Abnormally Expressed Circular RNAs as Novel Non-Invasive Biomarkers for Hepatocellular Carcinoma: A Pair-wise Meta-Analysis

Yanlin Jiang
The First Affiliated Hospital, and College of Clinical Medicine of Henan University og Science and Technology
Mengmeng Shang
The Family Planning Guidance Station of Luanchuan County
Shizhen Dong
The First Affiliated Hospital, and College of Clinical Medicine of Henan University of Science and Technology
Menglu Chen
The First Affiliated Hospital, and College of Clinical Medicine of Henan University of Science and Technology
Xiaoli Wang
The First Affilicate Hoepital, and College of Clinical Medicine of Henan University of Science and Technology
Yongchao Chang (zero6666888@163.com)
The First Affiliated Hospital, and College of Clinical Medicine of Henan University of Science and Technology https://orcid.org/0000-0002-5149-5424

Research article

Keywords: circRNA; hepatocellular carcinoma; diagnosis; prognosis; meta-analysis

Posted Date: August 15th, 2019

DOI: https://doi.org/10.21203/rs.2.12985/v1

Abstract

Background: In the recent literature, dysregulated circular RNAs (circRNAs) have been extensively investigated in hepatocellular carcinoma (HCC). This study strives to evaluate the diagnostic as well as the predictive 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 (OS) 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 approach was employed to evaluate the impacts of publication bias. Results: A sum of 21 eligible types of research was incorporated. The pooled sensitivity, specificity and area under the curve (AUC) of abnormally expressed circRNAs in distinguishing HCC from non-cancer controls were estimated to be 0.78 (95% confidence interval (CI): 0.69–0.85), 0.80 (95% CI: 0.74–0.86) and 0.86, respectively. Survival analyses expressed that the down-regulated circRNA expression signature correlated perfectly with HCC survival (hazard ratio (HR) = 0.42, 95% CI: 0.19–0.91, p = 0.028; I2 = 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; I2 = 91%; p = 0.000). Moreover, abnormally expressed circRNAs were intimately linked with tumor size, differentiation grade, microvascular invasion, metastasis, TNM stage, and serum AFP level in patients with HCC. Stratified analysis based on sample type, control source, and expression status also yielded robust results. Conclusions: Abnormally expressed circRNA signatures show immense potential as novel non-invasive biomarker(s) in complementing HCC diagnosis and prognosis.

Background

Hepatocellular carcinoma (HCC), which is a prevalent digestive system cancer, continues to be the prime cause 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 worldwide [2]. Although the technological advances for HCC treatment in recent years have vastly improved the clinical outcomes of patients with GC, the 5-year survival rate is very low [3]. It was discovered that for individuals with HCC, the average life span is 3–9 months [4]. The sensitivity and specificity of the currently used blood biomarkers such as alpha fetal protein (AFP), Protein Induced by Vitamin K Absence or Antagonist-II (PIVKA-II), and alpha-fetoprotein heterogeneity are not satisfactory for HCC detection [5]. For prognosis monitoring, no biomarkers were well developed. Thus, it was essential for us to develop and examine the novel efficient biomarkers for HCC.

Non-coding RNAs perform crucial roles in cancer biology, providing objectives for cancer intervention. As a new class of endogenous noncoding RNAs, circular RNAs (circRNAs) are a series of functional non-coding transcripts initiated from either joining exons, introns or both. [6]. Unlike linear RNAs, circRNAs actualize covalently closed continuous loop structures, characterized by stability, abundance and specific expression in different tissues as well as cells during maturation [6, 7]. CircRNAs act as key regulators in a broad scope 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 carried out this meta-analysis, aiming to evaluate the diagnostic and prognostic utilities of circRNAs expression signature in HCC.

Methods

Study Selection

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

Selection Criteria

Studies were in compliance with the following criteria: (1) Studies were limited to those which evaluated the diagnostic or prognostic or clinicopathological features of circRNA(s) in HCC patients; (2) the TP (true positive), FP (false positive), FN (false negative), and TN (true negative) values for diagnosis, or estimated HR (hazard ratio) values with 95% CIs for survival, were either available among studies or

could be extracted indirectly; (3) cases were definitely diagnosed with pathological evidence; and (4) the specimens were obtained prior to any radiotherapy or chemotherapy treatments. Irrelevant papers were excluded according to the following criteria: (1) Studies with insufficient data to form the 2 × 2 table for diagnosis, or the HRs with 95% CIs for survival were unavailable; (2) studies were rated as low quality; and (3) 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 basic information covered comprised facts like the author's first name, year of publication, design of the research, ethnicity, sample capacity, pathologic data of the population study, 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

The quality of the studies for diagnosis was assessed by the Quality Assessment for Studies of Diagnostic Accuracy II (QUADAS II) checklist [31]. The tool comprised of two domains that included "risk of bias" and "applicability concerns", which contained 7 queries concerning patient selection, index tests, reference standards, flow, and timing. The answer of risk for bias could be rated as "no" (0 score), "yes" (1 score), or "unclear" (0 score). 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 8 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 evaluation was carried out established on the Stata 12.0 program (Stata Corporation, College Station, TX, USA). Heterogeneity among studies was assessed by employing Chi^2 (Chi-square) and Inconsistency I^2 (I-square) tests. Either $p < 0.05$ in the Chi^2 test or $I^2 >$ 50% were both regarded as studies with 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. The random effect 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. Due to publication, bias was recognized by Deek's funnel plot, Begg's and Egger's tests, and P <0.05 was situated to denote statistically significant differences. If at any instance bias was noticed, the trim and fill approaches were employed to evaluate the impacts of bias on the overall joint 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 writers separately reviewed the titles and abstracts of 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 8 studies with 712 HCC cases and 788 controls assessed the diagnostic performance of circRNAs in HCC, and 11 studies included 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-action* were 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 compliance with the QUADAS-II criteria, whereby studies were assessed for patient selection, index test, reference standard, flow and timing [31]. As reported by Figure 2, all included 8 publications for diagnosis were judged as low risk for applicability concerns, and 3 studies were assessed with bias in patient selection, or index test, or reference standard, and received rated QUADAS scores equal to 3 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 6, and thus they were all included in the final synthesis.

