Original Article

Relevance between HLA-DP gene rs2281388 polymorphism and hepatocellular carcinoma risk

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Abstract: Purpose: We carried out this study to find out the relevance between rs2281388 T/C polymorphism of human leukocyte antigen (HLA) gene and hepatocellular carcinoma (HCC) risk in Chinese Han population. Methods: The method of polymerase chain reaction (PCR) was applied to amplify the genomic DNA. Then the PCR products were sequenced to test the HLA-DP gene rs2281388T/C polymorphism of the case and control groups. Odds ratios (ORs) and 95% confidence interval (95% CIs) were utilized to evaluate the potential correlation between rs2281388 variants and HCC risk. Results: We analyzed the rs2281388 polymorphism distribution among the clinical pathological features. The results showed that there existed a significant statistic correlation between rs2281388T/C polymorphism of HLA-DP gene and HBsAg feature, and no significant correlation was found between rs2281388 and other clinical features. Further analysis showed that the TT genotype of rs2281388 was significantly correlated with HCC risk, and the same to T allele, but there was no significant difference of CT genotype distribution in case and control groups. Conclusion: TT genotype and T allele of HLA-DP gene rs2281388 polymorphism may increase the risk of HCC.

Keywords: HLA-DP gene, single nucleotide polymorphism, hepatocellular carcinoma

Introduction

Hepatocellular carcinoma (HCC) is the mainly primary liver cancer (85%-90%) [1, 2]. China is a high-incidence country of HCC [3], and the incidence is higher than other countries. HCC seriously affects the individuals’ health, thence many researches have investigated the pathogenesis of it. As we all know, the occurrence of HCC is a complicated biological process, and the pathogenesis has not been totally explained by now. Recent studies demonstrated that the occurrence of HCC was affected by combined effects of many factors, such as cirrhosis, viral hepatitis and other chemical carcinogen, environment and inheritance factors [4-8], and the risk of suffering HCC is mainly determined by genetic background.

Human leukocyte antigen (HLA) complex is the human major histocompatibility complex (MHC). HLA gene is located at 6p21. 31, has the length of 3600kb, occupies about 0.1% of the human genome bases, and consists by a series of related gene sites [9]. As a human genetic marker with high polymorphisms [10, 11], HLA is an important system which controls the body immunity and immune regulation, determines the body histocompatibility and involves in the differentiation of T-cell. Researches have proved that HLA, one of the most important areas of genetic predisposition, is related to many diseases. At present, a number of studies have confirmed that a lot of polymorphism sites of the gene are linked to the occurrence of many kinds of diseases, including liver cancer, gastric cancer, breast cancer, hypertension, leukemia, tuberculosis and lung cancer etc [12-20]. Moreover, a lot of studies focused on the correlation between HLA polymorphisms and hepatitis virus infection [6, 21-24]. According to the reports, rs2281388 polymorphism, which located at the downstream of HLA-DPB1, has a significant association with chronic hepatitis B (CHB) [25], while the research about rs2281388 and HCC was
not observed. So we carried out this study to investigate the relevance between rs2281388T/C polymorphism and the occurrence of HCC in northern China, which will help for revealing the pathogenesis of HCC.

Materials and methods

Specimen source

139 HCC patients who have been confirmed by two pathologists in Shandong Provincial Hospital, 92 in men and 47 in women. The mean age of these people is 54.9±14.6. 150 healthy individuals came from the health check-up center, 95 in men and 55 in women, average age of 52.9±14.6, and had no tumor history, hepatitis, cirrhosis and other liver diseases. Clinical features of all participants were detailed in Table 1. All of the research objects belong to Chinese Han population with no blood relationship. All participants signed the written informed consent and provided whole blood after 12 hours fast. The study was approved by the Review Boards of Shandong Provincial Hospital, China.

DNA extraction and primer synthesis

2 ml anticoagulant was put in peripheral venous blood which preserved in -20°C. The whole blood genetic DNA extraction kit was used to extract DNA (Shanghai Xin Ran Biological Technology Co. Limited), according to the manufacturer’s protocol. The primers were designed by Primer Premier 5.0. Upstream primer: 5'-TCT CTG CAA TAC CCT CAA TGA CTG-3'; Downstream primer: 5'-CAA TGG TGA GCA GAC TGC AAA TC-3'. The primers were synthesized by Beijing Sunbiotech Co. Limited.

