Original Article

Prognostic role of dysregulated circRNAs in patients with non-small cell lung cancer: a meta-analysis

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Background: Lung cancer is the leading cause of cancer incidence and mortality. Non-small cell lung cancer (NSCLC) accounts for the vast majority of lung cancer, which lacks comprehensive prognostic biomarkers to predict the prognosis of patients. This research was performed to assess the potential prognostic role of circular RNAs (circRNAs) in patients with NSCLC.

Methods: We searched the following databases: PubMed, Web of Science, Embase, and Ovid MEDLINE(R) up to May 20, 2019 to identify studies which explored the association between circRNAs and NSCLC. Newcastle-Ottawa Scale (NOS) was applied to assess the quality of the included studies. Pooled hazard ratios (HRs) and the corresponding 95% confidence interval (CI) were calculated to assess the prognostic value of circRNAs in patients with NSCLC. Subgroup analyses were performed to explain heterogeneity among the included studies. Publication bias was estimated using Begg's funnel plot. Sensitivity analysis was performed to test the stability of pooled results.

Results: A total of 19 eligible studies including 1,650 NSCLC patients were included in this research. Pooled results indicated that the up-regulated expression of circRNAs was significantly associated with worse prognosis of patients with NSCLC (HR =2.08, 95% CI: 1.81–2.40).

Conclusions: Our finding indicated that circRNAs could serve as prognostic biomarkers in patients with NSCLC. However, further large-scale prospective studies about the clinical significance of circRNAs are of great need in order to obtain conclusive results.

Keywords: Circular RNA (circRNA); prognosis; non-small cell lung cancer (NSCLC); meta-analysis

Introduction

Lung cancer is the most frequent diagnosed cancer and the leading cause of cancer death worldwide, with nearly 2.1 million new cases and 1.8 million deaths in 2018 (1). Lung cancer is composed of small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) according to histopathological type, among which NSCLC accounts for over 85% of all cases (2). Much progress has been achieved in the diagnosis and treatment of NSCLC in recent years, but the 5-year survival rate of NSCLC patients remains unsatisfied, which merely varies from 4–17% depending on

the stage and regional differences (3).

Circular RNAs (circRNAs), a novel class of endogenous non-coding RNAs (ncRNAs), were characterized by their abundance, highly conservation, and stable covalently closed continuous loop without free 5' cap and 3' tail (4-7). CircRNAs were defined as the by-products of splicing mistakes without biological functions in the past several decades (8). More and more circRNAs have been detected in different cancer tissues with the widely application of high-throughput sequencing technology and bioinformatic approaches (9). Recent studies demonstrated that circRNAs could play a role in a series of biological processes such as microRNA sponging, RBP sponging and mRNA regulation which make effect to carcinogenesis (7,10-12). However, these studies determining the prognostic roles of circRNAs in NSCLC patients were limited by their small sample size. This meta-analysis aimed to evaluate the prognostic value of circRNAs in NSCLC patients.

Methods

Search strategy

This meta-analysis was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (13). Two authors independently searched in PubMed, Web of Science, Embase, and Ovid MEDLINE(R) up to May 20, 2019 using the following search term: ("lung squamous cell carcinoma" OR "lung adenocarcinoma" OR "large cell lung cancer" OR "non-small cell lung cancer" OR NSCLC) AND ("circular RNAs" OR "circular RNA" OR circRNAs OR circRNA) AND (prognosis OR prognostic OR survival).

Inclusion and exclusion criteria

The inclusion criteria were as follows: (I) studies detected circRNAs expression in NSCLC patients; (II) studies investigated the association between circRNAs expression and NSCLC patients' prognosis; and (III) studies presented hazard ratio (HR) and corresponding 95% confidence interval (CI) or Kaplan-Meier (K-M) curve.

The exclusion criteria were as follows: (I) duplicate publications; (II) reviews, case reports, or letters; (III) studies no published in English; (IV) studies which sample size <35; and (V) studies without available data.

Data extraction

Two authors independently extracted the data from all eligible studies. The following information were extracted from the included studies: first author, publication year, country, sample size, sample types, circRNAs studied, detection methods, circRNAs expression level, cut-off value, number of patients in low expression groups, number of patients in high expression groups, outcome, follow-up period, multivariate analysis, and HR and 95% CI for overall survival (OS). Engauge Digitizer 4.1 software and Tierney's protocol (14) were applied to extract the HR and 95% CI indirectly from K-M curves if the studies did not present complete survival data.

Quality assessment

Two authors assessed the quality of the included studies independently using Newcastle-Ottawa Scale (NOS) (15). Total scores of NOS were ranged from 0 to 9, and studies with scores greater than 6 were considered as high quality studies.

