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META-ANALYSIS

Abnormally expressed circular RNAs as novel non-invasive biomarkers for hepatocellular carcinoma: A meta-analysis

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Abstract

BACKGROUND

Circular RNAs (circRNAs) are a newly discovered class of endogenous non-coding RNAs that may have roles in cancer genesis and development. In the recent literature, dysregulated circRNAs have been extensively investigated in hepatocellular carcinoma (HCC). Whether or not circRNAs are of clinical value for the management of HCC has not been characterized.

AIM

To meta-analyze the diagnostic and prognostic value of abnormally expressed circRNAs in HCC.

METHODS

Eligible studies were sourced from PubMed, EMBASE, and CNKI online databases. Data on patients' clinical characteristics, including diagnostic efficacy and overall survival, were extracted. The diagnostic and prognostic parameters were respectively synthesized using the bivariate meta-analysis model and multivariate Cox hazard regression analysis based on Stata 12.0. The trim and fill method was adopted to assess the possible effects from publication bias.

RESULTS

A total of 21 eligible studies were included. The pooled sensitivity, specificity, and area under the curve of abnormally expressed circRNAs in distinguishing HCC from non-cancer controls were 0.78 (95%CI: 0.69–0.85), 0.80 (95%CI: 0.74–0.86), and 0.86, respectively. Survival analyses showed that the down-regulated circRNA expression signature correlated perfectly with HCC survival

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[hazard ratio (HR) = 0.42, 95%CI: 0.19–0.91, P = 0.028; I^2 = 92.7%, P = 0.000], whereas the HCC cases with high circRNA levels had significantly poorer prognoses than those of patients with low circRNA levels (HR = 2.22, 95%CI: 1.50–3.30, P = 0.000; I^2 = 91%, P = 0.000). Moreover, abnormally expressed circRNAs were intimately associated with tumor size, differentiation grade, microvascular invasion, metastasis, TNM stage, and serum alpha fetal protein level in patients with HCC. Stratified analysis based on sample type, control source, and expression status also yielded robust results.

CONCLUSION
Abnormally expressed circRNA signatures show immense potential as novel non-invasive biomarker(s) for HCC diagnosis and prognosis.

Key words: CircRNA; Hepatocellular carcinoma; Diagnosis; Prognosis; Meta-analysis

Core tip: Current studies investigating the clinical significance of circular RNAs (circRNAs) in hepatocellular carcinoma (HCC) were conducted using single-center and small-scale design, and the findings were controversial. We collected and analyzed the up-to-date clinical data on the significance of circRNAs in the diagnosis and prognosis of HCC. The results indicated that circRNAs may be novel indicators for the prognosis and diagnosis of HCC.

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INTRODUCTION

Hepatocellular carcinoma (HCC), one of the most common cancers of the digestive system, remains one of the leading causes of cancer deaths worldwide[1]. In China, the incidence of HCC was shown to have increased remarkably over the past decades, which has resulted in great health and economic burdens[2]. Although the technological advances for HCC treatment in recent years have vastly improved the clinical outcomes of patients with HCC, the 5-year survival rate is very low[3]. Particularly for patients with advanced HCC, the median survival time was shown to be only 3–9 mo[4]. The sensitivity and specificity of the currently used blood biomarkers such as alpha fetoprotein (AFP) and protein induced by vitamin K absence or antagonist-II (PIVKA-II) are not satisfactory for HCC detection[5]. For prognosis monitoring, no biomarkers were well developed. Therefore, it is necessary to investigate novel effective biomarkers for HCC.

Non-coding RNAs play important roles in cancer biology, providing potential targets for cancer intervention. As a new class of endogenous noncoding RNAs, circular RNAs (circRNAs) are a series of functional non-coding transcripts generated from the backsplicing of exons, introns, or both[6]. Unlike linear RNAs, circRNAs form covalently closed continuous loop structures, characterized by stability, abundance, and specific expression in different tissues and cells during development[6,7]. CircRNAs act as key regulators in a wide range of biological processes, including the initiation and progression of several types of cancer[8,9]. CircRNAs are aberrantly expressed in cancer tissues, especially in HCC, suggesting that these molecules could be novel biomarkers for HCC diagnosis and prognosis[10-30]. Whether or not circRNAs are of clinical value for the diagnosis of HCC must be clarified. Herein, we conducted this meta-analysis, aiming to assess the diagnostic and prognostic utility of circRNA expression signature in HCC.

MATERIALS AND METHODS

Study selection

The international online databases including PubMed, EMBASE, EBSCO, Biomed central, and CNKI were searched for eligible studies indexed until May 1, 2018. The searching items were: ("liver cancer" or "liver neoplasms [MeSH Terms]" or "hepatocellular carcinomas [MeSH Terms]") and ("circular RNA [MeSH Terms]" or "circRNA" or "hsa circ") and ("prognosis" or "prognoses [MeSH Terms]" or "prognostic factors [MeSH Terms]" "HR" or "hazard ratio" or "overall survival" or "OS" or "survival [MeSH Terms]" or "disease-free survival" or "DFS" or "EFS" or "event-free survival" or "progression-free survival" or "PFS") or ("diagnosis [MeSH Terms]" or "diagnoses [MeSH Terms]" or "sensitivity and specificity [MeSH Terms]" or "ROC" or "ROC curve [MeSH Terms]" or "AUC"). The attached reference list of literature was also manually searched to increase the search sensitivity.

Selection criteria

Studies were selected in compliance with the following criteria: (A) Studies were limited to those which evaluated the diagnostic or prognostic or clinicopathological features of circRNA(s) in HCC patients; (B) the true positive, false positive, false negative, and true negative values for diagnosis, or estimated hazard ratio (HR) values with 95%CIs for survival, were either available among studies or could be extracted indirectly; (C) cases were definitely diagnosed with pathological evidence; and (D) the specimens were obtained prior to any radiotherapy or chemotherapy treatments. Irrelevant papers were excluded according to the following criteria: (A) Studies with insufficient data to form the 2 × 2 table for diagnosis, or the HRs with 95%CIs for survival were unavailable; (B) Studies were rated as low quality; and (C) Basic studies, reviews, meta-analyses, comments, letters or case reports, *etc*. were also excluded.

