

Functional polymorphisms in microRNAs and susceptibility to liver cancer: a meta-analysis and meta-regression

B.S. Wang, Z. Liu, W.X. Xu and S.L. Sun

Department of General Surgery,
Shengjing Hospital of China Medical University, Shenyang, China

Corresponding author: B.S. Wang
E-mail: cmu2h_wbs@126.com

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ABSTRACT. MicroRNAs (miRNAs) are small non-coding RNA molecules that play a fundamental role in controlling a variety of biological functions. Emerging evidence has shown that common genetic polymorphisms in miRNAs may be associated with the development of liver cancer; however, several individually published studies showed inconclusive results. This meta-analysis aimed to derive a more precise estimation of the association between functional polymorphisms in miRNAs and susceptibility to liver cancer. A literature search of PubMed, Embase, Web of Science, and China BioMedicine (CBM) databases was conducted on articles published before May 1, 2012. Crude odds ratios with 95% confidence intervals were calculated. Fourteen case-control studies were included with a total of 6824 liver cancer patients and 7674 healthy controls. Nine single nucleotide polymorphisms in miRNAs were assessed, including miR-146a G>C (rs2910164), miR-499 T>C (rs3746444), miR-218 A>G (rs11134527), miR-let-7c Ins/Del (rs6147150), miR-106b-25 A>G (rs999885), miR-34b/c T>C (rs4938723), miR-196a-2 C>T (rs11614913), miR-920 Ins/Del (rs16405), and miR-122 Ins/Del (rs3783553). The meta-analysis

results showed that miR-let-7c*Del, miR-34b/c*C, and miR-122*Del variants may be associated with increased liver cancer risk. Conversely, miR-920*Del variant may decrease the risk of liver cancer. However, miR-146a G>C, miR-196a-2 C>T, miR-499 T>C, and miR-218 A>G polymorphisms showed no significant association with liver cancer risk. In conclusion, the current meta-analysis suggests that miR-let-7c*Del, miR-34b/c*C and miR-122*Del variants may be associated with increased liver cancer risk, while miR-920*Del variant may be a protective factor against liver cancer.

Key words: Liver cancer; Single nucleotide polymorphism; MicroRNA; Meta-analysis

INTRODUCTION

Liver cancer is the sixth most frequently diagnosed cancer and the third leading cause of cancer death worldwide, with an estimated 748,000 new liver cancer cases and 696,000 deaths during 2008 (Thun et al., 2010; Jemal et al., 2011). The major risk factors for liver cancer include chronic hepatitis B virus (HBV) and hepatitis C virus infections, dietary aflatoxin exposure, alcohol-related cirrhosis, fatty liver disease, obesity, smoking, diabetes, and iron overload (Liu et al., 2012a). Although chronic HBV infection has been recognized as the most important causal factor for liver cancer in humans, only a small fraction of HBV-persistent carriers eventually develop liver cancer, suggesting that genetic variations may play roles in the carcinogenesis of liver cancer after HBV infection (Wang et al., 2012b). Therefore, the role of common genetic variations is increasingly recognized as an important factor in determining an individual's susceptibility to liver cancer (El-Serag and Rudolph, 2007).

MicroRNAs (miRNAs) are small non-coding RNA molecules with approximately 23 nucleotides, which act as post-transcriptional regulators of gene expression and have been implicated in the initiation and progression of various cancers (Kim et al., 2012; Wang et al., 2012a). Therefore, it has been hypothesized that genetic polymorphisms in miRNAs can be functional and may be associated with the development of liver cancer (Ryan et al., 2010). Numerous case-control studies have demonstrated that single nucleotide polymorphisms (SNPs) in miRNAs, including rs2910164 (G>C) in miR-146a, rs11134527 (A>G) in miR-218, rs11614913 (C>T) in miR-196a-2, rs999885 (A>G) in miR-106b-25, and rs3783553 (Ins/Del) in miR-122, may contribute to increased susceptibility to liver cancer (Xu et al., 2008; Gao et al., 2009; Qi et al., 2010; Liu et al., 2012b; Zhang et al., 2012). However, some other studies have suggested that functional polymorphisms in miRNAs are not associated with susceptibility to liver cancer (Akkiz et al., 2011bc; Zhang et al., 2011; Zhou et al., 2012). A recent meta-analysis of 9 case-control studies by Wang et al. (2012c) revealed that two common SNPs rs2910164 in miR-146a and rs11614913 in miR-196a-2 were not associated with the risk of liver cancer. Similar results were obtained from the other two previous meta-analyses, which focused on the effects of rs2910164 and rs11614913 polymorphisms on cancer susceptibility (Qiu et al., 2011; Xu et al., 2011a). Conversely, a meta-analysis based on 15 independent case-control studies conducted by Guo et al. (2012) suggested that rs11614913 in miR-196a-2 may contribute to increased liver cancer risk. In view of these conflicting results from previous

studies and the insufficient statistical power in the previous meta-analyses, we performed the present meta-analysis to update previous meta-analyses, as well to provide a more comprehensive and reliable conclusion on the association between functional polymorphisms in miRNAs and susceptibility to liver cancer.

