

Upregulation of circRNA_100395 sponges miR-142-3p to inhibit gastric cancer progression by targeting the PI3K/AKT axis

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Received June 17, 2020; Accepted January 20, 2021

DOI: 10.3892/ol.2021.12680

Abstract. Gastric cancer (GC) has a high morbidity and mortality, hence it is very important to elucidate the molecular pathogenesis mechanism of GC progression in order to find new treatment strategies. The present study aimed to explore the biological function of circular RNA_100395 (circRNA_100395) in GC. The expression level of circRNA_100395 in GC tissues, as well as normal epithelial cells and various gastric cancer cell lines, was detected using reverse transcription-quantitative PCR. Cell Counting Kit-8, EdU assay, flow cytometry and Transwell assays were performed to investigate cell proliferation, apoptosis, migration and invasion, respectively. A dual-luciferase reporter assay was performed to detect the correlation between circRNA_100395 and micro (mi)RNA-142-3p. Western blotting was performed to elucidate the potential regulatory mechanism. circRNA_100395 expression was found to be increased in GC tissues and cell lines. However, miR-142-3p expression was significantly reduced. Besides, low expression levels of circRNA_100395 were associated with poor tumor differentiation, advanced Tumor-Node-Metastasis stage, lymph node metastasis and shorter overall survival time. Moreover, overexpression of circRNA_100395 suppressed cell proliferation, increased the apoptosis rate and suppressed cell invasion and migration by inhibiting the PI3K/AKT signaling pathway. These findings also showed that miRNA-142-3p rescued the antitumor effects induced by circRNA_100395-overexpression. circRNA_100395-overexpression had antitumor effects via regulating the miR-142-3p signaling pathway, which might be a promising treatment target for GC.

Introduction

Gastric cancer (GC) is a malignant tumor and is associated with high morbidity and mortality (1). According to the latest

statistics, there were >1,000,000 new cases of GC and an estimated 783,000 deaths from GC worldwide in 2018 (2,3). Radical gastrectomy and perioperative chemotherapy are the standard treatments for GC. However, a large number of patients are diagnosed during advanced stages, with missed opportunity for surgery as early diagnosis is difficult due to the lack of effective diagnostic biomarkers and most patients are asymptomatic (3). In previous years, there has been major progress in molecular-targeted therapy and immunotherapy (4,5); however, the overall survival time for patients with GC is <20%. Consequently, it is imperative to identify effective diagnostic or therapeutic biomarkers for GC, and to further elucidate the potential regulatory mechanisms involved in GC progression.

Studies have identified that circular RNAs (circRNAs) are a specific type of non-coding RNA, with a closed-loop structure to prevent digestion by RNase R (6,7). Research into the regulatory role of circRNAs is gaining considerable interest. Notably, emerging evidence has shown that circRNAs play a regulatory role in various biological processes via sponging microRNAs (miRNAs) (7-9). Yu *et al* (9) indicated that circRNA_104718 acted as a tumor promoter gene in hepatocellular carcinoma by regulating the miRNA-218-5p/Thioredoxin domain-containing protein 5 signaling pathway. Besides, another study revealed that circRNA_0084043 could sponge miR-153-3p, upregulate Snail protein expression and promote malignant melanoma progression (10). Nevertheless, the biological function of circRNAs in GC progression has not been fully elucidated.

circRNA_100395, also called circRNA_0015278, is derived from chr1:173726114-173744981. Notably, circRNA_100395 has been reported to play an important role in the progression of various cancer types (11-13). Li *et al* (11) demonstrated that circRNA_100395 could regulate the cellular phenotype and the malignant capacity in ovarian cancer via regulating the miR-1228/p53 axis. Another study showed that the upregulation of circRNA_100395 significantly suppressed liver cancer cell proliferation (12). However, the biological role of circRNA_100395 in GC remains unclear. Hence, the present study aimed to explore the regulatory role of circRNA_100395 in GC progression and further elucidate its possible mechanism in GC, which may provide evidence for a promising novel diagnostic and therapeutic biomarker for GC.

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Key words: circRNA_100395, miR-142-3p, gastric cancer, cell proliferation, invasion, migration

Materials and methods

Tissue samples. A total of 54 paired GC and normal tissue samples were obtained from patients undergoing radical gastrectomy from January 2013 to January 2015 at Hospital Affiliated 5 to Nantong University, Taizhou People's Hospital (Taizhou, China). The inclusion criteria were: i) Age 18-80 years; ii) provision of written informed consent; iii) and pathologically confirmed GC as assessed by veteran independent pathologists that were independent to the present study. The exclusion criteria of the patients were: i) Other malignant diseases; and ii) patients who had received previous neoadjuvant chemotherapy or radiotherapy. The clinicopathological features of the patients enrolled in the study were assessed and recorded. The study was approved by The Ethical Committees of Taizhou People's Hospital (Taizhou, China) (approval no. KY2020-06511). All the patients provided written informed consent before enrollment.

Cell culture. The 293T cells, normal epithelial cell lines (GES-1) and human gastric cancer cell lines (HGC-27 MKN45, NCI-N87, SNU-5 and AGS) were obtained from the American Type Culture Collection. All the cells were incubated in RPMI-1640 media and supplemented with 10% FBS (both Gibco; Thermo Fisher Scientific, Inc.) and incubated at 37°C in a 5% CO₂ incubator.

RNA transfection. The pcDNA3.1-circRNA_100395 and pcDNA3.1 vector (NC control) were brought from Invitrogen company (Thermo Fisher Scientific, Inc.). HGC-27 and AGS cells were seed in a 12-well plate and incubated overnight at 37°C with 5% CO₂. At 80% confluency, these cells were transfected with 1 µg of pcDNA3.1-circRNA_100395 or pcDNA3.1 vector respectively using Lentiviruses (multiplicity of infection of 30) following the manufacturer's protocol. Forty-eight hours later, these cells were collected for subsequent experimentation.

