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Original Article

NFKB1 -94ins/del ATTG polymorphism increases osteosarcoma risk in a Chinese Han population

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Abstract: Osteosarcoma is one of the most common bone malignancies. The Nuclear factor-κB1 (NFKB1) gene plays an important role in the pathogenesis of osteosarcoma. The objective of this study aimed to detect the potential association between NFKB1 -94 ins/del ATTG polymorphism and osteosarcoma susceptibility in Chinese Han population. We recruited 220 osteosarcoma patients and 222 cancer-free controls in this case-control study. The NFKB1 -94 ins/del ATTG polymorphism by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Patients with ID genotype and II genotype showed higher risk of osteosarcoma than individuals with DD genotype (OR=1.54, 95% CI 1.00-2.44, *P*=0.05; OR=1.72, 95% CI 1.01-2.93, *P*=0.04), respectively. Subjects with ID or II genotype also showed increased risk of osteosarcoma (OR=1.60, 95% CI 1.04-2.47, *P*=0.03). In addition, I allele was significantly associated with osteosarcoma risk (OR=1.31, 95% CI 1.01-1.71, *P*=0.04). We also found that this polymorphism was significantly associated with advanced osteosarcoma risk (OR=3.43, 95% CI 1.61-7.36, *P*=0.001) and metastatic osteosarcoma risk (OR=2.33, 95% CI 1.22-5.03, *P*=0.01). In conclusion, our findings indicate that osteosarcoma is associated with the NFKB1 promoter -94ins/del ATTG polymorphism.

Keywords: Osteosarcoma, Nuclear factor-κB, polymorphism, genetics

Introduction

Osteosarcoma is one of the most common bone malignancies, which occurs most frequently in adolescents [1]. Although the neoadjuvant therapy with aggressive surgical resection has improved the prognosis, the treatment of osteosarcoma is still unsatisfactory for the risk of local relapse and the development of pulmonary metastasis [2]. Therefore, a specific genetic biomarker which can identify high risk patients is critical to the effective prevention and treatment of osteosarcoma.

Nuclear factor κB (NF-κB) is a important pleiotropic transcription factor. NF-κB could regulate the innate and adaptive immune response and inflammation [3]. Five members of this transcription factor, such as RELA (p65), RELB, REL (c-Rel), NF-κB1 (p105) and NF-κB2 (p100), have been identified. NF-κB1 (p105) and NF-κB2 (p100) are pro-forms proteolytically processed to p50 and p52 [4]. The human NFKB1 gene is located in chromosome 4q24, which encodes a 50 kDa DNA-binding protein [5]. The previous study found a -94 insertion (ins)/deletion (del) ATTG polymorphism in NFKB1 gene promoter [6]. This polymorphism is reported to be associated with several types of cancer risks, such as lung cancer, hepatocellular carcinoma, colorectal cancer, and breast cancer [7-10]. However, no study is conducted to assess the association between this polymorphism and osteosarcoma risk. Therefore, we performed a case-control study to investigate the NFKB1 -94ins/del ATTG polymorphism and risk of osteosarcoma in a Chinese Han population.

Methods

Study participants

A hospital-based case-control study was conducted in 220 osteosarcoma patients and 222 cancer-free controls between 2012 and 2014 from Shanghai Changzheng Hospital and

Table 1. Characteristics of the cases and controls

Characteristics	Case	Control	*P* value
Gender			
Male	119	113	0.50
Female	101	109	
Age (years)			
≤20	122	119	0.69
>20	98	103	
Enneking stage			
I+II	125	NA	NA
III	95	NA	
Tumour metastasis			
Yes	121	NA	NA
No	99	NA	
Tumor location			
Extremities	143	NA	NA
Other	77	NA	

Cancer Hospital, Fudan University. All subjects were ethnically homogeneous Chinese Han population and from the city of Shanghai and its surrounding region. All patients underwent a series of examinations of pathologic stages by board-certified pathologists. The controls were randomly selected from healthy individuals who underwent routine physical examination in the same area during the same time period as the case study. Controls had no individual history of cancer. Information on individuals was gathered from both cases and controls. The study protocol was approved by the Institutional Review Boards of the hospital. Written consent for publication has been obtained from all patients about whom personal information is contained.