Investigations of Heterogeneity

In the overall diagnostic meta-analysis, the Chi^2 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 effect model.

Overall Diagnostic Performance

The summary receiver-operating characteristic (SROC) curve was employed to assess the diagnostic efficacy of circRNA profiling in distinguishing HCC from non-tumorous controls. The pooled sensitivity (Figure 3A), specificity (Figure 3B), PLR (Positive Likelihood Ratio), NLR (Negative Likelihood Ratio), DOR (Diagnostic Odds Ratio) (Figure 3C) and 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 in the abnormally expressed circRNA signature in HCC, wherein the signature covered up-regulated circRNAs and was negatively correlated with the 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 profiling (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

Evaluation of the link between circRNA expression and clinicopathological elements in HCC also produced 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 depended on sample type and revealed that the tissue-based circRNA testing expressed a slightly higher diagnostic efficacy in confirming HCC than the overall pooled analysis (AUC: 0.88 vs. 0.86; DOR: 15.17 vs. 15.59). Different effects were also observed in different expressed circRNAs, wherein up-regulated circRNA profiling yielded a better diagnostic performance than down-regulated circRNAs (AUC: 0.97vs. 0.81; DOR: 11.48 vs. 8.75). Moreover, the analysis grouped by control type expressed that circRNA profiling 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

Influence analysis was performed in both the diagnostic and prognostic effect sizes. As exemplified by Figure 5, 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 profiling (Egger's test, $p = 0.606$, Figure 6B). Nevertheless, the funnel plot expressed evidence of a publication bias in the effects of up-regulated circRNA profiling (Egger's test, $p = 0.001$, Figure 6C), and the trim and fill technique was employed to detect the outcome of bias [33]. As indicated in Figure 6D, the filled funnel plots identified 5 imputed studies, but the effect was slightly altered before and after adjustment (variance = 0.187, $p = 0.005$ vs. variance = 0.287, $p = 0.000$).

Discussion

As stated earlier, hepatocellular carcinoma, (HCC) is among the causes of cancer death in digestive system tumors [1–3]. There are numerous reviewed biomarkers for HCC, which include 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 types of covalently closed circular non-coding RNAs, have lately been determined as key regulators in cell growth and function in HCC [35]. Numerous researches indicate that a significant amount 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 8 studies were included, covering 712 HCC cases. The combined ROC curve showed 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 (diagnostic odds ratio), a key parameter used in meta-analyses of diagnostic test accuracy studies, was estimated to be 14.59 and expressed the powerful capability of circRNA signatures in discriminating HCC from con-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 accumulative number of original researches 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 notably linked 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 impacts of heterogeneous studies on the generation of heterogeneity among combined effects. Additionally, biases from publications appeared in one of our pooled prognostic analyses. Nevertheless, our further assessment through the use of a nonparametric trim and fill approach affirmed that the joint precision is not subjected to the unprinted 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 perform 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.

Abbreviations

AUC: area under the curve; circRNA:circular RNA; DOR: diagnostic odds ratio; DST: digestive system tumors;; HCC: hepatocellular carcinoma; HC: healthy control; HR: hazard ratio; NOS: Newcastle-Ottawa Scale; NLR: negative likelihood ratio; OS: overall survival; QUADAS: Quality Assessment for Studies of Diagnostic Accuracy; PLR: positive likelihood ratio;

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

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

Competing interests

All authors have no competing interests to declare.

Funding

None

Authors’ contributions

Conceived and designed the experiments: YCC & YLJ; Data extraction: MMS & SZD; Analyzed the data: MLC & XLW; Wrote the paper: YLJ. All authors read and approved the final manuscript.

Acknowledgements

None

References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A: **Global cancer statistics, 2012**. *CA: a cancer journal for clinicians* 2015, **65**(2):87-108.
2. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J: **Cancer statistics in China, 2015**. *CA: a cancer journal for clinicians* 2016, **66**(2):115-132.

