RESEARCH ARTICLE

XRCC1 and ADPRT Polymorphisms Associated with Survival in Breast Cancer Cases Treated with Chemotherapy

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Abstract

Aim: To investigate whether XRCC1 and ADPRT polymorphisms might be associated with outcomes of breast cancer. Methods: A prospective study was conducted with a total of 335 breast cancer patients undergoing chemotherapy consecutively collected from Jan. 2005 to Jan. 2008. Genotyping of XRCC1 and ADPRT polymorphisms was conducted by PCR-RFLP assay. Results: All 335 patients were followed up until death or the end of Jan. 2012, with a median follow-up period of 38.8 (2-64) months. It was shown that the variant genotype of XRCC1 399Gln/Gln was strongly significantly associated with a decreased risk of death from breast cancer, with an HR (95% CI) of 0.52 (0.28-0.91). Similarly, individuals carrying the ADPRT 762Ala/Ala demonstrated longer survival compared to ADPRT 762 Val/ Val, with an HR (95% CI) of 0.58 (0.31-0.97). Individuals with combination genotypes of XRCC1 399Gln allele and ADPRT 762Ala/Ala presented with a longer survival, the HR (95% CI) being 0.56 (0.32-0.97). Conclusion: We found a significant association between XRCC1399Gln/Gln and ADPRT 762Ala/Ala polymorphisms and clinical outcomes. These two genotypes could be used as a surrogate markers of clinical outcome in glioma cases receiving chemotherapy.

Keywords: Breast cancer - XRCC1 - ADPRT - polymorphism - outcome

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Introduction

Breast cancer is the most common cancer in women. It is estimated 1.38 million new cancer cases diagnosed in 2008 (23% of all cancers), and ranks second overall (10.9% of all cancers). It is now the most common cancer both in developed and developing regions with around 690 000 new cases estimated in each region (population ratio 1:4) (IARC, 2008). In China, breast cancer is the most common type of cancer among women, with an incidence of 14.2/105, it is also one of the most leading causes of death in Chinese women (IARC, 2008). Although the major prognostic factors for breast cancer is the cancer stage, there are differences in survival among breast cancer patients with similar disease stages. Recently, a number of studies have revealed that polymorphisms in certain genes, especially DNA repair genes, have a role in tumor response to chemotherapy and thus survival variability among individual patients (Ang et al., 2011; Giovannetti et al., 2011; Liu et al., 2012; Wu et al., 2012). Therefore, better understanding of prognostic factors would be helpful for individualizing breast cancer treatment.

The DNA repair gene products can recognize and repair damaged DNA induced by autologous cells or environmental agents. Impaired DNA repair ability can increase genomic instability and tumorigenesis. Thus, single nucleotide polymorphisms in DNA repair genes can impair DNA repair mechanisms (Qiao et al., 2002). Studies have shown that DNA repair gene polymorphisms may promote cancer progression and thus develop more aggressive tumors (Kudo et al., 2012). These DNA repair gene polymorphisms may lead to different responses to platinum-based chemotherapy by affecting the gene products' capacity to remove anthracycline -DNA adducts (Kang et al., 2012).

X-ray repair cross-complementing group 1 (XRCC1) and adenosine diphosphate ribosyl transferase (ADPRT) are two important genes in the DNA repair process. ADPRT specifically binds to DNA strand breaks, where it is autoactivated and recruits the XRCC1-Ligase IIIα complex to stimulate BER, causing XRCC1 to interact with ADPRT to recruit other partner proteins such as DNA polymerase β (Ploβ) to execute BER (Masson et al., 1998; Keith et al., 2003). For this reason, functional variants of XRCC1 and ADPRT may alter BER function and affect the outcome of breast cancer. Thus, we conducted a prospective study in 516 breast cancer patients to investigate whether XRCC1 and ADPRT polymorphisms were associated with outcomes of breast cancer.

Materials and Methods

Study Subjects

A prospective study design was conducted in our study.

