Received: 24 March 2021 | Revised: 2 April 2021 | Accepted: 3 April 2021

DOI: 10.1002/jcla.23851

RESEARCH ARTICLE

WILEY

Circular RNA hsa_circ_0001874 is an indicator for gastric cancer

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Funding information
National Natural Science Foundation of China, Grant/Award Number: 81702367 and 81772279; the Affiliated Hospital of Medical School of Ningbo University Youth Talent Cultivation Program, Grant/Award Number: FYQMKY202001

Abstract

Background: Recent studies have indicated that circular RNAs (circRNAs) are novel endogenous RNAs whose 5′ and 3′ ends are covalently linked and play critical roles in gastric carcinogenesis. However, the significance of circRNA hsa_circ_0001874 in gastric cancer (GC) is still unclear.

Methods: Therefore, we first detected hsa_circ_0001874 levels in GC cell lines and tissues and analyzed their potential correlation with clinicopathological factors. Then, a receiver operating characteristic (ROC) curve was established to evaluate its clinical value. Finally, we further predicted the biological functions of this molecule by bioinformatics analysis.

Results: Our data showed that as an indicator, hsa_circ_0001874 expression was significantly decreased in 78.02% (71/91) of the GC patients. Combined with clinicopathological factors, the hsa_circ_0001874 level was strongly associated with cell differentiation ($p < 0.001$), tumor stage ($p = 0.005$), invasion ($p = 0.024$), lymphatic metastasis ($p = 0.023$), and CEA level ($p < 0.001$) in GC tissues. The area under the curve (AUC) was up to 0.673, with a sensitivity and specificity of 61.54% and 68.13%, respectively. Bioinformatics analysis showed that hsa_circ_0001874 harbors miR-593-5p, miR-103a-3p, and miR-107 seed sequences to regulate these three miRNAs and downstream target genes and exert its various biological functions in the carcinogenesis and progression of GC.

Conclusion: In summary, these data suggest that hsa_circ_0001874 is an indicator of GC and plays a significant role in gastric carcinogenesis and progression.

KEYWORDS
bioinformatics analysis, circRNAs, gastric cancer, hsa_circ_0001874

1 | INTRODUCTION

Gastric cancer (GC) is a common malignancy and the fourth leading cause of mortality worldwide.[1] The majority of GC patients are diagnosed at an advanced stage and lack typical symptoms, leading to a poor prognosis. It not only seriously endangers people's health, but also creates a huge economic burden on society. Although there were many studies on GC, the molecular mechanism of GC initiation and progression is still unclear.[2,3] Therefore, it is important to explore biomolecules and their functions in

large-scale data analyses, which will help improve the diagnosis and treatment of GC.

Circular RNAs (circRNAs) are special endogenous circular RNA molecules with 5′ and 3′ ends covalently linked by alternative splicing.[4] Circular RNAs have various biological functions and show stability, abundance, tissue-specific exhibition, and high conservation.[5,6] Accumulating evidence suggests that circRNAs play key roles in cell physiological processes such as cell proliferation, differentiation, and apoptosis.[7,8] Moreover, some circRNAs with dysregulated expression can affect the occurrence and progression of cancers by many ways, especially acting as miRNA sponges.[4]

Hsa_circ_0001874 is an aberrantly expressed circRNA identified in our GC microarray.[5] Its gene symbol is BICD cargo adaptor 2 (*BICD2*), and it is located at chr9:95500310–95500614 and is 304 nts in length. However, the importance of hsa_circ_0001874 in GC is still unknown. Therefore, we first detected the hsa_circ_0001874 expression levels in GC cells and tissues and analyzed its clinicopathological correlation. Then, a receiver operating characteristic (ROC) curve was established to evaluate its clinical value. Finally, we further predicted its potential biological functions by bioinformatics methods. Our results showed that hsa_circ_0001874 is an indicator of gastric carcinogenesis and progression.