Besides, the AGS cells were further transfected with 100 pmol miR-142-3p mimics or NC at 37°C with 5% CO₂ for 48 h using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). Transfected cells were collected 48 h after incubation for subsequent experiments.

Reverse transcription-quantitative (RT-q)PCR. According to the manufacturer's protocols, TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) was used to extract total RNA. Subsequently, total RNA (1 µg) was transcribed to cDNA via using PrimeScript RT Master Mix (Takara Biotechnology Co., Ltd.) according to the manufacturer's protocol. The temperature and duration of RT were: 37°C for 30 sec, followed by 85°C for 5 sec and 4°C for 10 min. The cDNA (10 ng) was subjected to qPCR (Bio-Rad Laboratories, Inc.) using SYBR Premix Ex Taq II kit (Takara Biotechnology Co., Ltd.), and the reaction volume was 10 µl. The thermocycling conditions used were as follows: 95°C for 30 sec, followed by 40 cycles of 95°C for 5 sec and 60°C for 40 sec. Analysis of circRNA_100395 and miR-142-3p expression was performed using the 2^{-ΔΔCq} method (14). The primers sequences are shown in Table I.

In vitro cell proliferation assay. For Cell Counting Kit (CCK)-8 assay, the cells were collected and incubated with CCK-8 kit (Dojindo Molecular Technologies, Inc.) for 2 h, and

the absorbance (OD value) at 450 nm was then determined to assess cell viability. For EdU assay, the cells were stained using the EdU cell proliferation kit (Guangzhou RiboBio Co., Ltd.) according to the manufacturer's instructions, and results were captured using a fluorescence microscope.

Apoptosis. The Annexin V-FITC/PI apoptosis detection kit (Nanjing KeyGen Biotech Co., Ltd.) was used to assess apoptosis rate through BD FACSCelesta™ (BD Biosciences), and the total apoptosis (early + late) rate were analyzed using FlowJo software v.10 (Flow Jo, LLC).

Transwell assay. In brief, for cell migration assay, RPMI-1640 media supplemented with 20% FBS (Gibco; Thermo Fisher Scientific, Inc.) was added into the lower chamber, while 40,000 cells were suspended in RPMI-1640 media without FBS and added into the upper chamber. After 48 h, the cells trapped on the Transwell chamber membrane were fixed with 4% methanol for 10 min, stained with crystal violet for 10 min at room temperature, and finally captured with a light microscope. However, for cell invasion assay, the filter membranes were precoated with Matrigel (Corning, Inc.) at 37°C for 30 min, and other steps were performed as described in the migration assay.

Prediction of downstream molecules regulated by circRNA-100395. A publicly available bioinformatic database Starbase v.2.0 (15) was utilized to predict the downstream microRNAs of circRNA-100395.

Dual-luciferase reporter assays. The test (293T) cells were co-transfected with either the pmirGLO-circRNA_100395-wild-type or pmirGLO-circRNA_100395-mutant plasmid along with either miR-142-3p mimics or miR-142-3p NCs (all Shanghai GenePharma Co., Ltd.) using Lipofectamine® 3000 Transfection reagent (Invitrogen; Thermo Fisher Scientific, Inc.). A dual-luciferase reporter system (Promega Corporation) was used 48 h later to measure the luciferase gene activity. Firefly luciferase activity was normalized to that of *Renilla* luciferase. The related sequences are shown in Table I.

Western blotting. The transfected HGC-27 and AGS cells were collected and lysed with RIPA buffer (Beyotime Institute of Biotechnology). Subsequently, 40 µg/lane of proteins were loaded and resolved using 10% SDS-PAGE and transferred onto polyvinylidene fluoride membranes. Then, the membranes were blocked with 10% BSA (Beijing Solarbio Science & Technology Co., Ltd.) for 1 h at room temperature. Subsequently, the membranes were incubated with primary antibodies: PTEN (cat. no. ab267787), PI3K (cat. no. ab154598), AKT (cat. no. ab8805), p-AKT (cat. no. ab250676) and GAPDH (cat. no. ab9485; all 1:1,000 dilution; Abcam) overnight at 4°C. Subsequently, proteins were incubated with the goat anti-rabbit IgG H&L (HRP; 1:2,000 dilution; cat. no. ab6721; Abcam) for 1 h at room temperature. The membranes were washed with 0.05% Tween-20 (TBS/Tween) 3 times. Finally, the protein bands were then analyzed with super sensitive ECL luminescence reagent (Dalian Meilun Biology Technology Co., Ltd.). The proteins expression levels were detected using a chemi-luminescence detection system with Quantity One software v.3.0 (Sigma-Aldrich; Merck KGaA).

Table I. Sequences of oligomers and primers used in the present research.

Primer	Sequence, 5'-3'
circRNA_100395	
forward	AGTGATGTGGCCCCTACAAG
circRNA_100395	
reverse	CCACTGGAGACCACTGGTTG
miR-142-3p forward	AGCGTGTAGTGTTCCTACTT
miR-142-3p reverse	GTTGTGGTTGGTTGGTTTGT
GAPDH forward	CCTCCGTGTCCCCACT
GAPDH reverse	GCCTGCTTACCACCTTC
U6 forward	CTCGCTTCGGCAGCACA
U6 reverse	AACGCTTCACGAATTGCGT
miR-142-3p mimic	UGUAGUGUUUCCUACUUUAUGGA
NC mimics	UGUACGUACGUUCGUACCGUGA

miR, microRNA; NC, negative control.

