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ARTICLE

Upregulation of miR-29a-3p suppresses cisplatin resistance in gastric cancer via targeting ATG7 to restrain autophagy and induces apoptosis

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Abstract

Background Drug resistance and relapse are the main reasons for malignant gastric cancer. miR-29a-3p is a tumor suppressor in gastric cancer. However, the antitumor function and regulatory mechanism of miR-29a-3p in gastric cancer remain largely unknown. Thus, the aims of this study are investigation of the role of lncRNA DANCR and the molecular mechanism of miR-29a-3p in gastric cancer.

Methods The expression of miR-29a-3p and autophagy-related gene 7 (ATG7) was detected by qRT-PCR. Survival curve was performed by Kaplan-Meier methods. The cell proliferation and apoptosis were measured by Cell counting kit-8 (CCK-8) assay and Annexin-V FITC/PI staining. And the autophagy-related proteins and apoptosis-related proteins were analyzed by western blotting. Besides, the relationship between miR-29a-3p and ATG7 were determined by dual-luciferase reporter system. The xenograft tumor mice were established to detect the effects of miR-29a-3p *in vivo*. Immunohistochemistry was used to detect the LC3II level, and the TUNEL staining was used to calculate apoptotic cells.

Results We found that low expression of miR-29a-3p, and low expression of ATG7 in gastric cancer tissues and cell lines. And low expression of miR-29a-3p positively correlated with poor survival of gastric cancer patients. Moreover, upregulation of miR-29a-3p inhibited cell proliferation, autophagy, and induced cell apoptosis and sensitive to cisplatin. And we predicated and proved that miR-29a-3p inhibited cell proliferation, autophagy, and induced apoptosis and cisplatin-sensitivity by targeting ATG7.

Conclusion Our study demonstrated the anti-tumor role of miR-29a-3p in gastric cancer, and indicated the regulatory mechanism of miR-

29a-3p on autophagy of gastric cancer.

Introduction

Gastric cancer is the fifth diagnosed malignant tumor and third reason cause of the cancer-related deaths, which leads 784,000 global deaths in 2018 [1]. The risk factors of gastric cancer include Helicobacter pylori infection, age, high salt diet, and lack of fruit and vegetables consumption [2]. In China, gastric cancer is the second reason leads cancer-related death, about 679,000 new cases have been diagnosed each year and more than 80% patients diagnosed at an advanced stage [3]. Gastric cancer and its histological are confirmed using CT, endoscopic ultrasound, PET, and laparoscopy [4]. The patients have been treated with differential approaches according to the histological stages, such as early stage of gastric cancer patients mostly treat with endoscopic resection, advanced stage of gastric cancer patients commonly treat with surgery or adjuvant chemotherapy [5]. Cisplatin is the first-line chemotherapeutic drug for advanced gastric cancer patients, however, drug resistance acquisition is the major cause for high death rate for gastric cancer patients [6]. Despite cisplatin resistance has been widely found in gastric cancer, the molecular mechanism of cisplatin resistance remains unclear.

Autophagy is a cellular response to stress and maintains the intracellular homeostasis via regulating a series of autophagy-related genes (ATGs) expression, and modulating the protein and organelles degeneration [7]. Autophagy is a conserved catabolic process includes autophagosomes formation and autophagolysosomes formation, which initiation from the double-membrane cytosolic vesicles wrap the cytoplasm, damaged organelles, abnormal proteins, and other components, then the outer membranes fuse with lysosomal membranes to form autophagolysosomes, and the intracellular cargoes are degraded to support cellular metabolism [8, 9]. Autophagy acts as a major role in malignant procession of multiple tumors [10, 11]. Autophagy exhibits dual regulatory effects on tumorigenesis [12, 13]. In some cases, induction of autophagy accelerates neoplastic cell death to inhibit tumorigenesis [13, 14], oppositely, promotion of autophagy facilitates neoplastic cell survival and proliferation [15, 16]. In other cases, induction of autophagy promotes neoplastic cells resistance to the stimulation of hypoxia [17], metabolites [18] and therapeutic drugs [19, 20]. Thus, exploring the function and regulatory mechanism of autophagy are significant in study of HCC pathogenesis. Previous study has been demonstrated that inhibiting autophagy is vital for overcoming chemoresistance and radioresistance in glioblastoma [21]. In addition, it also reports that fusobacterium nucleatum enhances chemoresistance to colorectal cancer by inducing autophagy [22]. In gastric cancer, it also has been found suppressing autophagy inhibits chemo-resistance [23]. However, the regulatory mechanism of autophagy in gastric cancer remains unclear.