3. Siegel RL, Miller KD, Jemal A: **Cancer statistics, 2016**. *CA: a cancer journal for clinicians* 2016, **66**(1):7-30.
4. Medavaram S, Zhang Y: **Emerging therapies in advanced hepatocellular carcinoma**. *Experimental hematology & oncology* 2018, **7**:17.
5. Park SJ, Jang JY, Jeong SW, Cho YK, Lee SH, Kim SG, Cha SW, Kim YS, Cho YD, Kim HS *et al*: **Usefulness of AFP, AFP-L3, and PIVKA-II, and their combinations in diagnosing hepatocellular carcinoma**. *Medicine* 2017, **96**(11):e5811.
6. Salzman J: **Circular RNA Expression: Its Potential Regulation and Function**. *Trends in genetics : TIG* 2016, **32**(5):309-316.
7. Salzman J, Gawad C, Wang PL, Lacayo N, Brown PO: **Circular RNAs are the predominant transcript isoform from hundreds of human genes in diverse cell types**. *PloS one* 2012, **7**(2):e30733.
8. Sheng JQ, Liu L, Wang MR, Li PY: **Circular RNAs in digestive system cancer: potential biomarkers and therapeutic targets**. *American journal of cancer research* 2018, **8**(7):1142-1156.
9. Cui X, Wang J, Guo Z, Li M, Li M, Liu S, Liu H, Li W, Yin X, Tao J *et al*: **Emerging function and potential diagnostic value of circular RNAs in cancer**. *Molecular cancer* 2018, **17**(1):123.
10. Cai H, Hu B, Ji L, Ruan X, Zheng Z: **Hsa_circ_0103809 promotes cell proliferation and inhibits apoptosis in hepatocellular carcinoma by targeting miR-490-5p/SOX2 signaling pathway**. *American journal of translational research* 2018, **10**(6):1690-1702.
11. Chen D, Zhang C, Lin J, Song X, Wang H: **Screening differential circular RNA expression profiles reveal that hsa_circ_0128298 is a biomarker in the diagnosis and prognosis of hepatocellular carcinoma**. *Cancer management and research* 2018, **10**:1275-1283.
12. Fu L, Wu S, Yao T, Chen Q, Xie Y, Ying S, Chen Z, Xiao B, Hu Y: **Decreased expression of hsa_circ_0003570 in hepatocellular carcinoma and its clinical significance**. *Journal of clinical laboratory analysis* 2018, **32**(2).
13. Fu L, Yao T, Chen Q, Mo X, Hu Y, Guo J: **Screening differential circular RNA expression profiles reveals hsa_circ_0004018 is associated with hepatocellular carcinoma**. *Oncotarget* 2017, **8**(35):58405-58416.
14. Gong Y, Mao J, Wu D, Wang X, Li L, Zhu L, Song R: **Circ-ZEB1.33 promotes the proliferation of human HCC by sponging miR-200a-3p and upregulating CDK6**. *Cancer cell international* 2018, **18**:116.
15. Guo W, Zhang J, Zhang D, Cao S, Li G, Zhang S, Wang Z, Wen P, Yang H, Shi X *et al*: **Polymorphisms and expression pattern of circular RNA circ-ITCH contributes to the carcinogenesis of hepatocellular carcinoma**. *Oncotarget* 2017, **8**(29):48169-48177.
16. Han D, Li J, Wang H, Su X, Hou J, Gu Y, Qian C, Lin Y, Liu X, Huang M *et al*: **Circular RNA circMTO1 acts as the sponge of microRNA-9 to suppress hepatocellular carcinoma progression**. *Hepatology* 2017, **66**(4):1151-1164.
17. Huang XY, Huang ZL, Xu YH, Zheng Q, Chen Z, Song W, Zhou J, Tang ZY, Huang XY: **Comprehensive circular RNA profiling reveals the regulatory role of the circRNA-100338/miR-141-3p pathway in hepatitis B-related hepatocellular carcinoma**. *Scientific reports* 2017, **7**(1):5428.
18. Li S, Gu H, Huang Y, Peng Q, Zhou R, Yi P, Chen R, Huang Z, Hu X, Huang Y *et al*: **Circular RNA 101368/miR-200a axis modulates the migration of hepatocellular carcinoma through HMGB1/RAGE signaling**. *Cell cycle (Georgetown, Tex)* 2018, **17**(19-20):2349-2359.
19. Li Y, Dong Y, Huang Z, Kuang Q, Wu Y, Li Y, Li M: **Computational identifying and characterizing circular RNAs and their associated genes in hepatocellular carcinoma**. *PloS one* 2017, **12**(3):e0174436.
20. Qin M, Liu G, Huo X, Tao X, Sun X, Ge Z, Yang J, Fan J, Liu L, Qin W: **Hsa_circ_0001649: A circular RNA and potential novel biomarker for hepatocellular carcinoma**. *Cancer biomarkers : section A of Disease markers* 2016, **16**(1):161-169.
21. Shang X, Li G, Liu H, Li T, Liu J, Zhao Q, Wang C: **Comprehensive Circular RNA Profiling Reveals That hsa_circ_0005075, a New Circular RNA Biomarker, Is Involved in Hepatocellular Crcinoma Development**. *Medicine* 2016, **95**(22):e3811.
22. Weng Qiaoyou CM, Li Maoquan , Zheng Yong-Fa , Shao Guoliang , Fan Weijun, Xu Xi-Ming , Ji Jiansong: **Global microarray profling identifed hsa_ circ_0064428 as a potential immune-associated prognosis biomarker for hepatocellular carcinoma**. *J Med Genet* 2018, **0**:1-7.
23. Xu L, Zhang M, Zheng X, Yi P, Lan C, Xu M: **The circular RNA ciRS-7 (Cdr1as) acts as a risk factor of hepatic microvascular invasion in hepatocellular carcinoma**. *Journal of cancer research and clinical oncology* 2017, **143**(1):17-27.
24. Xu Y, Yao Y, Zhong X, Leng K, Qin W, Qu L, Cui Y, Jiang X: **Downregulated circular RNA hsa_circ_0001649 regulates proliferation, migration and invasion in cholangiocarcinoma cells**. *Biochemical and biophysical research communications* 2018, **496**(2):455-461.
25. Yao Z, Luo J, Hu K, Lin J, Huang H, Wang Q, Zhang P, Xiong Z, He C, Huang Z *et al*: **ZKSCAN1 gene and its related circular RNA (circZKSCAN1) both inhibit hepatocellular carcinoma cell growth, migration, and invasion but through different signaling pathways**. *Molecular oncology* 2017, **11**(4):422-437.
26. Yu J, Xu QG, Wang ZG, Yang Y, Zhang L, Ma JZ, Sun SH, Yang F, Zhou WP: **Circular RNA cSMARCA5 inhibits growth and metastasis in hepatocellular carcinoma**. *Journal of hepatology* 2018, **68**(6):1214-1227.

27. Zhang X, Luo P, Jing W, Zhou H, Liang C, Tu J: **circSMAD2 inhibits the epithelial-mesenchymal transition by targeting miR-629 in hepatocellular carcinoma**. *OncoTargets and therapy* 2018, **11**:2853-2863.
28. Zhang X, Qiu S, Luo P, Zhou H, Jing W, Liang C, Tu J: **Down-regulation of hsa_circ_0001649 in hepatocellular carcinoma predicts a poor prognosis**. *Cancer biomarkers : section A of Disease markers* 2018, **22**(1):135-142.
29. Zhang X, Zhou H, Jing W, Luo P, Qiu S, Liu X, Zhu M, Liang C, Yu M, Tu J: **The Circular RNA hsa_circ_0001445 Regulates the Proliferation and Migration of Hepatocellular Carcinoma and May Serve as a Diagnostic Biomarker**. *Disease markers* 2018, **2018**:3073467.
30. Zhong L, Wang Y, Cheng Y, Wang W, Lu B, Zhu L, Ma Y: **Circular RNA circC3P1 suppresses hepatocellular carcinoma growth and metastasis through miR-4641/PCK1 pathway**. *Biochemical and biophysical research communications* 2018, **499**(4):1044-1049.
31. Whiting PF, Rutjes AW, Westwood ME, Mallett S, Deeks JJ, Reitsma JB, Leeflang MM, Sterne JA, Bossuyt PM: **QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies**. *Annals of internal medicine* 2011, **155**(8):529-536.
32. Stang A: **Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses**. *European journal of epidemiology* 2010, **25**(9):603-605.
33. Lin N, Lin Y, Fu X, Wu C, Xu J, Cui Z, Lin D: **MicroRNAs as a Novel Class of Diagnostic Biomarkers in Detection of Oral Carcinoma: a Meta-Analysis Study**. *Clinical laboratory* 2016, **62**(3):451-461.
34. Huang TS, Shyu YC, Turner R, Chen HY, Chen PJ: **Diagnostic performance of alpha-fetoprotein, lens culinaris agglutinin-reactive alpha-fetoprotein, des-gamma carboxyprothrombin, and glypican-3 for the detection of hepatocellular carcinoma: a systematic review and meta-analysis protocol**. *Systematic reviews* 2013, **2**:37.
35. Yao R, Zou H, Liao W: **Prospect of Circular RNA in Hepatocellular Carcinoma: A Novel Potential Biomarker and Therapeutic Target**. *Frontiers in oncology* 2018, **8**:332.
36. Wang M, Yang Y, Xu J, Bai W, Ren X, Wu H: **CircRNAs as biomarkers of cancer: a meta-analysis**. *BMC cancer* 2018, **18**(1):303.
37. Qiu LP, Wu YH, Yu XF, Tang Q, Chen L, Chen KP: **The Emerging Role of Circular RNAs in Hepatocellular Carcinoma**. *Journal of Cancer* 2018, **9**(9):1548-1559.