Statistical analysis. Results are shown as mean \pm standard deviation. All statistical analyses were performed using SPSS 17.0 (SPSS Inc.) and GraphPad Prism 7.0 (GraphPad Software Inc.). Comparisons between two groups were performed using unpaired Student's t-tests and χ^2 tests. The data among data among three or more groups were analyzed using one-way ANOVA followed by Fisher's least significant difference post hoc test or Tukey's multiple comparisons test. The Kaplan-Meier curve was generated and the log-rank tests were performed using GraphPad Prism software. All experiments were repeated at least three times. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

circRNA_100395 is downregulated in GC tissues. As shown in Fig. 1A, circRNA_100395 expression was downregulated in GC tissues compared with paired normal samples. The GC samples with low expression of circRNA_100395 accounted for 74.1% (40/54) (Fig. 1B). Statistical analysis revealed that circRNA_100395 expression was significantly associated with differential status ($P < 0.001$), vascular invasion ($P = 0.001$), tumor size ($P = 0.001$), N stage ($P = 0.003$) and TNM stage ($P = 0.031$) (Table II). Similarly, the patients with GC with low expression of circRNA_100395 showed poor tumor differentiation ($P < 0.0011$, Fig. 1C), advanced TMN stage ($P = 0.0001$; Fig. 1D) and lymph node metastasis ($P = 0.0015$, Fig. 1E), suggesting that circRNA_100395 may play a suppressor role in the development of GC. Moreover, the Kaplan-Meier curve showed that patients with low expression of circRNA_100395 had shorter survival times compared with patients with high expression of circRNA_100395 (Fig. 1F). Therefore, circRNA_100395 might be considered as a tumor suppressor in GC progression.

Upregulation of circRNA_100395 inhibits GC cell proliferation. As shown in Fig. 2A, circRNA_100395 was downregulated in GC cell lines compared with normal epithelial cells (GES-1). HGC-27 and AGS cells showed the lowest expression and so were selected for further experiments. The results of RT-qPCR

Table II. Association between the clinicopathological data and circ_100395 expression in gastric cancer (n=54).

Characteristics	Value, n	circ_100395 expression		P-value
		High	Low	
Sex				
Male	29	14	15	0.785
Female	25	13	12	
Age, years				
<60	21	10	11	0.876
≥ 60	33	15	18	
Differential status				
Moderate/well	19	13	6	<0.001
Undifferentiated/poorly	35	7	28	
Vascular invasion				
Negative	22	14	8	0.001
Positive	32	6	26	
Tumor size, cm				
≤ 5	25	16	9	0.001
> 5	29	6	23	
N stage				
N0	24	17	7	0.003
N1-N3	30	9	21	
TNM stage				
I-II	23	15	8	0.031
III-IV	31	11	20	

TNM, Tumor-Node-Metastasis; circ, circular.

revealed that after transfection with pcDNA3.1-circRNA_100395 plasmids, the circRNA_100395 expression in HGC-27 and AGS cells were significantly increased compared with the control group, suggesting the successful construction of the circRNA_100395-overexpressed GC cell models (Fig. 2B and C). Besides, the CCK-8 assay revealed that the upregulation of circRNA_100395 significantly suppressed HGC-27 and AGS cell proliferation (both $P < 0.001$; Fig. 2D and E). Similarly, the EdU incorporation assay also demonstrated that there were fewer EdU-positive HGC-27 and AGS cells in the OE-circRNA_100395 group compared with the OE-vector group (Fig. 2F and G). Therefore, circRNA_100395-overexpression could significantly suppress cell proliferation in GC.

Upregulation of circRNA_100395 promotes apoptosis and suppresses cell migration and invasion. Herein, overexpression of circRNA_100395 was found to significantly inhibit the GC cell proliferation rate. However, it was not clear whether apoptosis contributed to the proliferation inhibition caused by circRNA_100395-overexpression. Therefore, flow cytometry experiments were performed to evaluate the apoptosis rate induced by circRNA_100395-overexpression. The findings showed that the total apoptosis rate of HGC-27 cells in the circRNA_100395-overexpression group was higher compared with the OE-vector group ($P < 0.001$; Fig. 3A). Similarly, upregulating circRNA_100395 expression could induce

