Prognostic and clinicopathological significance of circular RNA circ-ITCH expression in cancer patients: a meta-analysis

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Abstract

Background: Circular RNAs are a class of noncoding RNAs, and several members of them have been reported to be capable of regulating various biological processes and predicting the outcome of disease. Among them, circular RNA circ-ITCH has been identified to be aberrantly expressed and associated with disease progression in diverse cancers. However, the relationship between circ-ITCH expression and clinicopathological features, as well as prognosis of cancers, remains inconclusive.

Material and methods: Up to April 30th, 2020, relevant articles were searched in PubMed, Web of Science, Cochrane library, Embase, CNKI and Wanfang databases. A meta-analysis was performed to investigate the clinical significance of circ-ITCH in cancers by systematically summarizing all eligible literatures.

Results: A total of 1 456 patients from 12 studies were included in this meta-analysis. The results showed that cancer patients with low circ-ITCH expression were more susceptible to develop lymph node metastasis (OR=2.26, 95% CI:1.63-3.13, *P*=0.000), and were associated with larger tumor size (OR=2.69, 95%CI:1.76-4.12, *P*=0.000), advanced TNM stage (OR=2.57, 95%CI:1.63-4.06, *P*=0.000), as well as poor overall survival (OS) (HR=2.51, 95%CI:2.09-3.02, *P*=0.000, univariate analysis; HR=2.69, 95%CI:1.82-3.96, *P*=0.000, multivariate analysis).

Conclusion: Low circ-ITCH expression was significantly associated with aggressive clinicopathological features and unfavorable outcome in various cancers. Therefore, circ-ITCH may serve as a molecular therapy target and a prognostic marker in cancers.

Background

Circular RNAs (circRNAs) are a class of newly discovered noncoding RNAs with a covalently closed configuration that exist in various organisms [1]. Similar to other non-coding RNAs, circRNAs were initially considered as byproducts of splicing errors. With the advances in the field of high-throughput sequencing, increasing number of circRNAs and their functions have been characterized [2]. Functionally, circRNAs were found to be implicated in various biological processes by acting as sponges of microRNA or protein, by regulating protein function or locations, or by undergoing cap-independent translation [3]. In particular, the essential role of circRNAs in cancer development has been illustrated. Several circRNAs have been reported to be aberrantly expressed and exert oncogenic or tumor-suppressor function in cancers [4, 5] Furthermore, significant association between the expression of circRNAs and the progression of cancers has been found, indicating the potential of circRNAs to serve as biomarker for predicting outcome of cancers [6–9].

Recently, circular RNA Itchy E3 ubiquitin protein ligase (circ-ITCH), a novel circular RNA originated from exons of gene itchy E3 ubiquitin protein ligase (ITCH), located on chromosome 20q11.22, was reported to be lower expressed in several cancers [10]. So far, circ-ITCH has been proved to be implicated in prostate cancer [11, 12], ovarian cancer [13–15], bladder cancer [16], breast cancer [17], lung cancer [18], gastric cancer [19], hepatocellular carcinoma [20], glioma [21] and multiple myeloma [22]. Although the correlation between circ-ITCH expression and cancer progression has been investigated by these studies above, most individual studies have been limited by inconsistent conclusions or small sample sizes. Thus, we performed this quantitative meta-analysis by systematically evaluating the relationship between circ-ITCH expression and the clinicopathological parameters as well as prognosis of cancers with all eligible articles.

Materials And Methods

Publication search

Our literature search was performed following the preferred reporting items for systemic reviews and meta-analysis (PRISMA) statement criteria [23]. A comprehensive electronic search was performed in PubMed, Web of Science, Cochrane library, Embase, CNKI and Wanfang databases updated to April 30th, 2020. The keyword during the literature search was “circular RNA ITCH” or “circ-ITCH” or “cir-ITCH” or “circular RNA Itchy E3 ubiquitin protein ligase”. The search strategy in PubMed was *“circular RNA ITCH [All Fields] OR circ-ITCH [All Fields] OR cir-ITCH [All Fields] OR circular RNA Itchy E3 ubiquitin protein ligase [All Fields]*”. The search strategy in Web of Science was *“TS = (circular RNA ITCH OR circ-ITCH OR cir-ITCH OR circular RNA Itchy E3 ubiquitin protein ligase)*”. In addition, the citation lists of retrieved articles were screened manually for potential eligible studies.

