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CircAGFG1 Promotes Ovarian Cancer Progression Through the miR-409-3 p/ZEB1 Axis

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Abstract

Objectives: Circular RNAs (circRNAs) serve a crucial regulatory role in ovarian cancer (OC). Circular RNA ArfGAP with FG repeats 1 (circAGFG1) has been shown to be involved in promoting the progression of several cancers, containing triple-negative breast cancer, esophageal cancer and colorectal cancer. However, the function of circAGFG1 in OC is unclear. **Methods:** Quantitative real-time reverse transcription PCR (RT-qPCR) was conducted to determine the expression levels of circAGFG1 and miR-409-3p. The proliferation and metastasis of cells were determined by colony formation assays, EdU assays, transwell assays and wound healing assays. In addition, a dual-luciferase reporter assay was performed to validate the mechanism between circAGFG1, miR-409-3p, and ZEB1. **Results:** Our data suggested that circAGFG1 was significantly overexpressed in OC tissues compared to normal ovarian epithelial tissues. Overexpression of circAGFG1 was correlated with intraperitoneal metastasis, tumor recurrence and advanced stage. Additionally, circAGFG1 overexpression revealed a poor prognosis in OC patients. Knockdown of circAGFG1 suppressed the proliferation, invasion and migration of OC cells. Mechanistically, circAGFG1 acted as a sponge of miR-409-3p to enhance the expression level of zinc finger E-box binding homeobox 1 (ZEB1), thereby conferring OC cell proliferation, invasion and migration. Importantly, re-expression of ZEB1 effectively reversed the effects of circAGFG1 knockdown on OC cells. **Conclusions:** In summary, our study indicated that circAGFG1 may act as a prognostic biomarker and potential therapeutic target for patients with OC.

Keywords

circAGFG1, miR-409-3p, ZEB1, ovarian cancer, progression

Abbreviations

CircRNAs, circular RNAs; OC, ovarian cancer; RT-qPCR, quantitative real-time reverse transcription PCR; ZEB1, zinc finger E-box binding homeobox1; TF, transcription factor; EMT, epithelial–mesenchymal transition; ATCC, the American Type Culture Collection; EdU, 5-ethynyl-20-deoxyuridine

Introduction

Ovarian cancer (OC) is the primary cause of cancer-related death among cancers of the Female reproductive system, and its prognosis is still poor worldwide.[1] A high metastasis rate contributes to the rapid progression of ovarian cancer and causes a huge number of deaths.[2] The five-year rate of overall survival in OC patients is relatively low.[3] Until now, there has been no effective strategy for ovarian cancer metastasis. In particular, the detailed mechanism of OC metastasis remains unclear. Thus, it is necessary to explore novel and valuable targets for the treatment of OC patients, especially in terms of metastasis.

Nearly 75% of the human genome is transcribed into RNA, while only about 3% is transcribed into protein-coding mRNAs.

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Non-coding RNAs (ncRNAs) can be divided into several classes depending on their length, shape, and location. Among them, microRNA (miRNA), long ncRNA (lncRNA) and circular RNA (circRNA) are the major type ncRNAs that play a key role in cancers.[4–7] Circular RNAs (circRNAs), characterized by covalently closed-loop structures, have enormous advantages as noninvasive biomarkers of tumors due to their high stability and evolutionary conservation feature.[8–10] Recently, quite a lot of studies have revealed that circRNAs are involved in the malignant progression of cancer.[11,12] What's more, circRNA function in cancer metastasis has been investigated in many studies.[13–15] For example, Zhi et al showed that circ102049 serves as a key regulatory molecules of colorectal cancer metastasis.[16] Xu et al reported that circIKBKB confers breast cancer bone metastasis by sustaining NF-κB/bone remodeling factor signaling aix.[17] Additionally, Ai et al circFOXO3 regulates KDM2A expression level by targeting miR-214 to induce tumor growth and metastasis in oral squamous cell carcinoma. Thus, functional circRNAs may be vital for OC prognosis and novel therapeutic targets.

Zinc finger E-box binding homeobox 1 (ZEB1) has been reported to serve as a transcription factor (TF) that regulates numerous biochemical processes, including invasion, metastasis and chemoresistance, through binding with particular proteins.[18,19] Moreover, ZEB1 is overexpressed in several kinds of tumors, such as cervical cancer, pancreatic cancer, osteosarcoma, lung cancer, gastric cancer, colorectal cancer, and breast cancer.[20] A previous study showed that ZEB1 regulates the expression of vimentin to induce tumorigenesis and metastasis in hepatocellular carcinoma.[21] Moreover, silencing of DNAJB9 inhibits the metastasis ability of triple-negative breast cancer cells via inducing FBXO45-mediated ZEB1 degradation.[22] In breast cancer cells, ZEB1 confers miR-99b/let-7e/miR-125a axis cluster confers invasion and metastasis in esophageal squamous cell carcinoma.[23] Although people have considered that ZEB1 may play a vital function in tumor metastasis, the specific mechanism of ZEB1 in ovarian cancer is still ambiguous and needs further study.

In our study, we explored circAGFG1 expression in our ovarian cancer clinical samples and cell lines. The functional roles of circAGFG1 in cell lines were further explored. Moreover, the detailed molecular mechanisms of circAGFG1 and ZEB1 were investigated. In summary, this study might be helpful for identifying effective treatments for OC.

Methods

Clinical Samples

Thirty OC tissues and 30 normal ovarian epithelial tissues were obtained from the department of Obstetrics and Gynecology of the First Affiliated Hospital, School of Clinical Medicine of Guangdong Pharmaceutical University. These were tissues collected between 2016 and 2018 and the tissues were stored in liquid nitrogen. None of the patients had received preoperative chemotherapy or radiation. Verbal informed consent was obtained from the study participants. The study was approved by the Ethics Committee of our hospital in 2021 (approval number: 2021-87).

