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Meta Analysis

Association between miR-499 rs3746444 and the susceptibility of hepatocellular carcinoma

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Abstract: Considering the inconsistent association between miR-499 rs3746444 and the risk of hepatocellular carcinoma (HCC), it is critical to carry out a meta-analysis in order to produce a precise result. Therefore, a meta-analysis was performed. All of the potential eligible studies were screened based on the following databases, including PubMed, EMBASE, Medline and China National Knowledge Internet (CNKI) up to December 2015. The associations between miR-499 rs3746444and HCC susceptibility was quantified using odds ratios (ORs) with its 95% confidence intervals (CIs). Nine case-control articles were included in the analysis. A total of 2593 cases and 3259 controls were included. The pooled OR was 1.27 (95% CI: 1.10 – 1.48, $P = 0.002$) which suggested thatmiR-499 rs3746444 was significantly associated with an increased the risk of HCC. Subgroup analysis was performed by ethnicity, miR-499 rs3746444 was significantly associated with an increased the risk of HCC in Asians (OR = 1.31, 95% CI = 1.10-1.55; $P = 0.002$). However, no significant result was found in Caucasians (OR = 1.10, 95% CI = 0.84-1.44; $P = 0.49$). In addition, miR-499 rs3746444 was significantly associated with an increased the risk of HCC in subjects with HBV infection OR = 1.31, 95% CI = 1.09-1.58; $P = 0.004$). This study suggested that miR-499 rs3746444 might play an important role in the development of HCC.

Key words: Hepatocellular carcinoma, Single nucleotide polymorphism, MicroRNAs.

Introduction

Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related death worldwide in men (1). Most of the burden of HCC is in developing countries, especially in East Asia and Africa with high incidence (>20 per 100,000 individuals) (2). The Insidious onset of early-stage HCC makes it difficult to diagnose, and a number of HCC patients have lost opportunity of surgery at the time of diagnosis due to intrahepatic or distant metastasis (3).

MicroRNAs (miRNAs) are endogenous, non-coding small regulatory RNAs of 19 to 24 nucleotides (nt) that bind to complementary target sites in the 3' untranslated region (UTR) of mRNAs, resulting in translational repression and/or mRNA destabilization (4). Mature miRNAs are derived from pri-miRNA precursors composed of hundreds or thousands of nt that constitute monocistronic or polycistronic transcriptional units (5). More attention has been paid to those factors which affect the disease in its early stage and it is estimated that genetic risk factors play important role in HCC (6). As a result of this, the identification of genetic risk factors enabled us to understand the disease mechanism in a sensible way.

Considering the inconsistent association between miR-499 rs3746444 and the risk of HCC (7-15), it is critical to carry out a meta-analysis in order to produce a consistent result. Therefore, a meta-analysis on all eligible related studies was performed to evaluate the association between miR-499 rs3746444and HCC susceptibility.

Materials and Methods

Search Strategies

All of the potential eligible studies were screened based on the following databases, including PubMed, EMBASE, Medline and China National Knowledge Internet (CNKI) up to December 2015 through advanced searching strategies. Three Mesh terms, "Hepatocellular carcinoma", "Single nucleotide Polymorphism", and "miR-499", were used to search for relevant articles. Systematic searching was performed using the combination of "Hepatocellular carcinoma", "Single nucleotide Polymorphism", and "miR-499" with their other eligible similar terms. Other additional studies were screened manually from the retrieved articles.

Study Selection and Data Extraction

The following four criteria were used to determine the inclusion of studies: (1) studies assessing the association between pmiR-499 rs3746444and HCC susceptibility; (2) case-control studies were based on human beings; (3) sufficient information were accessible, for instance, the study sample size for each research group, allele or genotype frequencies, effect sizes, and other useful information; (4) the diagnose of HCC should meet the clinical criterion set by the guidelines.

A predesigned data collection form was used by two independent reviewers in order to collect the following data: the name of first author, year of publication, country of research, ethnicity of study population, numbers of the case and control groups, HBV infection

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status. Finally, relevant studies were selected and key data were collected by two independent reviewers.

