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The clinical significance of microRNA-122 in predicting the prognosis of patients with hepatocellular carcinoma
A meta-analysis validated by the Cancer Genome Atlas dataset

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Abstract
Background: Although the prognostic value of microRNA-122 (miR-122) for hepatocellular carcinoma (HCC) patients have been evaluated by numerous studies, the results of them were not completely consistent. The present study aims to comprehensively evaluate the predicting value of miR-122 on the prognosis of patients with HCC based on all eligible literatures.

Methods: Numerous electronic databases (MEDLINE, Embase, Pubmed, Google Scholar, and China Biology Medicine disc) were applied to retrieve relevant studies. Overall survival (OS) and progression-free survival (PFS) were used as primary endpoints. All statistical analyses were performed by RevMan software version 5.3.5 and STATA software version 14.1. In addition, the results of this meta-analysis were validated by an independent dataset from the Cancer Genome Atlas (TCGA).

Results: A total of 11 studies containing 1124 patients were included in this meta-analysis. The pooled results showed that low miR-122 expression in HCC tissues significantly associated with unfavorable OS (hazard ratio [HR] = 1.48, 95% confidence interval [CI] 1.22–1.80, $P < .001$) and PFS (HR = 1.54, 95% CI 1.28–1.85, $P < .001$) in patients with HCC. However, the expression level of miR-122 in blood did not have the ability in predicting OS (HR = 0.75, 95% CI 0.44–1.28, $P = .29$) and PFS (HR = 0.84, 95% CI 0.58–1.20, $P = .33$) of HCC. Subgroup analysis further indicated that low expression of miR-122 in tumor tissues predicted poor OS in HCC patients who received curative liver resection (HR = 2.00, 95% CI 1.08–3.70, $P = .03$). Analysis using TCGA dataset suggested that low miR-122 expression in HCC tissues was significantly associated with OS (HR = 1.61, 95% CI 1.13–2.27, $P = .008$) other than PFS (HR = 1.30, 95% CI 0.96–1.75, $P = .09$).

Conclusion: Low miR-122 expression in HCC tissues was a reliable indicator for predicting the OS of HCC patients who underwent curative resection. Owing to the disagreement between this meta-analysis and the TCGA dataset, the predictive value of miR-122 in tissues for PFS needs to be verified by future well-designed studies with large sample size.

Abbreviations: HCC = hepatocellular carcinoma, LT = liver transplantation, miRNA = microRNA, NOS = Newcastle–Ottawa scale, OS = overall survival, PFS = progression-free survival, RFA = radiofrequency ablation, TACE = transarterial chemoembolization, TCGA = the Cancer Genome Atlas.

Keywords: hepatocellular carcinoma, meta-analysis, microRNA-122, prognosis

1. Introduction

Hepatocellular carcinoma (HCC) is the main type of liver cancer and a serious health problem worldwide.[1,2] Approximately 750,000 new cases are identified and account for half a million deaths are caused by HCC each year.[3] Nowadays, multiple modalities such as hepatectomy, liver transplantation (LT), radiofrequency ablation (RFA), transarterial chemoembolization (TACE), and sorafenib can be used to treat HCC patients distributed in different stages.[1,4] However, the long term prognosis of HCC is still unsatisfactory. In spite of numerous potential etiologies of HCC carcinogenesis including hepatitis virus infection (HBV and HCV), alcohol abuse, cirrhosis caused by inflammation or chronic liver damage, intake of aflatoxin B1, and metabolic syndrome have been identified by previous epidemiological studies,[5–7] the molecular mechanisms involved in the initiation and progression of HCC remain far from being fully understood.[8,9] Therefore, elucidating the precise mechanisms of HCC and identifying some essential molecules are helpful to predict the prognosis early and develop new therapeutic strategies.

MicroRNA (miRNA) is a major type of endogenous noncoding RNA with 19 to 25 nucleotides in length. They negatively regulate genes at posttranscriptional level and participated in almost all biological processes.[10,11] In addition, miRNAs are highly stable and are reliably detected in various clinical samples such as tissue, blood, body fluid and even excreta, and are therefore considered to be ideal biomarker candidates.[12] microRNA-122 (miR-122), a mammalian liver-specific miRNA, has been reported to play crucial roles in the control of diverse aspects of hepatic function and dysfunction, including lipid metabolism,[13,14] injury caused by drug or alcohol,[15,16] viral infection,[17–19] and hepatocarcinogenesis.[20–22]

Previous studies have reported that miR-122 was commonly downexpressed in HCC tissues when compared with adjacent paired nontumorous tissues and functioned as a tumor suppressor.[23–25] Multiple mechanistic researches have revealed that artificially upregulated miR-122 obviously suppressed the proliferation, metastasis, and drug resistance of HCC tumor cells via targeting GALNT10,[23] PKM2,[24] Snail1/2,[25] and so on.[26,27]

In addition, many clinical studies suggested aberrantly expressed miR-122 in HCC tissues was associated with various clinical features such as tumor size, disease stage, venous invasion, and pathological differentiation,[23–25] and the different miR-122 expression in peripheral blood of HCC patients was also correlated with the platelet count, albumin level, alanine transaminase level and disease stage.[28,29] For the relationship between the miR-122 expression and prognostic outcomes of patients with HCC, the results from previous studies were not completely consistent. Jin[25] and Xu et al,[24] respectively reported that downregulation of miR-122 in HCC tissues was an independent risk factor for the prognosis of HCC patients who received curative resection, together with other well-demonstrated risk factors such as tumor size, vascular invasion, and disease stage. While Gyongyosi et al[30] found that there was no significant association between the expression level of miR-122 in fine-needle aspiration biopsy tissues and the prognosis of patients with advanced HCC. Compared with tissues, the correlations between the prognosis of patients with HCC and the miR-122 expression levels in blood were more controversial. Although Cho et al[28] identified that high blood miR-122 levels significantly predicted poor overall survival (OS) of patients who underwent RFA, and the combination of miR-122 and tumor stage resulted in a larger area under the receiver operating characteristic (ROC) curve for predicting 1-year OS in patients who accepted RFA than these 2 parameters alone. However, other negative or opposite results were also reported by previous studies.[29,31–33] Therefore, the clinical significance of miR-122 in predicting the prognosis of patients with HCC is still unclear. In present study, we collected all eligible evidence and conducted a comprehensive meta-analysis to evaluate the relationship between the prognosis of patients with HCC and the miR-122 expression levels in tissue and blood, respectively. Furthermore, the tissue results of this meta-analysis were validated by an independent microarray dataset from The Cancer Genome Atlas (TCGA).

2. Materials and methods

2.1. Ethics statement

Owing to all data in this meta-analysis were extracted from previously published studies, special ethical approval and patient consent were unnecessary.

2.2. Search strategy

Multiple electronic databases including Cochrane Library, MEDLINE, Embase, Pubmed, BioMed Central, Google Scholar, and China Biology Medicine disc were used to retrieve relevant studies which investigated the value of miR-122 on predicting the prognostic outcomes of HCC patients. The literature searching was censored on February 28, 2018. Following keywords were used during the literature searching process: (microRNA-, miRNA-, miR-122) and (hepatic or liver) and (carcinoma or tumor or neoplasm or cancer or malignancy) and (prognostic or prognosis or survival or recurrence or relapse or progression or metastasis). And the reference lists of relevant reviews, meta-analyses, and original studies were manually screened to acquire additional studies.

