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Original Article

Correlation of rs1799793 polymorphism in ERCC2 and the clinical response to platinum-based chemotherapy in patients with triple negative breast cancer

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Abstract: Background: Polymorphisms of DNA repair genes may affect the repair capacity of DNA damages and cause different responses towards chemotherapy. Excision repair cross-complementing group 2 (ERCC2) plays an important role in the nucleotide excision repair. Objectives: The aim of this study was to investigate the association between ERCC2 single nucleotide polymorphisms (SNPs) and the response to platinum-based chemotherapy among patients with triple negative breast cancer. Methods: In total, 60 triple negative breast cancer patients treated with platinum-based chemotherapy were studied. The clinical, pathological and treatment data of them were collected. Sequenom's MassARRAY system was used in the detection of the SNPs of ERCC2. Finally, the association between genotypes and different clinical responses among patients was analyzed. All of the patients received a platinum-based chemotherapy for 4 cycles in median and achieved an overall response rate of 66.7%, showing a comparative good response towards platinum-based chemotherapy among triple negative breast cancer. Fifty-three of the 60 patients had got the results of ERCC2 rs1799793 polymorphisms after MassARRAY detection. Results: The proportion of GG genotype and GA genotype was 81.1% and 18.9% respectively. The response rate of the rs1799793 GG genotype group was 69.8%, while the GA genotype group only had a response rate of 30.0%. It turned out that the GG genotype was associated with better response towards platinum-based chemotherapy (P=0.030). Conclusions: ERCC2 rs1799793 polymorphism may be associated with the clinical sensitivity of platinum-based chemotherapy and could be a potential predictive biomarker for triple negative breast cancer patients treated with platinum compounds.

Keywords: Excision repair cross-complementing group 2, single nucleotide polymorphisms, triple negative breast cancer, platinum-based chemotherapy, response

Introduction

Triple-negative breast cancer refers to any breast cancer that does not express the genes for estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (Her2) [1]. It possesses specific biological behaviors and clinicopathological characteristics. It is highly invasive with poor prognosis and is likely to recur locally and metastasis distantly. endocrinotherapy and anti-HER-2 target treatment are invalid. At present, no standard therapy has bee available. Platinum drugs could crosslink with DNA double strands, leading to DNA double strands break, inhibiting DNA replication and transcription and eventually causing carcinogenesis and cell death. Patients with triple-negative breast cancer are abnormal in a variety of genes and signal transduction pathways and have defects in DNA repair. Consequently, platinum drugs might be more efficacious in treatment of triple negative breast cancer compared with alternative agents [2]. Previous findings revealed that DNA repair genetic polymorphism may affect DNA repair ability, thereby impacting the efficacy of individualized chemotherapy [3].

Excision repair cross-complementing group 2 (ERCC2) coding protein is involved with nucleotide excision repair and genetic transcription as evolutionary conservative DNA helicase, and

Table 1. PCR primer sequence of ERCC2 SNPs

SNPs	PCR primer
rs238406	
Forward primer	ACGTTGGATGCAGTACCAGCATGACACCAG
Reverse primer	ACGTTGGATGTACCTGTCCTGCCTCCCTC
rs1799793	
Forward primer	ACGTTGGATGTATCAGCGGCGACGGGGAG
Reverse primer	ACGTTGGATGCACCTGGCCAACCCCGTG

plays a pivotal role in DNA repair [4]. In this clinical trial, 60 patients with triple negative breast cancer underwent platinum-based chemotherapy and their clinicopathological data and follow-up information were analyzed, aiming to preliminarily to investigate the correlation between the single nucleotide polymorphisms of ERCC2 gene and the sensitivity of females with triple negative breast cancer towards platinum-based chemotherapy.

Materials and methods

Clinical data

Study subjects: All 60 subjects were diagnosed with advanced or locally advanced triple negative breast cancer and admitted to our hospital between July 2009 and July 2011. All included cases were diagnosed by histological or cytological examinations. Prior to treatment, measurable target lesions were detected by clinical or imaging tests. ECOG (Eastern Cooperative Oncology Group) scores ranged from 0 to 1. No severe damage was observed in vital organs. Blood routine examination, liver and renal function test and electrocardiogram yielded normal outcomes. The expected survival was longer than three months.

Treatment: All patients were treated with platinum-based chemotherapy for 2 to 3 cycles and their therapeutic efficiency was evaluated according to RECIST 1.0 (Response Evaluation Criteria in Solid Tumors).