MATERIAL AND METHODS

Literature search

Relevant studies published before May 1, 2013 were identified through a search in PubMed, Embase, Web of Science, and China BioMedicine (CBM) databases using the following terms: (“genetic polymorphism” or “polymorphism” or “SNP” or “single nucleotide polymorphism” or “gene mutation” or “genetic variants”) and (“liver neoplasms” or “liver cell adenoma” or “hepatocellular carcinoma” or “hepatic neoplasms” or “liver cancer” or “hepatic cancer” or “liver tumor” or “hepatic tumor” or “hepatic carcinogenesis”) and (“microRNAs” or “microRNA” or “miRNAs” or “miRNA” or “primary microRNA” or “primary miRNA” or “pri-miRNA” or “small temporal RNA” or “pre-miRNA”). The references from the eligible articles or textbooks were also reviewed to find other potential sources. Disagreements were resolved through discussions between the authors.

Inclusion and exclusion criteria

Studies included in our meta-analysis had to meet the following criteria: a) case-control studies focused on the association between functional polymorphisms in miRNAs and liver cancer risk; b) all patients diagnosed with liver cancer were confirmed by pathological or histological examinations; c) published data about the frequencies of alleles or genotypes were sufficient. Studies were excluded when they were: a) not a case-control study on the association between functional polymorphisms in miRNAs and liver cancer risk; b) duplicates of previous publications; c) publications with incomplete data; d) meta-analyses, letters, reviews, or editorial articles. If more than one study by the same author using the same case series was published, either the study with the largest sample size or the most recently published study was included.

Data extraction

Data from the published studies were extracted independently by two authors and entered on a standardized form. For each study, the following characteristics and numbers were collected: the first author, year of publication, country, language, study design, ethnicity of subjects, numbers of subjects, gender ratio, mean age, detecting sample, genotype method, allele and genotype frequencies of SNP, and evidence of Hardy-Weinberg equilibrium (HWE). In cases of conflicting evaluations, disagreements were resolved through discussions between the authors.

Quality assessment of studies included

Two authors independently assessed the quality of studies included according to the

modified STROBE-quality score systems (Gallo et al., 2012). Forty assessment items related to quality appraisal were used in this meta-analysis with scores ranging from 0 to 40. The studies included were classified into three levels based on their scores: low quality (0-19), moderate quality (20-29), and high quality (30-40). Disagreements were also resolved through discussions between the authors.

Statistical analysis

Crude odds ratios (ORs) with 95% confidence intervals (95%CI) were calculated under five genetic models: allele, dominant, recessive, homozygous, and heterozygous models. The statistical significance of the pooled ORs was examined using the Z-test. Between-study variations and heterogeneities were estimated using the Cochran Q-statistic with $P < 0.05$ as a cutoff to identify statistically significant heterogeneity (Higgins and Thompson, 2002). We also quantified the effects of heterogeneity by using the I^2 test (ranging from 0 to 100%), which represents the proportion of inter-study variability that can be contributed to heterogeneity rather than to chance (Zintzaras and Ioannidis, 2005). A significant Q-test with $P < 0.05$ or $I^2 > 50\%$ indicated that heterogeneity between studies existed, and then, the random-effect model (DerSimonian Laird method) was conducted for the meta-analysis; otherwise, the fixed-effect model (Mantel-Haenszel method) was used. To explore the potential sources of heterogeneity, multivariate meta-regression analysis was used (Ioannidis et al., 2008). Subgroup analysis was performed by the ethnicity and genotype method. Sensitivity analysis was performed by omitting each study in turn to assess the quality and consistency of the results. Begger funnel plots were used to detect publication bias. The Egger linear regression test was also used to evaluate publication bias (Peters et al., 2006). We tested whether genotype frequencies were in HWE using the χ^2 test. All the P values were two-sided. All analyses were calculated using the STATA Version 12.0 software (Stata Corp., College Station, TX, USA).

