

Original Article

Interleukin-6 gene-174 G/C polymorphism is correlated with susceptibility of osteosarcoma in Chinese population

Jianbing Niu¹, Xiulian Zhao², Qingsheng Liu¹, Guangyu Liu³

¹Department of Bone and Joint Surgery, Jining No. 1 People's Hospital, No 6, Jian Kang Road, Jining 272011, Shandong Province, China; ²Department of Kidney and Medical, Jinxiang People's Hospital, No. 117, Jin Feng East Road, Jinxiang 272200, Shandong Province, China; ³Department of Traumatology, Linyi People's Hospital, Linyi 276003, Shandong Province, China

Received December 18, 2015; Accepted March 30, 2016; Epub August 15, 2017; Published August 30, 2017

Abstract: Background: Interleukin-6 (IL-6) is an angiogenic chemokine that plays a potent role in both development and progression of many human malignancies. Patients and methods: A total of 232 subjects, including 116 patients with osteosarcoma and 116 healthy controls, were recruited in this study. Interleukin-6 gene-174 G/C polymorphisms were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Results: Significant differences of genotype distribution were observed between osteosarcoma cases and controls at the IL-6-174 G/C genotypes. Compared with the homozygote GG, the heterozygous GC genotype was associated with significantly increased risk for osteosarcoma (OR = 1.95, 95% CI = 1.15-3.75, P = 0.011); the CC genotype was associated with increased risk for osteosarcoma (OR = 1.78, 95% CI = 1.25-3.96, P = 0.013). GC and CC combined variants were associated with increased risk for osteosarcoma compared with the GG genotype (OR = 1.89, 95% CI = 1.29-3.81, P = 0.022). Moreover, the genotype CC of IL-6-174 G/C carried a higher risk of osteosarcoma metastasis and later Enneking stages, compared with the GG genotype. Conclusion: Our results showed that the IL-6 gene-174 G/C genotype was associated with increased risk for development and metastasis of osteosarcoma in Chinese Han population.

Keywords: IL-6, 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 spe-

cific genes and pathways may play an important role in the pathogenesis of osteosarcoma. Interleukin-6 (IL-6), a phosphorylated glycoprotein containing 185 amino acids, is a multifunctional protein principally involved in different physiologic and pathophysiologic processes including proliferation and differentiation and plays a pivotal role in acute phase response and in the control of the balance between pro-inflammatory and anti-inflammatory pathways [6]. It has emerged in literature as one of the most important regulators of the cytokine-related tumor biology [7], involved in key steps of tumor development, such as proliferation, apoptosis, angiogenesis, and differentiation [8-11].

It has been found that IL-6 promoter polymorphisms were associated with various cancer risks such as esophageal cancer, prostate cancer, colorectal cancer, oral cancer and cervical

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Table 1. Characteristics of the osteosarcoma patients and controls

Characteristics	Cases (%)	Controls (%)	P value
Number of subjects	116	116	
Age (years), (mean \pm SD)	22.1 (\pm 8.4)	21.2 (\pm 9.9)	0.246
Gender (male/Female)	64/52	59/57	0.148
Tumor location			
Long tubular bones	86 (74.1)		
Axial skeleton	30 (25.9)		
Pathological fracture			
Yes	25 (21.6)		
No	91 (78.4)		
Metastasis			
Yes	43 (37.1)		
No	73 (62.9)		
Enneking stages			
I	25 (21.6)		
II	74 (63.8)		
III	17 (14.6)		

*Chi-square (χ^2) test.

cancer [12-16]. However, there is no any report on investigating IL-6 polymorphism of osteosarcoma patients. The aim of our study was to investigate the possible association between the polymorphisms of Interleukin-6 gene-174 G/C and risk of osteosarcoma in Chinese Han population.

Material and methods

Study population

We conducted a hospital-based case-control study. A total of 232 subjects, including 116 osteosarcoma cases and 116 age- and gender-matched healthy controls, were recruited in this study between February 2009 and December 2015 in Linyi People's Hospital. All of them were histologically/pathologically confirmed by two experienced pathologists. All the healthy controls had been under the health screening, and their clinical characteristics were matched to the gender and age distribution with the osteosarcoma cases, as outlined in **Table 1**. 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 Linyi People's 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

Genomic DNA was isolated from 20 g/L ethylenediaminetetra-acetic acid (EDTA) or sodium citrate anticoagulated 5 ml venous blood by the commercially available Qiagen kit (QIAGEN Inc.) and stored at 4°C. The polymerase chain reaction (PCR) combined with the restriction fragment length polymorphism (RFLP) was used to determine the IL-6 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.