2.3. Inclusion and exclusion criteria

Inclusion criteria:

(1) only included the patients suffered from HCC;
(2) the miR-122 expression in tissues or blood were measured;
(3) the relationship between the miR-122 expression and survival outcomes was investigated;
(4) the hazard ratio (HR) and 95% confidence interval (CI) were reported directly or sufficient information was supplied for calculating HR and 95% CI.

Exclusion criteria:

(1) included patients suffered from other types of cancers or benign diseases;
(2) the study only conducted on animal model or tumor cell lines;
(3) studies lack of control group, no data could be extracted or the studies published as abstracts, reviews, conference reports, letters, expert opinions, or editorials.

2.4. Endpoints and quality assessment

OS and progression-free survival (PFS) was used as the primary endpoints in this study. OS was defined as the time at which the clinical sample was obtained to the date of death or last follow-up. PFS was considered as the time between the clinical sample was captured and documentation of the first tumor recurrence or deterioration.

The Newcastle–Ottawa scale (NOS)[34] was employed to assess the quality of each included study. Three main characteristics, namely selection, comparability, and outcome, were judged and a possible score of 0 to 9 was distributed to each study. The study with an NOS score more than 6 was considered to be of high quality.

2.5. Literature selection and data extraction

After removing duplicated studies, 2 reviewers (YZ and WJ) evaluated the titles and abstracts of identified articles independently and excluded the irrelevant ones. Then the full-text of potentially eligible studies were carefully examined to confirm the final inclusion. Any disagreements were resolved by discussion with a third reviewer (YGL).

The useful information from all enrolled studies was extracted by 2 independent reviewers (WJ and QL). Relevant items including first author, publication year, ethnicity, sample type, miR-122 assay method, cut-off value, HR, and corresponding 95% CI of OS or PFS. If a study reported the survival results of univariate and multivariate analyses at the same time, the later one was recorded

since it was more precise due to accounting for confounding factors. If the authors showed the prognosis results only using survival curves, the HR and 95% CI were calculated by the method recommended by Tierney et al.[35] Finally, extracted data forms were crosschecked to rule out any discrepancy.

2.6. Statistical analysis

All of the statistical analyses were performed using RevMan software version 5.3.5 (Cochrane Collaboration, Oxford, UK) and STATA software version 14.1 (StataCorp, College Station, TX). The pooled HR and 95% CI of OS and PFS were employed to test the prognostic value of miR-122 expression on HCC. Heterogeneity of combined HRs was assessed using the Cochran Q test and Higgins I-square (I^2) statistic. If the P value was less than .05 and/or I^2 greater than 50%, the random-effect model (DerSimonian and Laird method)[36] was used. Otherwise, the fixed-effect model (Mante–Haenszel method)[37] was performed. The subgroup analysis was employed to decrease the heterogeneity among studies. Publication bias was measured by the funnel plot, Begg, and Egger bias indicator tests. Moreover, the stability of results was examined using sensitivity analysis. All tests were 2-sided, and P value less than .05 was considered statistically significant.

2.7. The analysis of TCGA dataset

The miR-122 expression and corresponding survival data for TCGA HCC were downloaded from the UCSC Xena website (https://xenabrowser.net/heatmap/).[38] Unpaired Student t test was used to analyze the difference of miR-122 expression between tumor and nontumor tissues. In line with most enrolled studies,[23–25,30,32,39] the miR-122 expression levels in tumor tissues were dichotomized into high and low expression groups based on the median value. Survival curves of OS and PFS were plotted using the Kaplan–Meier method and were tested with the log-rank test. P value less than .05 was considered statistically significant. Statistical analyses were performed in Prism version 5.0 (GraphPad Software).

3. Results

3.1. Process of study selection and characteristics of eligible studies

A detailed flow diagram about the process of study selection was displayed in Figure 1. Briefly, 569 studies were yield by comprehensively searching electronic database and manually screening reference lists of relevant studies. After carefully removing duplication, browsing titles and abstracts, and assessing full-text, 559 studies were excluded and remaining 10 articles[23–25,28–33,39] which met our eligibility criteria were included. Since Cho et al[28] investigated the prognosis value of blood miR-122 in patients who underwent RFA and resection respectively in a single article. So a total of 11 studies were collected to perform final quantitative analysis.

Among these studies, 9[23–25,28,29,32,33,39] out of 11 were conducted in Asia and 2[30,31] in Europe. Four studies[23–25,30]
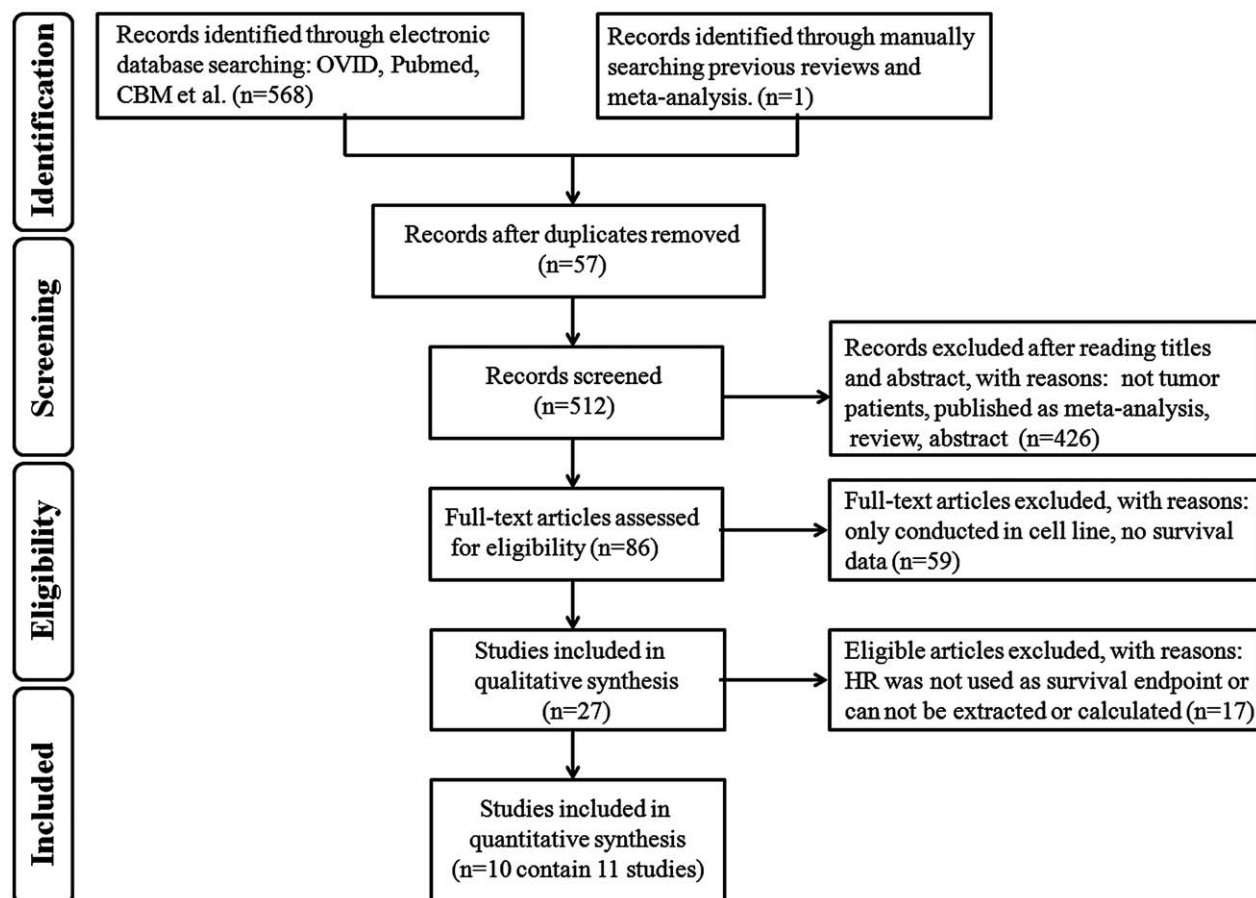

