

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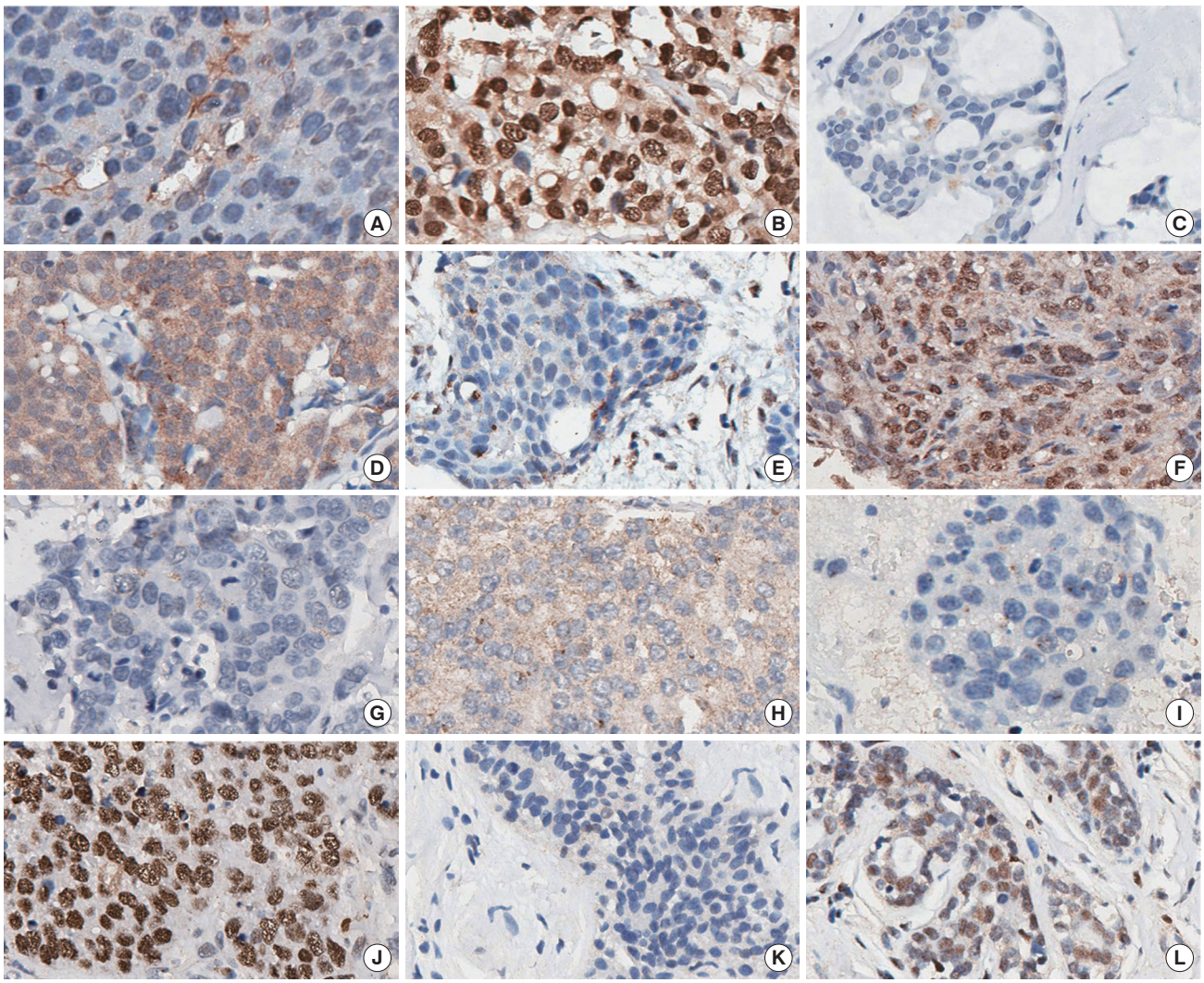

Figure 1. Expression of different DNA damage response proteins, (immumohistochemical stain, ×10). BRCA1 negative nuclear staining (A) and positive nuclear staining (B). Microcephalin 1 negative cytoplasmic staining (C) and positive cytoplasmic staining (D). Checkpoint kinase 2 negative nuclear staining (E) and positive nuclear staining (F). RAD51 recombinase negative cytoplasmic staining (G) and positive cytoplasmic staining (H). Poly (ADP-ribose) polymerase 1 negative nuclear staining (I) and positive nuclear staining (J). ATM serine/threonine Kinase negative nuclear staining (K) and positive nuclear staining (L).