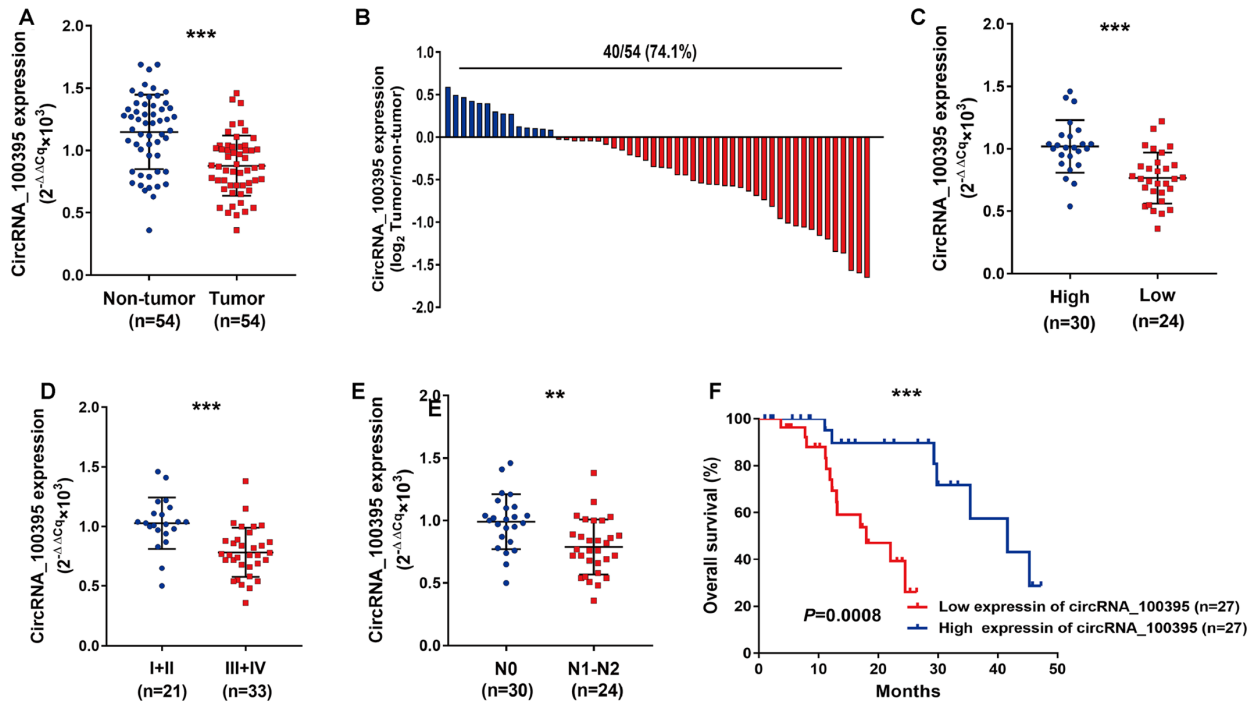


Figure 1. Expression pattern of circRNA_100395 in GC. (A) Expression of circRNA_100395 in GC tissues. (B) circRNA_100395 was downregulated (74.1%, 40/54) in GC. Relationship between circRNA_100395 expression and (C) tumor differentiation, (D) TNM stage and (E) lymph node metastasis. (F) Kaplan-Meier curves of overall survival of patients with GC. ** $P < 0.01$ and *** $P < 0.001$. circRNA, circular RNA; GC, gastric cancer.

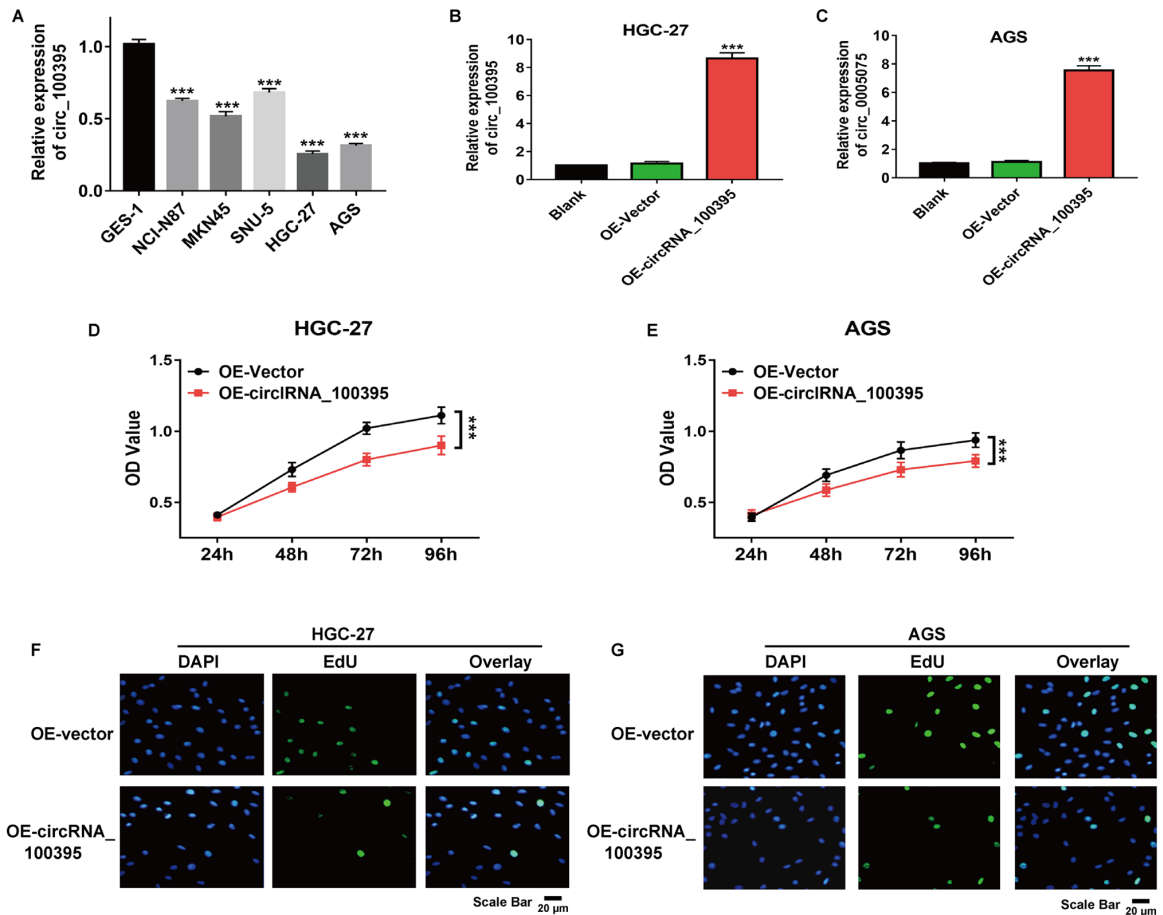


Figure 2. Upregulation of circRNA_100395 inhibits GC cell proliferation. (A) Expression of circRNA_100395 in GC cells. Successful generation of circRNA_100395-overexpression in (B) HGC-27 and (C) AGS cells. Cell proliferation of (D) HGC-27 and (E) AGS with circRNA_100395-overexpression determined via a Cell Counting Kit-8 assay. EdU assay of (F) HGC-27 and (G) AGS cells after upregulating circRNA_100395 expressions. *** $P < 0.001$ vs. GES-1 cells or OE-Vector. circRNA, circular RNA; GC, gastric cancer; OE, overexpression.