Figure 1. The flow diagram illustrating the process of studies selection. CBM = China Biology Medicine.

Table 1

The main characteristics of included studies.

Study	Year	Country	Tumor type	Treatment method	Detected sample	Assay method	Expression in tumor	Cut-off value	Clinical relationship	Sample number (low/high)	Follow-up, mo	Survival endpoints	HR source	Independent risk factor	NOS score
Jin	2017	China	HCC	Resection	Tissue	qRT-PCR	Decreased	Median	Size, venous invasion, AJCC stage	122 (66/56)	55	OS/DFS	Reported (m)	Yes	9
Kim	2017	South Korea	HCC	TACE	plasma	qRT-PCR	NR	ROC	NR	161 (NR)	79	OS/PFS	Reported (m)	No	9
Kevin	2016	China	HCC	LT	Plasma	qRT-PCR	Decreased	Youden index	NR	62 (34/28)	135	OS/DFS	Reported (m)	No	8
Wu	2015	China	HCC	Resection	Tissue	ISH	Decreased	Median	HBV infection, size, venous invasion, differentiation	142 (70/72)	80	OS	SC	NR	7
Cho*-1	2015	South Korea	HCC	RFA	Plasma	qRT-PCR	Increased	75th percentile	PT, ALB, stage	57 (43/14)	96	OS/DFS	Reported (m)	Yes	9
Cho-2	2015	South Korea	HCC	Resection	Plasma	qRT-PCR	Increased	75th percentile	NR	63 (38/25)	80	OS/DFS	SC	No	7
YQ Xu	2015	China	HCC	Resection	Serum	qRT-PCR	NR	Median	NR	122 (61/61)	41	OS	Reported (u)	NR	7
QR Xu	2015	China	HCC	Resection	Tissue	qRT-PCR	Decreased	Median	Differentiation, TNM stage, PVTT	44 (35/9)	32	OS	Reported (m)	Yes	9
Gyöngyösi	2014	Italy	HCC	Sorafenib	FNAB	qRT-PCR	NR	Median	NR	20 (10/10)	40	OS/PFS	Reported (u)	NR	7
Liu	2014	China	HCC	TACE	Serum	qRT-PCR	Decreased	Median	NR	136 (68/68)	49	OS	Reported (u)	NR	8
Koberle	2013	Germany	HCC	Resection, RFA, Sorafenib, LT	Serum	qRT-PCR	Comparable	25th percentile	ALT, AST, GGT, MELD score	195 (NR)	80	OS	Reported (u)	No	8

* Cho et al investigated the prognostic value of miR-122 in HCC patients underwent radiofrequency ablation (Cho-1) and curative resection (Cho-2).

ALB = albumin, ALT = alanine transaminase, DFS = disease-free survival, FNAB = fine-needle aspiration biopsy, HCC = hepatocellular carcinoma, HR = hazard ratio, ISH = in situ hybridization, LT = liver transplantation, (m) = multivariate analysis, NOS = the Newcastle–Ottawa scale, NR = not reported, OS = overall survival, PFS = progression-free survival, qRT-PCR = quantitative real-time polymerase chain reaction, RFA = radiofrequency ablation, ROC = receiver operating characteristic, SC = survival curve, TACE = transarterial chemoembolization, (u) = univariate analysis.

investigated miR-122 predicted value in HCC tissues while other 7 studies[28,29,31–33,39] tested in bloods. Almost all studies measured miR-122 expression using quantitative polymerase chain reaction (qRT-PCR), except Wu et al employed in situ hybridization, and their results were consistent with the qRT-PCR results after verification.[23] Five studies[23–25,28,39] investigated the miR-122 prognostic value on HCC patients who accepted curative resection, and remaining 6 studies focused on the miR-122 prognostic value on LT,[33] RFA,[28] TACE,[29,32] or sorafenib,[30,31] respectively. Nine studies reported survival results directly,[24,25,29–33,39] and 2 studies showed survival results using survival curves.[23,28] With regard to the etiologies of HCC, 8 studies[23–25,28,29,32,33] included patients suffered from HBV infection, 2[30,39] included patients suffered from HBV or HCV infection, 1[31] included patients suffered from HCV or alcoholic hepatitis. The sample size in each study was ranged from 20 to 195 with median of 122. All studies were of high quality due to the NOS score being not less than 7. Other detailed information of included studies were listed in Table 1.

3.2. Relationship between the expression of miR-122 in tumor tissues and survival outcomes of HCC

Four studies[23–25,30] included 328 patients evaluated the predictive value of miR-122 expression in tissues on OS of HCC. The results of meta-analysis with no obvious heterogeneity ($I^2 = 34\%$, $P = .21$) indicated that the low miR-122 expression in tissues was significantly associated with poor OS in HCC, with the pooled HR of 1.48 (95% CI 1.22–1.80, $P < .001$). With respect to the relationship between miR-122 expression level in tumor tissues and PFS of HCC patients, the pooled analysis based on 2 studies[25,30] also showed that low miR-122 expression in tissues was significantly associated with unfavorable PFS of HCC patients (HR = 1.54, 95% CI 1.28–1.85, $P < .001$). Similarly, this result has no heterogeneity ($I^2 = 0\%$, $P = .50$) (Fig. 2).

3.3. Relationship between expression of miR-122 in blood and survival outcomes of HCC

A total of 7 studies[28,29,31–33,39] including 796 patients investigated the relationship between blood miR-122 expression level and OS. As a significant heterogeneity ($I^2 = 27\%$, $P = .23$) existed in the result of meta-analysis, a random-effect model was employed to calculate the relationship between miR-122 expression level in blood and OS of HCC patients. The synthesized results suggested there was no significant difference of OS between the high and low blood miR-122 expression groups (HR = 0.75, 95% CI 0.44–1.28, $P = .29$). For the PFS, the pooled results from 4 studies[28,29,33] also suggested that miR-122 expression levels in blood have no significant association with the PFS of HCC (HR = 0.84, 95% CI 0.58–1.20, $P = .33$), this result was not heterogeneous ($I^2 = 0\%$, $P = .41$) (Fig. 3).