Cell Culture and Transfection

OC cell lines (A2780 and SKOV3) were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). A2780 and SKOV3 ovarian cancer cells were cultured with DMEM supplemented with 10% FBS (Gibco, USA), 100 U/mL penicillin, and 100 mg/mL streptomycin. For constructing circAGFG1 silencing cell lines, shRNAs targeting circAGFG1 and the corresponding negative control were obtained from Jiangsu Saisofi Biotechnology Co., Ltd (Wuhan, China). MiR-409-3p mimics and inhibitors were purchased from GeneCopoeia (Guangzhou, China). Transfection assays was performed by using Lipofectamine 2000 (Invitrogen, USA) following the manufacturer's instructions.

Quantitative Real-Time PCR (qRT-PCR)

As a previous study reported, TRIzol reagent was used to extract RNA.[24] SYBR Green reagent (Takara) was mixed with specific primers to conduct qRT-PCR following a previous study.[24]

Colony Formation Assay

Following a previous study,[24] OC cells (A2780 and SKOV3) were seeded in 6-well plates for 2 weeks. Then, the OC cells clones were fixed with 4% formaldehyde and stained with 0.5% crystal violet. The ImageJ was then used to calculate the number of colonies.

5-Ethynyl-20-Deoxyuridine (EdU) Assay

Briefly, stably transfected A2780 and SKOV3 OC cells were cultured in a 24-well plate (5×10^6/per well) and incubated with 300 μL of 50 μM EdU per well for 120 minutes at 37 ° C. Then, the above cells were fixed with 4% formaldehyde at 25 °C for half hours. Then, 150 μL of 2 mg/mL glycine was added to each well and washed with 500 μL of PBS three times to decolorize the plate. After decolorization, 500 μL of 0.5% Triton X-100 was added to each plate well and incubated for 15 minutes. The plates were washed with PBS three times and reacted with 400 μL of Apollo solution for 30 minutes at 37 °C under dark conditions. The above plates were washed with 0.5% Triton X-100 (500 μL) four times. Next, 500 μL of methanol was used to wash the plate three times, followed by washing with PBS solution. Then, the cells were incubated with 300 μL of Hoechst 33342 for half an hour at 25 °C in the condition of dark. The images were visualized with a fluorescence microscope (Olympus, Corporation).

Transwell Assay

Transwell experiments were performed in a transwell cell incubator (Corning, New York, USA) to investigate cell migration and invasion ability following a previous study.[25]

Wound Healing Assay

As previous study reported,[25] stably transfected A2780 and SKOV3 OC cells were seeded in 6-well plates without FBS at 37 °C, and a linear scratch was left in the center of the well plate using a 10-μL micropipette tip. Images were obtained at 0 and 24 hours of incubation with an Olympus BX51 microscope (Olympus Corporation). The experiments were conducted in triplicate.

Luciferase Reporter Assays

According to previously described methods,[24] the circAGFG1-wt, circAGFG1-mut, ZEB1-wildtype or ZEB1-mutation sequence was inserted into the pMIR-reporter (Promega, Madison, USA). Then, plasmids and miR-409-3p mimics or its corresponding controls were then transfected into A2780 and SKOV3 cells. Two days later, the relative luciferase activities were measured by using the Dual-Luciferase Reporter Assay Kit (Promega, USA).

Statistical Analysis

Data are shown as the mean ± standard deviation. Quantitative results were based on repeating the experiment from at least three. All statistical analyses were conducted using GraphPad Prism 8.0 software. The Student's t test or one-way analysis of variance was used to examine the differences between two groups and three groups, respectively. $P < 0.05$ was considered as statistically significant.

Results

circAGFG1 Overexpression was Correlated With Poor Prognosis in Ovarian Cancer

To examine the potential function of circAGFG1 in OC, qRT-PCR was performed to examine the gene expression of circAGFG1 in OC tissues and normal ovarian epithelial tissue. The data indicated that circAGFG1 was significantly overexpressed in OC tissues (Figure 1A). Moreover, circAGFG1 expression was higher in OC tissues with advanced stage (stage III + IV) and metastasis (Figure 1B and C). Consistently, circAGFG1 expression was overexpressed in OC cell lines compared with normal ovarian epithelial cells (Figure 1D). We further examined the correlation between circAGFG1 and the clinical characteristics. As displayed in Supplemental Table S1, circAGFG1 was significantly positively correlated with advanced stage, intraperitoneal metastasis and tumor recurrence. Subsequently, we analyzed the overall survival rates and recurrence-free survival rates. The results suggested that upregulation of circAGFG1 was associated with a poor survival rate, indicating that circAGFG1 may act as a valuable biomarker for OC patients (Figure 1E and F).