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 Interleukin-6 gene-174 G/C 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. The P-value less than 0.05 was considered statistically significant.

Results

Population characteristics

Characteristics of osteosarcoma cases and healthy controls were showed in **Table 1**. No significant difference was detected in the age and gender distribution between two groups ($P > 0.05$). The mean age (\pm SD) for case and control groups was 22.1 (8.4) and 21.2 (9.9) years,

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Table 2. Association between the IL-6-174 G/C genotype and osteosarcoma risk

Polymorphisms	Cases (N = 116) (%)	Controls (N = 116) (%)	OR (95% CI)	P-value*
-174 G/C rs1800795				
GG	46 (39.7)	78 (67.2)	1	
GC	54 (46.6)	20 (17.2)	1.95 (1.15-3.75)	0.011
CC	16 (13.7)	18 (15.5)	1.78 (1.25-3.96)	0.013
GC+CC	70 (56.4)	38 (39.8)	1.89 (1.29-3.81)	0.022
G	146 (62.7)	176 (74.5)	1	
C	86 (37.3)	56 (25.5)	1.82 (1.23-3.95)	0.023

OR, odds ratio; CI, confidence interval. *P-value is < 0.05.

Table 3. Correlations between IL-6-174 G/C genotypes and clinicopathological features of patients with osteosarcoma

Genotypes	-174 G/C rs1800795				P value
	n	GG	GC	CC	
Gender	46	54	16		
Male	64	25	28	11	0.136
Female	52	21	26	5	
Tumor location					
Long tubular bones	86	32	41	13	0.215
Axial skeleton	30	14	13	3	
Pathological fracture					
Yes	25	11	9	5	0.142
No	91	35	45	11	
Metastasis					
Yes	43	11	18	14	0.026*
No	73	35	36	2	
Enneking stages					
I	25	6	17	2	0.038*
II	74	38	32	4	
III	17	2	5	10	

*Chi-square (χ^2) test.

respectively. Our study included 116 osteosarcoma cases, including 64 males and 52 females, and 116 healthy controls, including 59 males and 57 females. Additionally, there were 86 patients (74.1%) with long tubular bones and 30 patients (25.9%) patients with axial skeleton. Regarding the clinical stage, 21.6% of patients were in stage I, and 63.8% were in stage II, whereas 14.6% of patients presented III stage. The control population was consistent with the Hardy-Weinberg Equilibrium (HWE) for the polymorphisms in Interleukin-6 gene-174 G/C.

Distributions of IL-6 gene-174 G/C genotypes and risk of osteosarcoma

The genotype and allele frequencies of the IL-6 gene-174 G/C polymorphisms for all the studied variations are shown in **Table 2**.

There were significant differences in the genotype and allele frequencies of IL-6-174 G/C (rs1800795) genotypes between osteosarcoma

cases and controls. Compared with the IL-6 rs1800795 homozygote GG, the heterozygous GC genotype was associated with significantly increased risk for osteosarcoma (OR = 1.95, 95% CI = (1.15-3.75), P = 0.011); the CC genotype was associated with increased risk for osteosarcoma (OR= 1.78, 95% CI = 1.25-3.96, P = 0.013). GC and CC combined variants were associated with increased risk for osteosarcoma compared with the GG genotype (OR = 1.89, 95% CI = 1.31-4.01, P = 0.029).

Distributions of interleukin-6 gene-174 G/C genotypes and clinicopathological Characteristics

The relationships between the IL-6 gene-174 G/C genotypes polymorphisms and clinicopathological parameters were calculated. The results are given in **Table 3**. For IL-6-174 G/C, the genotype CC frequency in tumor metastasis patients was greater compared to patients without tumor metastasis, and the difference in frequency distribution between genotypes reached significance (P = 0.032). The similar result was found with respect to Enneking stages. No significant difference was observed with respect to gender, pathological fracture and tumor location and the IL-8 rs4073 genotypes.

