Review Article

Accuracy Evaluation of Circular RNA in Diagnosing Lung Cancer in a Chinese Population

Zhihao Xiao, Xinglei Chen, Xiaodan Lu, Xuexin Zhong, and Yihui Ling

Institute for Chemical Carcinogenesis, Guangzhou Medical University, Guangzhou, China

Correspondence should be addressed to Yihui Ling; ling_yh@163.com

Received 13 May 2019; Revised 17 August 2019; Accepted 5 September 2019; Published 20 October 2019

Academic Editor: Chiara Nicolazzo

Copyright © 2019 Zhihao Xiao et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Circular RNA (circRNA) is a class of recently discovered noncoding RNA. circRNAs can be used as a potent noninvasive biological marker of cancer owing to their varying expression levels among different cancers. This meta-analysis was performed to assess the accuracy of circRNAs in diagnosing lung cancer. A total of eight studies identified through searching the PubMed, Web of Science, Cochrane Library, and Embase from inception to March 20, 2019 were eligible for this meta-analysis. The pooled sensitivity, specificity, positive likelihood ratios, negative likelihood ratios, and diagnostic odds ratio were 0.77 (95% confidence interval (CI): 0.73–0.80; $I^2 = 8.98\%$), 0.76 (95% CI: 0.69–0.82; $I^2 = 63.12\%$), 3.17 (95% CI: 2.43–4.14; $I^2 = 33.18\%$), 0.31 (95% CI: 0.26–0.37; $I^2 = 20.36\%$), and 10.26 (95% CI: 6.87–15.31; $I^2 = 97.18\%$), respectively. The area under the receiver operating characteristic curve was 0.78 (95% CI: 0.74–0.81). The study confirmed the use of circRNAs in diagnosing lung cancer in a Chinese population.

1. Introduction

Circular RNA (circRNA) is a circular single-strand RNA first discovered in plants [1, 2] and abundant in human cells. In some cases, the abundance of circRNAs exceeds that of associated linear mRNAs by more than tenfold [3]. CircRNAs lack free ends, 5’ cap, and 3’ poly(A) tail and are more stable than linear RNAs with their ends joining in a circle via phosphodiester bonds [4]. CircRNAs have many functions including regulating gene transcription and translation via binding to miRNAs, interacting with proteins, and being directly translated [5]. Recent studies suggested significant advantages of circRNAs in diagnosing cancers owing to their prevalence, stability, specificity, and conservatism [6].

Experiments proved that circRNAs were associated with proliferation, apoptosis, invasion, and migration of tumor cells, and the expression varied with tumor cells, thus helping in diagnosing and predicting the prognosis of tumors. Li et al. [7] found that Hsa_circ_0000096 was significantly downregulated in gastric cancer tissues and cell lines and was associated with the invasion and staging of tumors. Ma et al. [8] reported that circRNA-000284 was significantly higher in cervical cancer cells than in cervical epithelial cells and could serve as a biological marker. Li et al. [9] considered circHIPK3 as the novel therapeutic target of bladder carcinoma. Some meta-analyses summarizing the role of circRNAs on diagnosis and prognosis were reported [10–13]. Huang et al. [10] investigated the prognostic and diagnostic significance of the expression of circRNAs in patients with hepatocellular carcinoma. Wang et al. [11], Li et al. [12], and Ding [13] performed a meta-analysis on the value of circRNAs as a biological marker of tumors. However, they just explored the role of circRNAs in diagnosing tumors but not specially or independently diagnosing lung cancer.

Lung cancer accounts for 25% of cancer-related deaths worldwide, which is much higher compared with other cancers such as breast carcinoma, prostatic carcinoma, and colorectal carcinoma. The survival rate of patients with lung cancer is relatively low, possibly due to the lack of early testing methods [14]. The role of circRNAs in lung cancer was first reported in 2018 by Qu et al. [5] who found that hsa_circ_00013958 promoted the proliferation, invasion, and
metastasis of lung adenocarcinoma cells. A large number of recent studies explored the relationship between circRNAs and lung cancer [15–21].