RESULTS

Characteristics of the studies included

Overall, 148 potentially relevant studies were identified by searching electronic databases. According to the inclusion criteria, 14 case-control studies (Gao et al., 2009; Chen et al., 2010; Qi et al., 2010; Akkiz et al., 2011a,b,c; Xu et al., 2008, 2011b; Liu et al., 2012b; Xiang et al., 2012; Yu et al., 2012; Zhang et al., 2011, 2012; Zhou et al., 2012) were included in this meta-analysis and 134 were excluded. The flow chart of the study selection process is shown in Figure 1. A total of 14,516 subjects were involved in this meta-analysis, including 6824 liver cancer patients and 7674 healthy controls. The publication years of the studies considered ranged from 2008 to 2012. All patients diagnosed with liver cancer were confirmed by pathological examinations. The sources of controls in all studies included were based on the general population (population-based). The DNA samples used for examination of genetic polymorphisms in miRNAs were extracted from blood in all studies included. Genotyping methods included direct DNA sequencing, TaqMan, primer-introduced restriction analysis-polymerase chain reaction (PIRA-PCR), PCR-ligase detection reaction (PCR-LDR) and PCR-restriction fragment length polymorphism (PCR-RFLP). The HWE test was conducted on the

genotype distribution of the controls in all studies included. None of the studies deviated from the HWE (all $P > 0.05$). All quality scores of studies included were higher than 20 (moderate-high quality). The characteristics and methodological quality of the studies included are summarized in Table 1.

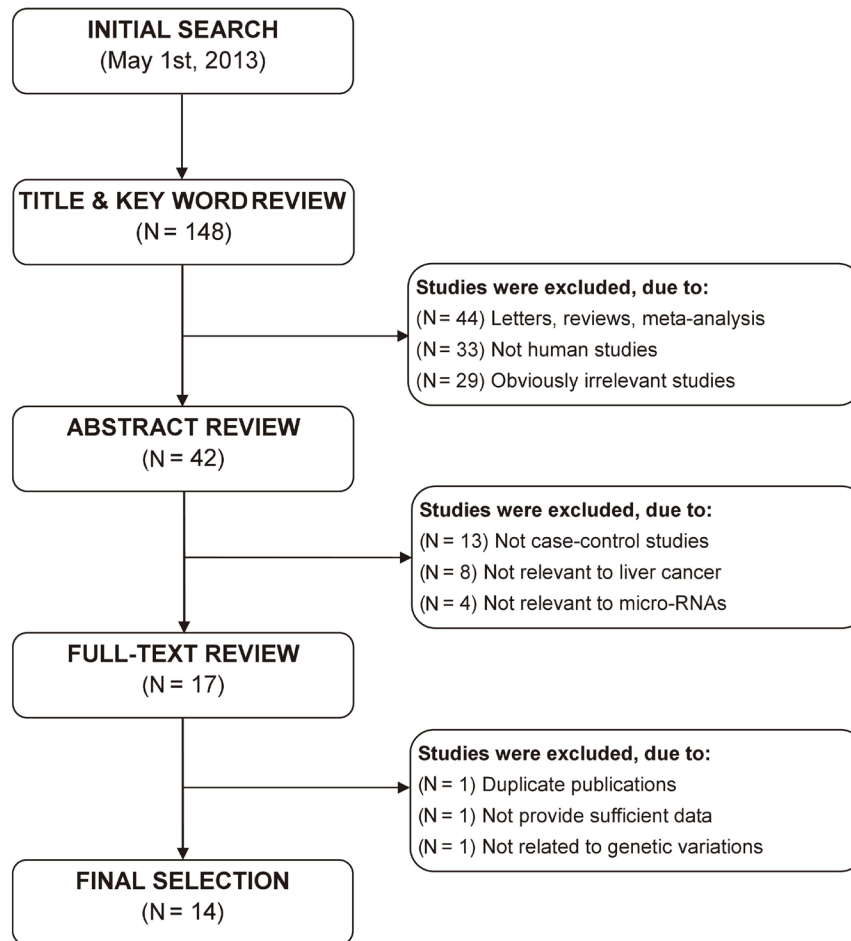


Figure 1. Flow chart of literature search and study selection. Fourteen case-control studies were included in this meta-analysis.

Quantitative data synthesis

Nine SNPs in miRNAs were addressed, including miR-146a G>C (rs2910164), miR-499 T>C (rs3746444), miR-218 A>G (rs11134527), miR-let-7c Ins/Del (rs6147150), miR-106b-25 A>G (rs999885), miR-34b/c T>C (rs4938723), miR-196a-2 C>T (rs11614913), miR-920 Ins/Del (rs16405), and miR-122 Ins/Del (rs3783553). A summary of the meta-analysis findings of the association between genetic polymorphisms in miRNAs and liver cancer risk is provided in Table 2.

Table 1. Characteristics and methodological quality of the studies included.