Inclusion and exclusion criteria

Studies were considered eligible if they fulfilled the inclusion criteria as follows: (1) articles investigated the association between circ-ITCH and progression of cancers; (2) the expression of circ-ITCH in cancerous tissues was measured; (3) cancer patients were divided into high/low groups according to the expression of circ-ITCH; and (4) related clinicopathological parameters and/or prognostic results were described. Exclusion criteria of present meta-analysis are following: (1) duplicated publications; (2) reviews, letters, comments, conference articles; (3) articles irrelevant to the present study; or (4) studies without available data.

Data extract

Two investigators (Xiao-Dong Sun, Chen Huan) performed the data extraction from the eligible studies independently. Discrepancies were resolved by discussion with a third investigator (Da-Wei Sun) to reach a consensus. The following items were collected from each included study: first author, year of publication, origin of patients, cancer type, number of patients, detecting method of circ-ITCH expression, cutoff value for grouping, number of patients with lymph node metastasis (LNM), larger tumor size and advanced TNM stage in each group, follow-up period, survival analytical method (multivariate

or univariate), and hazard ratio (HR) with 95% confidence interval (CI) for overall survival (OS). When the prognosis was plotted as Kaplan-Meier curve, the software Engauge Digitizer version 4.1 (http://digieizer.sourceforge.net/) was applied to digitize the data, and HR with 95% CI was calculated as described [24].

Quality assessment

The methodological quality of the included studies was assessed with Newcastle-Ottawa Scale (NOS) criteria, which is scored based on subject selection, comparability of subject, clinical outcome [25]. The final scores of NOS range from 0 to 9, and studies with scores ≥ 6 were considered to be of high quality.

Data analysis

Meta-analyses were conducted using Stata SE12.0 (Stata Corporation, College Station, Texas). The heterogeneity among the included studies was assessed through χ^2-based Q test and I^2 statistics. When the I^2 value > 50% and/or $P < 0.10$, indicating that the heterogeneity was significant, a random-effect model was applied; otherwise, the fixed-effect model was adopted. The Begg's and Egger's test were used to estimate the potential publication bias. In addition, funnel plots were used to present the distribution of included studies' results when more than 8 studies were included. Sensitivity analysis was also performed to evaluate the effect of an individual study on the overall pooled ORs and HRs. All tests were two-sided, P values < 0.05 was considered as statistically significant.

Results

Literature information

The flow diagram for literature screening and selection was shown in Fig. 1. A total of 106 records were retrieved by searching the databases, and 94 articles were excluded on the basis of the inclusion and exclusion criteria. Finally, 12 articles comprising 1 456 patients were identified as eligible and included in the present meta-analysis.

Study characteristics

The main characteristics of eligible studies were summarized in Table 1. These 12 enrolled articles were published between 2017 and 2020 with sample sizes ranging from 20 to 288. Most of the populations were from China and divided into high or low group based on the median value of circ-ITCH expression. The expression of circ-ITCH was detected with the method of quantitative reverse transcription-polymerase chain reaction (qRT-PCR) in all 12 populations. According to the Newcastle-Ottawa Scale (NOS) criteria, all of the included studies got scores ≥ 6, indicating their high methodological quality.

Table 1
Characteristics of included studies in this meta-analysis.

First author [ref.]	Year	Cancer	Country	Sample size	Detection methods	Cut-off value	Clinicopathological features	HR, (95% CI)	Data source	Follow-up time (months)	NOS score
Zhou et al. [22]	2020	multiple myeloma	China	92	qRT-PCR	median	NA	OS(U), 2.70(1.15–6.25)	direct	median 24.5	7
Li et al. [18]	2019	lung cancer	China	190	qRT-PCR	median	□□□	OS(U), 2.27(1.39–3.57)	curve	up to 36	8
Liang et al. [15]	2019	ovarian cancer	China	122	qRT-PCR	NA	NA	OS(U), 2.86(1.43–5.88) OS(M), 4.74(1.40–16.13)	direct	median 30	6
Wang et al. [11]	2019	prostate cancer	China	52	qRT-PCR	median	□	OS(U), 3.85(1.92–8.33)	curve	up to 70	8
Huang et al. [12]	2019	prostate cancer	China	364	qRT-PCR	median	□	OS(U), 2.50(1.49–4.17) OS(M), 2.41(1.42–4.08)	direct	up to 60	8
Ghasemi et al. [19]	2018	gastric cancer	Iran	30	qRT-PCR	median	□□□	NA	NA	NA	7
Wang et al. [17]	2018	breast cancer	China	91	qRT-PCR	median	□□□	OS(U), 2.44(1.30–4.55)	curve	up to 100	8
Luo et al. [14]	2018	ovarian cancer	China	77	qRT-PCR	median	NA	OS(U), 4.76(1.52–14.29) OS(M), 4.07(1.16–14.29)	direct	median 28	7
Hu et al. [13]	2018	ovarian cancer	China	20	qRT-PCR	NA	NA	OS(U), 2.27(1.09–4.76)	curve	up to 140	6
Ma et al. [21]	2018	glioma	China	60	qRT-PCR	0.36*	NA	OS(U), 3.33(1.14–4.55) OS(M), 2.33(1.20–5.43)	direct	up to 80	7
Yang et al. [16]	2018	bladder cancer	China	70	qRT-PCR	NA	□□	OS(U), 1.89(1.05–3.33)	curve	1–60	7
Guo et al. [20]	2017	hepatocellular carcinoma	China	288	qRT-PCR	median	NA	OS(U), 2.22(1.47–3.45)	direct	up to 90	7

