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High expression of miR-21 is not a predictor of poor prognosis in all patients with hepatocellular carcinoma

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Abstract. MicroRNA (miR)-21 has been identified as a novel biomarker of hepatocellular carcinoma (HCC). However, the prognostic value of miR-21 expression in HCC remains controversial. The aim of the present study was to investigate the value of high expression of miR-21 in predicting the prognosis of HCC. Following a search through the PubMed, Science Citation Index, EMBASE and CNKI databases, a total of 9 studies investigating the expression of miR-21 in HCC and the association between high expression of miR-21 and prognosis of HCC were identified. It was observed that high expression of miR-21 was not associated with poor overall survival of all patients with HCC (P=0.52). However, high expression of miR-21 was found to be correlated with poor prognosis of HCC patients undergoing curative resection (hazard ratio = 2.36; P<0.01). It was also demonstrated that high expression of miR-21 was correlated with tumor size >5 cm [odds ratio (OR)=1.53; P=0.04], venous invasion (OR=4.86; P=0.01), TNM stage (OR=3.44; P<0.01) and liver cirrhosis (OR=2.12; P=0.03). It was concluded that miR-21 cannot be considered as a factor complementary to α-fetoprotein, microvascular invasion and advanced tumor stage in predicting the prognosis of all HCC patients. Higher expression of miR-21 may be a promising biomarker associated with certain clinicopathological characteristics of HCC, such as tumor size, venous invasion, TNM stage and liver cirrhosis.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common types of cancer worldwide, and ranks second as a cause of cancer-related mortality in China (1). The majority of the patients are diagnosed at an intermediate or advance stage owing to lack of surveillance. Currently, the Barcelona Clinic Liver Cancer (BCLC) staging system is the most common staging system for HCC. BCLC divides HCC into three stages, namely early, intermediate and advanced (2,3). According to the BCLC recommendations, intermediate and advance HCC are not candidates for curative resection, indicating a relatively poor prognosis of these patients (4). Therefore, it is crucial to identify new biomarkers for early diagnosis and prediction of prognosis of HCC.

MicroRNAs (miRs) are a class of non-coding RNAs (18-25 nucleotides) that bind to the 3'-untranslated region and regulate translation at the post-translational level (5). More than 3,700 miRs have been registered in the miRBase to date (6). Over the past decades, miRs have been found to play key roles in tumorigenesis, cancer progression and metastasis. In particular, miR-21 expression has been found to be upregulated in breast, lung and cervical cancer, as well as in HCC (7), and numerous studies have investigated the association between miR-21 expression and the prognosis of HCC (8-10). miR-21 is considered as a promising biomarker and treatment target in HCC, and several systematic reviews have investigated the association between miR-21 expression and the prognosis of HCC. Wang *et al* investigated the diagnostic and prognostic value of miR-21 in cancer, and observed that high expression of miR-21 was significantly correlated with poor prognosis of cancer patients; however, only two studies in their analysis reported survival data from HCC patients (11). Yan *et al* reported that miR-21 maybe complementary to α-fetoprotein (AFP) in the diagnosis of HCC, and that high expression of miR-21 indicated poor prognosis of HCC (12); however, only 4 studies were included in their review, which limits the reliability of their conclusions. Taking into consideration that different investigators reported inconsistent information on the role of miR-21 in HCC, the present systematic review was performed to investigate the value of miR-21 expression in predicting the prognosis of HCC.

Data collection methods

Search strategy and study selection. 'miR-21' or 'microRNA-21' and 'hepatocellular carcinoma' or 'HCC' were used as mesh terms to search the PubMed, Science Citation

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Table I. Quality assessment of the included studies.