Statistical analysis

Statistical analyses were analyzed using STATA 12.0 software. Heterogeneity test was performed by I-squared statistics. If I-squared value >50% and P value <0.05, there was significant heterogeneity among the included studies, and a random effects model was applied to estimate the pooled results; otherwise, a fixed effects model was performed. Pooled HR and corresponding 95% CI were calculated to assess the prognostic value of circRNAs in patients with NSCLC. Sensitivity analysis was performed to test the stability of pooled results. Publication bias was estimated using Begg's funnel plot. Statistical significance was defined as P value <0.05.

Results

Search strategy results

A total of 136 articles were identified from PubMed, Web of Science, Embase, and Ovid MEDLINE (R). After removing 85 duplicate publications, 51 studies were included for further screening. After screening the titles, abstracts, and

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┌─────────────────────────────┐
│ Records (n=136) retrieved from │
│ PubMed (n=32), Web of       │
│ Science (n=31), Embase (n=42),│──────┐
│ Ovid MEDLINE (R) (n=31)     │      │   ┌────────────────────────┐
└─────────────────────────────┘      └──▶│ Duplicates removed      │
              │                          │ (n=85)                  │
              ▼                          └────────────────────────┘
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│ Articles after duplicates   │──────┐
│ removed (n=51)              │      │   ┌────────────────────────┐
└─────────────────────────────┘      └──▶│ Records excluded by     │
              │                          │ screening titles and    │
              ▼                          │ abstracts (n=25)        │
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│ Full text articles assessed │──────┐
│ for eligibility (n=26)      │      │   ┌────────────────────────┐
└─────────────────────────────┘      └──▶│ Articles excluded by    │
              │                          │ screening full text (n=7)│
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│ Studies included in         │
│ meta-analysis (n=19)        │
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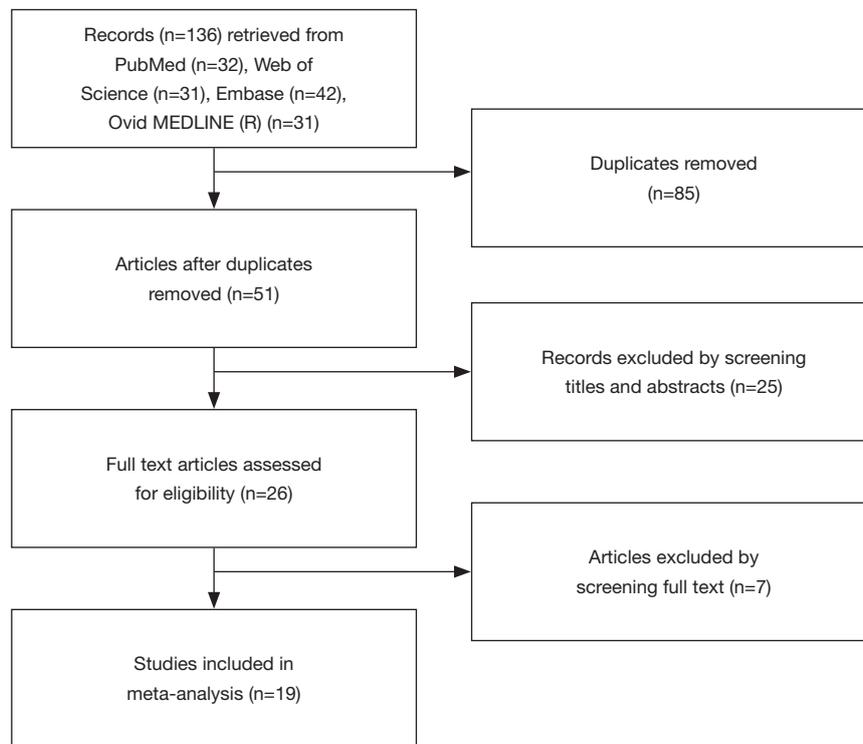
Figure 1 Flowchart showing the process of literature search and selection.

full texts, 19 eligible studies (16-34) were finally included in current meta-analysis based on the inclusion and exclusion criteria (*Figure 1*).

Main characteristics and quality assessment of eligible studies

In total, nineteen studies (16-34) comprised of 16 circRNAs were included in this meta-analysis. The main characteristics and quality assessment of the included studies (16-34) were summarized in *Table 1*. Publication year of all included studies (16-34) ranged from 2017 to 2019. The expression of circRNAs in all included studies (16-34) was detected by quantitative real-time polymerase chain reaction (qRT-PCR). Follow-up period ranged from 41 to 94 months. Eleven studies (18-21,23-26,28-30) directly provided HRs and 95% CI; moreover, we calculated the HRs and 95% CI for the other 8 studies (16,17,22,27,31-34). Furthermore, we assessed the quality of each included studies and the scores ranged from 6–8, which suggests the included studies are in a high quality.

