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Review Article

Clinicopathological and prognostic values of miR-148b in hepatocellular carcinoma: a meta-analysis

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Abstract: Background: Previous studies show the prognostic value of miR-148b for hepatocellular carcinomas (HCCs), but its predictive value remains controversial. Methods: Here we investigated the correlation between miR-148b expression and HCCs, as well as the clinicopathological characteristics using meta-analysis. We comprehensively searched PubMed, EMBASE and Cochrane databases until April of 2016. Finally, only six articles reporting-miR-148b expression in HCCs were included. Results: The pooled risk ratio (RR) and 95% confidence interval (CI) of miR-148b expression is 1.24 (0.86, 1.79) with no statistical significance at T stage, but is 2.97 (1.73, 5.10) in vein invasion with statistical significance. Giventhe AJCC stage, the pooled RR and its 95% CI is 1.80 (1.34, 2.41) for miR-148b expression with statistical significance, butis 1.42 (0.95, 2.11) for tumorgrade with no statistical significance. As for overall survival, the pooled RR and its 95% CI is 1.40 (1.25, 1.58) with statistical significance. Conclusions: Low expression of miR-148b shows a significant value for prognosis of HCC.

Keywords: miR-148b, HCC, meta-analysis

Background

Hepatocellular carcinoma (HCC) is a global health problem. In 2008, HCC was diagnosed in more than 500,000 people and caused about 500,000 mortalities [1]. Carcinogenesis of HCC is a multistep process through accumulation of genetic and epigenetic alterations. Althoughthe risk factors for HCC have been well characterized, its molecular pathogenesis is largely unknown [2, 3].

MicroRNAs (miRNAs) are small non-coding RNAs that regulate the expressionsof target gene by binding to the 3'-untranslated region (3'-UTR) of their target mRNAs and result in either mRNA degradation or translationalrepression. MiRNAs play essential roles in many bioprocesses, including cell proliferation, apoptosis, differentiation and stress resistance [4, 5]. The miR-148/152 family has three members (miR-148a, miR-148b and miR-152), and after maturation, they have the same seed sequence of about 8 nucleotides, which are processed from the pre-miR-148/152 family that owns a common stem-loop structure [6]. Therefore, the miR-148/152 members might play important roles in cellular bioprocesses by binding to the 3'UTR of the target mRNAs via their mutual seed sequence.

MiR-148b is downregulated in various cancers, including colon, oral, pancreatic and gastric cancers [7, 8], indicating it plays akey role as a tumor-suppressor miRNA. MiR-148b hasthe potential ability to suppress tumors in HCC patients and has a prognostic value in clinical evaluations. However, it is unclear whether the-miR-148b expression is associated with the prognosis of HCC. A comprehensive analysis of the various outcomes is warranted. Here we present a meta-analysis evaluating the prognostic value of miR-148b expression in HCC. We aimed to estimate the correlations of miR-148b with prognostic prediction and overall survivalin HCC patients.

Materials and methods

Two authors carried out the search independently. All relevant articles on PubMed, Embase and Cochrane databases were searched using different combinations of keywords "miR-

