Original Article
Association between XRCC1 and ERCC2 gene polymorphisms and development of osteosarcoma

Zimin Wang[1], Na Wu[2]

[1]Key Laboratory of People's Liberation Army, Institute of Orthopedics, PLA General Hospital, Haidian, Beijing 100853, China; [2]Medical Insurance Office, PLA General Hospital, Haidian, Beijing 100853, China

Abstract: We conducted a case-control study to investigate the association of three SNPs in XRCC1 gene (Arg194Trp, rs1799782; Arg280His rs25489; Arg399Gln rs25487) and one SNP in ERCC2 gene (Lys751Gln rs13181) with the susceptibility to osteosarcoma. Between May 2012 and May 2014, patients (n = 194) who were newly diagnosed with histopathologically confirmed osteosarcoma and 275 control subjects were selected from the First People's Hospital of Shangqiu. The XRCC1 Arg194Trp, Arg280His and Arg399Gln and ERCC2 Lys751Gln polymorphisms were genotyped by polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism (RFLP). We found that individuals with the Gln/Gln genotype and rg/Gln+ Gln/Gln genotype of XRCC1 Arg399Gln had an increased risk of osteosarcoma when compared with those carrying the wide-type genotype, and the adjusted OR (95% CI) was 2.55 (1.29-5.09) (P = 0.003) and 1.50 (1.01-2.25) (P = 0.04), compared to the Arg/Arg genotype. However, the XRCC1 Arg194Trp and Arg280His and ERCC2 Lys751Gln gene polymorphisms did not influence the risk of osteosarcoma. In conclusion, our study suggests that the XRCC1 Arg399Gln is significantly associated with the development of osteosarcoma.

Keywords: XRCC1, ERCC2, polymorphism, osteosarcoma

Introduction

Osteosarcoma is the most leading bone malignancy in adolescents. However, there exists a second incidence peak among individuals which aged above 60 years. It is well known that the osteosarcoma is a caused by a complex, multistep, and multifactorial process, such as ionizing radiation, family history and virus infection [1]. However, not all individuals who expose to the same risk factors of osteosarcoma would develop this cancer, which suggested that some hereditary factors play a key role in the susceptibility to this cancer. The concept of genetic factors being involved in the development of osteosarcoma has led to many studies on genetic determinants for this cancer in the recent decade [2-5].

It is well known that the DNA strand breaks and base damage that can result in severe mutations leading to cancer. The base excision repair (BER) and the nucleotide excision repair (NER) pathways are two of the most important DNA repair process involved in maintaining genome integrity. Small base lesions and DNA single strand breaks resulting from oxidation and alkylation damage could be repaired through the BER pathway [6-9]. As one of the most important proteins in BER, XRCC1 is closely related to BER pathway coordination through serving as a molecular scaffold for most other members of the BER short-patch pathway [7, 8]. In addition to BER, bulky adduct lesions induced by smoking chemical carcinogens can be repaired through the nucleotide excision repair (NER) pathway [9]. Xeroderma pigmentosum groups B and D (ERCC2/XPD) proteins are involved in the NER pathway by functioning as ATP-dependent DNA helicases responsible for opening the DNA strand around the lesion site to remove certain DNA cross-links, UV photolesions, and bulky chemical adducts [9]. Currently, few studies reported the association between XRCC1 and ERCC2 gene polymorphisms and development of osteosarcoma. Therefore, we conducted a case-control study to investigate the association of three
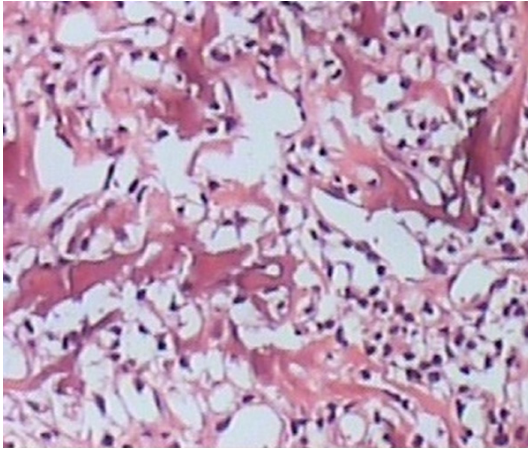
Figure 1. Chondroblastic osteosarcoma.

