Association between *ERCC1* and *ERCC2* gene polymorphisms and chemotherapy response and overall survival in osteosarcoma

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ABSTRACT. We aimed to evaluate the influence of four SNPs in *ERCC1* and *ERCC2* on the response to cisplatin-based treatment and on clinical outcome in patients with osteosarcoma. We identified 186 patients with osteosarcoma diagnosed between April 2009 and April 2011 who were eligible for inclusion in our study. Genotyping of *ERCC1* rs11615, rs3212986, and rs2298881; and *ERCC2* rs1799793 and rs13181 was conducted by a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. By conditional logistic regression analysis, patients carrying the CC genotypes of *ERCC1* rs11615 and rs2298881 were shown to be more likely to have good response to chemotherapy when compared with patients carrying wild-type genotypes; the ORs (95%CIs) were 2.56 (1.02-7.35) and 3.01 (1.07-9.71), respectively. By Cox regression analysis, individuals carrying the CC genotype of *ERCC1* rs11615 were associated with longer overall survival time and decreased risk of death from osteosarcoma; the hazards

ratio (95%CI) was 0.32 (0.07-0.98). In summary, our results suggested that the *ERCC1* rs11615 and rs2298881 polymorphisms play important roles in the response to chemotherapy mediated by the DNA repair pathway and in the clinical outcome of osteosarcoma.

Key words: *ERCC1*; *ERCC2*; Polymorphism; Clinical outcome; Osteosarcoma

INTRODUCTION

Osteosarcoma is a rare bone cancer yet is the most common primary bone tumor in children and adolescents (Mirabello et al., 2009; Moore and Luu, 2014). It has been reported that osteosarcoma often develops during periods of rapid skeletal growth, and that over 50% of tumors occur in the long bones (Hattinger et al., 2010). Neoadjuvant chemotherapy followed by definitive resection with subsequent adjuvant chemotherapy is used as a common therapy method for localized osteosarcoma, because chemotherapy can eradicate the micrometastatic disease (Bruland et al., 2005; Wesolowski and Budd, 2010). Neoadjuvant treatment for osteosarcoma including cisplatin with doxorubicin, methotrexate, and ifosfamide is frequently used (Bacci et al., 2005). However, inter-individual differences in treatment outcomes are observed between patients and even those receiving similar treatment (Hattinger et al., 2010). It has been reported that genetic polymorphisms involved in response to chemotherapeutic agents could influence survival and treatment toxicity following chemotherapy; therefore, the identification of predictive genetic markers might allow for improved drug selection and treatment outcomes.

Deficiencies in DNA repair capacity have been suggested to play an important role in affecting the response to cisplatin (Goode et al., 2002; Martin et al., 2008). DNA repair processes include at least four pathways that act on specific types of DNA damage. The nucleotide excision repair (NER) pathway repairs bulky lesions and is associated with tumor progression and the response to platinum-based chemotherapy (Reed, 1998; Stoehlmacher et al., 2004). There are two important enzymes in the NER pathway that are associated with resistance to cisplatin: excision repair cross-complementation group 2 (ERCC2) and excision repair cross-complementation group 1 (ERCC1).

Several previous studies have investigated the influences of the *ERCC1* and *ERCC2* genes on cisplatin response in many cancers including non-small cell lung cancer, esophageal cancer, and ovarian cancer (Schena et al., 2012; Rumiato et al., 2013). However, for osteosarcoma, the results have been inconsistent (Caronia et al., 2009; Hao et al., 2012; Yang et al., 2012; Li et al., 2014a). Therefore, we aimed to evaluate the influence of four SNPs in *ERCC1* and *ERCC2* on the response to cisplatin-based treatment and the clinical outcome in patients with osteosarcoma.

MATERIAL AND METHODS

Patients

We identified 186 patients with osteosarcoma diagnosed between April 2009 and April 2011 who were eligible for inclusion in our study. We included all patients with osteosarcoma who were treated with cisplatin-based chemotherapy at the Second Affiliated Hospital of Inner Mongolia Medical University. All samples were obtained with written informed consent from

patients or their parents. Ethical approval of the study was granted by the Ethics Committee of the Second Affiliated Hospital of Inner Mongolia Medical University.

Assessment of treatment outcome

The demographic and clinical characteristics of the included patients were obtained from the medical records. The response to chemotherapy was classified by the response evaluation criteria from European Organization for Research and Treatment of Cancer. The response to chemotherapy was assessed after receiving treatment, and patients were divided into good and poor responders. Patients showing more than 90% necrosis were considered to exhibit good response. Overall survival (OS) was calculated from the date of the beginning of treatment to an event or death. Patients without an event or death at the time of the analysis were recorded at the date of the last follow-up.

All the patients were followed-up until May 2014, with a median follow-up time of 38.5 months (range: 3 to 60 months). All patients were followed up by telephone or by clinic attendance every four weeks until death or the end of study.

