The Ser326Cys polymorphism of hOGG1 is associated with intrahepatic cholangiocarcinoma susceptibility in a Chinese population

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Abstract: Objective: Intrahepatic cholangiocarcinoma is a rare disease whose etiology is far from clear, the Ser326Cys polymorphism in human 8-hydroxyguanine glycosylase (hOGG1) has been shown associated with various cancers, however, the association of Ser326Cys (rs1052133) polymorphism and intrahepatic cholangiocarcinoma susceptibility has not been clarified. The purpose of this study is to investigate whether this polymorphism is related to the genetic susceptibility of intrahepatic cholangiocarcinoma. Methods: A total 150 patients and 150 normal people were included in this study, the Ser326Cys polymorphisms in each group were genotyped using PCR-RFLP method. Results: We found that individuals carrying Cys/Cys genotype were exposed to higher risk of intrahepatic cholangiocarcinoma (OR=2.924, 95% CI=1.475-5.780) compared with the individuals with wild type genotype Ser/Ser. Further analysis revealed that male individuals carrying Cys/Cys genotype also had increased risk (OR=2.762, 95% CI=1.233-6.173), whereas no significant difference was observed in female group. Conclusions: Therefore, our data indicates that the Ser326Cys (rs1052133) polymorphism is associated with intrahepatic cholangiocarcinoma susceptibility, and it shows preference in male population.

Keywords: hOGG1, polymorphism, intrahepatic cholangiocarcinoma

Introduction

Intrahepatic cholangiocarcinoma (ICC) is a subtype of a family of aggressive cholangiocarcinomas, tumors that arise from the epithelial of intrahepatic bile ducts [1]. It is a relatively rare neoplasm which for example only accounts for 4%-10% of primary hepatic carcinomas [2, 3]. Thanks to the popularization of new diagnostic techniques such as magnetic resonance cholangiopancreatography (MRCP) and endoscopic retrograde cholangiopancreatography (ERCP), the detection rate of intrahepatic cholangiocarcinoma slightly increased in recent years. Despite the improvements of early diagnosis and treatment of intrahepatic cholangiocarcinoma that achieved in last decades, intrahepatic cholangiocarcinoma is still considered to have very poor prognosis than hepatocellular carcinoma. Surgery is the only curative approach; nevertheless, it is not always practicable. It can be rapidly fatal, associated with median survival between 3.1 and 7.7 months in case of unresectable tumor. Additionally, the etiology of intrahepatic cholangiocarcinoma is still unclear but, generally, like many other types of cancer, it might result from environmental risk factors acting on certain susceptible populations [4].

As cells are constantly challenged by environmental factors and intracellular stresses that cause damages to DNA double helix, the normal cell functions are highly dependent on DNA repair processes. Challenged by reactive oxygen species (ROS), the chemical structure of guanine is often modified to form 8-oxoguanine, which can be removed by a base excision repair process. 8-Oxoguanine glycosylase (OGG1), a DNA glycosylase, plays a key role in this pro-
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cess. Human OGG1 (hOGG1) is a gene that broadly expressed in various organs such as lung, liver, stomach and prostate, etc. Recent studies have shown that OGG1 may be associated with cancer risk in BRCA1 and BRCA2 mutation carriers [5], which underlies its potential roles in the diagnosis and prognosis of cancer.

Recent advances in molecular biology have prompted single nucleotide polymorphisms (SNPs) to come into the sight of tumor diagnosis, prognosis and even treatment. So far, the most extensive studied functional polymorphism of hOGG1 is Ser326Cys, which refers to the replacement of Serine at codon 326 with Cysteine. It has been shown that hOGG1 Ser326Cys polymorphism is associated with a variety of cancers [6-9]. However, its role in intrahepatic cholangiocarcinoma has not been reported. The present study was designed attempting to analysis whether hOGG1 Ser326Cys plays a role in the etiology of intrahepatic cholangiocarcinoma. In this case-control study, we genotyped 150 patients and 150 normal people, the frequencies of Ser326Cys polymorphism in each group were compared.

