Association study of miR-149 rs2292832 and miR-608 rs4919510 and the risk of hepatocellular carcinoma in a large-scale population

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Abstract. Polymorphisms in pre-microRNAs (miRNAs) or mature miRNAs may influence miRNA processing or target binding, thus contributing to tumorigenesis and cancer development. The present study aimed to evaluate whether miR-149 rs2292832 (C>T) and miR-608 rs4919510 (G>C) are associated with the risk and clinical characteristics of hepatocellular carcinoma (HCC) in a large-scale population. miR-149 rs2292832 and miR-608 rs4919510 were genotyped in a total of 993 patients with HCC and 992 unrelated healthy subjects by Sequenom MassARRAY. The results showed that, compared with the reference CC genotype, the TC+TT genotype of miR-149 was more highly associated with HCC [CC vs. TC+TT: Odds ratio (OR)=1.384, 95% confidence interval (CI)=1.013-1.892, P=0.041], and was also associated with an increased risk of hepatitis B virus (HBV)-associated HCC (CC vs. TC+TT: OR=1.453, 95% CI=1.034-2.042, P=0.031). However, no significant association between miRNA-608 rs4919510 and the risk of HCC/HBV-associated HCC was found. In addition, these two SNPs were shown not to be correlated with a range of clinical characteristics. The present study may provide an indicator for identification of the high risk of HCC in patients.

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Introduction

Hepatocellular carcinoma (HCC) is one of the most common types of cancer worldwide, particularly in China (1). According to the latest Chinese cancer registration report, liver cancer accounts for the fourth highest morbidity and the second highest mortality rate of any type of cancer (2). In addition to hepatitis B (HBV) infection, other factors, including hepatitis C (HCV) infection, aflatoxin B1 exposure, genetic factors and excessive alcohol consumption also contribute to oncogenesis and development of HCC (3,4). However, the genetic factors are poorly understood and require further exploration. Furthermore, due to the low diagnostic accuracy and poor prognosis of HCC, investigation of highly efficient genetic biomarkers is essential.

MicroRNAs (miRNAs) are a class of small non-coding RNA molecules (~22 nucleotides in length), which, as critical post-transcriptional regulators, modulate gene expression or function through binding to the 3'-untranslated region of targets. miRNAs have been predicted to regulate half of all protein-coding genes in mammals (5). Single nucleotide polymorphisms (SNPs) in pri-, pre- or mature miRNAs, particularly in the seed regions, may influence the expression or target site selection of miRNAs, and thus they may be involved in a wide range of biological processes and increase the risk of cancer (6,7). Therefore, SNPs in miRNAs may be regarded as biomarkers for diagnosis and/or prognosis of cancers.

miR-149 rs2292832 (C>T) and miR-608 rs4919510 (G>C) have been predicted to be capable of influencing miRNA activities (8). miR-149 rs2292832 and mir-608 rs4919510 are involved in p53 signaling (9,10), and studies have confirmed that they are linked to the susceptibility to certain types of cancer, including lung (11), gastric (12) and breast cancer (13),

as well as head and neck squamous cell carcinoma (14) and colorectal cancer (12,15). In particular, a previous study showed that miR-149 rs2292832 was associated with the risk of HCC in Korean individuals (16). No confirmation of this association in large and other ethnic populations has been reported, to the best of our knowledge. In addition, no studies have been performed to investigate the association between miR-608 rs4919510 and the risk of HCC to the best of our knowledge. Therefore, the present study aimed to determine whether these two polymorphisms were associated with HCC in a large-scale Chinese population.

Materials and methods

Patients. The final analysis in the present study consisted of 993 cases of HCC and 992 controls from an Eastern Chinese population sample collected from Huashan Hospital (Shanghai, China), Eastern Hepatobiliary Surgery Hospital (Shanghai, China) and CMC Institute of Health Sciences (Taizhou, China) as described previously (17). All patients were diagnosed by pathological or imaging evidence, while all controls were healthy without a family history of cancer or other serious diseases. Clinical characteristics collected included age, gender, family history, smoking status, alcohol use, serum α fetoprotein (AFP) levels, hepatitis B surface antigen (HBsAg) status, HBV-DNA titer, alanine transaminase (ALT) levels, aspartate aminotransferase (AST) levels, total bilirubin levels, tumor number/size and tumor grade. This study was approved by the Human Research Review Committee of Huashan Hospital, Fudan University (Shanghai, China). All patients or their families provided written informed consent.