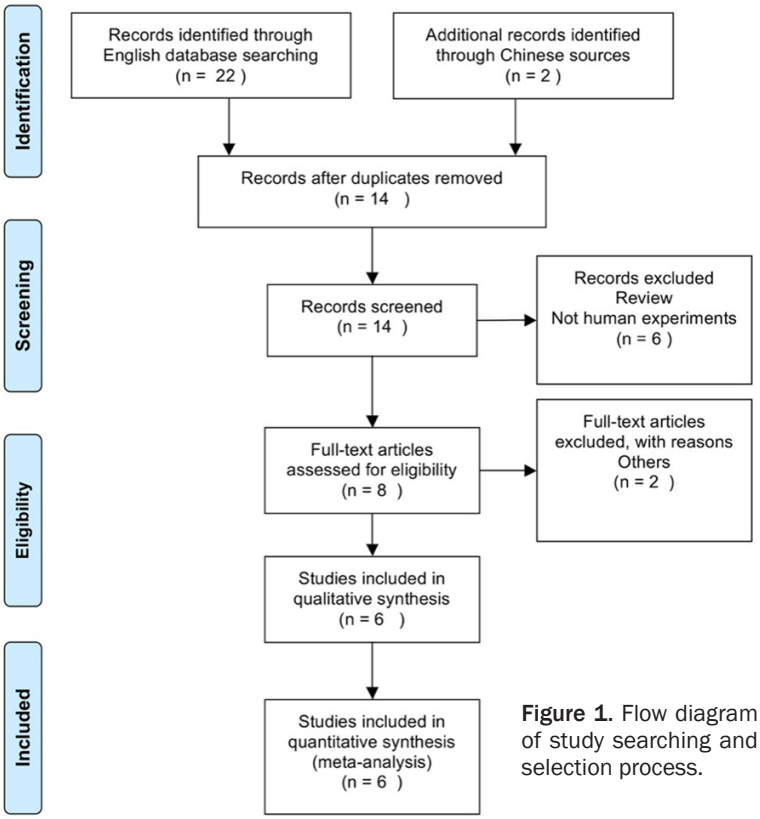

Figure 1. Flow diagram of study searching and selection process.

148b", "miR-148", "hepatocellular carcinoma" and "liver tumor". The titles and abstracts of potential references were carefully examined to exclude irrelevant studies. The remaining articles with the topic of interest were reviewed in depth for their relevance.

Selection criteria

The inclusion criteria were (a) focus on hepatocellular carcinoma, (b) description of correlations of miR-148b expression withtumor node metastasis (TNM) stages and clinicopathological characteristics. The exclusioncriteria were (a) reviews or letters; (b) insufficient data to determine the HR/RR and CI of HCC.

The quality of each included study was determined based on six key points: clear definitions of (a) study population and origin of country, (b) study design, (c) outcome assessment, (d) cutoff of miR-148b expression and (e) miR-148b assessment method, as well as (f) sufficient follow-up time.

Data extraction

All data were extracted by two authors in dependently. The quality of each included article was assessed according to the Newcastle-Ottawa Scale (NOS) [9]. Data tables were generated to extract all relevant data from texts, tables and figures, including author, year of publication, country, patient number, medium follow-up, TNM stage, vein invasion, follow-up duration, and positive rate of miR-148b expression. If anarticle provided the HR/RR with 95% CI, we used the data; otherwise, we calculated the HR and 95% CI using Kaplane Meier survival curves and Engauge Digitizer 4.1 (http://digitizer.sourceforge.net/). To reach a consensus, we resolved any disagreement on a conflicting study through complete discussion.

Statistical analysis

HR/RRs with 95% CIs were pooled according to the status of miR-148b expression. Heterogeneity across the included studies was assessed with a forest plot and the inconsistency statistic (I^2). The heterogeneity among studies was measured using the Q and I^2 tests. $P < 0.1$ and $I^2 \geq 50\%$ indicated significant heterogeneity [10]. In case of no significant heterogeneity among studies, the pooled HR/RRs of each study were calculated by afixed-effects model with Mantel-Haenszel method; otherwise, arandom-effects model with Inverse Mantel-Haenszel method was adopted. The pooled HR/RRs ofoverall survivalwere calculated by a fixed-effects model with Inverse Variance method. All calculations were performed on RevMan 5.3 (Nordic Cochrane Centre, Copenhagen, Denmark). All CIs had 2-sided probability coverage of 95%. $P < 0.05$ was considered significant.

Results

Search results

Figure 1 shows the meta-analysis search strategy and selection process. In all, 24 studies in the first search seemed to be potentially rele-

Table 1. Characteristics of included studies

No.	Included Studies	Countires	Number	Sex (male/female)	T Stage (T1-2/T3-4)	Vein invasion (+/-)	AJCC Stage (I/II- III/IV)	Grade (well + Moderate/poor)	Liver cirrhosis (+/-)	HBV infection (+/-)
1	Zhiyong Zhang 2014	China	156	100/56	85/71	36/120	91/65	105/51	142/12	132/24
2	Katayoun Ziari 2015	Iran	101	66/35	55/47	20/81	58/43	63/38	93/8	N/A
3	Yasan Sadeghian 2015	Iran	96	60/36	50/46	22/74	57/39	N/A	30/66	N/A
4	Feng Wang 2015	China	76	66/10	33/43	N/A	29/47	42/34	58/18	55/21
5	Yuqing Hao 2015	China	60	22/10	34/26	21/39	N/A	27/33	N/A	47/13
6	Jun-gang Zhang 2015	China	40	27/13	15/25	N/A	N/A	20/20	N/A	25/15