Materials and methods

Study population and blood sample collection

This study included 150 intrahepatic cholangiocarcinoma patients (80 male and 70 female, age 63.5±14.6) and 150 normal people (80 male and 70 female, age 62.3±9.3) in the First Affiliated Hospital of Nanjing Medical University from July 2009 to November 2014. Definitive diagnoses were made by Computed Tomography (CT), Magnetic Resonance Imaging (MRI), MRCP or ERCP in all patients, 90 intrahepatic cholangiocarcinoma patients have been confirmed by biopsy or postoperative histopathology. The normal people in control group were free from severe diseases of heart, lung and kidney as well as cancers. There was no blood relationship between individuals in each group. We requested the subjects in this study to fill a questionnaire that include social-demographic characteristics, age, sex, personal habits, disease history and so on, and informed consent was obtained from all the subjects. 2-3 ml blood samples were collected in EDTA-An-ticoagulant tubes and stored in a -80°C refrigerator.

Isolation of genomic DNA

A phenol-chloroform based DNA isolation protocol was used to extract genomic DNA from blood samples. Briefly, the blood samples were digested by protease K and RNase A at 55°C for 3 h, followed by adding equal volume of Tris-phenol and chloroform/isoamylalcohol (24:1) to each sample. Then, the samples were centrifuged at 2500 rpm for 15 min; the aqueous phase in each sample was transferred into a new tube. Equal volume of absolute ethanol was added to each tube to precipitate the DNA, followed by twice wash with 75% ethanol. The DNA were dissolved in TE buffer and stored in -20°C.

Genotyping of hOGG1 Ser326Cys polymorphism

The Ser326Cys polymorphism was assessed by a PCR-RFLP method as described elsewhere [7]. The primer set for PCR amplification is forward 5’-CTGTTCAGTGCCGACCTGCGCCGA-3’ and reverse 5’-ATCTTGTTGTGCAAACTGAC-3’. A single base mismatch was introduced in the forward primer so as to form an Mbo I site in wild type allele. After digested by Mbo I for 3 h at 37°C. PCR products were subjected to agarose gel electrophoresis. The wild type allele presents a 224 bp band and a 23 bp band due to the artificial MboI site, whereas the mutant allele presents a 247 bp band only. The polymorphisms detected in gel were confirmed by sequencing.

Statistical analysis

All the data were presented as mean ± S.D; comparisons between two groups were performed by student’s t test. Hardy Weinberg equilibrium test and the gene frequency between groups were compared by χ² test. A two tailed p value less than 0.05 was considered to be statistically significant. Odds ratio (OR) and 95% confidence intervals (CI) were used to determine the risk of intrahepatic cholangiocarcinoma of each genotypic groups. The OR and CI were calculated by logistic regression. The statistical analyses were conducted with SPSS 11.0 software.
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Results

PCR-RFLP results of hOGG1 Ser326Cys polymorphism

We performed PCR-RFLP analysis to detect the Ser326Cys polymorphism, as expected and shown in Figure 1, the PCR products that been through Mbo I digestion of a mutant Cys/Cys genotype only presented a 247 bp band, whereas the wild type Ser/Ser genotype should presented a double band of 224 bp and 23 bp. The 23 bp fragment is too small to be presented in the electrophoresis image, therefore, only a 224 bp fragment was seen. The heterozygous Ser/Cys genotype presented both 224 bp and 247 bp band.

General characteristics of the case and control groups

Firstly, we compared the age and sex between case group and control group, no statistical difference were observed (age: t=1.335, P=0.185, sex: χ²=0.214, P=0.644). The genotype distribution frequencies of Ser326Cys (rs1052133, c/g) in the normal people of Han population of Jiangsu province china are 24.7% (Ser/Ser), 48.6% (Ser/Cys) and 26.7% (Cys/Cys), respectively. χ² test results confirmed the distribution frequency of Ser326Cys polymorphism in control group was correspond with Hardy-Weinberg equilibrium (P=0.853).

The association between hOGG1 Ser326Cys polymorphism and intrahepatic cholangiocarcinoma susceptibility

Next we evaluated the genotype and allele frequencies of hOGG1 in case group and control group. As shown in Table 1. The number of patients who carried a Cys/Cys genotype in case group was greater than control group. The frequencies of genotype at the hOGG1 rs-1052133 polymorphism site were 12.7% (Ser/Ser), 47.3% (Ser/Cys), 40% (Cys/Cys). The statistical difference of genotype distribution in case group and control group was observed (χ²=9.813, P=0.0074). We then performed logistic regression analyses to compare the risk of intrahepatic cholangiocarcinoma in individuals who were Ser/Cys and Cys/Cys genotype carriers. We found that Ser/Cys and Cys/Cys genotypes possessed 1.894-(OR=1.894, 95% CI=0.994-3.597) and 2.924-fold (OR=2.924, 95% CI=1.475-5.780) risk of intrahepatic cholangiocarcinoma, respectively, compared with Ser/Ser genotype. We further analyzed the allele frequencies of hOGG1 polymorphism; we observed that the number of Cys alleles in case groups was also significantly greater than control group. The risk of intrahepatic cholangiocarcinoma in people with Cys allele was significantly higher than those who carries Ser allele (OR=1.68, 95% CI=1.215-2.331).

