The association between micro-RNA gene polymorphisms and the development of hepatocellular carcinoma in Egyptian patients

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Abstract

Introduction: The development and progression of hepatocellular carcinoma (HCC) is a multistage process involving the deregulation of genes that are crucial to cellular processes. Multiple risk factors are correlated with HCC. MicroRNA is differentially expressed in the development of different types of malignancies, including hepatic malignancy. Single nucleotide polymorphisms (SNPs) are the most common sequence variation in the human genome. SNPs in miRNAs may affect transcription, processing, or target recognition and result in malignant disease. The aim of the study was to determine the association between microRNA gene polymorphisms and the development of HCC in Egyptian patients.

Material and methods: This study included 200 individuals who were matched in age and sex. Tumour staging was done using the BCLC staging system. Quantification and genotyping of microRNA were performed.

Results: Among the 200 patients, 2 groups were described: group I included 90 HCC patients with a male majority (72.2%), and group II comprised 110 controls. Three microRNA SNPs were assayed in both patients and controls. There was a significant association between rs10061133 miR-499b and the risk of HCC. The genotypes GG or G allele were significantly associated with an increased risk of HCC (GG: OR = 2.91, 95% CI: 1.23–4.22, p = 0.013; G allele: OR = 1.79, 95% CI: 1.12–2.15, p = 0.026) compared with the genotype of AA or AG or A allele.

Conclusions: There is an association between the miRNA SNPs and the susceptibility to HCC, to explore some roles and mechanisms of SNPs within miRNAs in the occurrence and development of HCC.

Key words: microRNA, gene polymorphisms, hepatocellular carcinoma.

Introduction

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer worldwide. It is also the fifth leading cause of cancer-relat-

ed deaths worldwide, mainly because it is usually diagnosed at an advanced stage due to difficult early diagnosis. Therefore, to reduce HCC-related mortality and enable early diagnosis, new diagnostic and prognostic biomarkers for HCC are crucial [1]. Multiple risk factors are correlated with HCC, including hepatitis B or C virus (HBV and HCV, respectively) infection, alcohol abuse, aflatoxin exposure, metabolic diseases, and nonalcoholic steatohepatitis. HBV infection is an important cause of HCC, but only a fraction of infected patients develop HCC during their lifetime. This suggests that some genetic factors play a role in HCC development [2].

HCC development and progression is a multistage process involving the deregulation of genes that are crucial to cellular processes, such as cell cycle control, cell growth, apoptosis, and cell migration. As important genetic factors for HCC, Wang *et al.* identified alterations in the regulation of multiple oncogenes or tumour suppressor genes leading to the activation of tumourigenesis-related pathways [3].

MicroRNAs (miRNAs) are a group of naturally occurring, small, non-coding, single-stranded RNA molecules that have been identified in many organisms. They are approximately 20 nucleotides long and function as regulators of gene expression at the post-transcriptional level [4]. They regulate gene expressions in various eukaryotic systems by base pairing with the 3′-untranslatedregion of their target mRNAs, leading to mRNA cleavage or translational repression [5]. MiRNAs play crucial roles in many physiological and pathological conditions, including cell growth and development, cellular differentiation, proliferation, cell death, and metabolism. Numerous studies have demonstrated that alteration in miRNA genes and/or their expression plays a critical role in cancer development because it affects the regulation of proto-oncogene or tumour suppressor gene expression [6]. Bioinformatics data have indicated that a single miRNA could bind to as many as 200 different target transcripts. It has been conjectured that miRNAs regulate the expression of approximately one-third of the protein-coding miRNAs [7].

Single nucleotide polymorphisms (SNPs) are the most common sequence variations in the human genome. SNPs in miRNAs may affect transcription, processing, or target recognition, thereby resulting in malignancy. Abnormal expression of miRNA has been correlated with several pathophysiological events, including HCC development. Therefore, it was hypothesized that genetic polymorphisms in miRNAs are associated with HCC development [8]. MiRNAs play essential roles in HCC progression by directly contributing to cell proliferation, evasion of apoptotic cell death, and metastasis, by targeting many critical protein-coding genes [9].