3.4. Subgroup analysis of OS

In order to decrease the heterogeneity among studies and further recognize the prognostic value of miR-122 in depth, the subgroup analyses of OS were performed based on 5 criteria including treatment method, miR-145 assay method, HR resource, survival analysis type, and cut-off value (Table 2). For treatment method, the results showed that the expression level of miR-122 in tumor tissues instead of in blood was significantly associated with OS of HCC patients who underwent curative liver resection (Fig. 4). The expression level of miR-122 in blood was significantly associated with the OS of patients who received TACE (Fig. 4). However, these results need to be cautiously interpreted since it was derived from 2 studies, and 1 of them only provided the univariate analysis result which was inconsistent with the multivariate result. For other subgroup analysis, the miR-122 expression level in HCC tissues was still significantly associated with OS in different subgroups which were stratified based on miR-145 assay method and HR resource. And there was still no relationship between the miR-122 expression level in HCC patients' blood and OS after dividing patients into different
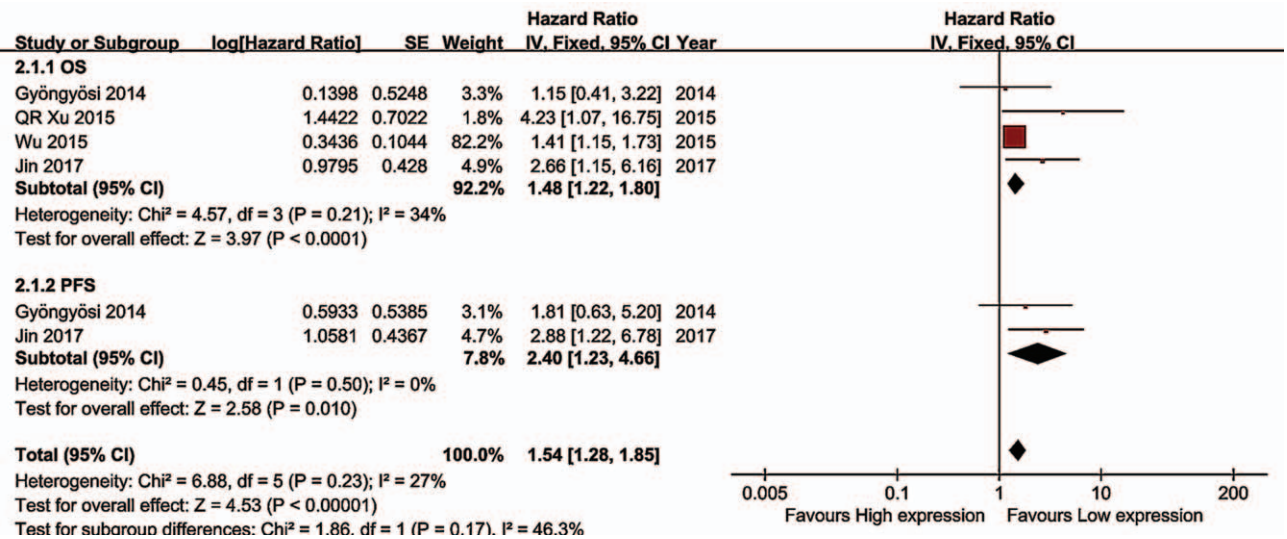
Figure 2. Frost plot of the association between the miR-122 expression level in tissues and survival outcomes of patients in various tumors. OS = overall survival, PFS = progression-free survival.

subgroups based on survival analysis type, cut-off value, and HR resource.

3.5. The validation of the tissue results by TCGA HCC dataset

In present study, the miR-122 expression levels in HCC tissues and the corresponding OS and PFS data extracted from the TCGA were used to confirm the results of this present meta-analysis. In this dataset, the miR-122 expression level was tested in 372 HCC and 50 nontumorous tissues by microarray IlluminaHiSeq platform, the unpaired t test result showed that

the miR-122 expression in HCC tissues was significantly lower than that in adjacent nontumor tissues ($P < .001$) (Fig. 5A). Which was consistent with the results reported by relevant enrolled studies. Subsequently, these patients were allocated into high and low groups based on the median value of miR-122 expression and a survival analysis was performed. The result showed the low miR-122 expression in HCC tissues was significantly predicted poor OS of HCC patients (HR = 1.61, 95% CI 1.13–2.27, $P = .008$) (Fig. 5B), this result was similar with the pooled results of this meta-analysis. However, although the patients with low miR-122 expression had worse PFS rate when compared with the high miR-122 expression patients, the
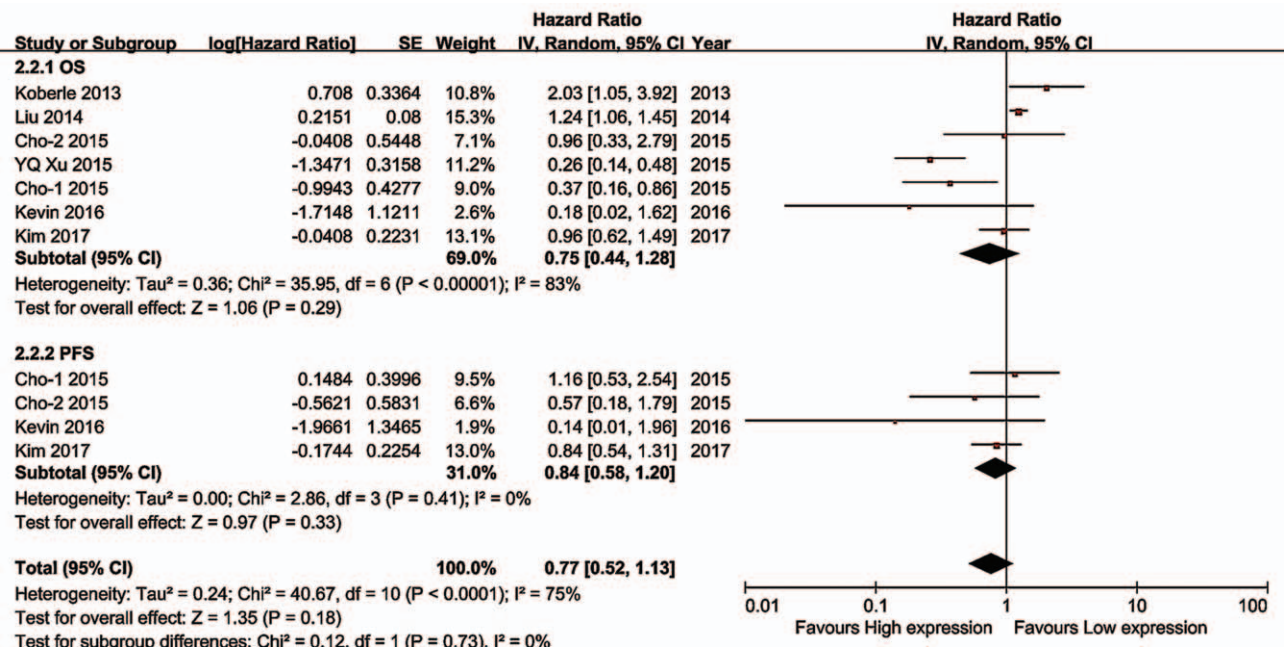

Figure 3. Frost plot of the association between the miR-122 expression level in blood and survival outcomes of patients in various tumors. OS = overall survival, PFS = progression-free survival.