Tables

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 II Score
Fu 2017 [12]	Chinese	107	107	CH & LC	Tissue	hsa_circ_0003570	Decreased	qRT-PCR	12.24	*GAPDH*	0.70	4
Yao 2017 [22]	Chinese	102	102	Adjacent non-cancerous liver tissue	Tissue	circZKSCAN1	Decreased	qRT-PCR	Unclear	*GAPDH*	0.834	4
Shang 2016 [19]	Chinese	30	30	Adjacent nontumorous tissue	Tissue	hsa_circ_0005075	Increased	qRT-PCR	0.000586	*GAPDH*	0.94	6
Qin 2016 [18]	Chinese	89	89	Paired adjacent liver tissues	Tissue	Hsa_circ_0001649	Decreased	qRT-PCR	0.00079	*β*-actin	0.63	6
Chen 2018 [11]	Chinese	30	30	Para-tumorous tissues	Tissue	hsa_circ_0091582	Increased	qRT-PCR	Unclear	*GAPDH*	0.679	5
Chen 2018 [11]	Chinese	30	30	Para-tumorous tissues	Tissue	hsa_circ_0128298	Increased	qRT-PCR	Unclear	*GAPDH*	0.664	5
Chen 2018 [11]	Chinese	48	48	Para-tumorous tissues	Tissue	hsa_circ_0128298	Increased	qRT-PCR	Unclear	*GAPDH*	0.668	5
Fu 2017 [17]	Chinese	102	129	Para-tumorous and CH tissues	Tissue	hsa_circ_0004018	Decreased	qRT-PCR	0.531	*GAPDH*	0.848	5
Zhang 2018 [20]	Chinese	104	52	Healthy control	Plasma	hsa_circ_0001445	Decreased	qRT-PCR	Unclear	*GAPDH*	0.862	5
Zhang 2018 [20]	Chinese	104	57	LC	Plasma	hsa_circ_0001445	Decreased	qRT-PCR	Unclear	*GAPDH*	0.672	5
Zhang 2018 [20]	Chinese	104	44	CH	plasma	hsa_circ_0001445	Decreased	qRT-PCR	Unclear	*GAPDH*	0.764	5
Li 2017 [16]	Chinese	80	80	Non-cancer tissue	Tissue	CircRNA pattern	/	qRT-PCR	Unclear	Unclear	0.988	3
Li 2017 [16]	Chinese	20	20	Non-cancer tissue	Tissue	CircRNA pattern	/	qRT-PCR	Unclear	Unclear	0.976	3

AUC: area under the curve; CH: chronic hepatitis, LC: liver cirrhosis; QUADAS: Quality Assessment for Studies of Diagnostic Accuracy

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 & 95% CI Extraction	*p* Value (Survival)	NOS Scores
Cai 2018 [10]	China	78	Unclear	Tissue	hsa_circ_0103809	Increased	OS	Unclear	Indirectly	0.001	6
Zhong 2018 [27]	China	47	7, 15, 16, 9	Tissue	circC3P1	Decreased	OS	Unclear	Indirectly	0.030	6
Li 2018 [30]	China	51	I–II: 24, III–IV: 27	Tissue	circRNA101368	Increased	OS	Unclear	Directly	0.001, 0.033	7
Weng 2018 [29]	China	120	I–III: 60, 14, 46	Tissue	hsa_circ_0064428	Increased	OS	Unclear	Indirectly	0.033	7
Chen 2018 [11]	China	78	Unclear	Tissue	hsa_circ_0128298	Increased	OS	Median: 37 months	Directly	0.009, 0.014	8
Gong 2018 [13]	China	64	12, 22, 17, 13	Tissue	circ-ZEB1.33	Increased	OS	Unclear	Indirectly	0.015, 0.019	7
Yu 2018 [24]	China	208	I: 62, II–III: 101	Tissue	cSMARCA5	Decreased	OS	Unclear	Directly	0.001, 0.021	7
Huang 2017 [15]	China	80	I–II: 43, III–IV: 37	Tissue	hsa_circRNA_100338	Increased	OS	5 years	Indirectly	<0.01	8
Han 2017 [28]	China	116	Unclear	Tissue	circMTO1	Decreased	OS	Unclear	Indirectly	0.0023	7
Guo 2017 [14]	China	1800	Unclear	Tissue	circ-ITCH	Decreased	OS	Unclear	Directly	<0.001	6
Zhang 2018 [25]	China	77	I–II: 34, III–IV: 43	Tissue	hsa_circ_0001649	Decreased	OS	Unclear	Directly	0.015, 0.011	6
Xu 2018 [23]	China	76	I–II: 23, III–IV: 53	Tissue	hsa_circ_0001649	Decreased	/	/	/	/	/
Zhang 2018 [26]	China	86	Early: 38, Late: 48	Tissue	circsMaD2	Decreased	/	/	/	/	/