DNA extraction. Genomic DNA was extracted from whole blood using the AxyPrep™ Blood Genomic DNA Miniprep kit (Axygen Biosciences, Union City, NJ, USA). Electrophoresis and concentration determination were performed for all DNA samples to ensure the accuracy of subsequent experiments. The concentration determination was conducted by NanoDrop 2000c Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). After NanoDrop software was opened and nucleic acid application was selected, 1 μl H_2O was pipetted on the pedestal for blank measurement, and then 1 μl DNA sample was loaded onto the lower optical surface to measure its concentration. DNA samples with distinct strips in the electrophoresis gel and a concentration >10 ng/μl were selected for further genotyping by Sequenom MassARRAY (Sequenom, San Diego, CA, USA).

Genotyping. The SNPs, miR-149 rs2292832 (C>T) and miR-608 rs4919510 (G>C), were genotyped using Sequenom MassARRAY technology. Polymerase chain reaction (PCR) primers were designed using MassARRAY Assay Design software version 3.1 (Sequenom), and synthesized by Shanghai Invitrogen Biotechnology Co., Ltd. (Shanghai, China). The primer sequences are listed in Table I. Sequencing was performed using the MassARRAY Analyzer Compact system (Sequenom) and analyzed by TYPER 4.0 (Sequenom).

Statistical analysis. The Statistical Package for Social Sciences (version 13.0; SPSS, Inc., Chicago, IL, USA) and Excel

Table I. Selected two SNP sites in the miRNAs.

SNP ID	Substitutes	miRNA	SNP location	Chromosome start-stop site	Amplification primers	Extension primer
rs2292832	C/T	hsa-mir-149	241395503 (stem-loop structure)	chr2:241395418-241395506	ACGTTGGATGAACTCGCCCAGCCGGCCC ACGTTGGATGTCTTCACTCCCGTGCTTGTC	GACCTGCGTTGTTCC
rs4919510	G/C	hsa-mir-608	102734778 (mat)	chr10:102734742-102734841	ACGTTGGATGATGGAAGCTCTTGGAGATGC ACGTTGGATGAAGATCCACTGGGCCAAGGT	GGAGATGCCTTTTTAAACG

SNP, single nucleotide polymorphism; miRNA, microRNA; hsa, *Homo sapiens*.

Table II. General characteristics in patients with HCC and controls, n (%) or mean ± standard deviation.

Characteristic	Cases (n=993)	Controls (n=992)	P-value
Age (years)	54.63±11.26	59.55±11.63	<0.001[a]
Gender			
Male	816 (82.2)	720 (72.6)	<0.001[a]
Female	177 (17.8)	272 (27.4)	
Smoking status			
Never	663 (67.7)	523 (52.7)	<0.001[a]
Ever	317(32.3)	469 (47.3)	
Alcohol status			
Never	733 (74.6)	731 (73.7)	0.628
Ever	249 (25.4)	261 (26.3)	-
HBsAg (n=938)			
Negative	179 (18.9)	-	-
Positive	768 (81.1)	-	-
Tumor size (n=536)			
<5 cm	218 (40.3)	-	-
≥5 cm	323 (59.7)	-	-
Tumor number (n=535)			
Single	477 (88.3)	-	-
Multiple	63 (11.7)	-	-
Tumor grade (n=382)			
I-II	85 (22.0)	-	-
III-IV	301 (78.0)	-	-
Serum level of tumor markers			
ALT (U/l, in 986 subjects)	58.51±86.21	-	-
AST (U/l, in 982 subjects)	62.18±81.12	-	-
AFP			
<20 μg/l	361 (37.2)	-	-
≥20 μg/l	609 (62.8)	-	-
(μg/l, in 402 subjects)	127.32±289.25 (0.7-1210)	-	-
HBV-DNA (IU/ml, in 450 subjects)	$1.749x10^6$±$5.430x10^6$ (1000-$6.9x10^7$)	-	-

[a]P<0.05 between the cases and controls. HCC, hepatocellular carcinoma; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; ALT, alanine transaminase; AST, aspartate aminotransferase; AFP, α fetoprotein.

