Introduction

Hepatocellular carcinoma (HCC) is one of the common malignant tumors globally, which is the fifth most prevalent cancer and the third cause of cancer-related deaths worldwide (Llovet et al., 2003; Parkin et al., 2005; Parikh et al., 2007). The estimated annual incidence of cases exceeds 600,000 (Parkin et al., 2005; But et al., 2008). In China, HCC has been the second cause of cancer-related deaths since the 1990s (Chen et al., 2010; Zeng et al., 2012). Many environmental and genetic factors have been approved to be associated with susceptibility to HCC (Thorgeirsson et al., 2002; Bosch et al., 2004; Cavaco et al., 2003; Taniguchi et al., 2003; Kaya et al., 2005; Pechandova et al., 2006; Chinn et al., 2007; Wu et al., 2007; Yu et al., 2011). However, the association between the MDR1 gene c.4125A>C variant and susceptibility to cancer including HCC, have not been analyzed. Therefore, the objective of this study was to detect the distribution of MDR1 gene c.4125A>C polymorphism and to evaluate its association with susceptibility to HCC in Chinese population.

Materials and Methods

Study population

This present case-control study consisted of 689 HCC patients and 680 cancer-free controls from January 2009 to December 2011 at the institute of liver disease of People’s Liberation Army, Beijing Military General Hospital. All subjects were unrelated Han Chinese living in China. Health subjects were randomly selected from health screening program participants to exclude those with a family history of liver-related tumours.

MDR1 gene SNPs were associated with susceptibility to HCC (Wu et al., 2007; Chen et al., 2009; Chen et al., 2011). MDR1 is a polymorphic gene and more than 50 SNPs have been reported (Hoffmeyer et al., 2000; Ambudkar et al., 2003; Cavaco et al., 2003; Taniguchi et al., 2003; Kaya et al., 2005; Pechandova et al., 2006; Chinn et al., 2007; Wu et al., 2007; Yu et al., 2011). However, the association between the MDR1 gene c.4125A>C variant and susceptibility to cancer including HCC, have not been analyzed. Therefore, the objective of this study was to detect the distribution of MDR1 gene c.4125A>C polymorphism and to evaluate its association with susceptibility to HCC in Chinese population.

Abstract

Objective: The objective of this study was to evaluate the association of MDR1 gene polymorphisms with susceptibility to hepatocellular carcinoma (HCC). Methods: A total of 689 HCC patients and 680 cancer-free subjects were enrolled. Human MDR1 gene polymorphisms were investigated by created restriction site-polymerase chain reaction (CRS-PCR) and DNA sequencing methods. Multiple logistic regression models were applied to estimate the association between MDR1 gene polymorphisms and susceptibility to HCC. Results: We detected a novel c.4125A>C polymorphism and our findings suggested that this variant was significantly associated with susceptibility to HCC. A significantly increased susceptibility to HCC was noted in the homozygote comparison (CC versus AA: OR=1.621, 95% CI 1.143-2.300, χ²=7.4095, P=0.0065), recessive model (CC versus AC+AA: OR=1.625, 95% CI 1.167-2.264, χ²=8.3544, P=0.0039) and allele contrast (C versus A: OR=1.185, 95% CI 1.011-1.389, χ²=4.4046, P=0.0358). However, no significant increase was observed in the heterozygote comparison (AC versus AA: OR=0.995, 95% CI 0.794-1.248, χ²=0.0017, P=0.9672) and dominant model (CC+AC versus AA: OR=1.106, 95% CI 0.894-1.369, χ²=0.8560, P=0.3549). Conclusions: These findings suggest that the c.4125A>C polymorphism of the MDR1 gene might contribute to susceptibility to HCC in the Chinese population. Further work will be necessary to clarify the relationship between the c.4125A>C polymorphism and susceptibility to HCC on larger populations of diverse ethnicity.

Keywords: HCC - multidrug resistance 1 gene - single nucleotide polymorphisms - susceptibility - association analysis
with a history of cancer and other medical diseases. Clinical characteristics data as well as related risk factors, including gender, age, smoking, drinking, serum α-FP levels, family history of HCC and HBV serological markers, were summarized (Table 1). The present study was approved by the independent ethics committee of institute of liver disease of People’s Liberation Army (Beijing Military General Hospital) and written informed consent was obtained from all subjects of the study.