NOS: Newcastle-Ottawa Quality Assessment Scale

Table 3. Study quality and bias in the retrospective cohort studies assessed via the Newcastle-Ottawa Scale (NOS) 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 2018 [10]	1	1	1	1	1	1	0	0
Zhong 2018 [27]	1	1	1	1	1	1	0	0
Li 2018 [30]	1	1	1	1	2	1	0	0
Weng 2018 [29]	1	1	1	1	2	1	0	0
Chen 2018 [11]	1	1	1	1	1	1	1	1
Gong 2018 [13]	1	1	1	1	2	1	0	0
Yu 2018 [24]	1	1	1	1	2	1	0	0
Huang 2017 [15]	1	1	1	1	1	1	1	1
Han 2017 [28]	1	1	1	1	2	1	0	0
Guo 2017 [14]	1	1	1	1	1	1	0	0
Zhang 2018 [25]	1	1	1	1	1	1	0	0

Table 4. Associations between circRNA expression and clinicopathological factors in patients with HCC.

	Included Studies	Chi^2	Poole P
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 focal (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

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

Analyses	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
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

Figures

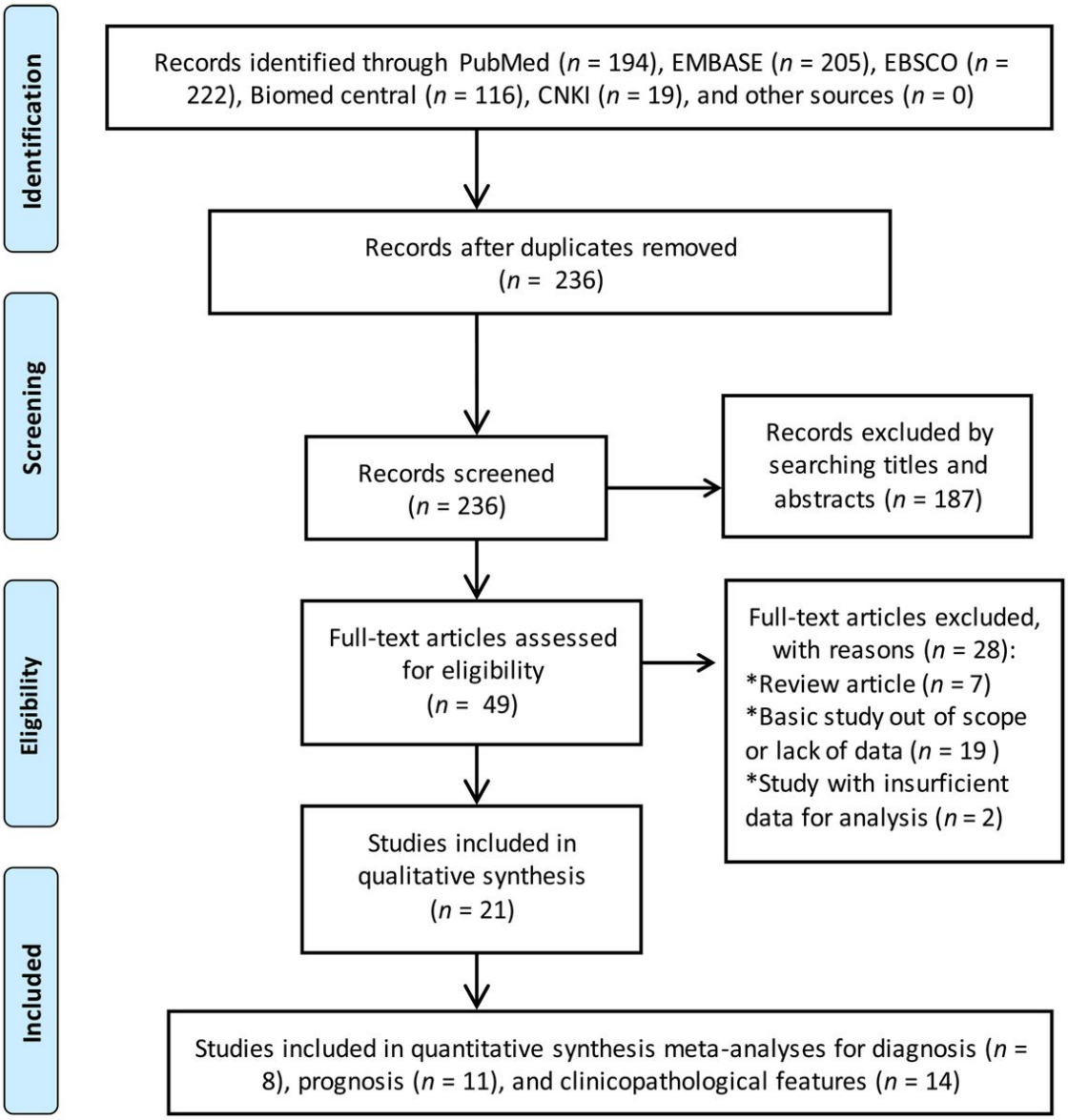

Figure 1

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

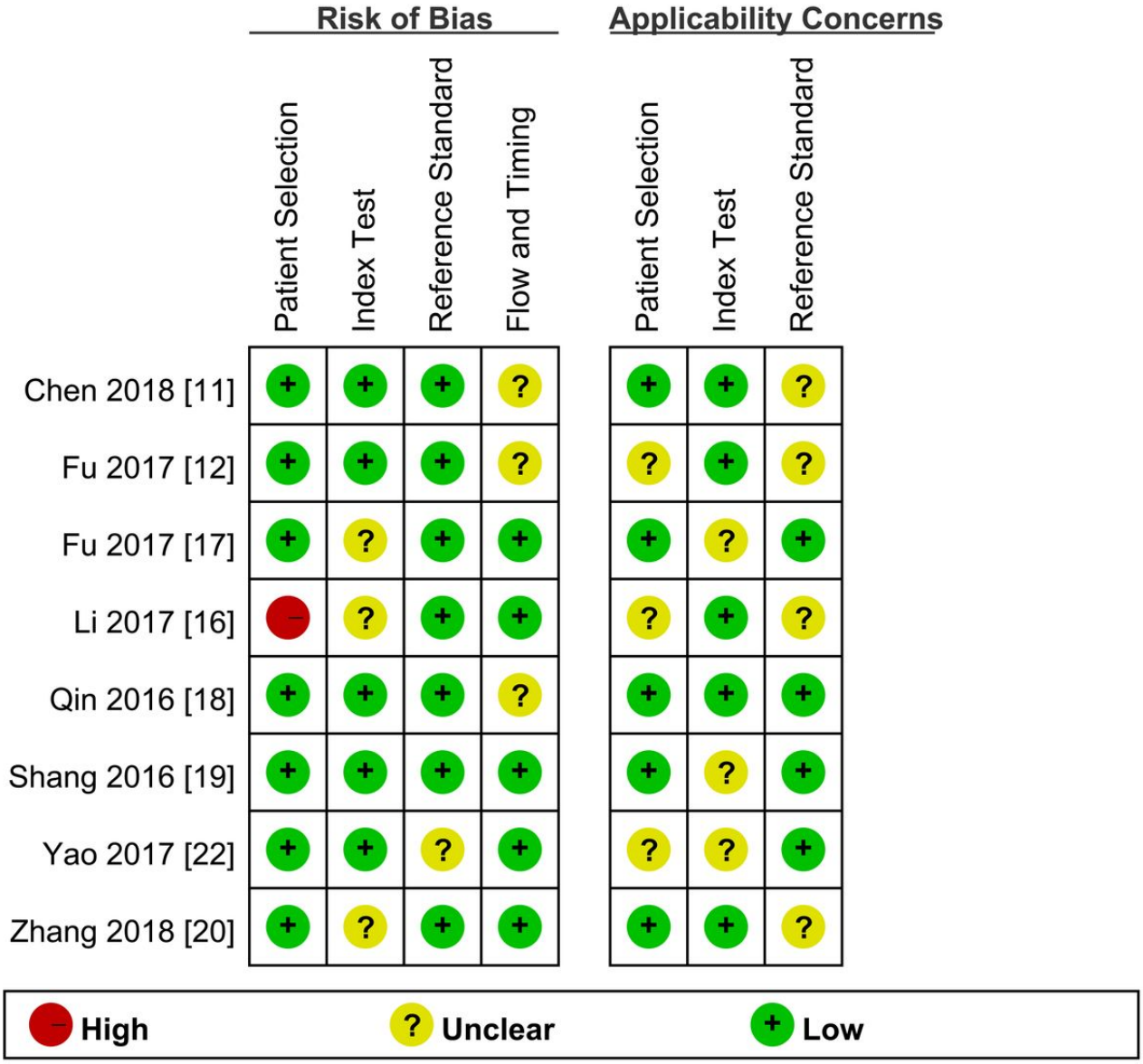

Figure 2

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

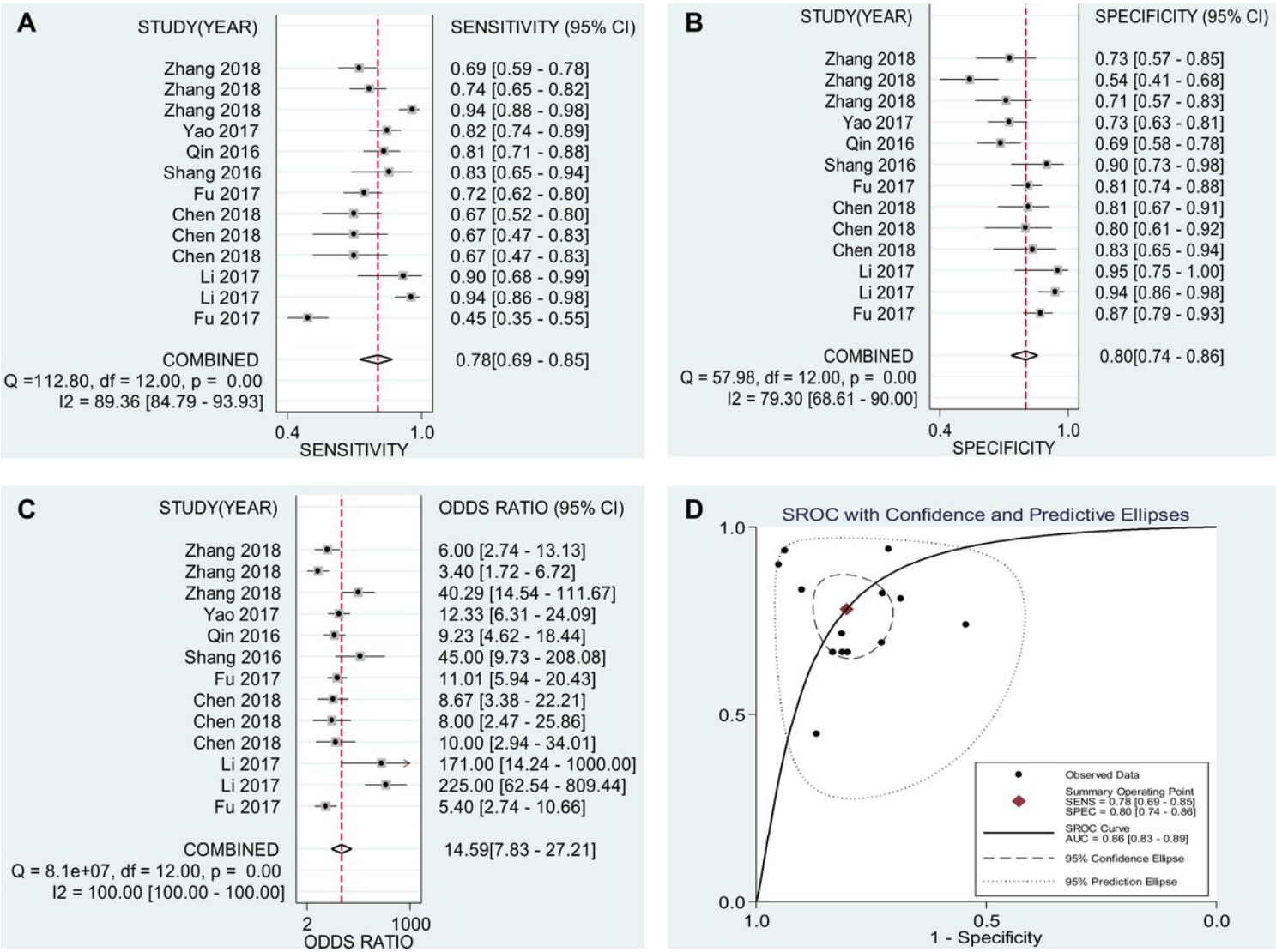

Figure 3

(A) Forest plots of the combined sensitivity, (B) specificity, (C) diagnostic Odds Ratio (DOR), and (D) area under the curve (AUC) among the 8 diagnostic studies.