circAGFG1 Sponged miR-409-3p in OC Cells

Next, we explored the molecular mechanism by which circAGFG1 induces metastasis in OC cells. Although circAGFG1 has been reported in other types of cancers to induce malignant progression through function as a miRNA sponge, how it played a role in ovarian cancer is still unclear. As previous researches reported, the function of circRNAs as "miRNA sponges" has been extensively explored.[26] Then, we conducted a nuclear/cytoplasmic fractionation assay and detected that circAGFG1 was principally located in the cytoplasm of the both A2780 and SKOV3 OC cell lines (Figure 2A and B). To explore the function and mechanism of circAGFG1, we designed shRNAs targeting circAGFG1 in OC cell lines (A2780 and SKOV3) and confirmed their effect (Figure 2C and D). Bioinformatic analysis (starBase 2.0 analysis) suggested that miR-409-3p was a possible target. The results of qRT-PCR showed that miR-409-3p expression levels were remarkably upregulated upon circAGFG1 knockdown in OC cells (Figure 2E). Additionally, circAGFG1 expression was significantly negatively associated with miR-409-3p in OC tissues, indicating a regulatory relationship between circAGFG1 and miR-409-3p (Figure 2F). To confirm their interaction, a luciferase reporter assay was performed with a wt- or mut-circAGFG1 reporter. In A2780 and SKOV3 cell lines, miR-409-3p mimics inhibited the activity of circAGFG1 wt- reporter (Figure 2G and H). In addition, a pull-down assay suggested that miR-409-3p directly pulled down circAGFG1 in A2780 and SKOV3 cell lysates (Figure 2I). Thus, our data demonstrated that circAGFG1 sponges miR-409-3p in OC cells.

circAGFG1 Conferred OC Progression by Suppressing miR-409-3p

The next, the function of circAGFG1 in OC was further explored. We carried out CCK8, EdU and colony formation assays to measure the effect of circAGFG1 on tumor cell proliferation. The data suggested that circAGFG1 silencing inhibited the proliferation ability of A2780 and SKOV3 cells (Figure 3A–E). To explore the role of circAGFG1 in metastasis, a transwell assay was conducted. As shown in Figure 3F and G, knockdown of circAGFG1 inhibited the migration ability of OC cells. The wound healing assay suggested that, compared to the indicated control OC cells, the number of migratory cells was markedly decreased upon silencing of circAGFG1, and transfection of miR-409-3p inhibitors rescued these effects (Figure 3H and I). Thus, circAGFG1 promotes OC cell progression by sponging miR-409-3p.

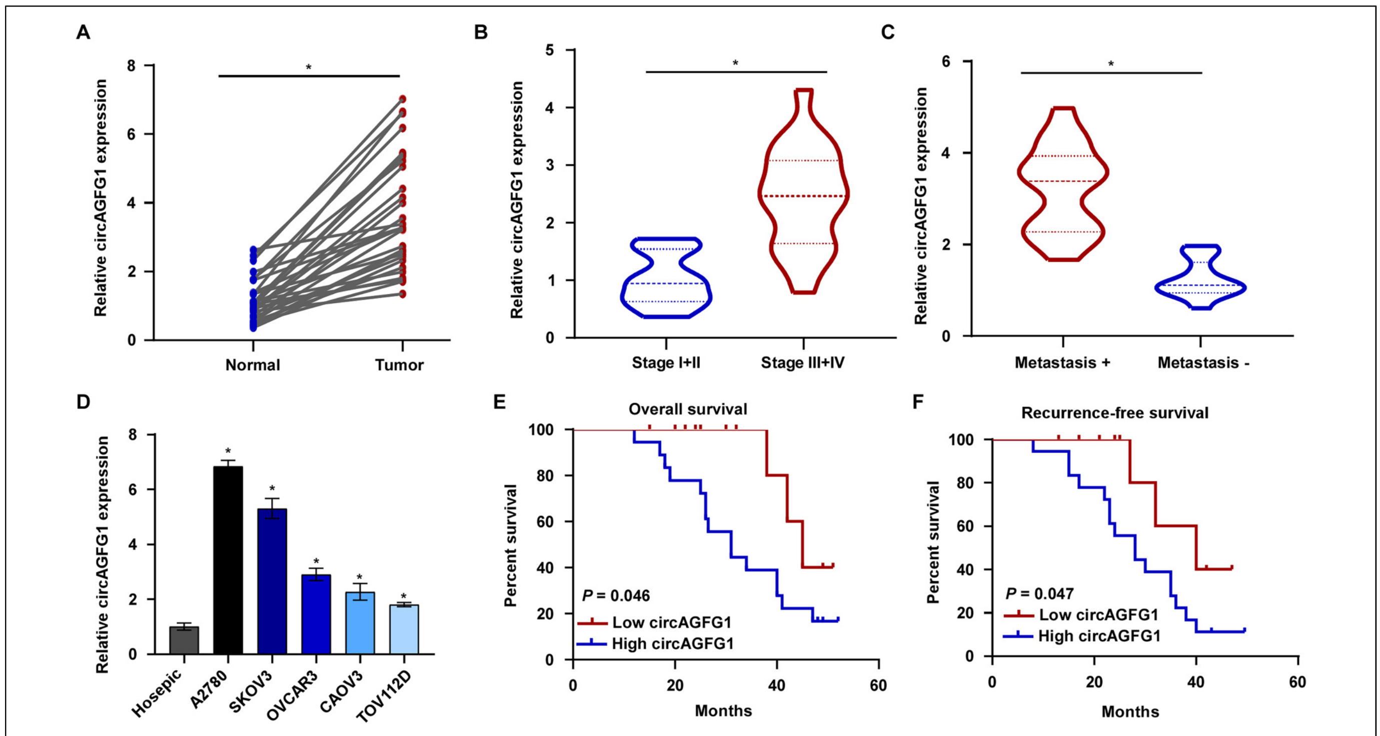

Figure 1. circAGFG1 up-regulation is correlated with poor prognosis in OC. (A) circAGFG1 expression was explored in 30 pairs of OC tissues and normal epithelial ovarian tissues controls. (B) Expression levels of circAGFG1 in OC tissues with different stages. (C) Expression levels of circAGFG1 in OC tissues with distant metastasis or not. (D) Expression levels of circAGFG1 in OC cell lines and normal epithelial ovarian cell. (E, F) Overall survival rate and recurrence-free survival rate in OC patients were determined using the Kaplan–Meier curve. $^{*}P < 0.05$, ns indicates no significance. Each error bar represents the mean ± SD of three independent experiments.