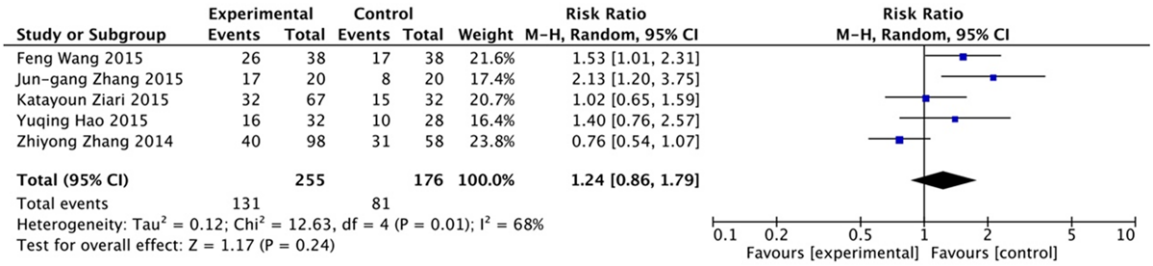

Figure 2. Meta analysis of miR-148b expression and T stage. Vertical line indicated no difference between the compared two groups. Squares indicated point estimates of risk ratio (RR) in each individual study, the size of the squares indicated the weight of the corresponding study in the meta-analysis, 95% CIs of point estimates were shown by horizontal lines. Pooled RR and its 95% CI were shown by diamond shape. (It was the same in **Figures 3-8**).

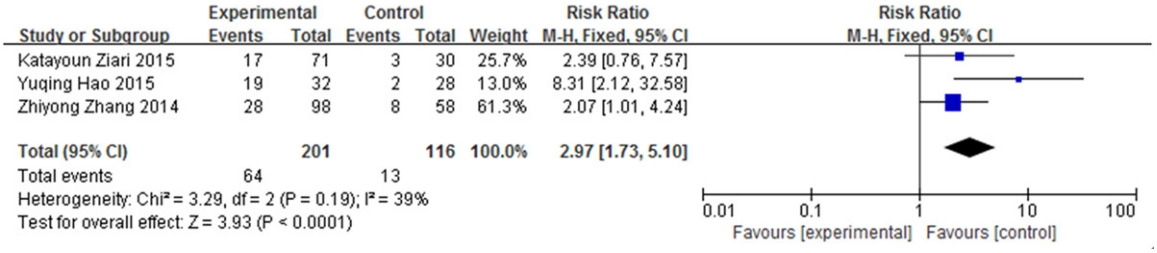

Figure 3. Meta analysis of miR-148b expression and vein invasion.

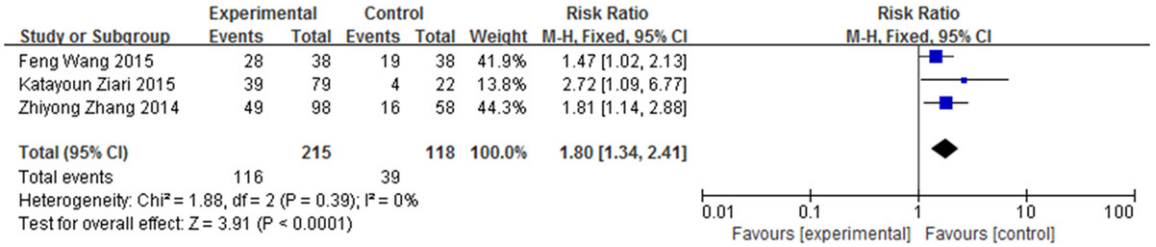

Figure 4. Meta analysis of miR-148b expression and AJCC stage.

vant. Ten duplicates were removed, and 5 studies were excluded (not human experiment) after initial screening of titles and/or abstracts. And 1 review and 2 studies not providing enough data for analysis were excluded. The remaining 6Chinese or English articles were included in the meta-analysis (**Figure 1**).