First author	Year	Country	Ethnicity	Number		Source		Sample	Genotype method	Gene	SNP ID	STROBE score
				Case	Control	Case	Control					
Zhou et al.	2012	China	Asian	186	483	HB	PB	Blood	PCR-RFLP	miR-146a miR-499	rs2910164 rs3746444	35/40
Zhang et al.	2012	China	Asian	302	513	HB	PB	Blood	PCR-RFLP	miR-218	rs11134527	33/40
Yu et al.	2012	China	Asian	270	270	HB	PB	Blood	Direct DNA sequencing	let-7c	rs6147150	29/40
Xiang et al.	2012	China	Asian	100	100	HB	PB	Blood	PCR-RFLP	miR-146a miR-499	rs2910164 rs3746444	34/40
Liu et al.	2012	China	Asian	1300	1344	HB	PB	Blood	TaqMan	miR-106b-25	rs999885	28/40
Zhang et al.	2011	China	Asian	963	852	HB	PB	Blood	PIRA-PCR	miR-146a miR-499	rs2910164 rs3746444	31/40
Xu et al.	2011	China	Asian	501	548	HB	PB	Blood	PIRA-PCR	miR-34b/c	rs4938723	30/40
Akkiz et al.	2011	Turkey	Caucasian	185	185	HB	PB	Blood	PCR-RFLP	miR-196a-2	rs11614913	32/40
Akkiz et al.	2011	Turkey	Caucasian	222	222	HB	PB	Blood	PCR-RFLP	miR-499	rs3746444	32/40
Akkiz et al.	2011	Turkey	Caucasian	222	222	HB	PB	Blood	PCR-RFLP	miR-146a	rs2910164	32/40
Oi et al.	2010	China	Asian	361	391	HB	PB	Blood	PCR-LDR	miR-196a-2	rs11614913	27/40
Chen et al.	2010	China	Asian	256	367	HB	PB	Blood	Direct DNA sequencing	miR-920	rs16405	29/40
Gao et al.	2009	China	Asian	1477	1673	HB	PB	Blood	TaqMan	miR-122	rs3783553	30/40
Xu et al.	2008	China	Asian	479	504	HB	PB	Blood	PCR-RFLP	miR-146a	rs2910164	26/40

SNP = single nucleotide polymorphism; HB = hospital-based; PB = population-based; PCR = polymerase chain reaction; RFLP = restriction fragment length polymorphism; PIRA = primer-introduced restriction analysis; LDR = ligase detection reaction.

Table 2. Meta-analysis of the associations between functional polymorphisms in miRNAs and susceptibility to liver cancer.

SNP	Allele model (M allele vs W allele)			Dominant model (MM + WM vs WW)			Recessive model (MM vs WW + WM)			Homozygous model (MM vs WW)			Heterozygous model (MM vs WM)		
	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
miR-146a G>C	0.93	0.82-1.05	0.230	0.89	0.70-1.13	0.339	0.90	0.79-1.04	0.152	0.82	0.64-1.07	0.140	0.92	0.80-1.07	0.269
miR-499 T>C	1.12	0.86-1.46	0.403	1.08	0.81-1.44	0.607	1.17	0.79-1.72	0.431	1.21	0.72-2.01	0.473	1.10	0.89-1.35	0.381
miR-218 A>G	1.05	0.85-1.28	0.665	1.15	0.85-1.57	0.368	0.94	0.63-1.40	0.749	1.04	0.67-1.64	0.850	0.88	0.58-1.34	0.551
miR-16-7c Ins/Del	1.59	1.23-2.06	<0.001	1.66	1.18-2.34	0.003	2.09	1.22-3.57	0.007	2.51	1.43-4.41	0.001	1.70	0.96-3.01	0.068
miR-106b-2.5 A>G	0.99	0.86-1.13	0.841	0.98	0.84-1.15	0.794	1.02	0.65-1.61	0.938	1.01	0.64-1.60	0.967	1.03	0.65-1.65	0.888
miR-34b/c T>C	1.26	1.05-1.51	0.012	1.37	1.08-1.75	0.011	1.29	0.88-1.90	0.195	1.50	0.99-2.25	0.052	1.11	0.74-1.68	0.603
miR-196a-2 C>T	0.84	0.53-1.33	0.461	0.83	0.52-1.35	0.457	0.76	0.35-1.64	0.479	0.70	0.27-1.81	0.456	0.91	0.68-1.23	0.548
miR-920 Ins/Del	0.72	0.57-0.91	0.007	0.51	0.31-0.85	0.010	0.72	0.52-0.99	0.046	0.46	0.27-0.78	0.004	0.80	0.57-1.13	0.209
miR-122 Ins/Del	1.26	1.13-1.39	<0.001	1.43	1.15-1.78	0.001	1.32	1.14-1.52	<0.001	1.61	1.28-2.04	<0.001	1.25	1.08-1.46	0.003

SNP = single nucleotide polymorphism; OR = odds ratio; 95%CI = 95% confidence intervals; W = wild allele; M = mutant allele; MM = mutant homozygote; WM = heterozygote; MM = mutant homozygote.