BRIT1, cytoplasmic localization was observed. Nuclear staining of BRIT1 was observed occasionally, but it was not considered in our study. For ATM and PARP-1, nuclear localization was observed. For CHEK2 and BRCA1, nuclear localization was mainly examined, cytoplasmic staining was also not considered in our study. Table 3 summarizes the expression status of different markers in three groups. ATM expression was similar in these groups, while the positive expression of CHEK2 was more frequently seen in *BRCA2*-associated cancers (84.6%) than BRCA1 (51.6%) and non-*BRCA1/2* (53.4%) breast cancers ($p=0.040$). The proportion of positive cytoplasmic staining of RAD51 in *BRCA2* tumors (69.2%) was much higher than in *BRCA1* (34.8%) and non-*BRCA1/2* (37.1%) tumors. BRCA1 expression was significantly reduced in non-*BRCA1/2* (71.9%) tumors versus *BRCA1* (51.9%) and *BRCA2* (40.0%) tumors ($p=0.008$). Positive nuclear staining for PARP-1 in *BRCA1* (56.3%) and *BRCA2* (53.8%) mutated breast cancers were higher than non-*BRCA1/2* (30.8%) mutated breast cancer ($p=0.003$).

The results of multivariate regression analysis of DNA damage repair biomarkers and clinicopathologic findings are presented in Tables 4 and 5. For familial breast cancers, positive cytoplasmic BRIT1 expression was associated with *BRCA1* genetic mutations. High nuclear grade, ER negative, and

Table 3. DNA repair proteins expression in three groups

Protein	*BRCA1* mutation No. (%)	*BRCA2* mutation No. (%)	Non-*BRCA1/2* mutation No. (%)	*p*-value*	*p*-value†	*p*-value‡	*p*-value§
BRIT1				0.020	0.007	0.735	0.045
Positive	16 (64.0)	4 (36.4)	38 51 (39.2)				
Negative	6 (36.0)	7 (56.4)	59 80 (60.8)				
BRCA1				0.024	0.042	0.035	0.008
Positive	13 (48.1)	6 (60.0)	36 (28.1)				
Negative	14 (51.9)	4 (40.0)	92 (71.9)				
CHEK2				0.087	0.859	0.040	0.356
Positive	16 (51.6)	11 (84.6)	71 (53.4)				
Negative	15 (48.4)	2 (15.4)	62 (46.6)				
RAD51				0.070	0.833	0.036	0.274
Positive	8 (34.8)	9 (69.2)	46 (37.1)				
Negative	15 (65.2)	4 (30.8)	78 (62.9)				
PARP-1				0.012	0.007	0.092	0.003
Positive	18 (56.3)	7 (53.8)	41 (30.8)				
Negative	14 (43.8)	6 (46.2)	92 (69.2)				
ATM				0.423	0.267	0.416	0.738
Positive	5 (16.1)	11 (84.6)	31 (25.6)				
Negative	26 (83.9)	2 (15.4)	90 (74.4)				

BRIT1 = microcephalin 1; CHEK2 = checkpoint kinase 2; RAD51 = RAD51 recombinase; PARP-1 = poly (ADP-ribose) polymerase 1; ATM = ATM serine/threonine kinase.
*The *p*-value between *BRCA1* and *BRCA2* and non-*BRCA1/2* mutation; †The *p*-value between *BRCA1* and non-*BRCA1/2* mutation; ‡The *p*-value between *BRCA2* and non-*BRCA1/2* mutation; §The *p*-value between *BRCA1/2* and non-*BRCA1/2* mutation.

Table 4. Multivariate regression logistic analysis for DNA repair proteins associated with *BRCA1/2* mutation

Protein	*BRCA1*		*BRCA2*		*BRCA1/2*	
	Hazard ratio	*p*-value	Hazard ratio	*p*-value	Hazard ratio	*p*-value
BRIT1	7.709	0.002	0.182	0.080	2.521	0.047
BRCA1	2.042	0.230	4.232	0.107	1.969	0.152
CHEK2	0.657	0.487	8.039	0.095	1.182	0.729
RAD51	0.308	0.107	5.707	0.037	0.909	0.840
PARP-1	3.032	0.058	2.383	0.305	3.071	0.018
ATM	0.589	0.398	0.455	0.514	0.421	0.116

BRIT1 = microcephalin 1; CHEK2 = checkpoint kinase 2; RAD51 = RAD51 recombinase; PARP-1 = poly (ADP-ribose) polymerase 1; ATM = ATM serine/threonine kinase.

