Genetic association between CTLA-4 variations and osteosarcoma risk: case-control study

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Received October 25, 2015; Accepted January 21, 2016; Epub June 15, 2016; Published June 30, 2016

Abstract: Objective: It was reported that cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), as a member of immunoglobulin superfamily, might involve in the development of osteosarcoma (OS). The objective of this study was to assess the association of CTLA-4 rs231775 and rs5742909 variations with OS risk in Chinese Han population. Methods: This case-control study recruited 122 OS patients and 131 healthy controls. Controls were age- and gender-matched with cases. CTLA-4 rs231775 and rs5742909 variations were genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to represent the relative risk of OS. Results: Frequencies of rs231775 and rs5742909 genotypes had no significant differences between case and control groups (P>0.05). But significant differences were observed between cases and controls respectively in the two polymorphisms (P=0.038, P=0.015). Correlation analysis under five genetic models indicated that rs231775 might increase the risk of OS, although the association had no statistical significance (P>0.05). Meanwhile, rs5742909 distinctly increased the risk of OS under T vs. C model (P=0.043, OR=1.582, 95% CI=1.012-2.475). No significant association was discovered under other models between rs5742909 and OS risk (P>0.05). Conclusion: CTLA-4 rs5742909 significantly associated with the risk of OS, but not rs231775.

Keywords: CTLA-4 gene, osteosarcoma, variations

Introduction

Osteosarcoma (OS) is one of the most common malignancy tumor in the bone. It usually occurs in adolescent and young adults [1]. It mainly affects the proximal end of tibia or humerus, or distal end of femur. OS has high metastasis rate, disable rate and fatality rate, the prognosis is very poor [2]. The exploration of OS pathogenesis will contribute to the therapy of OS. Previous evidence showed that the development of OS is a complex disease and may be influenced by multiple genetic and environmental factors [3-7]. Among these risk factors, genetic factor is the decisive factor. However, the mechanisms of OS are largely unknown. Recent years, tumor immunity becomes a hot point in cancer research. T cells and natural killer (NK) cells might participate in the antitumor response [8, 9]. Cytotoxic T-lymphocyte antigen-4 (CTLA-4) is a member of immunoglobulin superfamily, usually locals on the surface of T cells. T cells play a central role in cell mediated immunity. CD28 is the molecule to activate the T cells. While the sequence and structure of CTLA-4 has high homology with CD28. But CTLA-4 transmits a negative signal to T cells [10, 11]. CD28 and CTLA-4 are the receptors for the costimulatory molecules B7. Vahlenkamp et al. showed that the over-expression of CTLA4-B7 will increase the apoptosis of T cells, and then lead to the onset of feline immunodeficiency [12]. Besides, CTLA4-B7 might mediate the T cells immune responses via take part in the cell cycle regulation [13]. It is reported that CTLA-4 involved in the occurrence of OS [14] and the reducing of CTLA-4 could prevent the metastasis of OS [15].
Variations in **CTLA-4** gene might alter the expression and structure even the function of the protein, and then lead to disorders. Polymorphisms in exon and promoter regions might greatly change the expression of the gene. Rs231775 (exon1, c.49A>T) and rs5742909 (promoter, c.-319C>T) is the two widely studied single nucleotide polymorphisms (SNPs) of **CTLA-4** gene [16-18]. So in present study, we selected the two SNPs of **CTLA-4** gene to explore the association between **CTLA-4** variations and OS risk in Chinese Han population.

### Materials and methods

#### Subjects

This study was approved by the local ethic committee. Participants understood this study and signed the written informed consent form. The sample collection followed the Helsinki declaration. Controls were matched with cases in age and gender. All of the participants were unrelated Chinese Han population.

122 OS patients who were newly diagnosed by two pathologists in Hanxing Worker General Hospital during Jun 2011 to Jun 2015 were enrolled as cases. X ray, computed tomography (CT) magnetic resonance imaging (MRI) and pathological biopsy were used for the diagnosis of OS. OS patients included 74 males and 48 females, with the mean age of 19.2±7.94 (10-30 years old). 131 healthy controls were recruited from the community of Handan. Control group included 79 males and 52 females, with the mean age of 18.3±8.75 (aged 12-27 years). All of the participants did not receive radiotherapy and/or chemotherapy before the investigation. Individuals who had diabetes, cardiovascular disease, autoimmune diseases and other tumors and are over 30 years-old were excluded from this study. Characteristics of all participants were obtained by a questionnaire and medical records.

