RESEARCH ARTICLE

Positive Association Between miR-499A>G and Hepatocellular Carcinoma Risk in a Chinese Population

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Abstract

A case-control study of the association of miR-499A>G rs3746444 with risk of hepatocellular carcinoma (HCC)was conducted. Patients with HCC and healthy control subjects were recruited for genotyping of miR-499A>G using duplex polymerase-chain-reaction with confronting-two-pair primer(PCR-RFLP) analysis. The MiR-499 GG genotype was associated with a decreased risk of HCC as compared with the miR-499 AA genotype (adjusted OR=0.74, 95%CI=0.24-0.96). Similarly, the GG genotype showed a 0.45-fold decreased HCC risk in a recessive model. The MiR-499 G allele was significantly associated with decreased risk of HCC among patients infected with HBV in a dominant model (OR=0.09, 95%CI= 0.02-0.29). In conclusion, the MiR-499A>G rs3746444 polymorphism is associated with HCC risk in the Chinese population, and may be useful predictive marker for CAD susceptibility.

Keywords: miR-499A>G - hepatocellular carcinoma - susceptibility - Chinese population

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Introduction

Primary liver cancer is the third leading cause of death from cancer worldwide, and it is reported that almost 748,300 new cancer patients per year (IARC, 2008). Hepatocellular carcinoma (HCC) accounts for 80% of all primary liver cancer (Perz et al., 2006). Although chronic hepatitis B virus and hepatitis C virus infections, aflatoxin B1, alcohol and nonalcoholic steatohepatitis are regarded as the main carcinogenic mechanism, only a few of these patients with these risk factors develop HCC during their lifetime, suggesting the etiology of HCC is not well clarified (El Serag et al., 2011). Thus some genetic factors may contribute to the carcinogenic mechanism.

MicroRNAs (miRNAs) are small non-coding and single-stranded RNA molecules, with 22 nucleotides in length. MiRNAs target the 3' untranslated region of mRNA, and functions as a post-transcriptional regulator (Lu et al., 2009). MiRNAs are transcribed from endogenous DNA and processed from primary transcript to hairpin precursor, and consist of two strands, including leading strand used for production of mature miRNA and the degraded passanger strand. Mature miRNAs participate into posttranscriptional gene expression by base pairing with target mRNAs of protein-coding genes (Bartel et al., 2004), and thus induce cleavage of mRNA or the repression of productive translation or even the destabilization and reduction in mRNA concentration (Bartel et al., 2004). MiRNAs play an important role in gene regulation, and physiologic and pathologic process, such as tumorignesis, proliferation, apoptosis and metabolism (Aumiller and Forstemann, 2008; Johnnidis et al., 2008; Zhou et al., 2010). Although their biologic functions are still unclear, and recent studies indicated that miRNAs have a role in tumor suppressors and oncogenes (Bartel et al., 2004; Ryan et al., 2010). A common single nucleotide polymorphism in premiRNA, rs3746444 in miR-499A>G, have been reported to be the biomarker of several cancers, such as breast cancer, gastric cancer and cervical squamous cell cancer (Okubo et al., 2010; Gao et al., 2011; Zhou et al., 2011; Srivastava et al., 2012). However, the association between rs3746444 in miR-499A>G polymorphism and risk of HCC are controversial (Xu et al., 2008; Zhou et al., 2011; Xiang et al., 2012). Therefore, the aim of our study was to confirm the association of rs3746444 in miR-499A>G with risk of HCC.

Materials and Methods

Search strategy and eligibility criteria

Between October 2008 and December 2011, a total of 185 HCC patients were included who were diagnosed by liver biopsy, or by the findings of at least two radiological tests of HCC including abdominal ultrasound, magnetic resonance imagine (MRI), hepatic angiography and contrast enhanced dynamic computed tomography, or by increased alpha-fetoprotein (AFP of ≥200 ug/ml). Patients who had secondary or recurrent tumors and a history of other malignant tumors were excluded. 203 cancer-free controls who took the physical examination center in the Tumor Hospital Affiliated to Zhengzhou University were selected, and controls who had clinical liver diseases were excluded.

