Original Article

Association between CTLA-4 rs231775 polymorphism and hepatocellular carcinoma susceptibility

Zhao Liu1*, Zhao Song1*, Jing Sun2, Fenglei Sun1, Chuanzhi Li1, Jiuzheng Sun1, Liyou Xu1

1Department of Hepatobiliary and Pancreatic Surgery, Jinan Central Hospital Affiliated to Shandong University, Jinan 250013, Shandong, China; 2Department of Radiology, Jinan Central Hospital Affiliated to Shandong University, Jinan 250013, Shandong, China. *Equal contributors.

Received January 23, 2015; Accepted March 22, 2015; Epub November 1, 2015; Published November 15, 2015

Abstract: Aims: Our study aimed to investigate the association of cytotoxic T-lymphocyte antigen-4 (CTLA-4) rs231775 polymorphism with hepatocellular carcinoma (HCC) susceptibility. Methods: Genotypes distribution of the control was tested by Hardy-Weinberg Equilibrium (HWE). CTLA-4 rs231775 polymorphism was analyzed in 80 patients with HCC and 78 healthy controls by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method, and the expression level of CTLA-4 in the serum of all subjects was detected using enzyme linked immunosorbent assay (ELISA) kit. Odd ratio (OR) with 95% confidence interval (CI) were calculated by chi-squared test to determine the correlation of CTLA-4 rs231775 polymorphism and the risk of HCC. Results: The genotypes frequencies of the control group were in accordance with HWE. The frequencies of genotype AA and allele A in CTLA-4 rs231775 polymorphism were significantly higher in cases than the control group (AA vs. GG: OR=2.81, P=0.043; A vs. G: OR=1.63, P=0.022). Meanwhile, the expression level of CTLA-4 was remarkably higher in cases compared with the controls. The association analysis indicated that AA genotype carriers exhibited highest level of CTLA-4 (P<0.01). Conclusions: The genotype AA and allele A of CTLA-4 rs231775 polymorphism may have negative effects on HCC by modifying the expression and functions of CTLA-4.

Keywords: Hepatocellular carcinoma, CTLA-4, polymorphism

Introduction

Liver cancer is one of malignant tumors with the highest incidence all over the world [1]. Cause-of-death statistics of Chinese residents reveal that liver cancer is the second leading cause of death among the malignant tumors. The incidence of hepatocellular carcinoma (HCC) in China is the highest worldwide, and HCC brings serious threat to the health of human beings [2]. However, the pathogenesis of HCC has not been well clarified.

Cytotoxic T-lymphocyte antigen-4 (CTLA-4) encoded by CTLA-4 belongs to the immunoglobulin supergene family. Its gene located on chromosome 2 in q33, which is expressed on the surface of T cells in form of dimers [3]. The function of CTLA-4 is mainly to transmit inhibitory signals so as to negatively regulate the proliferation of T cells and block the immune responses [4]. Genetic variations or expression disorders of CTLA-4 may lead to an attenuated inhibitory effect on the activation of T cells, meanwhile autoimmune injury was activated [5, 6]. In recent years, studies concerning the relationship between CTLA-4 polymorphisms and some diseases have attracted extensive attention. Previous studies have indicated that CTLA-4 polymorphisms are closely related to various autoimmune diseases such as idiopathic dilated cardiomyopathy (IDCM), lupus erythematosus (LE) and thyroid diseases [7-9]. Just as we have known, CTLA-4 polymorphism exists a mutation from A to G on exon2 as rs231775, the relationship between CTLA-4 rs231775 polymorphism with various diseases has been exported, such as gastric cancer [10], colorectal cancer [11], hepatocellular carcinoma and cervical cancer [12]. But the association of CTLA-4 rs231775 polymorphism with HCC risk is hardly reported.

In present study, a case-control model included 80 cases and 78 controls were conducted. The CTLA-4 rs231775 polymorphism was checked...
by PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) method. The result about the relevance of CTLA-4 rs231775 polymorphism with HCC risk provides a theoretical basis for the risk prediction and the treatment of HCC.