Statistical Analysis

The associations between miR-499 rs3746444and HCC susceptibility was quantified using odds ratios (ORs) with its 95% confidence intervals (CIs). The pooled ORs and 95% CIs were estimated by allelic models. Statistical heterogeneity among individual studies was inferred by Q test and I^2 statistic. These two heterogeneity tests were used to calculate the variability among individual studies and the combined I^2 metric was derived to assess the percentage of variation. If the *P* value of Q test was less than or equal to 0.05 and I^2statistic result was greater than or equal to 50% ($P_h \leq 0.05$ and $I^2 \geq 50\%$), then significant heterogeneity was presented in these studies. Consequently, a random effects model was appropriate for meta-analysis. Furthermore, Subgroup analyses were performed by ethnic groups (Caucasian and Asian) and HBV status in order to explore the effects of ethnicity and HBV on the association between gene polymorphism and the susceptibility to HCC. Publication bias was indicated by the funnel plot and plot asymmetry was confirmed by the rank correlation test. If the *P* value of rank correlation test was greater than 0.05, then there was no significant evidence suggesting publication bias and a symmetrical inverted funnel was approximately presented in the plot, otherwise it was not. The robustness of these statistical results was evaluated by the sensitivity analysis. A two-sided *P* value of 0.05 was selected as the significant level and all of the statistical analyses were performed using Reviewer Manager software (Version 5.1).

Results

Study Inclusion and Characteristics

A total of 17 articles were identified initially based on the predefined searching strategies. Then 3 of the 17 articles were excluded because of duplication and 14 articles were screened manually for potential available information. After that, 5 of 14 articles were further excluded for several reasons. Consequently, 9 case-control articles were included in the analysis (Figure1).

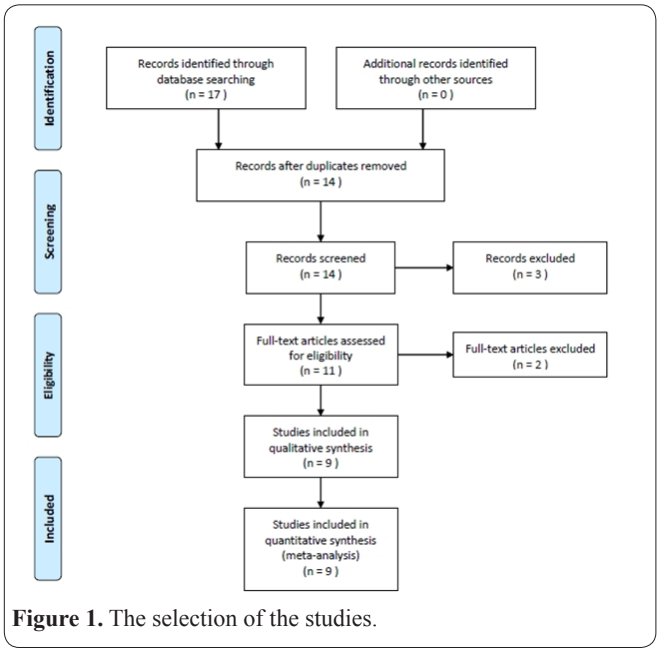

Figure 1. The selection of the studies.

The detailed characteristics of these studies were presented in the Table 1. All the research subjects came from Europe and Asia. A total of2593 cases and 3259 controls were included.

Meta-Analyses Results

The results of meta-analysis are presented in Figure 2. The pooled OR was 1.27 (95% CI: 1.10– 1.48, *P*= 0.002) which suggested thatmiR-499 rs3746444 was significantly associated with an increased the risk of HCC. Subgroup analysis was performed by ethnicity,

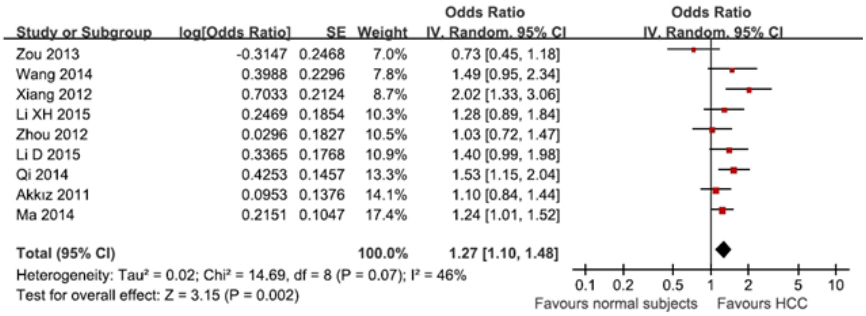

Figure 2. The forest plot ofmiR-499 rs3746444and HCC susceptibility.