Genotyping and quality control

The blood samples were collected from each enrolled subjects. The genomic DNA was extracted from peripheral venous blood using the Axygen DNA isolation kit (Axygen, USA). DNA fragments containing the polymorphism were amplified with the forward primer 5'-TGG GCA CAA GTC GTT TAT GA-3' and 5'-CTG GAG CCG GTA GGG AAG-3'. The PCR reaction was carried out in a 20 ml reaction mixture containing 1× Phusion High-Fidelity PCR Master Mix (Thermo Scientific, Finland) and 0.25 mM of each primer. The PCR cycle consisted of an initial denaturation step at 98°C for 30 s, followed by 35 cycles of denaturation (98°C for 5 s), annealing (65°C ffor 5 s) and extension (72°C for 5 s), and a final extension at 72°C for 5 min. The 281 bp (deletion allele) or 285 bp (insertion allele) products generated were then digested using PflMI (Van91I) restriction enzyme (Thermo Scientific, Finland). The wildtype (deletion) genotype did not contain PflMI (Van91I) restriction site, hence the PCR product of 281 bp remained undigested. The insertion variants were cleaved by PflMI (Van91I) restriction enzyme into two fragments of 240 bp and 45 bp. Heterozygotes showed all three bands. Ten percent of the samples were subjected to randomly repeated blind assays and all results were consistent.

Statistical analysis

All statistical analyses were performed by the Statistical Package for Social Sciences for Windows software (Windows version release 18.0; SPSS, Inc., Chicago, IL, USA). The frequencies of allele and genotype in cases and controls were calculated by gene counting method. Differences between cases and controls in demographic characteristics and frequencies of genotypes were evaluated by using chi-square (χ^2) test. Differences were considered significant when $P<0.05$.

Results

The cohort of 220 osteosarcoma patients contained slightly more men (n=119) than women (n=113), and 222 controls consisted of 113 men and 109 women. Between case and control, the age and gender were well balanced. The characteristics of cases and controls are listed in **Table 1**.

The genotype and allele frequencies of NFKB1 -94ins/del ATTG polymorphism were shown in **Table 2**. Patients with ID genotype and II genotype showed higher risk of osteosarcoma than individuals with DD genotype (OR=1.54, 95% CI 1.00-2.44, *P*=0.05; OR=1.72, 95% CI 1.01-2.93, *P*=0.04), respectively. Subjects with ID or II genotype also showed increased risk of osteosarcoma (OR=1.60, 95% CI 1.04-2.47, *P*=0.03). In addition, I allele was significantly associated with osteosarcoma risk (OR=1.31, 95% CI 1.01-1.71, *P*=0.04).

In order to determine the association between the polymorphism of NFKB1 -94ins/del ATTG

Table 2. Distribution of *NFKB1* -94 ins/del ATTG polymorphism

Genotype/allele	Case	Control	OR (95% CI)	*P* value
DD	46	66	1 (Reference)	
ID	114	106	1.54 (1.00-2.44)	0.05
II	60	50	1.72 (1.01-2.93)	0.04
II+ID	174	156	1.60 (1.04-2.47)	0.03
D	206	238	1 (Reference)	
I	234	206	1.31 (1.01-1.71)	0.04

Table 3. Association of *NFKB1* -94 ins/del ATTG polymorphism with clinicopathological characteristics