MiRNAs are detectable and remarkably stable in clinical samples, such as blood, serum, plasma, urine, and faeces. Furthermore, miRNAs are resistant to endogenous miRNase activity, extreme pH, high temperature, and multiple freeze–thaw cycles. These findings suggest that miRNAs can serve as a promising biomarker in cancer detection [10]. In one study, an analysis by racial descent revealed the association of miR-499a rs3746444 polymorphism with a decreased risk of HCC in the Asian population but no significant risk association in the Caucasian population [11]. MiR-499a may play an important role in HCC pathogenesis by regulating the ets1 proto-oncogene, which plays a fundamental role in extracellular matrix degradation, a process required for tumour cell invasion and migration [12]. MiR-449b has been reported to play a tumour-suppressive role in various human cancers and an important role in critical developmental processes [13].

To our knowledge, few studies have reported the effect of miRNA variants, namely miRNA-196a2 (rs11614913), miR-499a (rs3746444), and miR-499b (rs10061133), on the risk of HCC in the Egyptian population. Therefore, the current study aimed to identify the possible association between the polymorphisms of these miRNA variants and HCC in a sample of the Egyptian population and to correlate these polymorphisms with disease outcome.

Material and methods

Subjects

This was a case-control study including 200 participants, who were recruited from the Outpatient Clinics and Inpatient Department, National Liver Institute-Menoufia University, Egypt. Ninety of these participants were diagnosed with HCC by clinical examination, laboratory results, and triphasic computed tomography (CT) findings. The remaining 110 participants were randomly selected healthy subjects matched for age, sex, and risk factors that may affect HCC development, namely smoking status and the presence of diabetes mellitus and hypertension. They were enrolled as a control group.

The study was approved by the Ethics Committee of the National Liver Institute (Menoufia University, Egypt), and all patients and control subjects provided a written informed consent for participation.

Exclusion criteria

The exclusion criteria for participants were as follows: having undergone previous treatment for HCC

or any antiviral therapy for HCV or HBV infection; presence of any associated malignancies other than HCC; history of liver transplantation; or presence of autoimmune disease, chronic inflammatory disease, or chronic liver disease not related to HCV, e.g. fatty liver, bilharziasis, or alcoholic cirrhosis.

Clinical examination

A complete history was taken, including the history of HCV infection, haematemesis, melena, sclerotherapy, abdominal enlargement, jaundice, lower limb swelling, and encephalopathy. Clinical examination was performed for the presence of jaundice, lower limb oedema, hepatomegaly, splenomegaly, and ascites.

Abdominal ultrasonography (US) was performed for all participants, and triphasic CT was performed for those with focal lesions or elevated α-fetoprotein (AFP) levels. US and triphasic CT were performed to assess HCC grade, liver size and echo pattern, the presence of periportal fibrosis, spleen size, and the presence of ascites or any other abnormalities in the abdomen. Doppler studies of hepatic veins, the inferior vena cava, and the portal vein were also performed. The Barcelona Clinic Liver Cancer (BCLC) staging system was used to determine the stage of HCC.

Laboratory investigation

From each participant, 10 ml of venous blood was drawn and aliquoted into vacutainer tubes (EDTA tubes, plain tubes, and sodium citrate 3.5% tubes). Sera from the samples in plain tubes were separated by centrifugation and stored at –80°C until use for PCR.

Complete blood count (CBC) was performed on a Sysmex XT 1800 (Germany) using EDTA samples. Liver function tests were performed on an Integra 800 Auto analyser (Roche-Germany Catalogue number; M, 87432). AFP was performed on an Elecsys E411 (Switzerland). Anti-HCV and anti-HBsAg antibodies were determined using an Elecsys E411 (Switzerland). Reverse transcription quantitative real-time PCR (RT-qPCR) for HCV-RNA was performed using RT-PCR kits (Roche Diagnostic, Mannheim, Germany) according to the manufacturer's instructions. The lower detection limit was < 10 IU/ml.

