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Association of miR-149C>T and miR-499A>G polymorphisms with the risk of hepatocellular carcinoma in the Chinese population

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ABSTRACT. We investigate the potential association of miR-149C>T and miR-499A>G polymorphisms and the risk of hepatocellular carcinoma (HCC). A matched case-control study of 152 cases and 304 controls were conducted. The miR-149C>T and miR-499A>G genotypes were analyzed using duplex polymerase chain reaction with restricted fragment length polymorphism. HCC patients were more likely to be smokers and drinkers, have hepatitis B and C virus infections, and a family history of cancer. The miR-149 CC genotype was associated with a reduced risk of HCC, while the miR-499 GG genotype was associated with an increased risk of HCC. However, we did not find that the miR-149 CC and miR-499 GG genotypes were associated with risk of HCC, and no interaction was found between miR-149C>T and miR-499A>G polymorphisms and hepatitis B virus infection. In conclusion, the miRNA-149C>T and miR-499A>G polymorphisms were found to play an important role for HCC risk in China. This finding could be useful in identifying people at high risk for the disease for early intervention.

Key words: MicroRNA; Polymorphisms; Hepatocellular carcinoma

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors in China, and it is estimated that there were 292,966 new cases diagnosed and 266,830 deaths annually (IARC, 2008). Hepatitis B (HBV) infection is an important reason of HCC, and a national survey reported roughly 7.18% of serum hepatitis B surface antigen (HBsAg) carriers in the general population in China (Liang et al., 2009; Li et al., 2013). But only a fraction of infected patients develop HCC during their lifetime, which suggests that some genetic factors may play a role in tumor development.

MicroRNAs (miRNAs) are small non-coding single-stranded RNA molecules of approximately 22 nucleotides that regulate target genes, and have been involved in a wide range of biochemical pathways in the cells of various eukaryotic organisms (Lim et al., 2005; Wilfred et al., 2007). It was reported that two common miRNA, miR-149C>T and miR-499A>G, are associated with various cancers, such as esophageal cancer, cervical cancer and prostate cancer (He et al., 2012). Moreover, a recent meta-analysis reported that the miR-149C>T polymorphism is associated with susceptibility to hepatitis B virus-related HCC in the Chinese population (Xu et al., 2013), and two case-control studies indicated that miR-499A>G variants were associated with susceptibility to HCC (Xiang et al., 2012; Hu et al., 2013). However, few studies have evaluated the interaction between miR-149C>T and miR-499A>G and HBV infection.

Therefore, we conducted a case-control study to investigate the association of polymorphisms in miR-149C>T and miR-499A>G with susceptibility to HCC in a Chinese population.

MATERIAL AND METHODS

Study population

From March 2010 to December 2011, a total of 152 cases with HCC diagnosed at Huashan Hospital of Fudan University were enrolled in the study. All the patients were newly diagnosed and confirmed by histopathological examination. Patients with a history of cancer were excluded. The control group consisted of 304 individuals who were randomly selected from individuals who came to our hospital for regular health check-up, excluding those with a history of cancer and a normal liver function. The clinical stage of HCC cases was evaluated on the basis of the tumor-necrosis-metastasis (TNM) classification system. The HBsAg and anti-hepatitis C virus (HCV) antibody were tested by microparticle enzyme immunoassays using commercial assay kits, which were used to determine the infection status of hepatitis B or hepatitis C. The demographic characteristics and clinical characteristics of subjects were collected from medical records. Our study was approved by the Medical Ethics Committee of Huashan Hospital of Fudan University, and informed consent was obtained from all participants.

DNA extraction and genotyping

The participants were asked to provide 5 mL peripheral blood samples, which were stored at -20°C. Genomic DNA was extracted from peripheral blood samples, purified using high-salt buffer methods, and diluted to 100 ng/μL with 1X Tris-ethylenediaminetetraacetic

acid buffer. The miR-149C>T and miR-499A>G genotypes were analyzed using duplex polymerase chain reaction with restriction fragment length polymorphism (PCR-RFLP). The two regions were amplified using the following PCR conditions: 95°C for 5 min, followed by 30 cycles of 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s and annealing at 64°C for 30 s, with a final extension at 72°C for 10 min. Primers and probes of the ten SNPs for PCR amplification were designed by the Sequenom® Assay Design 3.1 software. Each PCR (20 µL) contained 200 ng DNA template, 200 µM dNTP, 1 U Taq DNA polymerase, and 200 µM primers, as well as 1.5 mM $MgCl_2$. PCR for the miR-149C>T polymorphism was performed to amplify the 263-bp product with the primers forward: 5'-CTG GCT CCG TGT CTT CAC TC-3' and reverse: 5'-TGA GGC CCG AAA CAC CCG TA-3'; PCR for the miR-499A>G polymorphism 146-bp product was performed using the primers forward: 5'-CAA AGT CTT CAC TTC CCT GCC A-3' and reverse: 5'-GAT GTT TAA CTC CTC TCC ACG TGA TC-3'. Ten percent of cases and controls were randomly selected to repeat analysis to confirm consistency, and the consistency rate was 100%.