Table 5. Multivariate regression logistic analysis for clinicopathologic factors associated with *BRCA1/2* mutation

Characteristic	*BRCA1*		*BRCA2*		*BRCA1/2*	
	Hazard ratio	*p*-value	Hazard ratio	*p*-value	Hazard ratio	*p*-value
Nuclear grade	8.307	0.030	2.021	0.435	3.665	0.057
ER	0.068	0.006	1.639	0.756	0.177	0.032
PR	3.231	0.278	0.678	0.781	1.709	0.545
HER2	0.810	0.001	0.000	0.998	0.034	0.002
Ki-67	0.647	0.639	1.211	0.803	0.871	0.828
CK5/6	2.032	0.364	0.000	0.999	1.185	0.815

ER = estrogen receptor; PR = progesterone receptor; HER2 = human epidermal growth factor receptor 2.

HER2 negative breast cancers also had an elevated risk for *BRCA1* mutation. Positive expression of cytoplasmic RAD51 was the only risk factor associated with *BRCA2* genetic mutation. When we included *BRCA1* and *BRCA2* cases together for multivariate analysis, tumors with positive expression of BRIT1 and PARP-1 had a higher probability of *BRCA1/2* genetic mutation. ER negative and HER2 negative were also risk factors associated with *BRCA1/2* genetic mutation.

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DISCUSSION

Many previous studies found different expressions of DNA damage repair proteins among *BRCA1*, *BRCA2*, and non-*BRCA1/2* mutated breast cancers [4,5]. Our study focused on Chinese familial breast cancer patients. The selection of these patients was based on the age of diagnosis and family history. Tissue microarray and immunohistochemistry technologies were applied to analyze the expression status of six DNA damage repair biomarkers of familial breast cancers. The association between pathologic characteristics and *BRCA1/2* mutation status was also analyzed. The collective data enrich the understanding of the tumor biology of Chinese familial breast cancers and different factors associated with *BRCA1/2* mutations among high-risk breast cancer patients.

Firstly, we analyzed the association between different mutation status and clinicopathologic findings. *BRCA1* mutation cancers demonstrated higher tumor grade, and higher prevalence of ER negative, PR negative, and HER2 negative cases. These findings were similar with other studies [17,18]. In addition, *BRCA1*-associated tumors also had higher Ki-67 proliferation index and higher expression of basal marker CK5/6 [19].

Among six biomarkers associated with DNA damage response and repair, some could help us to understand the tumor biology of these cancers. Presently, the cytoplasmic expression of BRIT1 in *BRCA1* mutation patients was higher than *BRCA2* or non-*BRCA1/2* mutation group ($p=0.007$) (Table 3). In normal tissue cells, BRIT1 is mainly located in the nucleus where it serves as a DNA damage response protein, which can regulate the recruitment of repair proteins and trigger the ATM/ATR damage response signaling cascades. For most breast cancers, the staining of BRIT1 changed from a predominant location in the nucleus to both nucleus and cytoplasm or the cytoplasm only. The high cytoplasmic and low nuclear expression of BRIT1 associated with high tumor grade and ER negative status suggests an aggressive biologic behavior and poor prognosis of breast cancer patients [6]. These pathological features were also common in *BRCA1* mutation cancers and this underlines the association between high cytoplasmic expression of BRIT1 and *BRCA1* mutation. However, the mechanism of such translocation of BRIT1 is still unclear.

Nuclear expression of BRCA1 in our specimens was similar in *BRCA1* and *BRCA2* tumors, but was even lower in non-*BRCA1/2* tumors ($p=0.008$) (Table 3). Other studies have described reduced BRCA1 expression in BRCA1 mutation breast cancers and non-BRCA1 familial breast cancers, and even in sporadic cases [20,21]. This means that even without genetic mutation other mechanisms, such as epigenetic loss of BRCA1 function at the level of transcription or promoter hypermethylation, can lead to BRCA1 alternation in non-mutation cases [22,23]. Moreover, reduced expression of the positive regulator of BRCA1 can also decrease BRCA1 expression in non-*BRCA1/2* mutation breast cancers [5,6]. This phenomenon also shows that reduced expression of BRCA1 protein may play an important role in mammary carcinogenesis, not only in BRCA1-associated breast cancers, but also in sporadic cases. However, from the diagnostic point of view, we believe that the expression of BRCA1 protein cannot be used as a method to distinguish between BRCA1 mutation positive breast cancer and mutation negative cancer, whether the latter is familial breast cancer or a sporadic case.