2 | MATERIALS AND METHODS

2.1 | Specimen collection

Ninety-one pairs of GC tissues and matched paracarcinoma tissues (3 cm away from the edge of the tumor) were obtained from the Affiliated Hospital of the Medical School of Ningbo University, China, between 2015 and 2019. All tissues were collected from surgical patients and stored in the refrigerator at −80°C, submerged in RNA-fixer Reagent (Bioteke). The tissues were finally diagnosed by pathology, and the tumor clinical stages were assessed according to the tumor-node-metastasis (TNM) staging system (8th ed.). The histological grades are based on the National Comprehensive Cancer Network clinical practice guidelines of oncology (V.3.2017). All patients assigned the informed consent.

2.2 | Cell culture

Cells were provided by the Chinese Academy of Sciences Biochemistry and Cell Biology Research . The culture conditions were RPMI-1640 medium (Life Technologies) with 10% fetal bovine serum at 37°C with 5% CO_2.

2.3 | qRT-PCR analysis

RNA was extracted using TRIzol reagents (Ambion) and then reverse-transcribed into cDNA via the GoScript Reverse Transcription (RT) System (Promega). Real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was performed with GoTaq qPCR Master Mix (Promega). We selected glyceraldehyde 3-phosphate dehydrogenase (GAPDH) to normalize the hsa_circ_0001874 levels. The primers were as follows: 5′-ATACCCGTTGGCTCTCCTGC-3′ (sense) and 5′-CGTGTGTAAGCCCACCCTGA-3′ (antisense) for hsa_circ_00001874; and 5′-ACCCACTCCTCCACCTTTGAC-3′ (sense) and 5′-TGTTGCTAGCCAAATTCGTTA-3′ (antisense) for GAPDH. Hsa_circ_00001874 levels were analyzed using the ΔCt method.[9] Each experiment was repeated independently at least two times. DNA sequencing was used to confirm the sequence of qRT-PCR products (Figure S1).

2.4 | Bioinformatics analysis

TargetScan and miRanda were used to predict and annotate the miRNA sponge function.[10] Kyoto Encyclopedia of Genes and Genomes pathway (KEGG) and Gene Ontology (GO) analyses of hsa_circ_0001874/miRNA pathways were performed by DIANA-miRPath software.[10] The circRNA-miRNA-mRNA network was drawn by Cytoscape software (https://cytoscape.org). The common downstream targets of the three miRNAs were identified based on the Venny 2.1 software.

2.5 | Statistical analysis

Statistical analyses were conducted by the Statistical Package for the Social Sciences (SPSS) 20.0 software (SPSS). Student's test and one-way analysis of variance (ANOVA) were the main statistical methods used in this study. A p-value < 0.05 was considered statistically significant.

3 | RESULTS

3.1 | Hsa_circ_0001874 expression was decreased in GC

To verify whether hsa_circ_0001874 was aberrantly expressed in gastric carcinogenesis, we first detected hsa_circ_0001874 levels in the normal human gastric epithelial cell line GES-1 and two GC cell lines (BGC-823 and SGC-7901). Our results showed that hsa_circ_0001874 expression was significantly decreased in the two GC cell lines compared with the GES-1 cell line (Figure 1A). Then, we detected its expression levels in GC tissues. It is that hsa_circ_0001874 was also significantly decreased in 78.02% (71/91) of the GC tissues compared with their paired paracarcinoma tissues (p < 0.001; Figure 1B,C). The area under the ROC curve (AUC) was 0.673 (95% confidence interval [CI]: 0.595–0.751, p < 0.001; Figure 1D), with a sensitivity and specificity of 61.54% and 68.13%, respectively.