MicroRNAs (miRNAs) are a class of noncoding RNAs with 20-22 nt in length, it binds with 3'-untranslated regions (3'-UTR) of target gene mRNA to inhibiting its expression. The regulatory manner of miRNAs. Accumulation evidences indicate that miRNAs play crucial roles in pathology and progression of gastric cancer. Multiple miRNAs have been identified involving in tumor growth, progression, and prognosis [24-26]. Of interest, miRNAs act important roles in chemo-resistance by modulating autophagy [27]. miR-29a-3p has been found acts as a tumor suppressor in gastric cancer [28, 29]. However, the role and regulatory mechanism of miR-29a-3p in modulating chemoresistance in gastric cancer entirely unknown.

Therefore, we explored the functions of miR-29a-3p on autophagy and chemoresistance in gastric cancer. Furthermore, the potential target gene of miR-29a-3p was identified, and the underly mechanism of miR-29a-3p regulated autophagy and chemoresistance through modulating downstream gene ATG7 was investigated *in vivo* and i*n vitro.*

Material and methods

2.1 Specimens

A total sixty paired cancer tissues and paracancerous tissues were harvested from April 2012 and June 2015 in our hospital. All patients had accepted DDP therapy prior to surgery, and the patients who with failure with tumor controlling were confirmed as DDP-resistance patients. Samples underwent surgical resection and frozen in liquid then stored at -80℃ for subsequent experiments. All participants were informed and signed the informed consent, and this study was approved by the Ethics Committee of our hospital.

2.2 Cell culture and transfection

Human gastric cancer cell lines BGC823 and SGC7901, human normal gastric mucosal epithelial cell line GES-1 and 293T cells were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA) and maintained in RPMI-1640 medium supplemented with 10% FBS (Hyclone, South Logan, UT, USA) and 1% penicillin/streptomycin solution (Invitrogen, Carlsbad, CA, USA) at 37℃ in a 5% CO_2 humidified incubator. In addition, the cisplatin resistant GC cell lines BGC823/DDP and SGC7901/DDP were established according to the previous described [30]. Briefly, BGC823 and SGC7901 cells were persistently stimulated with incrementally concentration of cisplatin from 0 μM to 2 μM. Then, BGC823/DDP and SGC7901/DDP cells were cultured in a complete medium supplementary with 0.1 μM cisplatin.

Overexpression and silence of miR-29a-3p was archived by transfection with miRNA mimic and inhibitor, respectively. And overexpression of ATG7 was performed using pcDNA3.1 plasmid. All oligonucleotides were designed and synthesized from Ribobio (Guangzhou, China). Cells were transfected with oligonucleotides or plasmids using Lipofectamine 2000 reagents (Invitrogen, Carlsbad, CA, USA) following the manual of manufacturer.

2.3 Cell count-kit 8 (CCK-8) assay

Cell viability was detected using a CCK-8 assay (Beyotime, Shanghai, China) according to the manufacturer's suggestion. Briefly, cells were seed in 96-well plates at density of 4 x 10^3 cells/well and transfected with oligonucleotides for 48 h, then BGC823 and BGC823/DDP and their parental cells were stimulated with increasing concentration of cisplatin (0, 1, 5, 10, 22 μM) for another 48 h. After that, 10 μL CCK-8 solution was added into each well and incubated at 37℃ for 2 h. After 2 h of treatment, the absorbance was detected a 450 nm using a microplate reader (Invitrogen, Carlsbad, CA, USA). And the IC50 values of DDP for BGC823 and BGC823/DDP were calculated.