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The serum hepatitis B surface antigen (HBsAg) and anti-HCV antibody were tested to determine the infection of hepatitis B or hepatitis C by microparticle enzyme immunoassay by commercial assay kits. All subjects were investigated with questionnaire to investigate the demographic characteristics, history of cancer and alcohol and tobacco consumption. The clinical characteristics were collected from medical records, including tumor differentiation, tumor size, metastasis, cirrhosis, child-pugh class, chemotherapy and surgery from medical records.

DNA collection and genotyping

To determine the association between common potentially functional miR-499A>G polymorphisms and HCC risk, we genotyped miR-499A>G. DNA was extracted from buffy-coat fractions with a TIANamp blood DNA kit provided by Tiangen Biotech (Beijing, China). Duplex polymerase-chain-reaction with the confronting-two-pair primer (PCR-RFLP) analysis was performed to determine the genotype of polymorphism of miR-499A>G. PCR of miR-499A>G polymorphism was performed using the following primers to amplify a 146-bp fragment: forward 5'-CAA AGT CTT CACTTC CCT GCC A-3' and reverse 5'-GAT GTT TAA CTC CTC TCC ACG TGATC-3'. The following cycling conditions were used: 95 °C for 5 min, followed by 30 cycles of 94 °C for 60 s, 62 °C for 60 s and 72 °C for 60 s, with a final extension at 72 °C for 10 min.

Statistically analysis

Continuous variables were presented as mean ± SD and analyzed using independent sample t-test. Categorical variables were presented as n of subjects (%) and analyzed using χ^2-test. The Hardy-Weinberg equilibrium and between-group comparison of genotype distribution were analyzed using χ^2-test. Odds ratios (OR) and their corresponding 95% confidence intervals (CI) were used to assess the association of polymorphism of miR-499A>G with the risk of HCC. All statistical analyses were performed using SPSS® version 11.0 (SPSS Inc., Chicago, IL, USA) for Windows®.

Results

The study included 185 HCC patients (115 males/70 females; mean age 58.7±8.1 years) and 203 healthy control subjects (136 males/67 females; mean age 57.6±8.8 years). Baseline characteristics of the study population are shown in Table 1. Patients with HCC were significantly more likely to be drinker, have family history of cancer, HBsAg

Table 1. Selected Characteristics of Cases and Controls

Characteristics	Case N=185	%	Control N=203	%	*P*-value
Age (mean±SD), years	58.7±8.1		57.6±8.8		0.1
Gender					
Male	115	62.3	136	67.1	0.32
Female	70	37.7	67	32.9	
Smoking status, %					
Smokers	49	26.6	46	22.8	0.38
Non-smokers	136	73.4	157	77.2	
Drinking status, %					
Drinkers	63	33.8	50	24.5	0.04
Non-drinkers	122	66.2	153	75.5	
Family history of cancer, %					
Yes	14	7.8	2	0.98	0.001
No	171	92.2	201	99.02	
HBsAg, %					
+	73	39.4	18	8.9	<0.001
-	112	60.6	185	91.1	
Anti-HCV, %					
+	12	6.7	1	0.4	0.001
-	173	93.3	202	99.6	

Table 2. Distribution of miR-499A>G Genotypes in Cases and Controls

miR-499A>G rs3746444	MAFs		Cases	%	Controls	%	*P* value	*P* for HWE
	In dbSNP	In controls						in controls
AA	0.1809	0.1895	136	73.7	139	68.3	0.19	0.06
AG			44	23.6	52	25.5		
GG			5	2.7	13	6.2		

Table 3. Adjusted OR (95% CI) of miR-499A>G Polymorphisms

miR-499A>G rs3746444	Controls	HCC patients	OR(95% CI)[1]	*P* value	HBV patients	OR(95% CI)[1]	*P* value
Allele frequency							
A	171	180	-		68	-	
G	62	49	0.73(0.45-1.13)	0.18	17	0.65(0.34-1.25)	0.22
General genotype							
AA	123	136	-		54	-	-
AG	48	44	0.76(0.36-1.14)	0.36	14	0.65(0.28-1.24)	0.23
GG	14	5	0.32(0.08-0.98)	0.02	3	0.43(0.09-1.85)	0.26
Dominant genotype							
AA	123	128	-		54	-	
AG+GG	62	49	0.73(0.46-1.16)	0.22	17	0.09(0.02-0.29)	<0.001
Recessive genotype							
AA+AG	171	180	-		68	-	
GG	14	7	0.45(0.14-1.22)	0.09	3	0.62(0.31-1.21)	0.13

[1]Adjusted for sex, age, smoking status, drinking status and family history of cancer

and Anti-HCV ($P < 0.05$ for all comparisons; Table 1).