ZEB1 Upregulation by circAGFG1-Induced miR-409-3p Suppression Conferred EMT

For further explore the detailed mechanism of the circAGFG1/miR-409-3p axis, we explored the direct target. StarBase bioinformatics analysis indicated that ZEB1 is a potential target of miR-409-3p. To confirm the predicted results, we carried out luciferase reporter assays. miR-409-3p mimics significantly decreased the activity of the wt-ZEB1 reporter (Figure 4A and B). Moreover, the expression level of ZEB1 was negatively associated with miR-409-3p in OC tissues (Figure 4C). In addition, we found that circAGFG1 knockdown inhibited the expression level of ZEB1, which was repressed by transfection of miR-409-3p inhibitors (Figure 4D). Therefore, circAGFG1 upregulated ZEB1 expression by inhibiting miR-409-3p. ZEB1 is a transcription factor (TF) that induced epithelial-mesenchymal transition (EMT) and the metastasis ability of cells in different cancers, which is significantly correlated with ovarian cancer development.[27] Thus, we further explored whether circAGFG1 regulated EMT and promoted OC progression. We explored the effects of circAGFG1 on the expression of EMT-related genes (ZEB1, E-cadherin, vimentin and FN1). The results revealed that circAGFG1 silenced reduced ZEB1, vimentin and FN1 expression but upregulated E-cadherin expression (Figure 4E–L).

Discussion

In our research, we demonstrated that circAGFG1 expression positively correlated with the proliferation and metastasis ability of ovarian cancer. In our in vitro results, circAGFG1 accelerated ovarian cancer progression by repressing miR-409-3p to upregulate ZEB1. Our research indicated that circAGFG1 may be regarded as a prognostic biomarker and novel treatment target for ovarian cancer.

Recently, the abnormal expression of circRNAs has been reported in diverse kinds of cancers.[27] Tang et al indicated that circINTS4 confers chemotherapy resistance in breast cancer via competitive binding miR-129-5p/POM121 signaling axis.[28] Moreover, Chen et al reported that CircSCAP interacts with SF3A3 to restrain the malignant progression of non-small cell lung cancer via activating p53 signaling.[29] circAGFG1 is a poorly studied circRNA, and no studies have been reported in ovarian cancer. The function and detailed mechanisms of circAGFG1 in OC remain unknown. Now, we demonstrated that circAGFG1 is overexpressed in OC tissues and predicts an unfavorable prognosis in OC patients. Additionally, circAGFG1 induced the proliferation, invasion and metastasis of OC cells. We are the first study to report the role of circAGFG1 in malignant progression of ovarian cancer. Moreover, our study is also the first to research circAGFG1/ miR-409-3p/ZEB1 axis in cancer.