2.4 RNA extraction and quantitative PCR

Trizol reagent (Life Technologies, Gaithersburg, CA, USA) was used to extract the total RNA from cells and tissues following the standard protocol. The high-capacity cDNA reverse transcription kit (mRNA) (Thermo Fisher Scientific, Waltham, MA, USA) was used to synthesize cDNA. And qPCR was performed using TaqMan Universal PCR Master Mix (Thermo Fisher Scientific,

Waltham, MA, USA) based on the opinion of manufacturer and carried on using a 7900HT Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA). U6 and β-actin were used to normalize the gene expression. And the relative expression was analyzed with the $2^{-\Delta\Delta Ct}$ method. The primers in this study as following,

2.5 Western blotting

Total proteins were isolated from cells and tissues using a RIPA reagent (Beyotime, Shanghai, China) following the standard manual. After measuring the concentration of protein using g Bradford protein concentration assay kit (Beyotime, Shanghai, China), proteins were separated by 10% SDS-PAGE gel and transferred into PVDF membranes, the membranes then blocked with 5% non-fat milk and incubated with primary antibodies including ATG7 (1:1000, ab133528, Abcam, Carlsbad, CA, USA), LC3II (1:3000, ab51520, Abcam, Carlsbad, CA, USA), Bcl-2 (1:1000, ab32124, Abcam, Carlsbad, CA, USA), Bax (1:2000, ab32503, Abcam, Carlsbad, CA, USA), caspase 3 (1:5000, ab32351, Abcam, Carlsbad, CA, USA), and GAPDH (1:1000, p30008, Abmart, Shanghai, China) at 4℃ overnight. After that, the membranes were probed with HRP-conjugated secondary antibody at 37℃ for 1 h. Finally, the bands were visualized using enhanced chemiluminescence (ECL) kit (Bio-Rad, Hercules, CA, USA) following the protocol of manufacturer.

2.6 Dual-luciferase activity reporter gene analysis

The sequences of 3' untranslated region (UTR) of ATG7 were amplified and inserted into pGL3 reporter plasmids (ATG7-WT), and "UGGUGCU" instead of "ACCACGA" in 3'-UTR of ATG7 inserted into pGL3 reporter plasmids (ATG7-MUT). Wild and mutant type pGL3 reporter plasmids were co-transfected with miR-29a-3p mimic or miR-29a-3p inhibitor and their negative control NC mimic and NC inhibitor into 293T cells for 48 h. Then, the luciferase activity was measured using a dual-luciferase reporter gene system (Promega, Madison, WI, USA).

2.7 Animal experiments

The animal experiments were used to investigate the function of miR-29a-3p *in vivo* and approved by the Ethic Committee of our hospital. a total ten four to six-week-old male mice were transplanted with 1 x 10^7 BGC823 cells and randomly divided into three groups, including miR-29a-3p antagomir + DDP (25 mg/kg) group (n=6), NC antagomir + DDP (25 mg/kg) group (n=6), and control group (n=6). After 24 h cell transplanted, mice were orally accepted control vehicle and 25 mg/kg DDP daily, and 100 nmol/kg miR-29a-3p antagomir and NC antagomir injected into mice via tail vein injection every three days for four weeks. After 28 days, the mice were sacrificed and tumors were separated for subsequent experiments.

2.8 Immunohistochemistry (IHC) assay

The expression of ATG7 was analyzed by IHC assay following previous work [31]. Briefly, after the paraffin sections were deparaffinized with xylene and hydrated with gradient concentration of ethanol. The sections were incubated with primary anti- ATG7 (1:500, ab133528, Abcam, Carlsbad, CA, USA) antibodies overnight at 4 °C. Then, the sections were incubated with HRP-conjugated secondary antibody at room temperature for 30 min. finally, each section was probed with diaminobenzidine for 2 min. The positive cells were observed and imaged under a fluorescence microscopy (Leica, Wetzlar, Germany).