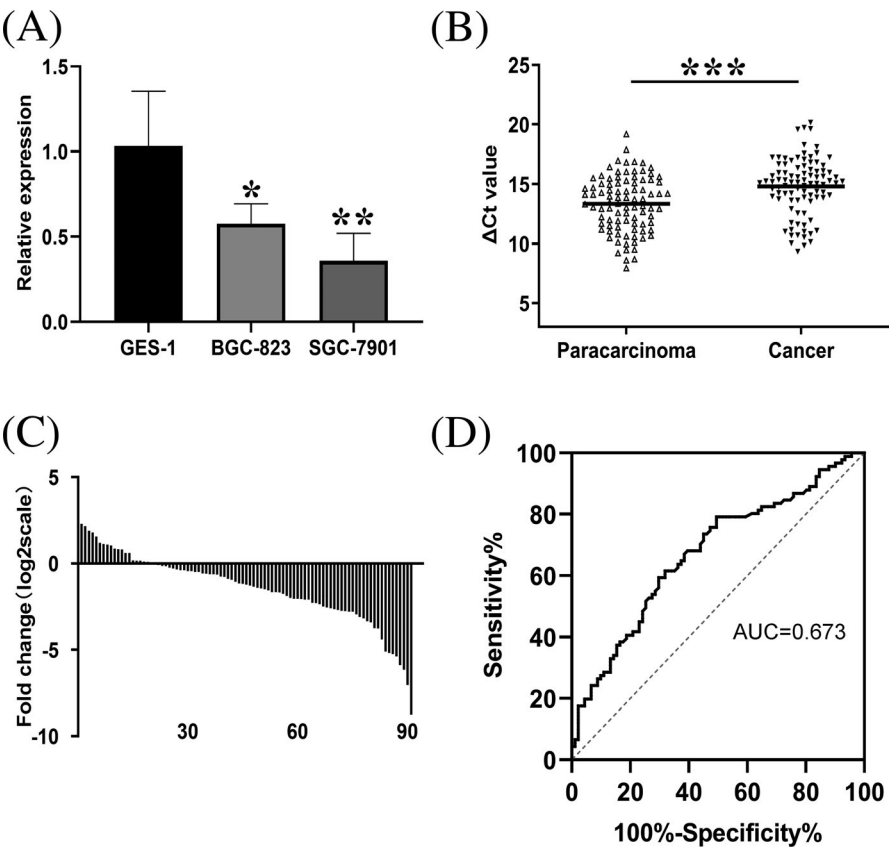

FIGURE 1 Aberrant expression of hsa_circ_0001874 in GC. (A) Relative expression levels of hsa_circ_0001874 in GES, BGC-823, and SGC-7901. (B) Hsa_circ_0001874 levels in 91 GC and marched paracarcinoma tissues. (C) Hsa_circ_0001874 expression levels were decreased in 78.02% (71/91) GC tissues. (D) ROC curve of hsa_circ_0001874 in distinguishing GC tissues from paracarcinoma tissues (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$)

3.2 | Clinicopathological correlation of hsa_circ_0001874

Hsa_circ_0001874 expression levels in GC tissues were negatively correlated with GC patient cell differentiation grade ($p < 0.001$), tumor stage ($p = 0.005$), invasion ($p = 0.024$), lymphatic metastasis ($p = 0.023$), and tissue CEA level ($p < 0.001$) (Table 1).

3.3 | Annotation of hsa_circ_0001874 biological function

Bioinformatics analysis indicated that hsa_circ_0001874 harbors miR-593-5p, miR-103a-3p and miR-107 seed sequences (Figure 2A). These three miRNAs regulate 104, 3627, and 3747 downstream target protein genes, respectively. A Venn diagram revealed the number of common targets of this three miRNAs (Figure 2B). A map comprising hsa_circ_0001874, three miRNAs, and their common targets was constructed to show their interaction (Figure 2C). GO and KEGG results indicated that the hsa_circ_0001874/miRNA axis regulates downstream target protein genes to exert its various biological functions involved in multiple biological processes and signaling pathways, such as protein complex regulation, gene expression, the cell cycle, the p53 signaling pathway, and metabolic processes (Figure 3A,B). All these factors will affect the carcinogenesis and progression of GC.

