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Original Article

BRCA1 gene mutation rate in breast cancer patients and its clinical significance

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Abstract: Breast cancer commonly occurs in females with increasing incidence. BRCA1 is one tumor suppressor gene and participates in DNA injury and repair. The dysfunction of BRCA1 impairs DNA and elevates the risk of breast or ovarian cancer. This study thus investigated the mutation rate of BRCA gene and its implication for prognosis. A total of 116 breast cancer patients were collected for peripheral blood. Leukocyte DNA was extracted to test germ-line mutation of BRCA1. Cancer tissues were also extracted for DNA, from which somatic mutation of BRCA1 was tested, in addition to MLPA approach. From clinical and pathological parameters, their correlation with BRCA1 gene mutation along with patient prognosis wasanalyzed. In addition, Kaplan-Meier method was used to analyze the survival rate. The overall mutation rate of BRCA1 gene in patients is 20.7%. Among those the mutation rate was 57.1% in all patients with inherited breast/ovarian cancer, significantly higher than those rates in sporadic breast cancer. Further analysis of clinical pathology parameters revealed no significant correlation between BRCA1 gene mutation and patient's age, menstrual status and metastasis. Kaplan-Meier method analyzed patient's diagnosis showed better RFS and OS within five years in patients with BRCA1 mutation compared to wild type ones. By sequencing and MLPA test, we found significantly higher mutation rate of BRCA1 gene in those patients with family history of breast/ovarian cancer compared to those in sporadic cancer patients. Meanwhile those breast cancer patients with BRCA1 mutation had more favorable prognosis.

Keywords: Breast cancer, BRCA1, gene mutation, RFS, OS, prognosis

Introduction

Breast cancer is the rank one malignant tumor in females worldwide [1]. In US there are about 230 000 women diagnosed as breast cancer each year, occupying 29% of all female specific cancer [2]. Due to the advancement of early screening for breast cancer and treatment approach, both treatment efficacy and survival rate were remarkably increased [3, 4]. However, due to complex pathology of breast cancer, significant variance exists in prognosis and clinical treatment efficacy among breast cancer patients. Therefore, the study of the genes that are closely related with treatment efficacy and prognosis and structural/function change have significance for improving efficacy of breast cancer [5].

BRCA1 is one tumor suppressor gene that encodes one protein containing 1 863 amino acids. Previous study showed that BRCA1 gene participated in activation of gene transcription, DNA injury and repair, plus the regulation of cell apoptosis and cycle [6]. The existence of hereditary pathological mutation in BRCA1 gene elevates the risk of breast cancer and ovarian cancer [7]. However, only 10% of all breast cancer patients carry such hereditary mutation [8]. Few studies have been performed regarding the incidence of BRCA1 gene mutation in sporadic breast cancer patients, nor did its role in occurrence or progression of breast cancer, or in the treatment/prognosis of patients [9]. Therefore this study aimed to investigate the mutation of BRCA1 gene in sporadic breast cancer patients, and its implication in patient's clinical treatment and prognosis.

Research objects and methods

Major equipment and reagent

Blood DNA extraction kit (Qiagen, US); Tissue DNA extraction kit (Qiagen, US); Ultra-micro

spectrometry (Thermo, US); Alkaline phosphatase (TAKARA, Japan); Nucleotide exonuclease (TAKARA, Japan); Big Dye II (ABI); BRCA-MLPA test reagent (MRC, US); ABI 3500 gene analyzer (ABI, US).

Research objects

A total of 116 breast cancer patients (average age = 51±6.2 years) who were admitted in Nanfang Hospital, Southern Medical University from March 2014 to March 2016. All patients were diagnosed with primary breast cancer by tissue biopsy and chest CT. All patients received chemo- or radio-therapy before recruitment. Breast cancer tissues were collected from patients. Fresh tissues were snap-frozen in liquid nitrogen for further experiments. 30 healthy volunteers (average age = 49±7.3 years old) were recruited meantime. This study was approved and supervised by the ethical committee. All involved individuals have signed consent forms.