The frequency distribution of miR-499A>G rs3746444 was shown in Table 2. In control subjects, the minor allele frequencies (MAFs) were consistent with published MAFs (available at http://www.ncbi.nlm.nih.gov/snp/), and in Hardy–Weinberg equilibrium, which suggested no population stratification and sample bias. We did not find significant difference in the frequency distributions of different genotypes of miR-499A>G between cases and controls.

We further analyzed the effects of the tested genotypes under different genetic models (Table 3). Using the miR-499 AA genotype as the reference genotype, GG was significantly decreased risk of HCC (adjusted OR=0.74, 95%CI=0.24-0.96). Similarly, GG genotype showed 0.45-fold decreased HCC risk in a recessive model. In the stratified analyses, we found G allele was significantly associated with decreased risk of HCC among patients infected with HBV in a dominant model (OR=0.09, 95%CI= 0.02-0.29).

Discussion

Genetic polymorphisms may be involved in multistage of hepatocarcinogenesis, and play a role in susceptibility to the development of HCC (Akkiz et al., 2011). Identification of genetic polymorphisms could clarify the pathophysiologic mechanism of carcinogenesis. Based on the genetic information, we determine the disease etiology in terms of genetic determinants to be used for identifying the high-risk individuals and perform targeting therapy to the individual's genetic make-up. In our study, we carried out a case-control study to investigate the association of miR-499A>G polymorphism and HCC risk.

MiRNAs are short nucleotide RNAs that may influence mRNA stability and translation (Kloosterman et al., 2006). Previous experimental studies have indicated that miRNAs can act as either tumor suppressors or oncogenes for cancers (Kloosterman and Plasterk, 2006; Osada and Takahashi, 2007; Stefani and Slack, 2008), and are related to several diseases, such as heart disease, neurological disorders (Dimmeler and Nicotera, 2013; Ouyang et al., 2013). SNPs are the most common source of genetic polymorphism in the human genome. The miR-499A>G rs3746444 located in its corresponding 3p mature miRNAs regions may influence both the bind-ing of 3p mature miRNAs to target mRNAs and pre-miRNA maturation of 5p and 3p miRNAs (Hu et al., 2009). Previous experimental study has indicated MiRNA-499 plays an important role in tumor biology and progression and various cancers (Liu et al., 2010; Srivastava et al., 2010). However, the results are controversial. A previous meta-analysis did not find significant association between the polymorphism of miR-499A>G rs3746444 and cancer risk (Qiu et al., 2012). The inconsistence of the results may be induced by different selection criteria of controls, ethnic variation, sample size and selection bias.

Three studies reported the association between miR-499A>G rs3746444 polymorphisms and HCC risk (Akkiz et al., 2011; Zhou et al., 2011; Xiang et al., 2012). However, the results of these studies are inconsistent. Xiang reported hsa-mir-499 polymorphism was associated with susceptibility to HBV-related HCC in Chinese population (Xiang et al., 2012), while two study conducted in China and Turkey did not find a significant association between miR-499A>G rs3746444 polymorphisms and HCC risk (Akkiz et al., 2011; Zhou et al., 2011). Our study found the miR-499A>G rs3746444 polymorphisms may alter the expression of mature miRNAs or their activities to target mRNA, and thus reduce cancer risk by variable mechanisms. Further large sample studies are still warranted to verify their association.

The current study has several limitations. First, the study was conducted in a single hospital in China, and may not be representative of China as a whole. Secondly, HCC is induced by multiple genes and environmental factors, which should be considered in further studies.

In conclusion, the present study shows that miR-499A>G rs3746444 polymorphism is associated with HCC risk in Chinese population. Further large-scale studies are required to elucidate whether miR-499A>G rs3746444 interact with environmental factors in the development of HCC.

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