(Microsoft Corporation, Redmont, WA, USA) was applied to conduct the data analysis. Binary logistic regression was used to evaluate the correlation between the risk of HCC and the two SNPs adjusted to smoking, alcohol use and other confounding factors. Odds ratios (ORs) and 95% confidence intervals (CI) were also calculated in order to estimate the relative risk. Pearson's χ^2 test was performed to verify whether the sample obeyed the Hardy-Weinberg Equilibrium by comparing the observed genotype frequencies with the expected ones. The association between clinicopathological characteristics and genotypic/allelic frequencies in the patients with HCC was also conducted by Pearson's χ^2 test. For the other quantitative variables which had heterogeneity of variance or non-normal distributions, analysis of variance or nonparametric tests were applied. All statistical tests were two-sided and probability levels <0.05 were used as a criterion of significance.

Results

General characteristics of the subjects. As Eastern China, including Jiangsu and Zhejiang provinces, is one of the main high-prevalence regions for HCC (2), a total of 993 patients with HCC and 992 healthy subjects were enrolled from the aforementioned provinces in the present study. The overview of the enlisted samples is presented in Table II. In terms of the distributions of age, gender and smoking status, significant differences between the cases and controls were identified: The control group had a higher age, higher female ratio and higher smoking ratio.

miRNA SNPs and the risk of HCC. Genotyping for rs2292832 and rs4919510 was successful in 1,928 and 1,985 subjects, respectively. The genotype distributions of

Table III. Association between genotypes/alleles of two miRNA SNPs and the risk of HCC.

Genotypes	Controls n (%)	HCC patients			HCC patients with HBV		
		n (%)	OR (95% CI)[a]	P-value[a]	n (%)	OR (95% CI)[a]	P-value[a]
miR-149 rs2292832	n=984	n=944			n=729		
CC	92 (9.3)	104 (11.0)	1.000		81 (11.1)	1.000	
TC	414 (42.1)	386 (40.9)	1.023 (0.837-1.250)	0.827	307 (42.1)	0.957 (0.768-1.193)	0.696
TT	478 (48.6)	454 (48.1)	1.401 (1.007-1.950)	0.046[b]	341 (46.8)	1.419 (0.990-2.034)	0.057
Dominant model (CC vs. TC+TT)			1.384 (1.013-1.892)	0.041[b]		1.453 (1.034-2.042)	0.031[b]
Recessive model (CC+TC vs. TT)			0.956 (0.791-1.155)	0.641		0.893 (0.726-1.100)	0.287
C	598 (0.3)	594 (0.3)	1.000		468 (32.1)	1.000	
T	1370 (0.7)	1294 (0.7)	1.103 (0.954-1.274)	0.186	988 (67.9)	0.869 (0.742-1.018)	0.082
miR-608 rs4919510	n=992	n=993			n=768		
GG	318 (32.1)	304 (30.6)	1.000		232 (30.2)	1.000	
GC	497 (50.1)	500 (50.3)	1.019 (0.790-1.315)	0.884	393 (51.2)	0.970 (0.733-1.282)	0.830
CC	177 (17.8)	189 (18.9)	0.958 (0.775-1.186)	0.695	143 (18.6)	0.946 (0.749-1.195)	0.641
Dominant model (GG vs. GC+CC)			0.953 (0.779-1.166)	0.643		1.049 (0.840-1.309)	0.674
Recessive model (GG+GC vs. CC)			1.036 (0.814-1.318)	0.774		0.991 (0.760-1.291)	0.944
G	1133 (0.6)	1108 (0.6)	1.000		856 (55.8)	1.000	
C	851 (0.4)	878 (0.4)	1.023 (0.896-1.170)	0.733	678 (44.2)	1.013 (0.875-1.173)	0.861