**Subjects and methods**

**Research subjects**

A case-control study was carried out. 80 patients with HCC by pathological examination were set in case group from Jinan Central Hospital, and 78 individuals who had a health examination in same hospital during the same period had no tumors or genetic diseases belonged to the control group. An age gap of ± 2 years existed among individuals in the two groups. Furthermore, the subjects in two groups had the similar native place, gender and ethnicity, as well as parallel life and eating habits, and had no genetic connections. Our research protocol was supported by Research Ethics Committee of the hospital. Written consents were acquired from every subject.

**Sample preparation**

5 ml peripheral venous blood was collected from each subject with TRANK 303 heparin vacuum blood collection tube. The expression level of CTLA-4 in serum was tested by enzyme linked immunosorbent assay (ELISA) kit.

**DNA extraction**

Genome DNA of peripheral venous blood from all subjects was extracted by using the conventional phenol-chloroform method. The purity and concentration of DNA were tested by an ultraviolet spectrophotometer and then stored at -20°C refrigerator.

**Genotyping**

PCR primers were designed by Premier 5.0 according to gene sequence of exon 1 in CTLA-

**Statistical analysis**

The data analysis was performed with SPSS 18.0 software. The genotypes frequencies of the controls were checked whether the distribution was in accordance with Hardy-Weinberg Equilibrium (HWE). Odds ratio (OR) with 95% confidence interval (CI) were used to evaluate the association of CTLA-4 rs231775 polymorphism with the risk of HCC, which was calculated by chi-squared test. In present study, the relationship between CTLA-4 rs231775 polymorphism and the expression of CTLA-4 in serum was analyzed with linear regression analysis. P<0.05 indicates significant difference.
**Table 2.** The comparison of genotypes and alleles frequencies of CTLA-4 rs231775 polymorphism in case and control group

<table>
<thead>
<tr>
<th>CTLA-4 rs231775</th>
<th>Case group (n=80, %)</th>
<th>Control group (n=78, %)</th>
<th>ORs (95% CIs)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>29 (36.3)</td>
<td>38 (48.7)</td>
<td>1 (Ref.)</td>
<td>-</td>
</tr>
<tr>
<td>GA</td>
<td>36 (45.0)</td>
<td>33 (42.3)</td>
<td>1.43 (0.73-2.81)</td>
<td>0.30</td>
</tr>
<tr>
<td>AA</td>
<td>15 (18.7)</td>
<td>7 (9.0)</td>
<td>2.81 (1.01-7.78)</td>
<td>0.043</td>
</tr>
<tr>
<td>G</td>
<td>94 (58.8)</td>
<td>109 (69.9)</td>
<td>1 (Ref.)</td>
<td>-</td>
</tr>
<tr>
<td>A</td>
<td>66 (41.2)</td>
<td>47 (30.1)</td>
<td>1.63 (1.02-2.59)</td>
<td>0.039</td>
</tr>
</tbody>
</table>

**Results**

**HWE examination**

The examination result showed that the genotypes frequencies of CTLA-4 rs231775 polymorphism in control group were satisfied with HWE (P>0.05).

**Analysis of CTLA-4 rs231775 polymorphism**

The digestion results showed AA genotype with a 162 bp fragment, AG genotype with three fragments of 162 bp, 88 bp and 74 bp, and GG genotype with 88 bp and 74 bp fragments.

**Analysis of CTLA-4 level in the serum and CTLA-4 rs231775 polymorphism**

The CTLA-4 level in the serum of subjects in the two groups is shown in Table 1. The result revealed that the expression level of CTLA-4 was significantly higher in cases compared with the control group (P<0.05). In the same time, we performed an analysis of the relationship between the expression level of CTLA-4 and genotypes distribution of CTLA-4 rs231775 polymorphism case group. The result showed that in case group, the expression level of CTLA-4 was highest in the AA genotype carriers compared with AG and GG (P<0.01) (Figure 1).