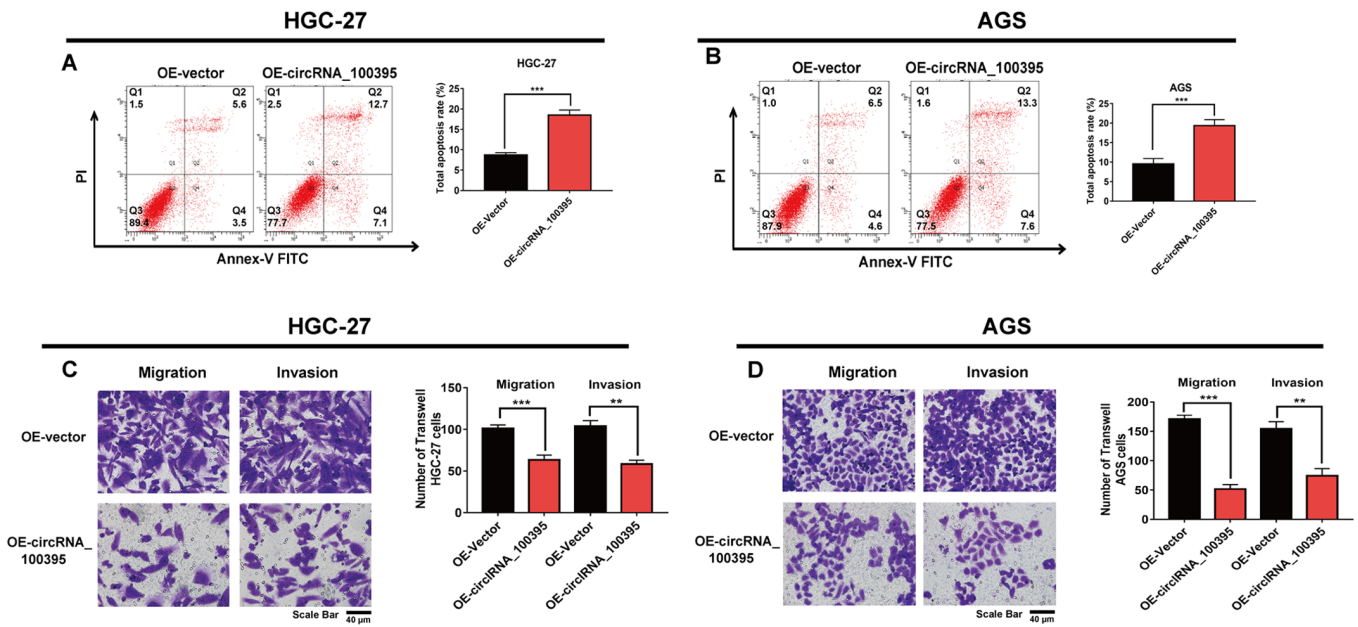


Figure 3. Upregulation of circRNA_100395 increases apoptosis rate and inhibits cell invasion and migration in gastric cancer. Apoptosis flow cytometry analysis of (A) HGC-27 and (B) AGS cells following circRNA_100395 upregulation. (C) Cell migration and (D) invasion images of HGC-27 and AGS cells. ** $P < 0.01$ and *** $P < 0.001$ vs. respective control. circRNA, circular RNA; OE, overexpression.

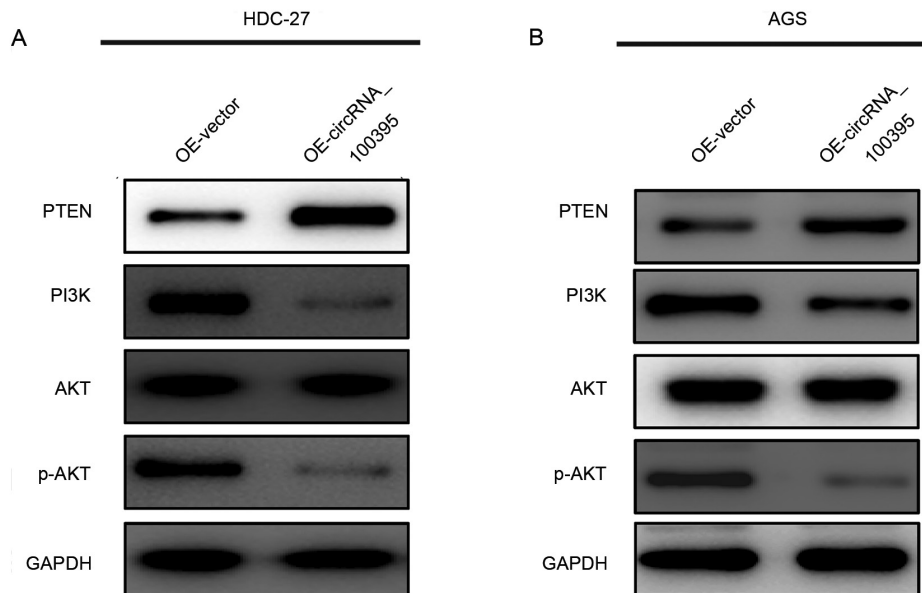


Figure 4. Upregulation of circRNA_100395 inhibits the PTEN-PI3K/AKT signaling pathway. Western blot analysis of PTEN, PI3K, total AKT and p-AKT expression in (A) HGC-27 and (B) AGS cells when circRNA_100395 was overexpressed. circRNA, circular RNA; OE, overexpression; p-, phosphorylated.

more apoptosis in AGS cells compared with in the OE-vector group (Fig. 3B).

