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Original Article

Association between IL-8 rs4073 polymorphisms and osteosarcoma risk in Chinese population: a case control study

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Abstract: Background: Interleukin-8 (IL-8) is an angiogenic chemokine that plays a potent role in both development and progression of many human malignancies. Patients and methods: A hospital-based case-control study was conducted among 109 patients with osteosarcoma and 109 healthy controls to investigate the possible association between the IL-8 rs4073 polymorphisms and the risk of osteosarcoma. Results: Significant differences of genotype distribution were observed between osteosarcoma cases and controls at the IL-8 rs4073 genotypes. Compared with the IL-8 -251T/A homozygote TT, the heterozygous TA genotype was associated with significantly increased risk for osteosarcoma (OR = 1.96, 95% CI = 1.28-3.75, P = 0.014); the AA genotype was associated with increased risk for osteosarcoma (OR = 1.68, 95% CI = 1.11-3.98, P = 0.025). TA and AA combined variants were associated with increased risk for osteosarcoma compared with the TT genotype (OR = 1.76, 95% CI = 1.19-3.86, P = 0.012). Moreover, the genotype AA of IL-8 -251T/A carried a higher risk of osteosarcoma metastasis and later Enneking stages, compared with the TT genotype. Conclusion: Our results showed that the IL-8 rs4073 genotype was associated with increased risk for development and metastasis of osteosarcoma in Chinese Han population.

Keywords: IL-8, osteosarcoma, single-nucleotide polymorphism, susceptibility

Introduction

Osteosarcoma is a malignant neoplasm that mainly arises in the metaphyses of long bones, such as the distal femur, proximal tibia, and proximal humerus [1]. Osteosarcoma mainly occurs in children and adolescents and accounts for 20% of all primary sarcomas in bone tumor [2-4]. Current treatment for osteosarcoma consists of en bloc resection with wide margin, aggressive adjuvant chemotherapy, and radiotherapy. Despite advancements in treatments over the past few decades, relapse and metastasis may occur, amounting to 30% of these patients, and the 5-year survival rate of osteosarcoma is still low than 50% [5]. This low survival rate has been attributed to the lack of understanding of the etiopathogenesis of osteosarcoma.

Molecular epidemiology studies suggested that single nucleotide polymorphisms (SNPs) in specific genes and pathways may play an important role in the pathogenesis of osteosarcoma. Interleukin-8 (IL8) is a member of the CXC chemokine family [6]. It is a major mediator of inflammation, acting as a chemoat-tractant for neutrophils, basophils and T-cells, and is a potent angiogenic factor [7]. IL-8 is encoded by the IL-8 gene which was localized to 4q12-q13, consisting of four exons, three introns, and the proximal promoter region [8]. The IL-8 gene polymorphisms at positions -251 (rs4073) and +781 (rs2227306) are known to affect IL-8 expression [9-11].

Previous studies have revealed that SNPs of the IL-8 gene were associated with several diseases, such as respiratory syncytial virus bronchiolitis, acute respiratory distress syndrome and gastric cancer [12, 13]. However, there is no any report on investigating IL-8 polymorphism of osteosarcoma patients. The aim of our study was to investigate the possible asso-

Table 1. Characteristics of the osteosarcoma patients and controls

Characteristics	Cases (%) N = 109	Controls (%) N = 109	*P* value *
Mean Age (years)	21.9 (±10.3)	22.4 (±12.3)	0.162
≤20	64 (58.7)	59 (54.1)	
>20	45 (41.2)	50 (45.9)	
Gender			
Male	60 (55)	56 (51.4)	0.281
Female	49 (45)	53 (48.6)	
Tumor location			
Long tubular bones	98 (89.9)		
Axial skeleton	11 (10.1)		
Pathological fracture			
Yes	25 (22.9)		
No	84 (77.1)		
Metastasis			
Yes	42 (38.5)		
No	67 (61.5)		
Enneking stages			
I	15 (13.7)		
II	76 (69.7)		
III	18 (16.6)		

*Student's t-test and the chi-square (χ^2) test.

ciation between the polymorphisms of IL-8 -251 rs4073 and risk of osteosarcoma in Chinese Han population.

Material & methods

Study population

The case-control study is composed of 109 osteosarcoma specimens in total from Tianjin Hospital between January 2010 and January 2015. All of them were histologically/pathologically confirmed by two experienced pathologists. The control group comprised 109 healthy volunteers for the general health checkup in our hospital during the same period. All the healthy controls had been under the health screening, and their clinical characteristics were matched to the sex and age distribution with the osteosarcoma cases, as outlined in **Table 1**. After obtaining written informed consent, 8 mL of peripheral blood was collected for DNA extraction. Each participant was interviewed using a standard questionnaire by a trained nurse, to collect medical histories, demographic characteristics. The present study was performed with strict protocol under the Ethics Committee of Tianjin Hospital. All the specimens we recruited were of Chinese Han ethnicity and were filtered based on their clinical characteristics. Before the assay, we obtained a written informed consent from each participant in our study.

