RESEARCH ARTICLE

Association of Four ERCC1 and ERCC2 SNPs with Survival of Bone Tumour Patients

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Abstract

Aim: SNPs of ERCC1 and ERCC2 genes have been found to be associated with response to platinum therapy in different clinical settings. In the current study, we investigated the relationship of SNPs in ERCC1 and ERCC2 to cisplain response and survival in osteosarcoma patients. Methods: 267 consecutive patients diagnosed with osteosarcoma between January 2003 to January 2005 were followed up until the end of January 2010. ERCC1 Asn118Asn, ERCC1 Gln504Lys, ERCC2 Asp312Asn and ERCC2 Lys751Gln polymorphisms were detected based upon the Sequenom MassARRAY platform. Results: For ERCC1 Asn118Asn, the variant genotype T/T was strongly significantly associated with a higher event free survival when compared with the wild-type C/C, with an adjusted OR (95% CI) of 0.39 (0.14-0.95). ERCC2 751 A/A genotype showed increased event free survival of osteosarcoma (HR=0.44; 95%CI=0.10-0.87). However, we did not find significant association of ERCC1 Gln504Lys and ERCC2 Asp312Asn polymorphisms with prognosis of osteosarcoma. Conclusion: We first report associations of four SNPs, ERCC1 Asn118Asn, ERCC1 Gln504Lys, ERCC2 Asp312Asn and ERCC2 Lys751Gln, with risk of death from osteosarcoma in a Chinese population, indicating ERCC1 118T/T and ERCC2 A/A may be used as surrogate markers for clinical outcome of osteosarcoma treatmetn with cisplain.

Keywords: ERCC1 - ERCC2 - polymorphisms - osteosarcoma - survival - cisplain treatment - China

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Introduction

Cancer that originates in the bone, termed primary bone cancer, is rare and typically occurring during the children and adolescent growth spurt, with a second smaller peak in the elderly. Osteosarcoma is one of the most frequent bone tumors, with a 3-5/105 population per year worldwide, but this tumor accounted for about 6% of all cancer diagnosed under the age of 20 years (American Cancer Society et al., 2007; Lu et al., 2011).

The five years survival of osteosarcoma is below 20% after surgery, but the chemotherapy after surgery have dramatically improved the survival to 55% to 70% after years (Le et al., 2007). The methotrexate, adriamycin, and cisplatin from the backbone of most standard treatment protocols as a triple-drug regimen (MAP). However, patients who have similar clinical characteristics usually showed variable response rate to chemotherapy, with inferior 5-year survival of 40% to 70% (Le et al., 2007). This implies the therapeutic efficacy has a remarkable interindividual variability.

Since DNA kinking is the major feature of oxaliplatin-DNA adducts that block DNA replication and lead to cancer cell death (Faivre et al., 2003; Reed, 2005), which is recognized and repaired by the nucleotide excision repair (NER) pathways. It is reported the interindividual difference in the NER capacity may influence the efficacy of cisplatin -based chemotherapy and clinical outcomes of the cancer patients. SNPs in the ERCC1 and ERCC2 genes have been found to be associated with the platinum response in different clinical studies. In particular, the polymorphisms of ERCC1 and ERCC2 were reported to be associated with increased survival and better prognosis in several cancer, such as non-small-cell lung cancer, colorectal cancer, gastric cancer, nasopharyngeal cancer, bladder cancer, and breast cancer (Baek et al., 2006; Rajaraman et al., 2008; Cao et al., 2011; Ishibashi et al., 2011; Sun et al., 2011; Rouissi et al., 2011).

Few studies clarified the association of SNPs in ERCC1 and ERCC2 with bone tumor. Only one study conducted in Spain indicated the prediction role of ERCC1 and ERCC2 on the response to cisplain chemotherapy in osteosarcoma patients (Caronia et al., 2009). In the current study, we investigated the relationship of SNPs in ERCC1 and ERCC2 to cisplain response and survival in osteosarcoma patients.