Detection of miRNA

RNA extraction and genotyping of miRNA polymorphisms by SNP assay

RNA extraction and cDNA synthesis: For total RNA extraction, 200 µl of serum samples obtained from all patients and control subjects was used. RNA was extracted using the miRNa Easy Extraction Kit (Qiagen, Valencia, USA) according to the manufacturer's instructions. The concentration of the extracted RNA was determined using a spectrophotometer. Reverse transcription to obtain cDNA was performed using the miRNa Easy Plasma Reverse Transcription Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. The extracted samples were stored at –80°C until use for PCR-restriction fragment length polymorphism (RFLP).

Genotyping of miRNAs by PCR-RFLP

PCR-RFLP was used to determine the SNP in the genes of miR-196a2 rs11614913, miR-499a rs3746444 [3], and miR-499b (rs10061133) [14].

PCR was performed using a 100-ng DNA template, 1.0-µl of 10 × PCR buffer, 0.25 Taq DNA Polymerase, 0.2 mN dNTPs (Invitrogen, CA, USA), and 200 nM of each primer in a total volume of 10 µl. The following PCR conditions were used: initial melting at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s and annealing at 64°C for 30 s, and final extension at 72°C for 10 min.

The PCR products were digested with restriction enzymes (miR-196a2rs11614913with MspI, miR-499 rs3746444 with BclI, and miR-499b rs10061133with BsmAI) by incubating at 37°C for 4 h then electrophoresed on 2% agarose gels containing 0.5 µg/ml ethidium bromide. The gel was visualized under UV illumination, and the PCR product sizes of 149 bp, 146 bp, and 173 bp for miR-196a-2 rs11614913, miR-499 rs3746444, and miR-499b rs10061133, respectively, were identified.

The primers used for genotyping in Table I were presented.

Statistical analysis

The collected data were tabulated and analysed by SPSS statistical package version 21 on an IBM-compatible computer. Quantitative data are expressed as mean and standard deviation (mean ± SD), and qualitative data are presented as frequencies and percentages. Quantitative data were analysed using Student's *t*-test for normally distributed variables and using the Mann-Whitney *U* test for non-normally distributed variables. Analysis of variance (F-test) was used for comparing more than 2 groups of normally distributed variables. Qualitative data are expressed as number and percentage and were analysed using the χ^2 test. Conditional logistic regression analysis was performed to analyse the association between miRNA polymorphisms and the risk of HCC, and the results are expressed as odds ratio (OR) and 95% confidence interval (CI).

Table I. The primers used for genotyping

miRNA	Primer sequence	Restriction enzyme
miR-196a2 (rs11614913):		
Forward	5'- CCCCTTCCCTTCTCCTCCAGATA-3'	MspI
Reverse	5- CGAAAACCGACTGATGTAACTCCG-3'	
miR-499a T>C (rs3746444):		
Forward	5'-CAAAGTCTTCACTTCCCTGCCA-3'	BclI
Reverse	5'-GATGTTTAACTCCTCTCCACGTGATC-3	
miR-499b A>G (rs10061133):		
Forward	5'-GGT ATC CAG AGC ACT TCA TTG ACA-3'	*Bsm*AI
Reverse	5'-GATGTTTAACTCCTCTCCACGTGATC-3	

P-values < 0.05 were considered to indicate a significant difference.

Results

Two hundred subjects were enrolled in this case-control study, including 90 patients diagnosed with HCC as the study group and 110 healthy subjects as the control group. The HCC and control groups included 65 (72.2%) and 85 (77.3%) male patients, respectively. Twenty-nine (32.2%) HCC patients and 32 (29.1%) control subjects had diabetes mellitus; 17 (18.9%) HCC patients and 15 (13.6%) control subjects had hypertension; and 31 (34.4%) HCC patients and 39 (35.5%) control subjects were smokers. Eighty-one (90%) and 9 (10%) HCC patients tested positive for HCV and HBsAg, respectively. General demographic and clinicopathological characteristics of HCC patients and control subjects are listed in Table II.