[a]ORs and P-values were all obtained after adjustment regarding to age, gender, smoking status and alcohol use status. [b]P<0.05 compared with the controls or between the comparison specified. miRNA, microRNA; SNP, single nucleotide polymorphism; HCC, hepatocellular carcinoma; OR, odds ratio; CI, confidence interval; HBV, hepatitis B virus.

Table IV. Comparison of genotype/allele frequencies of two miRNA polymorphisms in male subjects.

Genotypes	Controls n (%)	HCC patients			HCC patients with HBV		
		n (%)	OR (95% CI)[a]	P-value[a]	n (%)	OR (95% CI)[a]	P-value[a]
miR-149 rs2292832	n=717	n=772			n=606		
CC	56 (7.8)	83 (10.8)	1.000		65 (10.7)	1.000	
TC	309 (43.1)	318 (41.2)	1.062 (0.845-1.333)	0.608	257 (42.4)	0.996 (0.779-1.274)	0.976
TT	352 (49.1)	371 (48.0)	1.684 (1.134-2.501)	0.010[b]	284 (46.9)	1.649 (1.079-2.519)	0.021[b]
Dominant model (CC vs. TC+TT)			1.631 (1.120-2.374)	0.011[b]		0.605 (0.404-0.906)	0.015[b]
Recessive model (CC+TC vs. TT)			0.965 (0.778-1.198)	0.749		0.911 (0.721-1.151)	0.434
C	421 (27.4)	484 (31.3)	1.000		386 (31.9)	1.000	
T	1013 (72.6)	1060 (68.7)	0.881 (0.747-1.040)	0.135	824 (68.1)	0.856 (0.716-1.024)	0.089
miR-608 rs4919510	n=720	n=816			n=640		
GG	227 (31.5)	241 (29.5)	1.000		185 (28.9)	1.000	
GC	361 (50.2)	415 (50.9)	1.044 (0.783-1.391)	0.770	333 (52.0)	0.976 (0.716-1.332)	0.880
CC	132 (18.3)	160 (19.6)	0.933 (0.730-1.191)	0.577	122 (19.1)	0.898 (0.689-1.170)	0.424
Dominant model (GG vs. GC+CC)			0.922 (0.731-1.162)	0.491		0.903 (0.703-1.162)	0.428
Recessive model (GG+GC vs. CC)			1.072 (0.817-1.406)	0.618		1.016 (0.757-1.365)	0.914
G	815 (56.6)	897 (55.0)	1.000		703 (54.9)	1.000	
C	625 (43.4)	735 (45.0)	1.047 (0.900-1.219)	0.552	577 (45.1)	1.017 (0.867-1.194)	0.834

[a]ORs and P values were obtained after the adjustment of age, gender, smoking status and wine status. [b]P<0.05 compared with the controls or between the comparison specified. miRNA, microRNA; HCC, hepatocellular carcinoma; OR, odds ratio; CI, confidence interval; HBV, hepatitis B virus.

Table V. Comparison of genotype/allele frequencies of two miRNA polymorphisms in female subjects.