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Breast cancer patients were consecutively recruited at the First Affiliated Hospital of Sun-Yat Sen University from Jan. 2005 to Jan. 2008. All hospital patients with newly diagnosed primary breast cancer were asked to participate within one month after diagnosis, and all cases were histologically or clinically confirmed. Informed consent was obtained before each interview and blood draw. A total of 361 patients were included in our hospital, and only 335 patients agreed to participate in this study, with a participation rate of 92.8%.

Patients who received anthracycline-based chemotherapy were included in our study. Anthracycline-based chemotherapy included cyclophosphamide (C), the anthracycline agent (E or A), and/or 5-fluorouracil (F), (CEF and CAF regimens) combined with radiotherapy.

Genotyping

DNA samples were obtained from stored blood samples using Qiagen standard protocols (Shanghai, China). Genotyping for XRCC1 Arg194Trp, XRCC1Arg399Gln and ADPRT Val762Al polymorphisms was done by PCR-RFLP assay according to a previous study (Guo et al., 2008). The forwards and reverse primers of XRCC1 Arg194Trp were 5'-GCC AGG GCC CCT CCT TCA A-3' and 5'-TAC CCT CAG ACC CAC GAG T-3', respectively; For XRCC1 Arg399Gln, primers were 5'-TTG TGC TTT CTC TGT GTC CA-3' and 5'-TCC TCC AGC CTT TTC TGA TA-3', respectively; For XPD Lys751Gln, they were 5'-GCC CGC TCT GGA TTA TAC G-3' and 5'-CTA TCA TCT CCT GGC CCC C-3', respectively. Polymerase chain reaction was carried by an initial melting step of 5 min at 94°C, 35 cycles of denaturation for 30s at 94°C, annealing for 30 s at 63°C, and extension for 45s at 72°C, followed by a 5 min final extension at 72°C. The PCR products were then digested with restriction endonucleases. For quality control, 10% of samples was randomly selected for sequencing. These results of the quality control analysis were confirmed to 100% concordance.

Statistical Analysis

All analyses were performed with SPSS Version 16.0 software (SPSS Inc., Chicago, IL, USA). The Kaplan-Meier method was adopted to estimate survival curves, and the log-rank test was used to compare patients' survival time between genotype groups. Cox's proportional hazard model was used to assess the associations between XRCC1 and ADPRT polymorphism and survival. Primary death from breast cancer was defined as the failure event, and the time of survival as the time between diagnosis and death. The cause of death was determined by specialists based on clinical documents from hospital or reports by patients' family members. If a patient died of causes other than breast cancer, she was censored at the date of death. All surviving patients were censored at the date of last follow-up. Statistical significance was defined as a two-sided P value of less than 0.05.

Table 1. Characteristics of Included Cases in Our Studies

Variables	Cases	%	Patient deaths	%	Five-year survival rate (%)	P value
	N=335		N=159			
Age (mean±SD, years)	46.4±6.2		47.3±5.9			
<45	132	39.5	58	36.6	56	0.53
≥45	203	60.5	101	63.4	50.3	
Menopausal status						
Premenopausal	150	44.9	66	41.7	55.9	0.49
Postmenopausal	185	55.1	93	58.3	49.8	
First-degree family history of breast cancer						
No	305	91.1	138	86.5	54.9	0.89
Yes	30	8.9	21	13.5	28	
Lymph node metastasis(UICC)						
Node-negative	211	63.1	80	50.5	62	<0.05
Node-positive	124	36.9	79	49.5	36.3	
Tumor size, mm						
0-20	136	40.5	49	30.6	64.1	<0.05
21-50	106	31.7	56	35.5	46.8	
>50	71	21.3	42	26.5	41	
Unknown	22	6.5	12	7.4	46	
Malignancy grade						
I	82	24.5	30	18.7	63.8	0.19
II	133	39.6	68	42.6	48.9	
III	82	24.6	45	28.6	44.8	
Unknown	38	11.3	16	10.1	57.6	
Estrogen receptor status						
Negative	172	51.4	74	46.3	57.2	0.47
Positive	163	48.6	85	53.7	47.6	
Progesterone receptor status						
Negative	159	47.4	73	45.6	54.3	0.75
Positive	176	52.6	86	54.4	50.9	

