Association Between Two Genetic Variants in miRNA and Primary Liver Cancer Risk in the Chinese Population

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MicroRNAs (miRNAs) play an important role in the growth and development of human beings. Single nucleotide polymorphisms (SNPs) within miRNA could change their production or affinity with target genes, thus leading to malignant diseases. This case-control study conducted in Western China aimed to explore the relationship between polymorphisms in *miR-146a* (rs2910164 G>C) and *miR-499* (rs3746444 T>C) and primary liver cancers in the Chinese population. 186 primary liver cancer cases and 483 healthy controls were genotyped using polymerase chain reaction–restriction fragment length polymorphism. No significant differences were observed between distributions of the two SNPs and susceptibility of primary liver cancer or diverse clinicopathologic features. However, we found that patients with genotype CG of the SNP in *miR-146a* tended to have earlier onset and better liver function than patients with genotype CC (average age: 49.9 vs. 54.9, $p=0.038$; average Child-Pugh grade: 5.55 vs. 6.15, $p=0.021$), and further analysis showed that patients who had at least one G allele were diagnosed at an earlier age (average age: 49.6 vs. 54.9, $p=0.022$) and had better liver function (average Child-Pugh grade: 5.60 vs. 6.15, $p=0.026$). Our data suggested lack of association between the two SNPs and primary liver cancer risk, though, interestingly, the *miR-146a* SNP may influence the age of onset and Child-Pugh grade.

Introduction

PRIMARY LIVER CANCER IS the sixth most common cancer in the world (748,300 new cases per year), and the third most common cause of cancer deaths (695,900 deaths per year) (Jemal *et al.*, 2011). It is prevalent in Middle and Western Africa and in East and Southeast Asia, especially in China, where almost half of the cases and deaths occurred, owing to high prevalence of chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections (Yuen *et al.*, 2009). Epidemiological evidence suggested that the most dominant risk factor for liver cancer is chronic infection of HBV and HCV. Moreover, alcohol consumption and flavacin contamination of foodstuffs also could contribute to primary liver cancer (El-Serag *et al.*, 2007). Further, genetic alterations were also proven to be involved in the etiology and prognosis of primary liver cancer in recent years (Thorgeirsson and Grisham, 2002; Hagymási and Tulassay, 2008).

MicroRNAs (miRNAs) are small noncoding functional RNAs of ~22 nucleotides (Bartels and Tsongalis, 2009). They function as negative gene regulators (Ambros, 2004; Bartel, 2004), complementarily binding to hundreds of mRNA tar-

gets implicated in the regulation of almost every biological process (Ambros, 2003; Zamore and Haley, 2005). Alteration of miRNA expression was observed in initiation and progression of human cancers: *miR-15a* and *miR-16* in chronic lymphocytic leukemia (Calin *et al.*, 2008); *miR-145* in colon carcinoma, lung, breast, and prostate (Iorio *et al.*, 2005; Akao *et al.*, 2006, 2007; Bandrés *et al.*, 2006; Yanaihara *et al.*, 2006); and *miR-21* in glioblastoma (Chan *et al.*, 2005). In the pathogenesis of primary liver cancer, many miRNAs were found to be involved, for example, *let-7*, *miR-1*, *miR-101*, *miR-122* and *miR-124* were downregulated while *miR-17-5p*, *miR-106b-25*, *miR-143*, and *miR151* were upregulated, and they targeted genes such as *c-Myc*, *HDAC4*, *p21*, and *Cyclin G1* (Huang and He, 2011). Single nucleotide polymorphisms (SNPs), the most abundant form of DNA variation in the human genome, may have significant effects on miRNA generation. Sequence variations within miRNA can alter the secondary structure of the miRNA precursor, thus disturbing miRNA maturation (Xu *et al.*, 2008) and, consequently, changing the production output or the binding to targets, and finally altering protein expression, contributing to cancer susceptibility (Bartels and Tsongalis, 2009). To date, many

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studies have indicated that SNPs in pre-*miRNA* or *miRNA* could increase the risk of human diseases and lead to phenotypic differences (Bartels and Tsongalis, 2009; Hu *et al.*, 2009; Peng *et al.*, 2009), and thus were expected to be used for cancer diagnosis, staging, and prognosis (Ludwig and Weinstein, 2005).