	Controls	HCC patients			HCC patients with HBV		
Genotypes	n (%)	n (%)	OR (95% CI)[a]	P-value[a]	n (%)	OR (95% CI)[a]	P-value[a]
miR-149 rs2292832	n=267	n=172			n=123		
CC	36 (13.5)	21 (12.2)	1.000		16 (13.0)	1.000	
TC	105 (39.3)	68 (39.5)	0.941 (0.612-1.447)	0.782	50 (40.7)	0.949 (0.462-1.948)	0.886
TT	126 (47.2)	83 (48.3)	0.895 (0.474-1.689)	0.731	57 (46.4)	0.825 (0.500-1.360)	0.451
Dominant model (CC vs. TC+TT)			0.924 (0.512-1.670)	0.794		1.053 (0.540-2.052)	0.879
Recessive model (CC+TC vs. TT)			0.968 (0.649-1.444)	0.872		0.836 (0.526-1.330)	0.451
C	177 (33.2)	110 (32.0)	1.000		82 (33.3)	1.000	
T	357 (66.8)	234 (68.0)	0.978 (0.724-1.321)	0.883	164 (66.7)	1.086 (0.768-1.535)	0.642
miR-608 rs4919510	n=272	n=177			n=128		
GG	91 (33.5)	63 (35.6)	1.000		47 (36.7)	1.000	
GC	136 (50.0)	85 (48.0)	0.928 (0.528-1.633)	0.797	60 (46.9)	0.928 (0.480-1.794)	0.825
CC	45 (16.5)	29 (16.4)	1.053 (0.681-1.628)	0.818	21 (16.4)	1.149 (0.694-1.901)	0.590
Dominant model (GG vs. GC+CC)			1.072 (0.709-1.622)	0.742		1.170 (0.725-1.886)	0.521
Recessive model (GG+GC vs. CC)			0.909 (0.532-1.554)	0.728		0.877 (0.469-1.639)	0.681
G	318 (58.5)	211 (59.6)	1.000		154 (60.2)	1.000	
C	226 (41.5)	143 (40.4)	0.940 (0.708-1.248)	0.668	102 (39.8)	0.891 (0.640-1.239)	0.492

[a]ORs and P values were obtained after adjusting for age, gender, smoking status and wine status. [b]P<0.05 compared with the controls or between the comparison specified. miRNA, microRNA; HCC, hepatocellular carcinoma; OR, odds ratio; CI, confidence interval HBV, hepatitis B virus.

Table VI. Clinicopathological characteristics and genotype/allele frequencies of miR-149 and miR-608 polymorphisms in patients with HCC, n (%) or mean ± standard deviation.

Indexes		Genotype		P-value	Allele		P-value
Tumor size							
miR-149 rs2292832	CC	TC	TT	0.720	C	T	0.430
<5 cm	23 (11.0)	88 (41.9)	99 (47.1)		134 (31.9)	286 (68.1)	
≥5 cm	40 (13.1)	129 (42.3)	136 (44.6)		209 (34.3)	401 (65.7)	
miR-608 rs4919510	GG	GC	CC	0.514	G	C	0.257
<5cm	57 (26.1)	111 (50.9)	50 (22.9)		225 (51.6)	211(48.4)	
≥5cm	98 (30.3)	160 (49.5)	65 (20.1)		356 (55.1)	290 (44.9)	
Tumor focus number							
miR-149 rs2292832	CC	TC	TT	0.650	C	T	0.344
Single	57 (12.6)	193 (42.5)	204 (44.9)		307 (33.8)	601 (66.2)	
Multiple	6 (9.8)	24 (39.3)	31 (50.8)		36 (29.5)	86 (70.5)	
miR-608 rs4919510	GG	GC	CC	0.430	G	C	0.205
Single	140 (29.4)	239 (50.1)	98 (20.5)		519 (54.4)	435 (45.6)	
Multiple	15 (23.8)	31 (49.2)	17 (27.0)		61 (48.4)	65 (51.6)	
Tumor grade							
miR-149 rs2292832	CC	TC	TT	0.971	C	T	0.908
I-II	11 (13.6)	34 (42.0)	36 (44.4)		56 (34.6)	106 (65.4)	
III-IV	40 (13.8)	117 (40.5)	132 (45.7)		197 (34.1)	381 (65.9)	
miR-608 rs4919510	GG	GC	CC	0.818	G	C	0.538
I-II	27 (31.8)	42 (49.4)	16 (18.8)		96 (56.5)	74 (43.5)	
III-IV	86 (28.5)	153 (50.7)	63 (20.8)		325 (53.8)	279 (46.2)	
AFP							
miR-149 rs2292832	CC	TC	TT	0.460	C	T	0.264
<20 μg/l	36 (10.4)	134 (38.6)	177 (51.0)		206 (29.7)	488 (70.3)	
≥20 μg/l	64 (11.1)	242 (42.1)	269 (46.8)		370 (32.2)	780 (67.8)	
miR-608 rs4919510	GG	GC	CC	0.355	G	C	0.684
<20 μg/l	103 (28.5)	192 (53.2)	66 (18.3)		398 (55.1)	324 (44.9)	
≥20 μg/l	194 (31.9)	295 (48.4)	120 (19.7)		683 (56.1)	535 (43.9)	
Total bilirubin							
miR-149 rs2292832	CC	TC	TT	0.831	-	-	-
	19.49±36.99	18.03±16.51	18.64±28.17		-	-	
miR-608 rs4919510	GG	GC	CC	0.609	-	-	-
	17.87±15.41	18.69±27.83	17.87±15.41		-	-	
Direct bilirubin							
miR-149 rs2292832	CC	TC	TT	0.884	-	-	-
	9.58±28.65	8.36±12.65	8.51±20.00		-	-	
miR-608 rs4919510	GG	GC	CC	0.564	-	-	-
	10.01±29.18	8.63±20.14	7.95±10.91		-	-	
Indirect bilirubin							
miR-149 rs2292832	CC	TC	TT	0.847	-	-	-
	9.98±8.87	9.71±5.18	9.71±6.94		-	-	
miR-608 rs4919510	GG	GC	CC	0.416	-	-	-
	10.14±8.79	9.65±6.87	10.00±5.58		-	-	
ALT (U/l)							
miR-149 rs2292832	CC	TC	TT	0.456	-	-	-
	54.43±88.63	60.95±88.86	55.45±77.07		-	-	

