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A Meta-Analysis of the Prognostic and Clinicopathological Significance of circZFR in Human Gastrointestinal Cancers

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Abstract

Background: Studies of gastrointestinal (GIT) cancers have shown that circZFR could be involved in the development and progression of various GIT cancers. However, small sample sizes limit the clinical significance of these studies. Here, a meta-analysis was conducted to ascertain the actual involvement of circZFR in the development and prognosis of GIT cancers. **Methods:** PubMed, Embase, Web of Science, and the Cochrane Library were searched up to December 31, 2023. Hazard ratios (HRs) or odds ratios (ORs) with 95% confidence intervals (CIs) were pooled to evaluate the association between circZFR expression and overall survival (OS). Publication bias was measured using the funnel plot and Egger's test. **Results:** 10 studies having 659 participants were enrolled for meta-analysis. High circZFR expression was associated with poor OS (HR = 1.4, 95% CI: 1.20, 1.70). High circZFR expression also predicted larger tumor size (OR = 4.38, 95% CI 2.65, 7.25), advanced clinical stage (OR = 5.33, 95% CI 3.10, 9.16), and tendency for distant metastasis (OR = 2.89, 95% CI: 1.62, 5.11), but was not related to age, gender, and histological grade. **Conclusions:** In summary, high circZFR expression was associated with poor OS, larger tumor size, advanced stage cancer and tendency for distant metastasis. These findings suggested that circZFR could be a prognostic marker for GIT cancers.

Keywords

CircZFR, Gastrointestinal, Prognostic, Significance, Meta-Analysis

1. Background

In humans, approximately 93% of the genome can be transcribed into RNA yet less than 2% are capable of being translated into proteins. The rest are termed non-coding RNAs [1]. Among these are circular RNAs (circRNAs) [2] [3] characterized by highly conserved closed loop structures that lack a free 5 cap and 3' tail, making them resistant to degradation by exonucleases. While most circRNAs are derived from exons and found in the cell cytoplasm, their mechanism of formation remains largely unknown [4].

CircRNAs primarily carry out their biological activities by acting as competing endogenous RNAs (ceRNAs), helping to sponge miRNAs, control transcription, and translation and carry out other epigenetic tasks. For instance, upregulation of circCDR1as in gastric cancer suppresses miR-7 activity which leads to more aggressive oncogenic phenotype mediated by PTEN/PI3K/AKT pathway [5]. Various studies have demonstrated their ability to regulate aging [6], diabetes [7], and various tumors [8] [9] [10]. In tumors, the involvement of circRNAs has been demonstrated in tumor development, proliferation, and metastasis [11] [11]. Recent studies have also demonstrated their involvement in tumor resistance to chemotherapy [12] [13].

Circular RNA zinc finger RNA-binding protein (Circ-ZFR) is a transcription product of zinc finger RNA-binding protein (ZFR) gene mapped to chromosome 5p13.3. Studies of gastrointestinal (GIT) cancers have shown that it could be involved in the development and progression of various GIT cancers such as hepatocellular carcinoma (HCC) [14], gastric cancer (GC) [15], and colorectal cancer (CRC) [16] among others. While the majority of these studies have demonstrated its oncogenic property, their small sample sizes limit their clinical significance. In this study, we sought to conduct a meta-analysis of all these studies to ascertain the actual involvement of circZFR in the development and prognosis of GIT cancers.

2. Methods

Records search strategy

This meta-analysis was conducted according to the 2020 updated Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) [17]. A comprehensive database search was conducted by two independent reviewers (CCB and ET) in PubMed, Embase, Web of Science, and the Cochrane Library up to December 31, 2023. The key items in the search strategy were: "circZFR" OR "circ_ZFR" OR "circ-ZFR" OR "circRNA ZFR" OR "circular RNA ZFR" OR "circ_0072088" OR "circ_0072083" OR "Circ_103809" OR "circRNA_103809" OR "Hsa_circRNA_103809" OR "Circular RNA hsa_circRNA_103809". Additionally, references of included articles were manually searched for relevant articles, and a general search on google and google scholar were conducted for articles missed in the database search.