**The relationship of CTLA-4 rs231775 polymorphism with HCC susceptibility**

We made a comparison in genotypes and alleles frequencies of CTLA-4 rs231775 polymorphism between the cases and controls. The result demonstrated that frequencies of homozygous genotype AA and allele A were remarkably higher in case group than controls (18.7% vs. 9.0%, 41.2% vs. 30.1%). We also found that AA genotype and A allele might be risk factors for HCC (AA vs. GG: OR=2.81, 95% CI=1.01-7.78; A vs. G: OR=1.63, 95% CI=1.02-2.59) (Table 2).

**Discussion**

The occurrence and development of HCC is a complex biological process influenced by many factors. The environmental factors are the external causes, whereas genetic susceptibility is an important internal cause and largely determines the risk degree of developing liver cancer among individuals [13, 14]. Human genes have abundant polymorphisms. Genetic mutations may lead to abnormal expressions of corresponding proteins. As a result, each individual may react differently to toxics or carcinogens, and thus may have different liver cancer susceptibility [15, 16]. In recent years, abundant publications have reported genetic variants are related with HCC susceptibility. According to the study of Li et al., genetic variant of CYP2C19 may be an important risk factor for HCC in China Han population, but not in patients with HBV infection [17]. A meta-analysis from Xiao et al. demonstrated the homozyous null genotypes of glutathione-S-transferase genetic polymorphisms GSTM1 and GSTT1 significantly increased the risk of HCC in Chinese [18].

CTLA-4 is expressed in activated T cells, and is the homologue of CD28 in structure [5]. CTLA-4 can participate as a negative costimulatory molecule in the activation process of T cells and regulate immune responses of the body [19, 20]. CTLA-4 polymorphisms can modify the function of CTLA-4, and are related to a variety of autoimmune diseases [21-23]. CTLA-4 rs231775 polymorphism is derived from a Thr to Gly mutations in the signal peptide which may lead to subtle changes in subcellular location of CTLA-4 mature proteins. In the other hand, the interaction between nascent peptide fragments and molecular chaperones was influenced by genetic variants of CTLA-4, which modified the function of CTLA-4 [24, 25].

Many reports have shown that CTLA-4 rs231775 polymorphism is correlated with the function of CTLA-4 [26-28]. The G allele of CTLA-4 +49G/A polymorphism is associated with the generation process of CTLA-4 protein by endoplasmic reticulum. The increasing fre-
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frequency of G allele can reduce the generation and expression of glycosylated proteins in CTLA-4 +49G/A polymorphism. As a result, the regulation of T cells proliferation is weakened, which will damage the inhibitory function of CTLA-4 [29].

In present study, the result showed that the case group had an obviously higher frequencies of genotype AA and allele A than the controls, which implied that AA genotype and A allele of CTLA-4 rs231775 polymorphism might be related to the increasing risk of HCC. In addition, this study also showed that AA genotype had an apparent association with the expression of CTLA-4 in the serum in cases, suggesting that the CTLA-4 rs231775 polymorphism might result in disorders of cellular immune function by affecting secreting of CTLA-4.

In conclusion, a predicted possible mechanism of CTLA-4 rs231775 polymorphism for HCC susceptibility may be as follows: the replacement of Thr to Gly in the signal peptide on CTLA-4 rs231775 polymorphism may change the expression level and function of CTLA-4 and as a result, the polymorphism affects the individuals susceptibility for HCC. However, our study did not consider effects of the environmental factors on the pathogenesis of HCC and the sample size was relatively small, which may influence the reliability of our results. Therefore, further well-design research with large sample size was required to investigate the issue.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Liyou Xu, Department of Hepatobiliary and Pancreatic Surgery, Jinan Central Hospital Affiliated to Shandong University, 105 Jiefang Road, Jinan 250013, Shandong, China. E-mail: chongjj09@163.com

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