Discussion

To the best of our knowledge, this is the first study to investigate the relationship between the IL-6 gene promoter polymorphisms and osteosarcoma risk and overall survival. We assessed the association between the IL-6-174 G/C polymorphisms and risk of osteosarcoma in Chinese Han population and found the significant association between IL-6-174 G/C poly-

morphisms and risk of osteosarcoma. The genotype and allele distribution of polymorphisms IL-6-174 G/C genotypes were significantly different between case and control groups, indicating that IL-6-174 G/C might be related to osteosarcoma development. Moreover, our results showed the genotype CC frequency of IL-6-174 G/C genotypes in tumor metastasis patients was greater compared to patients without tumor metastasis, the similar result was found with respect to Enneking stages. These results indicated that the genotype CC of IL-6-174 G/C genotypes carried a higher risk of osteosarcoma metastasis and later Enneking stages, compared with the GG genotype.

Osteosarcoma is a common malignant tumor, which exists widely in the bone of children and adolescents [17]. It aroused people's concern universally owing to its highly malignant, facilely reversion, and readily metastases [18]. 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 [19]. IL-6 is a cytokine which has a great influence in cancer cells, involved in many processes, such as malignant differentiation of cancer cells, tumor growth, and microenvironment immunomodulation [20]. IL-6 is directly involved in the stimulation of tumor growth, by activation of several signaling pathways [21, 22].

In agreement with our findings, several studies reported a relationship between the IL-6-174 G/C gene polymorphism and human cancer. In 2008, Upadhyay et al conducted a case-control study and found that IL-6-174 G/C polymorphism was associated with squamous cell esophageal cancer risk [12]. A case-control study from Spain in 2003 suggested that IL-6-174 G/C polymorphism was associated with colorectal cancer risk [14]. Vairaktaris et al study found that IL-6-174 G/C polymorphism was strongly associated with oral cancer risk [15]. A hospital-based case-control study comprised 518 patients and 518 healthy controls found that the CC genotypes of the IL-6 gene polymorphisms (-174 G/C and -572 G/C) contribute a high risk of cervical cancer in a Chinese population [16].

In spite of interesting findings on the association of IL-6 polymorphisms with osteosarcoma risk, there were several limitations that need to be addressed regarding the present study. We did not collect lifestyle data for individual participants, e.g. on local environmental factors, diet, or level of physical activity, which potentially could interact with genetic variations in influencing overall risk of developing osteosarcoma. Besides, the relative small sample size might hide some weak gene-disease association and gene-environment interactions. Studies need to be performed in larger study groups to confirm our preliminary results.

In conclusion, our study provided the evidence of association between the IL-6-174 G/C polymorphisms and the risk of osteosarcoma and found the IL-6-174 G/C genotype was associated with increased risk for development and metastasis of osteosarcoma in Chinese Han population. The IL-6-174 G/C genotypes variant also modified the survival time in osteosarcoma patients.

Disclosure of conflict of interest

None.

Address correspondence to: Guangyu Liu, Department of Traumatology, Linyi People's Hospital, Linyi 276003, Shandong Province, China. Tel: +86 539 8011536; E-mail: jining1369@sina.com

References

- [1] Kramárová E, Stiller CA. The international classification of childhood cancer. *Int J Cancer* 1996; 68: 759-65.
- [2] Marina N, Gebhardt M, Teot L, Gorlick R. Biology and therapeutic advances for pediatric osteosarcoma. *Oncologist* 2004; 9: 422-41.
- [3] Fletcher CD, Unni KK, Mertens F. Pathology and genetics of tumours of soft tissue and bone. IARC; 2002.
- [4] Bai SB, Chen HX, Bao YX, Luo X, Zhong JJ. Predictive impact of common variations in DNA repair genes on clinical outcome of osteosarcoma. *Asian Pac J Cancer Prev* 2013; 14: 3677-80.
- [5] Chou AJ, Gorlick R. Chemotherapy resistance in osteosarcoma: Current challenges and future directions. *Expert Rev Anticancer Ther* 2006; 6: 1075-85.
- [6] Crosbie EJ, Einstein MH, Franceschi S, Kitchener HC. Human papillomavirus and cervical cancer. *Lancet* 2013; 382: 889-99.