Table 2
Subgroup analysis of overall survival in tissue and blood.

Subgroup	No. of studies	Total patients	Model	HR (95% CI)	P-value	HG I^2%	HG P-value
Tissue							
Treatment method							
LR	3	308	Random	2.00 (1.08, 3.70)	.03	54	.11
Sorafenib	1	20	ND	1.15 (0.41, 3.22)	.79	ND	ND
Assay method							
qRT-PCR	3	186	Fixed	2.20 (1.22, 3.96)	.008	23	.27
ISH	1	142	ND	1.41 (1.15, 1.73)	.001	ND	ND
HR resource							
Reported	3	166	Fixed	2.20 (1.22, 3.96)	.008	23	.27
SC	1	142	ND	1.41 (1.15, 1.73)	.001	ND	ND
Blood							
Treatment method							
LR	2	185	Random	0.46 (0.13, 1.65)	.24	77	.04
TACE	2	297	Fixed	1.20 (1.04, 1.40)	.01	14	.28
Ohters	3	314	Random	0.62 (0.14, 2.70)	.53	84	.002
Analysis type							
Univariate	4	516	Random	0.89 (0.40, 2.01)	.79	88	<.001
Multivariate	3	280	Random	0.54 (0.22, 1.32)	.18	64	.06
Cut-off value							
Median	2	258	Random	0.59 (0.13, 2.70)	.49	96	<.001
Others	5	538	Random	0.84 (0.44, 1.61)	.6	67	.02
HR resource							
Reported	6	733	Random	0.72 (0.40, 1.30)	.27	86	<.001
SC	1	63	ND	0.96 (0.33, 2.79)	.94	ND	ND

CI = confidence interval, HG = heterogeneity, HR = hazard ratio, ISH = in situ hybridization, LR = liver resection, ND = no data, qRT-PCR = quantitative real time polymerase chain reaction, SC = survival curve, TACE = transarterial chemoembolization.
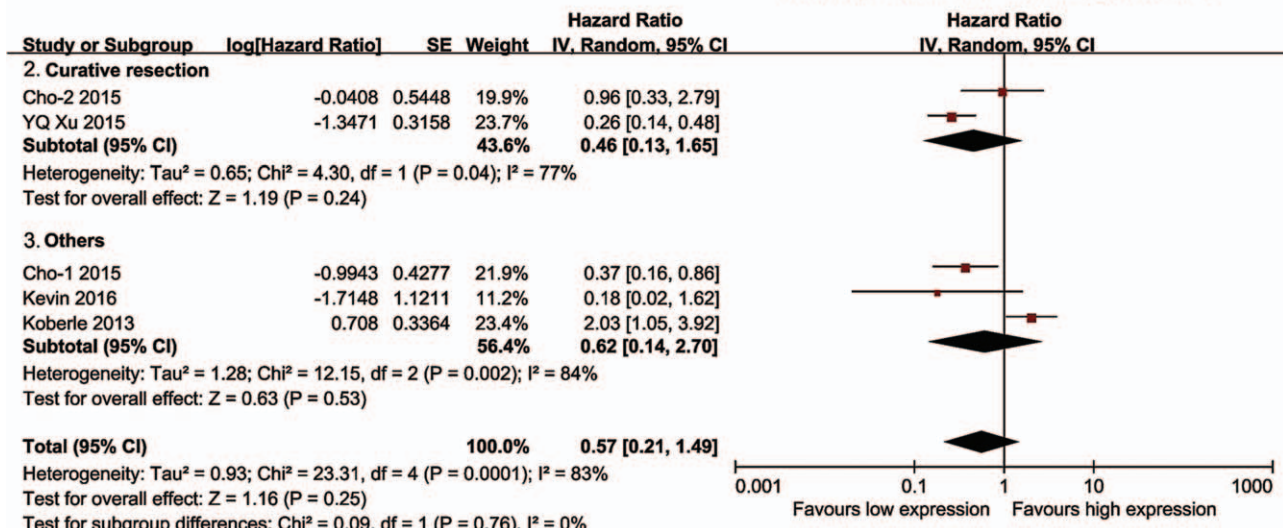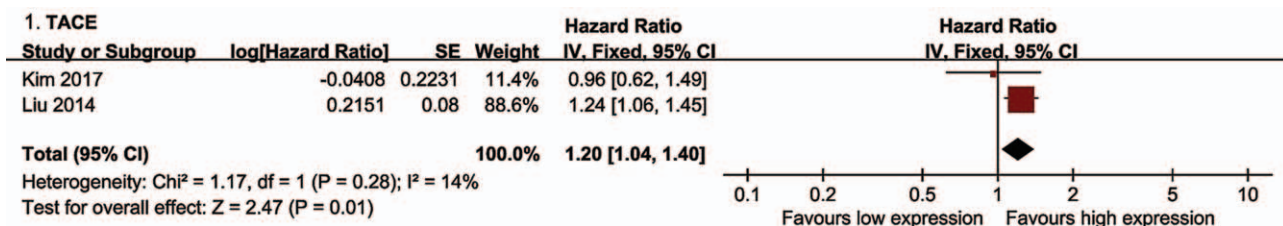

Figure 4. Subgroup analysis of blood overall survival based on treatment method. TACE; others including liver transplantation, radiofrequency ablation and sorafenib. TACE = transarterial chemoembolization.
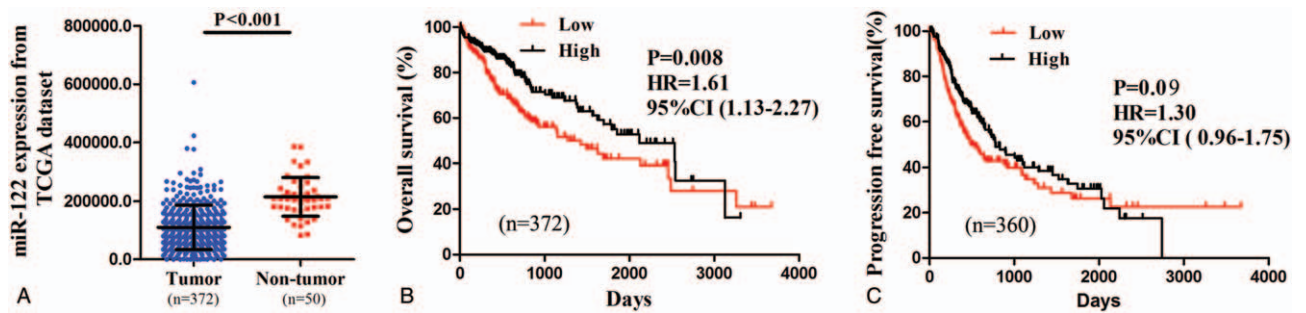
Figure 5. The tissue miR-122 expression level and corresponding survival outcomes of HCC in TCGA dataset. (A) miR-122 expression level in HCC tissues was significantly lower than that in adjacent non-tumor tissues. (B) Low miR-122 expression in HCC tissues significantly predicted unfavorable OS of HCC patients. (C) The relationship between the miR-122 expression and PFS did not achieve the statistical significance. HCC = hepatocellular carcinoma, OS = overall survival, PFS = progression-free survival, TCGA = transarterial chemoembolization.