SNPs in XRCC1 gene (Arg194Trp, rs1799782; Arg280His rs25489; Arg399Gln rs25487) and one SNP in ERCC2 gene (Lys751Gln rs13181) with the susceptibility to osteosarcoma.

Material and methods

Patients

A hospital-based case-control design was taken in this study. Between May 2012 and May 2014, patients (n = 194) who were newly diagnosed with histopathologically confirmed osteosarcoma (**Figure 1**) were selected from Key Laboratory of People's Liberation Army, Institute of Orthopedics, PLA General Hospital. The exclusion criteria were patients who had primary tumors other than osteosarcoma, tumors of an unknown origin, any histopathological diagnosis other than osteosarcoma or inadequate organ function. Finally, 172 patients were included into our study, and the participation rate was 88.66%.

A total of 275 subjects were randomly selected from individuals who received health check-up in the examination center of the First People's Hospital of Shangqiu during the same period. Control subjects who had a history of cancer were excluded from our study.

Demographic and related lifestyle factors of patients with osteosarcoma and control subjects were collected from the medical record or a self-designed questionnaire. The demographic and related lifestyle factors included age, sex, tobacco smoking, alcohol drinking habits, family history of cancer, Enneking stage, tumor location, histological type and tumor metasta-

sis. Written informed consent was obtained from each included subject prior to the study. The study was previously approved by the Institutional Research Ethics Committee of the First People's Hospital of Shangqiu.

DNA extraction and genotyping

Each patient and control subject was asked to provide 5 mL venous blood for genomic DNA extraction. The blood samples were kept at -20°C until use, with ethylenediaminetetraacetic acid (EDTA) 0.5 mg/mL used as an anticoagulant. DNA was extracted from peripheral blood samples using the TIANamp Blood DNA Kit (Tiangen, Beijing, China) according to instructions. The XRCC1 Arg194Trp, Arg280His and Arg399Gln and ERCC2 Lys751Gln polymorphisms were genotyped by polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism (RFLP). The PCR fragments of the investigated polymorphisms were subsequently digested with their specific restriction enzyme (**Table 1**). The PCR program was set as follows: an initial denaturation at 95°C for 5 min, 30 cycles of denaturation at 94°C for 60 s, annealing at 60°C for 60 s, and extension at 72°C for 60 s, and a final extension at 72°C for 10 min. The PCR products were digested with the MspI restriction endonuclease, and analyzed by electrophoresis in a 2% agarose gel stained with ethidium bromide (visualized under an UV light).

Statistical analysis

Differences in the distributions of demographic and related lifestyle as well as clinical characteristics and genotypes of IL-10 polymorphisms between patients and controls were calculated by the χ^2-test. Univariate logistic regression analysis was taken to analyze the association between demographic and related lifestyle characteristics of subjects and risk of osteosarcoma, and the results were expressed by Odd's ratios (ORs) and their 95% confidence intervals (CIs). The Hardy-Weinberg equilibrium (HWE) was calculated using the goodness-of-fit χ^2-test. Multiple logistic regression models were established to determine the association between XRCC1 Arg194Trp, Arg280His and Arg399Gln and ERCC2 Lys751Gln polymorphisms and risk of osteosarcoma, and the ORs and their 95% CIs were calculated for this association. All statistical analyses were performed using the SPSS statistical software package, version

Table 1. The primers, length of digested fragment and restriction enzymes of XRCC1 Arg194Trp, Arg280His and Arg399Gln and ERCC2 Lys751Gln

