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Vascular endothelial growth factor (*VEGF*) rs3025039 polymorphism is associated with increased risk of osteosarcoma.

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Abstract

Background: The role of vascular endothelial growth factor (*VEGF*) rs3025039 polymorphism on osteosarcoma risk was not fully clear. Thus, we did a case-control study to evaluate the association between *VEGF* rs3025039 polymorphism and osteosarcoma risk.
Method: This study included 242 patients with osteosarcoma and 253 controls. The genotyping was conducted using the matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS).
Results: The *VEGF* rs3025039 TT genotype was significant higher in the osteosarcoma group than in the control group (OR=2.42, 95%CI 1.00-5.85, P=0.04). The TT genotype and CT genotype was also significantky associated with osteosarcoma risk (OR=1.43, 95%CI 1.00-2.05, P=0.04). Under the allelic model, T allele of rs3025039 was significantly associated with higher osteosarcoma risk (OR=1.41, 95%CI 1.05-1.90, P=0.02). In addition, *VEGF* rs3025039 TT genotype was not associated with tumor location (OR=1.02, 95%CI 0.59-1.77, P=0.94) and metastasis (OR=1.76, 95%CI 0.90-3.43, P=0.10).
Conclusion: In conclusion, this study confirmed that *VEGF* rs3025039 polymorphism was significantly associated with higher risk of osteosarcoma.

Keywords: Vascular endothelial growth factor, Osteosarcoma, Genetic.

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Introduction

Osteosarcoma originates from primitive bone-forming mesenchymal cells and has been identified as an aggressive sarcoma of the bone [1]. Osteosarcoma has a wide range of histological appearances. Conventional osteosarcoma may be classified as osteoblastic, chondroblastic or fibroblastic, depending on the predominant type of extracellular matrix present [2]. Despite combined therapy, more than 30% of the patients showed the recurrence or metastatic disease during the first five years after diagnosis [3].

Vascular endothelial growth factor (*VEGF*) plays an important role in the maintenance of endothelial integrity, endothelial survival and the physiological function of endothelium [4]. Niu et al. suggested that knockdown of *VEGF*A by siRNA inhibited proliferation, migration, and invasion of U2OS cells [5]. Peng et al. demonstrated that *VEGF* silencing could suppress cells proliferation, promote cells apoptosis and reduce osteosarcoma angiogenesis through inactivation of *VEGF*/*PI3K*/*AKT* signaling pathway [6]. Han found that *VEGF* is related to the grade and metastasis of osteosarcoma [7]. The human *VEGF* gene is located on chromosome 6p21.3 with 7 introns and 8 exons and shows polymorphism [8]. The role of *VEGF* rs3025039 polymorphism on osteosarcoma risk was not fully clear. Thus, we did a case-control study to evaluate the association between *VEGF* rs3025039 polymorphism and osteosarcoma risk.

Methods

Study population

This study consisted of 242 patients with osteosarcoma. They were treated in the First Affiliated Hospital of Anhui Medical University between 2010 and 2017. The control group contained 253 healthy subjects who came from our hospital's physical checkup center during the same period of time. All control subjects had no history of cancer. Written permission was obtained from all the participants and the study was approved by the Research Ethics Committee of First Affiliated Hospital of Anhui Medical University.

Sample collection

Peripheral venous blood samples (10 mL) were collected from all patients using vacutainer tubes in the morning. Blood samples (5 mL) for genetic analyses were transferred into tubes

which contained ethylenediamine tetra-acetic acid (EDTA). Genomic DNAs were isolated using genomic DNA extraction kit (QIA amp DNA Blood Mini Kit, Qiagen, Berlin, Germany) under the manufacturer's instructions.

Genotyping method

The SNP genotyping was conducted using the matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) (Sequenom Inc., San Diego, CA, USA). The PCR reaction was performed in a 20 μl reaction mixture containing 40-160 ng DNA template, 0.25 units Taq DNA polymerase (Sangon Biotech), 250 μM each deoxyribonucleotide triphosphate (dNTP) (Sangon Biotech), 0.5 μM forward primer, 0.5 μM reverse primer, and 1X PCR buffer with 1.2 mM $MgCl_2$. The SNaPshot assay (Applied Biosystems) was performed to confirm genotypes of 6 DNA samples. All genotyping procedures were carried out in a double-blind manner and the whole assays were proved to be reliable.