Inclusion and exclusion criteria

The inclusion and exclusion criteria were as follows: Inclusion criteria: 1) Pa-

tients definitely diagnosed with HCC by histopathology; 2) studies that focused on clinical diagnostic or prognostic value of circZFR in HCC; 3) studies where circZFR was assigned to high expression group (high) or low expression group (low) based on its relative expression level; 4) studies that provided enough information on the correlation between circZFR expression level and overall survival (HRs with 95% CIs) or clinical characteristics (age, gender, stage, grade, and so on). Studies were excluded if: 1) they were duplicate publications; 2) focused on the structures or functions of circZFR, without any clinical diagnostic or prognostic information; 3) had non-extractable data; 4) and had no original data e.g. reviews and meta-analysis.

Data extraction and study quality assessment

Included studies were independently assessed in detail by two investigators (CCB and ET) for data extraction. Each investigator extracted data independently and any discrepancies were settled by consensus. None of the studies was an RCT. The baseline data extracted from each study were: 1) first author name and year of study, country, cancer type, clinical stage, tumor size, cut-off value, follow-up time, detection method, adjuvant therapy before surgery, survival analysis method, and outcome measure method; 2) hazard ratios (HRs) or odds ratios (ORs) with 95% confidence intervals (CIs) of circZFR for OS or clinicopathologic parameters. For studies that did not directly present HRs, the software Engauge Digitizer (version 4.1) was used to calculate it from the Kaplan-Meier curve. Study quality was assessed using the Newcastle-Ottawa Scale (NOS) [18].

Data synthesis and statistical analysis

The statistical analyses were conducted in Review Manager (RevMan 5.4). HRs or ORs with corresponding 95% CIs were used to describe the relationship between circZFR expression and the prognosis or clinical characteristics. The chi-squared test and I^2 statistics was used to assess the heterogeneity among studies. A value of $p < 0.05$, $I^2 > 50\%$ was considered to be study heterogeneity. Random effect model was used since the studies had varying methodologies. The funnel plot and Egger's test was used to estimate the potential publication bias. A P value of $p < 0.05$ was considered statistically significant.

3. Results

Study selection criteria

Thorough database search yielded 89 studies in total, with 49 duplicates that were promptly excluded. 3 studies were reviews and so excluded as well. The remaining 37 studies had their full-text articles extracted and thoroughly assessed. 24 of them did not have clinical analyses while 3 had unextractable data. These were all excluded leaving 10 studies [14] [15] [19]-[26] all from China for final inclusion in the meta-analysis. All the studies combined had a total of 659 participants. The selection flow chart is presented in **Figure 1**.

Description of included studies

Detailed information on the enrolled studies is presented in **Table 1**. Studies

