Review Article

Association between hsa-mir-149C>T (rs2292832) and susceptibility to hepatocellular carcinoma: a meta-analysis

Yuanyuan Zheng1*, Jingjing Li1*, Weiqi Dai1, Rong Zhang2, Jianrong Wang2, Wenxia Lu2, Yuqing Zhou3, Qin Yin3, Huerxidan Abudumijiti1, Rongxia Chen3, Kan Chen3, Sainan Li1, Tong Liu1, Fan Wang2, Yujing Xia1, Jie Lu1, Yingqun Zhou1, Chuanyong Guo1

1Department of Gastroenterology, Shanghai Tenth People’s Hospital, Tongji University School of Medicine, Shanghai 200072, China; 2Department of Gastroenterology, Shanghai Tenth People’s Hospital, Tongji University School of Medicine, Shanghai 200072, China; The First Clinical Medical College of Nanjing Medical University, Nanjing 210029, China; 3Department of Gastroenterology, Shanghai Tenth People’s Hospital, Tongji University School of Medicine, Shanghai 200072, China; The First Affiliated Hospital of Soochow University, Suzhou 215006, China. *Equal contributors.

Received October 15, 2015; Accepted July 10, 2016; Epub September 15, 2016; Published September 30, 2016

Abstract: miRNAs are a family of small non-coding RNAs that participate in diverse biological processes and play an important role in tumor progression. Single nucleotide polymorphisms miRNA genes may influence the expression and biological function of some miRNAs. In this meta-analysis, we calculated the odds ratio (OR) and 95% confidence interval (CI) for rs2292832 and its correlation with the risk of hepatocellular carcinoma (HCC) based on 1700 cases and 2044 controls. Our results suggest that rs2292832 is associated with the risk of HCC, TT/CT may play a conservative role in disease progression (T vs. C: OR=0.91, 95% CI=0.74-1.13, P=0.400, p_h=0.008; TT/CT vs. CC: OR=0.80, 95% CI=0.67-0.95, P=0.013, p_h=0.286; TT vs. CT/CC: OR=0.94, 95% CI=0.70-1.26, P=0.018; TT vs. CC: OR=0.77, 95% CI=0.62-0.95, P=0.014, p_h=0.068; CT vs. CC: OR=0.82, 95% CI=0.68-0.99, P=0.035, p_h=0.799; CT vs. CC/TT: OR=0.94, 95% CI=0.82-1.07, P=0.330, p_h=0.533).

Keywords: Hepatocellular carcinoma, hsa-mir-149C>T, rs2292832, miRNA polymorphism, meta-analysis

Introduction

Liver cancer is the fifth most frequent type of cancer, and accounts for almost 2.5% of deaths worldwide, together with other end-stage liver disease [1, 2]. Liver cancer can be divided into the following types [2, 3]: hepatocellular carcinoma (HCC), cholangiocarcinoma (CCA), combined or mixed HCC/CCA, hepatoblastoma, and others. HCC comprises most cases (70-85%) of liver cancer [1, 3]. With its rising incidence in many countries and a strong tendency towards hemorrhage, degeneration and necrosis [4], HCC mortality remains obstinately high. Early diagnosis can improve prognosis, so a proper process of surveillance with the aim of enhancing survival is actively being pursued. Currently, α-fetoprotein (AFP) serology, radiology and biopsy are the main diagnostic tests for HCC. AFP and ultrasound (US) surveillance shows a survival benefit over non-surveillance control, although for effective surveillance, AFP serology lacks adequate sensitivity and specificity [4, 5]. Radiology can help to assess the size, number, location and pathological changes in the nidus of tumors, however, contrast-enhanced US may confuse HCC and CCA [4], and contrast agents may have limitations in patients with renal insufficiency. Biopsy is considered a gold standard for diagnosis; it has some advantages in the diagnosis of small liver cancer, although it also has the risk of needle track seeding. Therefore, to screen for HCC as early as possible, optimized surveillance indicators are needed.