We hypothesized that the SNPs in *miR-146a* (rs2910164 G>C) and *miR-499* (rs3746444 T>C) are associated with primary liver cancer. To test this hypothesis, we genotyped the polymorphisms and assessed their relationship in the present case-control study (186 primary liver cancer cases and 483 healthy controls) in the Chinese population.

Materials and Methods

Study population

669 unrelated subjects who visited West China Hospital of Sichuan University, including 186 patients with primary liver cancer and 483 healthy individuals undergoing routine medical examination without any medical illness, were enrolled in this study between December 2008 and April 2010. All the patients were diagnosed either by histopathologic or imaging evidence. Informed consent for sample collection and subsequent analysis was obtained from each subject at recruitment. This study was approved by West China Hospital.

Genotyping assay of SNPs within *miRNA*

Genomic DNA was extracted from peripheral blood using Chelex-100, then genotyped using polymerase chain reaction (PCR)-restriction fragment length polymorphism, and confirmed by DNA sequencing analysis. The PCR mixture consisted of 5 pmol of each primer described previously (TAKARA) (Hu *et al.*, 2008), 1.5 mM MgCl₂, 200 μ M dNTP Mixture (Promega), 0.7 U *Taq* DNA Polymerase (Promega), 1 $\times$ PCR buffer, and 2.0 μ L exacted DNA; finally, the total volume of 25 μ L was reached by adding deionized water. Then, it was initially heated at 94°C for 8 min to activate the polymerase, and DNA amplification was achieved by 40 cycles of (denaturing in 94°C for 30 s, annealing for 30 s, and prolonging in 72°C for 30 s) in a PCR Amplifier (ABI 9700 cycler), the annealing temperature was 60°C for rs2910164 and 62°C for rs3746444. The final elongation step was maintaining 72°C for 8 min. The next step was digestion by restriction enzyme; it was performed with 2 μ L PCR product. For rs2910164, PCR products were digested overnight with 5 U *SacI* (MBI Fermentas) in a final volume of 7.5 μ L at 37°C, whereas for rs3746444, PCR products were digested with 5 U *BclII* (MBI Fermentas) in a final volume of 8 μ L at 65°C. Genotype calling was made by 3% agarose gel electrophoresis at 150 V for 1 h and photographing under ultraviolet light with Gel Doc (Bio-Rad). 600 ng PCR products were randomly selected for DNA sequencing (ABI 3130).

Liver function and tumor marker tests

Electrochemiluminescence was used for the quantitative determination of human serum alpha fetoprotein (AFP) by ROCHE-E170 (Roche). Alanine aminotransferase (ALT), glutamic-oxalacetic transaminase (AST), glutamyl transpeptidase (GGT), total bilirubin (TB) and serum albumin (ALB) were all tested by automatic biochemical analyzer ROCHE-P800 (Roche). Quantitation of HBV-DNA was performed

using real-time fluorescence quota PCR by ROCHE Light Cycler 480 (Roche). The quality control (Bio-Rad) and result audit were done by an experienced medical technologist. All assays were performed in the department of laboratory medicine in west China hospital of Sichuan University, which is a College of American Pathologists-certified lab.