4 | DISCUSSION

circRNAs are special endogenous circular RNA molecules that were once regarded as nonfunctional by-products and ignored by researchers. However, accumulating evidences have suggested that some circRNAs are aberrantly expressed and related to multiple pathological processes of gastric carcinogenesis, including tumorigenesis, development, and metastasis.[4,11] As crucial regulators, circRNAs have been found to exert their biological functions mainly acting as miRNA sponges.[12] Wang et al.[13] illustrated that the downregulated circ-ITCH expression in GC promoted proliferation, migration, invasion, and epithelial-mesenchymal transition (EMT) via a miR-199a-5p sponge mechanism. Zhang et al.[14] showed that upregulated circRNA_0005529 expression facilitates GC growth and metastasis by regulating the miR-527/Sp1 axis. Moreover, in a recent study, circPVT1 was discovered to regulate the chemoresistance and malignancy of GC by modulating cancer-related pathway through interacting with miR-152-3p.[15] Knockdown of circPVT1 expression significantly reduced DDP resistance and elevated cisplatin sensitivity in GC.[15] Similarly, circHN1, circMAN2B2, and hsa_circ_0023642 were also found to affect the growth and migration of GC cells by the similar ways.[16–18] All these circRNAs can serve as potential biomarkers and targets for the diagnosis and therapy of GC, which strongly suggests an important role of altered circRNAs in cancer pathophysiology.

TABLE 1 Relationship of Hsa_circ_0001874 expression levels (ΔC_t) in cancer tissues with clinicopathological factors of patients with gastric cancer

Characteristics	No. of case (%)	Mean ± SD	*p*-Value
Age (years)			
≥60	58 (63.7)	14.758 ± 2.518	0.818
<60	33 (36.3)	14.879 ± 2.165	
Gender			
Male	64 (70.3)	14.526 ± 2.455	0.089
Female	27 (29.7)	15.457 ± 2.107	
Tumor location			
Sinuses ventriculi	45 (49.4)	14.646 ± 2.604	0.496
Cardia	10 (11.0)	14.023 ± 1.959	
Corpora ventriculi	24 (26.4)	15.297 ± 1.411	
Others	12 (13.2)	15.048 ± 3.292	
Diameter (cm)			
≥5	46 (50.5)	14.841 ± 2.335	0.876
<5	45 (49.5)	14.762 ± 2.459	
Differentiation			
Well	24(26.4)	15.973 ± 1.255	**<0.001**
Poor	67 (73.6)	14.383 ± 2.555	
Stage			
Early	23 (25.3)	15.731 ± 1.411	**0.005**
Advanced	68 (74.7)	14.488 ± 2.567	
Borrmann type			
I and II	17 (25.0)	14.512 ± 3.513	0.972
III and IV	51 (75.0)	14.480 ± 2.208	
Pathological diagnosis			
Signet ring cell cancer	6 (6.6)	13.600 ± 2.356	0.203
Adenocarcinoma	85 (93.4)	14.887 ± 2.686	
Invasion			
T_1 and T_2	34 (37.4)	15.529 ± 2.079	**0.024**
T_3 and T_4	57 (62.6)	14.368 ± 2.466	
Lymphatic metastasis			
N_0	36 (39.6)	15.503 ± 2.199	**0.023**
N_{1-3}	55 (60.4)	14.344 ± 2.409	
Distal metastasis			
M_0	79 (86.8)	14.803 ± 2.318	0.993
M_1	12 (13.2)	14.797 ± 2.905	
Venous invasion			
Absent	50 (54.9)	14.856 ± 2.394	0.813
Present	41 (45.1)	14.736 ± 2.401	
Perineural invasion			
Absent	46 (50.5)	14.900 ± 2.392	0.693
Present	45 (49.5)	14.702 ± 2.400	
CEA (Tissue)			
Positive	70 (77.1)	15.323 ± 2.100	**<0.001**
Negative	21 (22.9)	12.523 ± 2.238	
CA19-9 (tissue)			
Positive	51 (56.0)	14.889 ± 2.105	0.696
Negative	40 (44.0)	14.691 ± 2.723	

Data were presented as mean ± standard deviation. A p -value < 0.05 was considered statistically significant (in bold).