2.9 TUNEL staining assay

Cell apoptosis was detected by Colorimetric TUNEL Apoptosis Assay Kit following the protocol of manufacturer. Briefly, the tissue slices were incubated with Biotin-dUTP solution at 37 °C for 60 min in the dark and then blocked with Reaction stop solution for 10 min. After that, sections were incubated with 50 μL Streptavidin-HRP solution at room temperature for 30 min. Then, sections were probed with 300 μL DAB solution at room temperature for 20 min. The apoptotic cells were observed and imaged under a fluorescence microscopy (Leica, Wetzlar, Germany).

2.10 Statistical analysis

Statistical analyses in this study were performed using GraphPad version 8.0 (GraphPad Software Inc., La Jolla, CA, USA). All values were presented as mean ± standard deviation (SD) from at least three independent experiments. The differences between two or multiple groups were performed using Student's t-test and one-way analysis of variance (ANOVA). The survival analysis was Kaplan-Meier methods and log-rank test. *P* value < 0.05 was considered as statistical significance.

Results

3.1 miR-29a-3p downregulated in gastric cancer and cell lines

Firstly, we examined the expression of miR-29a-3p in gastric cancer tissues and cell lines using the qPCR. The results indicated the expression of miR-29a-3p markedly lower in gastric cancer tissues compared to paracancerous tissues (Fig.1A). The sixty patients were separated into high miR-29a-3p expression group and low miR-29a-3p expression group according the median miR-29a-3p expression value. Then, the survival analysis exhibited that low miR-29a-3p expression associated with poor survival in gastric cancer patients (Fig.1B). Consistently, the lower miR-29a-3p expression was observed in gastric cancer cell lines compared to normal gastric mucosal epithelial cell (GES-1; Fig.1C). Of interest, miR-29a-3p lower expression in cisplatin-resistant cell lines (BGC823/DDP and SGC7901/DDP) compared to their parental cells (Fig.1C). Above results suggested miR-29a-3p acted as a tumor suppressor in gastric cancer and negatively associated with poor prognosis and chemoresistance.

3.2 Overexpression of miR-29a-3p inhibited cell viability and autophagy, but induces apoptosis and cisplatin-sensitivity

We next to detect the function of miR-29a-3p by gain-of-function experiments *in vitro*. Firstly, the CCK-8 assay revealed the inhibitory effects on cell viability of miR-29a-3p overexpression both in cisplatin-resistant and sensitive gastric cancer cells (Fig.2A-D). IC50 values of DDP for BGC823 and BGC823/DDP were decreased by miR-29a-3p upregulation (Fig.2E-F). We also found apoptosis was induced by miR-29a-3p upregulation both of BGC823 and BGC823/DDP cells (Fig.2G-H). Then, the pro-apoptotic markers Bax and caspase 3 expression was increased whereas the anti-apoptotic marker Bcl-2 expression was inhibited by miR-29a-3p upregulation both in cisplatin-resistant and sensitive gastric cancer cells (Figure 2I-J). In addition, the expression of autophagy marker LC3II had been analyzed, and the results showed significant inhibition effects of miR-29a-3p upregulation on LC3II expression compared with control both in BGC823 and BGC823/DDP cells (Fig.2K). These data indicated the negative function of miR-29a-3p on cell proliferation and chemoresistance, but exhibited the positive function on cell apoptosis.

