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Original Article

Association between XRCC3 Thr241Met polymorphism and risk of osteosarcoma in a Chinese population

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Abstract: The aim of this study was to investigate whether XRCC3 Thr241Met polymorphism could affect the development of osteosarcoma in a Chinese population. A total of 152 osteosarcoma patients and 304 health control subjects were included in our study. Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay was applied to assess the XRCC3 Thr241Met gene polymorphism. By conditional logistic regression analysis, we found that TT genotype of XRCC3 Thr241Met was associated with increased risk of osteosarcoma in codominant model (OR = 2.53, 95% CI = 1.28-5.39). Moreover, XRCC3 Thr241Met gene polymorphism was correlated with an elevated increased risk of osteosarcoma in dominant (OR = 1.55, 95% CI = 1.03-2.34) and recessive models (OR = 2.30, 95% CI = 1.16-4.56). In conclusion, we found that XRCC3 Thr241Met gene polymorphism was associated with increased risk of osteosarcoma in codominant, dominant and recessive models.

Keywords: XRCC3 Thr241Met, polymorphism, osteosarcoma, Chinese population

Introduction

Osteosarcoma is one of the most common bone malignancies, which often occurs in the distal femur, proximal tibia and humeral metaphysis. Osteosarcoma is more common in children and adolescents, and it is estimated that the annual incidence of this cancer is about 3.6/1,000,000 among children and adolescents [1]. It is well known that the development of osteosarcoma is a complex, multistep and multifactorial process, and many environmental and genetic factors are involved in the development of this cancer [2-4]. However, not all these cancer patients would develop osteosarcoma even when they have been exposed to similar risk factor, which suggests that genetic variations could influence the development of osteosarcoma. Several previous studies have reported that genetic factors may influence the development of osteosarcoma, such as RECQL5, GSTs, ABCB1 and VEGF genes [5-8].

X-ray repair cross-complementing group 3 is a member of DNA repair genes, and it is involved in the process of homologous recombination repair for DNA double-strand breaks so as to maintain the stability of genome [9]. *XRCC3* Thr241Met (rs861539) polymorphism is a common functional single-nucleotide polymorphism (SNP) at codon 241 in exon 7 with a C to T transition [10]. Previous studies reported that XRCC3 Thr241Met polymorphism could influence the risk of several kinds of cancers, such as prostate cancer, colorectal cancer, lung cancer, colorectal cancer and gastric cancer [11-14]. However, no previous study reported the association between XRCC3 Thr241Met polymorphism and risk of osteosarcoma. The aim of this study was to investigate whether XRCC3 Thr241Met polymorphism could affect the development of osteosarcoma in a Chinese population.

Materials and methods

Study population

Patients with pathologically proven osteosarcoma were included from the First Affiliated

Table 1. Characteristics of osteosarcoma patients and control subjects

Variables	Patients	%	Controls	%	χ^2 value	*P* value
Age, years						
< 20	94	61.84	182	59.87		
≥ 20	58	38.16	122	40.13	0.17	0.68
Gender						
Female	64	42.11	128	42.11		
Male	88	57.89	176	57.89	0.00	1.00
Enneking stage						
I-II	97	63.82				
III	55	36.18				
Tumor location						
Extremities	110	72.37				
Other	42	27.63				
Tumor metastasis						
Negative	94	61.84				
Positive	58	38.16				

Hospital of Xinxiang Medical University between January 2012 and December 2014. All the osteosarcoma patients were newly diagnosed and previously untreated. Clinical and pathological information of the osteosarcoma patients was obtained from medical records, such as tumor location, stage, histological type and tumor metastasis. A total of 176 osteosarcoma patients were included in our study, and finally 152 patients agreed to participate into this study with a participation rate of 86.36%. The controls group consisted of 304 subjects without cancers, and the controls were selected from patients who received health examination in our hospital during the same period. Two control subjects matched with one patient in terms of age and gender.

The socio-demographic characteristics of the cases and controls were interviewed using a standardized questionnaire, such as age, tobacco smoking, alcohol drinking and family history of cancer. All the cases and controls voluntarily participated in the study and gave their informed consent. The ethical committee of our hospital approved the study protocols.