Hsa_circ_0001874 is aberrantly expressed in gastric carcinogenesis. In this study, hsa_circ_0001874 expression levels were decreased in the GC cell lines and 78.02% of the GC tissues. Bioinformatics analysis revealed that hsa_circ_0001874 regulates abundant downstream target genes by sharing miRNA response elements with miR-593-5p, miR-103a-3p, and miR-107. KEGG and GO analyses indicated the hsa_circ_0001874/miRNA axis is related to various biological processes, pathophysiological mechanisms, and cancer signaling pathways, such as biosynthesis, gene expression, metabolic processes, and cancer pathways. This suggest that hsa_circ_0001874 plays crucial roles in gastric carcinogenesis and progression through the hsa_circ_0001874-miRNA-target gene axis.

Some clinicopathological variables are independent prognostic factors for GC patients. Previous studies showed that patients' 5-year survival rate in early stage was higher than 85%, whereas it was lower than 20% in advanced GC.[19] For advanced GC, TNM stage representing tumor (T), lymph node metastasis (N), and distant metastasis (M) in GC cancer tissues is good indicator for patient prognostic assessment. Clinical statistics showed that the 5-year survival rates were significantly different among different T and N categories. For GC patients in the T_1, T_2, T_3, T_{4a}, and T_{4b} categories, their 5-year survival rates were 93.9%, 98.7%, 51.8%, 32.6%, and 20.4%, respectively.[20] Similarly, in the N_0, N_1, N_2, N_{3a}, and N_{3b} categories, the 5-year survival rates were 81.3%, 61.0%, 48.3%, 35.9%, and 16.9%, respectively.[20] The degree of cell differentiation is also an important pathological factor associated with GC patient prognosis. The worse the differentiation was, the lower the overall survival rates were.[21] Moreover, as a common gastrointestinal tumor biomarker, CEA expression levels in tissue were directly correlated with GC prognosis.[22] The 5-year survival rates were 67.6% and 40.1% for negatively and positively stained tissues, respectively.[23] In the current study, the hsa_circ_0001874 expression levels in GC tissues were in related to GC patients' cell differentiation grade, tumor stage, invasion, lymphatic metastasis, and tissue CEA level. This implies that hsa_circ_0001874 is a potential indicator for clinical prognostic prediction.

Conclusively, our results suggested that hsa_circ_0001874 is an indicator of gastric carcinogenesis and progression.