Genotype	Primers (5'-3')	Restriction enzymes	Length of digested fragment
XRCC1 Arg194Trp	GCCCGCTCTGGATTATACG (Forward)	*Pvu*II	485 bp
	CTATCATCTCCTGGCCCCC (Reverse)		
XRCC1 Arg280His	CCAGTGGTGCTAACCTAATC (Forward)	*Rsa*I	201 bp
	CACTCAGCACCACTACCACA (Reverse)		
XRCC1 Arg399Gln	TTGTGCTTTCTCTGTGTCCA (Forward)	*Msp*I	615 bp
	TCCTCCAGCCTTTTCTGATA (Reverse)		
ERCC2 Lys751Gln	CCCCCTCTCCCTTTCCTCTG (Forward)	*Mbo*II	184 bp
	AACCAGGGCCAGGCAAGAC (Reverse)		

Table 2. Characteristics of patients with osteosarcoma and control subjects

Characteristics	Patients N = 172	%	Controls N = 275	%	χ^2 test	OR (95% CI)	*P* value
Gender							
Female	63	36.63	120	43.64		1.0 (Reference)	-
Male	109	63.37	155	56.36	2.15	1.34 (0.89-2.02)	0.14
Age, years							
< 20	108	62.79	191	69.45		1.0 (Reference)	-
≥ 20	64	37.21	84	30.55	2.12	1.35 (0.88-2.05)	0.15
Smoking habit							
No	147	85.47	241	87.64		1.0 (Reference)	-
Yes	25	14.53	34	12.36	0.44	1.21 (0.66-2.17)	0.51
Drinking habit							
No	140	81.40	235	85.45		1.0 (Reference)	-
Yes	32	18.60	40	14.55	1.29	1.34 (0.78-2.30)	0.26
Family history of cancer in the first relatives							
No	142	82.56	238	86.55		1.0 (Reference)	-
Yes	30	17.44	37	13.45	1.32	1.36 (0.77-2.37)	0.26
Enneking stage							
I-II	71	41.28					
III	101	58.72					
Histological subtype							
Osteoblastic	99	57.56					
Chondroblastic	31	18.02					
Fibroblastic	29	16.86					
Other	13	7.56					
Tumor location							
Extremities	115	66.86					
Other	57	33.14					

16.0 (SPSS Inc., Chicago, IL, USA). All tests were two-sided, with a significant level *P*-value < 0.05.

Results

The demographic, lifestyle and clinical characteristics of patients with osteosarcoma and control subjects are presented in **Table 2**. The mean age of patients with osteosarcoma and control subjects were 18.70±5.82 and 17.27±6.01 years, respectively. There were 63 (36.63%) females and 109 (63.37%) males in patients with osteosarcoma, and there were 120 (43.64%) females and 155 (56.36%) males in controls. As expected, the patients

Table 3. Genotype distributions of XRCC1 Arg194Trp, Arg280His and Arg399Gln and ERCC2 Lys-751Gln between patients and controls

Gene	Patients N = 172	%	Controls N = 275	%	Chi-square test	P value	P value for HWE In controls	Minor Allele Frequency In database	In controls
XRCC1 Arg194Trp									
Arg/Arg	124	72.09	208	75.64					
Arg/Trp	39	22.67	59	21.45					
Trp/Trp	9	5.23	8	2.91	1.75	0.42	0.14	0.1238	0.1364
XRCC1 Arg280His									
Arg/Arg	148	86.05	246	89.45					
Arg/His	20	11.63	29	10.55					
His/His	4	2.33	3	1.09	1.27	0.53	0.06	0.0671	0.06364
XRCC1 Arg399Gln									
Arg/Arg	72	41.86	143	52.00					
Arg/Gln	73	42.44	111	40.36					
Gln/Gln	27	15.70	21	7.64	8.78	0.01	0.93	0.2604	0.2782
ERCC2 Lys751Gln									
Lys/Lys	90	52.33	155	56.36					
Lys/Gln	66	38.37	103	37.45					
Gln/Gln	16	9.30	18	6.40	1.40	0.50	0.87	0.2366	0.2513

with osteosarcoma did not differ significantly from the controls in terms of gender (χ^2 = 2.15, P = 0.14), age (χ^2 = 2.12, P = 0.15), tobacco smoking (χ^2 = 0.44, P = 0.51), alcohol drinking (χ^2 = 1.29, P = 0.26) and family history of cancer in the first relatives (χ^2 = 1.32, P = 0.26). Seventy one (41.28%) patients were at Enneking stages I-II, and 101 (58.72%) were at stage III. Furthermore, 99 (57.56%) patients were osteoblastic histological type, 31 (18.02%) were chondroblastic type, and 29 (16.86%) were fibroblastic type. One hundred and fifteen (66.86%) patients showed tumor lociation at extemeities.