Association between circ-ITCH and clinicopathological parameters

As shown in Fig. 2, pooled meta-analysis was performed to estimate the relationship between circ-ITCH expression and clinicopathological features of cancers. Since there was no significant heterogeneity among these studies, fixed-effect model was exploited. The pooled OR with 95% CI indicated that cancer patients with low circ-ITCH expression were more susceptible to develop LNM (OR = 2.26, 95% CI: 1.63–3.13, P = 0.000), and were associated with advanced TNM stage (OR = 2.57, 95% CI: 1.63–4.06, P = 0.000) as well as larger tumor size (OR = 2.69, 95% CI: 1.76–4.12, P = 0.000), suggesting that low circ-ITCH level may serve as an indicator for aggressive clinicopathological features of cancer patients.

Association between circ-ITCH and OS

On one hand, 11 studies comprising a total number of 1 426 patients investigated the association between circ-ITCH expression and OS through univariate analysis. The fixed-effect model was used to assess the pooled HR and its 95% CI since no heterogeneity was found among these studies (I^2 = 0.0%, P = 0.900). We found that low circ-ITCH expression was significantly associated with poor OS (HR = 2.51, 95% CI: 2.09–3.02, P = 0.000) [Figure 3(a)]. Besides, subgroup meta-analysis was also conducted. The results showed that low circ-ITCH expression was a significant prognostic indicator of poor OS

for patients with different type of cancers: prostate cancer (HR = 2.88, 95% CI: 1.89–4.39, *P* = 0.000), ovarian cancer (HR = 2.85, 95% CI: 1.79–4.54, *P* = 0.000) and other types of cancers (HR = 2.34, 95% CI: 1.86–2.94, *P* = 0.000). Meanwhile, the significant association between low ITCH expression and unfavorable OS was also found despite the variation of sample size and different cutoff values (Table 2).

Table 2
Stratification analysis for the meta-analysis with overall survival (OS) in patients with cancers

Subgroup	No. of studies	No. of patients	Pooled HR (95% CI)	Heterogeneity	
				I^2 (%)	*P* value
Cancer type					
Prostate cancer	2	416	2.88(1.89–4.39)	0.0	0.345
Ovarian cancer	3	219	2.85(1.79–4.54)	0.0	0.558
Others	6	791	2.34(1.86–2.94)	0.0	0.884
Cut-off value					
Median	7	1154	2.53(2.03–3.16)	0.0	0.800
Others	4	272	2.46(1.76–3.43)	0.0	0.622
Sample size					
≥100	4	964	2.38(1.85–3.05)	0.0	0.900
<100	7	462	2.68(2.04–3.52)	0.0	0.673

On the other hand, 4 studies with a total number of 623 patients investigated the association between circ-ITCH expression and OS through multivariate analysis. Since there was no heterogeneity among these studies (I^2 = 0.0%, *P* = 0.671), the fix-effect model was used to assess the pooled HR and its 95% CI. We found that low circ-ITCH expression was also significantly associated with poor OS (HR = 2.69, 95% CI: 1.82–3.96, *P* = 0.000) [Figure 3(b)].

Sensitive analysis

To assess the robustness of our results, sensitivity analysis was conducted by omitting each individual included study. As illustrated in Fig. 4, removing any of the enrolled studies did not change the combined meta-analysis effect of circ-ITCH on the pooled ORs and HRs, indicating that our findings were relatively stable.