The meta-analysis results showed that miR-let-7c*Del variant was associated with increased liver cancer risk (Del allele vs Ins allele: OR = 1.59, 95%CI = 1.23-2.06, $P < 0.001$; Del/Del + Ins/Del vs Ins/Ins: OR = 1.66, 95%CI = 1.18-2.34, $P = 0.003$; Del/Del vs Ins/Ins + Ins/Del: OR = 2.09, 95%CI = 1.22-3.57, $P = 0.007$; Del/Del vs Ins/Ins: OR = 2.51, 95%CI = 1.43-4.41, $P = 0.001$) (Figure 2). Furthermore, miR-34b/c*C variant also showed significant association with increased liver cancer risk (C allele vs T allele: OR = 1.26, 95%CI = 1.05-1.51, $P = 0.012$; CC + TC vs TT: OR = 1.37, 95%CI = 1.08-1.75, $P = 0.011$). We also found that miR-122*Del variant could be a risk factor for liver cancer susceptibility (Del allele vs Ins allele: OR = 1.26, 95%CI = 1.13-1.39, $P < 0.001$; Del/Del + Ins/Del vs Ins/Ins: OR = 1.43, 95%CI = 1.15-1.78, $P = 0.001$; Del/Del vs Ins/Ins + Ins/Del: OR = 1.32, 95%CI = 1.14-1.52, $P < 0.001$; Del/Del vs Ins/Ins: OR = 1.61, 95%CI = 1.28-2.04, $P < 0.001$; Del/Del vs Ins/Del: OR = 1.25, 95%CI = 1.08-1.46, $P = 0.003$). Conversely, miR-920*Del variant was associated with decreased liver cancer risk (Del allele vs Ins allele: OR = 0.72, 95%CI = 0.57-0.91, $P = 0.007$; Del/Del + Ins/Del vs Ins/Ins: OR = 0.51, 95%CI = 0.31-0.85, $P = 0.010$; Del/Del vs Ins/Ins + Ins/Del: OR = 0.72, 95%CI = 0.52-0.99, $P = 0.046$; Del/Del vs Ins/Ins: OR = 0.46, 95%CI = 0.27-0.78, $P = 0.004$). However, miR-146a G>C, miR-196a-2 C>T, miR-499 T>C, and miR-218 A>G polymorphisms were not significantly associated with susceptibility to liver cancer (all $P > 0.05$) (Figure 3).

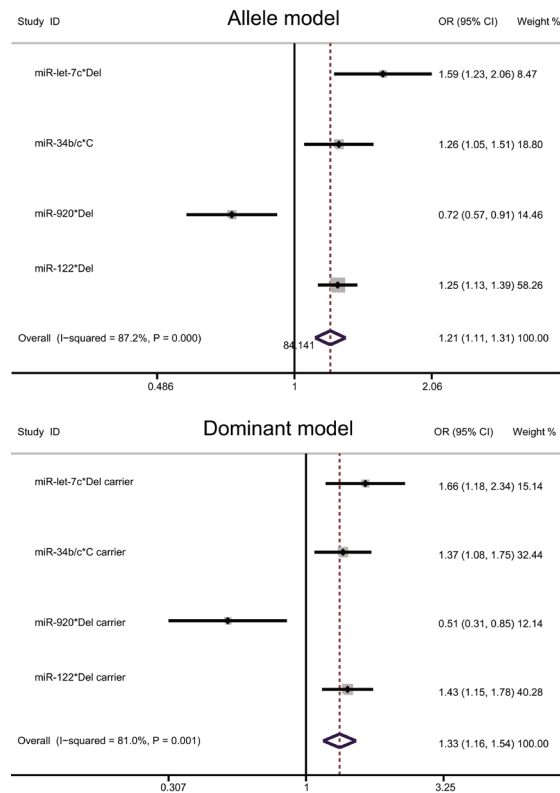


Figure 2. Forest plot of ORs for the associations of miR-let-7c Ins/Del, miR-34b/c T>C, miR-122 Ins/Del, and miR-920 Ins/Del with susceptibility to liver cancer.