3.3 ATG7 was a target of miR-29a-3p

Next, we further to excavate the mechanism of miR-29a-3p affected gastric cancer. The targets of miR-29a-3p were predicated using Starbase (http://starbase.sysu.edu.cn/index.php). The ATG7 is one of the candidates of miR-29a-3p and acts as an autophagy marker. Therefore, we focused on function of ATG7 in gastric cancer in this study. The binding sites of miR-29a-3p at the 3'UTR of ATG7 were illustrated in Fig.3A. A luciferase reporter gene assay was used to evaluate the relationship between miR-29a-3p and ATG7, which exhibited that co-transfection of miR-29a-3p mimic with ATG7-wt inhibited luciferase activity, but co-transfection of miR-95-3p inhibitor with ATG7-wt induced luciferase activity, and no changes co-transfection of miR-95-3p mimic or inhibitor with ATG7 -mut in 293T cells (Fig.3B). Additionally, the mRNA and protein levels of ATG7 were reduced by miR-29a-3p overexpression whereas increased by miR-29a-3p inhibition (Fig.3C-D). We also examined the expression of ATG7 in gastric cancer tissues and cell lines, and we found that ATG7 significantly increased in gastric cancer tissues compared with normal tissues as well as gastric cancer cell lines compared with GES-1 (Fig.3E-F). It also found increasing expression of ATG7 in cisplatin resistance cells than their parental cells in gastric cancer (Fig.3F). Our finding demonstrated that ATG7 is a direct target of miR-29a-3p.

3.4 miR-29a-3p contributed to cisplatin sensitivity by inhibiting ATG7 expression

Previous data clarified miR-29a-3p contributed to inhibition of cell proliferation and cisplatin resistance, and induce apoptosis in gastric cancer cells. Therefore, we employed the rescue experiments to reveal the opposite effects of ATG7 to miR-29a-3p. CCK-8 assay indicated that the cell viability was inhibited by miR-29a-3p upregulation, whereas the effects of miR-29a-3p upregulation were reversed by ATG7 upregulation both in cisplatin-resistant and sensitive cells (Fig.4A-B). IC50 values of DDP for BGC823 and BGC823/DDP were reduced by miR-29a-3p upregulation whereas increased by ATG7 upregulation (Figure 4C-D). Moreover, we found the promotion effects of miR-29a-3p upregulation on apoptosis were weaken by increasing of ATG7 in cisplatin-resistant and sensitive gastric cancer cells (Figure 4E). Furthermore, the inhibitory effects of miR-29a-3p upregulation on apoptosis related proteins were neutralized by ATG7 upregulation (Figure 4F). Besides, the expression of LC3 II was decreased by miR-29a-3p upregulation but increased by ATG7 overexpression (Fig.4G). Autophagic flux analysis indicated that the autophagic flux was inhibited by miR-29a-3p upregulation but increased by ATG7 overexpression (Fig.4H). These data revealed that miR-29a-3p contributed to inhibiting cell proliferation and cisplatin-resistance, and inducing apoptosis by directly binding to ATG7.

3.5 Overexpression of miR-29a-3p inhibited tumor growth and enhances cisplatin-sensitivity *in vivo*

We also investigate the antitumor effects of miR-29a-3p *in vivo*. The results indicated that tumor growth was repressed by cisplatin treatment, and the inhibitory effects of cisplatin on tumor growth were enhanced by miR-29a-3p upregulation (Fig.5A-D). QPCR results revealed that expression of miR-29a-3p was increased by cisplatin or miR-29a-3p activator (Fig.5E). Inversely, the expression of ATG7 was inhibited by cisplatin or miR-29a-3p activator (Fig.5E). IHC results revealed that LC3 II protein was inhibited by cisplatin, moreover, the inhibitory effects of cisplatin were enhanced by miR-29a-3p overexpression (Figure 5F-G). In addition, cell apoptosis was induced by cisplatin or miR-29a-3p activator (Figure 5F-G). Above results suggested that the

antitumor effects of miR-29a-3p, and miR-29a-3p enhanced cisplatin sensitivity *in vivo*.