Study	Selection of cohort	Comparability	Ascertainment of the outcome	Total score	(Refs.)
Wang *et al*	3	2	3	8	(25)
Huang *et al*	2	1	2	5	(26)
Wang *et al*	2	1	3	6	(27)
Wang *et al*	2	1	3	6	(28)
Liu *et al*	2	1	2	5	(29)
Gyöngyösi *et al*	2	1	3	6	(30)
Karakatsanis *et al*	3	1	3	7	(10)
Tomimaru *et al*	3	2	3	8	(9)
Tomimaru *et al*	2	1	3	6	(8)

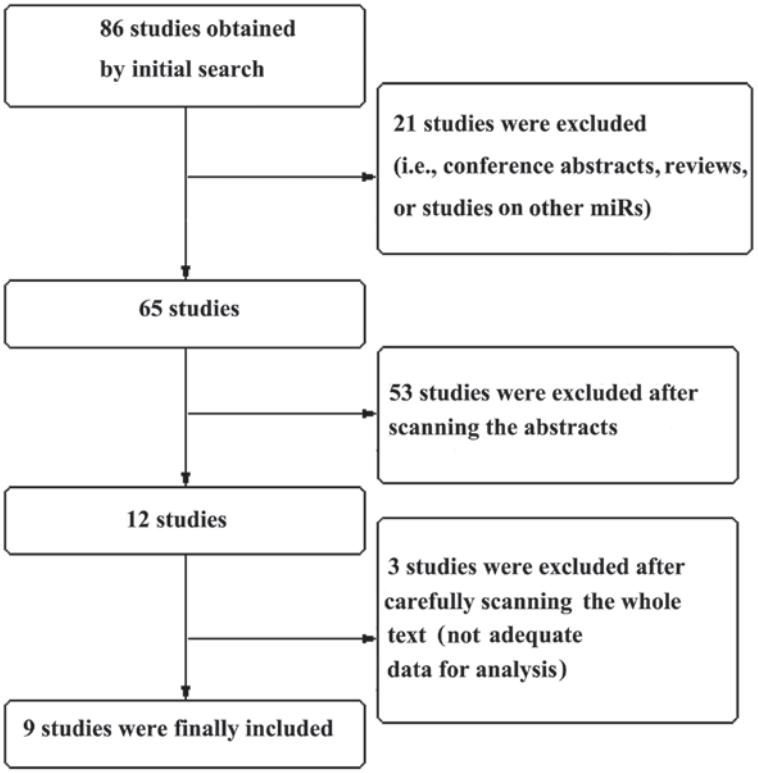

Figure 1. Flow diagram of study selection process. miR, microRNA.

Index, EMBASE and CNKI databases. There was no limitation in terms of publication year and region, but the language was limited to English. The cited references of the obtained articles were also scanned to identify additional relevant studies.

Studies were included if they met the following criteria: Studies on the role of miR-21 expression in patients with HCC; and studies with adequate data on survival outcome and clinical characteristics. Studies were not eligible if they were reviews, letters, or conference abstracts. Studies designed on animals or cell lines, and studies without adequate data for survival analysis, were also excluded. The study selection process is summarized in Fig. 1.

Quality assessment. Two investigators independently assessed the quality of the included studies; if a disagreement emerged, a third investigator was contacted. The quality of the studies included in the meta-analysis was evaluated based on the Newcastle-Ottawa scale (13). Three aspects were generally assessed: Selection of the cohort, comparability of the cohort and ascertainment of the outcome (Table I). A study with a score of >5 was considered as being of high methodological quality (14).

Data extraction. Two investigators independently extracted baseline characteristics from each study, including study name, publication year, country, patient number, tumor stage, specimens, RNA detection method and cut-off value of miR-21 expression. Survival data and clinical characteristics were also extracted. The survival outcome was measured by overall survival (OS) rate, and the clinical characteristics included tumor size, tumor number, differentiation, venous invasion,

Table II. Baseline characteristics of the included studies.