Statistical analysis

Genotype frequencies were assessed for Hardy-Weinberg equilibrium in the case and control groups. Dualvariate unconditional logistic regression analysis was performed to investigate the relationship between the risk of primary liver cancer and the two SNPs by adjusting for gender and age. Genotype and allele frequencies were compared for the two SNPs in patients versus controls using Chi-square test. Odds ratios (ORs) and 95% confidence intervals for the risk of primary liver cancer were also calculated simultaneously. Further, in a subsequent analysis of the association between the polymorphisms and clinical characteristics, Chi-square test was used when the data were qualitative, such as the presence of cancer embolus and satellite, single or multifocal, or differentiation degree. If the data were quantitative, analysis of variance was carried out when the data had homogeneity of variance, whereas nonparametric test was performed when the data had heterogeneity of variance or non-normal distributions. All statistical analyses were performed using SPSS version 17.0 software. A $p < 0.05$ was considered significantly different, and all statistical tests were two sided.

Results

Baseline characteristics of patients with primary liver cancer

This study involved 186 primary liver cancer patients and 483 healthy controls matched with patients by age and gender from Western China. Demographic and other baseline characteristics of patients with primary liver cancer are summarized in Table 1.

Distribution of genotype and allele frequencies and their association with primary liver cancer susceptibility

669 samples were genotyped from 186 patients with primary liver cancer and 483 healthy individuals. Genotype distributions of the two SNPs in both case and control groups were all in accordance with the Hardy-Weinberg equilibrium (all $p > 0.05$), suggesting the genetic equilibrium of population enrolled (Table 2).

As shown in Table 2, the frequencies of the genotype CC, CG, and GG of rs2910164 were 36%, 46.2%, and 17.7% in the patient group, and 32.7%, 52.6%, and 14.7% in the control group, and the allele frequencies were nearly the same in patient (C=59.1%, G=40.9%) and control (C=59.0%, G=41.0%) groups. As to the rs3746444, the frequencies of the genotype CC, CT, and TT were 2.2%, 22.0%, and 75.8% and 2.48%, 20.7%, and 76.8% in the case and control groups, respectively. The allele frequencies were also similar in patient (C=13.2%, T=86.8%) and control groups (C=12.8%, T=87.2%). The two SNPs within *miR-146a* and *miR-499* showed no significant difference between primary liver cancer patients and healthy controls in either genotype

TABLE 1. BASELINE DATA OF PATIENTS WITH PRIMARY LIVER CANCER (N=186)

	Patients, n (%) or mean $\pm$ SD
Age of onset	52.10 $\pm$ 15.20
Sex	
Male	154 (82.8)
Female	32 (17.2)
Serum level of tumor markers	
ALT (IU/L)	59.59 $\pm$ 56.39
AST (IU/L)	74.16 $\pm$ 59.72
GGT (IU/L)	199.33 $\pm$ 233.46
AFP (ng/mL)	727.9 (10.98–1210)
TB (μ M)	24.38 $\pm$ 30.48
Child-Pugh grade	5.83 $\pm$ 1.20
HBV-DNA (copies/mL)	9.810E03 (2.418E03–2.305E05)
Clinical pathological characteristics (n=49)	
Maximum diameter of tumor (n=41)	6.60 $\pm$ 3.20
Presense of satellite (n=34)	4 (8.2)
Presense of cancer embolus (n=37)	22 (44.9)
Number of lesions (n=40)	
Singlefocal	37 (75.5)
Multifocal	3 (6.1)
Histological differentiation (n=49)	
Well	5 (10.2)
Moderately	20 (40.8)
Poorly	24 (49.0)

For non-normal distributions, median (P₂₅–P₇₅) was used.
SD, standard deviation; TB, total bilirubin; HBV, hepatitis B virus; AFP, alpha fetoprotein.

($p=0.316$ and $p=0.907$, respectively) and allelic analysis ($p=0.964$ and $p=0.870$, respectively). With regard to the SNP rs2910164 G>C, OR of G versus C, CG versus GG, CC versus GG, and (CC+GG) versus GG were all extremely close to 1. As shown in Table 2, the conclusions for SNP rs3746444 T>C were the same. Our study had a power of 83.4% (rs2910164) and 96.33% (rs3746444) to detect a difference in the distribution of the SNPs within *miR-146a* and *miR-499* between case and controls.