#### Sample collection

5 mL peripheral blood was collected from every fasting subject in the morning, and put into the tubes which with EDTA, then stored in -80°C until to use.

#### Genotyping method

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was utilized to detect the genotypes of **CTLA-4** rs231775 and rs5742909 variations. Process of PCR-RFLP followed the previous study [19]. 10 samples from each genotype group were randomly selected to confirm the genotyping results of PCR-RFLP by direct sequencing using an ABI 3730xl genetic sequencer (Applied Biosystems, USA).

#### Statistical analysis

All of the calculations were performed by PASW 18.0. Significant level was set to 0.05 (two-side). Representativeness of the cases and controls were assessed by Hardy-Weinberg equilibrium (HWE) test. Differences of characteristics were analyzed by t test or χ² test. The frequencies of genotypes and alleles were calculated by direct counting. Differences of genotypes and alleles between cases and controls were detected by χ² test. Association between **CTLA-4** variants and the risk of OS was presented by odds ratios (ORs) and confidence intervals (CIs).


Results

Subject characteristics

Characteristics of the participant were shown in Table 1. The baseline characteristics, such as age, gender and family history of tumor had no significant differences between cases and controls.

Distributions of the variations

Genotype distributions of rs231775 and rs5742909 SNPs had a good goodness of fit, indicating the representativeness of the participants. The frequencies of rs231775 genotypes GG, GA and AA were 37.70%, 47.64%, 14.75% in cases and 48.85%, 42.75%, 8.40% in controls. Frequencies of CC, CT and TT genotypes of rs5742909 were 60.65%, 32.79%, 6.56% in case group and 74.05%, 22.90%, 3.05% in control group. The difference of the genotypes between case and control groups was not significant, respectively for rs231775 and rs5742909 (Table 2, P>0.05).

But the allele frequencies of the two SNPs were significantly different between cases and controls (Table 2, P<0.05). The minor alleles A and T had high frequencies in case group.

Association between CTLA-4 gene variations and OS risk

Associations of CTLA-4 gene rs231775 and rs5742909 SNPs with OS risk were assessed by five genetic models (Table 3). Positive association was observed between rs231775 and the risk of OS under five genetic models (OR=1.918, OR=1.218, OR=1.757, OR=1.195, OR=1.294). However, the associations had no statistical significance (P>0.05). Whilst, the data revealed that rs5742909 significantly associated with the risk of OS under T vs. C model (P=0.043, OR=1.582, 95% CI=1.012-2.475). But in the other contrast models, no significant association existed between rs-5742909 and OS risk (P>0.05).

Discussion

As the most common malignant bone tumor, OS usually originate from primitive transformed cells of mesenchymal origin. Children and adolescent are the high risk population of OS. Most OS could distantly metastasize to lung and has high recurrence rate. It has high mortality and morbidity, too. However, the exact mechanism of OS still unknown, and the therapy methods of OS cannot cure it. So the exploration of OS pathogenesis is necessary for the development of the therapy. It is reported that the occurrence and development of OS is affected by multiple factors. Recent years, the immunotherapy become a hot spot in the investigation of OS, and CTLA-4 is a potential candidate factor for OS [9]. But, up to now, the role of CTLA-4 in the onset of OS was unclear.

CTLA-4 gene, also known as CD152, located at chromosome 2q33, and contains 4 exons. Exon 1 to exon 4 respectively encodes the leader peptide sequence, ligand-binding site, transmembrane region, and the cytoplasmic tail [20]. CTLA-4 gene could express in variety of cells [21], but mainly in the activated T cells, and negatively regulate the T cell activation [22]. Previous studies showed that up-expression of CTLA4 might increase the risk of multiple diseases, including the occurrence and metastasis of the cancers [23, 24]. Meanwhile, evidence revealed that allelic variations and the abnormal expression pattern might lead to autoimmune diseases [24, 25]. Rs231775 and rs5742909 are the two widely studied variations respectively in the exon 1 region and promoter region in CTLA-4 gene. These two variations might alter the expression and function of CTLA-4 gene.