Study	Year	Country	Stage	Patient no.	Specimen	Method	Cut-off value	(Refs.)
Wang *et al*	2015	China	TNM I-IV	97	Serum	RT-PCR	Median	(25)
Huang *et al*	2015	China	TNM I-IV	112	Tissue	RT-PCR	Median	(26)
Wang *et al*	2014	China	TNM I-IV	119	Tissue	RT-PCR	Median	(27)
Wang *et al*	2014	China	TNM I-IV	30	Serum	RT-PCR	5-fold of control	(28)
Liu *et al*	2014	China	BCLC A-C	136	Serum	RT-PCR	Median	(29)
Gyöngyösi *et al*	2014	Italy	NR	20	Tissue	RT-PCR	Median	(30)
Karakatsanis *et al*	2013	Greece	TNM I-IV	60	Tissue	RT-PCR	3.07-fold of control	(10)
Tomimaru *et al*	2012	Japan	TNM I-IIIa	126	Plasma	RT-PCR	0.754	(9)
Tomimaru *et al*	2010	Japan	BCLC C	30	Tissue	RT-PCR	Median	(8)

TNM, tumor-node-metastasis; BCLC, Barcelona Clinic Liver Cancer; RT-PCR, reverse transcription-polymerase chain reaction.

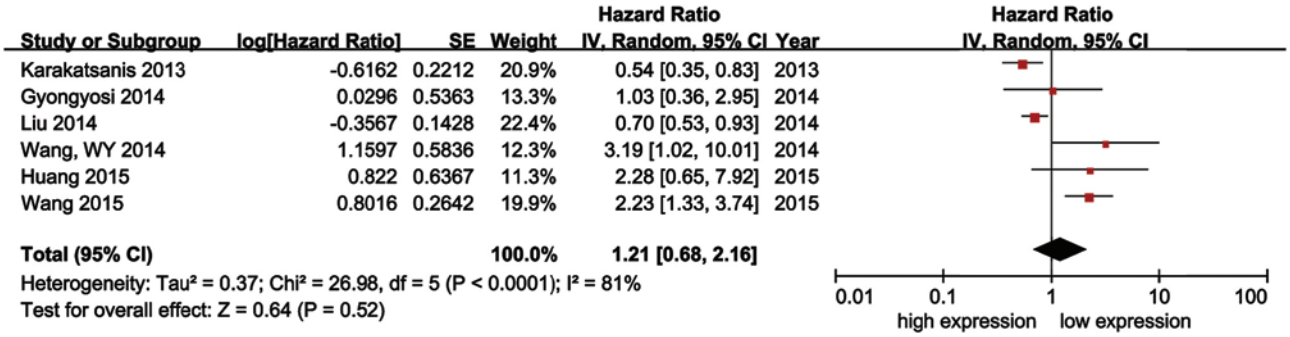

Figure 2. Analysis of correlation between miR-21 expression and overall survival of all patients with hepatocellular carcinoma. miR, microRNA; SE, standard error; CI, confidence interval.

TNM stage and liver cirrhosis. Differentiation was classified as high or low (moderate differentiation was classified as low), and the TNM stage was divided into stages I+II and III+IV.

Statistical analysis. Prognostic efficacy was measured by pooled hazard ratio (HR) of OS with 95% confidence interval (95% CI). If the HR of OS was not provided directly in the main text, it was calculated from Kaplan-Meier curves, as described by Tierney *et al* (15). The correlation between miR-21 expression and clinical characteristics was measured by odds ratio (OR) with 95% CI. All OR values were calculated by the Chi-square test using data provided by the investigations. Heterogeneity was assessed using Cochran's Q test and Higgins's I^2 statistics; P<0.1 was considered to indicate significant heterogeneity. If heterogeneity was detected, the random-effects model was used; otherwise, the fixed-effects model was adopted. Publication bias was detected by funnel plots. Statistical significance was set at P<0.05. All the statistics were performed by RevMan software, version 5.3 (Cochrane, London, UK).

Results

Characteristics of included studies. A total of 86 studies were obtained following an initial search through the PubMed, Science Citation Index, Embase and CNKI databases. A total of 21 studies were initially excluded (conference abstracts, reviews, systematic reviews, or studies investigating other miRs). A further 53 studies were excluded after scanning the abstracts, as they did not meet the inclusion criteria, and another 3 studies were excluded following a full-text review. Finally, 9 studies were included in the analysis. The baseline characteristics of the included studies are summarized in Table II.