Prognostic value of circRNAs in patients with NSCLC

A total of 19 studies (16-34) comprising 1,650 patients with NSCLC were included in the meta-analysis. There were 17 studies (16-18,20-32,34) indicating the association between the up-regulation of circRNAs and the poor prognosis of NSCLC patients. Since there was no significant heterogeneity among the above 17 studies (16-18,20-32,34) (I-squared value =0.0% & P value =0.752), we performed a fixed effects model to assess the pooled HR and corresponding 95% CI. As shown in *Figure 2*, the up-regulation of circRNAs was significantly associated with the poor prognosis of NSCLC patients (HR =2.08, 95% CI: 1.81–2.40). In other words, the up-regulated expression of circRNAs is a risk factor for NSCLC patients included in these 17 studies (16-18,20-32,34). As shown in *Figure 3*, the down-regulated expression of circRNAs might be a protected factor for NSCLC patients inversely as the pooled HR of the other 2 studies (19,33) exploring the effect of circRNAs down-regulation to the prognosis of NSCLC patients were 0.45 (95% CI: 0.27–0.73).

Table 1 The main characteristics and quality assessment of eligible studies in this meta-analysis

Author (ref.)	Year	Country	Sample size	Sample types	circRNAs	Detection methods	Expression level	Outcome	Cutoff value	Follow-up (months)	HR value	NOS scores
Yao JT (16)	2017	China	101	Tissues	circ_100876	qRT-PCR	Up	OS	Median	41	K-M curve	6
Ding LC (17)	2018	China	56	Tissues	circ_001569	qRT-PCR	Up	OS	Mean	60	K-M curve	6
Li YS (18)	2018	China	83	Tissues	circ_0016760	qRT-PCR	Up	OS	Mean	60	Provided	8
Liu TM (19)	2018	China	53	Tissues	circ_0001649	qRT-PCR	Down	OS	Mean	60	Provided	7
Qi Y (20)	2018	China	98	Tissues	circ_0007534	qRT-PCR	Up	OS	Mean	60	Provided	8
Qiu MT (21)	2018	China	89	Tissues	circPRKCI	qRT-PCR	Up	OS	Mean	90	Provided	8
Qu DH (22)	2018	China	80	Tissues	circ_0020123	qRT-PCR	Up	OS	Median	68	K-M curve	7
Su CY (23)	2018	China	128	Tissues	ciRS-7	qRT-PCR	Up	OS	Mean	60	Provided	8
Wang J (24)	2018	China	159	Tissues	circ_0067934	qRT-PCR	Up	OS	Median	60	Provided	8
Yan B (25)	2018	China	132	Tissues	ciRS-7	qRT-PCR	Up	OS	Median	94	Provided	8
Zhang XF (26)	2018	China	60	Tissues	ciRS-7	qRT-PCR	Up	OS	Median	84	Provided	8
Zong L (27)	2018	China	57	Tissues	circ_102231	qRT-PCR	Up	OS	Median	60	K-M curve	6
Zou QG (28)	2018	China	79	Tissues	circ_0067934	qRT-PCR	Up	OS	Median	80	Provided	8
An JC (29)	2019	China	59	Tissues	circ_0003645	qRT-PCR	Up	OS	Median	60	Provided	7
Liu G (30)	2019	China	80	Tissues	circ-FOXM1	qRT-PCR	Up	OS	Mean	60	Provided	8
Qin S (31)	2019	China	90	Tissues	circ-PVT1	qRT-PCR	Up	OS	Median	60	K-M curve	7
Wang T (32)	2019	China	80	Tissues	circP4HB	qRT-PCR	Up	OS	Median	80	K-M curve	7
Yao YS (33)	2019	China	94	Tissues	circ_0006427	qRT-PCR	Down	OS	Mean	60	K-M curve	7
Yao YS (34)	2019	China	72	Tissues	circ_0001946	qRT-PCR	Up	OS	Mean	60	K-M curve	7

HR, hazard ratio; NOS, Newcastle-Ottawa Scale; qRT-PCR, quantitative real-time polymerase chain reaction; OS; overall survival; K-M, Kaplan-Meier.

Sensitivity analysis

Sensitivity analysis was applied to test the stability of pooled results. As shown in *Figure 4*, the pool result of the 17 studies (16-18,20-32,34) in which circRNAs were up-regulated was not significantly affected by removing each eligible study. Regarding to the limited number of the other 2 studies (19,33) with down-regulation of circRNAs, there was no point to perform sensitivity analysis.

Publication bias

Publication bias was assessed by Begg's test. As shown in *Figure 5*, there was no obvious asymmetry in the Begg's funnel plot; and the P value for Begg's test was 0.837, which was greater than 0.05. Thus, there was no significant publication bias among the included studies.