In present case-control study, we found that the minor alleles A and T, respectively of

| Table 2. Genotypes and alleles distributions of rs231775 and rs5742909 |
|-----------------------------|-----------------------------|-----------------------------|
|                             | Case n=122                  | Control n=131                |
|                             | (%)                        | (%)                        |
| rs231775                    |                            |                            |
| GG                         | 46 (37.70)                 | 64 (48.85)                 |
| GA                         | 58 (47.64)                 | 56 (42.75)                 |
| AA                         | 18 (14.75)                 | 11 (8.40)                  |
| rs5742909                   |                            |                            |
| CC                         | 74 (60.65)                 | 97 (74.05)                 |
| CT                         | 40 (32.79)                 | 30 (22.90)                 |
| TT                         | 8 (6.56)                   | 4 (3.05)                   |
| C                          | 188 (77.05)                | 224 (85.50)                |
| T                          | 56 (22.95)                 | 38 (14.50)                 |
| P                          |                            |                            |
CTLA-4 variations and OS risk

Table 3. Association between CTLA-4 gene variations and OS risk

<table>
<thead>
<tr>
<th>Model</th>
<th>rs231775</th>
<th>rs5742909</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P (OR (95% CI)</td>
<td>P (OR (95% CI))</td>
</tr>
<tr>
<td>11 vs. 22</td>
<td>0.116 (1.918 (0.844-4.358))</td>
<td>0.141 (2.463 (0.716-8.471))</td>
</tr>
<tr>
<td>11 + 12 vs. 22</td>
<td>0.346 (1.218 (0.808-1.837))</td>
<td>0.104 (1.516 (0.916-2.509))</td>
</tr>
<tr>
<td>11 vs. 12 + 22</td>
<td>0.158 (1.757 (0.798-3.870))</td>
<td>0.212 (2.148 (0.631-7.313))</td>
</tr>
<tr>
<td>12 vs. 22</td>
<td>0.439 (1.195 (0.761-1.877))</td>
<td>0.147 (1.485 (0.868-2.540))</td>
</tr>
<tr>
<td>1 vs. 2</td>
<td>0.145 (1.294 (0.914-1.831))</td>
<td>0.043 (1.582 (1.012-2.475))</td>
</tr>
</tbody>
</table>

Notes: 1, minor allele; 2, major allele.

rs231775 and rs5742909, were significantly high in cases than that in controls. Our data showed that an allele as well as its carriers of rs231775 polymorphism might enhance the risk of OS under five genetic models, although the association had no statistical significance. The trend showed by our result was in accordance with the previous studies. A recent meta-analysis showed that G allele of rs231775 polymorphism might act as a protective factor for cancers including bone tumor [17]. Besides, other studies indicated that A allele of rs231775 might associate with the increased risk of OS [26, 27]. Additionally, our data demonstrated that rs5742909 polymorphism in promoter region of CTLA-4 gene might increase the susceptibility of OS in Han Chinese population. De Almeida ER et al. showed that T allele of CTLA-4 rs5742909 distinctly increased the expression of CTLA-4 [28]. Meanwhile, T allele of rs5742909 could increase the risk of acute rejection [29]. These evidences might support our result. But the study performed by Liu et al. did not find significant association between rs5742909 and the risk of OS in Chinese population [26]. This difference may be caused by the different region and include criteria of the participants.

In conclusion, rs231775 had no significant association with the risk of OS, and rs5742909 significantly associated with the susceptibility of OS under T vs. C model in Chinese Han population. This result might contribute to the diagnosis and therapy of OS. Although the subjects had a good representativeness, there still many limitations existed in our study. The small sample size and unadjusted results will influence the veracity of our results. The occurrence of OS is a complex process. A single gene cannot decide the development of the disease. So the gene-gene and gene-environment interactions should be considered in the study. Besides, polymorphism distribution exist ethnic difference, only one ethnicity included in our study cannot represent the overall results. Therefore, our results should be confirmed by further studies in future.

Disclosure of conflict of interest

None.

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References

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