Correlation between high miR-21 expression and prognosis of HCC. A total of 6 studies reported the HR of the OS of patients with HCC. Significant heterogeneity was detected among these 6 studies (I2=81%, P<0.1) and the random-effects model was used to estimate the overall effect. It was observed that high expression of miR-21 was not correlated with poor OS in HCC patients (HR=1.21; 95% CI: 0.68-2.16; P=0.52; Fig. 2). No publication bias was identified by the Begg's test (P>0.05; Fig. 3).

A total of 3 studies reported data on the association between the expression of miR-21 and the survival of HCC patients receiving curative resection. No significant heterogeneity was observed among these 3 studies and the fixed-effects model was used to estimate the overall effect. Subgroup analysis revealed that high expression of miR-21 was associated with poor OS (HR=2.36; 95% CI: 1.52-3.66; P<0.01) in HCC patients receiving curative resection (Fig. 4).

Association of high expression of miR-21 with clinical characteristics of HCC. It was observed that high expression of

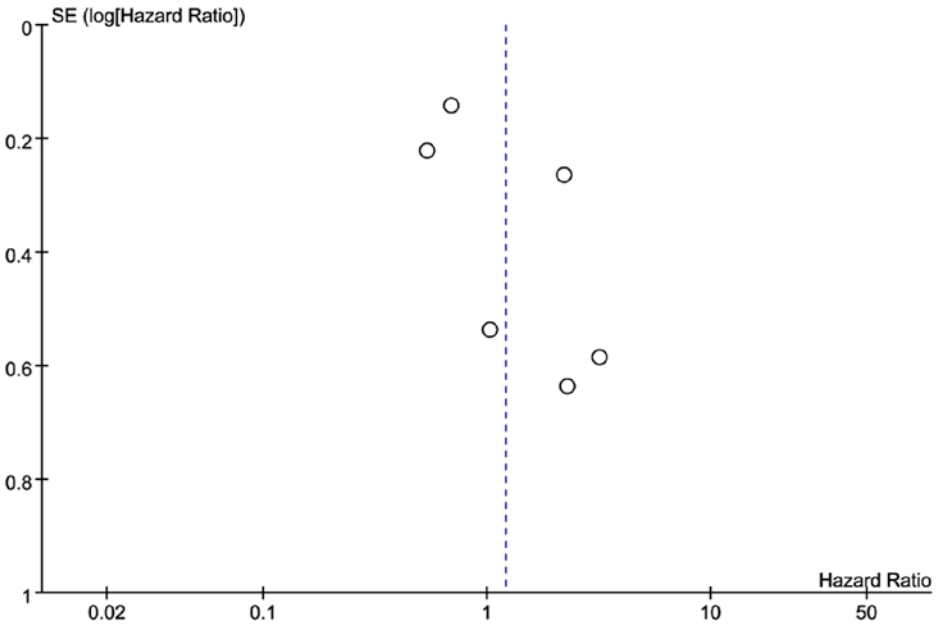

Figure 3. Funnel plot of overall survival. SE, standard error.