Discussion

The roles of miRNAs in cancer have been researched for many years. It regulates multiple tumors by modulating a wide range of biological processes. MiRNAs not only regulate mRNA, protein, but also regulate epigenetic modification in cancers. miR-29a-3p is a typical tumor suppressor in multiple solid cancers. For example, miR-29a-3p/IGF1R axis inhibits hepatocellular carcinoma by suppressing cell proliferation and migration [32]. miR-29a-3p suppresses tumor growth, proliferation, and invasion by targeting OTUB2 in papillary thyroid carcinoma [33]. miR-29a-3p acts as tumor suppressor in colorectal cancer by suppressing CDC42BPA [34]. Besides, miR-29a-3p has been found inhibits laryngocarcinoma through targeting promonin1 [35].

Here, we found that the downregulation of miR-29a-3p whereas elevation of ATG7 in gastric cancer tissues and cell lines. Low expression of miR-29a-3p associated with poor survival rate. The mechanism analysis indicated that upregulation of miR-29a-3p inhibited cell proliferation, but induced cell apoptosis and insensitive to cisplatin *in vitro* and *in vivo*. Previous studies have indicated that miR-29a-3p acts as a tumor suppressor in gastric cancer by regulating cell proliferation, migration, invasion, and apoptosis via differential pathways. However, it never reports the antitumor effects via enhancing cisplatin sensitivity and regulating autophagy in gastric cancer. In the present study, we firstly found miR-29a-3p functions as a tumor suppressor in gastric cancer not only inhibited cell proliferation and induced apoptosis, but also enhanced cancer cell sensitive to cisplatin and suppresses autophagy.

Autophagy is an evolutionarily conservative process of catabolism that regulates cell survival and death by modulating autophagy-associated genes to response to stress including hypoxia, nutrient deficiency, and energy exhaust [31]. Previous studies have demonstrated that autophagy plays as a tumor promoting role in HCC, such as, lncRNA HCG11 promotes the progression of HCC by acceleration of autophagy [10], and induction of autophagy enhances the progression of HCC and HCC cells resistance to sorafenib [32]. ATGs act as the vital roles in autophagosome formation and autophagic cargo delivery to the lysosome [33]. In most cases, lncRNAs regulate tumorigenesis and progression by regulating the transcriptional and post-transcriptional of ATGs. For example, lncRNA MALAT1 induces autophagy in gastric cancer through upregulating ATG5 expression [34]. In addition, lncRNA KCNQ1OT1 facilitates the progression of non-small cell lung cancer via activating autophagy by increasing ATG3 [35]. And lncRNA HCG11 induces autophagy in HCC by accelerating ATG12 expression [10]. It also reported that lncRNA CCAT1 activates autophagy in HCC by upregulating ATG7 [36]. ATG7 is an E1 enzyme that activates ATG8 and ATG12, and deliveries ATG8 and ATG12 to cognate E2 enzyme ATG3 or ATG10, and ATG7 is a part of ATG7-ATG8-ATG3 complex, which regulates autophagosome biogenesis and recruits the cargos of autophagy [37]. Here, we demonstrated that miR-29a-3p inhibited gastric cancer and enhanced cisplatin sensitivity by reducing autophagy via targeting ATG7.

5 Conclusion

In conclusion, our study demonstrated the antitumor function and regulatory mechanism of miR-29a-3p in gastric cancer by inhibiting autophagy and enhancing chemosensitivity.

Our finding provided a novel viewpoint on the regulation effects of miR-29a-3p in gastric cancer, and miR-29a-3p might act as a potential therapeutic target.