Data extraction

The baseline contents were collected independently by two trained authors. The information covered included data such as the name of the first author, year of publication, study design, ethnicity, sample size, pathologic data of the study population, circRNA signature, test methods, sensitivity, specificity, cut-off value setting, HR values with 95%CIs for survival, follow-up time, *etc*. Any disagreements which appeared during data summarization were resolved by group consensus, or the articles' authors were reached out to.

Study quality grading

Study quality for diagnostic articles was evaluated by the Quality Assessment for Studies of Diagnostic Accuracy II checklist[31]. The tool comprises two domains including "risk of bias" and "applicability concerns", containing seven questions regarding patient selection, index tests, reference standards, flow, and timing. The answer of risk for bias could be rated as "no" (0 points), "yes" (1 point), or "unclear" (0 points). The study quality for the case-control study was judged in line with the Newcastle-Ottawa Quality Assessment Scale (NOS) checklist[32], in which the assessment focuses on a total of eight items categorized in terms of study selection, comparability, and outcome, with a maximum judgment score of "9". An answer of "yes" receives a score of "1"; otherwise, no sores were awarded.

Statistical analysis

Statistical analyses were conducted based on the Stata 12.0 program (Stata Corporation, College Station, TX, United States). Heterogeneity among studies was assessed using chi-square and I^2 tests. Either $P < 0.05$ in the chi-square test or $I^2 > 50\%$ was regarded as significant heterogeneity. The diagnostic parameters were synthesized using the bivariate meta-analysis model, and HRs with 95%CIs were combined using multivariate Cox hazard regression analysis. A random-effects model was chosen when significant heterogeneity appeared in the pooled effect size. Sensitivity analysis was performed to trace the underlying outlier studies included in the pooled effects. The bias due to publication was detected by Deek's funnel plot and Begg's and Egger's tests, and $P < 0.05$ was set to indicate statistically significant differences. If publication bias appeared, the trim and fill method was adopted to assess the possible effects of bias on the overall pooled effects[33].

RESULTS

Study enrollment

Figure 1 presents the flowchart of the literature search procedure. Searching PubMed,

EMBASE, EBSCO, Biomed central, and CNKI databases, as well as other sources, resulted in an initial inclusion of 236 records after duplicates were removed. Two authors independently screened the titles and abstracts of the 236 publications, and 187 records were excluded because their study contents were unrelated to cirRNAs in HCC. The remaining articles were intensively evaluated for the full-text contents, and 28 of them were evaluated as review articles, or basic studies with irrelevant data, or relevant articles with insufficient information, which therefore were all eliminated. In the final stage, only 21 studies, including 8 publications for diagnosis[11-13,19-21,25,29], 11 for prognosis[10,11,14-18,22,26,28,30], and 14 for clinicopathological feature[11-13,15,17,18,22-24,26-30], were included in the quality assessment and quantitative synthesis.

Characteristics of included studies

Study characteristics are shown in Tables 1 and 2. All of the included studies were identified as case-control studies, in which eight studies with 712 HCC cases and 788 controls assessed the diagnostic performance of circRNAs in HCC, and eleven studies including 2719 cohorts focused on the evaluation of the prognostic value of cirNRAs. All HCC cases were reliably diagnosed based on histopathological methods. The control sources included chronic hepatitis, liver cirrhosis, para-tumorous tissues[11,20,21,25], and non-cancer/healthy individuals[19,29]. Amplification of circRNAs was enabled by using the qPCR test, and *GAPDH* or *β-actin* was used for normalization. The circRNA signatures for diagnosis included hsa_circ_0003570, circZKSCAN1, hsa_circ_0005075, Hsa_circ_0001649, hsa_circ_0091582, hsa_circ_0128298, hsa_circ_0004018, hsa_circ_0001445, and circRNAs panel sets. CircRNA profiles for prognosis contained hsa_circ_0001649, circ-ITCH, circMTO1, cSMARCA5, circC3P1, hsa_circRNA_100338, hsa_circ_0064428, circRNA101368, hsa_circ_0103809, and circ-ZEB1.33.

Methodological quality assessment

The quality and bias of all diagnostic studies were independently appraised by two authors in accordance with the QUADAS-II criteria, whereby studies were assessed for patient selection, index test, reference standard, flow, and timing[31]. As reported in Figure 2, all included eight publications for diagnosis were judged as low risk for applicability concerns, and three studies were assessed with bias in patient selection, or index test, or reference standard, and received rated QUADAS scores equal to three points. Evaluation of the quality of all case-control studies was enabled by applying the NOS checklist[32]. As shown in Table 3, all the included prognostic studies received rated NOS scores higher or equal to six, and thus they were all included in the final synthesis.

Investigations of heterogeneity

In the overall diagnostic meta-analysis, the chi-square and I^2 tests revealed significant substantial heterogeneity among pooled effects (Q = 49.403, df = 2.00, P = 0.000; I^2 = 95.95, 95%CI: 92.85–99.05). In line with the diagnostic effects, clear heterogeneity was also observed in the pooled prognostic effects for both the elevated (P = 0.000; I^2 = 91%) and down-regulated circRNA profiles (P = 0.000; I^2 = 92.7%). Thus, all weights were synthesized using a random-effects model.

Overall diagnostic performance

The summary receiver operating characteristic curve was employed to assess the diagnostic efficacy of circRNA profiles in distinguishing HCC from non-tumorous controls. The pooled sensitivity (Figure 3A), specificity (Figure 3B), positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR) (Figure 3C), and area under the curve (AUC) (Figure 3D) were estimated to be 0.78 (95%CI: 0.69–0.85), 0.80 (95%CI: 0.74–0.86), 3.97 (95%CI: 2.85–5.54), 0.27 (95%CI: 0.19–0.39), 14.59 (95%CI: 7.83–27.21), and 0.86, respectively.

Prognostic value

We found distinct prognostic value of the abnormally expressed circRNA signature in HCC, wherein the signature covered up-regulated circRNAs and was negatively correlated with the overall survival (OS) of patients with HCC (HR = 2.22, 95%CI: 1.50–3.30, P = 0.000) (Figure 4A), hinting that these circRNAs could be considered as independent prognostic biomarkers in HCC. Meanwhile, the significantly higher survival time (OS) was found in HCC patients with down-regulated circRNA profile (HR = 0.42, 95%CI: 0.19–0.91, P = 0.028) (Figure 4B), suggesting that circRNAs with decreased expression status were more prone to act as tumor suppressor genes in HCC.

