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Original Article

Correlation between *AEG-1* gene polymorphisms and osteosarcoma susceptibility

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Abstract: Aim: The objective of this study was to explore the relationship between single nucleotide polymorphisms (SNPs) of the astrocyte elevated gene-1 (*AEG-1*) gene (rs16896059 and rs1311) and osteosarcoma (OS) susceptibility in Chinese Han population. Methods: The case and control groups were evaluated by Hardy-Weinberg equilibrium (HWE). Genotype and allele frequencies of the two polymorphisms were obtained by direct calculation. Differences of the genotyes and alleles of these polymorphisms were assessed by Chi-square test. Relative risk of OS was represented by odds ratios (ORs) with 95% confidence intervals (CIs). Results: Frequencies of AA genotype and A allele of rs16896059 SNP between OS patients and healthy individuals were significantly different (P<0.05). AA genotype and A allele may be protective against OS onset respectively (OR=0.258, 95% CI=0.068-0.986; OR=0.628, 95% CI=0.401-0.985). However, there was no significant differences of the genotype and allele distributions of *AEG-1* gene rs1311 polymorphism between case and control groups (P>0.05). Conclusion: *AEG-1* gene rs16896059 polymorphism act as a protect factor for the occurrence of OS. But rs1311 had no significant association with the OS risk.

Keywords: Osteosarcoma, AEG-1, polymorphisms, susceptibility

Introduction

Osteosarcoma (OS) is a most common primary malignancy in bone tissues, particularly among children and adolescents. But there is a second incidence peak among individuals who is older than 60 years [1, 2]. Although the incidence of OS is not high, it has a rising trend recent years. Meanwhile the mortality of it is obviously high, and with the easy metastasis and recurrence the OS prognosis is very poor. Exploration of the OS etiology is essential, so as to provide a theoretical basis for the therapy method. Until now, etiology of OS remains unknown. But it was considered that OS is a complex disease. Development and progression of this disease is caused by an interaction of environmental factors and genetic susceptibility [3-5]. Recent evidence indicated that oncogenes, suppressor genes and DNA-repair genes may play critical roles in the determination of individual susceptibility to cancers. Polymorphisms in these genes possibly alter their expression and function, may increase or decrease carcinogen activation or detoxication and modulate DNA repair.

As an important oncogene, astrocyte elevated gene-1 (AEG-1, also known as MTDH and Lyric), was originally identified as an HIV-inducible gene in primary human fetal astrocytes [6]. *AEG-1* gene is widely over expressed in many malignant tumors [7-16]. AEG-1 participates in tumorigenesis, including cellular transformation, apoptosis inhibition, invasion, metastasis, angiogenesis and resistance to chemotherapeutic agents [17-24]. Up-regulation of AEG-1 promotes the growth, invasion and metastasis of cancers via several signaling pathways such as PI3K/AKT, NF-κB and MAPK pathways [25]. Polymorphisms are usually considered to be point mutations that can occur in coded or non-coded regions, thus the variant may or may not affect the function of the gene.

Table 1. Comparison of the clinical features between case and control groups

Variable	Cases n=104 (%)	Controls n=108 (%)	*P* value
Gender			0.926
Male	69 (66.35)	71 (65.74)	
Female	35 (33.65)	37 (34.26)	
Age			0.980
<20	82 (78.85)	85 (78.70)	
≥20	22 (21.15)	23 (21.30)	
Mean ± SD	24.6±12.8	24.1±13	
Injured History			0.944
Yes	65 (62.50)	68 (62.96)	
No	39 (37.50)	40 (37.04)	

In this study, we attempted to further investigate the potential role of *AGE-1* gene polymorphisms in OS development. Single nucleotide polymorphism (SNP), the most common polymorphism, is defined as genetic variation in a DNA sequence. Identification of SNPs in the host can potentially facilitate the evaluation of the susceptibility of cancer and predict progression of disease or response to treatment. Here, we investigated rs16896059 and rs1311 SNPs in *AEG-1* gene to determine whether a particular SNP may influence susceptibility to OS development.