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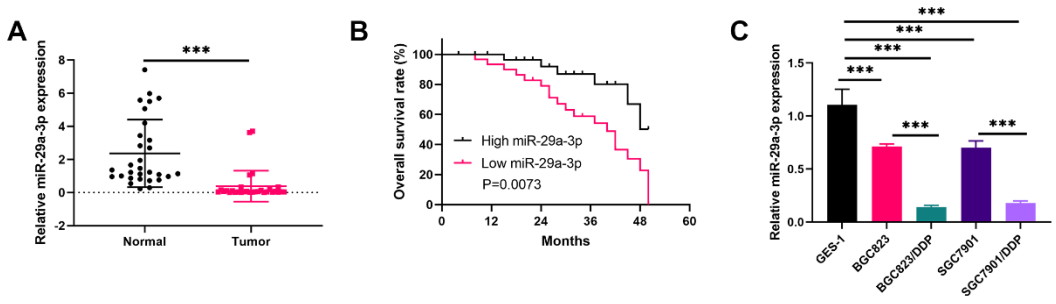

Figure 1 miR-29a-3p downregulated in gastric cancer and cell lines.

(A) QRT-PCR was used to detect the expression of miR-29a-3p in gastric cancer tissues compared with adjacent normal tissues.

(B) Overall survival curve exhibited survival time between high and low expression of miR-29a-3p for gastric cancer patients.

(C) QRT-PCR was used to detect the expression of miR-29a-3p in gastric cancer cell lines compared with gastric epithelial cell. ***P<0.001.