Materials and Methods

Patients

267 consecutive patients diagnosed with osteosarcoma between January 2003 to January 2005 from the Second

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Table 1. PCR Primers of Selected SNPs

Single nucleotide polymorphism	Forward	Reverse
ERCC1 Asn118Asn (rs11615)	CCAGAACACTGGGACAT	TCAGAGGATCAGGGACT
ERCC1 Gln504Lys (rs3212986)	ACAGTGCCCCAAGAGGAGAT	AGTCTCTGGGGAGGGATTCT
ERCC2 Asp312Asn (rs1799793)	ACCCTGTCTGGGTGCTAAGA	AATTCCTGGGACAAGAGTGC
ERCC2 Lys751Gln (rs13181)	CCCCCTCTCCCTTTCCTCTG	AACCAGGGCCAGGCAAGAC

Affiliated hospital of Inner Mongolia Medical University and Nanfang Hospital of Southern Medical University were enrolled in our study. All the patients were asked to provide blood samples, and informed consent were obtained from the patients or their relatives. Ethnical approval of the study was granted by the Ethics Committee of the Inner Mongolia Medical University.

All the patients received chemotherapy, including intravenous methotrexate (four courses of up to 14 gm^{-2} per day for 1 day), intravenous adriamycin (three courses at 25-30 mgm^{-2} per day for 3 days), and intra-arterial cisplatin (three courses at 35 mgm^{-2} per day for 3 days). After surgery, the adjuvant chemotherapy included methotrexate and alternate cylcles of intravenous cisplatin or adriamycin up to 48 weeks of treatment.

On the day of participating, all the patients were asked to provide their and their next of kins' telephone numbers and mailing addresses to enable our follow-up, and all the patients were followed up every one month until death.

Genotyping

DNA was extracted from the buffy-coat fractions with TIANamp blood DNA kit (Tiangen Biotech, Beijing, China). genotyping 4 SNPs (ERCC1 Asn118Asn, ERCC1 Gln504Lys, ERCC2 Asp312Asn and ERCC2 Lys751Gln) in was performed in a 384-well plate format on the Sequenom MassARRAY platform (Sequenom, San Diego, USA). Primers for polymerase chain reaction (PCR) amplification and single base extension (SBE) assays were designed by Sequenom Assay Design 3.1 software (Sequenom, San Diego, CA, USA) according to the manufacturer's instructions (Table 1).. For quality control, a 5% random sample of cases was genotyped twice by different researchers. The reproducibility was 100%.

Amplification was carried out in a total volume of 25 ml containing 0.25 mM of each primers, 0.02 mM dNTPs and 1 mM $MgCl_2$, 1.25U Taq polymerase and 5_ PCR buffer. All PCR samples divided into two tubes, one for the wild probe and another for the mutation probe. The PCR program initiated with a 1 min denaturation at 958C. The DNA was amplified by one cycle of 958C for 5 s and 50 cycles of 928C for 40 s, followed by elongation of 608C for 40s. In addition, to ensure the accuracy of this method, 20 randomly selected DNA samples were subjected to PCR-based direct DNA sequencing.

Statistical analysis

Stata 8.0 (StataCorp, College Station, USA) was used to perform statistical analyses. Continuous variables were expressed as mean±standard deviation (SD) while categorical variables were shown as frequencies and percentages. SNPs in ERCC1 and ERCC2 were assessed in terms of overall survival and event free survival by using regression analysis. The main endpoint of the study was overall survival, which calculated from the start of treatment to the date of last follow-up or death. Event-free survival was considered from tumor diagnosis to the first of disease recurrence, development of lung or bone metastasis or death. Survival curves were plotted by using the Kaplan and Meier method and compared with the log-rank test. The association between each genotype and survival was estimated by hazard ratios and their 95% confidence intervals by multivariate Cox's regression models. A P-value < 0.05 was considered as statistically significant.