Publication bias

In this meta-analysis, both Begg's and Egger's *P* value tests were used to assess the potential publication bias. No publication bias was found in most analyses, including the studies with LNM (*P* = 1.000, 0.685), TNM stage (*P* = 0.174, Begg's test), OS (*P* = 0.174, 0.101, multivariate analysis). Publication bias was found in the studies with TNM stage (*P* = 0.01, Egger's test), tumor size (*P* = 0.042, 0.020), and OS (*P* = 0.036, 0.017, univariate analysis). Besides, the funnel plots of OS from univariate analysis (Fig. 5) were largely symmetrical. Therefore, we speculate that most of our meta-analysis results are reliable.

Discussion

Recently, as increasing studies have demonstrated the participation of circRNAs in carcinogenesis, the potential of circRNA to predict cancer progression has been suggested due to the correlation between their expression and clinicopathological characteristics as well as outcome of cancers. circ-ITCH was a newly identified circRNA, and accumulating evidence has implied its tumor-suppressor role in diverse cancers functionally. For instance, by acting as a sponge for oncogenic miR-214 and miR-17, circ-ITCH significantly enhances expression of its ITCH linear isoform via competitive interacting with microRNAs, thereby inactivating Wnt/beta-catenin signaling in various cancers [17, 21, 26–28]. Meanwhile, circ-ITCH was also reported to act as competitive endogenous RNAs (ceRNAs) of other microRNAs, like microRNA-93-5p, miR-145, to execute its tumor suppressive activity in cervical cancer and ovarian carcinoma, respectively [13, 29]. Additionally, circ-ITCH was further found to inhibit tumorigenesis through other mechanism rather than acting as microRNA sponge. In melanoma, circ-ITCH suppress cancer cell proliferation via impairing glucose uptake of cancer cells [30]. Although the association between circ-ITCH expression and cancer progression has been evaluated in these studies, most individual results were limited by their limited sample sizes or inconstant conclusion. Here, we performed this meta-analysis to investigate the clinical and prognostic value of circ-ITCH in cancers.

We included 12 studies with a total of 1456 cancer patients in this meta-analysis. The pooled ORs with their 95% CIs showed that low circ-ITCH expression was significantly associated with larger tumor size, increased LNM and advanced TNM stage, indicating that low circ-ITCH expression was an indicator of aggressive clinicopathological parameters. Moreover, the pooled HRs with their 95% CIs showed that low circ-ITCH expression was also significantly correlated with poor OS, implying that low circ-ITCH expression may serve as an indicator of unfavorable prognosis of cancers. Meanwhile, it has been demonstrated that ectopic expression of circ-ITCH was capable of inhibiting cancer growth *in vivo* [11, 16], hinting that circ-ITCH might be a potential approach for cancer treatment. Taken together, circ-ITCH could serve as a biomarker for predicting the progression and outcome of cancers.

To the best of our knowledge, this is the first meta-analysis to investigate the clinical significance of circ-ITCH in cancer patients. Nevertheless, some limitations of this meta-analysis should be concerned. The primary concern is that most of the included studies were conducted based on the population from China, thus the results should be substantiated by additional studies in worldwide population. Secondly, publication bias was observed in the studies with OS via univariate analysis. For some studies, the OS results were not available, which may contribute to the publication bias. Based on these limitations above, the pooled results calculated in this meta-analysis may be just estimations.

Conclusion

In summary, our meta-analysis revealed that low circ-ITCH expression was significantly associated with larger tumor sizes, advanced TNM stage, increased LNM, as well as poor overall survival in cancers. Therefore, circ-ITCH may serve as a prognostic biomarker and a promising molecular therapy target in cancers.

Abbreviations

circ-ITCH: circular RNA Itchy E3 ubiquitin protein ligase; NA: not available; qRT-PCR: quantitative reverse transcription-polymerase chain reaction; OS: overall survival; U: univariate analysis; M: multivariate analysis; Curve: Kaplan-Meier curve.

Declarations

Acknowledgments

Not applicable.

Authors' Contributions

LGY designed this research, SXD and HC performed data analysis and wrote this paper, and SDW helped performing data analysis. All of the authors have reviewed and approved of the final manuscript. SXD and HC have contributed equally to this work.