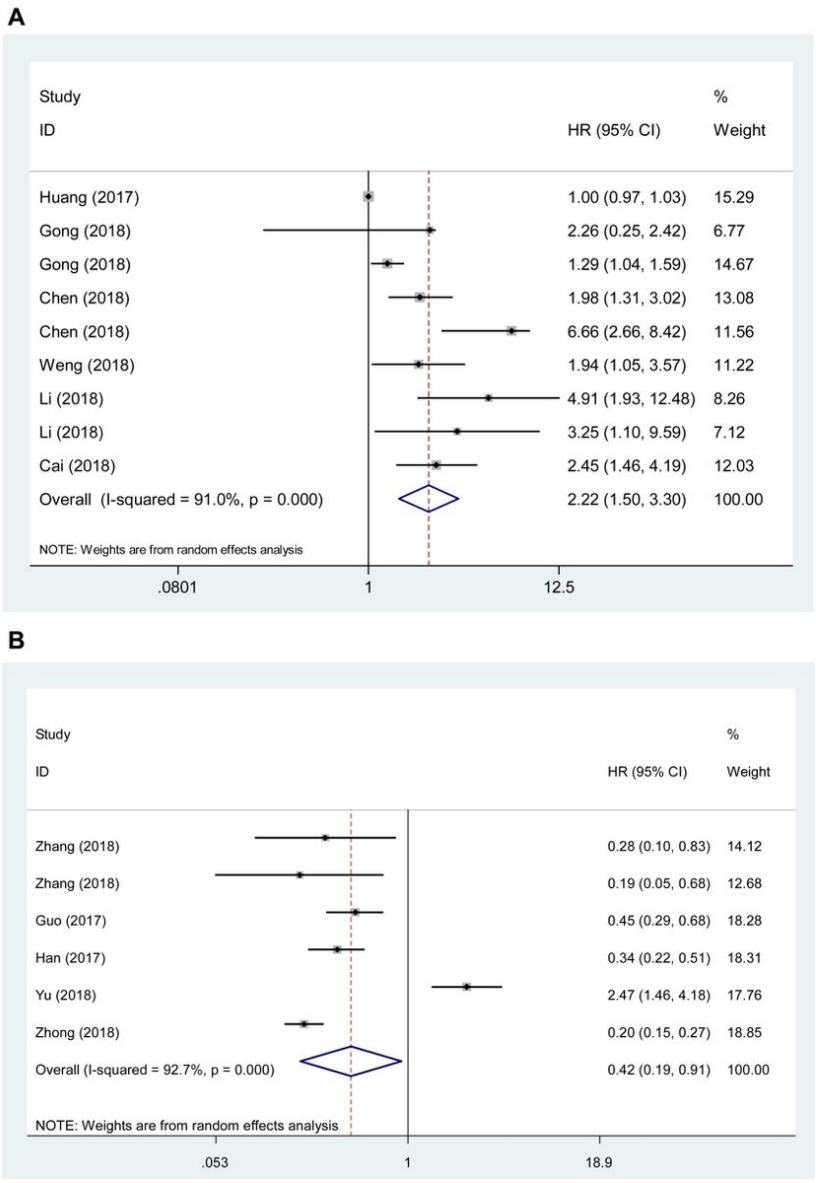

Figure 4

Forest plots of the combined hazard ratios (HRs) with 95% confidence intervals (CIs) respectively for the (A) up-regulated and (B) down-regulated circRNA profiles in predicting the overall survival (OS) of hepatocellular carcinoma (HCC) patients.

