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Investigation of *ERCC1* and *ERCC2* gene polymorphisms and response to chemotherapy and overall survival in osteosarcoma

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ABSTRACT. We assessed the role of single nucleotide polymorphisms (SNPs) in *ERCC1* and *ERCC2* genes in the clinical outcomes for osteosarcoma patients receiving cisplatin-based treatment. A perspective study was conducted on 260 patients with osteosarcoma during 2010 and 2011. A polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay was used to assess the *ERCC1* rs11615 and rs3212986, and the *ERCC2* rs1799793 and rs13181 gene polymorphisms. After adjustment for clinical variables, we found that the CC genotype of *ERCC1* rs11615 was significantly associated with better response to chemotherapy (OR = 2.87, 95%CI = 1.24-6.97). Our study found that those carrying the CC genotype of *ERCC1* rs11615 had a longer overall survival compared with the TT genotype, and the OR (95%CI) was 0.35 (0.12-0.92). In conclusion, our results suggest

that the *ERCC1* rs11615 polymorphism might influence the response to cisplatin-based chemotherapy and affect the clinical outcome for osteosarcoma patients.

Key words: *ERCC1*; *ERCC2*; Chemotherapy; Overall survival; Osteosarcoma; Single nucleotide polymorphism

INTRODUCTION

Osteosarcoma is a rare bone cancer derived from mesenchymal tissues; it occurs in the distal femur, proximal tibia, and humeral metaphysis. Osteosarcoma commonly occurs in children and adolescents, with an annual incidence of about 3/1,000,000 (Picci, 2007; Mirabello et al., 2009; Ottaviani and Jaffe, 2009). Patients receiving chemotherapy are reported to have improved survival compared with those receiving only surgical treatment (Kuhelj and Jereb, 2005). Despite advances in chemotherapy, survival rates differ greatly among individual patients. It is reported that more than 50% of osteosarcoma patients have a poor clinical outcome, and about 30% relapse locally or develop metastases (Chou and Gorlick, 2006; Salinas-Souza, 2010). Previous studies have reported that genetic polymorphisms affecting mechanisms could influence the response to chemotherapy and chemotherapy-related toxicity, and the survival time of cancer patients. Therefore, identification of predictive markers could play an important role in helping drug selection and improving treatment outcomes in chemotherapy.

Cisplatin is a platinum analog chemotherapy drug and is frequently used to treat various types of cancer. It binds to DNA to form DNA adducts and both intrastrand and interstrand crosslinks, and inhibits DNA replication (Marsh et al., 2009). DNA repair mechanisms play an important role in influencing the response to cisplatin. The main cisplatin-induced DNA damage is intrastrand crosslinks, and nucleotide excision repair (NER) is the main mechanism for repairing the DNA damage. The two enzymes involved in this pathway that are most closely associated with resistance to cisplatin are excision repair cross-complementation group 1 (*ERCC1*) and excision repair cross-complementation group 2 (*ERCC2*). Single nucleotide polymorphisms (SNPs) of the *NER* gene are correlated with response to cisplatin-based treatment in several kinds of cancer (Quintela-Fandino et al., 2006; Khrunin et al., 2010; Mathiaux et al., 2011). We aimed to conduct a study to assess the role of SNPs of *ERCC1* and *ERCC2* in the clinical outcome of osteosarcoma patients receiving cisplatin-based treatment.

MATERIAL AND METHODS

Patients

A perspective study was conducted on 260 patients with osteosarcoma during 2010 and 2011 in the Second Peoples' Hospital of Liaocheng. Peripheral blood specimens and demographic, medical, and family histories were obtained from sequentially ascertained, unrelated patients. Osteosarcoma patients were newly diagnosed and histopathologically and independently confirmed by two gynecologic pathologists. The Ethical Committee of the Second Peoples' Hospital of Liaocheng approved the study protocols, and all participants provided written informed consent according to the Declaration of Helsinki.

Treatment outcome assessment

The clinical and pathological information was collected from medical records, including Enneking stage, tumor location, histological type, tumor metastasis, and family history of cancer. The tumor response to chemotherapy was assessed histologically based on percentage of necrosis according to the response evaluation criteria in solid tumors (Duffaud and Therasse, 2000). Event was defined as death from any cause. Overall survival (OS) was defined as the time from the date of chemotherapy to death from any cause. Patients were followed-up until death or the end of the study (December 2014).