Clinicopathological association

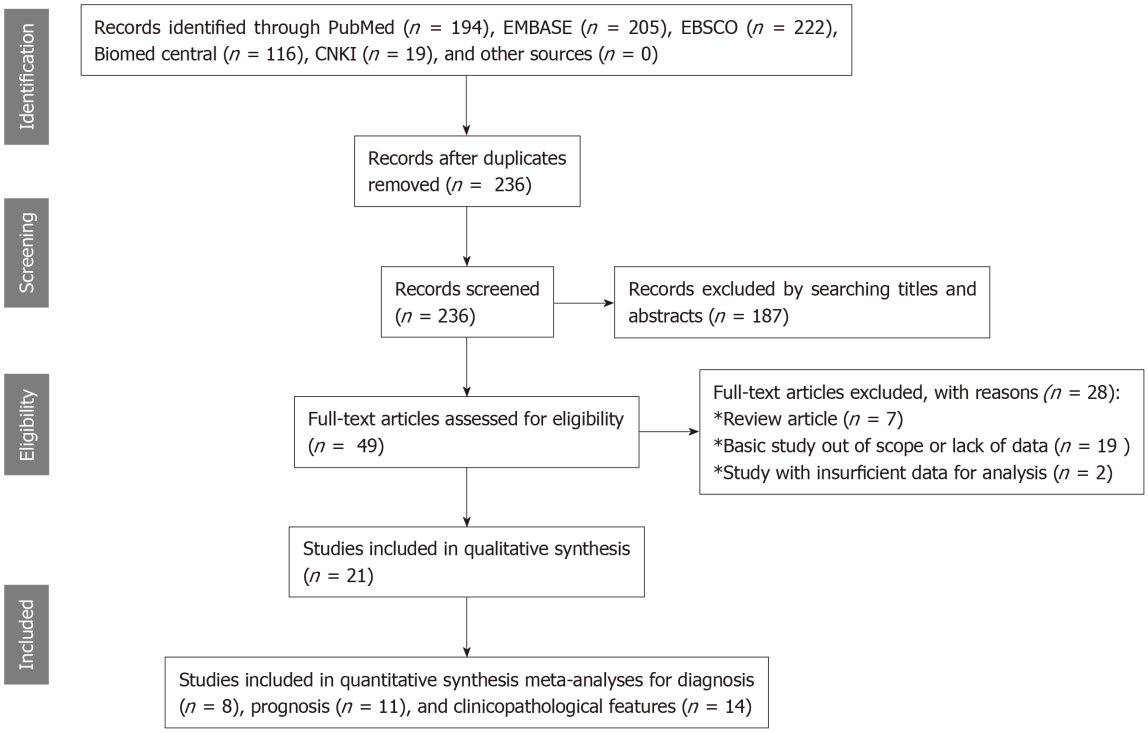

Figure 1 Study flow diagram for the diagnostic and prognostic meta-analyses.

Analysis of the association between circRNA expression and clinicopathological factors in HCC also yielded robust results. As shown in Table 4, significant associations were observed between the circRNA expression and alcoholism (pooled $P = 0.0323$), tumor size (pooled $P = 0.00012$), differentiation grade (pooled $P = 0.000$), microvascular invasion (pooled $P = 0.003744$), TNM stage (pooled $P = 0.000$), metastasis (pooled $P = 0.000$), and serum AFP level (pooled $P = 0.0115$).

Stratified analysis

The stratified analysis depending on sample type revealed that the tissue-based circRNA testing had higher diagnostic efficacy in confirming HCC than plasma-based analysis (AUC: 0.88 *vs* 0.72; DOR: 15.17 *vs* 8.93). Different effects were also observed in differentially expressed circRNAs, wherein up-regulated circRNA profile yielded a better diagnostic performance than down-regulated circRNAs (AUC: 0.97 *vs* 0.81; DOR: 11.48 *vs* 8.75). Moreover, the analysis grouped by control type showed that circRNA profiles could differentiate chronic hepatitis or cirrhosis from HCC, with an AUC of 0.84, sensitivity of 0.77, and specificity of 0.76; additionally, the circRNA expression signature was able to distinguish adjacent non-cancerous liver tissues from HCC samples, with an AUC of 0.73 and specificity of 0.75 (Table 5).

Sensitivity analysis

Sensitivity analysis was performed in both the diagnostic and prognostic effect sizes. As exemplified by Figure 4, one study[30] was identified as the outlier in the pooled prognostic effects of down-regulated circRNAs in HCC. After elimination of the outlier data and re-analysis of the effect, the I^2 dropped from 92.3% to 90%, indicating that included heterogeneous studies were a substantial cause of study heterogeneity. No outliers were detected in other pooled effects (Figure 5).

Publication bias

Publication bias was judged using different methods for different pooled effects. As shown in Figure 6A, no clear publication bias was detected in the combined diagnostic effects (Deek's funnel plot, $P = 0.446$), nor in the analysis of down-regulated circRNA profile (Egger's test, $P = 0.606$, Figure 6B). Nevertheless, the funnel plot showed evidence of publication bias in the effects of up-regulated circRNA profile (Egger's test, $P = 0.001$, Figure 6C), and the trim and fill method was applied to trace the possible impacts from bias[33]. As indicated in Figure 6D, the filled funnel plots identified five imputed studies, but the effect was slightly altered before and after adjustment (variance = 0.187, $P = 0.005$ *vs* variance = 0.287, $P = 0.000$).

Table 1 Characteristics of the included studies for diagnosis and clinicopathologic features