Description of studies

The flow diagram for screening and identification of relevantstudies is shown in **Figure 1**. **Table 1** shows the characteristics of the included studies [11-14], involvinga total of 529 patients. The TNM stage, tumor grade and vein invasion were reported in 6, 4 and 4 studies, respectively. The quality of the enrolled studies varied from 6 to 8, with a mean of 7.17. All of the studies utilized tissue quantitative reverse-transcription polymerase chain reaction (qRT-PCR) for miR-148b expression.

Correlation between miR-148b expression and clinicopathological characteristics of HCC

The correlations of miR-148b expression with overall T category, vein invasion, American Joint Committee on Cancer (AJCC) stage and tumor grade are illustrated in **Figures 2-5**. **Figures 6, 7** demonstrate the relationships of miR-148b withliver cirrhosis and hepatitis B virus (HBV) infection. **Figure 8** presents the correlation

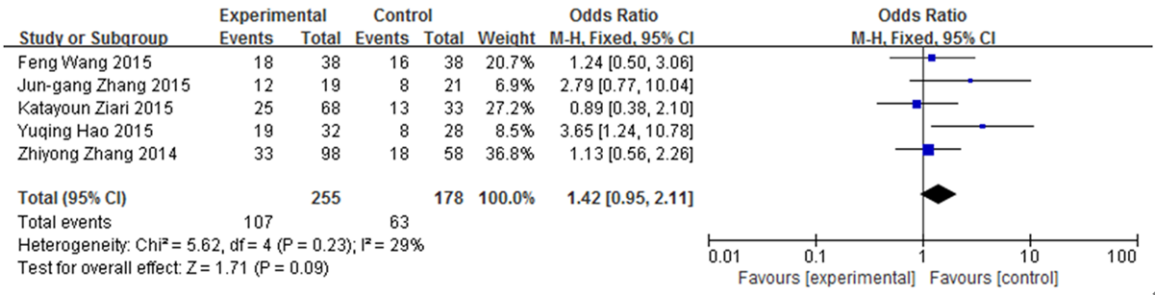

Figure 5. Meta analysis of miR-148b expression and tumor grade.

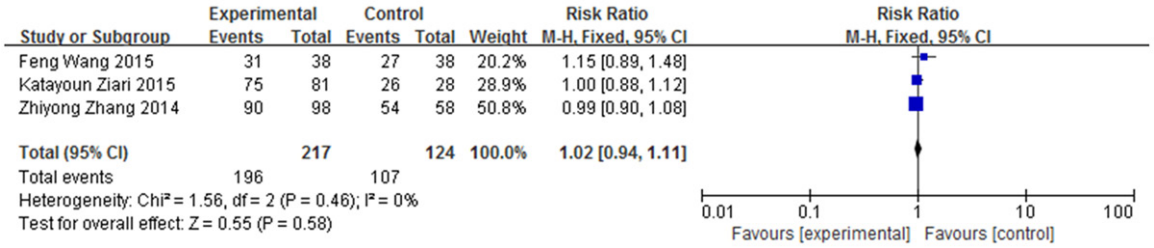

Figure 6. Meta analysis of miR-148b expression and liver cirrhosis.

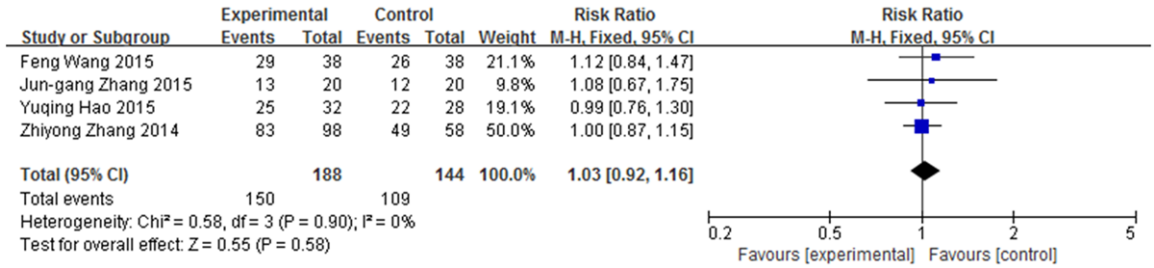

Figure 7. Meta analysis of miR-148b expression and HBV infection.