Statistical analysis revealed that circRNA_100395 expression in GC was associated with lymph node metastasis. Therefore, a Transwell assay was performed to confirm whether circRNA_100395 participated in regulating cell migration and invasion in GC. Fig. 3C shows that cell migration and invasion decreased after upregulating circRNA_100395 expression in HGC-27 cells compared with the OE-vector group. Similarly, Fig. 3D also demonstrated that fewer migratory and invasive AGS cells were observed after circRNA_100395-overexpression. Taken together, the upregulation of circRNA_100395

could inhibit cell proliferation, increase the apoptosis rate and suppress GC cell invasion and migration.

Antitumor effects of circRNA_100395 by regulating the PTEN-PI3K/AKT signaling pathway. The PI3K/AKT signaling pathway serves an important role in regulating cell proliferation, migration and invasion (16-18). Therefore, in the present study, the western blotting assay was performed to determine whether circRNA_100395 function as a tumor inhibitor via regulating this signaling pathway. As shown in Fig. 4A and B, the upregulation of circRNA_100395 could lead to increased expression of PTEN protein, accompanied with the decreased

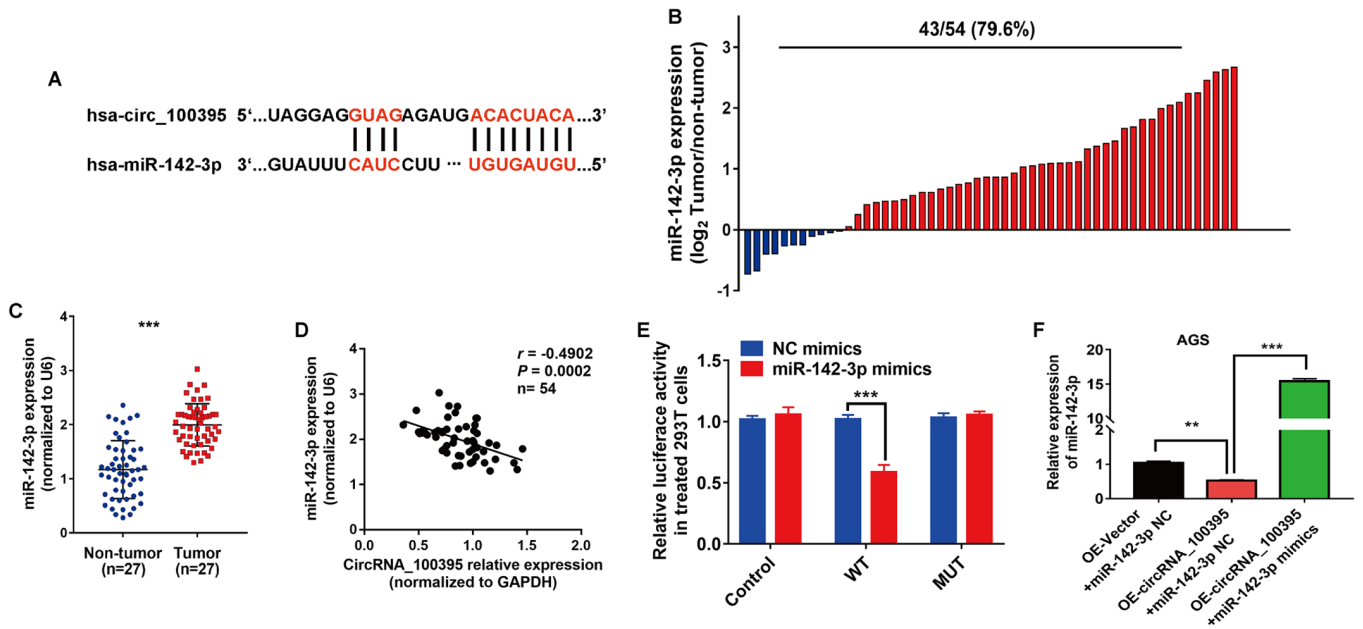


Figure 5. miR-142-3p is a downstream regulatory gene of circRNA_100395. (A) Predicted 3' untranslated region binding sites of circRNA_100395 on miR-142-3p. (B) miR-142-3p was significantly downregulated in patients with GC. (C) Expression of miR-142-3p in GC tissues. (D) Correlation between miR-142-3p and circRNA_100395 expression. (E) Relative luciferase activity of 293T cells after co-transfection. (F) Expression of miR-142-3p in circRNA_100395-overexpressed AGS cells after treatment with miR-142-3p inhibitors. ** $P < 0.01$ and *** $P < 0.001$ vs. respective control. circRNA, circular RNA; OE, overexpression; miR, microRNA; hsa, *Homo sapien*; NC, negative control.

expression of PI3K and phosphorylated (p-)AKT. Therefore, these results provided evidence that circRNA_100395 might promote apoptosis and suppress cell proliferation in GC by interfering with the PTEN-PI3K/AKT signaling pathway.