**DNA extraction and genotyping**

Blood samples were collected from peripheral venous blood of each subjects and genomic DNA was extracted using the standard method. The specific PCR primers were designed using Primer Premier 5.0 software. Primers, region, annealing temperature, product sizes and selected restriction enzymes were showed in Table 2. The PCR were carried out in a total volume of 20 μL solution containing 50ng template DNA, 1xbuffer (Tris-HCl 100 mmol/L, pH 8.3; KCl 500 mmol/L), 0.25 μmol/L primers, 2.0 mmol/L MgCl2, 0.25 mmol/L dNTPs, and 0.5U Taq DNA polymerase (Promega, Madison, WI, USA). The PCR conditions were as follows: 94°C for 5 min, 35 cycles at 94°C for 30 s, 54.6°C for 30 s, 72°C for 30 s, and a finally 72°C for 5 min.

Genotyping was performed using the created restriction site-polymerase chain reaction (CRS-PCR) method with one of the primers containing a nucleotide mismatch, which enables the use of restriction enzymes for discriminating sequence variations (Haliassos et al., 1989; Yuan et al., 2012; Yuan et al., 2012; Yuan et al., 2013). Each PCR amplified product was digested with 5 units restriction enzyme at 37°C for 10 h following the supplier’s manual and then electrophoresed on a 3% agarose gel and visualized under UV illumination. The DNA sequencing method was used to validate the CRS-PCR findings. Sequencing was analyzed using an ABI3730xl DNA Analyzer (Applied Biosystems, Foster City, CA).

**Statistical analyses**

The Statistical Package for Social Sciences software (SPSS, Windows version release 15.0; SPSS Inc.; Chicago, IL, USA) was used for all statistical analyses. The Chi-squared (χ²) test was performed to demonstrate differences in the Hardy–Weinberg equilibrium in all individuals, allele and genotype frequencies, and general characteristics between case and control groups. The multiple logistic regression models were analyzed to estimate the odds ratios (ORs) and 95% confidence intervals (95% CIs) of the association between MDR1 gene polymorphisms and susceptibility to HCC. P value<0.05 was defined as statistically significant.

**Results**

**General characteristics of the subjects**

This study was performed on 1369 subjects, including 689 HCC patients and 680 healthy controls. Their general characteristics of the subjects were summarized in Table 1. There was no significant difference between HCC patients and healthy controls in terms of gender and age distribution (P=0.6960 and P=0.7470, respectively). Additionally, no significant differences were detected in smoking and drinking status between the cases and controls (P=0.6946 and P=0.1720, respectively).

**Frequency of alleles and genotypes**

In the current study, through CRS-PCR and DNA sequencing methods, we detected a novel allelic polymorphism (c.4125A>C) within the exon28 of human MDR1 gene. Sequence analysis showed that the c.4125A>C polymorphism was caused by A to C mutations. This variant is a nonsynonymous mutation, causing Glutamic (Glu) to Alanine (Ala) acid replacement (p.Glu1211Ala, reference sequences GenBank ID: NG_011513.1, NM_000927.4 and NP_000918.2). The PCR products were digested with Rsal enzyme and performed into three genotypes, AA (199 and 22 bp), AC (221, 199 and 22 bp) and CC (221 bp, Table 2). The allelic and genotypic frequencies of c.4125A>C polymorphism were showed in Table 2.

<table>
<thead>
<tr>
<th>Characteristics Groups</th>
<th>cases (n=689)</th>
<th>% controls (n=680)</th>
<th>χ²-value</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>512</td>
<td>74.31</td>
<td>499</td>
<td>73.38</td>
</tr>
<tr>
<td>Female</td>
<td>177</td>
<td>25.69</td>
<td>181</td>
<td>26.62</td>
</tr>
<tr>
<td>Age(years) (mean±SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;55</td>
<td>392</td>
<td>56.89</td>
<td>381</td>
<td>56.03</td>
</tr>
<tr>
<td>≥55</td>
<td>297</td>
<td>43.11</td>
<td>299</td>
<td>43.97</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>367</td>
<td>53.27</td>
<td>355</td>
<td>52.21</td>
</tr>
<tr>
<td>No</td>
<td>322</td>
<td>46.73</td>
<td>325</td>
<td>47.79</td>
</tr>
<tr>
<td>Drinking</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>379</td>
<td>55.01</td>
<td>349</td>
<td>51.32</td>
</tr>
<tr>
<td>No</td>
<td>310</td>
<td>44.99</td>
<td>331</td>
<td>48.68</td>
</tr>
<tr>
<td>a-FP level (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;400 ng/ml</td>
<td>253</td>
<td>36.72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥400 ng/ml</td>
<td>436</td>
<td>63.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family history of HCC (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>67</td>
<td>0.0972</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>622</td>
<td>0.9028</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBV serological markers (n)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBs Ag( + )</td>
<td>173</td>
<td>25.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBs Ag( - )</td>
<td>516</td>
<td>74.89</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 1. Characteristics Between Hepatocellular Carcinoma (HCC) Cases and Healthy Controls**