Efficacy evaluation: Complete response (CR) and partial response (PR) were defined as being sensitive towards platinum-based chemotherapy. Stable disease (SD) and progressive disease (PD) were defined as be insensitive to platinum-based chemotherapy. CR and PR were utilized to calculate the overall efficacy.

Gene typing: A portion of 2 mL anticoagulation peripheral blood sample was collected from study subjects. Genomic DNA was extracted from peripheral blood using phenol-chloroform method. The extracted DNA sample was subject to quality testing by spectrophotometry, placed into 1.5 mL EP tube and stored in a freezer at -80°C for subsequent SNP typing analysis. The primer of PCR and single base was designed based upon the sequence of SNP sites (**Table 1**). Sequenom's MassARRAY system (Sequenom, U.S.) was employed to analyze the genotype of candidate SNP sites. For quality control, parallel repeated samples were established and blank samples were used as positive and negative samples during the detection of gene typing.

Statistical analysis

SPSS 13.0 software was utilized for data analysis. The correlation between ERCC2genotype and clinical efficacy was statistically analyzed by chi-square test. $P<0.05$ was considered as statistical significance.

Results

Clinical data

The median age was 46 years old (range: 26 to 66 years). Among 60 patients, 17 were accompanied by a family history of malignant tumors. Two patients suffered from carcinoma in situ, one pleomorphic carcinoma, two medullary carcinoma, one mixed ductal/lobular carcinoma and the remaining cases had infiltrative duct carcinoma. Clinical stages: two patients were in stage 0, four stage I, 28 stage II, 21 stage III and 5 stage IV. The median cycle of chemotherapy was four cycles, mainly consisting of combined chemotherapy. Thirty seven patients were treated with platinum drugs in combination with taxus agents, 11 platinum medication plus vinorelbine, 8 platinum drugs plus gemcitabine, 1 platinum drugs plus S1, 1 platinum drugs plus pemetrexed disodium and 2 were treated with platinum drugs alone. Fifteen cases were treated with neoadjuvant chemotherapy, 34 first-line chemotherapy, 8 second-line chemotherapy and the remaining 3 were third-line or above chemotherapy.

Genotyping

ERCC2 rs238406 and rs1799793 genotypes were distributed in Hardy-Weinberg equilibrium, suggesting that the cases enrolled in this study

Table 2. The distribution of clinicopathologic features in ERCC2 rs238406 genotypes

Characteristics	rs238406 genotype		*P* value
	GG	GT + TT	
Age/year	10	15	0.385
≤40	10	24	
>40			
Family history of malignancy			
Yes	2	14	0.033
No	18	25	
Histological subtype			
Invasive ductal carcinoma	17	37	0.322
Others	3	2	
Clinical stage			
0~II	11	22	0.915
III-IV	9	17	

Table 3. The distribution of clinicopathologic features in ERCC2 rs1799793 genotypes

Characteristics	Rs1799793 genotype		*P* value
	GG	GA	
Age/year	17	7	0.154
≤40	26	3	
>40			
Family history of malignancy			
Yes	12	2	1.000
No	31	8	
Histological subtype			
Invasive ductal carcinoma	39	9	1.000
Others	4	1	
Clinical stage			
0~II	22	5	1.000
III-IV	21	5	

are population-representative (χ^2=2.772, P=0.096; χ^2=0.575, P=0.448). Among all subjects, 59 patients presented with allelotype detected in ERCC2 rs238406 with gene frequency of 33.9% GG (20/59), 39.0% GT (23/59) and 27.1% TT (16/59).

Fifty three patients had allelotype detected in ERCC2 rs1799793 with a gene frequency of 81.1% GG (43/53) and 18.9% GA (10/53). No statistical significance was noted in the ERCC2 polymorphism among patients of different ages, pathological types and clinical stages (P>0.05). Compared with the patients without a family history of malignant tumor, those with a family history of cancer had a higher frequency of GT + TT genotype (P=0.034), as illustrated in **Tables 2** and **3**.

Genotyping and platinum-based chemotherapy efficacy

Following chemotherapy, 9 patients had CR, 31 PR, 15 SD and the remaining 5 PD. The overall efficacy of the platinum-based therapy was 66.7%. Patients' age, pathological type, clinical stages and with/without a family history of malignant tumors were not correlated with the clinical efficacy of chemotherapy (P>0.05).