Materials and methods

Study population

A total of 104 OS patients (aged 10-41 years old) and 108 healthy controls (aged 13-45 years old) were enrolled in this study. OS patients were diagnosed by histopathological examination at Qilu Hospital of Shandong University from 2005 to 2015. Healthy controls were recruited from the healthy check-up center of the same hospital during the same period. Control subjects were matched with patients in terms of age, gender and injured history. This study was approved by Review Boards of Qilu Hospital of Shandong University. All the participants were unrelated and signed the written informed consent.

Genomic DNA extraction and genotyping method

Gnomic DNA was extracted from 2 ml whole peripheral venous blood using the TIANamp DNA extraction kit (Tiagen Biotech Co., Ltd, China) according to the manufacturer's protocol.

PCR primers of *AEG-1* gene polymorphisms were designed by Primer Premier 5.0 and synthesize by Sangon Biotech Co., Ltd. PCR primer sequences were as follows: forward, 5'-CGT GAT AAG GTG CTG ACT GAT TC-3'; and reverse, 5'-CAG GAA ATG ATG CGG TTG TAA G-3'. PCR amplification was performed in a total volume of 25 μl, containing 100 ng genomic DNA, 2.5 μl of 10 × PCR buffer, 1.5 mM $MgCl_2$, 0.15 mM dNTPs, 25 pM of each primer, and 1 U of Taq DNA polymerase. PCR reaction conditions was as follows: an initial denaturation step at 94°C for 5 min, followed by 35 cycles of 30 s at 94°C, 30 s at 60°C, 30 s at 72°C and a final elongation at 72°C for 10 min. PCR products were sequenced by Sangon Biotech (Shanghai) Co., Ltd.

Statistical analysis

Hardy-Weinberg equilibrium (HWE) was used to detect the representativeness of the cases and controls. Differences of clinical features, genotypes and alleles between case and control groups were assessed by χ^2 test. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated by Chi-squared test. All statistical analyses were performed by PASW 18.0 statistical software. *P*-value less than 0.05 were considerate as statistically significant.

Results

General characteristics of subjects

General characteristics of the participants were shown in **Table 1**. Our data indicated that there were no significant differences between OS patients and cancer-free controls in age, gender and injured history.

Association of AEG-1 gene rs16896059 and rs1311 polymorphisms with OS risk

Genotype distributions of rs16896059 and rs1311 SNPs in case and control groups were all in accordance with HWE test.

The results of genotype distribution showed that AA genotype frequency of rs16896059 SNP was significantly higher in controls (*P*=

Table 2. Genotype frequency of *AEG-1* gene polymorphisms in the osteosarcoma (OS) cases and cancer-free controls

Genotype	Case n=104 (%)	Control n=108 (%)	OR (95% CI)	*P* value
rs16896059				
GG	65 (62.5)	56 (51.85)	-	-
GA	36 (34.62)	42 (38.89)	0.738 (0.417-1.307)	0.297
AA	3 (2.88)	10 (9.26)	0.258 (0.068-0.986)	0.036
rs1311				
TT	57 (54.81)	58 (53.70)	-	-
CT	37 (35.58)	43 (39.81)	0.876 (0.494-1.551)	0.649
CC	10 (9.62)	7 (6.48)	1.454 (0.518-4.083)	0.476

Table 3. Association between OS risk and allele frequencies of *AEG-1* gene polymorphisms

Allele	Case 2n=208 (%)	Control 2n=216 (%)	OR (95% CI)	*P* value
rs16896059				
G	166 (79.81)	154 (71.30)	-	-
A	42 (20.19)	62 (28.70)	0.628 (0.401-0.985)	0.042
rs1311				
T	151 (72.60)	159 (73.61)	-	-
C	57 (27.40)	57 (26.39)	1.053 (0.685-1.618)	0.814