**Table 2. Primer Pairs, PCR and CRS-PCR Analysis for Genotyping MDR1 Polymorphism**

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Primer sequences</th>
<th>Annealing temperature (°C)</th>
<th>Products (bp)</th>
<th>Region</th>
<th>Restriction enzyme</th>
<th>Genotype (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.4125A&gt;C</td>
<td>5'-CCCAATTTAATCTTACCTGT-3'</td>
<td>54.6</td>
<td>221</td>
<td>exon28</td>
<td>Rsal</td>
<td>AA:199,22 AC:221,199,22 CC:221</td>
</tr>
<tr>
<td></td>
<td>5'-GCTGTTAGAACATTTACTTGCAGTTC-3'</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PCR means polymerase chain reaction; CRS-PCR means created restriction site-PCR
Control group (n=680)
312 (0.4588) 303 (0.4456) 65 (0.0956) 927 (0.6816) 433 (0.3184)
Case group (n=689)
299 (0.4340) 289 (0.4194) 101 (0.1466) 887 (0.6437) 491 (0.3563)

AA             AC               CC                    A                     C

was associated with susceptibility to HCC. No similar
and demonstrated that the c.4125A>C polymorphism
polymorphism (c.4125A>C) by CRS-PCR methods
HCC, this study firstly found a novel MDR1 gene
Discussion

95% CI 0.894-1.369, χ² = 8.3561, p = 0.0153

OR=0.995, 95% CI 0.794-1.248, χ² = 0.0017, P = 0.9672

CC vs. AA 1.621(1.143-2.300) 7.4095 0.0065
AC vs. AA 0.995(0.794-1.248) 0.0017 0.9672
CC/AC vs. AA 1.106(0.894-1.369) 0.856 0.3549
CC vs. AC/AA 1.625(1.167-2.264) 8.3544 0.0039
C vs. A 1.185(1.011-1.389) 4.4046 0.0358

OR, odds ratio; CI, confidence interval; vs., versus; AA vs.
CC, Homozygote comparison; AC vs. CC, Heterozygote comparison; AA/AC vs. CC, Dominant model; AA vs. AC/CC,
Recessive model; A vs. C, Allele contrast

were shown in Table 3. The results from Chi-squared (χ²)
test suggested that the c.4125A>C polymorphism were
fitted with Hardy-Weinberg equilibrium in the studied
subjects (P>0.05). Allelic frequencies in HCC patients and
healthy controls were 64.37% and 68.16% for A allele, and
35.63% and 31.84% for C allele, respectively. Frequencies
of the AA, AC, and CC genotypes were 43.40%, 41.94%,
and 14.66% in HCC patients, while the frequencies of
these genotypes in healthy subjects were determined to
be 45.88%, 44.56%, and 9.56%. The genotypic and allelic
frequencies of HCC patients were significantly different
from those of the control subjects (χ²=8.3561, p = 0.0153
and χ²=4.4046, p = 0.0358, respectively).

MDR1 polymorphisms and susceptibility to HCC
The multiple logistic regression analysis showed
that the c.4125A>C polymorphism was significantly
associated with susceptibility to HCC (Table 4).
Significantly increased susceptibility to HCC were
found in the homozygote comparison (CC versus AA:
OR=1.621, 95% CI 1.143-2.300, χ²=7.4095, P = 0.0065),
recessive model (CC versus AC+AA: OR=1.625, 95%
CI 1.167-2.264, χ²=8.3544, P = 0.0039) and allele contrast
(C versus A: OR=1.185, 95% CI 1.011-1.389, χ²=4.4046,
P = 0.0358, Table 4). No significant associations were
detected in the heterozygote comparison (AC versus AA:
OR=0.995, 95% CI 0.794-1.248, χ²=0.0017, P = 0.9672)
and dominant model (CC+AC versus AA: OR=1.106,
95% CI 0.894-1.369, χ²=0.8560, P = 0.3549, Table 4).