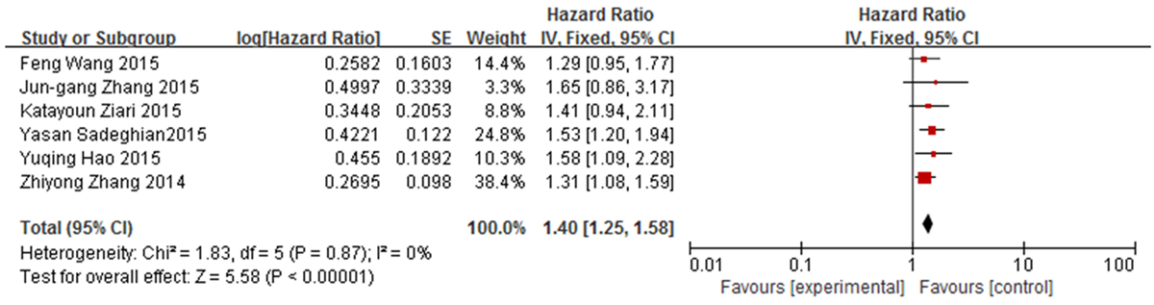

Figure 8. Meta analysis of miR-148b expression and overall survival in HCC.

between miR-148b expression andoverall survival.

Figure 2 demonstrates the category of low miR-148b expression compared to the category of high miR-148b expression with T stage in HCC. The random-effects model was applied, with the pooled RR and its 95% CI being 1.24 (0.86, 1.79), without statistical significance ($P > 0.05$).

Considering the vein invasion of low-miR-148b-expression patients compared to high-

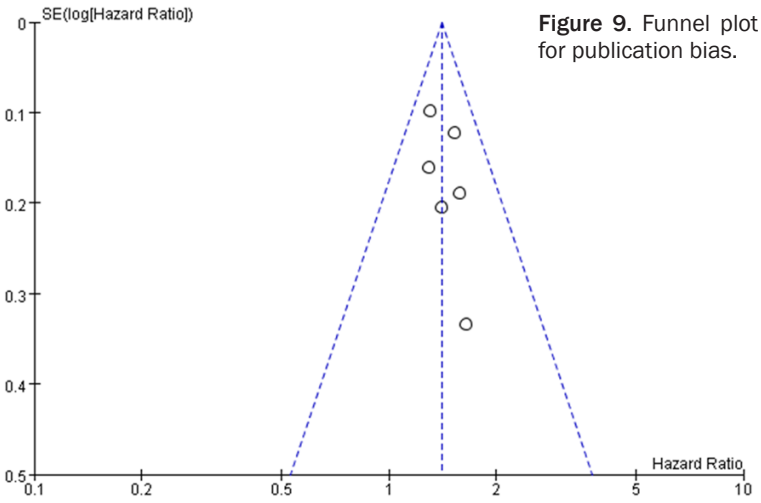

Figure 9. Funnel plot for publication bias.

miR-148b-expression patients, the pooled RR (95% CI) is 2.97 (1.73, 5.10) with statistical significance ($P < 0.05$) (**Figure 3**).

Consistently, the pooled RR (95% CI) is 1.80 (1.34, 2.41), with statistical significance ($P < 0.05$) for miR-148b downregulation in the AJCC stage (**Figure 4**).

Regarding tumor grade with miR-148b expression, we utilized the pooled RR (95% CI) was 1.42 (0.95, 2.11) with statistical significance ($P < 0.05$) in **Figure 5**, showing low miR-148b expression compared with the high expression in tumor grade.