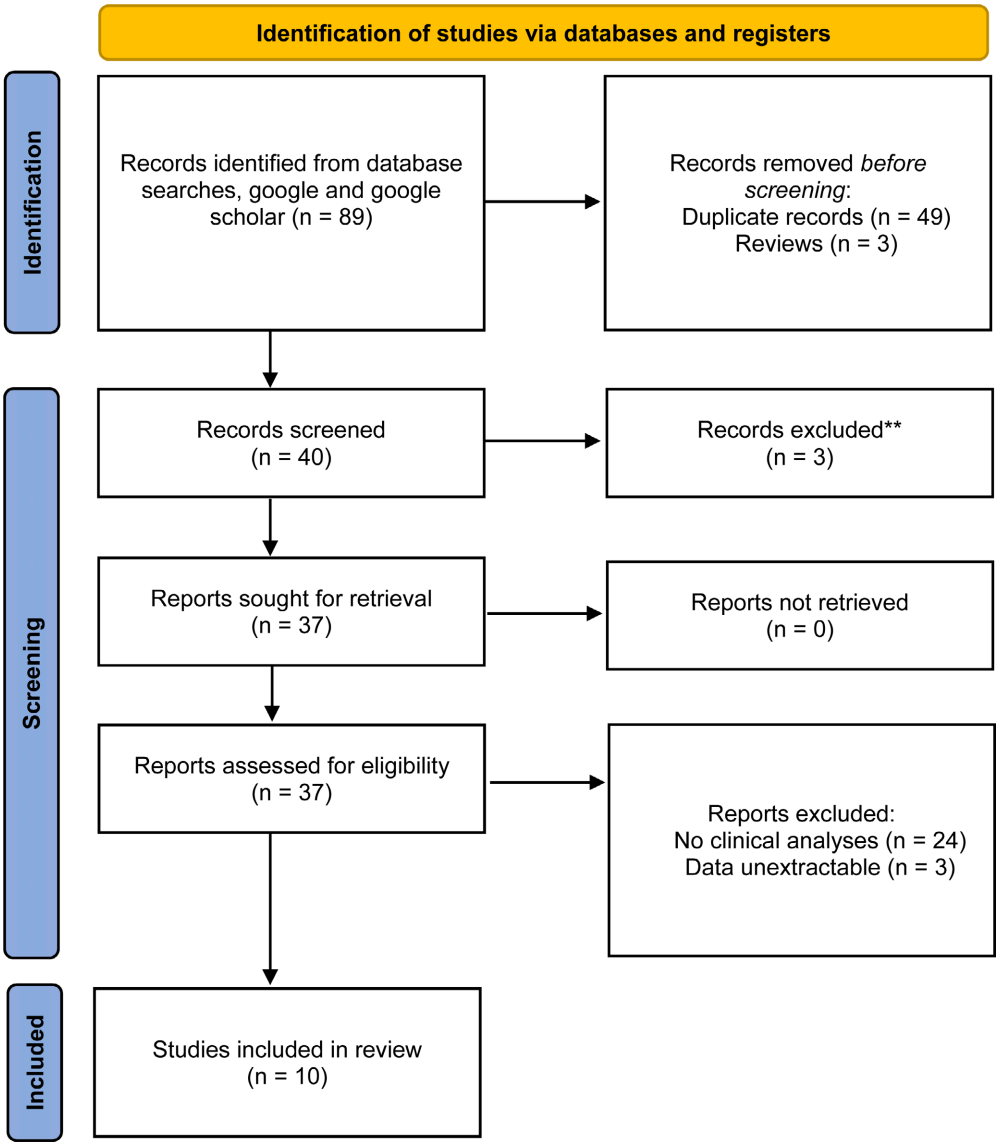

Figure 1. Flowchart of study selection criteria.

were published between 2017 to 2021, and all were conducted in China. CircZFR expression level was detected by quantitative real-time polymerase chain reaction (qRT-PCR) in all studies with the sample size ranging from 30 to 170. Analyses were both univariate and multivariate. Outcome measures were clinicopathological parameters (CP) and overall survival (OS). Only 4 studies mentioned the overall follow up time of the patients, all 60 months and more. Mean and median expression of circZFR were used as cut-off values. NOS score in all the studies was ≥ 7, indicating high overall quality of the studies.

Association between circZFR expression and OS

Four studies [15] [19] [20] [21] comprising 360 participants qualified for pooled OS analysis. The studies were generally homogeneous (I^2 = 0%, p = 0.94). The OS results indicated that high expression of circZFR was associated with relatively poor OS (HR = 1.4, 95% CI: 1.20, 1.70) (**Figure 2**).

Table 1. Summary of the main characteristics of included studies.

Author	Country	Cancer type	Clinical stage	Sample size	Cut off value	Follow up (months)	Detection method	Adjuvant therapy	Survival analysis	Outcome measure	NOS
Cedric, 2020	China	HCC	T1 - T4	62	Mean	-	qRT-PCR	None	Univariate	CP	7
Li, 2021	China	HCC	I-III	49	NA	-	qRT-PCR	None	Univariate	CP	7
Lin, 2021	China	HCC	I-IV	50	Median	60	qRT-PCR	None	Multivariate	OS, CP	9
Tan, 2019	China	HCC	-	80	Mean	60	qRT-PCR	None	Univariate	OS	8
Xu, 2021	China	HCC	I-IV	40	NA	-	qRT-PCR	None	Univariate	CP	7
Yang, 2019	China	HCC	I-IV	30	Median	-	qRT-PCR	None	Univariate	CP	7
Zhan, 2020	China	HCC	I-IV	60	NA	100	qRT-PCR	None	Univariate	OS, CP	9
Fang, 2020	China	ESCC	I-IV	58	Median	-	qRT-PCR	None	Univariate	CP	7
Huang, 2020	China	GC	-	60	NA	60	qRT-PCR	None	Univariate	OS	8
Zhang, 2017	China	CRC	I-IV	170	NA	-	qRT-PCR	None	Univariate	CP	7