Results

All 335 patients were followed up until death or the end of Jan. 2012, with a median follow-up period of 38.8 (2-64) months. During the follow-up, a total of 159 patients died. The mean age of patients was 46.4±6.2 years old. Most of the patients were grade II to III of malignancy. Patients without lymph node metastasis presented a longer survival rate than those with metastasis. Individuals with a smaller tumor size showed a longer survival rate.

Table 2 showed the frequencies of XRCC1 Arg194Trp, XRCC1Arg399Gln and ADPRT Val762Ala in cases. The associations between the SNPs and the risk of death from breast cancer were analyzed by Cox's proportional hazard model. For XRCC1Arg399Gln, the variant genotype Gln/Gln was strongly significantly associated with a longer survival when compared with the wide-type Arg/Arg, with HR (95% CI) of 0.52 (0.28-0.91). Individuals carrying the ADPRT 762Ala/Ala was associated with a better clinical

Table 2. Associations Between XRCC1 and ADPRT Gene Polymorphisms and Breast Cancer Prognosis

Genotypes	Cases	%	Patient deaths	%	Median overall survival (months)	HR (95% CI)[1]	P
	N=335		N=159				
XRCC1 Arg194Trp							
Arg/Arg	239	71.4	121	75.8	32.4	1.0 (Ref.)	-
Arg/Trp	69	20.5	30	18.6	34.5	0.85 (0.51-1.42)	0.53
Trp/Trp	27	8.1	9	5.6	38.8	0.66 (0.26-1.50)	0.29
XRCC1Arg399Gln							
Arg/Arg	152	45.3	83	52.4	31.5	1.0 (Ref.)	-
Arg/Gln	106	31.5	53	33.4	35.7	0.86(0.51-1.39)	0.68
Gln/Gln	78	23.2	23	14.2	41.3	0.52 (0.28-0.91)	<0.05
ADPRT Val762Ala							
Val/Val	136	40.6	76	47.6	33.5	1.0 (Ref.)	-
Val/Ala	132	39.3	61	38.5	33.6	0.83 (0.54-1.23)	0.35
Ala/Ala	67	20.1	22	13.9	42.4	0.58 (0.31-0.97)	<0.05

[1]Adjusted for age, lymph node metastasis (UICC) and tumor size

Table 3. Association of Combination of XRCC1Arg399Gln and ADPRT Val762Ala Genotypes with Breast Cancer Survival

Genotypes	No. of patients N=335	%	Patient deaths N=159	%	Median overall survival (months)		HR (95% CI)[1]	P
XRCC1Arg399Gln and ADPRT Val762Ala								
Arg/Arg and Val/Val	73	21.8	51	32.1	31.5		1.0 (Ref.)	-
Arg/Arg and Ala allele	79	23.6	32	20.1	34.2		0.58 (0.36-1.08)	0.052
Gln allele and Val/Val	63	18.8	25	15.7	33.9		0.61 (0.33-1.13)	0.061
Gln allele and Ala allele	120	35.8	51	32.1	39.8		0.56 (0.32-0.97)	<0.05

[1]Adjusted for age, lymph node metastasis (UICC) and tumor size

outcome and longer survival compared to ADPRT 762 Val/ Val, with HR (95% CI) of 0.58 (0.31-0.97). However, we did not find a significant decreased risk of death from breast cancer among patients with XRCC1 194Trp/Trp genotype.

We further identified the combination effect of XRCC1Arg399Gln and ADPRT Val762Ala genotypes on the prognosis of breast cancer. The results showed individuals with combination genotypes of XRCC1 399Gln allele and ADPRT 762Ala/Ala presented decreased risk of death from breast cancer, with the HR (95% CI) of 0.56 (0.32-0.97).