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Availability of data and materials

The data supporting this meta-analysis are from previously reported studies that have been cited. The processed data are available from the corresponding author upon request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Patop IL, Wust S, Kadener S. Past, present, and future of circRNAs. The EMBO journal. 2019, 38(16):e100836.
2. Wilusz JE. A 360 degrees view of circular RNAs: From biogenesis to functions. Wiley interdisciplinary reviews RNA. 2018, 9(4):e1478.
3. Kristensen LS, Andersen MS, Stagsted LVW, et al. The biogenesis, biology and characterization of circular RNAs. Nature reviews Genetics. 2019, 20(11):675-691.
4. Shang BQ, Li ML, Quan HY, et al. Functional roles of circular RNAs during epithelial-to-mesenchymal transition. Molecular cancer. 2019, 18(1):138.
5. Shan C, Zhang Y, Hao X, et al. Biogenesis, functions and clinical significance of circRNAs in gastric cancer. Molecular cancer. 2019, 18(1):136.
6. Chen Z, Zhang L, Han G, et al. A Meta-Analysis of the Diagnostic Accuracy of Circular RNAs in Digestive System Malignancy. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology. 2018, 45(3):962-972.
7. Huang X, Zhang W, Shao Z. Prognostic and diagnostic significance of circRNAs expression in hepatocellular carcinoma patients: A meta-analysis. Cancer medicine. 2019, 8(3):1148-1156.
8. Li F, Huang Q, Gong Z, et al. Diagnostic and prognostic roles of circ-SHPRH for solid cancers: a meta-analysis. Onco Targets Ther. 2019, 12:4351-4357.

9. Li C, Zhang L, Meng G, et al. Circular RNAs: pivotal molecular regulators and novel diagnostic and prognostic biomarkers in non-small cell lung cancer. Journal of cancer research and clinical oncology. 2019, 145(12):2875-2889.
10. Li Y, Ge YZ, Xu L, et al. Circular RNA ITCH: A novel tumor suppressor in multiple cancers. Technology in cancer research & treatment. 2019:117176.
11. Wang X, Wang R, Wu Z, et al. Circular RNA ITCH suppressed prostate cancer progression by increasing HOXB13 expression via spongy miR-17-5p. Cancer cell international. 2019, 19(1).
12. Huang E, Chen X, Yuan Y. Downregulated circular RNA itchy E3 ubiquitin protein ligase correlates with advanced pathologic T stage, high lymph node metastasis risk and poor survivals in prostate cancer patients. Artificial cells, nanomedicine, and biotechnology. 2019, 26(1):41-50.
13. Hu JH, Wang L, Chen JM, et al. The circular RNA circ-ITCH suppresses ovarian carcinoma progression through targeting miR-145/RASA1 signaling. Biochemical and biophysical research communications. 2018, 505(1):222-228.
14. Luo L, Gao Y, Sun X. Circ-ITCH correlates with small tumor size, decreased FIGO stage and prolonged overall survival, and it inhibits cells proliferation while promotes cells apoptosis in epithelial ovarian cancer. Cancer biomarkers: section A of Disease markers. 2018, 23(4):505-513.
15. Liang YH. The correlation between circ-itch expression level and the clinicopathological features and prognosis of patients with epithelial ovarian cancer and its effect on the proliferation and apoptosis of ovarian cancer cells. J Fujian Med Uni. 2019, 53(01):13-18.
16. Yang C, Yuan W, Yang X, et al. Circular RNA circ-ITCH inhibits bladder cancer progression by sponging miR-17/miR-224 and regulating p21, PTEN expression. Molecular cancer. 2018, 17(1).
17. Wang ST, Liu LB, Li XM, et al. Circ-ITCH regulates triple-negative breast cancer progression through the wnt/β-catenin pathway. Neoplasma. 2019, 66(2):232-239.
18. Li Z, Guo X, Gao S. Circ-ITCH correlates with less advanced tumor features as well as prolonged survival, and it inhibits cells proliferation but promotes apoptosis in non-small cell lung cancer. Translational Cancer Research. 2019, 8:1672-1679.
19. Ghasemi S, Emadi-Baygi M, Nikpour P. Down-regulation of circular RNA ITCH and circHIPK3 in gastric cancer tissues. Turk J Med Sci. 2019, 49(2):687-695.
20. Guo W, Zhang J, Zhang D, et al. Polymorphisms and expression pattern of circular RNA circ-ITCH contributes to the carcinogenesis of hepatocellular carcinoma. Oncotarget. 2017, 8(29):48169-48177.
21. Li F, Ma K, Sun MH, et al. Identification of the tumor-suppressive function of circular RNA ITCH in glioma cells through sponging miR-214 and promoting linear ITCH expression. American journal of translational research 2018, 10(5):1373-1386.
22. Zhou H, Zhang J, Chen B, et al. Potential of circular RNA itchy E3 ubiquitin protein ligase as a biomarker and treatment target for multiple myeloma. Translational Cancer Research. 2020, 9(1):335-345.
23. Moher D, Liberati A, Tetzlaff J, et al. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS medicine. 2009, 6(7):e1000097.
24. Tierney JF, Stewart LA, Ghersi D, et al. Practical methods for incorporating summary time-to-event data into meta-analysis. Trials. 2007, 8:16.
25. 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.
26. Wan L, Zhang L, Fan K, et al. Circular RNA-ITCH Suppresses Lung Cancer Proliferation via Inhibiting the Wnt/beta-Catenin Pathway. 2016, 2016:1579490.
27. Li F, Zhang L, Li W, et al. Circular RNA ITCH has inhibitory effect on ESCC by suppressing the Wnt/beta-catenin pathway. Oncotarget. 2015, 6(8):6001-6013.
28. Huang G, Zhu H, Shi Y, et al. cir-ITCH plays an inhibitory role in colorectal cancer by regulating the Wnt/beta-catenin pathway. PloS one. 2015, 10(6):e0131225.
29. Li J, Guo R, Liu Q, et al. Circular RNA Circ-ITCH Inhibits the Malignant Behaviors of Cervical Cancer by microRNA-93-5p/FOXK2 Axis. Reproductive sciences (Thousand Oaks, Calif) 2020.
30. Lin Q, Jiang H, Lin D. Circular RNA ITCH downregulates GLUT1 and suppresses glucose uptake in melanoma to inhibit cancer cell proliferation. The Journal of dermatological treatment. 2019:1-5.