Abbreviations: CRC: colorectal cancer; CP: clinicopathological parameters; ESCC: esophageal squamous; GC: gastric cancer; HCC: hepatocellular carcinoma; N/A: not available; NOS: Newcastle-Ottawa Scale; OS: overall survival; qRT-PCR: quantitative real-time polymerase chain reaction.

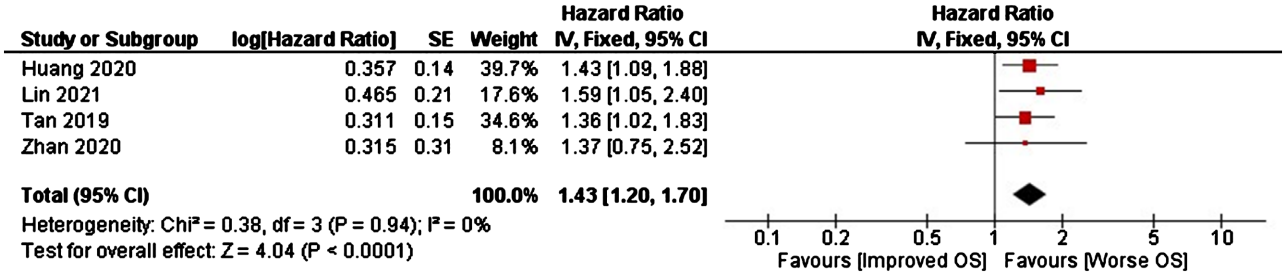

Figure 2. Forest plot evaluating the association between circZFR expression and OS.

Association between circZFR expression and clinicopathological parameters

Age, gender, tumor size, clinical stage, distant metastasis (DM), lymph node metastasis (LNM), and histology grade were the clinicopathological parameters analyzed to evaluate their correlation with circZFR expression (Table 2). Notably, six studies enrolled to explore the correlation between circZFR expression and tumor size, demonstrating that higher circZFR expression predicted larger tumor size (OR = 4.38, 95% CI 2.65, 7.25). Similarly, the upregulation of circZFR expression indicated advanced clinical stage (OR = 5.33, 95% CI 3.10, 9.16), and distant metastasis DM (OR = 2.89, 95% 1.62, 5.11) (Figure 3). Statistically insignificant association were found between circZFR expression and age (OR = 1.44, 95% CI 0.94, 2.22), gender (OR = 1.06, 95% CI 0.72, 1.57), and histological grade (OR = 1.75, 95% CI 0.52, 5.93) (Figure S1).

Publication bias analysis

The potential for publication bias was estimated using the funnel plot method and Egger's test. The results showed no significant publication bias as indicated

Table 2. Association between circZFR and other clinicopathological parameters.

Subgroup	Studies	Total participants	Odds ratio (95% CI)	P value	Model	Heterogeneity (I^2)
Age	7	479	1.44 (0.94 - 2.22)	0.09	Random	6%
Sex	8	519	1.06 (0.72 - 1.57)	0.77	Random	0%
Tumor size	6	397	4.38 (2.65 - 7.25)	0.0001	Random	0%
Clinical stage	6	397	5.33 (3.10 - 9.16)	0.00001	Random	0%
LNM stage	3	290	2.89 (1.62 - 5.11)	0.003	Random	0%
DM	2	232	2.09 (1.01 - 4.32)	0.05	Random	0%
Histology Grade	3	258	1.75 (0.52 - 5.93)	0.37	Random	73%

Abbreviations: CI: confidence interval; DM: distant metastasis; LNM: lymph node metastasis; OR: odds ratio.