Table 2. Characteristics of Patients with Osteosarcoma

Variables		All patients N=267	%
Mean age		13.6±5.2	
Range		4.5-36	
Gender	Female	92	34.5
	Male	175	65.5
Classification of osteosarcoma	Common type	214	80.3
	Chondroblastic	12	4.5
	Fibroblastic	16	6.1
	Anaplastic	5	2
	Small cell	9	3.3
	Telangiectatic	0	0
	Other	10	3.8
Location of tumor	Proximal	121	45.3
	Midshaft	2	0.7
	Distal	144	54
Response to treatment	Poor	124	46.3
	Good	143	53.7
Metastasis	No	134	50.3
	At diagnosis	57	21.4
	At follow up	76	28.3

Table 3. Genotype characteristics of the four SNPs

Single nucleotide polymorphism	Alleles	MAF[a]	HWE (P value)[b]	
		Case	From dbSNP	Case
ERCC1 Asn118Asn (rs11615)	C/T	0.346	0.36	0.29
ERCC1 Gln504Lys (rs3212981)	C/A	0.312	0.29	0.11
ERCC2 Asp312Asn (rs1799793)	G/T	0.332	0.38	0.18
ERCC2 Lys751Gln (rs13181)	G/A	0.241	0.23	0.37

MAF, Minor Allele Frequency; HWE, Hardy-Weinberg equilibrium

Results

All 267 patients were followed up until the end of January 2010. The median follow-up time was 44.3 about months. There was 4 patients loss to follow up due to immigrant to other cities, and a total of 119 patients were died during the 5 years follow-up. The demographic and clinic characteristics of included patients are shown in Table 2. The Mean age of patients were 13.6±5.2(ranged from 4.5 to 36). The percentage of good response to chemotherapy treatment was 53.7. About 21.4% of the patients showed metastasis at diagnosis, and 28.3% developed metastasis during the follow-up. Our study

Table 4. ERCC1 and XRCC3 Gene Polymorphism and Survival of Osteosarcoma

Gene	Patients N=267(%)	5-year survival rate (%)	HR (95%CI) of OS	HR (95%) of EFS
ERCC1 Asn118Asn				
C/C	118 (44.3)	49	1.0(Reference)	1.0(Reference)
C/T	113 (42.2)	58.2	0.82(0.50-1.33)	0.67(0.32-1.24)
T/T	36 (13.5)	68	0.57(0.23-1.23)	0.39(0.14-0.95)
T allele	149 (55.7)	60.6	0.91(0.50-1.43)	0.73(0.63-1.14)
ERCC1 Gln504Lys				
C/C	132 (49.4)	51.7	1.0(Reference)	1.0(Reference)
C/A	104 (38.8)	57	0.89(0.53-1.43)	0.77(0.32-1.53)
A/A	32 (11.8)	65.6	0.71(0.30-1.55)	0.65(0.43-1.76)
A allele	135 (50.6)	59	0.84(0.52-1.30)	0.72(0.43-1.67)
ERCC2 Asp312Asn				
G/G	124 (46.6)	52	1.0(Reference)	1.0(Reference)
G/T	108 (40.5)	58	0.86(0.52-1.41)	0.83(0.41-1.56)
T/T	34 (12.9)	59.9	0.84(0.37-1.76)	0.82(0.30-1.85)
T allele	143 (53.4)	58.4	0.86(0.53-1.25)	0.87(0.57-1.48)
ERCC2 Lys751Gln				
G/G	157 (58.7)	51	1.0(Reference)	1.0(Reference)
G/A	92 (34.4)	59.1	0.83(0.50-1.36)	0.73(0.35-1.73)
A/A	18 (6.9)	74.8	0.55(0.14-1.64)	0.44(0.10-0.87)
A allele	110 (24.1)	61.7	0.73(0.46-1.15)	0.61(0.38-0.95)

HR, Hazard Ratio; OS, overall survival; EFS: event free survival; The OS and EFS were adjusted for response to treatment and metastasis

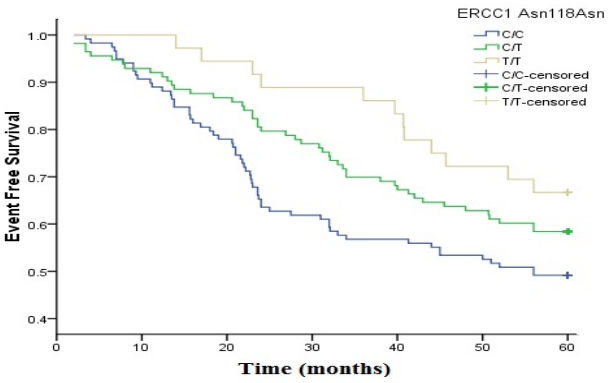

Figure 1. The Survival (in months) Among Difference Genotypes of ERCC1 Asn118Asn Polymorphism

showed the poor response to treatment and metastasis at diagnosis were associated with higher risk of death, with HR (95% CI) of 1.87 (1.23-4.59) and 2.33(1.15-5.98), respectively.