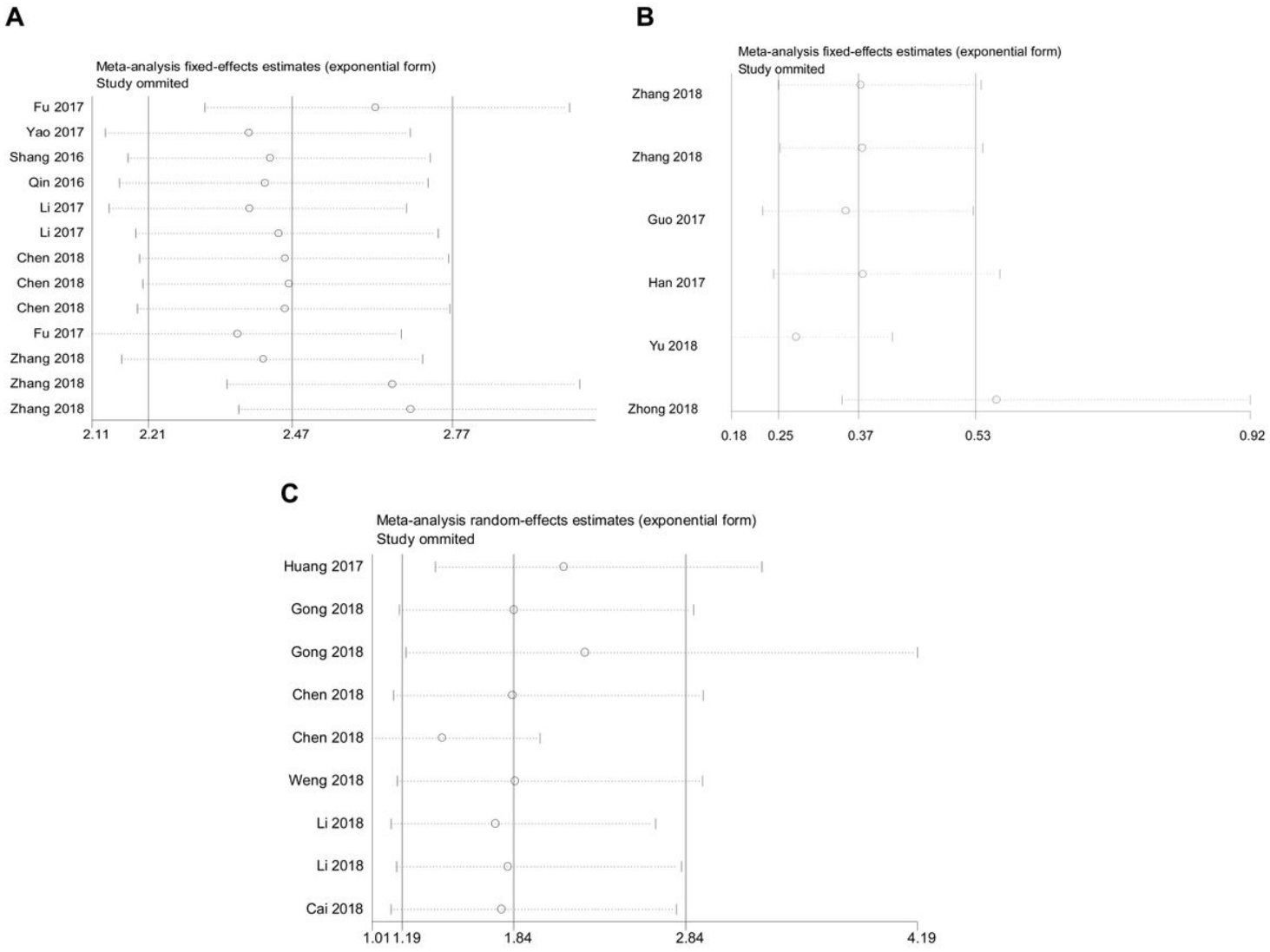

Figure 5

Sensitivity analysis of the outlier data for (A) the diagnostic studies, as well as (B) the down-regulated, and (C) up-regulated circRNA profiles in predicting the OS in HCC.

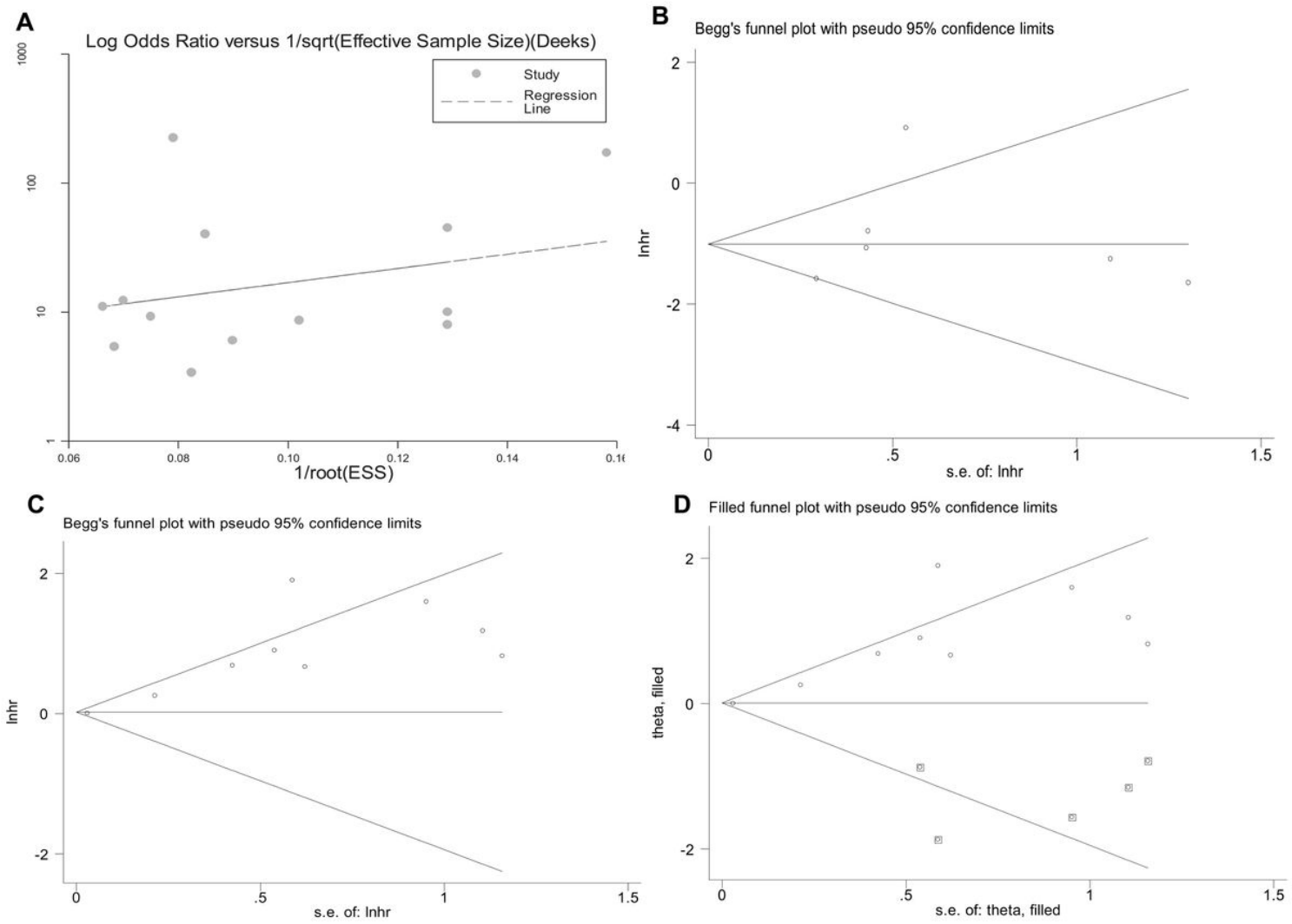

Figure 6

Publication bias judged by the Deek's funnel plot for the diagnostic meta-analysis (A), and Begg's test for (B) the down-regulated, and (C) up-regulated circRNA signatures in predicting the OS in HCC. (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, and the hollow circles in squares indicate the imputed studies.