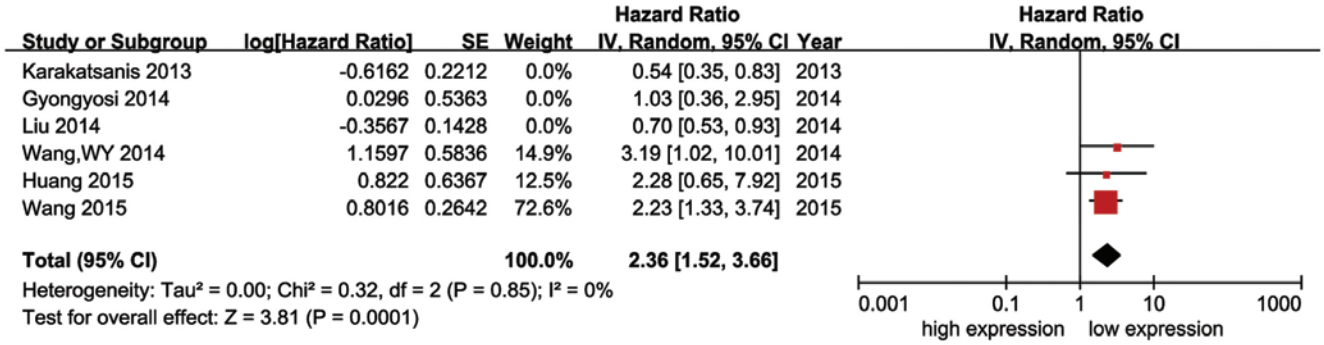

Figure 4. Subgroup analysis of high expression of miR-21 in hepatocellular carcinoma patients receiving curative resection. miR, microRNA; SE, standard error; CI, confidence interval.

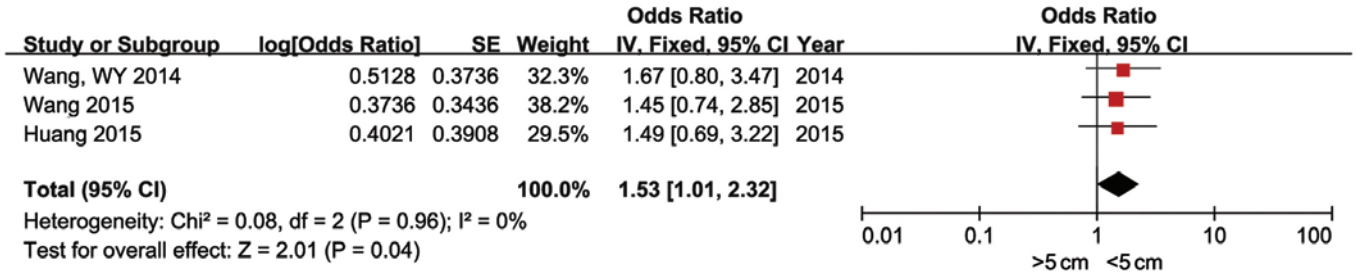

Figure 5. Correlation of high expression of miR-21 with tumor size. miR, microRNA; SE, standard error; CI, confidence interval.

miR-21 was correlated with tumor size >5 cm (OR=1.53; 95% CI: 1.01-2.32; P=0.04), venous invasion (OR=4.86; 95% CI: 1.47-16.08; P=0.01), TNM stage (OR=3.44; 95% CI: 1.56-7.59; P<0.01) and liver cirrhosis (OR=2.12; 95% CI: 1.07-4.23; P=0.03). However, high expression of miR-21 was not found to be significantly correlated with tumor number (P=0.44) or differentiation (P=0.83). Details on the association of high expression of miR-21 with the clinical characteristics of HCC are presented in Figs. 5-8.

Discussion

In the present analysis, higher miR-21 expression was not found to be significantly associated with poor prognosis of all HCC patients; however, high expression of miR-21 was found to be correlated with poor prognosis of HCC patients receiving curative resection. Zhou *et al* reported that miR-21 may be a potential prognostic biomarker for several carcinomas, including breast, colon and lung cancer, with an HR