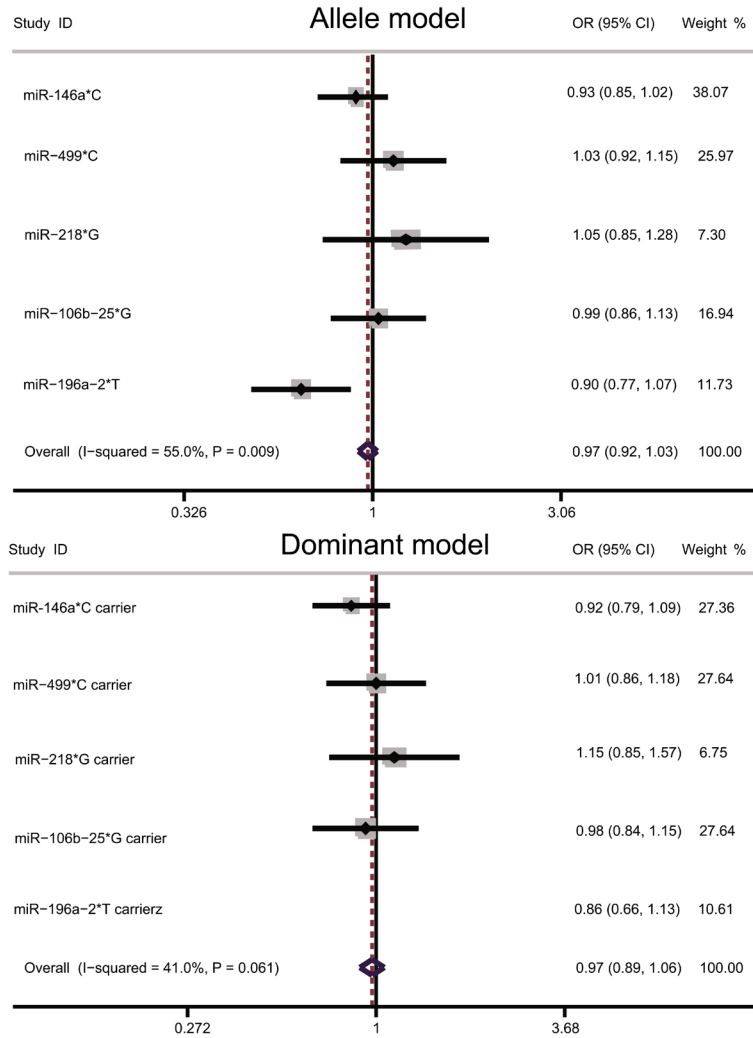


Figure 3. Forest plot of ORs for the associations of miR-146a G>C, miR-196a-2 C>T, miR-499 T>C, and miR-218 A>G with susceptibility to liver cancer.

In searching for possible factors that might have impacted the results, further subgroup analyses for two common SNPs miR-146a G>C and miR-499 T>C were performed based on the ethnicity and genotype method. The results also showed that there was no significant association between miR-146a G>C and miR-499 T>C polymorphisms and liver cancer risk (all $P > 0.05$) (as shown in Table 3).

Meta-regression and sensitivity analyses

Multivariate meta-regression analysis was used to explore the possible sources of heterogeneity among studies for miR-146a G>C and miR-499 T>C.

Table 3. Subgroup analyses for the associations of miR-146a G>C and miR-499 T>C with susceptibility to liver cancer.

Subgroup	Allele model (M allele vs W allele)			Dominant model (MM + WM vs WW)			Recessive model (MM vs WW + WM)			Homozygous model (MM vs WW)			Heterozygous model (MM vs WM)		
	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P	OR	95%CI	P
miR-146a G>C															
Asian	0.92	0.84-1.01	0.088	0.88	0.74-1.06	0.173	0.90	0.79-1.04	0.155	0.85	0.69-1.03	0.095	0.92	0.80-1.07	0.295
Caucasian	1.09	0.79-1.50	0.619	1.15	0.78-1.69	0.491	0.91	0.38-2.18	0.823	0.96	0.39-2.32	0.920	0.81	0.32-2.03	0.657
PCR-RFLP	0.88	0.78-1.00	0.055	0.82	0.66-1.02	0.072	0.88	0.72-1.06	0.182	0.71	0.54-0.93	0.013	0.94	0.77-1.15	0.546
PIRA-PCR	0.99	0.87-1.13	0.886	1.08	0.85-1.38	0.539	0.93	0.77-1.13	0.486	1.02	0.78-1.34	0.892	0.90	0.73-1.11	0.336
miR-499 T>C															
Asian	1.06	0.94-1.19	0.368	1.04	0.88-1.24	0.628	1.12	0.90-1.38	0.311	1.11	0.87-1.41	0.406	1.11	0.89-1.39	0.358
Caucasian	0.91	0.70-1.19	0.495	0.86	0.59-1.26	0.436	0.95	0.60-1.50	0.815	0.87	0.53-1.45	0.597	1.02	0.62-1.69	0.928
PCR-RFLP	1.18	0.93-1.35	0.250	1.11	0.87-1.41	0.610	1.22	0.84-1.75	0.298	1.23	0.83-1.83	0.302	1.18	0.79-1.77	0.413
PIRA-PCR	0.99	0.87-1.13	0.891	0.95	0.77-1.16	0.905	1.04	0.83-1.30	0.411	0.99	0.76-1.29	0.950	1.07	0.84-1.36	0.595

OR = odds ratio; 95%CI = 95% confidence intervals; W = wild allele; M = mutant allele; MM = mutant homozygote; MM = mutant homozygote; PCR = polymerase chain reaction; RFLP = restriction fragment length polymorphism; PIRA = primer-introduced restriction analysis.