DNA extraction and genotyping

The commercially available Qiagen Kit (Qiagen Inc., Valencia, CA, USA) was used to extract DNA from peripheral blood leukocytes. A polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay was applied to assess the *ERCC1* rs11615 and rs3212986, and *ERCC2* rs1799793 and rs13181 gene polymorphisms. PCRs were carried out in a Perkin-Elmer 9700 thermocycler with an initial denaturation step of 8 min at 94°C, followed by 30 cycles at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 1 min. PCR products were digested with *Nla*III restriction enzyme (New England Biolabs, Beverly, MA, USA). The resulting DNA fragments were electrophoresed on 3.5% agarose gel and visualized under UV light after ethidium staining.

Statistical analysis

We estimated the OR and 95% confidence interval for response rate (CR and PR) versus no response after chemotherapy. Survival duration was plotted using Kaplan-Meier curves and the prognostic values of each treatment based on the *ERCC1* and *ERCC2* gene polymorphisms were analyzed using a Cox log rank model, which was stratified according to center and adjusted for significant prognostic factors for survival (e.g., gender, age, histopathological type, disease stage, and family history of cancer). All analyses were performed using the IBM SPSS Statistics software, version 16.0 (IBM Corporation, Armonk, NY, USA). A P value less than 0.05 was considered to be statistically significant.

RESULTS

Patients' characteristics

The characteristics of the osteosarcoma patients are shown in Table 1. There were 116 females and 144 males, and their mean age was 18.40 ± 8.50 years. Of the 260 osteosarcoma patients, 188 were grade I-II, 20 had a family history of cancer, 133 were chondroblastic, 79 were osteoblastic, and 186 patients had tumors located at extremities.

Until the end of follow-up, 152 patients showed a response to chemotherapy, and 108 patients showed no response. After adjustment for clinical variables, the CC genotype of *ERCC1* rs11615 was significantly associated with better response to chemotherapy (OR =

2.87, 95%CI = 1.24-6.97) (Table 2). However, no significant associations were found between *ERCC1* rs3212986, *ERCC2* rs1799793, and *ERCC2* rs13181 polymorphisms and response to chemotherapy after adjustment for multiple comparisons.

Table 1. Clinical characteristics of osteosarcoma patients included.

Characteristics	N	%
Age (years)		
<20	152	58.46
≥20	108	41.54
Gender		
Females	116	44.62
Males	144	55.38
Grade		
I-II	188	72.31
III	72	27.69
Family history of cancer		
No	240	92.31
Yes	20	7.69
Histological type		
Osteoblastic	79	30.38
Chondroblastic	133	51.15
Fibroblastic	27	10.38
Mixed	21	8.08
Tumor location		
Extremities	186	71.54
Other	74	28.46

Table 2. Association between *ERCC1* and *ERCC2* polymorphisms and response to chemotherapy.

Gene	Cases	%	Responders	%	Non-responders	%	OR (95%CI)[1]	P value
ERCC1 rs11615								
TT	113	43.6	53	34.87	60	55.56	1.0 (Ref.)	-
TC	109	42.1	68	44.74	41	37.96	1.61 (0.91-2.86)	0.08
CC	38	14.3	31	20.39	7	6.48	2.87 (1.24-6.97)	0.007
ERCC1 rs3212986								
GG	118	45.5	63	41.45	55	50.93	1.0 (Ref.)	-
GT	120	46.2	72	47.37	48	44.44	1.21 (0.70-2.10)	0.46
TT	22	8.3	17	11.18	5	4.63	2.01 (0.72-6.14)	0.15
ERCC2 rs1799793								
GG	158	60.6	87	57.24	71	65.74	1.0 (Ref.)	-
GA	86	33.2	53	34.87	33	30.56	1.22 (0.69-2.16)	0.47
AA	16	6.2	12	7.89	4	3.70	1.56 (0.51-5.33)	0.39
ERCC2 rs13181								
AA	150	57.5	82	53.95	68	62.96	1.0 (Ref.)	-
AC	94	36.2	59	38.82	35	32.41	1.26 (0.72-2.21)	0.38
CC	16	6.3	11	7.24	5	4.63	1.45 (0.46-5.04)	0.48

[1]Adjusted for age, gender, grade, family history of cancer, histological type, and tumor location.

The association of the investigated polymorphisms with overall survival of osteosarcoma patients after adjustment for clinical variables is shown in Table 3. Our study found that those carrying the CC genotype of *ERCC1* rs11615 had a longer overall survival compared with the TT genotype, and the OR (95%CI) was 0.35 (0.12-0.92). However, *ERCC1* rs3212986, *ERCC2* rs1799793 and rs13181 did not significantly influence the overall survival in osteosarcoma.

Table 3. Association between *ERCC1* and *ERCC2* polymorphisms and overall survival of osteosarcoma patients.