The association between hOGG1 Ser326Cys polymorphism and intrahepatic cholangiocarcinoma susceptibility in two genders

To determine whether the increased risk of intrahepatic cholangiocarcinoma in hOGG1 Ser326Cys mutation population is related to sex, we calculated the odds ratios in each sex separately. The results were presented in Table 2. Interestingly, we found that the risk of Cys/Cys genotype in male population were 2.762-fold higher than Ser/Ser genotype (OR=2.762, 95% CI=1.233-6.173). Particularly, however, in female group, none of the mutant genotypes (Ser/Cys, Cys/Cys) possessed an increased risk of intrahepatic cholangiocarcinoma (Ser/Cys (OR=2.105, 95% CI=0.603-7.353, P=0.372), Cys/Cys (OR=4.448, 95% CI=0.898-13.334, P=0.243)).

Discussion

In this case-control study, we demonstrate that the hOGG1 Ser326Cys (rs1052133, c/g) is sta-
Polymorphism of hOGG1 and intrahepatic cholangiocarcinoma

Table 1. The association between hOGG1 Ser326Cys polymorphism and intrahepatic cholangiocarcinoma susceptibility

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Case group (n=150)</th>
<th>Control group (n=150)</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ser/Ser</td>
<td>19 (12.7)</td>
<td>37 (24.7)</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Ser/Cys</td>
<td>71 (47.3)</td>
<td>73 (48.6)</td>
<td>1.894 (0.994-3.597)</td>
<td>0.058</td>
</tr>
<tr>
<td>Cys/Cys</td>
<td>60 (40.0)</td>
<td>40 (26.7)</td>
<td>2.924 (1.475-5.780)</td>
<td>0.003</td>
</tr>
<tr>
<td>Ser/Cys+Cys/Cys</td>
<td>131 (87.3)</td>
<td>113 (75.3)</td>
<td>2.257 (1.230-4.149)</td>
<td>0.011</td>
</tr>
</tbody>
</table>

Table 2. The association between hOGG1 Ser326Cys polymorphism and intrahepatic cholangiocarcinoma susceptibility in two genders

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Male Case group (n=150)</th>
<th>Male Control group (n=150)</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ser/Ser</td>
<td>15 (18.8)</td>
<td>28 (35.0)</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Ser/Cys</td>
<td>28 (35.0)</td>
<td>27 (33.8)</td>
<td>1.93 (0.853-4.405)</td>
<td>0.151</td>
</tr>
<tr>
<td>Cys/Cys</td>
<td>37 (46.2)</td>
<td>25 (31.2)</td>
<td>2.76 (1.233-6.173)</td>
<td>0.017</td>
</tr>
<tr>
<td>Ser/Cys+Cys/Cys</td>
<td>65 (81.3)</td>
<td>52 (65.0)</td>
<td>2.43 (1.183-5.025)</td>
<td>0.020</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Female Case group (n=150)</th>
<th>Female Control group (n=150)</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ser/Ser</td>
<td>4 (5.7)</td>
<td>9 (2.9)</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Ser/Cys</td>
<td>43 (61.4)</td>
<td>46 (65.7)</td>
<td>2.10 (0.603-7.353)</td>
<td>0.372</td>
</tr>
<tr>
<td>Cys/Cys</td>
<td>23 (32.9)</td>
<td>15 (21.4)</td>
<td>3.4 (0.898-13.334)</td>
<td>0.107</td>
</tr>
<tr>
<td>Ser/Cys+Cys/Cys</td>
<td>66 (94.3)</td>
<td>61 (87.1)</td>
<td>2.43 (0.713-8.333)</td>
<td>0.243</td>
</tr>
</tbody>
</table>