Table III shows a comparison of laboratory data between the HCC and control groups. Compared with the control subjects, the HCC patients showed significantly increased aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, and AFP levels but significantly decreased albumin, prothrombin, and haemoglobin levels and platelet counts. No significant difference was observed in age or total leukocyte count between the groups.

In this study, 3 miRNA SNPs were assayed, namely miR-196a2 rs11614913 C>T, miR-499a rs3746444 T>C, and miR-499b rs10061133 A>G. A significant association was observed between miR-499b rs10061133 and the risk of HCC (Table IV). The GG genotype and G allele were significantly associated with an increased risk of HCC (GG: OR = 2.91, 95% CI: 1.23–4.22, $p = 0.013$; G allele: OR = 1.79, 95% CI: 1.12–2.15, $p = 0.026$) compared with the AA and AG genotypes and A allele.

In contrast, miR-499a rs3746444 T>C showed no statistically significant association with the risk of HCC with respect to the T or C allele and the TT, TC, or CC genotype. In addition, miR-196a2 rs11614913 C>T showed no significant association with the risk of HCC in terms of the C or T allele and the CC, TC, or TT genotype (Table IV).

Analysis of the association between miRNA SNPs and laboratory and clinicopathological data of HCC patients revealed no association between the risk factor genotypes of miR-499b rs10061133 and platelet counts or AFP, AST, ALT, total bilirubin, prothrombin, or serum albumin levels. Moreover, no relation was detected between the genotypes and tumour size, tumour number, BCLC score, or portal vein invasion (Table V).

Discussion

HCC is the most common liver tumour and the third leading cause of cancer-related mortality worldwide [15]. Among Egyptians, HCC accounts for 70.48% of all liver tumours [16]. HCC is a lethal tumour characterized by an aggressive course. Although early diagnosis of HCC is mandatory for the development of specific curative therapies [17], it is difficult.

Although many studies have been conducted on HCV involvement in hepatic carcinogenesis via the modulation of the Wnt/β-catenin signalling pathway, only a later study on an *in-vitro* model clarified the interactions between HCV proteins and Wnt/β-catenin [18].

In one study, the allele distribution at loci rs12979860 (C/T) rs8099917 (T/G), and HLA-DP (rs3077) were analysed in Chinese-origin patients with HBV infection, who tested negative for HBsAg [19]. The authors reported significantly more frequent HBsAg loss among HLA-DP1 (rs3077) AA genotype carriers than in rs179860CC and rs8099917GG allele carriers. The above-mentioned allele configurations activate the immunological response of the host, leading to non-cytolytic degradation of cccDNA in the hepatocyte nuclei [19].

SNPs could affect the biogenesis and functions of the host miRNAs. They also play an important role in cancer development via modulation of the biogenesis and functions of the host miRNAs

Table II. Demographic and clinicopathological characteristics of HCC patients and control subjects

Parameter	HCC patients (n = 90) n (%)	Control subjects (n = 110) n (%)	χ^2 or t-test	P-value
Age [years] Mean ± SD	58.2 ±10.4	49.6 ±11.6	1.65	0.35
Gender:				
Male	65 (72.2)	85 (77.3)	2.01	0.14
Female	25 (22.8)	25 (22.7)		
Diabetes mellitus:				
Yes	29 (32.2)	32 (29.1)	0.229	0.63
No	61 (67.8)	78 (70.9)		
Hypertension:				
Yes	17 (18.9)	15 (13.6)	1.02	0.31
No	73 (81.1)	95 (86.4)		
Smoking:				
Positive	31 (34.4)	39 (35.5)	0.02	0.88
Negative	59 (53.6)	71 (64.5)		
Viral infection:				
HCV	81 (90.0)	–	–	–
HBV	9 (10.0)			
AFP level:				
< 20 ng/ml	18 (20.0)	110 (100.0)	137.5	0
> 20–400 ng/ml	72 (80.0)	0 (0.0)		
BCLC score:				
A	12 (13.3)	–	–	–
B	36 (40.0)			
C	28 (31.1)			
D	14 (15.6)			
Tumour number:				
Single	62 (68.9)	–	–	–
Multiple	28 (31.1)			
Tumour size:				
< 5 cm	51 (56.7)	–	–	–
> 5 cm	39 (43.3)			
PVT:				
No	60 (66.7)	–	–	–
Yes	30 (33.3)			