P value did not achieve statistical significance (HR = 1.30, 95% CI 0.96–1.75, $P = .09$) (Fig. 5C).

3.6. Publication bias analysis

In this study, the potential publication bias was investigated using Begg and Egger test. Two funnel plots for OS of tissues and blood did not show obvious visual asymmetry (Fig. 6A and B), and the P values of the Egger test were also greater than .05 (OS of tissue, $P = .32$; OS of blood, $P = .17$) (Fig. 6C and D). Therefore, there was no significant publication bias in this meta-analysis.

3.7. Sensitivity analysis

Sensitivity analysis of OS in tissues and blood was performed to investigate the influence of individual study on pooled HRs. The result of OS in tissues showed that the HR and 95% CI in the study conducted by Wu et al were obviously different from other 3 studies (Fig. 7A). Then a new meta-analysis was performed after omitting this study, the result without any heterogeneity showed that the low tissue miR-122 expression was still associated with the poor OS of HCC (HR = 2.20, 95% CI 1.22–3.96, $P = .008$). In addition, the sensitivity analysis of OS in blood did not identify any study which would alter the pooled
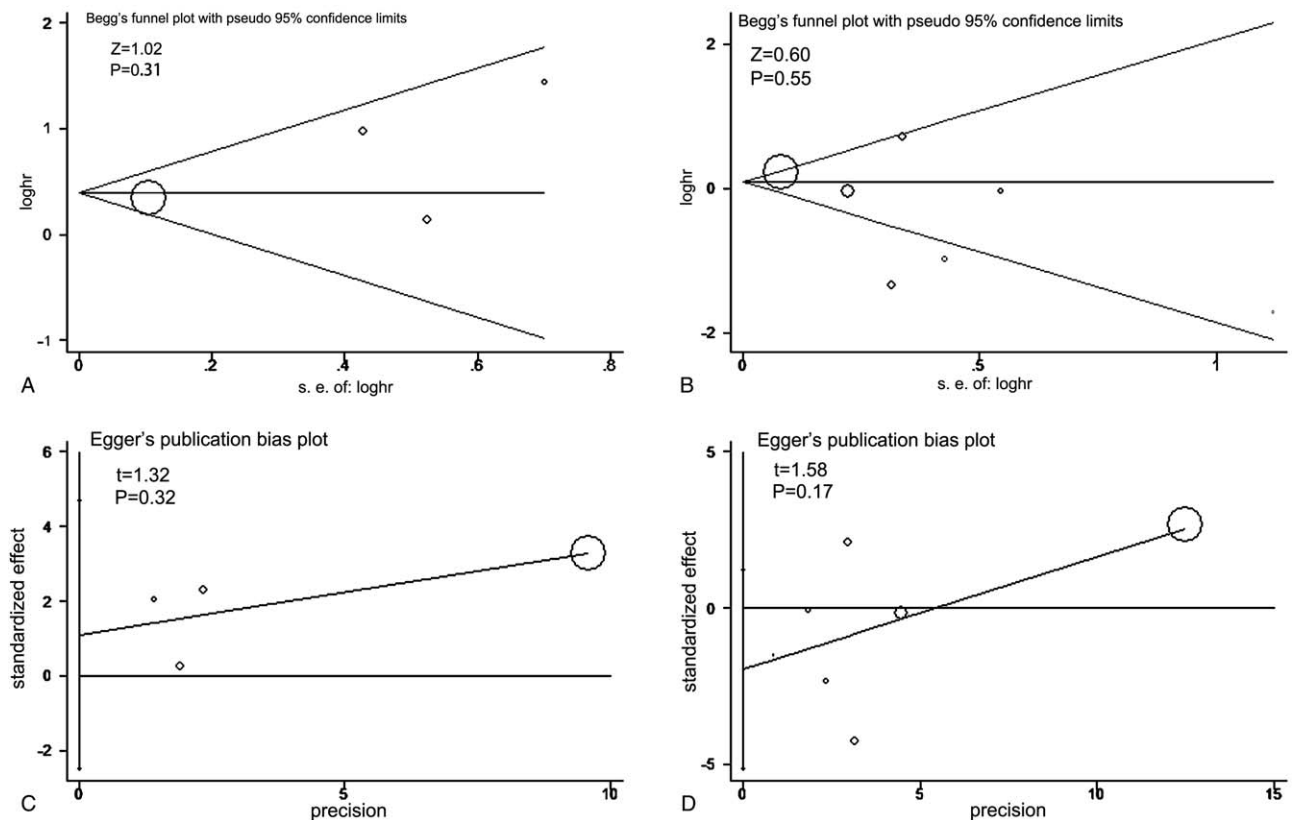

Figure 6. Begg funnel plot and Egger test were used to evaluate the publication bias. Begg funnel plot of OS of tissue (A) and blood (B), respectively. Egger test of OS of tissue (C) and blood (D), respectively. OS = overall survival.
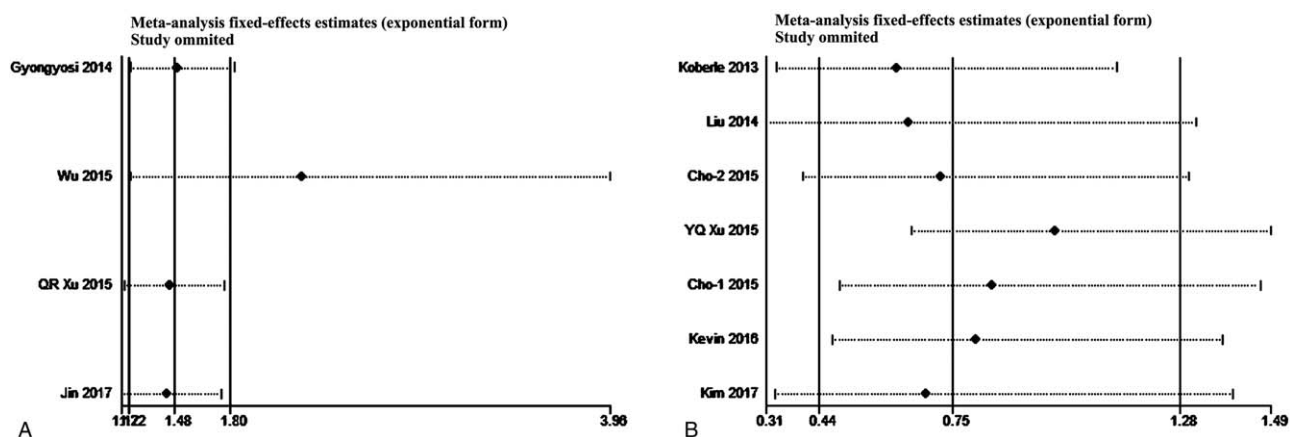
Figure 7. Sensitivity analysis of the relationship between miR-122 expression and overall survival of tissue (A) and blood (B), respectively.

results significantly (Fig. 7B). Taken together, the results of this meta-analysis were robust.