Table 1. The relevance between rs2281388T/C and clinical features of liver cancer

<table>
<thead>
<tr>
<th>Clinical features</th>
<th>Genotype</th>
<th>χ²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>CC (n=54)</td>
<td>CT+TT (n=85)</td>
</tr>
<tr>
<td>Clinical stage</td>
<td>I~II</td>
<td>29</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>III~IV</td>
<td>25</td>
<td>47</td>
</tr>
<tr>
<td>Differentiated degree</td>
<td>high</td>
<td>24</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>poor</td>
<td>30</td>
<td>41</td>
</tr>
<tr>
<td>Metastasis</td>
<td>yes</td>
<td>27</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>29</td>
<td>34</td>
</tr>
<tr>
<td>HBsAg</td>
<td>positive</td>
<td>21</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>negative</td>
<td>33</td>
<td>35</td>
</tr>
</tbody>
</table>

Results

HWE test

The genotype distribution of the HLA-DP polymorphism site rs2281388 in the healthy control was accorded with HWE ($\chi^2=0.533$, $P=0.465$), which showed that the community genetic inheritance of the control samples was balanced and it can represent the general population.

Relationship between rs2281388T/C polymorphism and the clinical features

According to the analysis of clinical stages, differentiated degree, metastasis and HBsAg, we found that HBsAg positive HCC patients were more than negative patients in the distribution
Correlation between rs2281388 T/C polymorphism and HCC risk

The results indicated that CT genotype exhibited no significant difference between case and control groups ($P>0.05$). In addition, there existed significant correlation between HCC risk and TT genotype (OR=2.121, 95% CI=1.084-4.148, $P=0.027$), and the same to T allele (OR=1.432, 95% CI=1.021-2.007, $P=0.037$). Above all, TT genotype and T allele might increase the risk of HCC (Table 2).

Discussion

HCC is one of the ten malignant tumors announced by the world health organization (WHO). About 700,000 new HCC cases occur and about one million people die of it every year. More than 50% of the annual newly increased HCC patients appear in China [1, 3]. In recent years, with the development of molecular biology, virology and genetics, the occurrence of HCC is widely regarded as the consequence of the long-term effect of various factors. Environment and genetical factors all involved in the process of HCC [26], such as virus, aflatoxin, alcohol, single nucleotide polymorphism (SNP), methylation etc. Not everyone who exposed to the carcinogenic factors will suffer the risk of liver cancer, there exists individual difference on HCC susceptibility. The study of HCC susceptibility will contribute to exploring the pathogenesis of HCC.

Amount of studies indicated that the chronic infection of Hepatitis B virus (HBV) is the most dangerous factor that leads to the occurrence of HCC in China [27], and more than 85% cases of HBV infection appear to be positive. Chinese population who infected the HBV has a higher risk of HCC. It is concluded that the host’s genetic predisposition plays an important role in the occurrence and development of HCC.

As we all know, there is an obvious relationship between HLA complex protein and the immune reaction. Present studies showed that some HLA polymorphisms might influence the immune reaction of HBV infection [6, 21-24]. So we speculated that mutations of HLA could down regulate the human immune capacity. As a result, it is easier for HBV to infect the host.

In the present study, we provided preliminary evidence that the HBsAg feature had a significant statistic correlation with rs2281388 T/C polymorphism of HLA-DP gene. But there was no significant relationship between rs2281388 T/C polymorphism and other clinical stages ($P>0.05$). Thecomparison between two groups on rs2281388 T/C polymorphism

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>CC n (%)</th>
<th>CT n (%)</th>
<th>TT n (%)</th>
<th>C n (%)</th>
<th>T n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Health control</td>
<td>150</td>
<td>68 (45.33)</td>
<td>63 (42.00)</td>
<td>19 (12.67)</td>
<td>199 (66.33)</td>
<td>101 (33.67)</td>
</tr>
<tr>
<td>HCC</td>
<td>139</td>
<td>54 (38.85)</td>
<td>53 (38.13)</td>
<td>32 (23.02)</td>
<td>161 (57.91)</td>
<td>117 (42.09)</td>
</tr>
</tbody>
</table>

χ²: 1.00, 0.049, 4.915, 1.00, 4.354

P value: - , - , 0.825, 0.027, - , 0.037

OR (95% CI): - , - , 1.059 (0.636-1.766), 2.121 (1.084-4.148), - , 1.432 (1.021-2.007)

In summary, the TT genotype and T allele of rs2281388 significantly increased the risk of HCC. But the pathogenic principle of rs2281388 polymorphism in HCC is still unknown. Therefore, more comprehensive study with large sample size is needed to explore issue. Well designed research will contribute to sup-
plying accurate evidence on the pathogenic principle of rs2281388 polymorphism in HCC.

**Disclosure of conflict of interest**

None.

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**References**


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