miR-142-3p is a downstream regulatory gene of circRNA_100395 in GC. The prediction results of the public database (Starbase v.2) showed that miR-142-3p might be the downstream target gene of circRNA_100395, and possible binding sites between miR-142-3p and circRNA_100395 are shown in Fig. 5A. Subsequently, RT-qPCR was used to detect miR-142-3p expression in GC tissues and cell lines. The results revealed that miR-142-3p overexpression was found in 79.6% (43/54) of the GC tissues (Fig. 5B). Statistical analysis showed that miR-142-3p was significantly upregulated in GC compared with non-tumor tissue samples ($P < 0.001$; Fig. 5C). Moreover, Pearson's correlation analysis showed that circRNA_100395 expression was negatively correlated with miR-142-3p expression in GC ($r = -0.4902$, $P = 0.0002$; Fig. 5D). Similarly, the results of dual-luciferase reporter assays revealed that the luciferase activity was significantly lower in wild-type cells transfected with miR-142-3p mimics compared with NC mimics ($P < 0.001$; Fig. 5E). RT-qPCR showed that the expression of miR-142-3p in OE-circRNA_100395-overexpressing AGS cells was significantly inhibited compared with the OE-Vector + miR142-3p NC group ($P < 0.01$), while the addition of miR-142-3p mimics increased miR-142-3p expression compared with the OE-circRNA_100395+miR-142-3p NC group ($P < 0.001$) (Fig. 5F). Therefore, miR-142-3p was found to be a downstream gene of circRNA_100395 in GC.

miR-142-3p rescues the antitumor effects induced by circRNA_100395 overexpression. The results of the RT-qPCR

assay revealed that the expression level of miR-142-3p in circRNA_100395-overexpressed cells was up-regulated with the transfection of miR-142-3p mimics. It was reported that the addition of miR-142-3p significantly restored the cell proliferation and apoptosis rate (both $P < 0.001$; Fig. 6A and B). Besides, the addition of miR-142-3p significantly increased the migratory and invasive AGS cell number with OE-circRNA_100395 treatment ($P < 0.001$; Fig. 6C). Moreover, the PTEN-PI3K/AKT signaling pathway-related markers in GC cells were evaluated after the upregulation of miR-142-3p expression. As shown in Fig. 6D, the addition of miR-142-3p reduced the expression of PTEN protein compared with the control group. However, the expression of PI3K and p-AKT were increased in circRNA_100395-overexpressed GC cells. Therefore, these results showed that downregulation of circRNA_100395 could promote apoptosis and suppress cell proliferation in GC by regulating the miR-142-3p/PI3K/AKT axis.

Discussion

GC is one of the deadly malignant tumors globally, causing an estimated 783,000 deaths in 2018 (2). Currently, the major therapeutic strategies for patients with GC include surgery, chemotherapy and molecular targeting therapy (19-21). Nevertheless, most patients with GC are diagnosed with advanced GC, hence their overall survival time is shorter (22). Therefore, it is important to identify effective diagnostic and therapeutic biomarkers for early diagnosis and treatment of GC and elucidate the possible regulatory mechanisms of pathogenesis and progression.

Numerous studies have demonstrated that circRNAs could serve as regulatory factors in GC progression (23-25). Over the

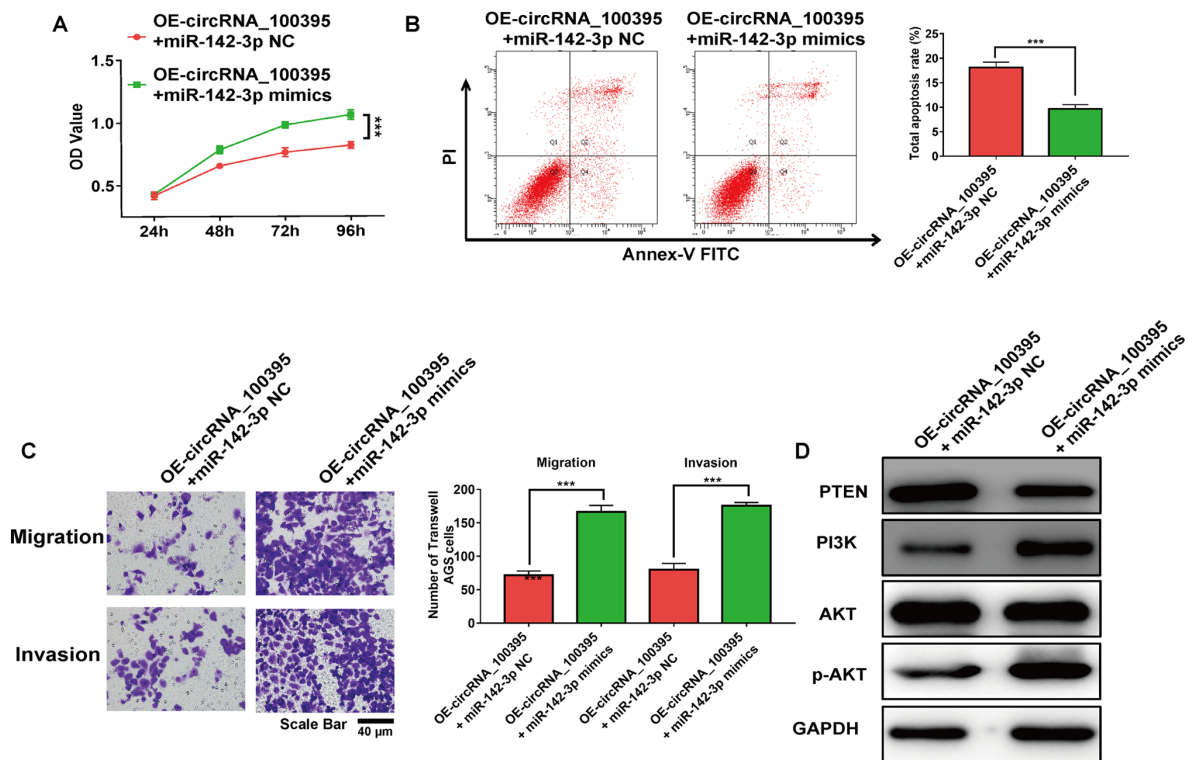


Figure 6. Upregulation of miR-142-3p rescues antitumor effects induced by overexpression of circRNA_100395. (A) Cell proliferation ability of circ_100395-overexpressed AGS cells. (B) Apoptosis rate of circ_100395-overexpressed AGS cells. (C) Cell migration and invasion of circ_100395-overexpressed AGS cells. (D) Expression of PI3K/AKT signaling pathway-related proteins in circ_100395-overexpressed AGS cells after the addition of miR-142-3p. *** $P < 0.001$ vs. respective control. circRNA, circular RNA; OE, overexpression; NC, negative control; p-, phosphorylated.