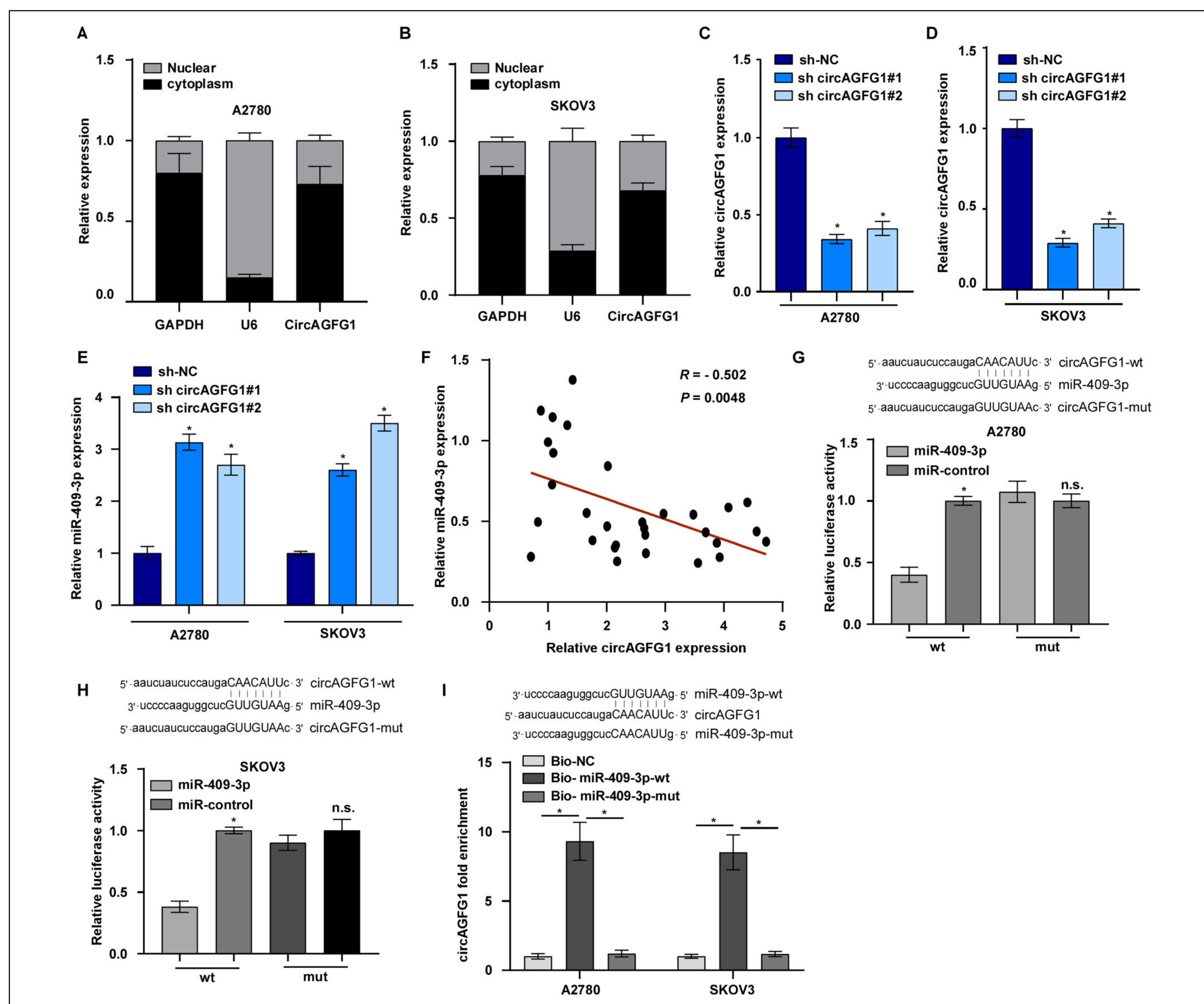

Figure 2. circAGFG1 sponges miR-409-3p in OC. (A, B) Nuclear/cytoplasmic fractionation analysis for circAGFG1 expression. (C, D) circAGFG1 expression was reduced by using two shRNAs targeting circAGFG1. (E) circAGFG1 knockdown caused upregulated expression of miR-409-3p (F) Negative correlation between circAGFG1 and miR-409-3p in OS tissues. (G, H) Luciferase reporter assay indicated circAGFG1-wt activity was impaired by miR-409-3p. (I) Biotin-wt-miR-93-3p interacted with AWPPH by pull-down assay. *$P < 0.05$, ns indicates no significance. Each error bar represents the mean ± SD of three independent experiments.

Our study revealed that circAGFG1 up-regulated ZEB1 to promote EMT progression and accelerated metastasis in ovarian cancer cells by sponging miR-409-3p. Therefore, circAGFG1 may act as a valuable predictor and biomarker of OC.

Current studies show that circRNAs are not only byproducts of pre-mRNA splicing, and abundant circRNAs have been reported to play an important function in numerous diseases, including cancer.[30] During the occurrence and development of cancers, aberrantly expressed circRNAs serve as oncogenes.[31] Recently, numerous studies have exerted their functions by sponging downstream target miRNAs and repressing their regulatory effects on target genes.[32] However, whether circAGFG1 has target miRNAs in ovarian cancer remains ambiguous. In this research, we found that circAGFG1 was located in the cytoplasm of ovarian cancer cells, indicted that circAGFG1 might serve as a miRNA sponge. After bioinformatics analysis, circAGFG1 was predicted to act as a sponge to bind miR-409-3p. Luciferase reporter assays and pulldown assays were conducted to examine their interaction. Additionally, miR-409-3p expression level was restrained by circAGFG1 in ovarian cancer cells. MiR-409-3p functions as a tumor inhibitor in diverse kinds of tumors, containing colon carcinoma, osteosarcoma and lung cancer.[33–35] In our research, we demonstrated that transfection with miR-409-3p inhibitors promotes the proliferation, migration and metastasis of ovarian cancer cells upon circAGFG1