Table VI. Continued.

Indexes	Genotype			P-value	Allele	P-value	
miR-608 rs4919510	GG	GC	CC	0.227	-	-	-
	61.23±86.06	56.76±85.23	58.78±89.34		-	-	
AST (U/l)							
miR-149 rs2292832	CC	TC	TT	0.106	-	-	-
	52.34±61.28	66.4±91.04	61.77±79.19		-	-	
miR-608 rs4919510	GG	GC	CC	0.888	-	-	-
	64.20±88.57	61.30±76.62	61.27±80.55		-	-	
HBV-DNA (IU/ml)							
miR-149 rs2292832	CC	TC	TT	0.526	-	-	-
	$8.72x10^5±1.59x10^6$	$2.27x10^6±7.38x10^6$	$1.42x10^6±3.61x10^6$		-	-	
miR-608 rs4919510	GG	GC	CC	0.456	-	-	-
	$2.92x10^6±8.33x10^6$	$1.23x10^6±3.24x10^6$	$1.10x10^6±2.76x10^6$		-	-	

miRNA, microRNA; HCC, hepatocellular carcinoma; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; ALT, alanine transaminase; AST, aspartate aminotransferase; AFP, α fetoprotein.

the two SNPs in the case and control groups conformed to the Hardy-Weinberg Equilibrium. Table III shows that the frequency distribution of the miR-149 rs2292832 genotype was significantly different between the patients with HCC and the control group following adjustment of age, gender, smoking and alcohol consumption status. Compared with that of the wild type CC, the TT genotype was associated with an increased risk of HCC (OR=1.401, 95% CI=1.007-1.950, P=0.046); however, the genotype was not correlated with that of HBV-associated HCC (OR=1.419, 95% CI=0.990-2.034, P=0.057). According to the genetic model analysis, in comparison with that of the wild-type CC, the TC+TT genotype was correlated with a higher risk of HCC (OR=1.384, 95% CI=1.013-1.892, P=0.041), as well as that of HBV-associated HCC (OR=1.453, 95% CI=1.034-2.042, P=0.031), indicating its dominant manner. Furthermore, further gender analysis (Table IV) implicated the association in males. Male subjects with the miR-149 TT genotype were associated with an increased risk of HCC/HBV-associated HCC as compared with the CC genotypes (HCC, OR=1.684, 95% CI=1.134-2.501, P=0.010; HBV-associated HCC, OR=1.649, 95% CI=1.079-2.519, P=0.021, respectively). However, in females, this association was not identified (Table V).