Genetic diagnosis has potential for screening the HCC-susceptible population, and may pro-
Association between hsa-mir-149C>T (rs2292832) and susceptibility to HCC

To date, we have seen major advances in diagnostic biomarkers [6-9] and drug therapy [10-15] for HCC, as well in other end-stage liver disease [16-18]. And genetic mutation may have great influence on the progress of HCC via different signalling pathway.

miRNAs are a family of endogenous non-coding RNAs of 19-25 bases. They participate in the translation and expression of hundreds of genes by combining specific mRNA sequences [19-21], thus having a close correlation with tumor occurrence, progression and prognosis. miRNAs participate in the regulation of many oncogenes and anti-oncogenes, and have a significant influence on a variety of tumors including HCC [14, 19, 20]. After intranuclear transcription, primary miRNAs (pri-miRNAs) are sheared into hairpin ring, structured precursor miRNAs (pre-miRNAs) by Drosha and transported to the cytoplasm by exportin-5/RanGTP. pre-miRNAs are matured by Dicer and combine with functional proteins to form RNA-induced silencing complexes, which regulate the translation and degradation of target mRNAs [21]. Single nucleotide polymorphisms (SNPs) in miRNA genes can contribute to dysfunction of miRNA processing or target binding by interfering pri-miRNAs, pre-miRNAs and mature miRNAs, which may influence tumorigenesis. Therefore, miR-SNPs have attracted our interest. rs2292832 is an SNP located on chromosome 2: 240456086, which is on the coding gene of hsa-mir-149 (chr2: 240456001-240456089 [+], miRBase), and the latter is modified into mature sequence hsa-mir-149-5p (hsa-miR-149) or hsa-mir-149-3p (hsa-miR-149*) to execute its specific biological function. There have been several case-control studies of hsa-mir-149C>T (rs2292832) and its association with HCC, although they have reported divergent opinions on whether it has an influence on the risk of HCC [22-26]. Here, we carried out a systematic analysis of this question.

Materials and methods

Search strategy

We searched Pubmed, EBSCO, Embase, Cochrane Library, Web of Science, Science Direct, Ovid, and Wiley Online Library, using the terms “(rs2292832 or miRNA-149 or microRNA 149) and (cancer or tumor or carcinoma)”. The latest data were from May 2015.

Inclusion and exclusion criteria

Duplicate studies were preliminarily excluded. Then, we ruled out studies that were: not about HCC or rs2292832 polymorphism; not in humans; not case-control original studies; or lacking detailed genotype and SNP phenotype data. Quality assessment was performed under a set of predetermined criteria [27, 28] (Table 1), articles were considered as “high quality” when scoring ≥ 12, and all the internalized studies in our meta-analysis were under the Hardy-Weinberg equilibrium expectation in control.

Data extraction

Data were collected by two independent investigators using the standard criteria in Table 1,
Association between hsa-mir-149C>T (rs2292832) and susceptibility to HCC

Table 2. Characteristics of case-control study

<table>
<thead>
<tr>
<th>Author</th>
<th>Year</th>
<th>Country</th>
<th>Ethnicity</th>
<th>HWE of control</th>
<th>Genotyping methods</th>
<th>Case/Control</th>
<th>Quality score</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kim</td>
<td>2012</td>
<td>Korean</td>
<td>Asian</td>
<td>0.345</td>
<td>PCR-RFLP</td>
<td>159/201</td>
<td>12</td>
<td>[23]</td>
</tr>
<tr>
<td>Kou</td>
<td>2014</td>
<td>Chinese</td>
<td>Asian</td>
<td>0.877</td>
<td>PCR-RFLP</td>
<td>270/532</td>
<td>12</td>
<td>[24]</td>
</tr>
<tr>
<td>Liu</td>
<td>2014</td>
<td>Chinese</td>
<td>Asian</td>
<td>0.054</td>
<td>PCR</td>
<td>327/327</td>
<td>15</td>
<td>[25]</td>
</tr>
<tr>
<td>Wang</td>
<td>2014</td>
<td>Chinese</td>
<td>Asian</td>
<td>0.863</td>
<td>Sequenom MassARRAY</td>
<td>944/984</td>
<td>16</td>
<td>[26]</td>
</tr>
</tbody>
</table>

Records identified through Pubmed [136], EBSCO [94], Embase [178], The Cochrane library [3], Web of science [104], Science direct [5451], Ovid [46] and Wiley online library [2322] up to May 2015.