To evaluate the association of primary liver cancer and the two SNPs without the interference of age and gender, dualvariate unconditional logistic regression analysis of the relationship between the two SNPs and the risk of primary liver cancer was performed adjusting for age and gender. In accordance with the Chi-square test, the distribution of the genotypes of the two SNPs showed no significant differences between cases and controls ($p=0.6\sim 0.8$).

Association between genotypes and phenotypes of primary liver cancer

AFP, HBsAg status, HBV-DNA, ALT, AST, GGT, and TB are all correlated with primary liver cancer and can reflect its progression and prognosis. Child-Pugh classification, developed in 1973, is a comprehensive assessment of liver function comprising quantitation of bilirubin, albumin, and prothrombin time, degree of ascites, and presence of hepatic encephalopathy, and it is widely used in prognosis of liver cancer. We investigated whether the mutations affected clinical features in 102 patients; the indicators above were obtained before treatment when the patients' disease was first diagnosed as primary liver cancer. In addition, we compared the age of onset among the genotypes of the two SNPs in all of the patients enrolled. No marker showed any

TABLE 2. GENOTYPE AND ALLELE DISTRIBUTION OF SINGLE-NUCLEOTIDE POLYMORPHISMS WITHIN *miR-146a* AND *miR-499* IN PRIMARY LIVER CANCER PATIENTS AND CONTROLS

Polymorphism	Patient, n (%)	Control, n (%)	χ^2	p	OR	95% CI
rs2910164 G>C						
Genotype						
CC	67 (36.0)	158 (32.7)	2.303	0.316		
CG	86 (46.2)	254 (52.6)				
GG	33 (17.7)	71 (14.7)				
CG versus GG			1.682	0.195	0.728	0.451–1.177
CC versus GG			0.128	0.720	0.912	0.552–1.507
Allele						
G	152 (40.9)	396 (41.0)	0.002	0.964	0.994	0.780–1.268
C	220 (59.1)	570 (59.0)				
rs3746444 T>C						
Genotype						
CC	4 (2.2)	12 (2.48)	0.195	0.907		
CT	41 (22.0)	100 (20.7)				
TT	141 (75.8)	371 (76.8)				
CT versus TT			0.130	0.718	1.079	0.715–1.629
CC versus TT			0.000	1.000	0.877	0.278–2.765
Allele						
C	49 (13.2)	124 (12.8)	0.027	0.870	1.030	0.722–1.469
T	323 (86.8)	842 (87.2)				

OR, odds ratio; CI, confidence interval.

TABLE 3. INDEXES CORRELATED WITH LIVER FUNCTION (N=102) AND AGE AT PRIMARY LIVER CANCER ONSET (N=186) WITH DIFFERENT GENOTYPES OF THE TWO SINGLE-NUCLEOTIDE POLYMORPHISMS WITHIN *miR-146a* AND *miR-499*