The results showed that none of these five potential heterogeneity factors explained heterogeneity (as shown in Table 4). Sensitivity analyses were performed to assess the influence of each individual study on the pooled ORs by omitting individual studies in turn. The analysis results suggested that no individual study significantly affected the pooled ORs of miR-146a G>C and miR-499 T>C polymorphisms under the allele model (Figure 4), indicating statistically robust results.

Table 4. Multivariate meta-regression analyses for the associations of miR-146a G>C and miR-499 T>C with susceptibility to liver cancer.

Heterogeneity factors	Coefficient	SE	Z	P	95%CI	
					UL	LL
Publication year						
miR-146a G>C	-0.02	0.07	-0.32	0.753	-0.16	0.12
miR-499 T>C	0.12	0.11	1.12	0.262	-0.09	0.334
Country						
miR-146a G>C	0.11	0.07	1.47	0.142	-0.04	0.25
miR-499 T>C	0.29	0.26	1.15	0.251	-0.21	0.79
Ethnicity						
miR-146a G>C	0.04	0.23	0.18	0.860	-0.42	0.50
miR-499 T>C	-0.63	0.95	-0.66	0.506	-2.48	1.23
Genotype method						
miR-146a G>C	-0.28	0.23	-1.18	0.237	-0.73	0.18
miR-499 T>C	-0.04	0.63	-0.06	0.952	-1.27	1.19
STROBE score						
miR-146a G>C	-0.03	0.04	-0.85	0.397	-0.10	0.04
miR-499 T>C	-0.01	0.07	-0.09	0.931	-0.14	0.13

SE = standard error; 95%CI = 95% confidence interval; UL = upper limit; LL = lower limit.

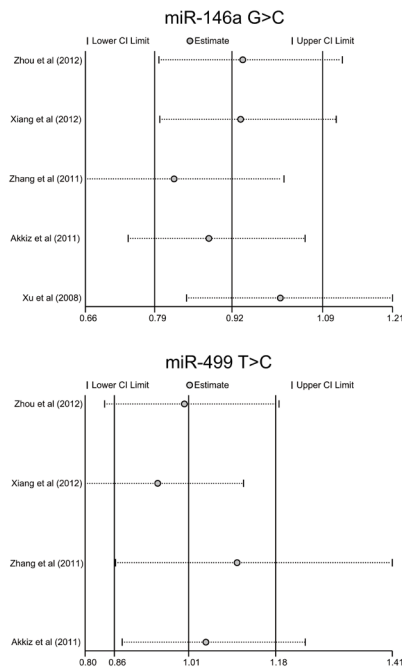


Figure 4. Sensitivity analysis of the pooled ORs on the associations of miR-146a G>C and miR-499 T>C with susceptibility to liver cancer under the dominant model. Results were computed by omitting each study in turn. The two ends of the dotted lines represent the 95%CI.

Publication bias

Publication bias within available research results may not be representative of all research results. Begger's funnel plot and the Egger linear regression test were performed to assess publication bias in the included studies. The shapes of the funnel plots of the association of miR-146a G>C and miR-499 T>C with liver cancer risk under the dominant model did not reveal any evidence of obvious asymmetry (Figure 5). The Egger test also did not indicate any statistically significant evidence of publication bias under the dominant model (miR-146a: $t = -1.12$, $P = 0.346$; miR-499: $t = 1.27$, $P = 0.331$).

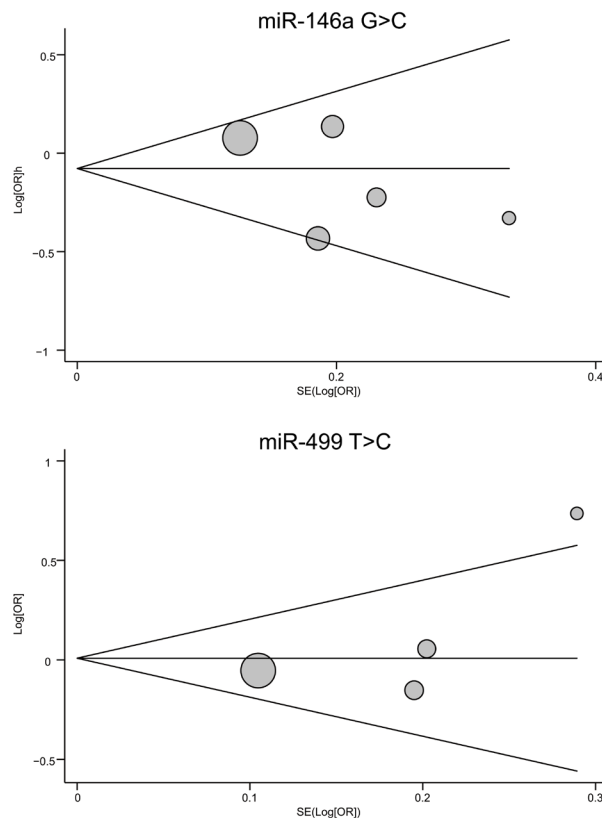


Figure 5. Begger's funnel plot of publication biases on the associations of miR-146a G>C and miR-499 T>C with susceptibility to liver cancer under the dominant model. Each point represents a separate study for the indicated association. Log[OR] = natural logarithm of OR. Horizontal line = mean magnitude of the effect.