As for miR-608 rs4919510, the frequency distributions of genotypes or alleles did not display statistically significant differences between the cases and the controls of HCC/HBV-associated HCC (P>0.05).

miRNA-499 and miR-608 polymorphisms and clinicopathological characteristics. Whether clinicopathological characteristics have an association with the distribution of genotypes/alleles was further analyzed. As shown in Table VI, no significant differences were discovered, implying that the two SNPs were uncorrelated with clinicopathological characteristics.

Discussion

Aberrant miRNA expression or function may alter a wide variety of physiological processes. SNPs in miRNA genes are able to influence the biogenesis and functions of their host miRNAs, and thus they participate in the susceptibility of developing complicated diseases (4). The present study identified for the first time, to the best of our knowledge, that rs2292832 in miR-149 had significant correlation with genetic predisposition to HCC and HBV-associated HCC in a large-scale Chinese population, while no correlation existed between miR-608 rs4919510 and the risk of HCC/HBV-associated HCC. Furthermore, the two SNPs lacked an association with the clinical characteristics recorded.

miR-149 functions as a tumor suppressor by promoting apoptosis through repression of Akt1 and E2F1 (as observed in HeLa cells and the Be2C neuroblastoma cell line) (18), by inhibiting epithelial-to-mesenchymal transition by targeting of Forkhead box M1 (in lung cancer cells) (19), or by targeting of the zinc finger and BTB domain containing 2 oncogene (in gastric cancer) (20). miR-149 is also a tumor oncogene regulator which is involved in the increased expression levels of myeloid cell leukemia sequence 1 (in human melanoma) (10). However, whether miR-149 is involved in the carcinogenesis and development of HCC remains unknown. To the best of our knowledge, there is only one study that has suggested an association between the miR-149 rs2292832 polymorphism and HCC/HBV-HCC, and the study used a small Korean sample (cases/controls = 159:201) (16). With a larger Chinese sample, the present study disclosed that individuals with the TC+TT genotype had a higher risk of HCC/HBV-associated HCC, compared with that of individuals with the CC genotypes. As the T variant in pre-miRNA-149 may prohibit the expression of mature miR-149 (14), the findings of the present study indicated that miR-149, as a tumor suppressor, was downregulated

in subjects with the TC+TT genotypes and thus resulted in an increased risk of HCC.

miR-608 is mapped in the 10q24 locus, at which a loss of heterozygosity exists in certain types of tumor, including colorectal, prostate, pancreatic and brain (21-23). A variant, rs4919510, is embodied in the mature sequence of miR-608, within an intron of sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain (semaphorin) 4G (24). A study predicted that common/variant miR-608 binds to its targets, including acyl-CoA dehydrogenase, CD4, growth hormone receptor, retinoid X receptor β and tumor protein p53 (involved in hepatocarcinogenesis), with different levels of energy and result in different biological activities (25). Furthermore, miR-605 may create a positive feedback loop by assisting the rapid accumulation of p53 in response to stress through interruption of the p53:Mdm2 interaction (9). Therefore, it was assumed that the variant may influence the risk of HCC, serving as a predictive biomarker. However, the results of the present study showed that miR-608 rs4919510 was not correlated with the risk of HCC/HBV-associated HCC, or clinical features, at least in the population studied.

In conclusion, to the best of our knowledge, the results of the present study demonstrated for the first time that the miR-149 rs2292832 polymorphism may influence genetic predisposition to HCC/HBV-associated HCC in the Chinese population, particularly in males, while the miR-608 rs4919510 polymorphism lacked such association in the studied population. The present study lays a foundation for further studies regarding the function of miR-149 in HCC by suggesting that miR-149 may be useful as an indicator for early detection of HCC risk.

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