DNA extraction and genotyping

The polymorphisms in the promoters of the IL-8 genes analyzed in this study are shown in **Table 2**. The polymerase chain reaction (PCR) combined with the restriction fragment length polymorphism (RFLP) was used to determine the IL-8 genotypes. Genomic DNA used for the assay was extracted from peripheral blood samples (96.5% of total samples) or exfoliated buccal cells (3.5% of total samples) as previously described [11]. For quality control, genotyping was repeated randomly in at least 5% of the samples, and two of the authors independently reviewed all results. PCR reactions were carried out in a total volume 10 μL containing 20 ng of genomic DNA, 0.25 mM of each Dntp (Ecogen, Biologia Molecular S.L.), 0.2 units of Taq polymerase (Biotools, Inc.) and 2.5 pmol of each primer in a 1×PCR buffer (Sigma-Aldrich Co.). The details of the primers and PCR conditions used for the amplification of IL-8 are shown in **Table 2**.

Statistical analysis

During the analysis, student t-test and chi-square (χ^2) test were performed to analysis the differences in the distribution of various considered characteristics as well as the differences of genotype frequencies between the osteosarcoma patients and the healthy controls, as appropriate. Similarly, the Hardy-Weinberg equilibrium (HWE) of each subject was examined by implying a two-sided chi-square (χ^2) test which was performed by comparison of observed and expected genotype frequencies. The IL-8 -251A/T polymorphisms genotypes related osteosarcoma risk was assessed by odds ratio (OR) and their corresponding respective confidence intervals 95% (CIs) value of the logistic regression, for both combined and respective genotype. We managed all the statistical analysis with the SPSS software version 19.0. A two-sided *P* value less

Table 2. Details of PCR Primer sequences and RFLPs conditions in our study

Gene	Polymorphism	SNP ID	Primer sequence	PCR Conditions
IL-8	-251T/A	rs4073	F: TCATCCATGATCTTGTTCTAA R: GGAAAACGCTGTAGGTCAGA	35 cycles: 95°C 40 s, 54°C 40 s, 72°C 60 s

Table 3. Association between IL-8 -251T/A (rs4073) gene and patients with osteosarcoma susceptibility

Polymorphisms	Cases (N = 109) (%)	Controls (N = 109) (%)	OR (95% CI)	*P*-value*
-251T/A rs4073				
TT	58 (53.2)	73 (67.0)	1	
TA	32 (29.4)	24 (22.0)	1.96 (1.28-3.75)	***0.014***
AA	19 (17.4)	12 (11.0)	1.68 (1.11-3.98)	***0.025***
TA+AA	41 (47.9)	36 (35.8)	1.76 (1.19-3.86)	***0.012***
T	148 (68.7)	170 (77.4)	1	
A	70 (31.3)	48 (22.6)	1.76 (1.25-4.34)	***0.017***

OR, odds ratio; CI, confidence interval. *Bold numbers indicate that the *P*-value is <0.05.

than 0.05 was considered to be statistically significant for all the analyses.

Results

Population characteristics

This study included 109 osteosarcoma patients and 109 healthy controls, age, gender, Enneking's stage, pathological fracture, metastasis and tumor location were summarized in **Table 1**. The mean age (±SD) for case and control groups was 21.9 (10.3) and 22.4 (12.3) years, respectively. Our study included 109 osteosarcoma cases, including 60 males and 49 females, and 109 healthy controls, including 56 males and 53 females. No significant difference was detected in the age and gender distribution between two groups (P>0.05). Additionally, there were 98 patients (89.9%) with long tubular bones and 11 patients (10.1%) patients with axial skeleton. Regarding the clinical stage, 13.7% of patients were in stage I, and 69.7% were in stage II, whereas 16.6% of patients presented III stage. The control population was consistent with the Hardy-Weinberg Equilibrium (HWE) for the polymorphisms in IL-8 -251A/T.

Distributions of IL-8 -251A/T genotypes and risk of osteosarcoma

The genotype and allele frequencies of the IL-8 -251T/A (rs4073) and IL-8 +781C/T (rs2227306) polymorphisms for all the studied variations are shown in **Table 3**. All genotype frequencies of the control group conformed to the Hardy-Weinberg equilibrium.

There were significant differences in the genotype and allele frequencies of IL-8 -251T/A (rs4073) genotypes between osteosarcoma cases and controls. Compared with the IL-8 rs4073 homozygote TT, the heterozygous TA genotype was associated with significantly increased risk for osteosarcoma (OR = 1.96, 95% CI = 1.28-3.75, P = 0.014); the AA genotype was associated with increased risk for osteosarcoma (OR = 1.76, 95% CI = 1.19-3.86, P = 0.012). TA and AA combined variants were associated with increased risk for osteosarcoma compared with the TT genotype (OR = 1.76, 95% CI = 1.26-4.34, P = 0.017). However, the genotype and allele frequencies of IL-8 rs2227306 polymorphisms in osteosarcoma patients were not significantly different from controls (P>0.05) as shown in **Table 3**.