HCC – hepatocellular carcinoma, HBV – hepatitis B virus, HCV – hepatitis C virus, PT – prothrombin time, AFP – α-fetoprotein, N – number, BCLC – Barcelona clinic liver cancer staging, PVT – portal vein thrombosis.

[20]. MiRNAs are differentially expressed in the development of different types of malignancies, including hepatic malignancy (Ventura and Jacks, 2009), which suggests that miRNAs play a role as new oncogenes or tumour-suppressor genes in carcinogenesis [21]. In 2008, miRNAs were first reported to be present in the serum of patients with large B cell lymphoma [22].

The current study aimed to identify the possible association between the miRNA SNP variants and HCC in a sample of the Egyptian population and to correlate these polymorphisms with disease outcome. For this purpose, the study groups were well matched for age and gender. The mean age of the HCC patients was 58.2 ±10.4 years, consistent with a previous finding that the incidence of HCC starts to increase after 45 years of age [23]. This statement is in agreement with the findings of Kuske *et al.*, 2012 in which they stated that males above 48 years old have high risk of HCC. In addition, most HCC patients were male, consistent with a previous report that male gen-

Table III. Comparison of routine laboratory data in the studied groups

Parameter	The studied groups		*P*-value
	HCC patients (*n* = 90) Mean ± SD	Control subjects (*n* = 110) Mean ± SD	
Age [year]	58.2 ±10.4	49.6 ±11.6	0.14
ALT [U/l]	43.8 ±21.3	29.2 ±4.1	0.006
AST [U/l]	57.2 ±23.1	31.4 ±7.1	0.001
Albumin [gm/dl]	3.41 ±0.62	4.61 ±1.04	< 0.01
T. Bil. [mg/dl]	3.21 ±2.35	0.95 ±0.22	0.008
PT (%)	67.2 ±12.4	96.4 ±5.2	< 0.001
Hb [g/dl]	12.6 ±2.9	14.3 ±2.1	0.01
Platelets [×10^3]	167 ±53	315 ±112	<0.001
TLC [×10^3]	7.82 ±3.44	8.52 ±2.16	0.16
AFP [ng/ml]	35.2 ±20.8	6.2 ±3.1	< 0.001

P-value < 0.05; significant, p < 0.01; highly significant and p > 0.05; non-significant. ALT – alanine aminotransferase, AST – aspartate aminotransferase, T. Bil. – total bilirubin, PT – prothrombin time, AFP – α-fetoprotein, N – number, Hb – hemoglobin, TLC – total leukocytic count, BCLC – Barcelona clinic liver cancer staging, HCC – hepatocellular carcinoma.

