Impact of common SNPs in *VEGF* gene on the susceptibility of osteosarcoma

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ABSTRACT. We conducted a case-control study to assess the role of vascular endothelial growth factor (*VEGF*) -634G/C, +936C/T, and +1612G/A genetic variations in the development of osteosarcoma in a Chinese population. This hospital-based case-control study examined 130 patients with osteosarcoma and 130 age- and gender-matched healthy controls from March 2011 and March 2013. Polymerase chain reaction-restriction fragment length polymorphism was applied to assess the *VEGF* -634G/C, +936C/T, and +1612G/A gene polymorphisms. Using conditional regression analysis, individuals carrying the TT genotype of *VEGF* +936C/T were found to be correlated with an elevated risk of osteosarcoma, with an adjusted odds ratio (95% confidence interval) of 2.70 (1.02-8.28). In conclusion, our study suggests that the TT genotype of *VEGF* +936C/T genetic variants is associated with an increased risk of osteosarcoma.

Key words: Polymorphism; Vascular endothelial growth factor; Osteosarcoma

INTRODUCTION

Osteosarcoma is derived from mesenchymal tissues, which is a rare bone cancer that often occurs in children and adolescents (Ritter and Bielack, 2010). The annual incidence of osteosarcoma is approximately 3 in 1,000,000 individuals (Mirabello et al., 2009; Ottaviani and Jaffe, 2009). The development of osteosarcoma is a complex, multistep, and multifactorial process, and many environmental and genetic factors play an important role in the carcinogenesis process (Bovee and Hogendoorn, 2010; Powers et al., 2010). Several studies have suggested that genetic factors are involved in the development of osteosarcoma, such as glutathione *S*-transferase, vascular endothelial growth factor (VEGF), interleukin factor, and DNA repair genes (Tang et al., 2014; Wang et al., 2014; Zhi et al., 2014; Han et al., 2015).

Angiogenesis is the formation of new blood vessels from the preexisting endothelium, and it is a discrete event in carcinogenesis that is associated with the aggressive potential of a tumor (Hanahan and Folkman, 1996; Nakamura, et al., 2005). Accumulating evidence suggests that tumor growth is associated with increased angiogenesis and that new blood vessel formation is a fundamental step in tumor development and expansion (Mariani et al., 2012). VEGF is an important promoter of angiogenesis and is encoded by the VEGF gene (Hicklin and Ellis, 2005). Previous studies reported that VEGF gene polymorphisms are associated with the development and progression of solid tumors (Roy et al., 2006; Kushner and Bautch, 2013). There are 3 common single-nucleotide polymorphisms in the VEGF gene affecting plasma VEGF levels, including -634G/C, +936C/T, and +1612G/A (Watson et al., 2000). We conducted this case-control study to assess the role of the *VEGF* -634G/C, +936C/T, and +1612G/A genetic variations in the development of osteosarcoma in a Chinese population.

MATERIAL AND METHODS

Patients

This hospital-based case-control study included 130 patients with osteosarcoma and 130 age- and gender-matched healthy controls from March 2011 and March 2013 in the Renmin Hospital of Wuhan University. Osteosarcoma patients were newly diagnosed and histopathologically confirmed independently by 2 gynecologic pathologists. The 130 controls were randomly selected from individuals who came to our hospital for regular health examination. The selection criteria for control subjects were: confirmation of lack of osteosarcoma, no history of any cancer, no family history of osteosarcoma in first-degree relatives, and a matched sex and age distribution with cases. All patients agreed to participate in the study and gave written informed consent according to the Declaration of Helsinki. The protocols of our study were approved by the Ethical Committee of the Renmin Hospital of Wuhan University. Demographic and clinical characteristics of patients and controls were obtained from medical records.