Table 1. Characteristics of included studies for miR-499 rs3746444.

No.	Author	Year	Country	Ethnicity	Cases	Controls	HBV infection	OR (95% CI)	Hardy-Weinberg equilibrium
1	Akkız	2011	Turkey	Caucasian	222	222	Reported	1.10(0.84-1.44)	Yes
2	Zhou	2012	China	Asian	186	483	NR	1.03 (0.72-1.47)	Yes
3	Xiang	2012	China	Asian	100	200	Reported	2.20 (1.33-3.06)	Yes
4	Zou	2013	China	Asian	185	203	Reported	0.73(0.45-1.18)	Yes
5	Qi	2014	China	Asian	314	406	Reported	1.53 (1.15-2.04)	Yes
6	Ma	2014	China	Asian	984	991	Reported	1.24 (1.01-1.52)	Yes
7	Wang	2014	China	Asian	152	304	Reported	1.49 (0.95-2.34)	Yes
8	Li XH	2015	China	Asian	266	266	NR	1.28 (0.89-1.84)	Yes
9	Li D	2015	China	Asian	184	184	Reported	1.40 (0.99-1.98)	Yes

NR, not reported.

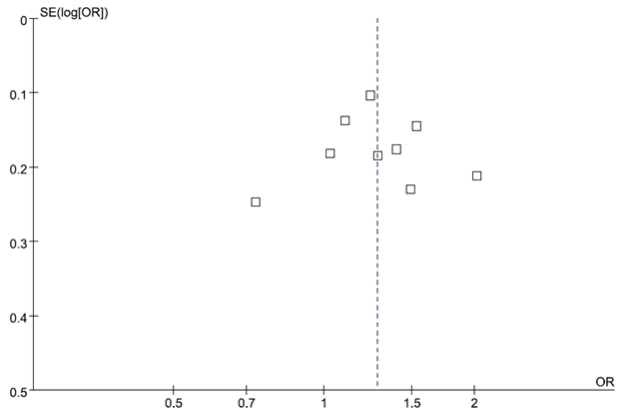

Figure 3. Funnel plot for publication bias.

miR-499 rs3746444 was significantly associated with an increased the risk of HCC in Asians (OR = 1.31, 95% CI = 1.10-1.55; *P* = 0.002). However, no significant result was found in Caucasians (OR = 1.10, 95% CI = 0.84-1.44; *P* = 0.49). In addition, miR-499 rs3746444 was significantly associated with an increased the risk of HCC in subjects with HBV infection OR = 1.31, 95% CI = 1.09-1.58; *P* = 0.004).

The funnel plots was constructed to assess the publication bias (Figure 3). *P* value of the rank correlation test for each polymorphism was greater than 0.05, which suggested there was no significant publication bias.

Discussion

HCC is one of the most common malignancies worldwide. There are no defined screening strategies, limited treatment options, high recurrence rates, and a very poor prognosis; as a consequence, HCC has been ranked the third leading cause of cancer-related death globally (16). Some studies have shown that multiple genomic changes occur during the development of HCC (17). Thus, it is important to study the role of genetic factors in hepatocarcinogenesis.

In this meta-analysis, we found that miR-499 rs3746444 was significantly associated with an increased the risk of HCC. Furthermore, Asians and HBV subjects with miR-499 rs3746444 had higher risk of HCC. There were many studies which reported the role of miR-499 rs3746444 in different diseases. Zhi et al. found that miR-499 rs3746444 may modulate the occurrence or prognosis in Chinese coronary artery disease (18). Yang et al. provided the first evidence that the SNP rs3746444 in pre-miR-499 could affect the inflammatory reaction in patients with rheumatoid arthritis (19). Hashemi et al. demonstrated that the hsa-mir-499 rs3746444, but not mir-146a rs2910164, polymorphism is associated with an increased rheumatoid arthritis risk in a sample of the Iranian population (20).

Some limitations should be acknowledged. Firstly, these results were based on unadjusted estimates that lack the original data from the eligible studies, which limited the evaluation of the effect of the gene-gene interaction during HCC development. Secondly, the subgroup analysis by ethnicity was limited by the small sample size in Caucasian. As a result of this, it is recommended that well-designed studies with large sample sizes should be performed. Genetic variants and other factors such as individual biological characteristics, environmental factors, particularly in Caucasian and Asian populations should be investigated together to assess the interaction between different factors which may significantly impact on the HCC development.

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