Study	Ethnicity	Patient size	Control size	Control source	Sample type	CircRNA Name	Expression status	Method	Cut-Off value	Control gene	AUC	QUADAS score
Fu *et al*[12], 2017	Chinese	107	107	CH & LC	Tissue	hsa_circ_0003570	Decreased	qRT-PCR	12.24	*GAPDH*	0.70	4
Yao *et al*[25], 2017	Chinese	102	102	Adjacent non-cancerous liver tissue	Tissue	circZKSCAN1	Decreased	qRT-PCR	Unclear	*GAPDH*	0.834	4
Shang *et al*[21], 2016	Chinese	30	30	Adjacent nontumorous tissue	Tissue	hsa_circ_0005075	Increased	qRT-PCR	0.000586	*GAPDH*	0.94	6
Qin *et al*[20], 2016	Chinese	89	89	Paired adjacent liver tissues	Tissue	Hsa_circ_0001649	Decreased	qRT-PCR	0.00079	*β*-actin	0.63	6
Chen *et al*[11], 2018	Chinese	30	30	Para-tumorous tissues	Tissue	hsa_circ_0091582	Increased	qRT-PCR	Unclear	*GAPDH*	0.679	5
Chen *et al*[11], 2018	Chinese	30	30	Para-tumorous tissues	Tissue	hsa_circ_0128298	Increased	qRT-PCR	Unclear	*GAPDH*	0.664	5
Chen *et al*[11], 2018	Chinese	48	48	Para-tumorous tissues	Tissue	hsa_circ_0128298	Increased	qRT-PCR	Unclear	*GAPDH*	0.668	5
Huang *et al*[17], 2017	Chinese	102	129	Para-tumorous and CH tissues	Tissue	hsa_circ_0004018	Decreased	qRT-PCR	0.531	*GAPDH*	0.848	5
Zhang *et al*[28],2018	Chinese	104	52	Healthy control	Plasma	hsa_circ_0001445	Decreased	qRT-PCR	Unclear	*GAPDH*	0.862	5
Zhang *et al*[29], 2018	Chinese	104	57	LC	Plasma	hsa_circ_0001445	Decreased	qRT-PCR	Unclear	*GAPDH*	0.672	5
Zhang *et al*[29], 2018	Chinese	104	44	CH	Plasma	hsa_circ_0001445	Decreased	qRT-PCR	Unclear	*GAPDH*	0.764	5
Han *et al*[16], 2017	Chinese	80	80	Non-cancer tissue	Tissue	CircRNA pattern	/	qRT-PCR	Unclear	Unclear	0.988	3
Han *et al*[16], 2017	Chinese	20	20	Non-cancer tissue	Tissue	CircRNA pattern	/	qRT-PCR	Unclear	Unclear	0.976	3

AUC: Area under the curve; circRNA: Circular RNA; CH: Chronic hepatitis; LC: Liver cirrhosis; qRT-PCR: Quantitative real-time PCR; GAPDH: Reduced glyceraldehyde-phosphate dehydrogenase; QUADAS: Quality Assessment for Studies of Diagnostic Accuracy.

DISCUSSION

HCC is among the most frequent causes of cancer death in digestive system tumors[1-3]. There are many investigated biomarkers for HCC, such as AFP, PIVKA-II, and the ratio of lens culinaris agglutinin-reactive alpha-fetoprotein to total AFP (AFP-L3/AFP)[34]. However, these biomarkers retain several limitations on their overall diagnostic efficacies[5,34]. In this respect, ideal noninvasive biomarkers are urgently needed to reinforce HCC detection. Circular RNAs (circRNAs), which are a group of covalently closed circular non-coding RNAs, have been recently identified as key regulators in cell development and function in HCC[35]. Accumulating investigations have shown that a large number of circRNAs are dysregulated in HCC[10-30], giving rise to the differential expression status and association in tumor diagnosis and prognosis. In the present study, we conducted diagnostic and prognostic meta-analyses and assessed the clinical significance of circRNA expression profiles in HCC.

A recently published meta-analysis showed that circRNAs are promising diagnostic biomarkers for tumors[36]. In our diagnostic meta-analysis, a total of eight studies were included, covering 712 HCC cases. The combined ROC curve showed

Table 2 Characteristics of the included studies for prognosis and clinicopathologic features

Study	Locale	Patient size	TNM stage (I/II/III/IV)	Sample type	CircRNA signature	Expression status	Survival indicator	Follow-up time	HR and 95%CI extraction	*P*-value (survival)	NOS scores
Cai *et al*[10], 2018	China	78	Unclear	Tissue	hsa_circ_0103809	Increased	OS	Unclear	Indirectly	0.001	6
Zhong *et al*[30], 2018	China	47	7, 15, 16, 9	Tissue	circC3P1	Decreased	OS	Unclear	Indirectly	0.030	6
Li *et al*[18], 2018	China	51	I–II: 24, III–IV: 27	Tissue	circRNA101368	Increased	OS	Unclear	Directly	0.001, 0.033	7
Weng *et al*[22], 2018	China	120	I–III: 60, 14, 46	Tissue	hsa_circ_0064428	Increased	OS	Unclear	Indirectly	0.033	7
Chen *et al*[11], 2018	China	78	Unclear	Tissue	hsa_circ_0128298	Increased	OS	Median: 37 months	Directly	0.009, 0.014	8
Gong *et al*[14], 2018	China	64	12, 22, 17, 13	Tissue	circ-ZEB1.33	Increased	OS	Unclear	Indirectly	0.015, 0.019	7
Yu et *al*[26], 2018	China	208	I: 62, II–III: 101	Tissue	cSMARCA5	Decreased	OS	Unclear	Directly	0.001, 0.021	7
Huang *et al*[17], 2017	China	80	I–II: 43, III–IV: 37	Tissue	hsa_circRNA_100338	Increased	OS	5 years	Indirectly	<0.01	8
Han *et al*[16], 2017	China	116	Unclear	Tissue	circMTO1	Decreased	OS	Unclear	Indirectly	0.0023	7
Guo *et al*[15], 2017	China	1800	Unclear	Tissue	circ-ITCH	Decreased	OS	Unclear	Directly	<0.001	6
Zhang *et al*[27], 2018	China	77	I–II: 34, III–IV: 43	Tissue	hsa_circ_0001649	Decreased	OS	Unclear	Directly	0.015, 0.011	6
Xu *et al*[23], 2018	China	76	I–II: 23, III–IV: 53	Tissue	hsa_circ_0001649	Decreased	/	/	/	/	/
Zhang *et al*[28], 2018	China	86	Early: 38, Late: 48	Tissue	circsMaD2	Decreased	/	/	/	/	/

circRNA: Circular RNA; OS: Overall survival; HR: Hazard ratio; NOS: Newcastle-Ottawa Scale; TNM: Tumor-node-metastasis.

that the circRNA expression profile had favorable sensitivity (0.78), specificity (0.80), and AUC (0.86) values in confirming HCC. Moreover, the respective PLR and NLR values were 3.97 and 0.27, which means that the circRNA signature achieved a ratio of nearly 4 between the true positive and false positive rates, and the probability of HCC patients that tested negative for circRNAs versus the probability of cases that tested positive had a ratio of 0.27. Importantly, the pooled DOR, a key parameter used in meta-analyses of diagnostic test accuracy studies, was estimated to be 14.59 and suggested the powerful capability of circRNA signatures in discriminating HCC from non-cancer cases. These encouraging findings suggest that circRNA expression signatures could be considered as important potential biomarkers for the diagnosis of patients with HCC.