Table IV. The miRNA SNPs among the studied groups

Polymorphism	HCC cases (*n* = 90) *n* (%)	Control subjects (*n* = 110) *n* (%)	OR (95% CI)	*P*-value
miR-196a2:				
rs11614913 C> T:				
Genotype:				
CC	34 (37.8)	43 (39.1)	1.00 (Ref.)	–
CT	44 (48.9)	56 (50.9)	1.02 (0.87–1.92)	0.45
TT	12 (13.3)	11 (10.0)	1.61 (0.97–2.08)	0.27
Allele:				
C	112 (62.2)	142 (64.5)	0.77 (0.59–1.22)	
T	68 (37.8)	78 (35.5)	0.98 (0.65–1.73)	0.33
miR-499a:				
rs3746444 T >C:				
Genotype:				
TT	34 (37.8)	43 (39.1)	1.00 (Ref.)	–
TC	41 (45.6)	51 (46.4)	0.89 (0.55–1.39)	0.472
CC	15 (16.7)	16 (14.5)	1.12 (0.89–1.61)	0.390
Allele:				
T	109 (60.5)	137 (62.3)		
C	71 (39.4)	83 (37.7)	1.06 (0.78–1.53)	0.261
miR-499b				
rs10061133 A>G:				
Genotype:				
AA	41 (45.6)	69 (53.6)	1.00 (Ref.)	–
AG	35 (38.9)	34 (40.9)	1.12 (0.96–1.72)	0.167
GG	14 (15.5)	7 (6.4)	2.91 (1.23–4.22)	0.013
Allele:				
A	117 (65.0)	172 (78.2)		
G	63 (35.0)	48 (21.8)	1.79 (1.12–2.15)	0.026

Table V. The relation of miR-499b rs10061133 genotype with laboratory and clinicopathological data in HCC patients

Parameter	miR-499brs10061133 genotype			*P*-value
	AA (*n* = 41)	AG (*n* = 35)	GG (*n* = 14)	
	Mean ± SD			
ALT [U/l]	54.3 ±19.2	49.3 ±7.1	51.5 ±14.8	> 0.05
AST [U/l]	53.1 ±8.7	56.2 ±12.3	47.6 ±7.2	> 0.05
T. Bil. [mg/dl]	3.54 ±1.23	3.66 ±1.41	4.32 ±2.01	> 0.05
PT (%)	75.6 ±11.2	69.2 ±7.2	61.2 ±8.1	> 0.05
Albumin [gm/dl]	2.68 ±0.81	2.06 ±0.14	2.33 ±0.56	> 0.05
Platelets	196 ±75	172 ±32	153 ±25	> 0.05
Parameter	**Number (%)**			***P*-value**
AFP:				
< 20 ng/ml	9 (22.0)	6 (17.1)	3 (21.4)	> 0.05
> 20 ng/ml	32 (78.0)	29 (82.9)	11 (78.6)	
Tumour size:				
< 5 cm	24 (58.5)	21 (60.0)	6 (42.9)	> 0.05
> 5 cm	17 (41.7)	14 (40.0)	8 (57.1)	
Tumour no:				
Single	28 (68.3)	25 (71.4)	9 (64.3)	> 0.05
Multiple	13 (31.7)	10 (28.6)	5 (35.7)	
BCLC score:				
A	6 (14.6)	4 (11.4)	2 (14.2)	> 0.05
B	15 (36.6)	15 (42.9)	6 (42.9)	
C	13 (31.7)	11 (31.4)	4 (28.6)	
D	7 (17.1)	5 (14.3)	2 (14.2)	
PVT:				
No	29 (70.7)	22 (62.9)	9 (64.3)	> 0.05
Yes	12 (29.3)	13 (37.1)	5 (35.7)	

P > 0.05; non-significant. ALT – alanine aminotransferase, AST – aspartate aminotransferase, T. Bil. – total bilirubin, PT – prothrombin time, AFP – α-fetoprotein, N – number, BCLC – Barcelona clinic liver cancer staging, PVT – portal vein thrombosis.

der is an important risk factor for HCC [24]. One reason for this could be the higher rate of exposure to risk factors in men than in women. Men are more commonly infected with HCV or HBV, cigarette smoke, and have higher stores of iron and carcinogenic substances than women.