The genotype distributions of XRCC1 Arg194-Trp, Arg280His and Arg399Gln and ERCC2 Lys751Gln were shown in **Table 3**. By chi-square test, there was significant difference in the genotype distributions of XRCC1 Arg399Gln between patients with osteosarcoma and control subjects (χ^2 = 8.78, P = 0.01). XRCC1 Arg194Trp, Arg280His and Arg399Gln and ERCC2 Lys-751Gln genotype distributions were found to be consistent with Hardy-Weinberg equilibrium in the control group, and the P values were 0.14, 0.06, 0.93 and 0.87, respectively. In the control group, the minor allele frequencies of the XRCC1 Arg194Trp, Arg280His and Arg399Gln and ERCC2 Lys751Gln were similar to those

given in the National Centre for Biotechnology Information database (http://www.ncbi.nlm.nih.gov/snp).

Using unconditional logistic regression analysis, we found that individuals with the Gln/Gln genotype of XRCC1 Arg399Gln had an increased risk of osteosarcoma when compared with those carrying the wide-type genotype, and the adjusted OR (95% CI) was 2.55 (1.29-5.09) (P = 0.003) (**Table 4**). Moreover, the Arg/Gln+ Gln/Gln genotype of XRCC1 Arg399Gln was correlated with a lower risk of osteosarcoma (OR = 1.50, 95% CI = 1.01-2.25, P = 0.04), compared to the Arg/Arg genotype. However, the XRCC1 Arg194Trp and Arg280His and ERCC2 Lys751Gln gene polymorphisms did not influence the risk of osteosarcoma.

Discussion

Osteosarcoma was associated with several environmental factors such as exposure radiation [1]. Carcinogenic compounds play an important role in causing direct or indirect DNA alteration. DNA repair is responsible for maintaining genomic stability in response to the assault of environmental carcinogens that causes DNA damage [10]. If left unrepaired, such DNA damage could cause mutation fixation and initiation of carcinogenesis. Up to now,

Table 4. Association between XRCC1 Arg194Trp, Arg280His and Arg399Gln and ERCC2 Lys751Gln and risk of osteosarcoma

Gene	Patients	%	Controls	%	OR (95% CI)	P value
XRCC1 Arg194Trp						
Arg/Arg	124	72.09	208	75.64	1.0 (Reference)	-
Arg/Trp	39	22.67	59	21.45	1.11 (0.68-1.80)	0.66
Trp/Trp	9	5.23	8	2.91	1.89 (0.63-5.77)	0.20
Arg/Trp+ Trp/Trp	48	27.9	67	24.36	1.20 (0.76-1.89)	0.40
XRCC1 Arg280His						
Arg/Arg	148	86.05	246	89.45	1.0 (Reference)	-
Arg/His	20	11.63	29	10.55	1.15 (0.59-2.18)	0.66
His/His	4	2.33	3	1.09	2.21 (0.37-15.24)	0.29
Arg/His+ His/His	24	13.96	32	11.64	1.25 (0.67-2.28)	0.45
XRCC1 Arg399Gln						
Arg/Arg	72	41.86	143	52	1.0 (Reference)	-
Arg/Gln	73	42.44	111	40.36	1.31 (0.85-2.01)	0.20
Gln/Gln	27	15.7	21	7.64	2.55 (1.29-5.09)	0.003
Arg/Gln+ Gln/Gln	100	58.14	132	48.00	1.50 (1.01-2.25)	0.04
ERCC2 Lys751Gln						
Lys/Lys	90	52.33	155	56.36	1.0 (Reference)	-
Lys/Gln	66	38.37	103	37.45	1.10 (0.72-1.68)	0.63
Gln/Gln	16	9.3	18	6.4	1.53 (0.69-3.35)	0.25
Lys/Gln+ Gln/Gln	82	47.67	121	43.85	1.17 (0.78-1.74)	0.43

[1]Adjusted for sex and age.

there are at least five known major DNA repair pathways were found, including above 150 human DNA repair genes, among which the base excision repair (BER) and the nucleotide excision repair (NER) are the most versatile and particularly significant in association with cancer risk [11].