Among 20 patients with ERCC2 rs238406 GG, platinum-based chemotherapy was effective for 12 cases and ineffective for 8. For 39 subjects with ERCC2 rs238406 GT + TT, platinum-based chemotherapy was effective for 27 and ineffective for 12. The effective rates of chemotherapy in patients with GG and GT + TT were 60.0% and 69.2% with no statistical significance (P=0.478). For patients with ERCC2 rs1799793 GG, chemotherapy was effective for 30 cases and ineffective for 13. For those with ERCC2 rs1799793 GA, chemotherapy exerted an efficacy for 3 and was ineffective for 7 cases. The effective rates in two groups achieved 69.8% and 30.0% with a statistical significance (P=0.030). The patients carrying GG genotype were more sensitive towards chemotherapy compared with their counterparts carrying GA genotype, as shown in **Table 4**.

Discussion

ERCC2, also known as xeroderma pigmentosum group D, is located at human chromosome 19q13.3 and participates in the nucleotide excision repair (NER) and genetic transcription as a pivotal element. NER is one of the most vital pathway of DNA repair. ERCC2, as an important element of the repairing process, encodes proteins that possess ATP-dependence DNA helicase activity, initiates the DNA double strands from 5'→3' direction, allows injured specific nuclease cutting damaged DNA bilaterally and participates in DNA repair caused by various factors [4]. Certain sites of ERCC2gene polymorphism is not only associated with the susceptibility of head and neck cancer, prostate cancer, bladder carcino-

Table 4. The association between ERCC2 SNPs and the response to platinum-based chemotherapy among patients with triple negative breast cancer

Genotype	Response rate/%	Group		*P* value
		CR + PR	SD + PD	
rs238406				
GG	60.0	12	8	0.458
GT + TT	69.2	27	12	
rs1799793				
GG	69.8	30	13	0.031
GA	30.0	3	7	

ma and breast cancer, but also correlated with the sensitivity to platinum drugs [5].

At present, ERCC2 single nucleotide polymorphisms related studies have been frequently in Western population whereas seldom conducted in Chinese population. Most investigations focus upon the susceptibility of malignant tumors. The clinical efficacy of platinum-based therapy has been investigated in patients suffering from lung and colon cancer and rarely seen in breast cancer cases. Until now, no studies focusing on the correlation between ERCC2 gene polymorphism and clinical efficacy of platinum-based chemotherapy in treatment of triple negative breast cancer have been performed yet.

In ERCC2 gene, a total of 6 SNPs are located in the exon region and codon 156,321 and 751 are most commonly seen. codon 156 site (rs238406) is nonsense mutation, whereas codon 312 and 751 sites (rs1799793 and rs13181) are non-synonymous variants, which are able to cause changes in coding amino acids (Lys751Gln and Asp312Asn). Jian Gu et al [6] demonstrated that Lys751Gln polymorphism is associated with the clinical efficacy of oxaliplatin in treatment of advanced colon cancer in Chinese Han population. Park et al [7] retrospectively analyzed 73 patients with metastatic colon cancer who received 5-fluracil/oxaliplatin therapy and found that the cases with 751Lys/Lys genotype had the highest remission rate (P=0.015). However, codon 312 of SNP was not significantly associated with clinical efficacy.

The correlation between ERCC2 genotypes and the remission rate of platinum-based chemotherapy remains debated in patients with lung cancer. Majority of Asp312Asn polymorphism-related studies revealed that the levels of DNA adduct in Asn individuals is higher compared with those in Asp individuals. Asn has a lower ability to repair allele. Lys751Gln-related investigations demonstrated that Gln individuals have a higher level of DNA adduct and lower repair ability [4, 8]. A variety of studies demonstrated that rs13181 polymorphism is correlated with clinical efficacy while no significant association is found between rs1799793 polymorphism and clinical efficacy [7, 9]. Previous studies also indicated that ERCC2 polymorphism is not correlated with the sensitivity to chemotherapy [10-13]. In this study, the triple negative breast cancer patients carrying rs1799793 GG genotype present with a higher clinical efficacy of platinum-based chemotherapy compared with their counterparts carrying rs1799793 GA genotype, which is inconsistent with previous findings [14], probably because ERCC2 gene SNPs play different roles in varying types and subtypes of malignant tumors.

Currently, extremely rare studies have been conducted to analyze the correlation between ERCC2 genotype polymorphism and clinical efficacy of breast cancer. Whether rs1799793 acts as a sensitive index evaluating the efficacy of platinum-based chemotherapy in treatment of triple negative breast cancer remains to be further investigated by large sample size studies. Alternative DNA repair genes may be additionally required. SNP sample is convenient to collect and the detection procedure is simple, which serves as a sensitive index evaluating the efficacy and prognosis triple negative breast cancer, thereby promoting the individualized selection of drugs and enhancing patients' clinical benefits.

Disclosure of conflict of interest

None.

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