4. Discussion

MiR-122, derived from chromosome 18 with 23 nucleotides in length, constitutes 70% of all miRNAs in mature hepatocytes and was considered as a mammalian liver-specific miRNA.[40,41] During the past several years, the relationships between the miR-122 expression levels in tumor tissues or peripheral blood of HCC patients and clinical characteristics as well prognostic outcomes were investigated by amount of studies.[23–25,28–33,39] However, the results of prognosis were not completely consistent among these studies. And to our best knowledge, no relevant meta-analysis of miR-122 in HCC was published up till now. Therefore, we aimed to comprehensively evaluate the predicting value of miR-122 on the survival outcomes of HCC patients in tissue and blood dimensions, respectively.

In this meta-analysis, a total of 11 studies[23–25,28–33,39] including 1124 patients were enrolled, the pooled results without obvious heterogeneity showed that the low miR-122 expression in tissues significantly predicted the poor OS and PFS of HCC patients, and the subgroup analysis based on treatment method further indicated that the low miR-122 expression in tissues significantly associated with the OS of patients who underwent curative liver resection rather than sorafenib. This inconsistency may be arised from the clinical heterogeneity, namely the disease stages were commonly different among the HCC patients who received different treatment methods. In these included studies, Jin et al[25] reported that the aberrantly expressed miR-122 in HCC tissues were significantly associated with the tumor size, vascular invasion, and tumor stage, and Xu et al[24] also found that the expression levels of miR-122 in tumor tissues were significantly related with tumor differentiation and tumor-node-metastasis stage, all these parameters were the well-demonstrated risk factors related to the prognosis of HCC.[42,43] Additionally, Jin, Xu, and Wu et al, respectively reported that miR-122 involved in the proliferation, apoptosis, and metastasis of HCC cell lines via regulating the expression of Snail1/2,[25] PKM2,[24] and GALNT10.[23] Taken together, miR-122 seemly played a vital role in the tumorigenesis and progression of HCC and maybe a promising biomarker for predicting the prognosis for HCC patients who underwent curative resection.

However, the pooled results from 7 studies showed that miR-122 expression levels in blood had no relationship with OS and PFS of patients with HCC, and almost all results of subgroup analyses were in line with this result except the treatment method. The subgroup analysis based on treatment method showed that low miR-122 expression in blood indicated poor OS of HCC patients who underwent TACE. Although this result had no obvious heterogeneity, the conclusion need to be cautiously interpreted. On the one hand, only 2 studies[29,32] with small sample size investigated the prognosis value of miR-122 in patients who underwent TACE, this result was not robust enough. On the other hand, and both of these 2 studies found that low miR-122 expression in blood was significantly associated with poor OS of patients received TACE in univariate analysis but not in multivariate analysis. However, Liu et al[32] only documented the result of univariate analysis instead of multivariate analysis. Therefore, we thought that the association between the blood miR-122 expression levels and the prognosis of patients who underwent TACE need to be further investigated in the future.

Compared with the result of OS in tissues, a more serious heterogeneity was existed in the result of OS in blood. This probably caused by the demographic and methodological difference among relevant studies.[28,29,31–33,39] These studies enrolled patients who distributed in different disease stages and accepted different treatment therapies including LR, RFA, LT, TACE, and sorafenib, while a number of published studies have been demonstrated that the disease stage and treatment method were the independent risk factors for HCC patients.[1,44] In addition, the cut-off value was also varied in these studies, 2 used median value,[32,39] 2 adopted 75th percentile,[28] 1 employed 25th percentile,[31] and remaining 2 determined by ROC curve.[29,33] Therefore, the prognostic value of miR-122 in blood also need to be confirmed by future well-designed studies.

Moreover, the results of meta-analysis of tissue were validated by an independent data downloaded in TCGA database. The results showed that miR-122 expression in HCC tissues was significantly higher than that in adjacent nontumor tissues. And the low miR-122 expression significantly predicted poor OS for HCC patients, which indicated that the results in our meta-analysis had capable application value in reality. However, the relationship between the miR-122 expression level and PFS did not reach the statistical significance. Meanwhile, considering the

pooled PFS results of tissues were derived from 2 studies with small sample size,[25,30] the effective value of miR-122 expression in tissues on predicting PFS of HCC patients need to be further studied by large size and well-designed studies in the future. Furthermore, we also demonstrated that there were no obvious publication bias and no potential studies which would influence the pooled results significantly, and each enrolled study had a high quality with NOS scores not less 7, all these evidence indicated that the results of this meta-analysis were stability.

As the first meta-analysis of miR-122, our study provided robust evidence about the prognostic value of miR-122 expression for patients with HCC, but some limitations need to be figured out. First, both of the number and sample size of enrolled studies were small, this would potentially compromise the robust of the pooled results. Second, this study analyzed the predicted value of miR-122 on the survival outcomes of HCC patients who belonged to different stage, the inconsistence among these patients must amplify the heterogeneity of the pooled results. Third, the HR and 95% CI of 2 enrolled studies were calculated using survival curves, which might bring several tiny statistical errors.

5. Conclusions

In conclusion, based on all eligible evidence published up till now, our study demonstrated that there was no significant association between the miR-122 expression levels in blood and the prognosis of patients with HCC. While low miR-122 expression in HCC tissues was a reliable indicator on predicting the OS of patients with HCC who underwent curative liver resection. Owing to the disagreement between our meta-analysis and TCGA dataset, the clinical value of tissue miR-122 in predicting the PFS of patients with HCC needed to be verified by further well-designed studies with large sample size.