Test of germ-line mutation

10 mL peripheral blood samples were collected from fasted patients by EDTA-containing tubes. Leukocytes were separated by centrifugation of blood samples. Blood DNA kit (Qiagen) was used to extract DNA samples from leukocytes. UV spectrometry was used to quantify DNA. Specific primers for exon sequence of BRCA1 gene was used to amplify the whole exon sequence of BRCA1 gene. PCR conditions were: 95°C pre-denature for 10 min, followed by 35 cycles each containing 95°C 30 s, 62°C 30 s and 72°C 60 s. PCR products were processed in 0.1 U shrimp alkaline phosphatase (SAP) and 0.1 U exonuclease (EXO) to remove unbounded nucleotide and non-specific amplification products. PCR products after digestion were then diluted in ultrapure water with 1:5 ratio. PCR sequencing was then performed using 1 μL diluted products. PCR sequencing was performed using Big Dye II and Taq polymerase under the following conditions: 95°C 3 min, followed by 32 cycles each containing 95°C 30 s, 50°C 30 s and 60°C 3 min, ended by 72°C elongation for 10 min. PCR sequencing products were precipitated by 35 μL absolute ethanol and centrifugation to remove excess ethanol. After air drying, DNA products were solved in 10 μL formamide. The mutation of BRCA1 gene was then analyzed on ABI 3500 analyzer [10].

MLPA assay for BRCA1 gene mutation

To test functional abnormality of BRCA1 caused by the heterogeneous deletion of long fragment, multiplex ligation-dependent probe amplification (MLPA) was used to test BRCA1 gene sequences in all research objects, using SALSA MLPA P002 BRCA1 mixed probes targeting MLPA of BRCA1 gene. The test procedures were: 5 μL leukocyte DNA was firstly denatured at 98°C for 5 min and was cooled down to 25°C. 1.5 μL BRCA1 probe mixture and 1.5 μL hybridization buffer were added into DNA samples. The hybridization was performed at 95°C for 1 min followed by 60°C incubation for 16 h. After hybridization, 25 μL ultrapure water, 3 μL Buffer A and 3 μL Buffer B, plus 1 μL ligase were sequentially added into hybridization products. The ligation was carried out at 54°C for 15 min followed by 98°C for 5 min. After cooling down to room temperature, 7.5 μL ultrapure water, 2 μL SALSA PCR Primer and 0.5 μL SALSA polymerase were sequentially added for mixture, followed by PCR under the following conditions: 95°C for 30 s, 60°C for 30 s and 72°C for 60 s for 35 cycles, followed by 72°C elongation for 20 min. The product was saved under 15°C. 0.5 μL product and 9 μL formamide were then mixed with 0.5 μL LIZ-600 for fragment analysis on ABI 3500 gene analyzer [11].

MLPA results of BRCA1 gene in all research objects were analyzed by Cofalyser (www.mlpa.com). Results were presented as relative peak (RP) and relative peak ratio (RPR). Deletion or repeated mutation of BRCA1 gene was identified when RPR value was less than 0.7 or higher than 1.3.

Analysis of somatic cell mutation of BRCA1 gene

Somatic DNA was extracted by DNA extraction kit (Qiagen) from breast cancer patients. The sequencing and MLPA analysis of BRCA1 gene were performed as abovementioned.

Statistical analysis

SPSS20.0 software was used for statistical analysis. Analysis of variance (ANOVA) or t-test

Table 1. Mutation of BRCA1 gene in breast cancer patients

Germ-line mutation		Somatic cell mutation	
Mutation type	N	Mutation type	N
60delAG	6	S451X (1352C>G)	1
M1775R (5324T>G)	2	E1134X (3400G>T)	1
C61G (181T>G)	3		
S451X (1352C>G)	4		
E1134X (3400G>T)	4		
E29X (87G>T)	2		
Del Exon 18	1		

Table 2. BRCA1 gene mutation in healthy individuals

Germ-line mutation		Somatic mutation	
Mutation type	N	Mutation type	N
M1775R (5324T>G)	2		

was used for comparison between groups. The representativeness of samples was tested by Hardy-Weinberg equilibrium. Both prognosis and survival rate were predicted by Kaplan-Meier product limit method. The correlation between BRCA1 gene mutation and prognosis of breast cancer patients was analyzed by univariate logistic regression. Results were presented as odd ratios (ORs) and 95% confidence intervals (Cis). A statistical significance was defined when $P<0.05$.