The allele and genotype distributions of polymorphisms in ERCC1 Asn118Asn, ERCC1 Gln504Lys, ERCC2 Asp312Asn and ERCC2 Lys751Gln were showed in Table 3. The minor allele frequencies among selected among cases were consistent with the MAF from NCBI SNP databases. Moreover, all the SNPs were in line with the Hardy-Weinberg equilibrium among cases (All the P value >0.05).

The associations between the SNPs and the risk of death from osteosarcoma were studies by using multivariate Cox's regression analysis (Table 4). For ERCC1 Asn118Asn, the variant genotype T/T was strongly significantly associated with a higher event free survival when compared with the wide-type C/C, with the adjusted OR (95% CI) of 0.39(0.14-0.95) (Figure 1). Furthermore, ERCC2 751 A/A genotype showed increased the event free survival of osteosarcoma (HR=0.44; 95%CI=0.10-0.87), and T allele genotype of ERCC2 Lys751Gln was significantly associated with low risk

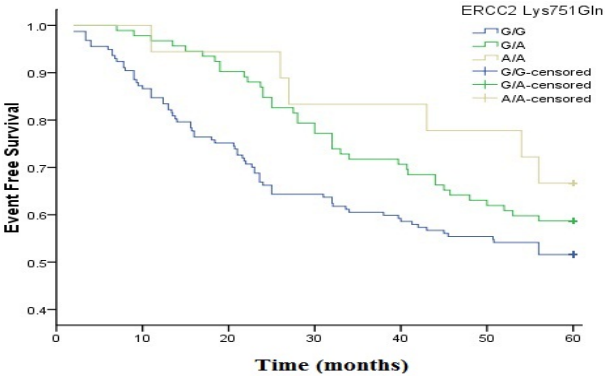

Figure 2. The Survival (in months) Among Difference Genotypes of ERCC2 Lys751Gln Polymorphism

of death from osteosarcoma (OR=0.61, 95%CI= 0.38-0.95) (Figure 2). However, we did not find significant association of ERCC1 Gln504Lys and ERCC2 Asp312Asn polymorphisms with prognosis of osteosarcoma.

Discussion

The drug resistance is a major problem in most solid tumors. Identification of predictive markers for response to chemotherapy is most warranted, since a subgroup of the patients could not benefit from chemotherapy. Pre-treatment identification the predictive gene polymorphism would be very helpful in the further treatment. Our study firstly published the associations of ERCC1 and ERCC2 polymorphisms and the prognosis of primary malignant osteosarcoma in Chinese populations, and implied ERCC1 118T/T and ERCC2 A/A are related to better event free survival of osteosarcoma.

ERCC1 and ERCC2 are potentially relevant to cancer because of their involvement in the process of nucleotide expcision repair (NER) (Goode et al., 2002). The NER pathway acts predominately on bulky DNA lesions and UV damage, and polymorphisms in ERCC1 and ERCC2 may change the function of NER in repairing DNA damage by chemotherapy. It is implied the High ERCC1 and ERCC2 levels are related to increased removal of cisplatin -induced DNA adducts and cisplatin resistance. Previous experimental and epidemiological studies showed the ERCC1 118C allele and ERCC2 751G allele are associated with higher mRNA levels and DNA single-strand break repair than ERCC1 118T allele and ERCC2 751A allele genotypes, and thus to induce the resistance to cisplatin-based chemotherapy which used to damage the DNA of cancer cells, and the polymorphisms in ERCC1 118T allele and ERCC2 751A allele are associated with decreased risk of death from several cancers, such as non-small-cell lung cancer, gastric cancer, nasopharyngeal cancer, and breast cancer (Baek et al., 2006; Rajaraman et al., 2008; Cao et al., 2011; Sun et al., 2011). Only one previous study reported the ERCC2 751G allele are associated with response to cisplatin chemotherapy and shorter event-free survival in osteosarcoma patients (Caronia et al., 2009). The results of our study are in line with previous studies, and indicated ERCC1 118T/T and ERCC2 751A/A are associated with better event free survival in osteosarcoma patients.