An increasing number of single studies have documented the prognostic value of circRNAs in HCC[10,11,14,16-18,22,26,28,30]. In our pooled analysis, we found that circRNAs with different expression statuses in HCC displayed distinct prognostic features. The down-regulated circRNA profile (hsa_circ_0001649, circ-ITCH, circMTO1, cSMARCA5, and circC3P1) was closely associated with favorable OS in patients with HCC, whereas the up-regulated circRNA signature (hsa_circRNA_100338, hsa_circ_0064428, circRNA101368, hsa_circ_0103809, and circ-ZEB1.33) was related to worse OS time in HCC. A newly published study has reviewed the oncogenic (tumor suppression) roles of single circRNAs in HCC[37], further evidencing our findings. These encouraging results showed that circRNA expression signatures may be developed as potential indicator(s) for predicting the OS of HCC patients. The clinicopathological value of the circRNA expression profile also manifested robust results; circRNAs were found to be markedly associated with alcoholism, tumor size, differentiation grade, microvascular invasion, TNM stage, metastasis, and serum AFP level, suggesting that abnormally expressed circRNAs are likely to be implicated in tumor progression in HCC as well.

Our study still retains many limitations. The overriding problem is the substantial heterogeneity which appeared among studies. The sensitivity analysis identified one study[30] as the outlier in the pooled prognostic effects of down-regulated circRNAs in HCC. Our analysis further confirmed the impact of heterogeneous studies on the

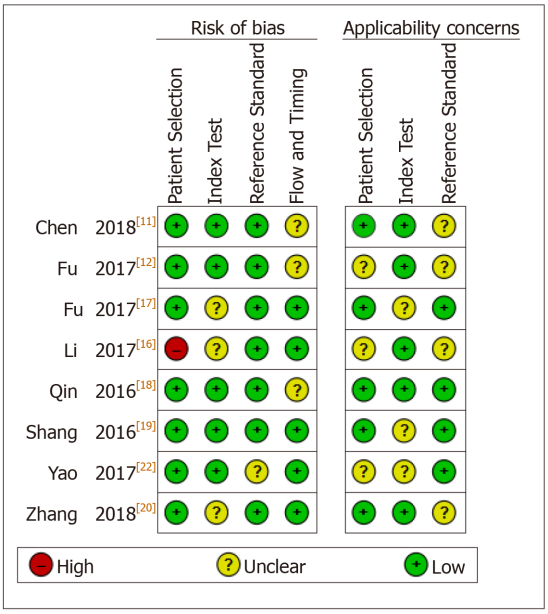

Figure 2 Study quality regarding the risk of bias and applicability concerns as assessed by the QUADAS II tool.

generation of heterogeneity among combined effects. Additionally, biases from publications appeared in one of our pooled prognostic analyses. Nevertheless, our further assessment using a nonparametric trim and fill procedure confirmed that the combined accuracy is not an artifact of unpublished negative studies. Consequently, the accuracy of all the pooled effects was shown to be relatively reliable.

In summary, our study shows evidence that abnormally expressed circRNAs may play a critical role in HCC progression and could serve as diagnostic and prognostic biomarkers for cases of HCC. Future in-depth research is required to further evaluate the utilities of single or panel circRNA(s) for HCC diagnosis and prognosis.

Table 3 Study quality and bias in the retrospective cohort studies assessed *via* the Newcastle-Ottawa Scale checklist

Study	Cohort selection				Comparability	Outcome ascertainment		
	Representativeness of the exposed cohort	Selection of the non-exposed cohort	Ascertainment of exposure	Demonstration that outcome of interest was not present at start of study	Comparability of cases and controls on the basis of the design or analysis	Assessment of outcome	Was follow-up long enough for outcomes to occur	Adequacy of follow up of cohorts
Cai *et al*[10], 2018	1	1	1	1	1	1	0	0
Zhong *et al*[30], 2018	1	1	1	1	1	1	0	0
Li *et al*[18], 2018	1	1	1	1	2	1	0	0
Weng *et al*[22], 2018	1	1	1	1	2	1	0	0
Chen *et al*[11], 2018	1	1	1	1	1	1	1	1
Gong *et al*[14], 2018	1	1	1	1	2	1	0	0
Yu et *al*[26], 2018	1	1	1	1	2	1	0	0
Huang *et al*[17], 2017	1	1	1	1	1	1	1	1
Han *et al*[16], 2017	1	1	1	1	2	1	0	0
Guo *et al*[15], 2017	1	1	1	1	1	1	0	0
Zhang *et al*[27], 2018	1	1	1	1	1	1	0	0

Table 4 Associations between circular RNA expression and clinicopathological factors in patients with hepatocellular carcinoma

Class	Included studies	χ^2	Pooled *P*-value
Gender	18	36.426	0.4487
Age	18	32.517	0.635
Smoking (yes *vs* no)	5	8.597	0.5707
Alcoholism	5	19.684	0.0323
Tumor size	13	57.979	0.00012
Tumor number (single *vs* multiple)	7	14.3614	0.4231
Encapsulation (incomplete/complete)	3	3.8078	0.7026
Differentiation grade (well/moderate/poor)	11	66.9698	1.97×10^{-6}
Microvascular invasion	3	19.261	0.003744
TNM stage	13	76.1066	2.51×10^{-7}
HBsAg	7	14.4284	0.418306
Serum AFP	12	42.4249	0.0115
Metastasis	12	79.8852	6.35×10^{-8}
ALT	3	5.4896	0.4827
AST	4	12.3545	0.1361
GGT	3	14.3614	0.4231
Cirrhosis (yes/no)	5	5.8236	0.8298

ALT: Alanine transaminase; AST: Aspartate transaminase; GGT: Gamma-glutamyl transpeptidase; AFP: Alpha fetoprotein; TNM: Tumor-node-metastasis; HbsAg: Hepatitis B surface antigen.