Figures

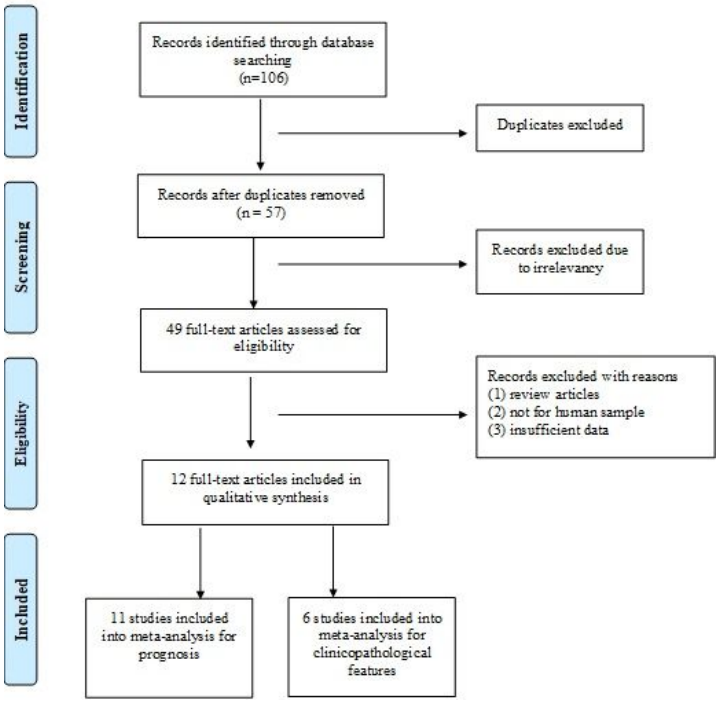

Figure 1

Literature selection process by following PRISMA guidelines in this meta-analysis.