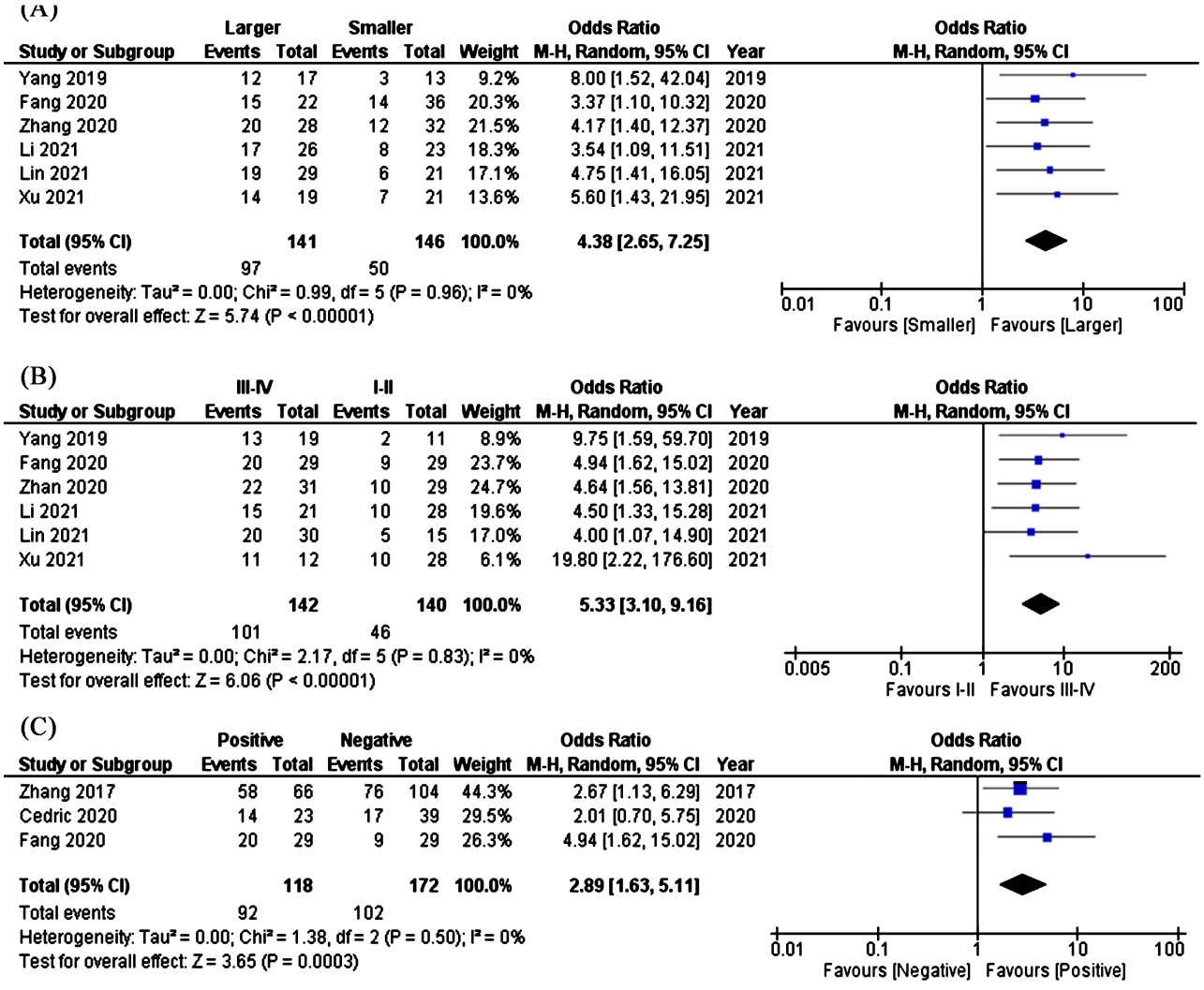

Figure 3. Forest plots evaluating the correlation between circZFR expression and clinicopathological characteristics of the patients that included; tumor size (a), clinical stage (b), and distant metastasis (c).

by the symmetrical distribution of study points in the funnel plot (**Figure S2**). Furthermore, Egger's test ($p = 0.172$) indicted no publication bias.