Statistical analysis

All statistical analyses were performed using the Statistical Package for Social Science software version 16.0 (SPSS Inc., Chicago, IL, USA). Continuous variables are reported as means ± standard deviation (SD), and categorical variables are reported as frequencies (N) and percentages (%). The χ^2 test was used to assess differences between cases and controls with regard to demographic characteristics. A goodness-of-fit χ^2 test was used to evaluate the Hardy-Weinberg equilibriums in controls. Conditional logistic regression was performed to analyze the association between miR-149C>T and miR-499A>G polymorphisms and risk of HCC, which were estimated by odds ratios (ORs) and their 95% confidence intervals (95%CIs). The significance levels of all tests were set at $P < 0.05$.

RESULTS

The demographic and clinical characteristics of HCC cases and controls are shown in Table 1. The mean age of patients and controls were 53.5 ± 9.4 and 53.0 ± 11.5 years, respectively. Patients with HCC were more likely to be smokers and drinkers, have hepatitis B and C virus infections, and a family history of cancer. With respect to clinical characteristics, 66.4% of the patients were at TNM stage III-IV, and 33.6% were at I-II stage.

The genotype frequencies of miR-149C>T and miR-499A>G polymorphisms between HCC patients and controls are shown in Table 2. The genotype distributions of miR-149C>T in controls were in accordance with Hardy-Weinberg equilibrium, and the P value was 0.29. However, the miR-499A>G genotype distribution in controls was not in line with Hardy-Weinberg equilibrium, and the P value was less than 0.001. The miR-149 CC genotype was associated with a reduced risk of HCC (adjusted OR = 0.43, 95%CI = 0.21-0.92), while the miR-499 GG genotype was associated with an increased risk of HCC (adjusted OR = 2.15, 95%CI = 1.28-4.17). For HCC patients with hepatitis B virus infection, we did not find that the miR-149 CC and miR-499 GG genotypes were associated with risk of HCC, and no interaction was found between miR-149C>T and miR-499A>G polymorphisms and hepatitis B virus infection.

Table 1. Characteristics in hepatocellular carcinoma (HCC) patients and control subjects.

Variables	Cases (N = 152)	%	Controls (N = 304)	%	χ^2 or t	P value
Age (years, mean ± SD)	53.5 ± 9.4		53.0 ± 11.5		0.46	0.32
Gender						
Female	113	74.3	226	74.3		
Male	38	25.0	78	25.7	0.01	0.91
Smoking						
No	93	61.2	208	68.4		
Yes	59	38.8	96	31.6	2.37	0.12
Drinking						
No	74	48.7	203	66.8		
Yes	78	51.3	101	33.2	13.91	<0.001
Hepatitis B						
No	63	41.4	283	93.1		
Yes	89	58.6	21	6.9	147.7	<0.001
Hepatitis C						
No	139	91.4	301	99.0		
Yes	13	8.6	3	1.0	17.13	<0.001
Family history of cancer						
No	165	92.2	367	99.7		
Yes	14	7.8	1	0.3	25.7	<0.05
TNM stage						
I-II	51	33.6				
III-IV	101	66.4				

Table 2. Comparison of genotype frequencies and odds ratios (ORs) of four miRNA polymorphisms between cases and controls.