Results

Mutation of BRCA1 gene in breast cancer patients

Mutations of BRCA1 gene of both somatic and germline cells were studied by sequencing on all research objects. We found a total of 6 types of germ-line mutation and 2 types of somatic cell mutations in BRCA1 gene among all breast cancer patients. 22 patients had germ-line mutation, while 2 patients had somatic mutation in BRCA1 gene. In all 30 healthy control people, one of them was found to have germline mutation of BRCA1 gene. Detailed mutation type and patient number were shown in **Tables 1** and **2**. Patients with heterogeneous exon 18 deletion showed no mutation by sequencing, but showing significantly lower probe signals specific for exon 18. Therefore, those patients had deletion of exon 18 of BRCA1 gene (**Figure 1**).

Correlation between BRCA1 gene mutation and clinical parameters in breast cancer patients

We analyzed all clinical information of research subjects as shown in **Table 2**. Among all 116 breast cancer patients, 24 of them had somatic or germ-line mutation. A total of 35 patients had family history of breast cancer or ovarian cancer. Among those 16 patients had at least one close relative(s) who were diagnosed as breast/ovarian cancer. 20 out of 35 patients had BRCA1 gene mutation, occupying 83.3% of all patients. From **Table 3** we known no significant correlation between patient's age, menstrual status, TNM stage of breast cancer, lymph node metastasis and distal metastasis ($P>0.05$ in all cases). However, in those patients with family history of breast/ovarian cancer, their mutation rate of BRCA1 gene was significantly that that of sporadic breast cancer patients ($P<0.05$).

BRCA1 gene mutation and prognosis of breast cancer patients

A 14-month follow-up was performed on all research subjects. With all 116 individuals, 43 of them had recurrence (37.1% rate). 49 patients died during the period (42.2% mortality). The survival rate of all included patients was analyzed and shown in **Tables 4** and **5**, from which one can analyze the progression free survival (RFS) within five years between breast cancer patients with mutant and wild type forms of BRCA1 gene. We found that 5-year RFS of BRCA1 mutant breast cancer patients was 75%, which was significantly higher than wild type patients ($P<0.05$). The 5-year overall survival (OS) rate in breast patients with mutant BRCA1 gene was 70.8%, which was not different from that in wild type patients ($P>0.05$).

Kaplan-Meier product limit method was used to predict RFS and OS of breast cancer patients. As shown in **Figure 2**, breast cancer patients with BRCA1 mutation had significantly higher 5-year RFS and 5-year OS compared to those of wild type BRCA1 patients.

Discussion

Previous studies showed the close correlation between BRCA1 genotype and occurrence of breast cancer. The inheritable mutation of

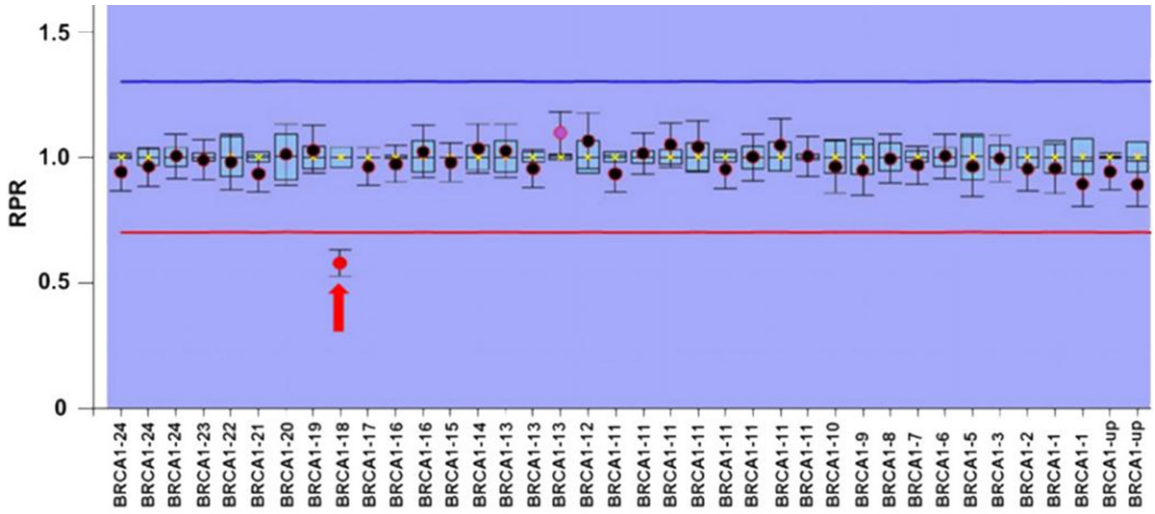

Figure 1. Illustration of exon 18 deletion in BRCA1 gene.