There are two limitations in our study. First, because of the rarity of osteosarcoma, we only had a limited number of cases, which would decrease the study power to find the difference, such as no significant association between ERCC1 Gln504Lys and ERCC2 Asp312Asn polymorphisms and survival of osteosarcoma. Second, we collected the cases from one hospital visitors, which may be a threat to the validity of the results. Further large sample studies are warranted in Chinese population.

Overall, as the first study to investigate the association of four SNPs, ERCC1 Asn118Asn, ERCC1 Gln504Lys, ERCC2 Asp312Asn and ERCC2 Lys751Gln, with risk of death from osteosarcoma in Chinese population, and out study found the ERCC1 118T/T and ERCC2 A/A are related to prognosis of osteosarcoma. Further studies in Chinese populations with large sample size are still warranted.

References

American Cancer Society (2007). Global Cancer Facts and Figures 2007. Atlanta, GA: American Cancer Society. Available at: http://www.cancer.org/downloads/STT/Global_Cancer_Facts_and_Figures_2007_rev.pdf. Oct. 2011.

Baek SK, Kim SY, Lee JJ, et al (2006). Increased ERCC expression correlates with improved outcome of patients treated with cisplatin as an adjuvant therapy for curatively resected gastric cancer. *Cancer Res Treat*, **38**, 19-24.

Caronia D, Patiño-García A, Milne RL, et al (2009).Common variations in ERCC2 are associated with response to cisplatin chemotherapy and clinical outcome in osteosarcoma patients. *Pharmacogenomics J*, **9**, 347-53.

Cao C, Zhang YM, Wang R, et al (2011). Excision repair cross complementation group 1 polymorphisms and lung cancer risk: a meta-analysis. *Chin Med J (Engl)*, **124**, 2203-8.

Faivre S, Chan D, Salinas R, et al (2003). DNA strand breaks and apoptosis induced by oxaliplatin in cancer cells. *Biochem Pharmacol*, **66**, 225-37.

Goode EL, Ulrich CM, Potter JD (2002). Polymorphisms in DNA repair genes and associations with cancer risk. *Cancer Epidemiol. Biomarkers Prev*, **11**, 1513-30.

Ishibashi K, Okada N, Tajima Y, et al (2011). Prediction of the efficacy of modified FOLFOX6 therapy according to the mRNA levels of thymidylate synthase (TS), excision repair cross-complementing-1 and -2(ERCC-1 and ERCC-2) and methylenetetrahydrofolate dehydrogenase(MTHFD) in the primary lesion of colorectal cancer. *Gan To Kagaku Ryoho*, **38**, 2220-3.

Le Deley MC, Guinebretiere JM, Gentet JC, et al (2007). Societe Francaise d'Oncologie Pediatrique (SFOP). SFOP OS94: a randomised trial comparing preoperative high-dose methotrexate plus doxorubicin to high-dose methotrexate plus etoposide and ifosfamide in osteosarcoma patients. *Eur J Cancer*, **43**, 752-61.

Lu XF, Yang WL, Wan ZH, et al (2011).Glutathione S-transferase polymorphisms and bone tumor risk in China. *Asian Pac J Cancer Prev*, **12**, 3357-60.

Rajaraman P, Bhatti P, Doody MM, et al (2008). Nucleotide excision repair polymorphisms may modify ionizing radiation-related breast cancer risk in US radiologic technologists. *Int J Cancer*, **123**, 2713-6.

Reed E (2005). ERCC1 and clinical resistance to platinum-based therapy. *Clin Cancer Res*, **11**, 6100-2.

Rouissi K, Bahria IB, Bougatef K, et al (2011). The effect of tobacco, XPC, ERCC2 and ERCC5 genetic variants in bladder cancer development. *BMC Cancer*, **11**, 101.

Sun JM, Ahn MJ, Park MJ, et al (2011). Expression of excision repair cross-complementation group 1 as predictive marker for nasopharyngeal cancer treated with concurrent chemoradiotherapy. *Int J Radiat Oncol Biol Phys*, **80**, 655-60.