Gene	Cases	%	Events	%	OR (95%CI)[1]	P value
ERCC1 rs11615						
TT	113	43.6	44	52.38	1.0 (Ref.)	-
TC	109	42.1	33	39.29	0.68 (0.38-1.23)	0.18
CC	38	14.3	7	8.33	0.35 (0.12-0.92)	0.02
ERCC1 rs3212986						
GG	118	45.5	41	48.81	1.0 (Ref.)	-
GT	120	46.2	37	44.05	0.84 (0.47-1.49)	0.52
TT	22	8.3	6	7.14	0.70 (0.21-2.08)	0.5
ERCC2 rs1799793						
GG	158	60.6	52	61.90	1.0 (Ref.)	-
GA	86	33.2	27	32.14	0.93 (0.51-1.70)	0.81
AA	16	6.2	5	5.95	0.93 (0.24-3.08)	0.89
ERCC2 rs13181						
AA	150	57.5	50	59.52	1.0 (Ref.)	-
AC	94	36.2	30	35.71	0.94 (0.51-1.68)	0.82
CC	16	6.3	4	4.76	0.67 (0.15-2.35)	0.49

[1]Adjusted for age, gender, grade, family history of cancer, histological type, and tumor location**.**

DISCUSSION

In the present study, we investigated the influence of *ERCC1* rs11615 and rs3212986, and *ERCC2* rs1799793 and rs13181 polymorphisms on treatment response and survival in osteosarcoma patients receiving cisplatin-based therapy. Our study found that the *ERCC1* rs11615 gene polymorphism was associated with response to chemotherapy, and this gene polymorphism could influence the overall survival of osteosarcoma patients.

Cisplatin is a commonly used chemotherapeutic agent, and exerts a cytotoxic effect by forming a variety of DNA lesions. Therefore, the DNA repair mechanism could play an important role in the response to cisplatin and the clinical outcome in cancers subject to chemotherapy. Enzymes in the NER pathway may play a key role in modifying susceptibility to cisplatin, and other DNA repair mechanisms, such as homologous recombination repair pathways, might also be involved in influencing the response to chemotherapy. Our study found that the *ERCC1* rs11615 gene polymorphism influenced the response to cisplatin-based chemotherapy, which suggests that this gene polymorphism could contribute to individual differences in response between patients.

Many epidemiologic studies have reported that *ERCC1* polymorphisms may influence the outcome of cisplatin treatment in gastric cancer, non-small cell lung cancer (NSCLC), ovarian cancer, and esophageal squamous cell carcinoma (Qi et al., 2013; Chen et al., 2014; Gao et al., 2014; Liu et al., 2014). Liu et al. (2014) conducted a cohort study to investigate the role of *ERCC1* gene polymorphism in response to chemotherapy and clinical outcome in gastric cancer, and did not find the *ERCC1* rs3212986 polymorphism to be associated with OS in gastric cancer. Gao et al. (2014) also conducted a cohort study to investigate the role of three SNPs of *ERCC1* in the clinical outcome of NSCLC, and found that the TT genotype of *ERCC1* rs11615 and the AA genotype of rs3212986 polymorphisms were associated with increased risk of death from NSCLC. Chen et al. (2014) showed that the *ERCC1* rs3212986 polymorphism showed a significant association with survival in epithelial ovarian cancer patients. However, another study found that *ERCC1* rs3212986 did not produce a significant difference in the response to

chemotherapy or overall survival in ovarian cancer (Qi et al., 2013).

Four previous studies have been conducted to investigate the association between *ERCC1* gene polymorphisms and osteosarcoma (Hao et al., 2012; Yang et al., 2012; Li et al., 2014; Goričar et al., 2015). Hao et al. (2012) investigated the association between *ERCC1* and *ERCC2* gene polymorphisms and clinical outcome in osteosarcoma, and found that the *ERCC1* rs11615 polymorphism was associated with clinical outcome. Yang et al. (2012) did not find an association between *ERCC1* polymorphisms and response to chemotherapy. Li et al. (2014) found no significant association between *ERCC1* polymorphisms and prognosis in osteosarcoma. Goričar et al. (2015) also did not find that *ERCC1* polymorphisms could be used as predictive factors for osteosarcoma patients receiving cisplatin-based chemotherapy. In contrast, our study suggested that *ERCC1* rs11615 was significantly associated with better response to chemotherapy and could affect the overall survival of osteosarcoma patients.

In conclusion, our results suggest that the *ERCC1* rs11615 polymorphism might influence the response to cisplatin-based chemotherapy and affect the clinical outcome in osteosarcoma patients.

Conflicts of interest

The authors declare no conflict of interest.

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