Intrahepatic cholangiocarcinoma is relatively rare, accounting for 20% to 25% of all cholangiocarcinomas; perihilar (50%-60%) or distal common bile duct (20%-25%) tumors are more common. Particularly, intrahepatic cholangiocarcinoma only accounts for less than 3% of the cancers in digestive system, and is characterized by later presentation of symptoms [1, 2]. Therefore little attention has been drawn with regard to its etiology, diagnosis and treatment. It is believed that primary sclerosing cholangitis, cholelithiasis, viral hepatitis, diabetes and unhealthy lifestyle (alcohol consumption, smoke etc.) were attributed to the onset of intrahepatic cholangiocarcinoma, however, only a small subgroup of patients are exposed to specific risk factors [1, 10]. Oxidative damages promote the chemical modifications of guanine to form an 8-Oxoguanine byproduct, which must be removed through a base excision repair process guided by OGG1. OGG1 has been demonstrated to have multiple effects in animal models, mice lacking OGG1 exhibit enhanced lung carcinogenesis [11] and higher probability to develop obesity as well as insulin resistance [12]. Single nucleotide polymorphism refers to the common variation of DNA sequence. Although that most of the SNP occur in non-coding regions, several mutations in the coding regions have been found. The most extensive studied polymorphism site of human OGG1 gene, Ser326Cys (rs1052133, c/g) has been proven to functionally related to the enzyme activity of hOGG1 [13, 14]. Recent studies demonstrated that hOGG1 Ser326Cys polymorphism is associated with the susceptibility of cancers in esophagus, stomach, and prostate and so on [15-18]. However, no study has analyzed the association between intrahepatic cholangiocarcinoma and this polymorphism. In our case-control study, we found that the number of patients carrying the mutant Cys/Cys genotype was increased, further logistic regression analysis revealed that the risk of Cys/Cys genotype is approximate 3-fold higher than Ser/Ser genotype. Our study showed much consistency with the previous case-control studies or Meta analyses [15, 19-21]. Nevertheless, it is reasonable to propose that individuals with mutant genotype hold a low hOGG1 activity therefore lowers the DNA repair ability with resultant increased risk of carcinogenesis.
An interesting observation in this study is that, when we separated the analysis by different gender groups, the high risk is biased toward male group. We found that in male, the mutant genotype Cys/Cys takes approximate 2.8-fold risk higher than wild type genotype, whereas the odds ratio of Ser/Cys and Cys/Cys genotypes in female group did not reach statistical significance (P=0.372 and 0.107, respectively). Several possible reasons might explain this deviation in two genders. First, because that intrahepatic cholangiocarcinoma is a relative low epidemic cancer, the cases in our study is limited. We only enrolled 70 female patients in our study; with a more expanded sample size, a more definitive analysis should possibly be carried out. The other possible reason is that different hormone conditions in each sex may alter the susceptibility to environmental factors which masks the effect of hOGG1 Ser326Cys polymorphism on intrahepatic cholangiocarcinoma risks. It is note-worthy that OGG1 deficient female mice are more prone to establish lung cancer rather than male mice [11]. It seems paradoxically related to our analyses. But further large sample sized clinical analysis in combination with animal studies are required to test the relevance of sex and the hOGG1 polymorphism associated intrahepatic cholangiocarcinoma risk in bile duct system.

Despite that majority of the studies have established the associations between the susceptibility of certain types of cancers and hOGG1 Ser326Cys polymorphism, conflicting results were still presented. In a case-control study on hepatocellular carcinoma (HCC) [18], they proposed that hOGG1 Ser326Cys is associated with its risk. In the contrary, two recent meta-analyses indicated that limited evidence support their associations [22, 23]. So far, no case-study or meta-analysis has been performed with regard to the association between hOGG1 Ser326Cys polymorphism and intrahepatic cholangiocarcinoma. Our results provided preliminary observation on their association with inevitable limitations. It should be noted that our study is single centered with limited sample size. In addition, as our study is retrospective, the existing selection bias may have influence on our results. Lessons learned from the studies on HCC suggested that our case-control study should be interpreted cautiously.

In summary, our case-control study unraveled the association of the Ser326Cys polymorphism and the susceptibility of intrahepatic cholangiocarcinoma. From the evidence we obtained, we can at least draw a conclusion that hOGG1 Ser326Cys polymorphism is a genomic risk factor of intrahepatic cholangiocarcinoma in male Han population of Jiangsu province, China. Because intrahepatic cholangiocarcinoma is a rare disease, a multi-center cooperated large scale study would enable us to gain more definitive understandings of the association of gene polymorphisms and its susceptibility.

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Disclosure of conflict of interest

None.

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