Blood samples and genotyping

All study subjects were asked to provide 5 mL peripheral venous blood. According to the manufacturer instructions, genomic DNA was extracted from peripheral venous blood samples using the TIANamp blood DNA kit (Tiangen Biotech, Beijing, China). Genotyping of *ERCC1* rs11615, rs3212986, and rs2298881; and *ERCC2* rs1799793 and rs13181 was conducted by a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. Probes and primers for *ERCC1* rs11615, rs3212986, and rs2298881; and *ERCC2* rs1799793 and rs13181 were designed using the Sequenom Assay Design 3.1 software (Sequenom®, San Diego, CA, USA) according to manufacturer instructions. The extension reactions were performed at 94°C for 30 s, 94°C for 5 s, followed by 40 cycles at 52°C for 5 s, and 80°C for 5 s, with a final incubation at 72°C for 3 min. For quality control, 10% of subjects were randomly selected, and the results of genotyping repeated samples showed 100% concordance.

Statistical analysis

Continuous variables are reported as means ± standard deviation (SD), and categorical variables were shown by N of subjects (%). Logistic regression analysis was used to analyze the association between *ERCC1* rs11615, rs3212986, and rs2298881; and ERCC2 rs1799793 and rs13181 polymorphisms and the response to chemotherapy, and the results were expressed by ORs and 95%CIs. Cox regression analyses were taken to analyze the associations between *ERCC1* rs11615, rs3212986, and rs2298881; and *ERCC2* rs1799793 and rs13181 polymorphisms and overall survival, and the results were expressed by hazards ratio (HR) and 95%CI. Two-tailed P values < 0.05 were regarded as indicating statistical difference. All statistical analyses were performed using the SPSS version 16.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS

The demographic and clinical characteristics of the study subjects are shown in Table 1.

The mean age of patients with osteosarcoma was 19.2 ± 9.4 years old. Of 186 patients with osteosarcoma, 107 (57.53%) were males, 127 (68.28%) were at tumor stage I-II, 122 (65.59%) had a tumor location in the long tubular bones, and 139 (74.73%) received limb salvage treatment.

Table 1. Demographic and clinical characteristics of patients with osteosarcoma.

Characteristics	N	%
Age		
≤20	98	52.69
>20	88	47.31
Gender		
Male	107	57.53
Female	79	42.47
Stage		
I-II	127	68.28
III-IV	59	31.72
Tumor location		
Long tubular bones	122	65.59
Axial skeleton	64	34.41
Therapy		
Amputation	47	25.27
Limb salvage	139	74.73

At the end of the follow-up, 112 (60.22%) patients showed good response to cisplatin-based chemotherapy. By conditional logistic regression analysis, patients carrying the CC genotypes of *ERCC1* rs11615 and rs2298881 were found to be more likely to have had good response to chemotherapy when compared with those carrying wild-type genotypes; the ORs (95%CIs) were 2.56 (1.02-7.35) and 3.01 (1.07-9.71), respectively (Table 2). However, no significant difference was found between *ERCC1* rs3212986, rs2298881, and *ERCC2* rs1799793 and rs13181 polymorphisms and patient response to chemotherapy.

Table 2. Association between *ERCC1* and *ERCC2* polymorphisms and response to chemotherapy in patients with osteosarcoma.

Genotypes	Good response (N = 112)	%	Poor response (N = 74)	%	Adjusted OR (95%CI)[1]	P value
ERCC1 rs11615						
TT	43	38.39	38	51.35	1.0 (Ref.)	-
TC	46	41.07	28	37.84	1.45 (0.73-2.90)	0.25
CC	23	20.54	8	10.81	2.56 (1.02-7.35)	0.03
ERCC1 rs3212986						
CC	54	48.21	40	54.05	1.0 (Ref.)	-
CA	45	40.18	29	39.19	1.15 (0.59-2.24)	0.66
AA	13	11.61	5	6.76	1.93 (0.59-7.45)	0.24
ERCC1 rs2298881						
AA	61	54.46	50	67.57	1.0 (Ref.)	-
AC	29	25.89	18	24.32	1.32 (0.62-2.84)	0.43
CC	22	19.64	6	8.11	3.01 (1.07-9.71)	0.02
ERCC2 rs1799793						
GG	65	58.04	48	64.86	1.0 (Ref.)	-
GA	32	28.57	19	25.68	1.24 (0.60-2.62)	0.53
AA	16	14.29	7	9.46	1.69 (0.60-5.23)	0.28
ERCC2 rs13181						
AA	63	56.25	46	62.16	1.0 (Ref.)	-
AC	34	30.36	21	28.38	1.18 (0.58-2.43)	0.62
CC	15	13.39	7	9.46	1.56 (0.54-4.90)	0.37

[1]Ajusted for gender, age, stage, tumor location, and therapy.

At the end of follow-up, 53 (28.49%) patients had died from all causes. By Cox regression analysis, individuals carrying the CC genotype of *ERCC1* rs11615 were associated with longer overall survival time and decreased risk of death from osteosarcoma, and the HR (95%CI) was 0.32 (0.07-0.98) (Table 3). However, we observed no association between *ERCC1* rs3212986, rs2298881, and *ERCC2* rs1799793 and rs13181 polymorphisms and overall survival in patients with osteosarcoma.