Table 3. Correlation between BRCA1 gene mutation and clinical parameters

Pathological feature	BRCA1 mutant		BRCA1 wild type		*P* value
	N	Ratio	N	Ratio	
Age (year)					
≥50	14	12.1%	60	51.7%	>0.05
<50	10	8.6%	32	27.6%	
Menopause					
Yes	13	11.2%	35	30.2%	>0.05
No	11	9.5%	57	49.1%	
Family history					
Yes	20	17.2%	15	36.2%	<0.05
No	4	3.4%	77	44.0%	
TNM stage					
I+II	13	11.2%	57	49.1%	>0.05
III	11	9.5%	35	30.2%	
Lymph node metastasis					
Yes	5	4.3%	22	19.0%	>0.05
No	19	16.4%	70	60.3%	
Distal metastasis					
Yes	7	6.0%	23	19.8%	>0.05
No	17	14.7%	69	59.5%	

BRCA1 gene is one important independent risk factor [12]. This is mainly due to the important role of BRCA1 gene in repairing of DNA mismatch. The dysfunction of BRCA1 may cause disruption of cellular DNA mismatch and repair mechanism, thus elevating the incidence of malignant tumors including breast cancer and ovarian cancer [13, 14]. This study firstly tested status of BRCA1 gene in breast cancer patients by sequencing and MLPA approach to collect all mutant information. We then statistically analyzed the correlation between BRCA1 gene mutation and clinical pathological indexes including patient's age, menstrual status, TNM stage, family history, lymph node metastasis and distal metastasis. Finally we analyzed the difference of 5-year RFS and OS between breast cancer patients with mutant or wild type form of BRCA1 gene.

We found that the incidence of germline and somatic mutation was 20.7% in all breast cancer patients. Meanwhile, we also showed that 83.3% of BRCA1 mutant carriers had family history of breast cancer or ovarian cancer, whilst only 16.7% BRCA1 mutant carriers were sporadic patients of breast cancer, indicating significant difference. Statistical analysis also found correlation between BRCA1 gene mutation and family history ($P<0.05$), but not with patient's age, TNM stage, lymph node metastasis and distal metastasis. Previous studies revealed significantly higher mutation rate of BRCA1 gene in breast cancer patients with family history of breast/ovarian cancer compared to those in sporadic cancer patients [15, 16], as consistent with this study.

The relationship between prognosis of breast cancer patients and BRCA1 gene mutation showed better 5-year RFS and OS in patients

Table 4. BRCA1 gene mutation and RFS of breast cancer patients

Pathological features	N	Recurrence	RFS	95% CI	*P* value
Age (years)					
≥50	74	25	66.2%	(51.3%, 84.7%)	>0.05
<50	42	18	57.1%	(38.7%, 70.1%)	
Menopause					
Yes	48	19	60.4%	(46.7%, 84.1%)	>0.05
No	68	24	64.7%	(42.7%, 72.5%)	
Family history					
Yes	35	26	25.7%	(19.1%, 54.7%)	<0.05
No	81	17	79.0%	(61.9%, 89.6%)	
TNM stage					
I+II	70	19	72.9%	(61.7%, 89.0%)	<0.05
III	46	24	47.8%	(19.1%, 54.7%)	
BRCA1 mutation					
Mutant	24	3	87.5%	(68.9%, 98.2%)	<0.05
Wild type	92	40	56.5%	(39.3%, 65.7%)	