Discussion

There are increasing evidences that the DNA repair genes polymorphisms induce inter-individual variability in chemotherapy, and the reduced DNA capacity from DNA repair genes contributed to the longer survival after treatment with chemotherapy in various cancers, such as non-small cell lung cancer, colorectal cancer, pancreatic cancer, and head and neck squamous cell carcinoma (Ang et al., 2011; Giovannetti et al., 2011; Liu et al., 2012; Wu et al., 2012). Thus, the use of DNA repair gene polymorphisms as predictive or prognostic markers holds clinical promise. However, evidence linking DNA repair gene polymorphisms and breast cancer survival in Chinese populations is lacking. The current study demonstrated that breast cancer patients with XRCC1399Gln/Gln polymorphisms had a longer survival time than XRCC1 399Arg/Arg genotype, carriers with and ADPRT 762Ala/Ala had relatively shorter survival times when compared with ADPRT 762Val/Val. However, no association was found between XRCC1 Arg280His and breast cancer prognosis.

The XRCC1 Arg399Gln and XRCC1 Arg194Trp polymorphisms are the two most common sequence variants among the three polymorphisms. Our study showed the XRCC1 Arg399Gln and XRCC1 Arg194Trp could affect the clinical outcome in breast cancer patients. The possible explanation is that they enhance DNA repair capacity. XRCC1 gene is thought to be involved in DNA single-strand break repair, and also plays an important role in the BER pathway (Brem et al., 2005; Nazarkina et al., 2007). The gene polymorphism in codon 399 may be related to DNA repair, and likely has an effect on protein function (Savas et al., 2004). Several studies have found an association of XRCC1-399 with the risk of various cancers, such as breast cancer, colorectal cancer, lung cancer, thyroid carcinoma, gastric cancer and breast cancer (Engin et al., 2011; Fard-Esfahani et al., 2011; Xu et al., 2011; Raabe et al., 2012; Zhao et al., 2012). Previous study showed an improved survival rate for breast cancer patients with XRCC1-399 Gln/Gln receiving chemotherapy (Bewick et al., 2009; Rodrigues et al., 2011). One study conducted in Canada reported XRCC1 Arg399Gln was an independent predictor for progression (Bewick et al., 2009), and another study showed a non-significant decreased risk of survival for breast cancer (Rodrigues et al., 2011). Similar results were found for the survival of non-small-cell lung cancer, colorectal cancer, gastric cancer and ovarian cancer (Khrunin et al., 2010; Engin et al., 2011; Tahara et al., 2011; Liao et al., 2012).

We found a light decreased survival of breast cancer among patients with ADPRT 762Ala/Ala genotypes, which were consistent with a previous analysis (Gonçalves et al., 2011). This Meta-analysis showed the over-expression of PARP1 may be associated with tumor sensitivity to cytotoxic treatment, essentially cyclophosphamide and/or anthracycline-based chemotherapy (Gonçalves et al., 2011). Our study is consistent with the recently reported positive correlation between PARP1 protein expression and response to neoadjuvant chemotherapy (Loibl et al., 2010; Von et al., 2010). Moreover, ADPRT polymorphism is reported to have a significant association with the prognosis of various human cancers, such as ovarian cancer, lung cancer and glioma (Nowsheen et al., 2011; Kase et al., 2011; Wysham et al., 2012). Further larger studies are needed to validate the association between ADPRT Val762Ala polymorphism and breat cancer prognosis.

In conclusion, this study is the first one to evaluate the associations between XRCC1 and ADPRT and breast cancer outcomes in a Chinese population. We found a significant association between XRCC1399Gln/Gln and ADPRT 762Ala/Ala polymorphisms and clinical outcomes. Further prospective studies incorporating larger numbers of patients are needed to validate these associations.

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