18 studies were reviewed for a detail assessment.

13 records excluded:
11: meta-analysis
2: meeting abstract

1 record excluded: Not match Hardy-Weinberg equilibrium in control

4 studies internalized in this meta-analysis.

Figure 1. Flow chart of study identification.

and they reached agreement on all items. A third reviewer participated in the discussion to resolve any problems when discrepancies occurred. The following data were obtained from the eligible studies (Table 2): authors’ surname; publication year; country and ethnicity of the study population; sources of case-control; specimens and genotyping methods used for each study; total number and genotype distribution of study population; p value for the control of Hardy-Weinberg equilibrium (HWE).

Statistical analysis

STATA version 12.0 (Stata Corporation, College Station, TX, USA) was used for data analysis, and helped us understand the association between rs2292832 polymorphism and the risk of HCC. We calculated the odds ratio (OR) and 95% confidence interval (CI) for different types of genetic model, as described previously [29, 30]: allele model (T vs. C); dominant model (TT/CT vs. CC); recessive model (TT vs. CT/CC); homozygous model (TT vs. CC); heterozygous model (CT vs. CC); and complete over-dominant model (CT vs. CC/TT). Subgroup analysis was performed for different countries.

Stata commands “metan” and “metagen” were used to calculate the pooled OR (P<0.05 was considered of statistical significance) and decipher the most plausible genetic model [29]. We used the χ² test to calculate the p value of HWE in each control group of included studies, and P<0.05 was considered to show deviation from HWE. We use the χ²-based Q test to check the heterogeneity among eligible studies, and heterogeneity was considered present at P<0.05. Then, we used the random-effects model (Der Simonian-Laird method), or else we used the fixed-effects model (Mantel-Haenszel method) to calculate combined OR [31, 32]. A sensitivity analysis was carried out to establish the contribution of each study to the overall heterogeneity when it occurred (Stata command: meta inf). To determine whether there was a publication bias, we used Egger’s test and Begg’s funnel plot for consultation [33], and P<0.05 for Egger’s test was considered to show significant publication bias.

Results

Study characteristics

We searched Pubmed, EBSCO, Embase, Cochrane Library, Web of Science, Science Direct,
Association between hsa-mir-149C>T (rs2292832) and susceptibility to HCC

Ovid, and Wiley Online Library, and retrieved 136, 94, 178, 3, 104, 5451, 46, and 2322 records, respectively (Figure 1). We excluded articles that had unrelated titles and/or abstracts. Next, meta-analyses and meeting abstracts were removed. Four studies including 1700 cases and 2044 controls were used in our systematic analysis [23-26]. As shown in Table 1, all the included studies were of high quality and the frequency departures of the control in these studies are under the expectation of HWE. One of these four studies [23] was from Korea, while the other three [24-26] were from China, and we carried out a subgroup analysis by country to determine population-based heterogeneity.

Meta-analysis

There were 1700 cases and 2044 controls in our systematic analysis. The random-effects model were used for the allele model ($p_h=0.008$) and recessive model ($p_e=0.018$), as they both had an overall $P$ value for heterogeneity of $<$

Figure 2. Forest plot of hepatocellular carcinoma susceptibility associated with miR149 rs2292832 polymorphism in different genetic models. A. Allele model: T versus C; B. Dominant model: TT/CT versus CC; C. Recessive model: TT versus CT/CC; D. Homozygous model: TT versus CC; E. Heterozygous model: CT vs. CC; F. Complete overdominant model: CT vs. CC/TT.
Association between hsa-mir-149C>T (rs2292832) and susceptibility to HCC

0.05, whereas the fixed-effects model was used for the dominant model ($p_d = 0.286$), homozygous model ($p_h = 0.068$), heterozygous model ($p_h = 0.799$) and complete over-dominant model ($p_h = 0.533$) (Figure 2). There was a significant decrease in the risk of HCC for mutant genes versus wild-type among TT/CT versus CC (OR=0.80, 95% CI=0.67-0.95, $P=0.013$), TT versus CC (OR=0.77, 95% CI=0.62-0.95, $P=0.014$), and CT versus CC (OR=0.82, 95% CI=0.68-0.99, $P=0.035$). There was no significant decrease in overall OR for T versus C (OR=0.91, 95% CI=0.74-1.13, $P=0.400$), TT versus CT/CC (OR=0.94, 95% CI=0.70-1.26, $P=0.672$), and CT versus CC/TT (OR=0.94, 95% CI=0.82-1.07, $P=0.330$) (Figure 2).