years, significant efforts have been made to elucidate the regulatory function of circRNA_100395 (11-13,26). Chen *et al* (13) demonstrated that overexpression of circRNA_100395 could sponge miR-1228 and regulate target transcription factor 21 expression, which inhibits the cell proliferation rate in lung cancer. Another study analyzed microarray data of 18 thyroid samples and identified circRNA_100395 as a promising biomarker for papillary thyroid carcinoma (26). However, it is still not clear whether circRNA_100395 is involved in GC progression. In the present study, RT-qPCR results showed that circRNA_100395 was upregulated in GC tissues. Statistical analysis showed that patients with GC with low expression of circRNA_100395 were more likely to have advanced TNM stage, advanced N stage, larger tumor size, poor differential status and shorter survival time. Therefore, circRNA_100395 might serve as a tumor inhibitor in GC.

To confirm the biological role of circRNA_100395 in GC development, a plasmid transfection assay was performed to construct circRNA_100395-overexpressed GC cells. CCK-8 and EdU assay results showed that circRNA_100395-overexpression significantly inhibited the proliferation ability of GC cells. Besides, the upregulation of circRNA_100395 induced apoptosis. Since the expression of circRNA_100395 was closely associated with advanced N stage, cell invasion and migration were analyzed using a Transwell assay. The results revealed that the cell invasion and migration with OE-circRNA_100395 was significantly inhibited compared with the control group. Therefore, circRNA_100395 was demonstrated to serve a significant role in regulating GC progression, including increasing the apoptosis rate, inhibiting

cell proliferation, migration and invasion. However, the underlying molecular mechanisms are not clear.

The PI3K/AKT signaling pathway has been identified to play an important role in the progression of various cancer types, including GC (27-29). Once the PI3K/AKT signaling pathway is activated, phosphorylation of AKT directly regulates the expression of a range of proteins involved in cell metabolism, proliferation, motility, invasion and migration. Studies have also revealed that circRNAs are significant regulatory factors in activating or silencing the PI3K/AKT signaling pathway (30,31). Peng *et al* (26) reported that circRNA_0010882 regulates the PI3K/AKT signaling pathway, and promotes cell proliferation in GC (32). Besides, circRNA_LARP4 has been identified as an miR-1323 sponge and tumor inhibitor in esophageal squamous cell carcinoma and regulates the PI3K/AKT signaling pathway (33). Consistently, the present results indicated that the upregulation of circRNA_100395 increased the expression of PTEN, and decreased the expression of PI3K and p-AKT. Taken together, in the present study, circRNA_100395 was proposed to exert antitumor effects by suppressing the PI3K/AKT signaling pathway.

miRNA sponging is a critical function of circRNAs in regulating cancer development (34). Li *et al* (29) revealed that the silencing of circRNA_ZNF609 suppressed cell proliferation by regulating miR-188 expression. Another study indicated that circRNA_CCDC66 regulated cisplatin resistance in GC via the miR-618/BCL2 axis (35). Based on the results of the present bioinformatics analysis, miR-142-3p was proposed as a downstream target gene of circRNA_100395.

Besides, miR-142-3p was found to be upregulated in GC tissues, and the expression of miR-142-3p was negatively correlated with circRNA_100395 expression. Moreover, the relationship between the circRNA_100395 and miR-142-3p was confirmed by dual-luciferase reporter assays. Numerous studies have identified that miR-142-3p plays a significant role in the progression of various cancer types (36,37). For example, Wang *et al* (33) provided evidence to show that miR-142-3p could act as a tumor suppressor in GC progression. In addition, miR-142-3p has been confirmed to promote prostate cancer progression by targeting the forkhead box protein O1 pathway (38). In the present study, the addition of miR-142-3p rescued the antitumor effects induced by overexpression of circRNA_100395. Therefore, these findings suggested that circRNA_100395 acts as a tumor suppressor by regulating the miR-142-3p/PI3K/AKT signaling pathway. However, the present study has some limitations. Firstly, the molecules acting downstream of the circRNA_100395/miR-142-3p axis need further confirm via bioinformatics analysis. Secondly, *in vivo* experiments are needed to further corroborate the present findings. That is, the untransfected and transfected cells were collected and planted in the nude mice, followed by recorded the tumor volume and weight during an observation period.

circRNA_100395 can serve as a tumor inhibitor in GC, and its low expression is associated with poor prognosis in patients with GC. Besides, the upregulation of circRNA_100395 significantly inhibits cell proliferation by regulating the miR-142-3p/PI3K/AKT axis. Overall, circRNA_100395 may be a potential therapeutic biomarker for GC.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ZC and GL designed the present study. ZC, GL, CH and XZ performed all the experiments, analyzed the data and prepared the figures. ZC, GL and CH drafted the initial manuscript. ZC and XZ reviewed and revised the manuscript. ZC, GL and CH confirmed the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Written informed consent was sought from the participants before the samples were obtained. Authority to carry out the present study was sought from the Ethical Committees of Taizhou People's Hospital (Taizhou, China) (approval no. KY2020-06511).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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