Table 5 Subgroup analysis conducted based on sample type, control type, and expression status among the diagnostic studies

Analysis	Included individual studies	Sensitivity 95%CI	Specificity 95%CI	PLR 95%CI	NLR 95%CI	DOR 95%CI	AUC	Heterogeneity
Sample type								
Tissue	10	0.73 (0.70–0.77)	0.82 (0.78–0.84)	4.03 (2.98–5.46)	0.29 (0.19–0.43)	15.17 (8.42–27.34)	0.88	I^2 = 73.9%, P = 0.0001
Plasma	3	0.79 (0.74–0.84)	0.65 (0.57–0.73)	2.33 (1.47–3.7)	0.28 (0.12–0.63)	8.93 (2.37–33.64)	0.72	I^2 = 87.3%, P = 0.0004
Expression status								
Up-regulated circRNAs	4	0.70 (0.62–0.78)	0.83 (0.76–0.89)	4.00 (2.71–5.91)	0.37 (0.28–0.50)	11.48 (5.90–22.33)	0.97	I^2 = 21.2%, P = 0.2832
Down-regulated circRNAs	7	0.74 (0.70–0.77)	0.75 (0.71–0.78)	2.75 (2.16–3.5)	0.33 (0.22–0.49)	8.75 (5.31–14.43)	0.81	I^2 = 70.4%, P = 0.0025
Control type								
Chronic hepatitis/cirrhosis *vs* HCC	3	0.77 (0.72–0.81)	0.76 (0.71–0.81)	3.08 (2.49–3.80)	0.32 (0.25–0.41)	10.89 (7.51–15.78)	0.84	I^2 = 0.0%, P = 0.5262
Adjacent non-cancerous liver tissue *vs* HCC	6	0.63 (0.57–0.68)	0.75 (0.69–0.81)	2.33 (1.44–3.75)	0.52 (0.40–0.69)	4.7 (3.12–7.08)	0.73	I^2 = 0.0%, P = 0.4953

AUC: Area under the curve, PLR: Positive likelihood ratio; NLR: Negative likelihood ratio; DOR: Diagnostic odds ratio; HCC: Hepatocellular carcinoma.

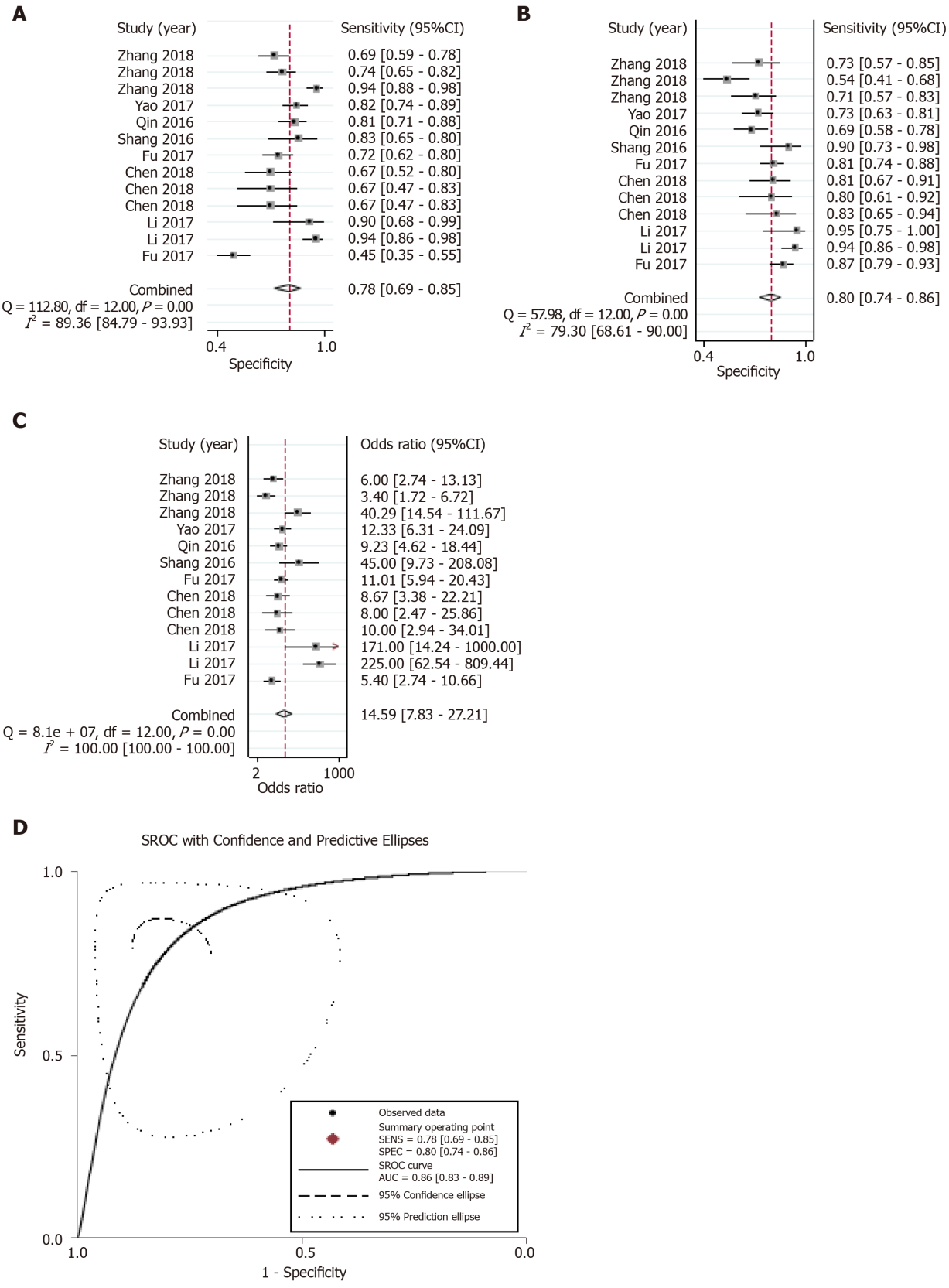

Figure 3 Overall diagnostic performance. A-D: Forest plots of the combined (A) sensitivity, (B) specificity, (C) diagnostic odds ratio, and (D) area under the curves among the eight diagnostic studies.