	rs2910164 G > C					rs3746444 T > C				
	CC (mean ± SD)	CG (mean ± SD)	GG (mean ± SD)	p-value	CC (mean ± SD)	CT (mean ± SD)	TT (mean ± SD)	p-value		
ALT (IU/L)	51.15 ± 27.55	70.85 ± 77.40	48.13 ± 27.38	0.815 ^a	101.7 ± 104.9	44.00 ± 24.81	61.49 ± 59.02	0.535 ^a		
AST (IU/L)	76.34 ± 55.72	76.57 ± 68.81	60.80 ± 37.58	0.648	86.33 ± 90.01	50.11 ± 26.78	79.05 ± 63.15	0.320 ^a		
GGT (IU/L)	180.4 ± 138.3	226.6 ± 290.5	167.4 ± 172.6	0.530	230.0 ± 202.1	144.1 ± 127.6	210.5 ± 240.2	0.511		
TB (μM)	20.07 ± 10.34	24.42 ± 31.21	36.03 ± 55.38	0.500 ^a	14.73 ± 9.616	17.73 ± 8.420	26.21 ± 33.74	0.489		
Child-Pugh grade	6.14 ± 1.35	5.54 ± 1.02	5.79 ± 1.12	0.069	5.500 ± 0.707	5.722 ± 1.018	5.861 ± 1.258	0.844		
AFP ^b (ng/mL)	441.2 (11.63–1210)	1048 (11.28–1210)	413.3 (3.590–1210)	0.577 ^a	389.7 (3.020–1210)	244.1 (13.11–1210)	977.7 (10.42–1210)	0.987 ^a		
HBV-DNA ^b (copies/mL)	9.810E03 (1.897E03–23628E05)	1.330E04 (2.435E03–2.405E05)	5.225E03 (1.470E03–6.995E05)	0.889 ^a	7.950E03 (0.000–0.590E04)	9.860E03 (5.990E03–5.380E05)	9.760E03 (2.380E03–2.390E05)	0.685 ^a		
Age of onset	54.89 ± 14.78	49.85 ± 14.38	49.00 ± 13.90	0.069	49.00 ± 19.90	51.03 ± 14.36	51.80 ± 14.62	0.901		

^aOwing to heterogeneity of variance or non-normal distributions, nonparametric test was used.^bOwing to non-normal distributions, median (Q_U–Q_L) was used to describe the distribution of AFP and HBV-DNA.

significant difference in the genotypes of the two SNPs (Table 3). Interestingly, we found that patients with genotype CG tended to had earlier onset and lower Child-Pugh grade, compared with CC (average age: 49.9 vs. 54.9, $p=0.038$; average Child-Pugh grade: 5.55 vs. 6.15, $p=0.021$). Further, when we compared genotype GG+CG with CC by analysis of variance, we found that patients who had at least one G allele were diagnosed at an earlier age (average age 49.6 vs. 54.9, $p=0.022$) and lower Child-Pugh grade (average Child-Pugh grade: 5.60 vs. 6.15, $p=0.026$; Table 4).

Association between genotypes and pathological features of primary liver cancer

A subset of 49 patients with hepatocellular cancer who underwent surgical resection also underwent a pathological examination at diagnosis. We analyzed the pathological features to reveal whether there was any relationship with the distribution of genotypes. The pathological features for comparison included cancer embolus, single or multifocal, differentiation, satellite (Chi-square test for all the previous indexes), and maximum diameter (nonparametric test: Kruskal–Wallis), which greatly affected prognosis. However, as shown in Table 5, no significant difference was found among the genotypes, suggesting lack of relationship of the two SNPs and the pathological characteristics.

Discussion

Primary liver cancer is a deadly cancer whose incidence is increasing worldwide; there is a growing understanding of the molecular mechanism of hepatocarcinogenesis, mainly the role of oncogenes (Zender *et al.*, 2010). In recent years, noncoding small RNAs, miRNAs, have been shown to participate in tumorigenesis, such as inflammation, cell cycle regulation, differentiation, apoptosis, and invasion (Farazi *et al.*, 2011). For instance, down-regulated *let-7* was found in nonsmall cell lung cancer patients (Johnson *et al.*, 2007) and was associated with poor prognosis (Yanaiharu *et al.*, 2006), whereas highly expressed *miR-21*, which was frequently in solid and hematological malignancies, resulted in a pre-B malignant lymphoid-like phenotype, and *miR-21* inactivation led to apoptosis and tumor regression (Medina *et al.*, 2010). Previous studies have demonstrated that alteration of *miR-146a* expression was found in various malignances, such as PTC, breast cancer, and pancreatic cancer (He *et al.*, 2005; Pallante *et al.*, 2006; Volinia *et al.*, 2006; Li *et al.*, 2010b). SNPs within miRNA could significantly change its binding and post-transcriptional regulation (Cai *et al.*, 2009) and