Table 5. BRCA1 gene and OS of breast cancer patients

Pathological features	N	Recurrence	RFS	95% CI	*P* value
Age (years)					
≥50	74	28	62.2%	(45.6%, 83.7%)	>0.05
<50	42	21	50.0%	(39.1%, 71.0%)	
Menopause					
Yes	48	21	56.3%	(41.3%, 82.9%)	>0.05
No	68	28	58.8%	(42.9%, 72.6%)	
Family history					
Yes	35	27	22.9%	(19.3%, 50.7%)	<0.05
No	81	22	72.8%	(61.6%, 89.8%)	
TNM stage					
I+II	70	20	71.4%	(62.9%, 88.6%)	<0.05
III	46	29	37.0%	(20.1%, 52.7%)	
BRCA1 mutation					
Mutant	24	5	79.2%	(67.9%, 97.5%)	<0.05
Wild type	92	44	52.2%	(39.6%, 58.5%)	

carrying mutant forms of BRCA1 gene, indicating better prognosis in breast cancer patients carrying BRCA1 gene mutation. Currently lots of medical researchers believed that BRCA1 genotype might provide significant implication for patient's treatment, in addition to its role to evaluate the risk of individuals for breast cancer or ovarian cancer [17, 18]. Clinical study has confirmed that PARP1 inhibitor could effectively elongate overall survival period of breast cancer patients having BRCA mutants [19]. Moreover, other study also showed better treatment efficacy in breast cancer patients carrying BRCA mutation after surgery or chemo-therapy, as compared to BRCA wild type patients. These data all suggested that inheritable mutation of BRCA1 gene elevated the risk of breast cancer in individuals, which, however, had better prognosis and longer survival span [20, 21].

This study performed sequencing and MLPA assays on BRCA1 gene in breast cancer patients to reveal the relationship between mutation of BRCA1 gene and clinical pathological parameters of patients, followed by the analysis of breast cancer patients with BRCA1 mutation. Our results revealed the mutation pattern of BRCA1 gene in breast cancer patients to some extents, in addition to patient's prognosis. Meanwhile the combined approach including both sequencing and MLPA can reflect BRCA1 gene mutations with different times. With continuous advancement of biotechnology, elevated speed and accuracy of DNA sequencing, in combined with targeted treatment [22], more treatment opportunities can be provided for breast cancer patients.

Conclusion

By sequencing and MLPA test, we found significantly higher rate of BRCA1 gene mutation in breast cancer patients having family history of breast cancer and ovarian cancer. Meanwhile we also showed better prognosis in breast cancer patients carrying BRCA1 mutation.

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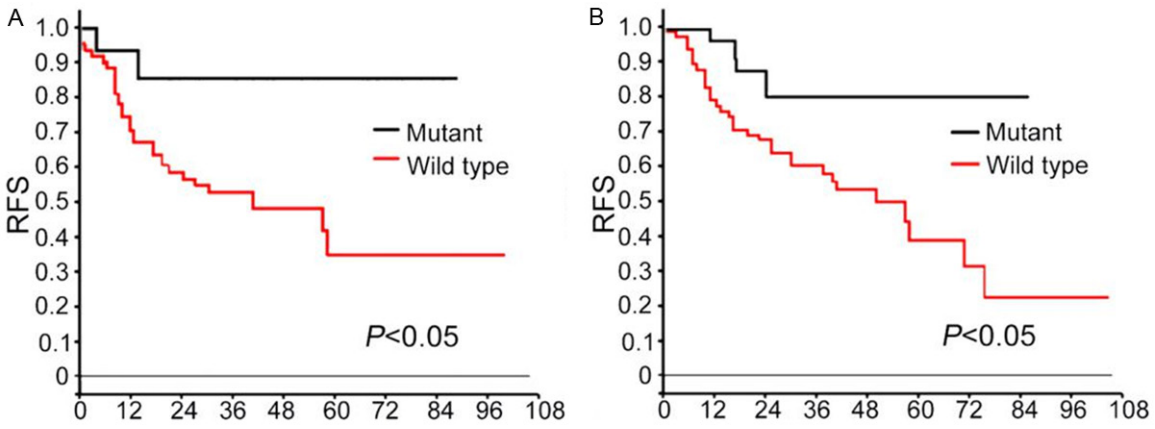

Figure 2. BRCA1 gene mutation and patient's survival rate. A. 5-year RFS of breast cancer patients; B. 5-year OS of breast cancer patients.

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Disclosure of conflict of interest

None.

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