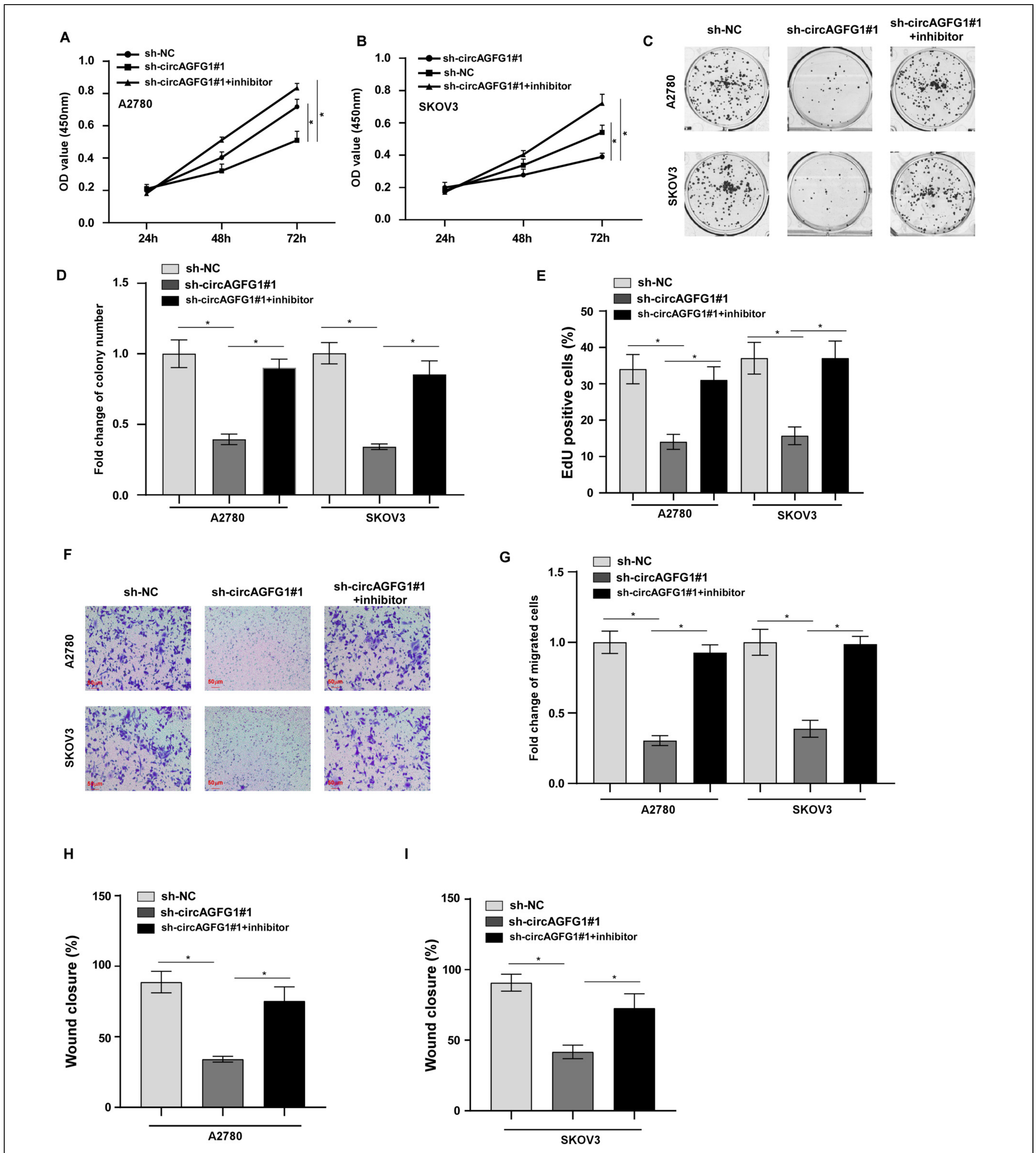

Figure 3. circAGFG1 promotes OC progression through suppressing miR-409-3p. (A, B) CCK8 assay was performed to test cell proliferation in the indicated cells. (C, D) colony formation assay was performed to explore cell proliferation in the indicated cells. (E) EdU incorporation assay was conducted to examine cell proliferation in the indicated cells. (F, G) Transwell assay was conducted to analyze migration. A2780 and SKOV3 cells were transfected with NC shRNA, sh circAGFG1 or sh circAGFG1 + miR-409-3p inhibitor. (H, I) Wound healing experiments was conducted in the indicated A2780 and SKOV3 cells. *$P < 0.05$, ns indicates no significance. Each error bar represents the mean ± SD of three independent experiments.