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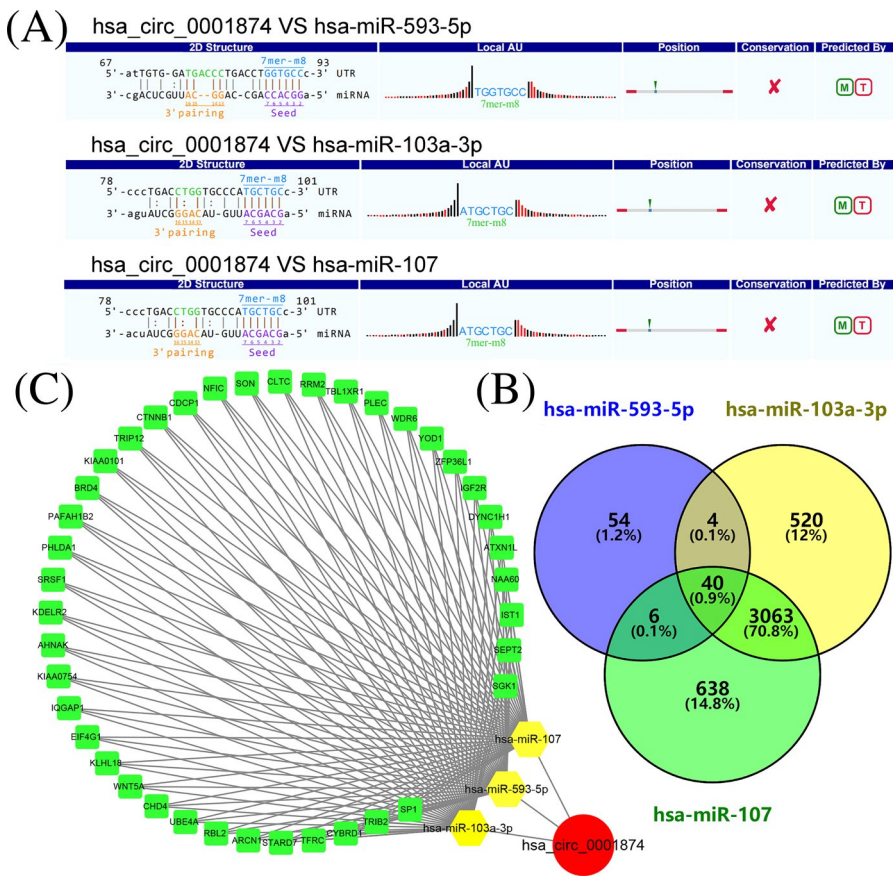

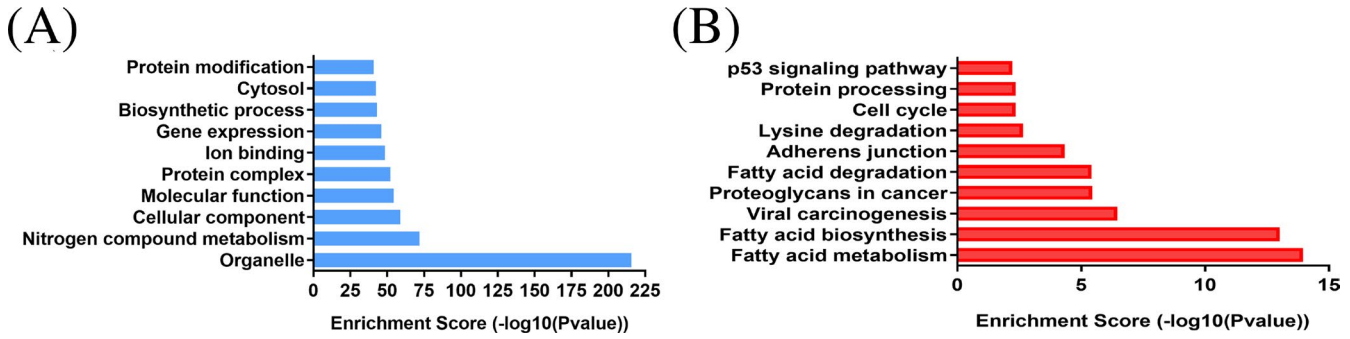

FIGURE 2 Prediction for hsa_circ_0001874-miRNA-target gene axis. (A) The interaction between hsa_circ_0001874 and miRNAs based on TargetScan and miRanda. (B) Venn diagram reveled the number of common downstream targets of hsa-miR-593-5p, hsa-miR-103a-3p, and hsa-miR-107. (C) The interaction network map of hsa_circ_0001874-miRNA-target gene axis

FIGURE 3 GO analysis and KEGG analysis of hsa_circ_0001874-miRNAs. (A) GO analysis. (B) KEGG analysis

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (Grant Nos. 81702367 and 81772279) and the Affiliated Hospital of Medical School of Ningbo University Youth Talent Cultivation Program (Grant No. FYQMKY202001).

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Ye Q, Qi C, Xi M, Ye G. Circular RNA hsa_circ_0001874 is an indicator for gastric cancer. *J Clin Lab Anal*. 2021;35:e23851. https://doi.org/10.1002/jcla.23851