DNA extraction and SNPs genotyping

Each participant was asked to provide 5 ml blood sample for DNA extraction. The commercially available Qiagen kit (QIAGEN Inc., Valencia, CA, USA) was used to extract DNA from peripheral blood leukocytes. Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay was applied to assess the XRCC3 Thr241Met gene polymorphism. Primers of XRCC3 Thr241Met were designed using Sequenom Assay Design 3.1 software, and the forward and reverse primers were 5'-TTGGGGCCTCTTTGAGA-3' and 5'-AACGGCTGAGGGTCTTCT-3', respectively. For PCR amplification, the standard program was used as follows: an initial denaturation step of 8 minutes at 94°C, followed by 30 cycles at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 1 minute. PCR products were digested with *Nla*III restriction enzyme. Length of PCR products was 205 bp for XRCC3 Thr241Met. The resulting DNA fragments were electrophoresed on 3.5% agarose gel and visualized under UV light after ethidum staining.

Statistical analysis

All analyses were performed using the IBM SPSS Statistics, version 16.0 (IBM Corporation, Armonk, NY, USA). The statistical differences between osteosarcoma patients and controls were analyzed by a Chi-squared test. Fisher's exact test was taken to assess the Hardy-Weinberg equilibrium (HWE) in controls. Conditional logistic regression analysis with adjusted OR and their associated 95% CIs was used to estimate the strength of association between the XRCC3 Thr241Met polymorphism and osteosarcoma risk. The most common control homozygote of XRCC3 Thr241Met was taken as reference for analysis. A two-tailed *P* value of < 0.05 was considered to be statistically significant.

Results

The characteristics of patients with osteosarcoma and controls were shown in **Table 1**. There were no significant differences between cases and controls in terms of sex (*P* value = 0.68) and age (*P* value = 1.00). For Enneking stage of 152 patients, 97 (63.82%) were stage

Table 2. Association between XRCC3 Thr241Met gene polymorphism and osteosarcoma risk

Model	XRCC3 Thr241Met	Patients	%	Controls	%	OR (95% CI)[1]	*P* value
Codominant	CC	66	43.42	166	54.61	1.0 (Ref.)	-
	CT	63	41.45	117	38.49	1.35 (0.87-2.10)	0.15
	TT	22	15.13	21	6.91	2.53 (1.28-5.39)	0.003
Dominant	CC	66	43.42	166	54.61	1.0 (Ref.)	-
	CT+TT	85	56.58	138	45.39	1.55 (1.03-2.34)	0.03
Recessive	CC+CT	129	84.87	283	93.09	1.0 (Ref.)	-
	TT	22	15.13	21	6.91	2.30 (1.16-4.56)	0.01

[1]Adjusted for sex and age.

Table 3. Stratification analysis of association between XRCC3 Thr241Met polymorphism in osteosarcoma risk by clinical characteristics

Variables	CC	%	CT+TT	%	OR (95% CI)[1]	*P* value
Enneking stage						
I-II	45	68.18	52	61.18	1.0 (Ref.)	
III	21	31.82	34	40.00	1.40 (0.68-2.92)	0.33
Tumor location						
Extremities	49	74.24	61	71.76	1.0 (Ref.)	
Other	17	25.76	25	29.41	1.18 (0.54-2.61)	0.65
Tumor metastasis						
Negative	43	65.15	51	60.00	1.0 (Ref.)	
Positive	23	34.85	35	41.18	1.28 (0.63-2.64)	0.46

[1]Adjusted for sex and age.

I-II osteosarcoma, and 55 (36.18%) were stage III osteosarcoma. For tumor location of these patients, 110 (72.37%) were extremities osteosarcoma and 42 (27.63%) were other osteosarcoma. For the tumor metastasis of these cases, 58 (38.16%) were positive osteosarcoma and 94 (61.84%) were negative osteosarcoma.