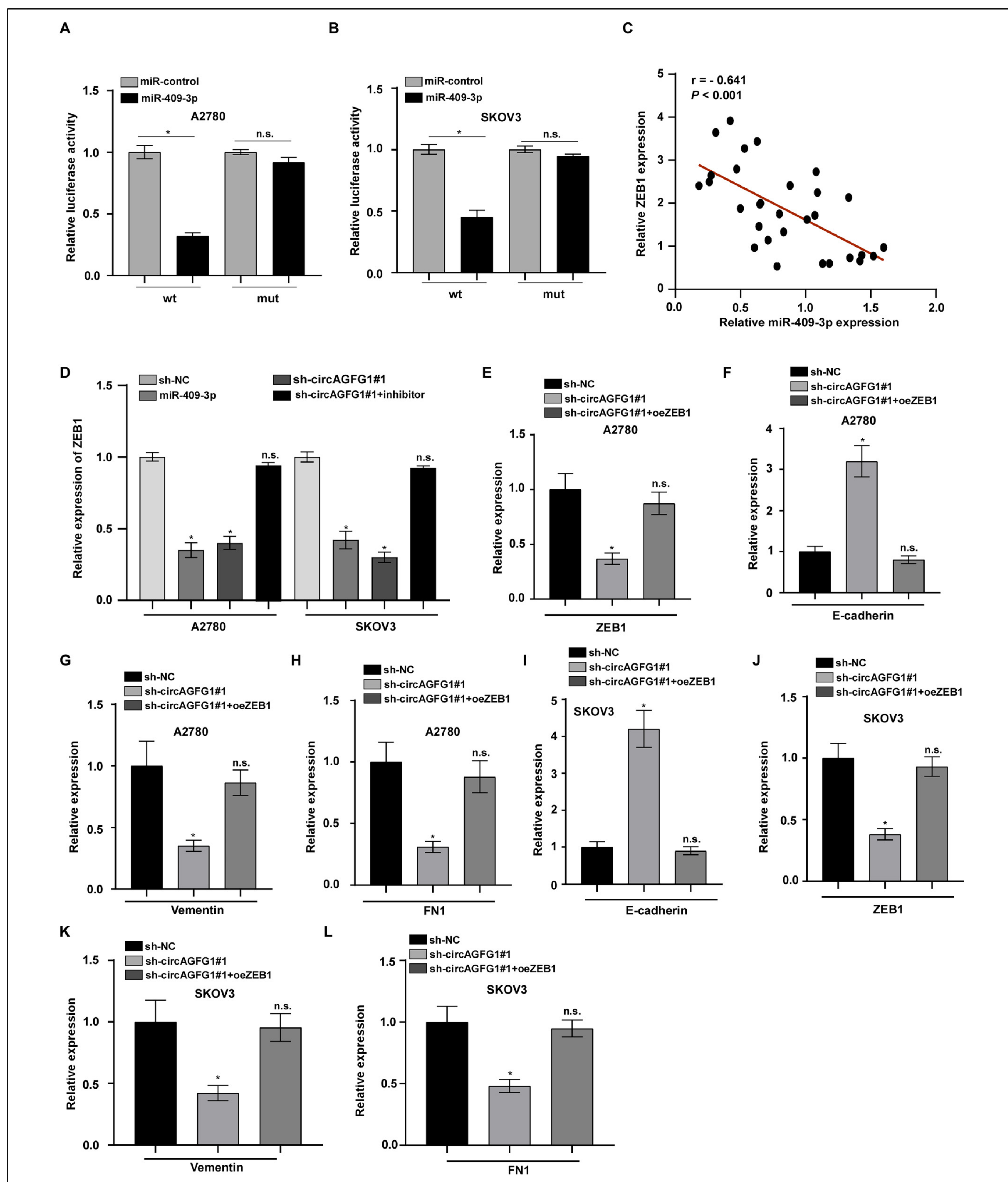

Figure 4. ZEB1 up-regulation by circAGFG1-induced miR-409-3p suppression promoted metastasis of OC cells. (A, B) Luciferase reporter assay showed miR-409-3p directly targeted ZEB1. (C) Negative correlation between ZEB1and miR-409-3p in OC tissues. (D) Expression of ZEB1 was measured by qRT-PCR in OC cells transfected with the indicated plasmids. (E–L) Expression of ZEB1 and its related genes (E-cadherin, Vementin and FN1) in the indicated cells. *$P < 0.05$, ns indicates no significance. Each error bar represents the mean ± SD of three independent experiments.

silence. Therefore, circAGFG1 induces ovarian cancer cell malignant progression by inhibiting miR-409-3p.

Subsequently, we indicated that miR-409-3p targets ZEB1. Inhibition of miR-409-3p by circAGFG1 upregulated ZEB1 expression. ZEB1 is a notorious transcription factor that serves a vital role in the metastasis of tumor cells.[36] Previous studies reported that ZEB1 induces epigenetic knockdown of E-cadherin by recruiting multiple chromatin remodeling enzymes from the promoter of E-cadherin.[37] Thus, ZEB1 confers the process of EMT. Our study showed that miR-409-3p inhibited ZEB1 expression, circAGFG1 increased the expression of ZEB1 and miR-409-3p could repress the positive effect of circAGFG1 on ZEB1. Further study indicated that after mutation of the miR-409-3p binding site in circAGFG1, circAGFG1 no longer regulated ZEB1 expression. These findings also underline the important roles of circAGFG1 and miR-409-3p in OC metastasis.

However, our study has limitation. Besides the ceRNA mechanism, circRNAs also regulated their biological roles via interactions with proteins, DNA or others. Thus, we could not exclude that circAGFG1 mediated cell metastasis in OC through other mechanisms. Further detailed mechanisms underlying the circAGFG1 function were need further exploration in the further. Moreover, in next studies, in vivo experiments need to be performed.

Conclusion

In brief, our study shows that circAGFG1 is significantly upregulated in OC patients, especially in metastatic OC patients, and promotes OC cell metastasis. Mechanistically, the circAGFG1/miR-409-3p/ZEB1 axis confers the metastasis of OC cells. These findings also suggested that circAGFG1 could function as a novel biomarker and a valuable therapeutic target for OC patients.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval

This study was approved by the Ethic Committee of The First Affiliated Hospital, School of Clinical Medicine of Guangdong Pharmaceutical University. The ethic approved number is 2021-87.

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Supplemental Material

Supplemental material for this article is available online.

Reference

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *CA Cancer J Clin*. 2022;72(1):7-33.
2. Cheng X, Wang J, Liu C, et al. Zinc transporter SLC39A13/ZIP13 facilitates the metastasis of human ovarian cancer cells via activating Src/FAK signaling pathway. *J Exp Clin Cancer Res*. 2021;40(1):199.
3. Roett MA, Evans P. Ovarian cancer: an overview. *Am Fam Physician* 2009;80(6):609-616.
4. Shirvani H, Ghanavi J, Aliabadi A, et al. MiR-211 plays a dual role in cancer development: from tumor suppressor to tumor enhancer. *Cell Signal*. 2023;101:110504
5. Pordel S, Khorrami M, Saadatpour F, et al. The role of microRNA-185 in the pathogenesis of human diseases: a focus on cancer. *Pathol Res Pract*. 2023;249:154729.
6. Fattahi M, Shahrabi S, Saadatpour F, et al. microRNA-382 as a tumor suppressor? Roles in tumorigenesis and clinical significance. *Int J Biol Macromol* 2023; 250:125863.
7. Faramin Lashkarian M, Hashemipour N, Niaraki N, et al. MicroRNA-122 in human cancers: from mechanistic to clinical perspectives. *Cancer Cell Int* 2023;23(1):29.
8. Shen T, Han M, Wei G, Ni T. An intriguing RNA species—perspectives of circularized RNA. *Protein Cell*. 2015;6(12):871-880.
9. Najafi S. The emerging roles and potential applications of circular RNAs in ovarian cancer: a comprehensive review. *J Cancer Res Clin Oncol*. 2023;149(5):2211-2234.
10. Najafi S. Circular RNAs as emerging players in cervical cancer tumorigenesis; A review to roles and biomarker potentials. *Int J Biol Macromol* 2022;1:206:939-953.
11. Chen B, Huang S. Circular RNA: an emerging non-coding RNA as a regulator and biomarker in cancer. *Cancer Lett* 2018;418:41-50.
12. Zhao Z-J, Shen J. Circular RNA participates in the carcinogenesis and the malignant behavior of cancer. *RNA Biol* 2017;14(5):514-521.
13. Mo Y, Wang Y, Zhang S, et al. Circular RNA circRNF13 inhibits proliferation and metastasis of nasopharyngeal carcinoma via SUMO2. *Mol Cancer* 2021;20(1):112.
14. Cen J, Liang Y, Huang Y, et al. Circular RNA circSDHC serves as a sponge for miR-127-3p to promote the proliferation and metastasis of renal cell carcinoma via the CDKN3/E2F1 axis. *Mol Cancer* 2021;20(1):19.
15. Zhang C-C, Li Y, Feng X-Z, Li DB. Circular RNA circ_0001287 inhibits the proliferation, metastasis, and radiosensitivity of non-small cell lung cancer cells by sponging microRNA miR-21 and up-regulating phosphatase and tensin homolog expression. *Bioengineered*. 2021;12(1):414-425.
16. Zhi Q, Wan D, Ren R, et al. Circular RNA profiling identifies circ102049 as a key regulator of colorectal liver metastasis. *Mol Oncol*. 2021;15(2):623-641.
17. Xu Y, Zhang S, Liao X, et al. Circular RNA circIKBKB promotes breast cancer bone metastasis through sustaining NF-kappaB/bone remodeling factors signaling. *Mol Cancer* 2021;20(1):98.