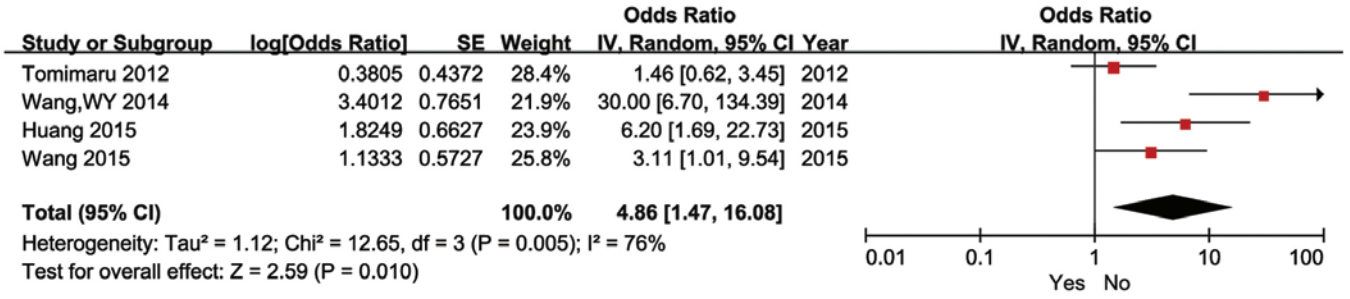

Figure 6. Correlation of high expression of miR-21 with venous invasion. miR, microRNA; SE, standard error; CI, confidence interval.

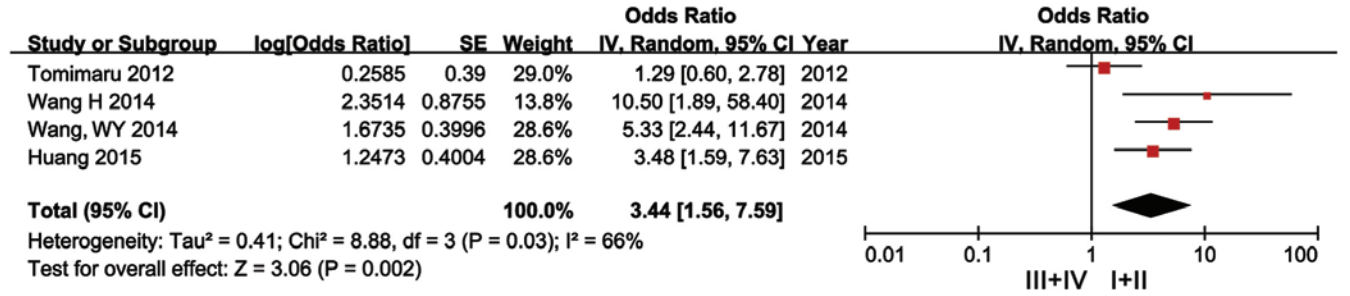

Figure 7. Correlation of high expression of miR-21 with TNM stage. miR, microRNA; SE, standard error; CI, confidence interval.

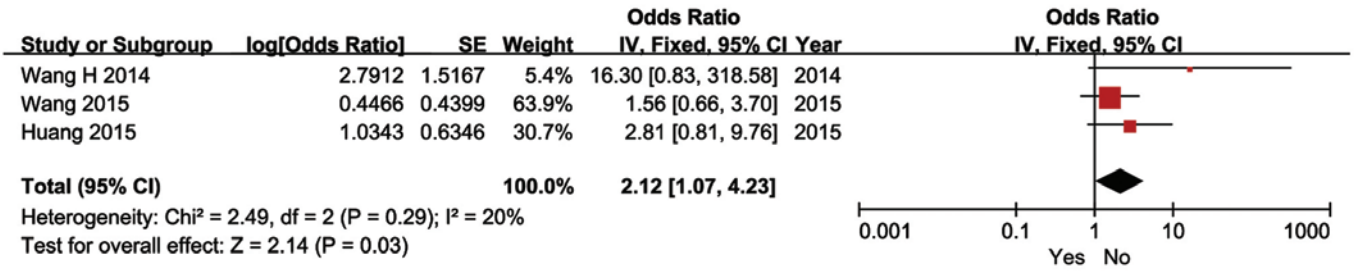

Figure 8. Correlation of high expression of miR-21 with liver cirrhosis. miR, microRNA; SE, standard error; CI, confidence interval.