2. Materials and Methods

2.1. Literature Search. Electronic databases, including PubMed, Web of Science, Cochrane Library, and Embase, were systematically searched from inception to March 20, 2019. The following search terms were used: circRNA, circular RNA, lung cancer, lung neoplasm, lung adenocarcinoma, non-small-cell lung cancer, NSCLC, pulmonary cancer, and pulmonary neoplasm. In addition, the reference lists of eligible studies were manually searched to guarantee the comprehensiveness of the literature.

2.2. Inclusion and Exclusion Criteria. The inclusion criteria were as follows: (1) studies analyzing the relationship between circRNAs and lung cancer, (2) studies providing data on the sensitivity and specificity, and (3) studies involving ≥30 patients and controls. The exclusion criteria were as follows: (1) repetitive research; (2) letters, editorials, commentaries, or abstracts; (3) studies involving ineligible patients or controls; (4) studies lacking data; or (5) studies in a non-English language. If the results came from the overlapping population, only the first study or the most complete study was included.

2.3. Data Extraction and Quality Assessment. Two reviewers (Xuexin Zhong and Zhihao Xiao) extracted data. The discrepancies were resolved by the third reviewer (Xiaodan Lu) if needed. The following information was extracted from each study: first author name, year of publication, country, sample size, sample type, sensitivity, specificity, area under the receiver operating characteristic (ROC) curve (AUC), testing methods, tumor staging, reference gene, and differential expression of circRNAs.

2.4. Statistical Analysis. Stata 12.0 software and Meta-DiSc 1.4 were used for statistical analysis. The sensitivity, specificity, positive likelihood ratio (PLR), negative likelihood ratio (NLR), diagnostic odds ratio (DOR), 95% confidential intervals (95% CI), summary ROC (SROC) curve, and AUC were calculated for quality assessment. The significance level was set as \( P < 0.05 \). The heterogeneity induced by the threshold effect of included studies was tested using the Spearman correlation analysis. Cochran’s Q and \( I^2 \) tests were used to assess the heterogeneity of data. \( I^2 > 50\% \) indicated significant heterogeneity. A subgroup analysis was performed based on sample type, cancer type, reference gene type, and differential expression of circRNAs. The potential source of heterogeneity by the nonthreshold effect was analyzed by regression analysis. A Fagan nomogram was used to calculate the post-test probabilities. Finally, publication bias was assessed.

3. Results

3.1. Search Results. The flowchart of the study selection process is shown in Figure 1. The review of the literature identified 388 studies, of which 209 repeated ones were excluded and the remaining 179 ones were screened based on titles and abstracts. Subsequently, 165 studies, including reviews, letters, conference abstracts, and 2 studies on hsa_circ_0102533 [22] and circFARSA [23], were further excluded, owing to the inability of constructing a 2 × 2 contingency table. A total of 8 studies [15–21, 24] and 10 eligible studies, involving 668 patients with lung cancer and 153 healthy controls, were assessed during this meta-analysis. The characteristics of eight studies are shown in Table 1. Surprisingly, all eight studies identified using search terms, inclusion criteria, and exclusion criteria were performed in China.
Table 1: Characteristics of eight studies included in the meta.