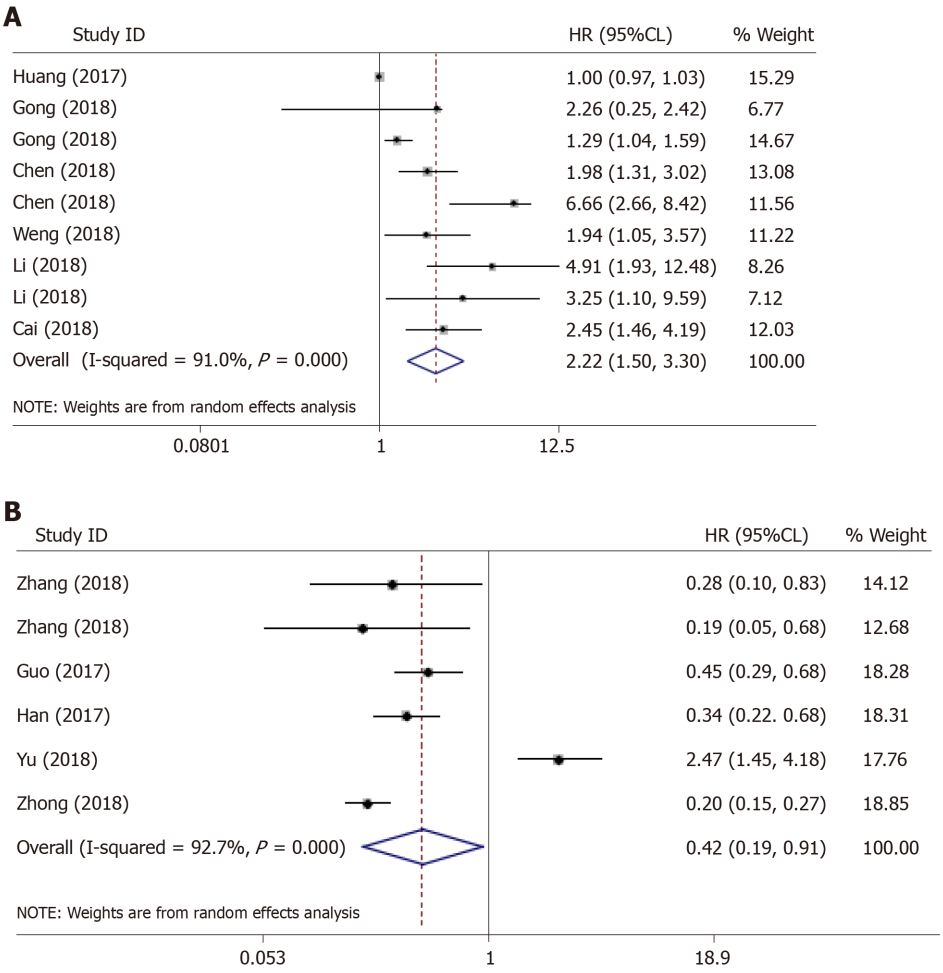

Figure 4 The outlier in the pooled prognostic effects of down-regulated circular RNAs in hepatocellular carcinoma. A and B: Forest plots of the combined hazard ratios with 95% confidence intervals for the (A) up-regulated and (B) down-regulated circular RNA profiles in predicting the overall survival of hepatocellular carcinoma patients.

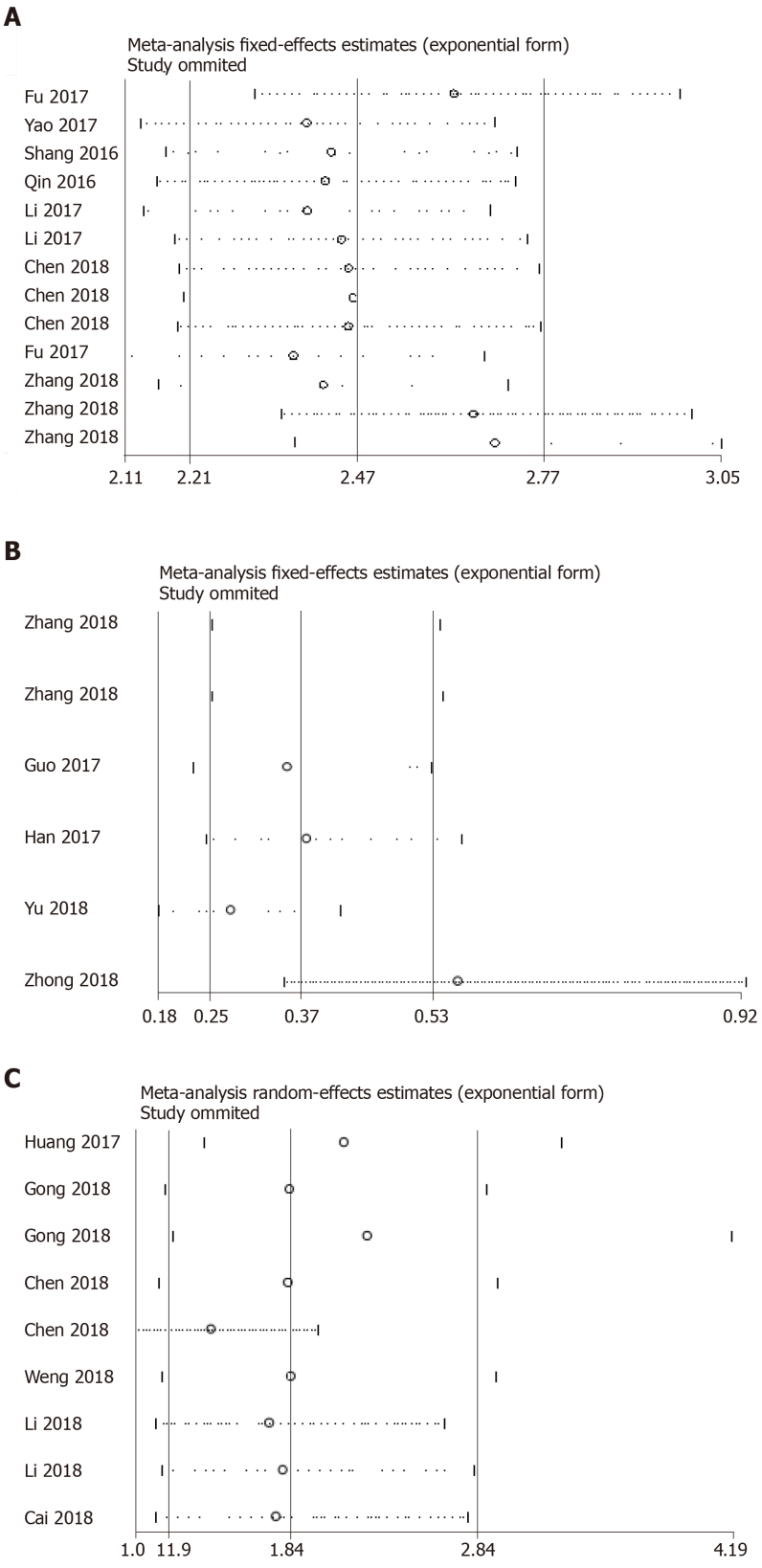

Figure 5 Sensitivity analysis of the outlier data. A: Diagnostic studies; B: Down-regulated; and C: Up-regulated circular RNA profiles in predicting the overall survival in hepatocellular carcinoma.