Discussion

To the best of our knowledge, as one of the most
important candidate gene for human cancers including
HCC, this study firstly found a novel MDR1 gene
polymorphism (c.4125A>C) by CRS-PCR methods
and demonstrated that the c.4125A>C polymorphism
was associated with susceptibility to HCC. No similar
studies have been reported in other cancers. As shown
in Table 3, the genotypic and allelic frequencies between
HCC patients and healthy subjects were statistically
associated with the risk of HCC (p=0.0153 and p=0.0358,
respectively). Besides, the C allele may increase the
risk of HCC (C versus A: OR=1.185, 95% CI 1.011-
1.389, P = 0.0358, Table 4). Our data suggested that the
CC genotype was strongly associated with increased
susceptibility to HCC compared to AA genotype and AC/
CC carriers (OR=1.621, 95% CI 1.143-2.300, P =0.0065
and OR=1.625, 95% CI 1.167-2.264, P =0.0339, Table
4). Results from this study suggested that the c.4125A>C
polymorphism of MDR1 gene would contribute to
susceptibility to HCC in the Chinese population. There
were several similar studies were reported the correlation
between the MDR1 gene SNPs and susceptibility to HCC
(Wu et al., 2007; Chen et al., 2009; Chen et al., 2011).
Most of these studies were focused on the C1236T,
G2677A/T and C3435T polymorphisms (Wu et al., 2007;
Chen et al., 2009; Chen et al., 2011), but not including the
c.4125A>C variant. Furthermore, the results from these
studies still remain inconsistent. Thus, further work will be
warranted to explain the role of the c.4125A>C and other
polymorphisms of MDR1 gene in susceptibility to HCC
and other cancers on larger diverse ethnic populations.

Acknowledgements

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30471541). The author(s) declare that they have no
competing interests.

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MDR1 single nucleotide polymorphism with prognosis of

Table 3. Genotypic and Allelic Frequencies of c.4125A>C Polymorphism in the Studied Subjects

<table>
<thead>
<tr>
<th>Groups</th>
<th>Genotypic frequencies</th>
<th>Allelic frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
<td>AC</td>
</tr>
<tr>
<td>Case group(n=689)</td>
<td>299(0.4340)</td>
<td>289(0.4194)</td>
</tr>
<tr>
<td>Control group(n=680)</td>
<td>312(0.4588)</td>
<td>303(0.4456)</td>
</tr>
</tbody>
</table>

χ² = 8.3561, p = 0.0153

Table 4. Relationship Between c.4125A>C Polymorphism of XRCC1 Gene and Hepatocellular Carcinoma (HCC) Risk

<table>
<thead>
<tr>
<th>Comparisons</th>
<th>Test of association</th>
<th>OR(95% CI)</th>
<th>χ²-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC vs. AA</td>
<td></td>
<td>1.621</td>
<td>7.4095</td>
<td>0.0065</td>
</tr>
<tr>
<td>AC vs. AA</td>
<td></td>
<td>0.995</td>
<td>0.0017</td>
<td>0.9672</td>
</tr>
<tr>
<td>CC/AC vs. AA</td>
<td></td>
<td>1.106</td>
<td>0.856</td>
<td>0.3549</td>
</tr>
<tr>
<td>CC vs. AC/AA</td>
<td></td>
<td>1.625</td>
<td>8.3544</td>
<td>0.0039</td>
</tr>
<tr>
<td>C vs. A</td>
<td></td>
<td>1.185</td>
<td>4.4046</td>
<td>0.0358</td>
</tr>
</tbody>
</table>

OR, odds ratio; CI, confidence interval; vs., versus; AA vs.
CC, Homozygote comparison; AC vs. CC, Heterozygote comparison; AA/AC vs. CC, Dominant model; AA vs. AC/CC,
Recessive model; A vs. C, Allele contrast
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