| Author     | Year | circRNAs            | Country | Sample type | No. of cases | No. of controls | AUC     | Sensitivity | Specificity | Testing method | TNM staging I-II | TNM staging III-IV | circRNA expression | References |
|------------|------|---------------------|---------|-------------|--------------|----------------|---------|-------------|-------------|----------------|------------------|------------------|-------------------|------------|
| Zhu et al. | 2017 | hsa_circ_0013958    | China   | Blood       | 30           | 30             | 0.794   | 0.667       | 0.933       | qRT-PCR         | 28               | 21               | Upregulated       | [24]        |
| Li et al.  | 2018 | hsa_circ_0079530    | China   | Tissue      | 92           | 92             | 0.756   | 0.762       | 0.721       | qRT-PCR         | —                | —                | —                 | [15]        |
| Zong et al.| 2018 | circRNAs_102231     | China   | Tissue      | 57           | 57             | 0.897   | 0.812       | 0.887       | qRT-PCR         | 30               | 27               | Upregulated       | [16]        |
| Zhang et al.| 2018| hsa_circ_0014130    | China   | Tissue      | 46           | 46             | 0.878   | 0.87        | 0.848       | qRT-PCR         | 36               | 10               | Upregulated       | [17]        |
| Zhang et al.| 2018| circ_FOXO3         | China   | Tissue      | 45           | 45             | 0.782   | 0.8         | 0.733       | qRT-PCR         | —                | —                | Downregulated     | [18]        |
| Li et al.  | 2018 | circ_PVT1          | China   | Blood       | 45           | 45             | 0.794   | 0.711       | 0.8         | qRT-PCR         | 31               | 37               | Upregulated       | [19]        |
| Chen et al.| 2019 | circRNAs_100146    | China   | Tissue      | 40           | 40             | 0.643   | 0.725       | 0.575       | qRT-PCR         | —                | —                | Upregulated       | [20]        |
| Liu et al. | 2019 | hsa_circ_0005962    | China   | Blood       | 153          | 54             | 0.73    | 0.719       | 0.7222      | qRT-PCR         | 96               | 55               | Upregulated       | [21]        |
|            |      | hsa_circ_0086414    | China   | Blood       | 153          | 54             | 0.78    | 0.7712      | 0.6667      | qRT-PCR         | 96               | 55               | Downregulated     |            |

Note: data not extracted; AUC: area under the receiver operating characteristic curve.
Study ID Specificity (95% CI)
Liu et al/2019 0.77 [0.70 - 0.84]
Li et al/2018 0.82 [0.71 - 0.91]
Zhu et al/2017 0.67 [0.47 - 0.83]
Liu et al/2019 0.72 [0.64 - 0.79]
Chen et al/2019 0.73 [0.56 - 0.85]
Li et al/2018 0.71 [0.56 - 0.84]
Zhang et al/2018 0.80 [0.65 - 0.90]
Zhang et al/2018 0.87 [0.74 - 0.95]
Zong et al/2018 0.81 [0.68 - 0.90]
Li et al/2018 0.76 [0.66 - 0.84]
Combined 0.77 [0.73 - 0.80]

Sensitivity
0.5 1.0

Specificity

Q = 24.40, df = 9.00, P = 0.00
I² = 63.12 [37.94 - 88.30]

Figure 2: Forest plots of the sensitivity and specificity for circRNAs in diagnosing lung cancer.

Study ID DLR positive (95% CI) Study ID DLR negative (95% CI)
Liu et al/2019 2.31 [1.57 - 3.41]
Li et al/2018 2.55 [1.77 - 3.65]
Zhu et al/2017 10.00 [2.56 - 39.06]
Liu et al/2019 2.59 [1.66 - 4.02]
Chen et al/2019 1.71 [1.13 - 2.56]
Li et al/2018 3.56 [1.93 - 6.57]
Zhang et al/2018 3.00 [1.81 - 4.98]
Zhang et al/2018 5.71 [2.86 - 11.41]
Zong et al/2018 6.57 [3.25 - 13.30]
Li et al/2018 2.69 [1.91 - 3.80]
Combined 3.17 [2.43 - 4.14]

DLR positive
1.1 39.1

DLR negative
0 1

Q = 22.80, df = 9.00, P = 0.01
I² = 33.18 [13.18 - 87.87]

Q = 11.30, df = 9.00, P = 0.26
I² = 20.36 [0.00 - 76.67]

Figure 3: Forest plots of the positive likelihood ratio and the negative likelihood ratio for circRNAs in diagnosing lung cancer.
3.2. Threshold Effect. The threshold effect was evaluated with the Spearman rank correlation. The Spearman correlation coefficient was 0.079 \((P = 0.829)\), suggesting no threshold effect.