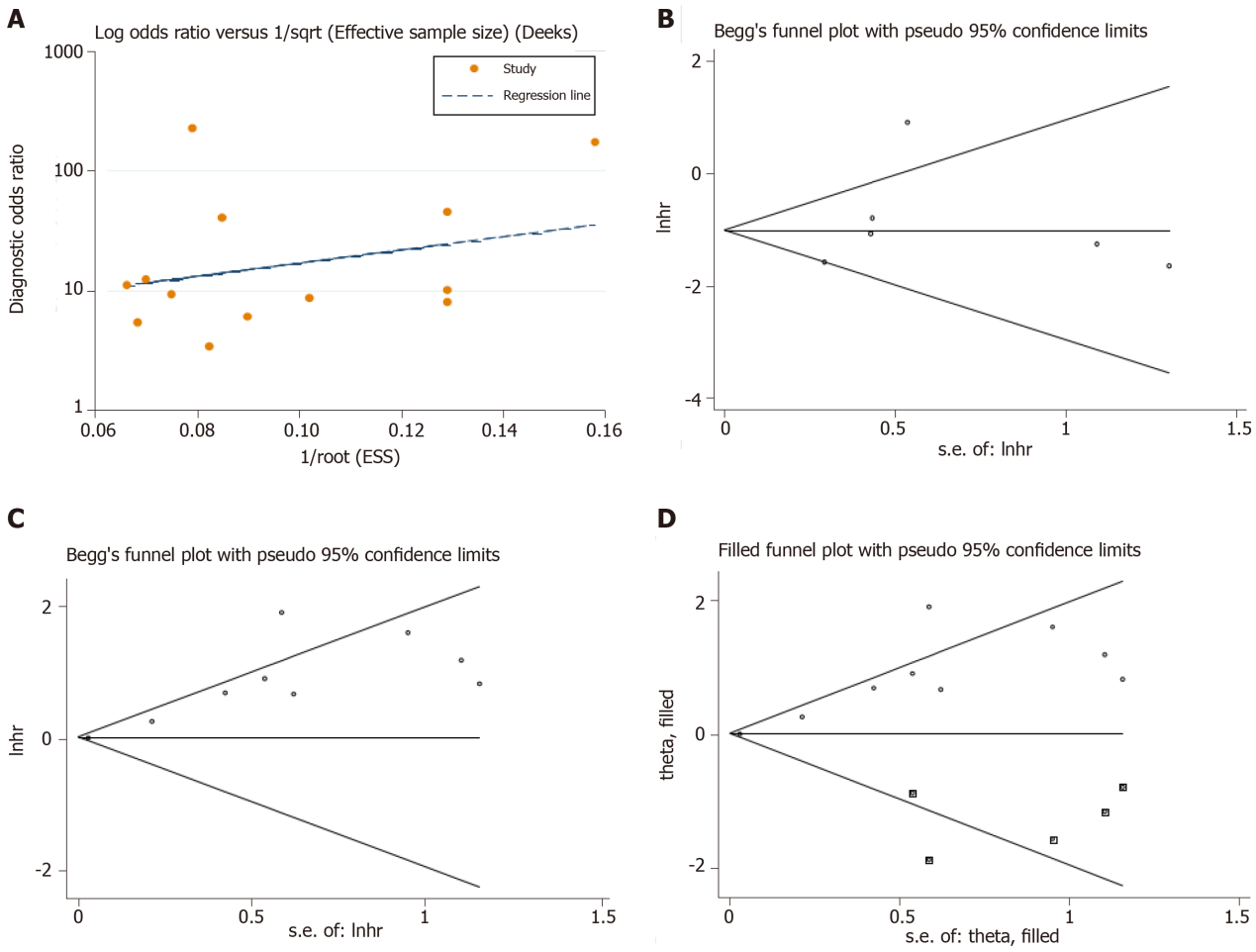

Figure 6 Publication bias judged by the Deek's funnel plot for the diagnostic meta-analysis. A-C: Begg's test for the down-regulated and up-regulated circular RNA (circRNA) signatures in predicting the overall survival in hepatocellular carcinoma; D: The trim and fill method performed to assess the possible effects of bias on the overall pooled effects of the up-regulated circRNA signature. The hollow circles in squares indicate the imputed studies.

ARTICLE HIGHLIGHTS

Research background

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide. At present, reliable biomarkers for HCC are unavailable, so it is necessary to investigate novel effective ones. The application of circular RNAs (circRNAs) in numerous tumors has been drawing considerable attention. However, the clinical value of circRNAs in HCC has not been determined.

Research motivation

We sought to provide evidence on the potential clinical value of abnormal circRNAs in HCC from the perspective of evidence-based medicine.

Research objectives

This meta-analysis was designed to reveal the clinicopathological, prognostic, and diagnostic features of circRNAs in HCC.

Research methods

We searched for articles in PubMed, EMBASE, and CNKI databases before May 2019. Studies reporting on the clinicopathologic, diagnostic, or prognostic significance of circRNAs in HCC were eligible for inclusion. The meta-analysis was performed with Stata and Meta-DiSc software, and the study quality was assessed in accordance with the Quality Assessment of Diagnostic Accuracy Studies-2 Checklist and the Newcastle-Ottawa Scale. According to the heterogeneity of the studies, a fixed- or random-effects model was used for pooling analysis.

Research results

A total of 21 studies were eligible for the meta-analysis. The results showed that the abnormality in the expression of circRNAs was of good significance in the diagnostic determination of HCC. The down-regulation of circRNAs was negatively correlated with HCC prognosis, while the up-

regulated circRNAs were positively related to the overall survival. The circRNAs were significantly associated with poor clinicopathological features in patients with HCC.

Research conclusions

This meta-analysis suggested that circRNAs may be a promising biomarker for the determination of diagnosis and prognosis of HCC in clinical practice.

Research perspectives

The results of this meta-analysis may contribute to a better understanding of the potential clinical application of abnormal circRNAs in HCC.

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