In subgroup analysis based on countries, there was a strong association between rs2292832 polymorphism and HCC susceptibility in Chinese people (Figure 2): TT/CT versus CC (OR=0.78, 95% CI=0.65-0.93, $P=0.007$, $p_h = 0.302$), TT versus CC (OR=0.73, 95% CI=0.58-0.90, $P=0.004$, $p_h = 0.136$), and CT versus CC (OR=0.81, 95% CI=0.66-0.98, $P=0.030$, $p_h = 0.692$). There was no association between polymorphism and HCC risk in the genetic models of T versus C (OR=0.84, 95% CI=0.69-1.02, $P=0.086$, $p_h = 0.042$), TT versus CT/CC (OR=0.89, 95% CI=0.77-1.03, $P=0.108$, $p_h = 0.080$), and CT versus CC/TT (OR=0.96, 95% CI=0.84-1.11, $P=0.600$, $p_h = 0.754$). There was only one study on Korean people, therefore, we did not perform a similar analysis on this population.

Genetic model optimization

The Stata command metagen, which is based on logistic regression, was used to screen the optimized genetic model. We set “OR\text{CT vs. CC} = \text{OR\text{TT vs. CC}}” as the null hypothesis and achieved a supportable result for the hypothesis: OR\text{CT vs. CC} = 0.801, 95% CI=0.66-0.966, $P=0.020$; OR\text{TT vs. CC} = 0.794, 95% CI=0.652-0.967, $P=0.022$; $p_d = 0.118$; $p_h = 0.095$=$0.05$. As the null hypothesis was established and both of the ORs≠1, we selected the dominant model as the most optimized for rs2292832 polymorphism in HCC [29].

Publication bias

Egger’s test and Begg’s funnel plot were used to appraise the publication bias of the meta-data. As shown in Figure 3, there was no significant asymmetry under all genetic models using Begg’s funnel plot. Similarly, Egger’s test also did not find obvious bias among all the genetic models: T versus C ($t=0.18$, $P=0.872$), TT/CT versus CC ($t=0.46$, $P=0.693$), TT versus CT/CC ($t=0.01$, $P=0.991$), TT versus CC ($t=0.53$, $P=0.652$), CT versus CC ($t=1.00$, $P=0.423$), and CT versus CC/TT ($t=0.78$, $P=0.517$).

Sensitivity analysis

Using the Stata command metainf, we assessed the statistical robustness by deleting one study at a time to calculate the OR, and compared it with the original pooled OR. The results show that no individual studies significantly influenced overall OR (Figure 4).

Discussion

Modulation of miRNAs may have a critical influence on the progression of HCC, by regulating cellular differentiation, proliferation, apoptosis, invasion and metastasis; thus, some of them should be considered as oncogenes or tumor suppressor genes, which may be potential targets for diagnosis and therapy [14, 34]. hsa-mir-149 acts as an oncogene or tumor suppressor gene in different types of carcinoma. Ke et al. [35] found that miR-149 acted as a tumor suppressor by inhibiting expression of forkhead box M1 in non-small-cell lung cancer. Wang [36] also found that miR-149 inhibited proliferation and cell cycle progression in human gastric cancer by partially targeting the zinc finger and BTB domain holding 2 oncogenes (ZBTB2). Others showed that miR-149 suppressed apoptosis by directly regulating PUMA expression or through adjusting the mitochondrial network, and may act as an oncogene [37]. There are also inconsistent results on the function of miR-149* in different carcinomas. It may induce apoptosis of HeLa cells and the Be2C neuroblastoma cell line by inhibiting Akt1 and E2F1 [38]. In contrast, miR-149* increases the expression of Mcl-1 by targeting glycogen synthase kinase-3α and resistance to apoptosis in melanoma cells, thus, it functions as an oncogene and p53-responsive miRNA [39]. There are still gaps in our knowledge of how miR-149 and miR-149* work in HCC. One study found that miR-149 inhibited the proliferation and tumorigenicity of HCC by targeting the AKT/mTOR pathway [40]. This reminds us that SNPs
Association between hsa-mir-149C>T (rs2292832) and susceptibility to HCC