3.3. Results of Meta-Analysis. Significant heterogeneity was assessed using the random-effects model \(I^2 > 50\%\). For the value of circRNAs in diagnosing lung cancer, the pooled sensitivity, specificity, PLR, NLR, and DOR were 0.77 \((95\% \text{ CI}: 0.73 - 0.80; I^2 = 8.98\%\), 0.76 \((95\% \text{ CI}: 0.69 - 0.82; I^2 = 63.12\%\), 3.17 \((95\% \text{ CI}: 2.43 - 4.14; I^2 = 33.18\%\), 0.31 \((95\% \text{ CI}: 0.26 - 0.37; I^2 = 20.36\%\), and 10.26 \((95\% \text{ CI}: 9.87 - 10.68; I^2 = 97.18\%\), respectively. AUC was 0.78 \((95\% \text{ CI}: 0.74 - 0.81\). Forest plots and SROC are shown in Figures 2–5. Fagan’s nomogram is shown in Figure 6. If the pretest probability was 20\%, the posttest probability increased to 44\%. The pretest likelihood ratio (LR) was 3\%, and the posttest LR decreased to 7\%. The NLR was 0.31. An LR scattergram was used to evaluate the clinical value of different diagnostic tests and divided into four quadrants (Figure 7). All 10 eligible studies were in the right lower quadrants, suggesting that circRNAs were useful in diagnosing lung cancer.

3.4. Regression Analysis. The \(I^2\) value was 96.84\%, suggesting significant heterogeneity. The sample type, cancer type, reference gene type, and differential expression of circRNAs were taken as potential causes of heterogeneity, and a metaregression analysis was performed. No significant causes for heterogeneity were found. The results are shown in Table 2.

3.5. Subgroup Analysis. A subgroup analysis was performed based on the sample type, sample size, cancer type, reference gene type, and differential expression of circRNAs. Although the sample size did not contribute to heterogeneity, the sensitivity, specificity, and DOR of blood samples were 0.72, 0.78, and 9.32, while the corresponding values of tissue samples were 0.80, 0.75, and 11.67, respectively. These findings suggested that tissue circRNAs were slightly superior to blood circRNAs in diagnosis. The sensitivity, specificity, and DOR of the non-small-cell carcinoma subgroup were 0.78, 0.70, and 9.66, while the corresponding values of the lung adenocarcinoma subgroup were 0.75, 0.80, and 12.54, respectively, suggesting the superiority of circRNAs in lung adenocarcinoma over those in non-small-cell carcinoma. The subgroup analysis of the reference gene was not conducted because only one study considered \(\beta\)-actin as the reference gene. The results are shown in Table 3.

3.6. Publication Bias. The publication bias of the included studies was tested using Deeks’ funnel plot, and significant differences in the slope rate \((P < 0.05)\) suggested the publication bias. The funnel plot was constructed with the Stata 12.0 software (Figure 8), and the results showed no publication bias \((P = 0.24)\).
4. Discussion

The present meta-analysis enrolled 8 studies from 2017 to 2019 and systemically reviewed 10 circRNAs in diagnosing lung cancer. The results showed that the AUC was 0.78, and the pooled sensitivity, specificity, and DOR were, respectively, 0.77 (95% CI: 0.73–0.80), 0.76 (95% CI: 0.69–0.82), and 10.26 (95% CI: 6.87–15.31). The findings suggested the diagnostic value of circRNAs for lung cancer. The included studies involved only a preliminary analysis of the role of one or two circRNAs in diagnosing lung cancer, with small sample size and sensitivity varying from 0.511 to 0.884 (lower than pooled sensitivity in six studies), specificity from 0.575 to 0.933 (lower than pooled specificity in six studies), and AUC from 0.643 to 0.897 (lower than or equal to AUC in this meta-analysis in five studies). The sensitivity, specificity, and AUC fluctuated largely among these studies, possibly due to the involvement of one or two circRNAs and small sample size.