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

Genotypes	Deaths (N = 53)	%	Survivors (N = 131)	%	Adjusted HR (95%CI)[1]	P value
ERCC1 rs11615						
TT	29	54.72	53	40.46	1.0 (Ref.)	-
TC	20	37.74	55	41.98	0.66 (0.32-1.39)	0.36
CC	4	7.55	23	17.56	0.32 (0.07-0.98)	0.03
ERCC1 rs3212986						
CC	28	52.83	60	45.80	1.0 (Ref.)	-
CA	21	39.62	56	42.75	0.85 (0.41-1.76)	0.59
AA	4	7.55	15	11.45	0.60 (0.13-2.14)	0.40
ERCC1 rs2298881						
AA	34	64.15	70	53.44	1.0 (Ref.)	-
AC	12	22.64	32	24.43	0.71 (0.29-1.66)	0.39
CC	7	13.21	29	22.14	0.49 (0.17-1.32)	0.13
ERCC2 rs1799793						
GG	34	64.15	78	59.54	1.0 (Ref.)	-
GA	12	22.64	32	24.43	0.89 (0.37-2.04)	0.76
AA	7	13.21	21	16.03	0.79 (0.26-2.17)	0.62
ERCC2 rs13181						
AA	33	62.26	77	58.78	1.0 (Ref.)	-
AC	16	30.19	42	32.06	0.89 (0.41-1.89)	0.74
CC	4	7.55	12	9.16	0.78 (0.17-2.82)	0.68

[1]Ajusted for gender, age, stage, tumor location, and therapy. HR = hazards ratio.

DISCUSSION

In this study, we conducted a case-control study to explore the association between the *ERCC1* rs11615, rs3212986, and rs2298881; and *ERCC2* rs1799793 and rs13181 polymorphisms and treatment response and overall survival in osteosarcoma. We observed that patients carrying the CC genotypes of *ERCC1* rs11615 and rs2298881 were more likely to have had good response to chemotherapy when compared with those with wild-type genotypes, and that individuals carrying the CC genotype of *ERCC1* rs11615 were associated with longer overall survival time and decreased risk of death from osteosarcoma.

Multiple previous studies have reported that *ERCC1* and *ERCC2* were associated with the response to cisplatin-based chemotherapy and clinical outcome in several cancers (Kamikozuru et al., 2008; Park et al., 2011; Cheng et al., 2012; Ren et al., 2012; Chen et al., 2013; Rumiato et al., 2013; Gao et al., 2014; Li et al., 2014b). Park et al. (2011) reported that the *ERCC1* rs3212986 polymorphism was correlated with treatment outcome of metastatic gastric cancer treated with cisplatin-based chemotherapy. Chen et al. (2013) evaluated the role of *ERCC1* in the clinical outcomes of patients with metastatic nasopharyngeal carcinoma (NPC) treated with cisplatin-based chemotherapy, and found that A allele of *ERCC1* rs3212986 are associated with increased risk of disease progression in these patients. Kamikozuru et al.

(2008) found that *ERCC1* rs11615 was negative associated with risk of death in patients with pancreatic cancer treated with platinum-based chemotherapy. Ren et al. (2012) indicated that AAT codons of *ERCC1* rs11615 might be a predictive marker for patients with non-small cell lung carcinoma (NSCLC) treated with platinum-based chemotherapy. Cheng et al. (2012) found that the C allele of *ERCC1* rs11615 polymorphism was associated with longer response to cisplatin-based chemotherapy in treatment of last-stage NSCLC than T allele. Gao et al. (2014) found that the *ERCC1* polymorphisms rs11615 and rs3212986 were negatively associated with response to chemotherapy and survival time of patients with advanced NSCLC. Li et al. (2014b) found that *ERCC1* rs11615 was positive associated with risk of death in patients with gastric cancer. Overall, the results from these studies are inconsistent. These inconsistencies might arise from differences in ethnicities or source of patients, types of tumors, disease stages, sample size, or by chance.

Similar inconsistencies exist with regard to the association between *ERCC1* and *ERCC2* polymorphisms and the clinical outcome of osteosarcoma, which has been examined in four previous studies (Caronia et al., 2009; Hao et al., 2012; Yang et al., 2012; Li et al., 2014b). Only one study reported similar results with ours (Hao et al., 2012); this group found that the *ERCC1* rs11615 polymorphism was associated with lower risk of death from osteosarcoma in a Chinese population. In contrast, the other three studies did not find that the *ERCC1* rs11615 and rs2298881 gene polymorphisms were associated with a response to chemotherapy and the clinical outcome of osteosarcoma (Caronia et al., 2009; Yang et al., 2012; Li et al., 2014b). Therefore, further studies with large sample sizes are required to confirm the association between *ERCC1* and *ERCC2* polymorphisms and treatment outcome of osteosarcoma identified herein.

In summary, our results suggest that the *ERCC1* rs11615 and rs2298881 polymorphisms play important roles in the response to chemotherapy mediated by DNA repair pathways, and in the clinical outcome of osteosarcoma. Further multicenter studies with larger sample sizes are greatly needed to confirm our results.

Conflicts of interest

The authors declare no conflict of interest.

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