Figure 3. Begg's funnel plot for publication bias test. A. Allele model: T versus C; B. Dominant model: TT/CT versus CC; C. Recessive model: TT versus CT/CC; D. Homozygous model: TT versus CC; E. Heterozygous model: CT vs. CC; F. Complete overdominant model: CT vs. CC/TT. Each point represent a separate study for the indicated study. OR means odds ratio, Log OR is the nature logarithm of OR, and the horizontal line represent size of effect.
**Figure 4.** Sensitivity analysis for the influence on pooled OR by each study. A. Allele model: T versus C; B. Dominant model: TT/CT versus CC; C. Recessive model: TT versus CT/CC; D. Homozygous model: TT versus CC; E. Heterozygous model: CT vs. CC; F. Complete overdominant model: CT vs. CC/TT. Each study was omitted to calculate the specific OR, which presented by circles above. Dashed lines were used to represent each 95% CI correspondingly.
Association between hsa-mir-149C>T (rs2292832) and susceptibility to HCC

on miR-149 gene may lead to changes in its regulation pathway in HCC, either by affecting expression of miR-149/miR-149* or some other unknown pathways. In the present study, we found that rs2292832 polymorphism may have a significant influence on the risk of HCC, especially in the Chinese population. Compared to people with wild-type CC, those with TT/CT genotype had a lower risk of HCC in the dominant model, which may be the optimized model selected by logistic regression. In the homozygous and heterozygous models, our results also suggest that wild-type CC may have an association with higher susceptibility to HCC compared to TT and CT genotypes. In contrast, there was no significant difference in the allele, recessive and complete over-dominant models. The random-effects model was used to calculate the OR in the allele model for its p=0.008, and we could not reduce the heterogeneity to an acceptable range, even through subgroup analysis, so we can not overlook the associated error. Therefore, a large case-control study and further investigation of the pathway for how rs2292832 mutation influences HCC progression are still required.

Our meta-analysis had some limitations. First, although 1700 cases and 2044 controls were included, the sample size was still too small because we only had four studies in the meta-analysis. Second, although we did not find a single study that had a significant influence on the overall OR based on sensitivity analysis, heterogeneity remains in our genetic models. Third, there exist the bias that brought by language and grey literatures we couldn’t avoid. Fourth, we did not take into account the environmental influence on individual gene expression because of a lack of relevant data. Lastly, we did not draw any reliable conclusion on whether different genotyping methods would have influenced the test results.

In conclusion, hsa-mir-149C>T (rs2292832) may have a significant influence on the risk of HCC, and TT/CT may play a conservative role in disease progression. This may help us find an earlier screening method for HCC, which can improve prognosis. Further studies on the rs2292832 polymorphism and its relationship with HCC are still required.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (grant number 81270515), the Chinese Foundation for Hepatitis Prevention and Control (grant numbers WBN20100021, CFHPC20131011), and the Shanghai Municipal Health Bureau Foundation (grant numbers 2011287, 2012-107).

Disclosure of conflict of interest

None.

Address correspondence to: Chuanyong Guo and Yingqun Zhou, Department of Gastroenterology, Shanghai Tenth People’s Hospital, Tongji University School of Medicine, Shanghai 200072, China. E-mail: guochuanyong@hotmail.com (CYG); yqzh02@163.com (YQZ)

References

Association between hsa-mir-149C>T (rs2292832) and susceptibility to HCC


Association between hsa-mir-149C>T (rs2292832) and susceptibility to HCC