TABLE 4. AGE OF ONSET AND IMPROVED CHILD-PUGH GRADE WITH GENOTYPES OF *miR-146a* SINGLE-NUCLEOTIDE POLYMORPHISM

Genotype	Age of onset (n=186)		Improved Child-Pugh grade (n=102)	
	Mean ± SD	p-value	Mean ± SD	p-value
CG	49.85 ± 14.38	0.038 ^a	5.54 ± 1.02	0.021 ^a
CC	54.89 ± 14.78	0.022 ^b	6.14 ± 1.35	0.026 ^b
CG+GG	49.64 ± 14.20		5.60 ± 1.04	

^aCG versus CC.^bCG+GG versus CC.

TABLE 5. CLINICALPATHOLOGICAL CHARACTERISTICS AND THE GENOTYPES OF TWO SINGLE-NUCLEOTIDE POLYMORPHISMS WITHIN *miR-146A* AND *miR-499* IN 49 PRIMARY LIVER CANCER PATIENTS

	rs2910164G>C (n (%))			p-value	rs3746444T>C (n (%))			p-value
	CC	CG	GG		CC	CT	TT	
Cancer embolus								
Presence	6 (27.3)	12 (54.5)	4 (18.2)	0.990	0 (0.00)	6 (27.3)	16 (72.7)	0.297
Absence	4 (26.7)	8 (53.3)	3 (20.0)		1 (6.67)	6 (40.0)	8 (53.3)	
Single or multifocal								
Single	10 (27.2)	20 (54.1)	7 (18.9)	0.758	1 (2.70)	10 (27.0)	26 (70.3)	0.939
Multifocal	1 (33.3)	1 (33.3)	1 (33.3)		0 (0.00)	1 (33.3)	2 (66.7)	
Differentiation								
Poorly	7 (29.2)	15 (62.5)	2 (0.02)	0.401	0 (0.00)	7 (29.2)	17 (70.83)	0.788
Oderately	6 (30.0)	8 (40.0)	6 (30.0)		1 (5.00)	6 (30.0)	13 (65.0)	
Well	1 (20.0)	3 (60.0)	1 (20.0)		0 (0.00)	2 (40.0)	3 (60.0)	
Satellite								
Presence	2 (50.0)	2 (50.0)	0 (0.00)	0.482	0 (0.00)	2 (50.0)	2 (50.0)	0.508
Absence	8 (26.7)	16 (53.3)	6 (20.0)		1 (3.33)	7 (23.3)	22 (73.3)	
Maximum diameter of tumor (mean±SD)	3.31±1.00	2.65±0.55	4.11±1.55	0.121	2.50	7.68±3.73	6.28±2.87	0.194

contribute to carcinogenesis. A SNP within *miR-196a2* was reported to be associated with susceptibility to hepatocellular carcinoma with cirrhosis (Li *et al.*, 2010a) and chronic HBV infection (Qi *et al.*, 2010) in Chinese population. Xu *et al.* (2011) found that a SNP in the promoter region of *miR-34b/c* contributed to primary hepatocellular carcinoma risk. We hypothesized that primary liver cancer progression is also affected by miRNA SNPs. Reports have demonstrated that SNPs within *miR-146a* and *miR-499* played an important role in carcinogenesis. Zeng *et al.* (2010) reported that subjects with the variant genotypes (GC+GG) of *miR-146a* SNP had a 58% increased risk of gastric cancer (OR=1.58), compared with CC genotype carriers. Pastrello *et al.* (2010) found that *BRCA1/BRCA2*-negative familial breast and ovarian cancer patients with C allele of *miR-146a* SNP had earlier onset. *miR-499* may play important roles in cardiac differentiation of human embryonic stem cells (Wilson *et al.*, 2010) and regulating mitochondrial fission machinery and apoptosis (Wang *et al.*, 2011). Variation of *miR-499* is associated with increased risk of dilated cardiomyopathy and breast cancer (Hu *et al.*, 2009; Zhou *et al.*, 2010). Variants of *miR-146a* and *miR-499* may be associated with primary liver cancer, which were assessed in this study.