The genotype frequencies of XRCC3 Thr241Met gene in osteosarcoma patients and controls were shown in **Table 2**. The genotype distributions of XRCC3 Thr241Met were in line with HWE in control group, and *P* value for HWE was 0.95. By χ^2 test, there were significantly differences in genotype frequencies of XRCC3 Thr241Met between patients and controls (χ^2 = 8.88, *P* value = 0.01). By conditional logistic regression analysis, we found that the TT genotype of XRCC3 Thr241Met was associated with increased risk of osteosarcoma in codominant model (OR = 2.53, 95% CI = 1.28-5.39). Moreover, XRCC3 Thr241Met gene polymorphism was correlated with an elevated increased risk of osteosarcoma in dominant (OR = 1.55, 95% CI = 1.03-2.34) and recessive models (OR = 2.30, 95% CI = 1.16-4.56).

By stratification analysis, we did not find significant association of XRCC3 Thr241Met polymorphism with Enneking stage, tumor location and tumor metastasis in the risk of osteosarcoma (**Table 3**). Moreover, no interaction was found between XRCC3 Thr241Met polymorphism and clinical characteristics (*P* for interaction > 0.05).

Discussion

Osteosarcoma was associated with several environmental factors, such as ionizing radiation [15]. Carcinogenic compounds exert their effect causing direct or indirect DNA alteration. Repair of DNA damage capacity which is under genetic control may be an important factor influencing osteosarcoma susceptibility. Polymorphisms in DNA repair genes resulting in variation of DNA repair efficiency may therefore be associated with osteosarcoma risk. In the present study, we firstly reported the impact of XRCC3 Thr241Met polymorphism in the susceptibility to osteosarcoma in a Chinese population.

Polymorphism in exon 7 of the XRCC3 gene results in an amino acid substitution at condon 241 could influence the enzyme function, and may affect its association with other DNA damage and repair proteins. Increasing studies have suggested that variations of XRCC3 Thr241Met gene may alter DNA repair capacity, and thus influence the susceptibility to carcinogens.

Numerous epidemiological studies have shown a strong association between XRCC3 Thr241Met polymorphism and the development of several kinds of cancers, such as breast cancer, colorectal cancer, lung cancer, gastric cancer and hepatocellular cancer [16-20]. Qureshi et al. conducted a study with 156 breast cancer patients and 150 controls in a Pakistani population, and they reported that XRCC3 Thr241Met polymorphism can be independent markers of breast cancer [16]. Nissar et al. investigated the role of XRCC3 Thr241Met gene polymorphism in colorectal cancer, and reported that CT and TT genotypes were associated with elevated risk of colorectal cancer [17]. Duan et al. conducted a meta-analysis with six individual studies, and they reported that XRCC3 Thr241Met polymorphism variant was significantly associated with hepatocellular cancer risk in the HBsAg (+) individuals [18]. However, some studies reported no association between XRCC3 Thr241Met gene polymorphism and cancer risk (Xing et al., 2014; Wang et al., 2014). Xing et al. reported that XRCC3 Thr241Met gene polymorphism may not be associated with development of lung cancer [19]. One meta-analysis study with 12 case-control studies reported that XRCC3 Thr241Met gene polymorphism was not associated with an increased gastric cancer risk [20]. The discrepancies existed in results from different studies may be caused by different cancer types, sample size and study design.

Current, no previous study reported the association between XRCC3 Thr241Met and risk of osteosarcoma. In our study, we found that XRCC3 Thr241Met gene polymorphism was associated with increased risk of osteosarcoma in codominant, dominant and recessive models. Further large sample size studies are greatly needed to confirm our results.

Some limitations should be mentioned in this study. First, since a hospital-based case control study was taken in our study, the selection bias cannot be avoidable and the subjects may not be representative of the general population. Second, sample size of this study is relatively small, which may not have enough statistical power to explore the real association. Finally, the population in our study was only from Chinese population, which reduces the possibility of confounding from ethnicity, so it does not permit extrapolation of the results to other ethnic groups.

In conclusion, our study firstly reported the role of XRCC3 Thr241Met gene polymorphism in the susceptibility to osteosarcoma, and we found that XRCC3 Thr241Met gene polymorphism was associated with increased risk of osteosarcoma in codominant, dominant and recessive models. Future studies using larger sample size and employing either similar or different analytic strategies may help to elucidate the impact of XRCC3 Thr241Met polymorphism in the risk of osteosarcoma.

Disclosure of conflict of interest

None.

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