RESEARCH ARTICLE

Association of MDR1 Gene Polymorphisms with Susceptibility to Hepatocellular Carcinoma in the Chinese Population

Yong-Qiang Ren*, Ju-Qiang Han, Jian-Biao Cao, Shao-Xiang Li, Gong-Ren Fan

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, χ^2=7.4095, P=0.0065), recessive model (CC versus AC+AA: OR=1.625, 95% CI 1.167-2.264, χ^2=8.3544, P=0.0039) and allele contrast (C versus A: OR=1.185, 95% CI 1.011-1.389, χ^2=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, χ^2=0.0017, P=0.9672) and dominant model (CC+AC versus AA: OR=1.106, 95% CI 0.894-1.369, χ^2=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

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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; Suriawinata et al., 2004; Farazi et al., 2006; Gomaa et al., 2008; Nault et al., 2011), while the reliable mechanism of the pathogenesis for HCC remains poorly understood.

Human multidrug resistance 1 gene (MDR1) has been suggested that it is an important candidate gene influencing susceptibility to various diseases, including HCC (Leonessa et al., 2003; Taniguchi et al., 2003; Jamroziak et al., 2004; Kurzawski et al., 2005; Sohn et al., 2006; Wu et al., 2007; Vander Borght et al., 2008; Yu et al., 2011). Several previous studies suggested that the 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.

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

*Institute of Liver Disease of People's Liberation Army, Beijing Military General Hospital, Beijing, China *For correspondence: yongqiang_ren@sina.com*

with a history of cancer and other medical diseases. Clinical characteristics data as well as related risk factors, including gender, age, smoking, drinking, serum a-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, 1×buffer (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., 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 (χ^2) 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 RsaI 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

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

Characteristics	Groups	cases (n=689)	%	controls (n=680)	%	χ^2-value	P-Value
Gender (n)							
	Male	512	74.31	499	73.38	0.1527	0.696
	Female	177	25.69	181	26.62		
Age(years) (mean±SD)		58.72±11.29		55.78±15.63			
	<55	392	56.89	381	56.03	0.1041	0.747
	≥55	297	43.11	299	43.97		
Smoking							
	Yes	367	53.27	355	52.21	0.1542	0.6946
	No	322	46.73	325	47.79		
Drinking							
	Yes	379	55.01	349	51.32	1.8652	0.172
	No	310	44.99	331	48.68		
a-FP level (n)							
	<400 ng/ml	253	36.72	-	-		
	≥400 ng/ml	436	63.28	-	-		
Family history of HCC (n)							
	Yes	67	0.0972	-	-		
	No	622	0.9028	-	-		
HBV serological markers (n)							
	HBs Ag(+)	173	25.11	-	-		
	HBs Ag(-)	516	74.89	-	-		

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

Polymorphism	Primer sequences	Annealing temperature (°C)	Products (bp)	Region	Restriction enzyme	Genotype (bp)
c.4125A>C	5'-CCCAATTTAAATTCTTACCTGT-3' 5'-GCTGTTAGAACTTTACTTTCAGTTC-3'	54.6	221	Exon28	RsaI	AA:199,22 AC:221,199,22 CC: 221

PCR means polymerase chain reaction; CRS-PCR means created restriction site-PCR

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

Groups	Genotypic frequencies			Allelic frequencies	
	AA	AC	CC	A	C
Case group(n=689)	299(0.4340)	289(0.4194)	101(0.1466)	887(0.6437)	491(0.3563)
Control group(n=680)	312(0.4588)	303(0.4456)	65(0.0956)	927(0.6816)	433(0.3184)
		χ^2=8.3561, p=0.0153		χ^2=4.4046, p=0.0358	

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

Comparisons	Test of association		
	OR(95% CI)	χ^2-value	P-value
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 (χ^2) 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 (χ^2=8.3561, p=0.0153 and χ^2=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, χ^2=7.4095, P=0.0065), recessive model (CC versus AC+AA: OR=1.625, 95% CI 1.167-2.264, χ^2=8.3544, P=0.0039) and allele contrast (C versus A: OR=1.185, 95% CI 1.011-1.389, χ^2=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, χ^2=0.0017, P=0.9672) and dominant model (CC+AC versus AA: OR=1.106, 95% CI 0.894-1.369, χ^2=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=0.0039, 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.

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