4. Discussion

Recent studies indicate that circRNAs possess potentials for cancer prognostic and treatment applications given their stability in body fluids such as plasma and serum, and specificity in certain cancers [27]. Circ ZFR is one of such circRNAs whose potential as a cancer driver gene has been verified in different studies. It is overexpressed in some GIT cancers [15] [20], while under expressed in others [26]. Analysis of its expression indicates strong association with certain clinicopathological characteristics in GIT cancers, making it a potential biomarker for prognostic prediction of these cancers.

In this meta-analysis, we assessed the association between CircZFR and GIT cancers. In the first step, we determined the correlation between circZFR expression and the overall survival (OS) of patients with GIT cancers. The pooled HR revealed that high circZFR expression was associated with poor OS. This was true whether the cut-off values of CircZFR expression were captured in mean or median in the original studies. Indeed, circZFR overexpression has been shown to promote cell proliferation, migration and invasion in GIT cancers such as esophageal squamous cell carcinoma [22], hepatocellular carcinoma [24] and gastric cancer [15] among others.

In relation to other major patient characteristics, we evaluated the association between circZFR expression and the patients' age, gender, tumor size, clinical stage, distant metastasis and histology grade. Our findings showed that higher circZFR expression was correlated with larger tumor size, advanced clinical stage, and distant metastasis. Statistically insignificant associations were noted in age, gender and histological grades. While circRNAs effect their biological functions by acting as miRNA molecular sponge, or regulating transcription of genes, and sometimes translation into proteins or small peptides, the function and mechanism of action of circZFR in promoting GIT cancers is still largely unclear and needs further research to unravel.

In terms of heterogeneity, the studies were largely homogenous as demonstrated by the I^2 values in the various forest plots. This is likely because all the studies were conducted in China and most had similar designs. Similarly, there was no publication bias as indicated by the symmetrical shape of the funnel plot and the result of the Egger's test. These findings improve the reliability of the meta-analysis.

This study had the following limitations that may affect interpretation. Firstly, all the included participants were from China, making generalization of results across different regions of the world difficult. Secondly, only four studies qualified for the prognosis meta-analysis, which greatly limited the wide application of the meta-analysis results. Finally, since many studies never reported HRs with their 95% CIs in the main articles, we extracted these values from the Kap-

lan-Meier curves. This could have an effect on their accuracy.

5. Conclusion

In summary, this meta-analysis demonstrated that upregulation of circ-ZFR expression is highly correlated with poor prognosis of GIT cancers. It is also correlated with certain clinicopathological parameters such as larger tumor size, advanced clinical stage, and distant metastasis among patients of Chinese origin. This demonstrates that circZFR could be a prognostic biomarker for GIT cancers. However, large-scale studies from different regions of the world will be required to verify these results.

Authors' Contributions

CCB designed the study, performed the literature retrieval, data analysis, interpretation and drafted the manuscript. ET contributed to the study methodology, performed literature retrieval and data analysis and reviewed the manuscript. ZY participated in the data analysis and assisted in creating the figures and reviewed the manuscript. JCT supervised the study. All authors read and approved the final manuscript.

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Availability of Data and Materials