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- [7] Lukaszewicz M, Mroczko B, Szmitkowski M. Clinical significance of interleukin-6 (IL-6) as a prognostic factor of cancer disease. *Pol Arch Med Wewn* 2007; 117: 247-51.
- [8] Crohns M, Saarelainen S, Laine S, Poussa T, Alho H, Kellokumpu-Lehtinen P. Cytokines in bronchoalveolar lavage fluid and serum of lung cancer patients during radiotherapy-association of interleukin-8 and VEGF with survival. *Cytokine* 2010; 50: 30-6.
- [9] Culig Z, Steiner H, Bartsch G, Hobisch A. Interleukin-6 regulation of prostate cancer cell growth. *J Cell Biochem* 2005; 95: 497-505.
- [10] Giannitrapani L, Soresi M, Balasus D, Licata A, Montalto G. Genetic association of interleukin-6 polymorphism (-174G/C) with chronic liver diseases and hepatocellular carcinoma. *World J Gastroenterol* 2013; 19: 2449-55.
- [11] Heffler LA, Grimm C, Ackermann S, Malur S, Radjabi-Rahat AR, Leodolter S, Beckmann MW, Zeillinger R, Koelbl H, Tempfer CB. An interleukin-6 gene promoter polymorphism influences the biological phenotype of ovarian cancer. *Cancer Res* 2003; 63: 3066-8.
- [12] Upadhyay R, Jain M, Kumar S, Ghoshal UC, Mittal B. Association of interleukin-6 (-174G > C) promoter polymorphism with risk of squamous cell esophageal cancer and tumor location: an exploratory study. *Clin Immunol* 2008; 128: 199-204.
- [13] Yang M, Li C, Li M. Association of interleukin-6 (-174 G/C) polymorphism with the prostate cancer risk: a meta-analysis. *Biomed Rep* 2014; 2:637-43.
- [14] Landi S, Moreno V, Gioia-Patricola L, Guino E, Navarro M, de Oca J. Association of common polymorphisms in inflammatory genes interleukin (IL)6, IL8, tumor necrosis factor alpha, NFkB1, and peroxisome proliferator-activated receptor gamma with colorectal cancer. *Cancer Res* 2003; 63: 3560-6.
- [15] Vairaktaris E, Yiannopoulos A, Vylliotis A, Yapijakis C, Derka S, Vassiliou S. Strong association of interleukin-6-174 G > C promoter polymorphism with increased risk of oral cancer. *Int J Biol Markers* 2006; 21: 246-50.
- [16] Shi WJ, Liu H, Wu D, Tang ZH, Shen YC, Guo L. Stratification analysis and case-control study of relationships between interleukin-6 gene polymorphisms and cervical cancer risk in a Chinese population. *Asian Pac J Cancer Prev* 2014; 15: 7357-62.
- [17] Boerman I, Selvarajah GT, Nielen M, Kirpensteijn J. Prognostic factors in canine appendicular osteosarcoma-a meta-analysis. *BMC Vet Res* 2012; 8: 56.
- [18] Jones KB, Salah Z, Del Mare S, Galasso M, Gaudio E, Nuovo GJ, Lovat F, LeBlanc K, Palatini J, Randall RL, Volinia S, Stein GS, Croce CM, Lian JB, Aqeilan RI. miRNA signatures associate with pathogenesis and progression of osteosarcoma. *Cancer Res* 2012; 72: 1865-77.
- [19] Teng JW, Yang ZM, Li J, Xu B. Predictive role of glutathione S-transferases (GSTs) on the prognosis of osteosarcoma patients treated with chemotherapy. *Pak J Med Sci* 2013; 29: 1182-6.
- [20] Zarogoulidis P, Yarmus L, Darwiche K, Walter R, Huang H, Li Z, Zaric B, Tsakiridis K, Zarogoulidis K. Interleukin-6 cytokine: a multi-functional glycoprotein for cancer. *Immunome Res* 2013; 9: 16535.
- [21] Ara T, Declerck YA. Interleukin-6 in bone metastasis and cancer progression. *Eur J Cancer* 2010; 46: 1223-31.
- [22] Gomes M, Coelho A, Araújo A, Azevedo A, Teixeira AL, Catarino R, Medeiros R. IL-6 polymorphism in non-small cell lung cancer: a prognostic value? *Tumour Biol* 2015; 36: 3679-84.