Statistical analysis

Data are presented as mean ± standard deviation (SD) or number (percentage). The chi-square test and Student's t-test were used to compare case and control groups, as appropriate. The statistical comparison between 2 groups was conducted using the t-test or the analysis of variance (ANOVA). Genotype distribution in the control group was tested by Hardy-Weinberg equilibrium (HWE). The differences in genotype and allele distribution between the patients and the controls are represented as odds ratio (OR) and 95% confidence interval (CI). P values for all tests are 2-tailed, and <0.05 was considered as statistically significant. Statistical analysis was conducted using SPSS 18 software (SPSS Inc., Chicago, IL, USA).

Results

Table 1 shows the demographic and clinical characteristics of 242 patients with osteosarcoma and 253 controls. Comparisons between the osteosarcoma group and the control group demonstrated that family history of cancer was significantly higher in patients with osteosarcoma than in controls (P<0.05). No statistical differences were seen in age and gender (all P>0.05).

Table 1. *Characteristics of the cases and controls.*

Characteristics	Case (n=242)	Control (n=253)	P value
Age at diagnosis	26.1 ± 16.6	28.1 ± 12.2	0.17
Sex			0.87
Male	118	122	
Female	124	131	
Family history of cancer			<0.01
Yes	198	142	
No	44	111	
Tumor location			
Extremities	210		
Non-extremities	32		
Metastasis			
Yes	68		
No	174		

The genotype and allele frequencies of the *VEGF* rs3025039 polymorphism in patients and controls are displayed in Table 2. The *VEGF* rs3025039 TT genotype was significant higher in the osteosarcoma group than in the control group (OR=2.42, 95% CI 1.00-5.85, P=0.04). The TT genotype and CT genotype was also significantly associated with osteosarcoma risk (OR=1.43, 95% CI 1.00-2.05, P=0.04). Under the allelic model, T allele of rs3025039 was significantly associated with higher osteosarcoma risk (OR=1.41, 95% CI 1.05-1.90, P=0.02). In addition, *VEGF* rs3025039 TT genotype was not associated with tumor location (OR=1.02, 95% CI 0.59-1.77, P=0.94; Table 3) and metastasis (OR=1.76, 95% CI 0.90-3.43, P=0.10; Table 3).

Table 2. *The genotype and allele frequencies of VEGF polymorphism in the cases and controls.*

Genotype	Case	Control	OR	95% CI	P value
CC	131	159	1.00	Reference	
CT	95	86	1.34	0.92-1.94	0.12
TT	16	8	2.42	1.00-5.85	0.04
TT+CT	111	94	1.43	1.00-2.05	0.04
C	357	404	1.00	Reference	
T	127	102	1.41	1.05-1.90	0.02

Table 3. *Relation of VEGF polymorphism and characteristics.*

Characteristics	TT+CT (n=111)	CC (n=94)	OR	95% CI	P value
Tumor location					
Extremities	49	42	1.00	Reference	
Non-extremities	62	52	1.02	0.59-1.77	0.94
Metastasis					
Yes	80	77	1.00	Reference	
No	31	17	1.76	0.90-3.43	0.10

Discussion

In the current study, we investigated the association between the *VEGF* rs3025039 polymorphism and osteosarcoma risk. The *VEGF* rs3025039 TT genotype was significant higher in the osteosarcoma group than in the control group. The TT genotype and CT genotype was also significantky associated

with osteosarcoma risk. Under the allelic model, T allele of rs3025039 was significantly associated with higher osteosarcoma risk. In addition, *VEGF* rs3025039 TT genotype was not associated with tumor location and metastasis.

Zhang et al. found that vascular endothelial growth factor overexpression predicted a worse prognosis for laryngeal cancer patients [9]. Zhao et al. indicated that high plasma/intratumoral *VEGF*-A level at baseline could predict poor treatment effect (depressed PFS and OS) of BEV-based chemotherapy in mCRC [10]. Cai et al. found that high level of *VEGF* is associated with poor outcomes in HCC patients treated with sorafenib [11]. Hui et al. indicated that the OS of the *VEGF*-positive group with ovarian cancer was significantly poorer than the *VEGF*-negative group [12]. Ma et al. suggested that both the *VEGF* +936C/T and -634G/C polymorphisms influence breast cancer susceptibility and tumor growth, instead of metastasis [13].

This study has some limitations. The selective bias was mostly controlled by the design of a hospital-based case-control study. As other case-control studies, this study raises concern about recall bias and residual confounding. The major difficult is still the inability to separate exposures to factors prior to clinical onset from exposures to factors after clinical onset.

In conclusion, this study confirmed that *VEGF* rs3025039 polymorphism was significantly associated with higher risk of osteosarcoma risk.

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