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

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Supplementary

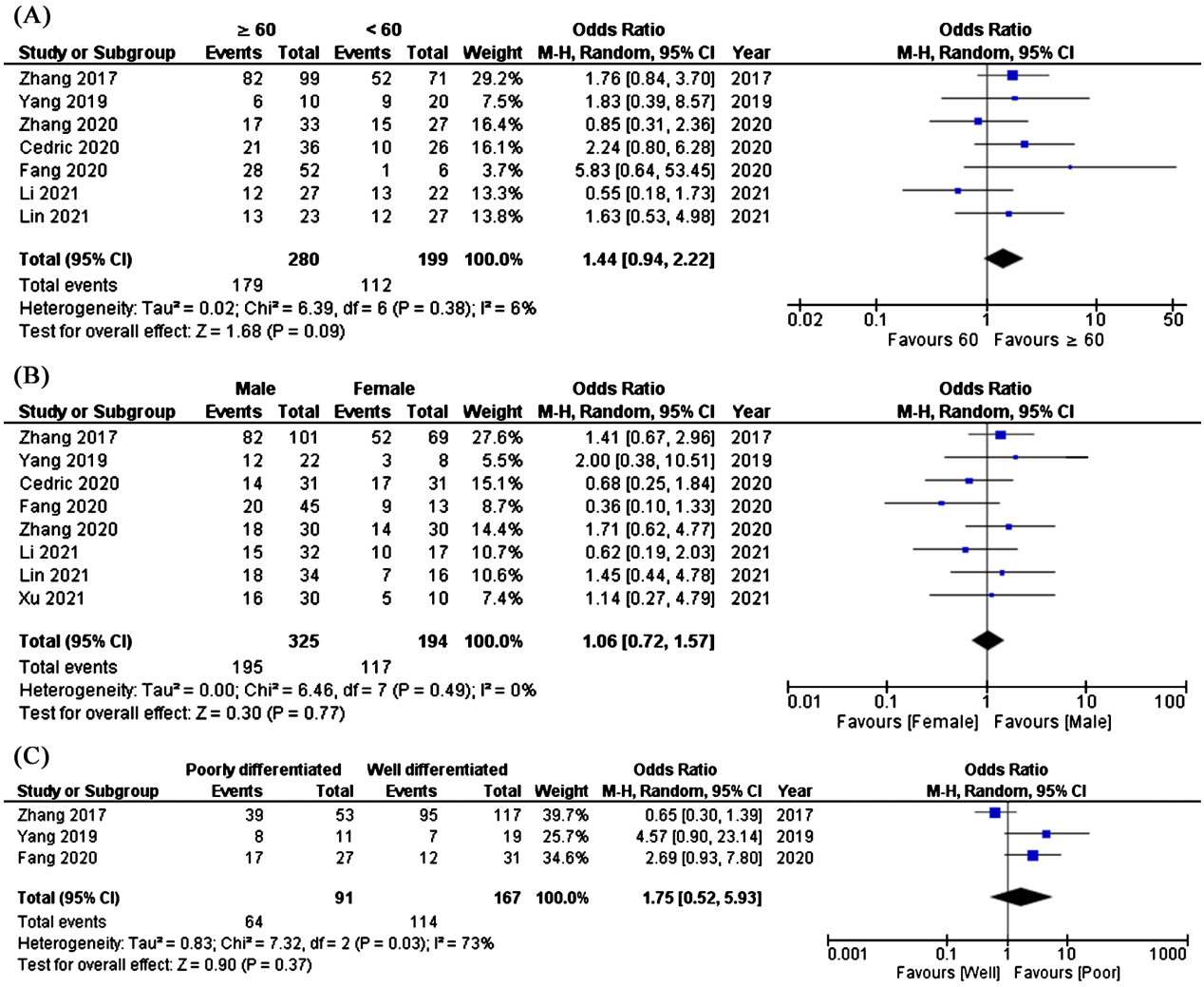

Figure S1. Forest plots of the association between circZFR expression and clinicopathological parameters, including patient age (a), gender (b), and tumor histology grade (c).

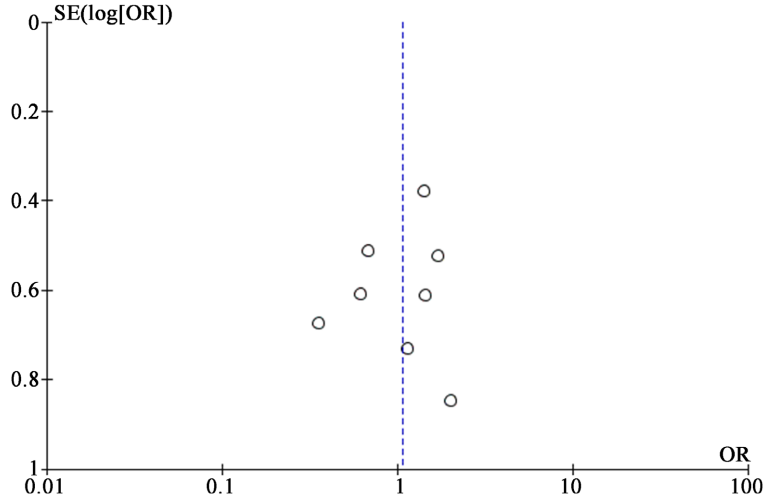

Figure S2. Funnel plot.