Genotype	Controls	%	HCC patients	%	Crude OR (95%CI)	Adjusted OR (95%CI)[1]	HCC cases with HBV infection	%	Crude OR (95%CI)	Adjusted OR (95%CI)[1]
miR-149C>T										
TT	113	37.2	67	44.1	1.0 (Ref.)	1.0 (Ref.)	40	44.9	1.0 (Ref.)	1.0 (Ref.)
CT	148	48.7	72	47.4	0.83 (0.54-1.29)	0.78 (0.50-1.03)	42	47.2	0.80 (0.47-1.36)	0.74 (0.41-1.24)
CC	43	14.1	13	8.6	0.48 (0.24-0.98)	0.43 (0.21-0.92)	7	7.9	0.46 (0.16-1.15)	0.38 (0.14-1.05)
C allele	191	62.8	85	55.9	0.76 (0.50-1.16)	0.74 (0.47-1.12)	49	55.1	0.72 (0.44-1.21)	0.69 (0.41-1.16)
miR-499A>G										
AA	218	71.7	98	64.5	1.0 (Ref.)	1.0 (Ref.)	59	66.3	1.0 (Ref.)	1.0 (Ref.)
AG	62	20.4	32	21.1	1.15 (0.68-1.92)	1.19 (0.72-2.15)	18	20.2	1.07 (0.55-2.01)	1.10 (0.58-2.08)
GG	24	7.9	22	14.5	2.04 (1.03-3.99)	2.15 (1.28-4.17)	12	13.5	1.85 (0.79-4.11)	1.93 (0.84-4.25)
G allele	86	28.3	54	35.5	1.40 (0.91-2.16)	1.49 (0.95-2.24)	30	33.7	1.29 (0.75-2.19)	1.34 (0.81-2.25)

[1]Adjusted for smoking and drinking status, hepatitis B and C virus infections and a family history of cancer.

DISCUSSION

We aimed to identify miRNAs that contribute to the risk of HCC in order to better predict individual risk and understand the pathogenesis of the disease. Only few studies have examined the association between miRNA-149C>T and miR-499A>G polymorphisms and HCC risk (Kim et al., 2012; Zou and Zhao, 2013). Zou and Zhao (2013) reported that miR-499A>G polymorphisms are associated with increased risk of HCC. Kim et al. (2012) reported that miR-149C>T and miR-499A>G are associated with HBV-related HCC. In the present study, we found that the miRNA-149C>T and miR-499A>G polymorphisms were associated with risk of HCC, whereas miRNA-149C>T and miR-499A>G had no interaction with hepatitis B virus infection.

Our study is the first to demonstrate that the miRNA-149C>T polymorphism is associated with the risk of HCC. MiR-149C>T polymorphisms have been widely investigated in various cancers, such as gastric cancer, lung cancer, colorectal cancer, nasopharyngeal carcinoma, and breast cancer (Luo et al., 2012; Wang et al., 2012; Øster et al., 2013; Wilting et al., 2013; Zhang et al., 2013). Two studies reported that miRNA-149C>T polymorphisms are associated with the development of HCC (Liu et al., 2010; Kim et al., 2012). Liu et al. (2010) used a comprehensive microarray analysis in human hepatoma cells, and reported that miR-149 is involved in HCV entry, replication, and propagation. Kim et al. (2012) conducted a study in Korea demonstrating that miR-149C>T polymorphisms are associated with decreased risk of HCC. Our study also indicated a significant association with HCC, which is in line with these previous studies. However, one study conducted in China indicated that miR-149 could not affect the cell cycle, but did inhibit cell proliferation (Cao et al., 2012). Genetic frequencies of specific genes are variable in different populations. Therefore, further studies are greatly warranted to confirm the association between miRNA-149C>T polymorphisms and HCC.

In our study, the miR-499A>G polymorphism was associated with risk of HCC. Several previous studies indicated that the miR-499A>G polymorphism was associated with susceptibility to hepatocellular carcinogenesis in various populations (Kim et al., 2012; Xiang et al., 2012; Zou and Zhao, 2013; Hu et al., 2013). However, a study conducted in Turkish population reported that the MiR-499A>G polymorphism did not have any major role in genetic susceptibility to hepatocellular carcinogenesis (Akkiz et al., 2011). A recent meta-analysis reported no significant association between miR-499A>G polymorphism and susceptibility to HCC (Wang et al., 2013), which was inconsistent with the results of our study. The inconsistency of findings on that association may be explained by differences in population background, source of control subjects, and sample size. Therefore, further large sample and multicenter studies are greatly needed to confirm the association between SNPs in miRNA and risk of HCC.

In conclusion, the miRNA-149C>T and miR-499A>G polymorphisms were found to play an important role for HCC risk in China, and no interaction was found between the miRNA-149C>T and miR-499A>G polymorphism and hepatitis B virus infection. SNPs in miRNA sequences can be used as a diagnostic biomarker for HCC. Further large sample studies are warranted to confirm the role of SNPs in miRNA sequences in the development of HCC.

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