In the present study, we genotyped *miR-146a* SNP (rs2910164 G>C) and *miR-499* SNP (rs3746444 T>C) in 186 primary liver cancer patients and 483 healthy controls in a Chinese population. Our results suggested that genetic polymorphism of *miR-146a* is associated with age of onset and Child-Pugh grade in primary liver cancer patients. The genotype GC may contribute to early onset of primary liver cancer and relatively good liver function. Further analysis indicated that patients with at least one G allele had earlier onset and better liver function. However, this variant seemed to lack association with risk of primary liver cancer and the pathological characteristics. Similarly, no association was found between the SNP *miR-499* and the risk of primary liver cancer and the clinicopathologic outcome, and, moreover, the age of onset and liver function.

As alteration of miRNAs could lead to changes of protein expression by complementarily binding to protein-coding

RNAs, polymorphisms of miRNAs, which can alter the expression and the sequence of miRNAs, are likely to make a significant contribution to phenotypic variation (Georges *et al.*, 2007). Reports showed that G to C variation in the *miR-146a* precursor could reduce production of mature miRNA (Jazdzewski *et al.*, 2008; Xu *et al.*, 2008). The variant was located in the middle of the stem hairpin, leading to a change from C: U pair (WT) to G: U (MT) mismatch (www.patrocles.org), thus making the secondary structure unstable and thereby altering the expression of mature *miR-146a*, as reported in previous studies (Zeng and Cullen, 2003; Duan *et al.*, 2007). The G allele of the precursor of *miR-146a* produced more mature *miR-146a* in hepatocellular carcinoma and PTC (Jazdzewski *et al.*, 2008; Xu *et al.*, 2008), but less mature *miR-146a* in familial breast/ovarian cancer (Shen *et al.*, 2008). Jazdzewski *et al.* (2009) reported that the genotype GC produced two additional mature miRNA from the passenger strands (*miR-146a**G and *miR-146a**C) of miRNA precursor, which could target different sets of genes. Further, upregulation of *miR-146a* could result in dramatic loss of Kit transcript and protein, a key tumor suppressor in the thyroid carcinogenesis pathway (He *et al.*, 2005). *miR-146a* can target TRAF6 and IRAK1, which are key adapter molecules downstream of the Toll-like and cytokine receptors in the signaling pathway that plays a crucial role in cell growth and immune recognition (Jazdzewski *et al.*, 2008). Xu *et al.* (2008) demonstrated that *miR-146a* can promote cell proliferation as well as colony formation. Therefore, we hypothesize that the variation of *miR-146a* affected the age at primary liver cancer onset and liver function via target selection and/or alteration of the expression of miRNA. However, the association between *miR-146a* SNP and the age of onset and liver function in primary liver cancer may also be coincidental in our study. The number of subjects analyzed was relatively small; replicate studies and further research are required to verify our conclusion.

In addition, our results indicated no significant difference between the distribution of genotypes and alleles of *miR-146a* SNP and the risk of primary liver cancer, in contrast to Xu *et al.*'s (2008) report, which showed that the genotype of *miR-*

146a SNP in HCC (hepatocellular carcinoma) cases was significantly different from that in control subjects ($p=0.026$). More than 90% primary liver cancer is hepatocellular carcinoma; the lack of consistency may be due to the cases we enrolled and differences in samples size and population-specific factors. They enrolled 479 HCC patients and 504 cancer-free controls who lived in Southern China, whereas the population we enrolled lived in Western China, which is a multi-nationality area. We suspect that different allele frequencies of different areas and ethnic groups may contribute to the discordance. Sample size of the present study is smaller, especially when analyzed in subgroups. Therefore, further large multi-center studies with different ethnic groups are warranted to assess the role of the *miR-146a* SNP in primary liver cancer risk.

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Disclosure Statement

The authors declare that they have no competing interests that are directly relevant to the content of this study.

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