In the present study, the levels of liver biochemical markers, namely ALT, AST, total bilirubin, and AFP, were significantly higher in the HCC patients than in the control subjects, consistent with the result of Thapa and Walia, who reported that aminotransferases, which are the most frequently used indicators of hepatocellular necrosis, are expected to be elevated in liver cell injury of any cause. Problems with the liver are reflected as a defect in bilirubin metabolism (e.g. reduced hepatocyte uptake, impaired bilirubin conjugation, and reduced hepatocyte bilirubin secretion) [25]. The normal serum levels of AFP reflect the normal rate of liver cell turnover, whereas increased AFP levels may be due to retro-differentiation of mature liver cells during regeneration, proliferation, or activation of relatively undifferentiated adult hepatocytes that have the AFP gene in an unrepressed state [26].

The current study revealed a significant association between the increased risk of HCC and several factors such as miR-499b rs10061133 A>G with the GG genotype or G allele (GG: OR = 2.91, 95% CI: 1.23–4.22, p = 0.013; G allele: OR = 1.79, 95% CI: 1.12–2.15, p = 0.026). By contrast, no statistically significant association was found between the risk of HCC and miR-499a rs3746444 T>C in terms of the T or C allele and the TT, TC, or CC genotype. Furthermore, miR-196a2 rs11614913 C>T showed no significant association with the risk of HCC in terms of the C or T allele and the CC, TC, or TT genotype.

Our finding is consistent with that of Wenshuai *et al.*, who found a statistically significant association between miR-449b rs10061133 and the risk of HCC. In their study, the G allele was significantly associated with the increased risk of HCC (G allele: OR = 1.234, 95% CI: 1.002–1.520,

p = 0.048) compared with the A allele. Therefore, miR-449b rs10061133 could be used in association with other significant miRNA SNPs as a prediction tool [27]. Iorio and Croce reported that miRNAs can modulate critical cellular functions, including cell proliferation, differentiation, and apoptosis as well as deregulation of genes that play important roles in tumourigenesis and cancer progression. Some miRNAs act as oncogenes or cancer suppressor genes [28]. Yu *et al.* revealed that individuals with the CC genotype inmiR-499 were approximately three-fold more susceptible to HCC (OR = 3.63, 95% CI: 1.545–8.532) than those with the TT genotype [29]. Chu *et al.* showed that miR-499 polymorphism along with smoking and alcohol consumption synergistically increase the risk of HCC [30], whereas Wang *et al.* reported that there is no association between miR-499 polymorphism and the risk of HCC [31].

Pingping *et al.* found that compared to the TT genotype, the TC and CC genotypes of miR-196a2 C>T polymorphism were associated with an increased risk of HCC, suggesting that variation in miR-196a2 C>T contributes to HCC development [17]. Wang *et al.* suggested that miR-146a C>G does not increase the risk of HCC [32].

Several studies have indicated that miRNAs are involved in various physiological processes, including cell proliferation, differentiation, metabolism, and apoptosis. The change in miRNA expression may be correlated with cancer pathogenesis [10, 33]. In particular, miR-196a2 C>T and miR-499 C>T have been reported to be associated with liver cancer, with the rs11614913 SNP in miR-196a2 [34] and the rs3746444 SNP in miR-499 likely to be associated with the risk of HCC [4, 35].

The discrepancies in the results of the above-mentioned studies may be due to differences in genetic background and gene–environment interactions involved in the aetiology of hepatocellular carcinogenesis or due to differences in patient populations and sample sizes, as stated by Pingping *et al.* [17].

In conclusion, this study shows an association between the miRNA499SNPs and the susceptibility to HCC, aiming to explore some roles and mechanisms of SNPs within miRNAs in the occurrence and development of primary liver cancer. To the best of our knowledge, no previous studies have elicited such a finding. We hope that our efforts and findings will facilitate the use of miRNA SNPs in the early detection of HCC and targeting for HCC therapy. Further prospective investigations with a large number of cases would allow us to evaluate miRNA-499 polymorphism in a variety of clinical settings, to help us better understand its role in HCC.

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Conflict of interest

The authors declare no conflict of interest.

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