0.036, **Table 2**), indicating a statistical association with the onset of OS (OR=0.258, 95% CI=0.068-0.986). Meanwhile, rs1311 genotypes had no obvious association with the occurrence of OS (*P*>0.05). As the **Table 3** shown, we observed a statistically significant difference of rs16896059 A allele frequency between case and control groups (*P*=0.042). A allele appears to be protective against OS development (OR=0.628, 95% CI=0.401-0.985). However, C allele distribution of rs1311 polymorphism appears to be insignificant between case and control groups (*P*>0.05), suggesting that the rs1311 variant may not relate to the OS occurrence.

Discussion

OS is a most common life-threatening malignancy originated from mesenchymal tissues. This tumor often occurs in children, adolescents and young adults. Besides, OS incidence is highly in males than that in females [26, 27]. With a high tendency of metastasis and recurrence, OS has a poor prognosis. Although the incidence of OS is not high, the mortality of it is high, and the morbidity and mortality has a rising trend recent years. Moreover, current therapy method for OS is not effective. Therefore, exploration of the OS pathogenesis is necessary. Despite many studies investigate the etiology of OS, but the mechanism of this disease remains unclear. Cumulative studies suggested that OS is a complex disease caused by the combined effects of genetic and environmental factors. Among these factors genetic background is an important determinant for OS development.

In order to certify the pathogenesis of OS, an extensive functional genomics research is needed which will discover new and unknown polymorphic loci for OS etiology. Recent studies indicate that *AEG-1* gene plays a critical role in tumor biology, and it is involved in a variety of tumor biological behaviors [28]. AEG-1 is first find in the study of HIV-1 infection in human astrocytes [29, 30]. AEG-1 is a single-pass transmembrane protein, and mainly presented in nucleolus. This protein is an extremely conservative protein in mammals. Soon after, it was found that AEG-1 expressed in almost all tissues. The expression level of AEG-1 is up-regulated in various cancer cells. Besides, AEG-1 over-expressed in metastatic breast cancer and promoted the adhesion of mammary tumor cells. AEG-1 protein is necessary for the proliferation, metastasis invasion and prognosis of tumors, and even the angiogenesis in tumor tissues [31, 32]. AEG-1 emerge as a potential regulator for many malignant tumors, and mediate by complex signaling pathway including PI3K/AKT, NF-κB and MAPK pathways [25]. AEG-1 protein also relates to the metastasis, invasion and drug tolerance in OS patients [33, 34].

AEG-1 protein is encoded by *AEG-1* gene which is located in chromosome 8q22.1. As we all know, polymorphisms in the genes may alter the expression and function of the corresponding protein, and then lead to disorders of the

organism. SNP, the most common polymorphism, play a crucial role in the occurrence and development of multiple diseases. As an important regulator of cancer progression, polymorphisms in *AEG-1* gene might lead to many cancers. However, there are few researches focus on the association of *AEG-1* gene polymorphisms and cancer risk.

To our knowledge, this is the first study which investigated the impact of *AEG-1* gene polymorphism in risk of OS. We selected rs16896059 and rs1311 two SNPs of *AEG-1* gene to explore the association with OS risk. Then we found that AA genotype and A allele of rs16896059 polymorphism were frequently observed in controls. The results indicated that AA genotype and A allele decreased the OS risk approximately 0.258 and 0.628 fold. That was partly according to the results in ovarian cancer [35]. But GA genotype of rs16896059 and all of the genotypes of rs1311 polymorphism had no significant association with the occurrence of OS.

Because the incidence of OS is low, so the sample size of this study is small. Besides, there only one ethnicity involves in our study. Although the participants of this study had a good representativeness, and no significant differences of clinical features between case and control groups, the results of this study was still insufficient to certify the pathogenesis of OS. Thus well designed studies with large sample size and more ethnicity are necessary in the future, so as to obtain more exact evidence to certify the pathogenesis of OS.

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Disclosure of conflict of interest

None.

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