The Spearman correlation coefficient was calculated to test the threshold effect and was 0.79 (P = 0.829), suggesting that the threshold effect was not the cause of heterogeneity. In addition, the regression analysis of sample type, lung cancer type, reference type, and differential expression of circRNAs showed that these factors did not cause heterogeneity. The causes of heterogeneity could not be directly found using the studies included in this analysis. Neither study was a randomized controlled study, possibly leading to heterogeneity. However, this hypothesis should be further verified. Additionally, the present meta-analysis included 8 studies and systematically reviewed the value of 10 different circRNAs in diagnosing lung cancer. The analysis revealed that the expression levels of these circRNAs were different, which might be one of the sources of heterogeneity and also a common problem in this type of analysis.

In conclusion, this systematic review of data extracted from eight studies showed the value of circRNAs in diagnosing lung cancer. These studies were primarily based on the testing of lung cancer tissues. Blood and exocrine secretions...
were less used, and clinical data were limited. Therefore, the relationship of circRNAs in the blood or exocrine secretions with lung cancer needs further investigation, and the finding might help in the development of molecular markers of diagnosis and prognosis. Further, the recent studies explored the role of a single circRNA in diagnosing and treating cancer using a small sample size; no multicenter and large-sample studies were reported. Hence, the diagnostic accuracy and stability of circRNAs need further elucidation.

## Conflicts of Interest

The authors declared that they have no conflict of interests.

## Acknowledgments

This study was supported by Guangdong Medical Research Fund Project (A2018164) and Guangdong Province General University Characteristic Innovation Project (2018KTSCX185).

## References

[1] S. Memczak, M. Jens, A. Elefsinioti et al., "Circular RNAs are a large class of animal RNAs with regulatory potency," *Nature*, vol. 495, no. 7441, pp. 333–338, 2013.

[2] H. L. Sanger, G. Klotz, D. Riesner, H. J. Gross, and A. K. Kleinschmidt, "Viroids are single-stranded covalently closed circular RNA molecules existing as highly base-paired rod-like structures," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 73, no. 11, pp. 3852–3856, 1976.

[3] W. R. Jeck, J. A. Sorrentino, K. Wang et al., "Circular RNAs are abundant, conserved, and associated with ALU repeats," *RNA*, vol. 19, no. 2, pp. 141–157, 2013.

[4] X. O. Zhang, H. B. Wang, Y. Zhang, X. Lu, L. L. Chen, and L. Yang, "Complementary sequence-mediated exon circularization," *Cell*, vol. 159, no. 1, pp. 134–147, 2014.

[5] S. Qu, Z. Liu, X. Yang et al., "The emerging functions and roles of circular RNAs in cancer," *Cancer Letters*, vol. 414, pp. 301–309, 2018.

[6] X. Wang and L. Fang, "Advances in circular RNAs and their roles in breast cancer," *Journal of Experimental & Clinical Cancer Research*, vol. 37, no. 1, p. 206, 2018.

[7] P. Li, H. Chen, S. Chen et al., "Circular RNA 0000096 affects cell growth and migration in gastric cancer," *British Journal of Cancer*, vol. 116, no. 5, pp. 626–633, 2017.

[8] H. B. Ma, Y. N. Yao, J. J. Yu, X. X. Chen, and H. F. Li, "Extensive profiling of circular RNAs and the potential regulatory role of circRNA-000284 in cell proliferation and invasion of cervical cancer via sponging miR-506," *American Journal of Translational Research*, vol. 10, no. 2, pp. 592–604, 2018.