DNA extraction and genotyping

Venous blood samples were collected from the forearm of each participant and then placed in EDTA anticoagulant and centrifuged at 2700 rpm for 10 min at room temperature. The collected supernatant was stored in a refrigerator maintained until further use. Polymerase

chain reaction-restriction fragment length polymorphism was used to assess the *VEGF* -634G/C, +936C/T, and +1612G/A gene polymorphisms. The primers for *VEGF* -634G/C, +936C/T, and +1612G/A were as follows: 5'-GTAGCAAGAGCTCCAGAGAGAAGT-3' (forward) and 5'-TGGACGAAAAGTTTCAGTGCGACG-3' (reverse) for *VEGF* -634G/C, 5'-CTCGGTGATTTAGCAGCAAG-3′ and 5′-CTCGGTGATTTAGCAGCAAG-3' for +936C/T, and 5'-CACATGCTGCACGCGCATCTC-3' and 5'-ACCCCAGGAAGGGGAGCAGGA-3' for +1612G/A. The amplification fragments for *VEGF* -634G/C, +936C/T and +1612G/A were 304, 208, and 217 base pairs, respectively. The restriction enzymes were BsmFI for *VEGF* -634G/C, NlaIII for *VEGF* +936C/T, and MnlI for *VEGF* +1612G/A. PCRs were carried out with an initial denaturation step of 8 min at 94°C, followed by 30 cycles at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 1 min. The resulting DNA fragments were separated by 3.5% agarose gel electrophoresis and visualized under UV light after ethidium staining.

Statistical analysis

Statistically significant differences between cases and controls for demographic characteristics were assessed by Student's *t*-test and c^2 test. Departures from Hardy-Weinberg equilibrium for the *VEGF* -634G/C, +936C/T, and +1612G/A genotypes were evaluated by comparing the expected frequencies to the observed genotype frequencies using χ^2 tests. Differences in genotypic frequencies between groups was assessed by the Pearson χ^2 test and calculating the odds ratio, 95% confidence intervals, and their corresponding P values. All analyses were performed using SPSS 16.0 software version 16.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

The distribution of the demographic characteristics in osteosarcoma patients and controls are shown in Table 1. No statistically significant differences were found between cases and controls in terms of gender and age at interview. Of the 130 osteosarcoma patients, 89 (68.46%) patients were stage I-II, 94 (72.31%) with tumors at the long tubular bones, while 28 (21.54%) showed tumor metastasis. The tumor size of osteosarcoma was approximately 8.2 ± 3.5 cm.

Table 1. Demographic characteristics in osteosarcoma patients and controls.

Characteristics	Osteosarcoma cases	%	Control subjects	%	χ^2 value	P value
Gender						
Female	54	41.54	54	41.54		
Male	76	58.46	76	58.46	0.00	1.00
Age						
<20	80	61.54	77	59.23		
≥20	50	38.46	53	40.77	0.15	0.70
Stage						
I-II	89	68.46				
III-IV	41	31.54				
Tumor location						
Long tubular bones	94	72.31				
Axial skeleton	36	27.69				
Tumor size, cm	8.2+3.5					
Tumor metastasis						
Positive	28	21.54				
Negative	102	78.46				

Genotype distributions of VEGF gene polymorphisms in osteosarcoma patients and controls are shown in Table 2. We found that the genotype frequencies of *VEGF* -634G/C, +936C/T, and +1612G/A were in Hardy-Weinberg equilibrium in the control group (Table 2). By conditional regression analysis, individuals carrying TT genotype of *VEGF* +936C/T were found to be correlated with an elevated risk of osteosarcoma, and the adjusted odds ratio (95% confidence interval) was 2.70 (1.02-8.28). However, the *VEGF* -634G/C and +1612G/A polymorphisms showed no significant association with the risk of osteosarcoma.

Table 2. Association between VEGF gene polymorphisms and risk of osteosarcoma.