Correlation of miR-148b expression to liver cirrhosis and HBV infection

Considering the relation between miR-148b expression and liver cirrhosis or HBV infection, the pooled RR (95% CI) was 1.02 (0.94, 1.11) without statistical significance ($P > 0.05$) for miR-148b expression with liver cirrhosis (**Figure 6**).

Consistently, the pooled RR (95% CI) is 1.03 (0.92, 1.15) with no statistical significance ($P < 0.05$) for miR-148b expression with HBV infection (**Figure 7**).

Overall survival and miR-148b expression

Moreover, the miR-148b expression of overall survival (low expression vs. high expression) is significantly correlated with the pooled RR (95% CI) is 1.40 (1.25, 1.58) ($P < 0.05$) (**Figure 8**).

Publication bias

The funnel plot does not show any evidence of obvious asymmetry for overall survival in comparison between high and low miR-148b expressions (**Figure 9**).

Discussion

HCC is the mostcommon primary liver cancer that is the third cause of tumor-associated mortality worldwide [15]. Dysregulation of miRNA contributes to tumor prognosis [16]. The frequent aberrant-miRNA expression implies a tumor suppressor or oncogenefunction [17]. MiR-148b has been suggested to be upregulated in ovarian cancer [18]. However, miR-148b is downregulated in pancreatic cancer [7]. These controversial findingsmay reflect the diverse roles of miR-148b in different types of cancers. Despite the low survival rate of HCC, identifying new prognostic markers and modifying staging systems can improve the prognostic assessment of HCC and clinically satisfy personalized prescriptionin particular by biomarkers that reflect tumor aggressiveness.

It is increasingly indicated that miR-148b is a major coordinator of malignancy or an independent prognostic marker since its expression is closely associated with tumor invasion and progression in both breast cancer and liver cancer [19]. The potential mechanism is partially due to the miR-148b regulation of WNT1/-catenin signaling pathway in the proliferation and invasionof HCC cells [17]. Another research also pointed out miR-148b could affect the expressions of EMT-related genes and increase the metastasis and angiogenesis of HCC by targeting neuropilin-1 [20].

For HCC patients, the association of the miR-148b expression and their prognosis remains unclear. A meta-analysis incorporating all available data from correlative studies is a reasonable solutionto this question. We conducted this study and found that HCC patients with low miR-148b expression had significantly more vein invasion, unfavorable AJCC stage and overall survival than those with high expres-

sion. Our findings indicate that a lower miR-148b expression is correlated with poor HCC prognosis. All these results confirm that miR-148b has a profoundly adverse prognostic impact on HCC patients.

To the best of our knowledge, this is the first study that comprehensively answers the impact of miR-148b status on the prognosis of HCC patients. However, there are several limitations. First, this meta-analysis was based on the dataindirectly extracted from the survival curves, which somehow compromised the precision of data. In addition, researchers might prefer to only report the positive results of the prognostic biomarker, which led to potential publication bias. In addition, few studies evaluated miR-148b simultaneously, which prevented the insightful explanation of mechanism. Further studies are warranted to complete the above information. Regardless of the above limitations, this comprehensive analysis statistically confirmed that HCC patients with abnormal miR-148b expression were associated with significant vein invasion, AJCC stage and overall survival. The results indicate that miR-148b downregulation may be an independent prognostic factor in HCC patients. It can be a prognostic marker and has predictive value for poor prognosis in HCC patients. In addition, miR-148b expression wasnot associated with liver cirrhosis or HBV infection in HCC patients.

Conclusions

The current evidence first shows that a decreased miR-148b expression is a negative predictor of survival in HCC patients. More larger-size multi-center studies are needed to present more reliable dataaboutthe clinical relevance and precise molecular explanation for the abnormal miR-148b expression.

Disclosure of conflict of interest

None.

Authors' contributions

BXJ wrote the first draft of the manuscript and contributed to the data collection and analysis. TLD and JZ contributed to the data collection. LYH and SLK participated in the manuscript drafting, revising, and study design. All authors read and approved the final manuscript.

Abbreviations

HCC, Hepatocellular Carcinoma; AJCC, American Joint Committee on Cancer; TNM, Tumor node metastasis.

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