[9] Y. Li, F. Zheng, X. Xiao et al., "CircHIPK3 sponges miR-558 to suppress heparanase expression in bladder cancer cells," *EMBO reports*, vol. 18, no. 9, pp. 1646–1659, 2017.
X. Huang, W. Zhang, and Z. Shao, “Prognostic and diagnostic significance of circRNAs expression in hepatocellular carcinoma patients: a meta-analysis,” *Cancer Medicine*, vol. 8, no. 3, pp. 1148–1156, 2019.

M. Wang, Y. Yang, J. Xu, W. Bai, X. Ren, and H. Wu, “CircRNAs as biomarkers of cancer: a meta-analysis,” *BMC Cancer*, vol. 18, no. 1, p. 303, 2018.

Y. Li, X. Zeng, J. He et al., “Circular RNA as a biomarker for cancer: a systematic meta-analysis,” *Oncology Letters*, vol. 16, no. 3, pp. 4078–4084, 2018.

H. X. Ding, Z. Lv, Y. Yuan, and Q. Xu, “The expression of circRNAs as a promising biomarker in the diagnosis and prognosis of human cancers: a systematic review and meta-analysis,” *Oncotarget*, vol. 9, no. 14, pp. 11824–11836, 2018.

I. Balgkouranidou, T. Liloglou, and E. S. Lianidou, “Lung cancer epigenetics: emerging biomarkers,” *Biomarkers in Medicine*, vol. 7, no. 1, pp. 49–58, 2013.

J. Li, J. Wang, Z. Chen, Y. Chen, and M. Jin, “Hsa_circ_0079350 promotes cell proliferation and invasion in non-small cell lung cancer,” *Gene*, vol. 665, pp. 1–5, 2018.

L. Zong, Q. Sun, H. Zhang et al., “Increased expression of circRNA_102231 in lung cancer and its clinical significance,” *Biomedicine & Pharmacotherapy*, vol. 102, pp. 639–644, 2018.

S. Zhang, X. Zeng, T. Ding et al., “Microarray profile of circular RNAs identifies hsa_circ_0014330 as a new circular RNA biomarker in non-small cell lung cancer,” *Scientific Reports*, vol. 8, no. 1, p. 2878, 2018.

Y. Zhang, H. Zhao, and I. Zhang, “Identification of the tumor-suppressive function of circular RNA FOXO3 in non-small cell lung cancer through sponging miR-155,” *Molecular Medicine Reports*, vol. 17, no. 6, pp. 7692–7700, 2018.

X. Li, Z. Zhang, H. Jiang et al., “Circular RNA circPVT1 promotes proliferation and invasion through sponging miR-125b and activating E2F2 signaling in non-small cell lung cancer,” *Cellular Physiology and Biochemistry*, vol. 51, no. 5, pp. 2324–2340, 2018.

L. Chen, A. Nan, N. Zhang et al., “Circular RNA 100146 functions as an oncogene through direct binding to miR-361-3p and miR-615-5p in non-small cell lung cancer,” *Molecular Cancer*, vol. 18, no. 1, p. 13, 2019.

X. X. Liu, Y. E. Yang, X. Liu et al., “A two-circular RNA signature as a noninvasive diagnostic biomarker for lung adenocarcinoma,” *Journal of Translational Medicine*, vol. 17, no. 1, p. 50, 2019.

X. Zhou, H. Y. Liu, W. Y. Wang, H. Zhao, and T. Wang, “Hsa_circ_0102533 serves as a blood-based biomarker for non-small-cell lung cancer diagnosis and regulates apoptosis in vitro,” *International Journal of Experimental Pathology*, vol. 11, no. 9, pp. 4395–4404, 2018.

D. Hang, J. Zhou, N. Qin et al., “A novel plasma circular RNA circFARSA is a potential biomarker for non-small cell lung cancer,” *Cancer Medicine*, vol. 7, no. 6, pp. 2783–2791, 2018.

X. Zhu, X. Wang, S. Wei et al., “hsa_circ_0013958: a circular RNA and potential novel biomarker for lung adenocarcinoma,” *The FEBS Journal*, vol. 284, no. 14, pp. 2170–2182, 2017.