Distributions of IL-8 -251A/T genotypes and clinicopathological characteristics

The relationships between the IL-8 -251A/T and +781C/T genotypes polymorphisms and clinicopathological parameters were calculated. The results are given in **Table 4**. For IL-8 -251T/A rs4073, the genotype AA frequency in tumor metastasis patients was greater compared to patients without tumor metastasis, and the difference in frequency distribution between genotypes reached significance (P = 0.021). The similar result was fond with respect to Enneking stages. No significant difference was observed with respect to age, gender, pathological fracture and tumor location and the IL-8 rs4073 genotypes.

Discussion

In current hospital based case-control study, we assessed the association between the polymorphisms of two SNPs of IL-8 rs4073 -251T/A

Table 4. Correlations between genotypes of IL-8 -251T/A (rs4073) and clinicopathological features of patients with osteosarcoma

Genotypes		-251T/A rs4073			
Variable	n	TT	TA	AA	*P* value
Age (years)		58	32	19	
≤20	64	26	13	13	0.452
>20	45	32	19	6	
Gender					
Male	60	21	17	16	0.352
Female	49	37	15	3	
Tumor location					
Long tubular bones	168	86	57	25	0.371
Axial skeleton	22	13	6	3	
Pathological fracture					
Yes	98	15	12	11	0.427
No	11	84	51	17	
Metastasis					
Yes	42	18	9	15	0.024*
No	67	40	23	4	
Enneking stages					
I	15	7	5	4	0.027*
II	76	47	23	6	
III	18	4	5	9	

*Student's t-test and the chi-square (χ^2) test.

and risk of osteosarcoma in Chinese Han population and found the significant association between IL-8 rs4073 -251T/A polymorphisms and risk of osteosarcoma. The genotype and allele distribution of polymorphisms rs4073 -251T/A of IL-8 genotypes were significantly different between case and control groups, indicating that rs4073 -251T/A of IL-8 might be related to osteosarcoma development.

Moreover, our results showed the genotype AA frequency of IL-8 -251T/A rs4073 in tumor metastasis patients was greater compared to patients without tumor metastasis, the similar result was fond with respect to Enneking stages. These results indicated that the genotype AA of IL-8 -251T/A carried a higher risk of osteosarcoma metastasis and later Enneking stages, compared with the TT genotype. To the best of our knowledge, our study is the first report to describe the possible role of two polymorphisms of IL-8 rs4073 -251T/A as a risk factor for osteosarcoma and found that IL-8 rs4073 genotype variations do influence susceptibility to osteosarcoma development and metastasis in the Chinese Han population.

Osteosarcoma is a common malignant tumor, which exists widely in the bone of children and adolescents [14, 15]. Up to now, inaugural mechanism of osteosarcoma was considered as a complex process and was not clear, but it was universally acknowledged that environment carcinogens could induce genomic polymorphism, such as oxidative stress, drinking, smoking, and ionizing radiation [16]. Interleukin-8, a member of the chemokine family, is produced by a wide range of normal cells including monocytes, neutrophils, fibroblasts, and endothelials cells, as well as by several types of tumor cells [17]. It was originally described as a chemoattractant for neutrophils and lymphocytes [18] and recently linked to cancer progression through its functions as mitogenic, motogenic, and angiogenic factor [13]. Recent studies revealed that IL-8 is overexpressed in a range of human cancers including nasopharyngeal [19], breast [20], and gastric cancers [21] and may, thus, constitute a risk factor in the development of solid tumors.

In agreement with our findings, several studies reported a relationship between the IL-8 -251 (T/A) gene polymorphism and human cancer. This polymorphism is associated with a high risk of occurrence of gastric cancer, a strong neutrophil infiltration, an increased risk of lymph node metastasis and a poor differentiation of tumors [18]. The IL-8 (-251) A allele was also associated with a higher risk of prostate cancer [22], colorectal cancer [23], and oral squamous cell carcinoma [24]. There is now compelling evidence that these correlations are the result of increased levels of IL-8 protein, which may impact cancer development via regulation of immune responses and pathways of tumor angiogenesis and cancer progression [24]. IL-8 is also an important chemoattractant, involved in the activation and migration of lymphocytes and neutrophils into tissues and,

thereby, is a major contributing factor involved in the initiation and amplification of the inflammatory response [18].

In conclusion, our study provided the evidence of association between the polymorphisms of IL-8 -251 rs4073 and the risk of osteosarcoma and found the IL-8 -251A/T genotype was associated with increased risk for development and metastasis of osteosarcoma in Chinese Han population.

Disclosure of conflict of interest

None.

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