Polymorphisms, which have an effect on the regulation of gene expression, can contribute to the differences between individuals in the susceptibility to a disease and its severity [12]. The regulation of DNA repair is a vital factor in the multistep process of carcinogenesis, and the XRCC1 and ERCC2 gene is an important part of the DNA repair machinery. In our study, we conducted a study to investigate the XRCC1 Arg194Trp, Arg280His and Arg399Gln and ERCC2 Lys751Gln polymorphisms and development of osteosarcoma. We found that the XRCC1 Arg399Gln significantly increased the osteosarcoma risk when compared with the wide-type genotype.

Previous studies have reported the association of XRCC1 Arg399Gln gene polymorphism with

the development of several kinds of cancers, such as breast cancer, ovarian cancer, glioma, prostate cancer, non-small cell lung cancer, chronic lymphocytic leukemia, cervical cancer and esophageal cancer [13-20]. Some studies reported that XRCC1 Arg399Gln polymorphism was associated with development of cancers. Malisic et al. investigated the impact of XRCC1 Arg399Gln polymorphisms on ovarian carcinoma risk in Serbian women, and they suggest that XRCC1 Arg399Gln polymorphisms may be biomarkers of susceptibility for ovarian carcinoma development [14]. Wang et al. conducted a study in a Chinese population, and they suggested that XRCC1 Arg399Gln polymorphisms contribute to increased risk of glioma [15]. However, some studies reported inconsistent study. Al Zoubi evaluated the association between XRCC1 Arg399Gln and susceptibility to breast cancer in a Jordanian population, and they reported that XRCC1 Arg399Gln did not show any significant correlation with susceptibility of breast cancer [13]. Kang et al. conducted a study in a Chinese population, and they did not find a significant association between XRCC1 Arg399Gln polymorphism and risk of

non-small cell lung cancer [17]. Morever, Mutlu et al. indicated that XRCC1 Arg399Gln polymorphism was not associated with the development of chronic lymphocytic leukemia [18]. Baijpai et al. did not find significant association between XRCC1 Arg399Gln polymorphism and susceptibility to cervical cancer [19]. Currently, no study reported the association between XRCC1 gene polymorphisms and osteosarcoma risk. Further studies in different ethnicities are greatly needed to confirm the results of our findings.

Our study has two major limitations. Firstly, the patients and control subjects were selected from a single hospital, which may not represent the general population; therefore, selection bias could not be avoided. However, the genotype distributions of the four SNPs were found to be consistent with Hardy-Weinberg equilibrium in the control group, which suggest that the subjects in our study could represent the general population. Second, the sample size is relatively small, and the statistical power of determining the association between polymorphisms in XRCC1 and ERCC2 and the risk of osteosarcoma could be limited. Therefore, further studies with larger sample sizes must be performed to validate our findings.

In conclusion, our study suggests that the XRCC1 Arg399Gln is significantly associated with the development of osteosarcoma. Further studies with larger sample sizes must be conducted in the future to confirm this association.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Na Wu, Medical Insurance Office, PLA General Hospital, NO.28 Fuxing Road, Haidian, Beijing 100853, China. Tel: +86-010-66936537; Fax: +86-010-66937419; E-mail: zhaozhijj@sina.com

References

[1] Ottaviani G, Jaffe N. The etiology of osteosarcoma. Cancer Treat Res 2009; 152: 15-32.

[2] He M, Wang Z, Zhao J, Chen Y, Wu Y. COL1A1 polymorphism is associated with risks of osteosarcoma susceptibility and death. Tumour Biol 2014; 35: 1297-305.