Characteristics	Case	II+ID	DD	OR (95% CI)	*P* value
Gender					
Male	119	93	26	1 (Reference)	
Female	101	81	20	1.13 (0.59-2.18)	0.71
Age (years)					
≤20	122	95	27	1 (Reference)	
>20	98	79	19	1.18 (0.61-2.28)	0.62
Enneking stage					
I+II	125	89	36	1 (Reference)	
III	95	85	10	3.43 (1.61-7.36)	0.001
Tumour metastasis					
Yes	121	88	33	1 (Reference)	
No	99	86	13	2.33 (1.22-5.03)	0.01
Tumor location					
Extremities	143	111	32	1 (Reference)	
Other	77	63	14	1.30 (0.64-2.61)	0.47

polymorphism and some clinicopathological features, we conducted stratified analyses for combined genotypes with the DD genotype versus the ID+II genotypes in osteosarcoma patients according to gender, age, Enneking stage, metastasis, and location. There was a significantly higher frequency of ID+II genotypes observed in metastatic osteosarcoma patients, compared to non-metastatic patients (OR= 2.33, 95% CI 1.22-5.03, *P*=0.01). In addition, subjects with ID or II genotype have an increased risk of osteosarcoma with stage III (OR=3.43, 95% CI 1.61-7.36, *P*=0.001). Results are listed in **Table 3**.

Discussion

Recently, a lot of evidence indicated that genetic factors played important roles in the development of osteosarcoma. Zhang et al. suggested that the rs454006 polymorphism of the PRKCG gene correlated to osteosarcoma susceptibility and might increase the risk of osteosarcoma [11]. Zhao et al. indicated that ARHGAP35 rs1052667 polymorphism may be associated with osteosarcoma risk [12]. Jiang and colleagues found that GRM4 gene polymorphism was associated with the susceptibility and metastasis of osteosarcoma in a Chinese Han population [13]. Additionally, many studies also suggested that genetic factors also had critical roles in the prognosis of osteosarcoma. For example, A G>A variation in the pre-miR-34a coding region was found to be associated with higher OS morbidity [14]. Patients carrying the VEGF +936 CC genotype and C allele were associated with a significantly decreased risk of presenting progressive disease or death from osteosarcoma [15].

In this case-control study, we analyzed the association between NFKB1 -94-ins/del ATTG polymorphism and osteosarcoma susceptibility in a Chinese Han population. Results of this study suggested that NFKB1 -94ins/del ATTG polymorphism was significantly associated with the risk of osteosarcoma. This result indicated that NFKB1 -94ins/del ATTG polymorphism involved in the pathogenesis of osteosarcoma in Chinese Han population. We also found that this polymorphism was significantly associated with advanced osteosarcoma risk and metastatic osteosarcoma risk. To our knowledge, this is the first study which investigates the association between NFKB1 -94ins/del ATTG polymorphism and osteosarcoma risk.

The association between NFKB1 -94ins/del ATTG polymorphism and other cancers were reported. Cheng et al. suggested that the NFKB1 -94 Ins promoter polymorphism increased the risk of hepatocellular carcinoma, and may be applied as a predictive factor for the clinical stage and tumor size in female hepatocellular carcinomapatients [9]. Shiels et al. indicated that NFKB1 -94ins/del ATTG polymorphism was associated with lung cancer risk [8]. A recent meta-analysis also found that NFKB1 promoter -94ins/del ATTG polymorphism was significantly associated with cancer risk. Stratified analyses

revealed a significant association between the polymorphism and ovarian, oral, and prostate cancers. Similar results were determined in an Asian population and not in a Caucasian population [16].

Several limitations should be noted. First, the number of cases and controls is relatively small, which may undermine the statistical power. Second, our sample is derived from the local region and the genetic background is different from other regions; thus, the conclusion should be interpreted with caution when applying to other ethnicities. Third, this was a hospital-based case-control study, so selection bias cannot be excluded and the participants may not be representative of the general population.

In conclusion, our findings indicate that osteosarcoma is associated with the NFKB1 promoter -94ins/del ATTG polymorphism.

Disclosure of conflict of interest

None.

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