18. Sanchez-Tillo E, Siles L, de Barrios O, et al. Expanding roles of ZEB factors in tumorigenesis and tumor progression. *Am J Cancer Res*. 2011;1(7):897-912.
19. Zhang P, Sun Y, Ma L. ZEB1: at the crossroads of epithelial–mesenchymal transition, metastasis and therapy resistance. *Cell Cycle*. 2015;14(4):481-487.
20. Yang Y, Ahn Y-H, Chen Y, et al. ZEB1 sensitizes lung adenocarcinoma to metastasis suppression by PI3K antagonism. *J Clin Invest*. 2014;124(6):2696-2708.
21. Qin Y, Yu J, Zhang M, Qin F, Lan X. ZEB1 promotes tumorigenesis and metastasis in hepatocellular carcinoma by regulating the expression of vimentin. *Mol Med Rep*. 2019;19(3):2297-2306.
22. Kim H-Y, Kim Y-M, Hong S. DNAJB9 suppresses the metastasis of triple-negative breast cancer by promoting FBXO45-mediated degradation of ZEB1. *Cell Death Dis* 2021;12(5):461.
23. Ma J, Zhan Y, Xu Z, et al. ZEB1 induced miR-99b/let-7e/miR-125a cluster promotes invasion and metastasis in esophageal squamous cell carcinoma. *Cancer Lett* 2017;398:37-45.
24. Li H, Zhang W, Niu C, et al. Nuclear orphan receptor NR2F6 confers cisplatin resistance in epithelial ovarian cancer cells by activating the Notch3 signaling pathway. *Int J Cancer* 2019;145(7):1921-1934.
25. Li H, Zhang W, Yan M, et al. Nucleolar and spindle associated protein 1 promotes metastasis of cervical carcinoma cells by activating Wnt/beta-catenin signaling. *J Exp Clin Cancer Res* 2019;38(1):33.
26. Bezzi M, Guarnerio J, Pandolfi PP. A circular twist on microRNA regulation. *Cell Res*. 2017;27(12):1401-1402.
27. Liang H, Yu T, Han Y, et al. LncRNA PTAR promotes EMT and invasion-metastasis in serous ovarian cancer by competitively binding miR-101-3p to regulate ZEB1 expression. *Mol Cancer* 2018;17(1):119.
28. Tang Q, Zhou F, Yang C, Dai J, Li J, He Y. CircINTS4 facilitates chemoresistance of TNBC by competitively binding miR-129-5p/POM121 axis. *J Oncol*. 2022;2022:2630864.
29. Chen D, Zhou H, Cai Z, et al. CircSCAP interacts with SF3A3 to inhibit the malignance of non-small cell lung cancer by activating p53 signaling. *J Exp Clin Cancer Res* 2022;41(1):120.
30. Yin Y, Long J, He Q, et al. Emerging roles of circRNA in formation and progression of cancer. *J Cancer*. 2019;10(21):5015-5021.
31. Zhu XP, Wang XL, Ma J, et al. Down-regulation of miR-1236-3p is correlated with clinical progression and unfavorable prognosis in gastric cancer. *Eur Rev Med Pharmacol Sci* 2018;22(18):5914-5919.
32. Hansen TB, Jensen TI, Clausen BH, et al. Natural RNA circles function as efficient microRNA sponges. *Nature* 2013;495(7441):384-388.
33. Chen J, Wang R, Lu E, Song S, Zhu Y. LINC00630 as a miR-409-3p sponge promotes apoptosis and glycolysis of colon carcinoma cells via regulating HK2. *Am J Transl Res*. 2022;14(2):863-875.
34. Liu S, Zhan N, Gao C, et al. Long noncoding RNA CBR3-AS1 mediates tumorigenesis and radiosensitivity of non-small cell lung cancer through redox and DNA repair by CBR3-AS1/miR-409-3p/SOD1 axis. *Cancer Lett* 2022;526:1-11.
35. Long Z, Gong F, Li Y, Fan Z, Li J. Circ_0000285 regulates proliferation, migration, invasion and apoptosis of osteosarcoma by miR-409-3p/IGFBP3 axis. *Cancer Cell Int*. 2020;20:481.
36. Ying X, Zhu J, Zhang Y. Circular RNA circ-TSPAN4 promotes lung adenocarcinoma metastasis by upregulating ZEB1 via sponging miR-665. *Mol Genet Genomic Med*. 2019;7(12):e991.
37. Zhang Y, Xu L, Li A, Han X. The roles of ZEB1 in tumorigenic progression and epigenetic modifications. *Biomed Pharmacother*. 2019;110:400-408.