Nuclear expression of CHEK2 was detected in the majority of *BRCA2* tumors (84.6%), but was less in *BRCA1* (51.6%) and non-*BRCA1/2* (53.4%) tumors ($p=0.004$) (Table 3). CHEK2 participates in a number of cellular activities like cell cycle checkpoint activation, induction of apoptosis or senescence, DNA repair, or tolerance of damage. CHEK2 can phosphorylate BRCA1 and BRCA2 to promote homologous recombination [24], and the decrease of downstream substrates can lead to increased expression of CHEK2, as we observed in *BRCA2* tumors. However, this situation was not present in *BRCA1* tumors. Abdel-Fatah et al. [25] found that in sporadic breast cancers, low nuclear CHEK2 protein level was associated with ER negative tumors. This might support the low CHEK2 expression that we observed in *BRCA1* mutation tumors, which appeared with more ER negative cases. Given the complex role of CHEK2 molecular, further study is required to understand the mechanism of interaction between CHEK2 and other DNA repair proteins in *BRCA1* and non-*BRCA1/2* tumors.

RAD51 is a key factor in DNA damage response and double-strand break repair. BRCA1 and BRCA2 are indispensable for RAD51 stimulation. *BRCA2* regulates both the intracellular localization and DNA binding ability of RAD51, and the transportation of RAD51 to the nucleus is defective in BRCA2 associated breast cancers [26]. Presently, the cytoplasmic expression of RAD51 was much higher in *BRCA2* tumors (69.2%) than other two groups ($p=0.036$) (Table 3), which means that RAD51 does not translocate from the cytoplasm to the nucleus where it functions as a DNA repair protein in *BRCA2* tumors. Similar findings were reported in another study [27].

In the base excision single-strand repair pathway, PARP-1 protein is an important nuclear enzyme that detects and initiates DNA repair [28]. When the homologous recombination repair pathway is compromised, especially in *BRCA1/2* muta-

tion breast cancer patients, PARP-1 repairs the DNA damage. Therefore, based on the hypothesis of synthetic lethality, PARP-1 inhibitor can be used in these patients to cause the death of tumor cells. However, not all cancer patients with *BRCA1/2* mutation respond to PARP-1 inhibitor; the low level of PARP-1 protein expression may be one reason [29]. Presently, the positive nuclear expression of PARP-1 was more frequent in *BRCA1* (56.3%) and *BRCA2* (53.8%) tumors than non-*BRCA1/2* tumors (30.8%) ($p = 0.003$) (Table 3). However, comparison with another study [5] revealed that a considerable number of *BRCA1* (43.8%) and *BRCA2* (43.8%) mutation breast cancers featured the low expression of PARP-1 among Chinese familial breast cancer patients. Low PARP-1 expression in tumor cells can reduce the therapeutic effect of PARP-1 inhibitor [30]. Therefore, the effectiveness of PARP-1 inhibitor for Chinese *BRCA1/2* mutation patients might be compromised by the relative high proportion of patients with a low nuclear expression of PARP-1 in *BRCA1/2* mutation breast cancers.

The level of expression of ATM kinase was similar among the different groups in our study, suggesting that ATM expression is not affected by different mutation status of familial breast cancers. This suggestion is not conclusive and data from more patients are needed to validate the present findings.

In summary, for Chinese familial breast cancers, a higher grade of invasive ductal cancer and negative ER/PR/HER2 status are associated with *BRCA1* mutations. These two findings underline the exclusive pathological characteristics of *BRCA1* tumors (high tumor grade, ER negative). Cytoplasmic RAD51 and nuclear CHEK2 expression were more frequently seen in *BRCA2* tumors. This is because the role of BRCA2 in the translocation of RAD51 from cytoplasm to nucleus and the feedback regulation of upstream CHEK2 phosphorylation. *BRCA1* tumors were characterized by the low expression of CHEK2 in the nucleus and by the high expression of BRIT1 in the cytoplasm compared to *BRCA2* tumors. Considering the complexity of the DNA damage response and repair pathways, the mechanism for the alteration of these proteins is still unknown. Surprisingly, a comparatively high proportion of low nuclear PARP-1 expression in Chinese familial *BRCA1/2* mutation breast cancers was discovered in our center, and the effectiveness of PARP-1 inhibitor in the Chinese population is still unknown. Further studies with selected control groups are necessary to validate our results in larger number of Chinese familial breast cancer patients and to explore the mechanism of alteration and translocation of different biomarkers in the DNA damage response and repair pathway.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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