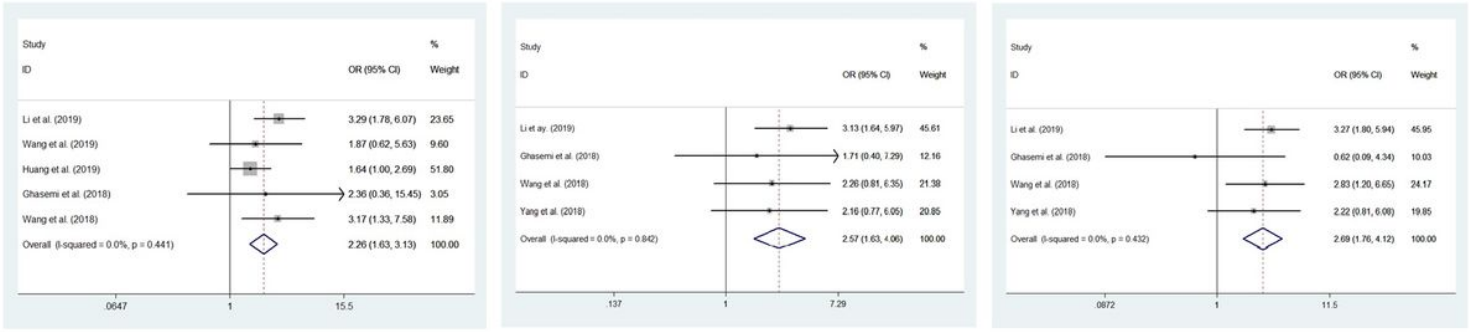

Figure 2

Forest plots of odds ratios (ORs) for the association between circ-ITCH expression and lymph node metastasis (LNM) (a), TNM stage (b), tumor size (c).

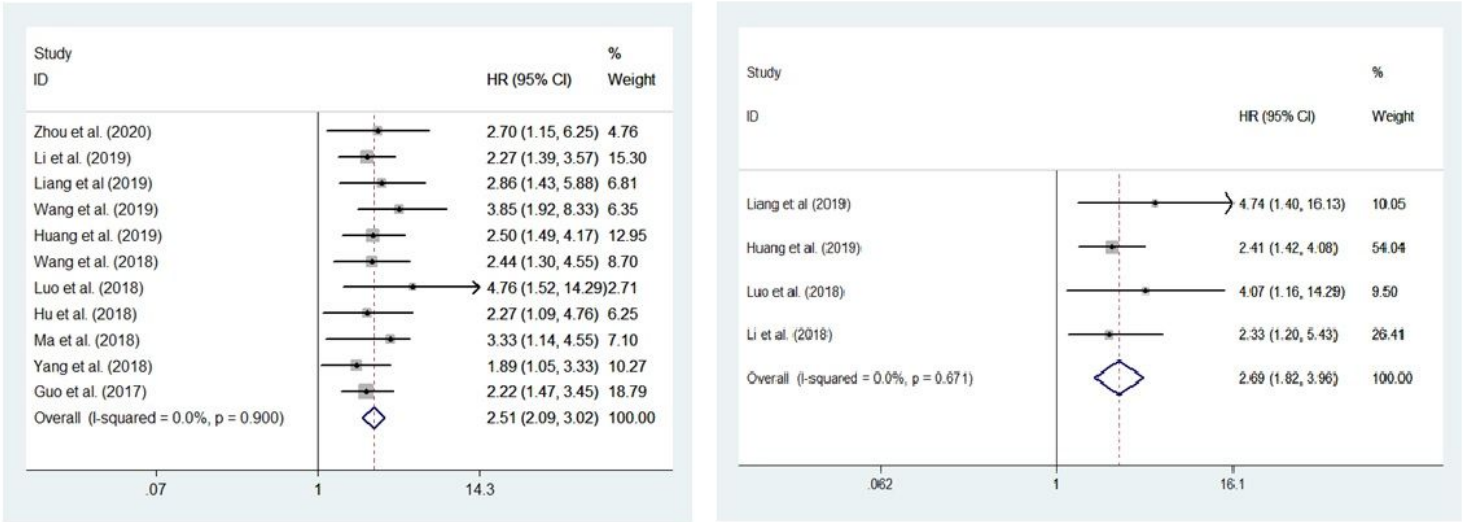

Figure 3

Forest plots of hazard ratios (HRs) for the association between circ-ITCH expression with overall survival (OS) from univariate analysis results (a), OS from multivariate analysis results (b).