[3] Zhang HF, Yan JP, Zhuang YS, Han GQ. Association between angiogenic growth factor genetic polymorphisms and the risk of osteosarcoma. Genet Mol Res 2015; 14: 10524-9.

[4] Wang J, Liu H, Liu X, Qi X. Effect of variation of FGF2 genotypes on the risk of osteosarcoma susceptibly: a case control study. Int J Clin Exp Med 2015; 8: 6114-8.

[5] Jin G, Wang M, Chen W, Shi W, Yin J, Gang W. Single nucleotide polymorphisms of nucleotide excision repair and homologous recombination repair pathways and their role in the risk of osteosarcoma. Pak J Med Sci 2015; 31: 269-73.

[6] Hoeijmakers JH. Genome maintenance mechanisms for preventing cancer. Nature 2001; 411: 366-374.

[7] Tudek B. Base excision repair modulation as a risk factor for human cancers. Mol Aspects Med 2007; 28: 258-275.

[8] Maynard S, Schurman SH, Harboe C, de Souza-Pinto NC, Bohr VA. Base excision repair of oxidative DNA damage and association with cancer and aging. Carcinogenesis 2009; 30: 2-10.

[9] Batty DP, Wood RD. Damage recognition in nucleotide excision repair of DNA. Gene 2000; 241: 193-204.

[10] Friedberg EC. DNA damage and repair. Nature 2003; 421: 436-440.

[11] Garfinkel DJ, Bailis AM. Nucleotide Excision Repair, Genome Stability, and Human Disease: New Insight from Model Systems. J Biomed Biotechnol 2002; 2: 55-60.

[12] Choi JK, Kim SC. Environmental effects on gene expression phenotype have regional biases in the human genome. Genetics 2007; 175: 1607-1613.

[13] Al Zoubi MS. X-ray repair cross-complementing protein 1 and 3 polymorphisms and susceptibility of breast cancer in a Jordanian population. Saudi Med J 2015; 36: 1163-7.

[14] Malisic EJ, Krivokuca AM, Boljevic IZ, Jankovic RN. Impact of RAD51 G135C and XRCC1 Arg399Gln polymorphisms on ovarian carcinoma risk in Serbian women. Cancer Biomark 2015; 15: 685-91.

[15] Wang L, Jiang YQ, Zhou MD, Jiang Z. Association between XRCC1 Arg399Gln polymorphism and glioma risk in a Chinese population: a case-control study. Int J Clin Exp Med 2015; 8: 10026-30.

[16] Yan J, Wang X, Tao H, Deng Z, Yang W, Lin F. Corrigendum: Meta-Analysis of the Relationship between XRCC1-Arg399Gln and Arg280His Polymorphisms and the Risk of Prostate Cancer. Sci Rep 2015; 5: 11967.

[17] Kang S, Ma Y, Liu C, Cao C, Hanbateer, Qi J, Li J, Wu X. Assoication of XRCC1 gene polymor-

phisms with risk of non-small cell lung cancer. Int J Clin Exp Pathol 2015; 8: 4171-6.

[18] Mutlu P, Elçi MP, Yıldırım M, Nevruz O, Çetin AT, Avcu F. Identification of XRCC1 Arg399Gln and XRCC3 Thr241Met Polymorphisms in a Turkish Population and Their Association with the Risk of Chronic Lymphocytic Leukemia. Indian J Hematol Blood Transfus 2015; 31: 332-8.

[19] Bajpai D, Banerjee A, Pathak S, Thakur B, Jain SK, Singh N. Single nucleotide polymorphisms in the DNA repair genes in HPV-positive cervical cancer. Eur J Cancer Prev 2015; [Epub ahead of print].

[20] Yun YX, Dai LP, Wang P, Wang KJ, Zhang JY, Xie W. Association of polymorphisms in X-ray repair cross complementing 1 gene and risk of esophageal squamous cell carcinoma in a Chinese population. Biomed Res Int 2015; 2015: 509215.