Genotype	Cases	%	Controls	%	Hardy-Weinberg equilibrium	OR (95%CI)[1]	P value
VEGF -634G/C							
GG	42	32.31	46	35.38		1.0 (Ref.)	-
GC	68	52.31	65	50.00		1.15 (0.65-2.04)	0.62
CC	20	15.38	18	14.62	0.51	1.22 (0.53-2.80)	0.61
GC+CC	88	67.69	83	64.62		1.16 (0.67-2.01)	0.57
VEGF+936C/T							
CC	67	51.54	79	60.77		1.0 (Ref.)	-
CT	47	36.15	44	33.85		1.26 (0.72-2.20)	0.39
TT	16	12.31	7	5.38	0.79	2.70 (1.02-8.28)	0.03
CT+TT	63	48.46	51	39.23		1.46 (0.86-2.46)	0.13
VEGF+1612G/A							
GG	41	31.54	46	35.38		1.0 (Ref.)	-
GA	61	46.92	60	46.15		1.14 (0.64-2.06)	0.64
AA	28	21.54	24	18.46	0.57	1.31 (0.62-2.76)	0.44
GA+AA	89	68.46	84	64.62		1.19 (0.69-2.06)	0.51

[1]Adjusted for gender and age.

DISCUSSION

In this study, we investigated the association between *VEGF* genetic polymorphisms and the risk of osteosarcoma in a Chinese population. Our results suggested that the TT genotype of *VEGF* +936C/T was significantly associated with an increased risk of osteosarcoma.

Angiogenesis plays an important role in the development and metastasis of tumors, and the VEGF is a potent regulator of angiogenesis involved in the carcinogenesis of solid tumors (Roy et al., 2006; Kushner and Bautch, 2013). It is well-known that the VEGF gene is located on chromosome 6p21.3, and the protein consists of 8 exons, which can be alternatively spliced to generate a family of proteins (Vincenti et al., 1996). VEGF gene polymorphisms may influence the gene expression and affect plasma VEGF levels (Renner et al., 2000; Watson et al., 2000). Previous studies reported that VEGF gene polymorphisms may influence the development of many solid tumors, such as esophageal cancer, colorectal cancer, renal cell carcinoma, and breast cancer (Yao et al., 2014; Jannuzzi et al., 2015; Ma et al., 2015; Qasim et al., 2015). Qasim et al. (2015) conducted a case-control study to investigate the role of the *VEGF* +936C/T and +404C/G polymorphisms on the risk of esophageal cancer, and they found a significant association between the *VEGF* +936C/T and +404C/G polymorphisms and esophageal cancer risk. Jannuzzi et al. (2015) found that +936C/T and +404C/G polymorphisms may not play a role in the development of colorectal cancer. Ma et al. (2015) demonstrated that the *VEGF* -2578C/A polymorphism may be associated with the prognosis of renal cell carcinoma patients. Yao et al. (2014) conducted a meta-analysis of 8 case-control studies and found that the *VEGF* -634G/C polymorphism does not appear to carry a risk factor for breast cancer. The discrepancies between these studies may have been caused by differences in types of tumors, selection of patients, and control and sample sizes.

For the correlation between VEGF gene polymorphisms and the risk of osteosarcoma, only 2 previous studies reported their association (Tie et al., 2014; Wang et al., 2014). Wang et al. (2014) conducted a case-control study in a Chinese population and found that the TT genotype of *VEGF* +936C/T genetic variants was related to an increased susceptibility to osteosarcoma. Tie et al. (2014) conducted a case-control study to assess the role of 5 common single-nucleotide polymorphisms in the VEGF gene in the development of osteosarcoma, and they found that the *VEGF* -2578C/A and -634G/C polymorphisms were associated with an increased risk of osteosarcoma. We also found that the TT genotype of *VEGF* +936C/T was associated with the pathogenesis of osteosarcoma. Further large-sample studies are greatly needed to confirm our findings.

In conclusion, our study suggests that the TT genotype of *VEGF* +936C/T genetic variants is associated with an increased risk of osteosarcoma, and further investigations of the role of VEGF gene polymorphisms on the risk of osteoporosis are needed.

Conflicts of interest

The authors declare no conflict of interest.

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