DISCUSSION

Human miRNAs, as endogenous small non-coding RNAs, play a fundamental role in controlling a variety of biological functions, such as developmental patterning, cell differentiation, cell proliferation, genome rearrangements and transcriptional regulation (He and Hannon, 2004; Kota et al., 2009). A growing body of evidence has demonstrated that miRNAs

possess the functions essential for normal development and cellular homeostasis, and therefore, dysfunction of these molecules has been associated with several human cancers, including liver cancer (Ryan et al., 2010). Thus, genetic mutations in miRNAs may contribute to its abnormal expression and could probably be linked to liver cancer risk (Negrini et al., 2009). Several miRNA genes have been studied in the aspect of correlations with genetic susceptibility to liver cancer, involving miR-146a, miR-499, miR-218, miR-let-7c, miR-106b-25, etc. (Croce, 2009). These miRNAs have long been recognized as targets of genomic lesions, which can frequently activate oncogenes and inactivate tumor suppressors in liver cancer cells such as amplification, deletion, and epigenetic silencing through critical functions downstream of oncogene or tumor suppressor signaling pathways (Ferracin et al., 2010). Some previous case-control studies and meta-analyses have suggested that common genetic variants in miRNAs may play important roles in the development of liver cancer (Gao et al., 2009; Qi et al., 2010; Xu et al., 2011a; Guo et al., 2012; Liu et al., 2012b; Zhang et al., 2012), while other investigations have not found any convincing evidence of these polymorphisms in increasing susceptibility to liver cancer (Akkiz et al., 2011b,c; Qiu et al., 2011; Xu et al., 2011a; Zhang et al., 2011; Wang et al., 2012c; Zhou et al., 2012). This controversy could be explained by several reasons, such as the differences in study designs, sample size, ethnicity of subjects, source of controls, genotype methods, etc. Considering this controversy, we performed the present meta-analysis to provide a more comprehensive and reliable conclusion on the association between functional polymorphisms in miRNAs and susceptibility to liver cancer.

In this meta-analysis, 14 case-control studies were included with a total of 6824 liver cancer cases and 7674 healthy controls. When all the eligible studies were pooled into the meta-analysis, the results indicated that miR-let-7c*Del, miR-34b/c*C and miR-122*Del variants were associated with increased liver cancer risk, while miR-920*Del variant was associated with a decreased liver cancer risk. Although the exact function of miRNAs in liver cancer is not yet clear, a possible reason could be that inherited mutations in miRNAs are associated with changes in their expression and function and could thereby explain inter-individual differences in susceptibility to liver cancer (Adams et al., 2007). However, miR-146a G>C, miR-196a-2 C>T, miR-499 T>C, and miR-218 A>G showed no significant association with liver cancer risk. These findings are partially consistent with most previous studies and meta-analyses, indicating that these polymorphisms may not be major determinants or promoting factors in the development of liver cancer in humans. In subgroup analysis based on the ethnicity and genotyping method for two common SNPs miR-146a G>C and miR-499 T>C, we also found no significant association between these polymorphisms and liver cancer risk.

Similar to other meta-analyses, our study also bears some limitations and drawbacks. First, there were only fourteen articles included in the present meta-analysis, so the sample size was relatively small and may not provide sufficient statistical power. Therefore, more studies with a larger sample size are still needed to provide a more representative statistical analysis. Second, as a type of retrospective study, a meta-analysis may encounter recall or selection bias, possibly influencing the reliability of our study results (Ioannidis and Lau, 1999). Third, our lack of access to the original data from the studies limited further evaluation of potential interactions between other factors and liver cancer risks, such as gene-environment and gene-gene interactions (Dennis et al., 2011).

In conclusion, our meta-analysis suggests that miR-let-7c*Del, miR-34b/c*C and miR-122*Del variants may be associated with increased liver cancer risk, while miR-920*Del

variant may be a preventive factor against liver cancer. These relationships have the potential to provide functional profiling of miRNA genes involved in the development of liver cancer. Our meta-analysis may also serve as a guide for further studies in the diagnosis and clinical therapy of liver cancer. Considering the limitations mentioned above, detailed studies are still needed to confirm our findings. Further studies investigating the effect of gene-environment interactions on liver cancer are also required.

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