Author contributions

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References

[1] Llovet JM, Schwartz M, Mazzaferro V. Resection and liver transplantation for hepatocellular carcinoma. Semin Liver Dis 2005;25:181–200.
[2] Torre LA, Bray F, Siegel RL, et al. Global cancer statistics, 2012. CA: Cancer J Clin 2015;65:87–108.
[3] Marquardt JU, Andersen JB, Thorgeirsson SS. Functional and genetic deconstruction of the cellular origin in liver cancer. Nat Rev Cancer 2015;15:653–67.
[4] Franssen B, Alshebeeb K, Tabrizian P, et al. Differences in surgical outcomes between hepatitis B- and hepatitis C-related hepatocellular carcinoma: a retrospective analysis of a single North American center. Ann Surg 2014;260:650–6.
[5] Laursen L. A preventable cancer. Nature 2014;516:S2–3.
[6] Liu J, Fan D. Hepatitis B in China. Lancet (London, England) 2007;369:1582–3.
[7] Liver1 EAFTSOTEASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. J Hepatol 2012;56:908–43.
[8] Edamoto Y, Hara A, Biernat W, et al. Alterations of RB1, p53 and Wnt pathways in hepatocellular carcinomas associated with hepatitis C, hepatitis B and alcoholic liver cirrhosis. Int J Cancer 2003;106:334–41.
[9] Wulfkuhle JD, Liotta LA, Petricoin EF. Proteomic applications for the early detection of cancer. Nat Rev Cancer 2003;3:267–75.
[10] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004;116:281–97.
[11] Chen K, Rajewsky N. The evolution of gene regulation by transcription factors and microRNAs. Nat Rev Genet 2007;8:93–103.
[12] Turchinovich A, Weiz L, Langheinz A, et al. Characterization of extracellular circulating microRNA. Nucleic Acids Res 2011;39:7223–33.
[13] Brandt S, Roos J, Inzaghi E, et al. Circulating levels of miR-122 and nonalcoholic fatty liver disease in pre-pubertal obese children. Pediatr Obes 2018;13:175–82.
[14] Raitoharju E, Seppala I, Lyytikainen LP, et al. Blood hsa-miR-122-5p and hsa-miR-885-5p levels associate with fatty liver and related lipoprotein metabolism – the Young Finns Study. Sci Rep 2016;6:38262.
[15] Chai FN, Zhang J, Xiang HM, et al. Protective effect of Coptisine from Rhizoma Coptidis on LPS/D-GalN-induced acute liver failure in mice through up-regulating expression of miR-122. ACS Synth Biol 2017; 98:180–90.
[16] Satishchandran A, Ambade A, Rao S, et al. MicroRNA 122, regulated by GRLH2, protects livers of mice and patients from ethanol-induced liver disease. Gastroenterology 2018;154:238–52.e7.
[17] Jopling CL, Yi M, Lancaster AM, et al. Modulation of hepatitis C virus RNA abundance by a liver-specific microRNA. Science (New York, NY) 2005;309:1577–81.
[18] Lanford RE, Hildebrandt-Eriksen ES, Petri A, et al. Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. Science (New York, NY) 2010;327:198–201.
[19] Anadol E, Schierwagen R, Elfimova N, et al. Circulating microRNAs as a marker for liver injury in human immunodeficiency virus patients. Hepatology (Baltimore, MD) 2015;61:46–55.
[20] Ambade A, Satishchandran A, Szabo G. Alcoholic hepatitis accelerates early hepatobiliary cancer by increasing stemness and miR-122-mediated HIF-1alpha activation. Sci Rep 2016;6:21340.
[21] Bai S, Nasser MW, Wang B, et al. MicroRNA-122 inhibits tumorigenic properties of hepatocellular carcinoma cells and sensitizes these cells to sorafenib. J Biol Chem 2009;284:32015–27.
[22] Nassirpour R, Mehta PP, Yin MJ. miR-122 regulates tumorigenesis in hepatocellular carcinoma by targeting AKT3. PloS One 2013;8:e79655.
[23] Wu Q, Liu HO, Liu YD, et al. Decreased expression of hepatocyte nuclear factor 4alpha (Hnf4alpha)/microRNA-122 (miR-122) axis in hepatitis B virus-associated hepatocellular carcinoma enhances potential oncogenic GALNT10 protein activity. J Biol Chem 2015;290:1170–85.
[24] Xu Q, Zhang M, Tu J, et al. MicroRNA-122 affects cell aggressiveness and apoptosis by targeting PKM2 in human hepatocellular carcinoma. Oncol Rep 2015;34:2054–64.
[25] Jin Y, Wang J, Han J, et al. MiR-122 inhibits epithelial-mesenchymal transition in hepatocellular carcinoma by targeting Snail1 and Snail2 and suppressing WNT/beta-cadherin signaling pathway. Int J Cancer 2017; 360:210–7.
[26] Lin CJ, Gong HY, Tseng HC, et al. miR-122 targets an anti-apoptotic gene, Bcl-w, in human hepatocellular carcinoma cell lines. Biochem Biophys Res Commun 2008;375:315–20.
[27] Fornari F, Gramantieri L, Giovannini C, et al. MiR-122/cyclin G1 interaction modulates p53 activity and affects doxorubicin sensitivity of human hepatocarcinoma cells. Cancer Res 2009;69:5761–7.
[28] Cho HJ, Kim JK, Nam JS, et al. High circulating microRNA-122 expression is a poor prognostic marker in patients with hepatitis B virus-related hepatocellular carcinoma who undergo radiofrequency ablation. Clin Biochem 2015;48:1073–8.
[29] Kim SS, Nam JS, Cho HJ, et al. Plasma micoRNA-122 as a predictive marker for treatment response following transarterial chemoembolization in patients with hepatocellular carcinoma. J Gastroenterol Hepatol 2017;32:199–207.
[30] Gyongyosi B, Vegh E, Jaray B, et al. Pretreatment microRNA level and outcome in sorafenib-treated hepatocellular carcinoma. J Histochem Cytochem 2014;62:547–55.
[31] Koberle V, Kronenberger B, Pleti T, et al. Serum microRNA-1 and microRNA-122 are prognostic markers in patients with hepatocellular carcinoma. Eur J Cancer (Oxford, England: 1990) 2013;49:3442–9.

[32] Liu M, Liu J, Wang L, et al. Association of serum microRNA expression in hepatocellular carcinomas treated with transarterial chemoembolization and patient survival. PloS One 2014;9:e109347.

[33] Ng KT, Lo CM, Wong N, et al. Early-phase circulating miRNAs predict tumor recurrence and survival of hepatocellular carcinoma patients after liver transplantation. Oncotarget 2016;7:19824–39.

[34] Stang A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. Eur J Epidemiol 2010;25:603–5.

[35] Tierney JF, Stewart LA, Ghersi D, et al. Practical methods for incorporating summary time-to-event data into meta-analysis. Trials 2007;8:16.

[36] DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986;7:177–88.

[37] Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst 1959;22:719–48.

[38] Goldman M, Craft B, Swatloski T, et al. The UCSC cancer genomics browser: update 2015. Nucleic Acids Res 2015;43:D812–7.

[39] Xu Y, Bu X, Dai C, et al. High serum microRNA-122 level is independently associated with higher overall survival rate in hepatocellular carcinoma patients. Tumour Biol 2015;36:4773–6.

[40] Wienholds E, Kloosterman WP, Miska E, et al. MicroRNA expression in zebrafish embryonic development. Science (New York, NY) 2005; 309:310–1.

[41] Chang J, Nicolas E, Marks D, et al. miR-122, a mammalian liver-specific microRNA, is processed from hcr mRNA and may downregulate the high affinity cationic amino acid transporter CAT-1. RNA Biol 2004; 1:106–13.

[42] Fu YP, Yi Y, Huang JL, et al. Prognostic nomograms stratify survival of patients with hepatocellular carcinoma without portal vein tumor thrombosis after curative resection. Oncologist 2017;22:561–9.

[43] Shim JH, Jun MJ, Han S, et al. Prognostic nomograms for prediction of recurrence and survival after curative liver resection for hepatocellular carcinoma. Ann Surg 2015;261:939–46.

[44] Llovet JM, Zucman-Rossi J, Pikarsky E, et al. Hepatocellular carcinoma. Nat Rev Dis Primers 2016;2:16018:1–23.