Discussion

Research on the functional roles of circRNAs in carcinogenesis is still in its infancy. There is some evidence to indicate that circRNAs could function as valuable biomarkers for cancer diagnosis and prognosis (12). The use of circRNAs as prognostic biomarkers has the following advantages: (I) circRNAs are more stable than linear RNAs; (II) circRNAs are superior to traditional biomarker in terms of organ specificity (35); (III) circRNAs can be easily detected in blood and body fluid (35).

To our knowledge, this meta-analysis is the first time to estimate association between the expression of circRNAs and the prognosis of patients with NSCLC. A total of 16 circRNAs from 19 eligible studies (16-34) comprising 1,650 NSCLC patients were included in this meta-analysis. After a fixed-effects model was performed to estimate pooled HR
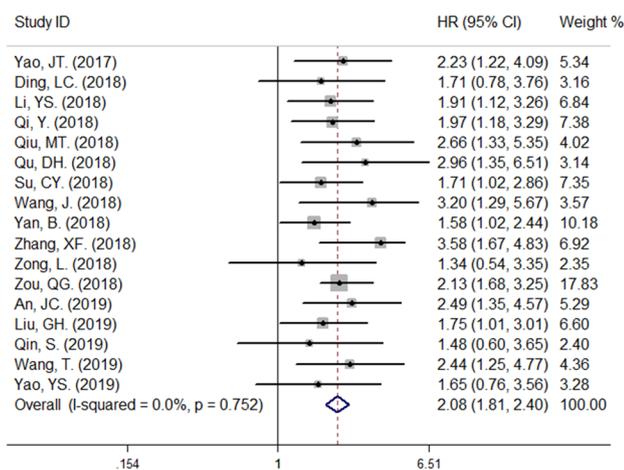
Figure 2 Forest plot of HR for the relationship between the expression of upregulated circRNAs and the prognosis of patients with NSCLC. HR, hazard ratio; NSCLC, non-small cell lung cancer.
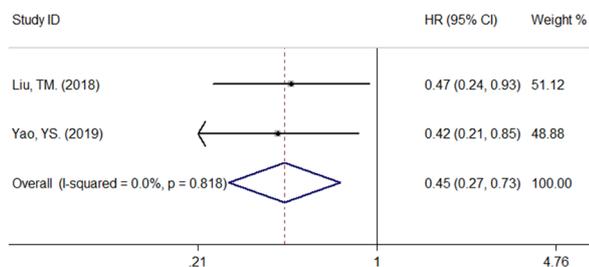
Figure 3 Forest plot of HR for the relationship between the expression of downregulated circRNAs and the prognosis of patients with NSCLC. HR, hazard ratio; NSCLC, non-small cell lung cancer.

Figure 4 Sensitivity analysis of meta-analysis for circRNAs in prognosis of patients with NSCLC. NSCLC, non-small cell lung cancer.
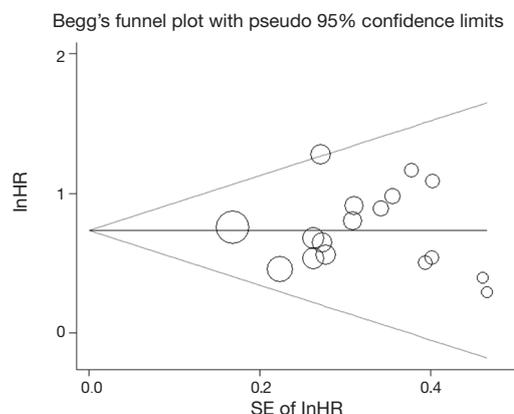
Figure 5 Funnel plot of publication bias. HR, hazard ratio.

and the 95% CI (pooled HR =2.08, 95% CI: 1.81–2.40), we became more convinced that up-regulated expression of circRNAs was significantly associated with poor prognosis of patients with NSCLC.

However, there were several limitations in this meta-analysis. Firstly, as HR and 95% CI values from 8 studies (16,17,22,27,31-34) were retrieved from the K-M curves, there could be some marginal error as numerical values were not reported explicitly in the full-text of those studies. Secondly, since we combined multiple types of circRNAs to assess their prognostic role in patients with NSCLC, the heterogeneity might be inevitable. Thirdly,

all the included studies of this meta-analysis just tested the expression of circRNAs among Chinese population, thus it is unclear whether our results might be applicable to other populations. Last but not least, we only enrolled 1,650 NSCLC patients from 19 studies (16-34) in this meta-analysis making it hard to widespread the clinical applicability of this meta-analysis because of the small sample size and the small number of studies.

Conclusions

The current meta-analysis might provide evidence that up-regulated expression of circRNAs is significantly associated with poor prognosis of NSCLC patients in Chinese

population. Therefore, circRNAs could be novel prognostic biomarkers for NSCLC patients. However, further large-scale prospective studies about the clinical significance of circRNAs are of great need in order to obtain conclusive results.

Acknowledgments

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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