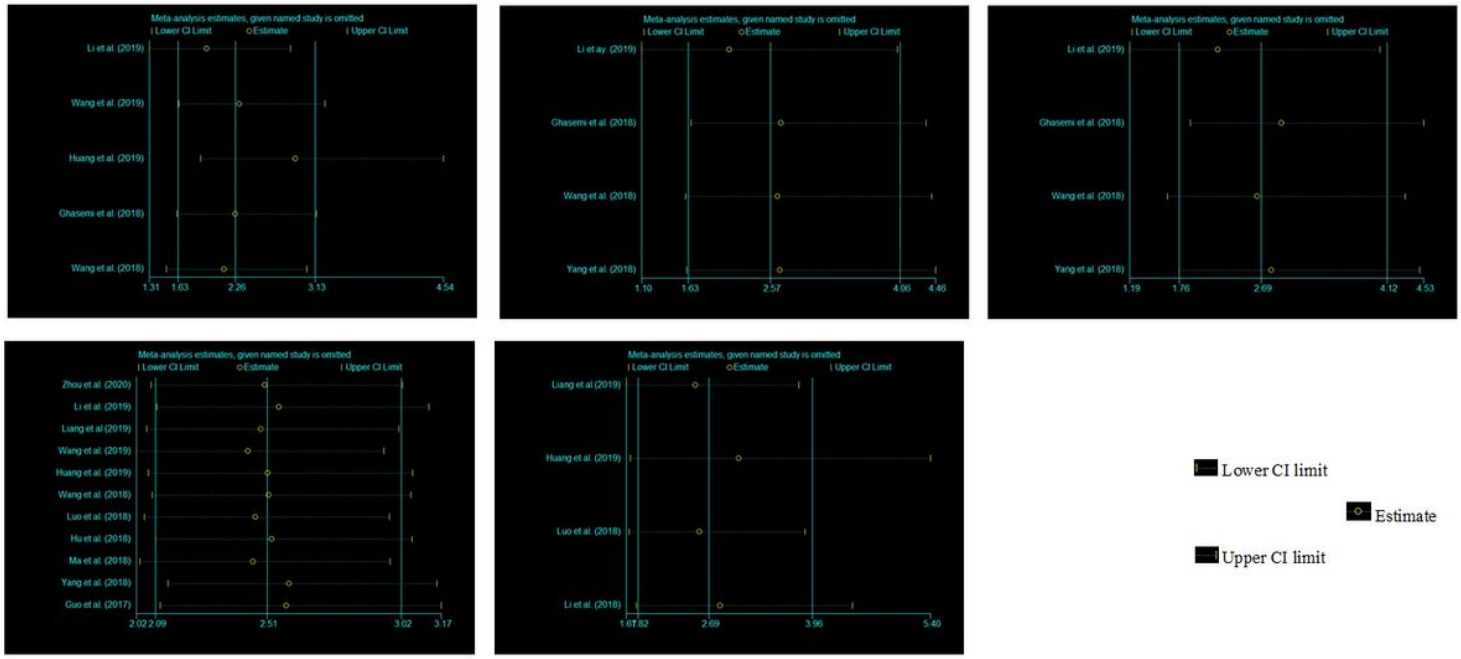

Figure 4

Sensitivity analysis between circ-ITCH expression and lymph node metastasis (LNM) (a), TNM stage (b), tumor size (c), overall survival (OS) via univariate analysis results(d), OS from multivariate analysis results (e).

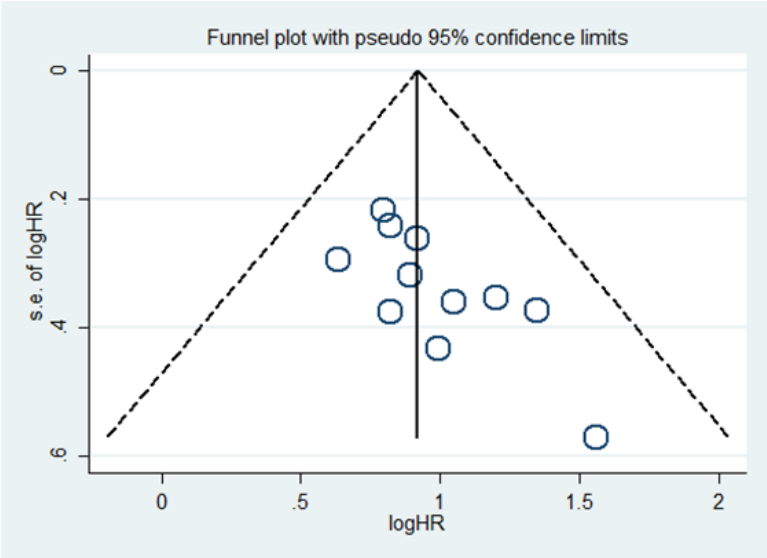

Figure 5

Funnel plots for the meta-analysis with overall survival (OS) from univariate analysis results.