of 1.91, and the subgroup analysis revealed that elevated miR-21 expression was associated with poor prognosis of liver cancer based on the results of 3 studies (16); however, those 3 studies differed in the pathological type of the liver cancer (2 studies enrolled patients diagnosed with cholangiocarcinoma, whereas1 enrolled patients with HCC). Thus, it is difficult to assess the prognostic value of miR-21 in HCC from that study. Subsequently, Wang *et al* investigated the value of miR-21 in cancer diagnosis and prognosis, and concluded that miR-21 may be a prognostic marker in digestive tract cancers with an HR of 5.77 (11), whereas only 2 studies were designed to explore the diagnostic and prognostic value of miR-21 in HCC, in which the small patient sample limited the reliability of the conclusions. Yan *et al* investigated the prognostic and diagnostic value of miR-21 in early HCC, based on the pooled results of sensitivity, specificity and area under the curve, and concluded that miR-21 may be a complementary factor in HCC diagnosis (12); in addition, the pooled results suggested that high expression of miR-21 was associated with poor OS of early HCC. That study by Yan *et al* only enrolled patients with early HCC; however, as our study enrolled patients with early and intermediate HCC, our patient sample was more representative of that patient population.

In our review, 6 studies with a total of 730 patients were eligible for analysis, covering all stages of HCC, and the larger patient sample in our analysis made the findings more reliable compared with the abovementioned studies. Subgroup analysis revealed that high expression of miR-21 was correlated with poor prognosis of HCC patients receiving curative resection. Previous studies demonstrated that miR-21 may promote liver regeneration following partial hepatectomy (17,18). It may be hypothesized that miR-21 can simultaneously promote liver regeneration in normal hepatocytes and tumor cells, resulting in poor prognosis of HCC patients receiving liver resection; however, the mechanism underlying the association of miR-21 expression and poor prognosis of HCC following resection requires further investigation.

On investigating the correlation between higher miR-21 expression and the clinical characteristics of HCC, it was observed that higher miR-21 expression was significantly correlated with tumor size >5 cm, venous invasion, TNM stage and liver cirrhosis. Yan *et al* also observed that higher expression of miR-21 was associated with HCC TNM stage (12),

which was consistent with our findings. However, compared with the findings of Yan *et al*, we found that more clinical characteristics were associated with higher miR-21 expression.

Considering the mechanism underlying the role of miR-21 in HCC, various studies have reported potential signaling of miR-21 in tumorigenesis, progression and metastasis of HCC. Verified targets of miR-21 include RASGRP1, BCL2, RPS7, PTEN, E2F1 and PDCD4 (6,19). Modulation of miR-21 has been shown to regulate the translation of PTEN; hence, miR-21 may play a key role in PTEN-dependent pathways involved in cancer cell growth, migration and invasion (20,21). Recently, miR-21 has been demonstrated to mediate sorafenib resistance of HCC by suppressing autophagy via the PTEN/Akt pathway (22). PDCD4 is a known tumor suppressor mediating the apoptosis of tumor cells and repressing the development of HCC. miR-21 was demonstrated to promote migration and invasion via the miR-21-PDCD4-AP-1 feedback loop in HCC (23), and Qiu *et al* reported that miR-21 may deregulate the expression of PDCD4 in patients with hepatitis B virus infection, and promote tumorigenicity (24). Taken together, these findings indicate that miR-21 may promote tumor cell growth, migration and invasion, and inhibit tumor cell apoptosis through various pathways, resulting in tumor lesions of a larger size, higher incidence of venous invasion and more advance stage.

The present study had certain limitations: First, heterogeneity was observed between studies included in the survival analysis and the test specimens of miR-21 differed among these studies, which increased the selection bias and decreased the reliability of our results. Second, the OR values were calculated from data provided in the main text, and most investigators had not provided the precise OR values in their studies. Third, the sample size of the studies included in our analysis was still comparatively small, and more well-designed studies are required to elucidate the prognostic value of miR-21 in HCC.

In conclusion, miR-21 cannot be considered as a factor complementary to AFP, microvascular invasion and advanced tumor stage in predicting the prognosis of HCC. However, higher expression of miR-21 may be a promising biomarker associated with certain clinicopathological characteristics of HCC, such as tumor size, venous invasion, TNM stage and liver cirrhosis.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JSL designed this study, PSY wrote the paper and performed data analysis. Both authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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