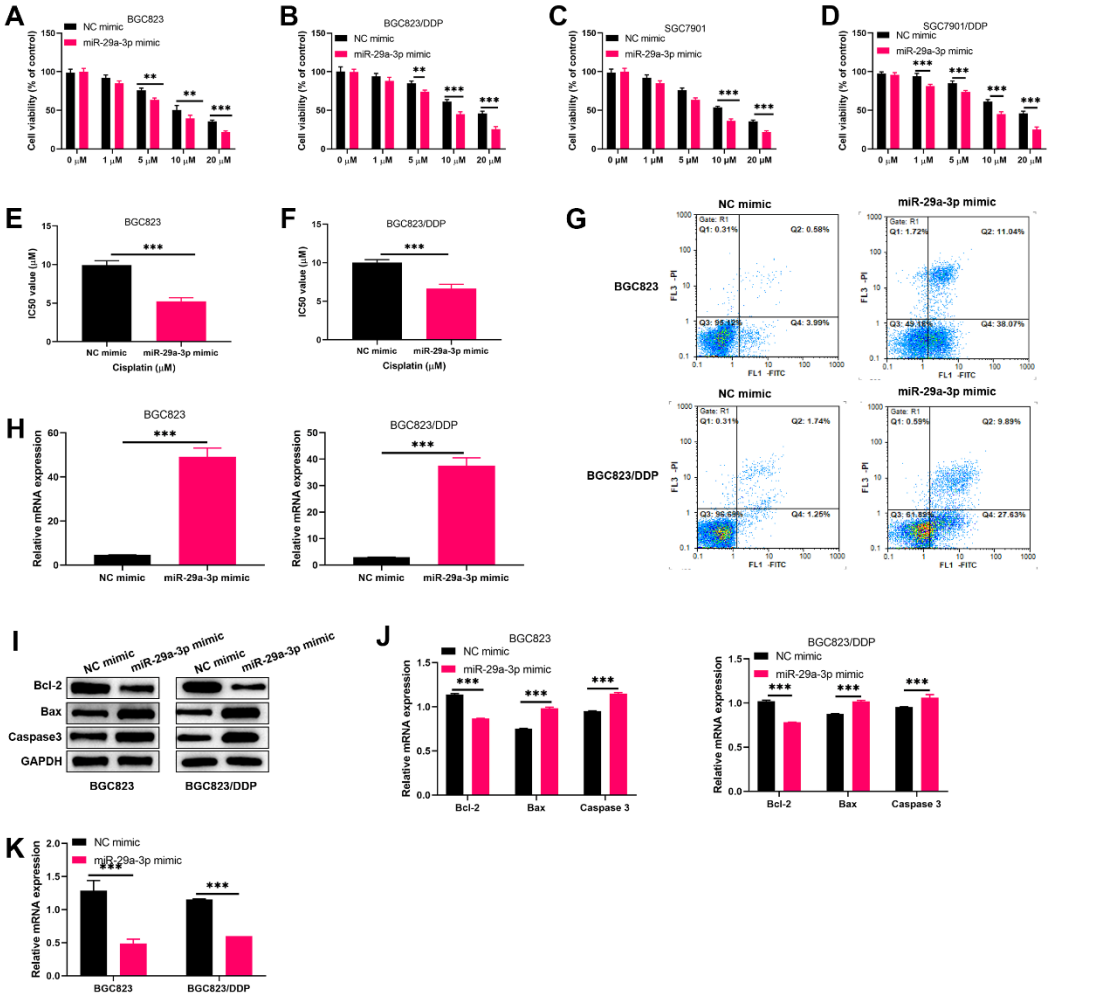

Figure 2 Overexpression of miR-29a-3p inhibited cell viability and autophagy, but induces apoptosis and cisplatin-sensitivity.

miR-29a-3p mimic and its negative control were transfected into BGC823/DDP and SGC7901/DDP and their parental cell lines, then, (A)-(D) CCK-8 assay was used to determine cell viability after stimulated with increasing does of cisplatin (0, 1, 5, 10, 20 μM). (E)-(F) IC50 values of BGC823/DDP and BGC823 cells were calculated according to the results of CCK-8 assay. (G) Annexin-V FITC/PI staining was used to detect the apoptotic cells. (I)-(K) QRT-PCR and western blot assays were used to measure the mRNA and proteins levels of Bcl-2, Bax, and caspase 3 both in BGC823/DDP and BGC823 cells. ***P*<0.01, ****P*<0.001.

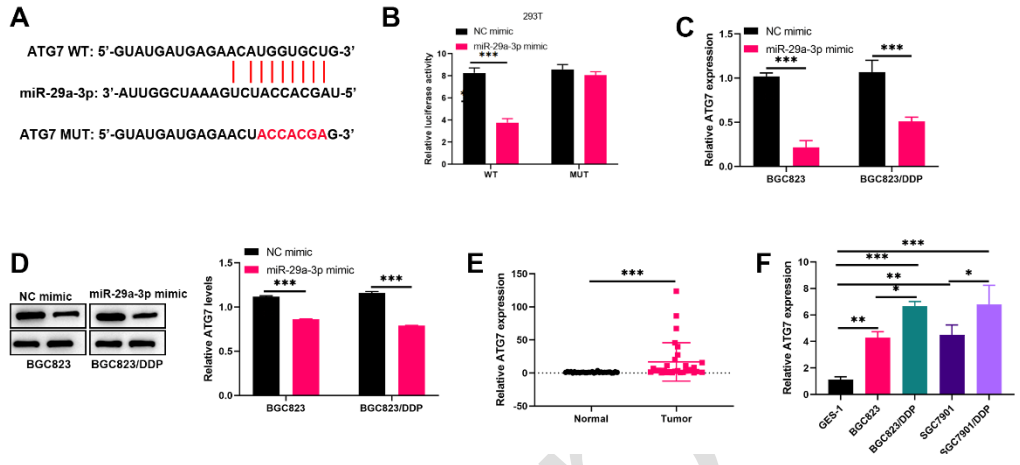

Figure 3 ATG7 was a target of miR-29a-3p.

(A) The binding sites of ATG7 at miR-29a-3p were predicated based on the Starbase online software.

(B) Dual-luciferase activity assay was used to detect the luciferase activity.

(C)-(D) QRT-PCR and western blot assays were used to measure the mRNA and proteins levels of ATG7.

(E) QRT-PCR was used to analyze the expression of ATG7 in gastric cancer tissues compared with adjacent normal tissues.

(F) QRT-PCR was used to determine the expression of ATG7 in gastric cancer cell lines compared with gastric epithelial cell. **P*<0.05, ***P*<0.01, ****P*<0.001.

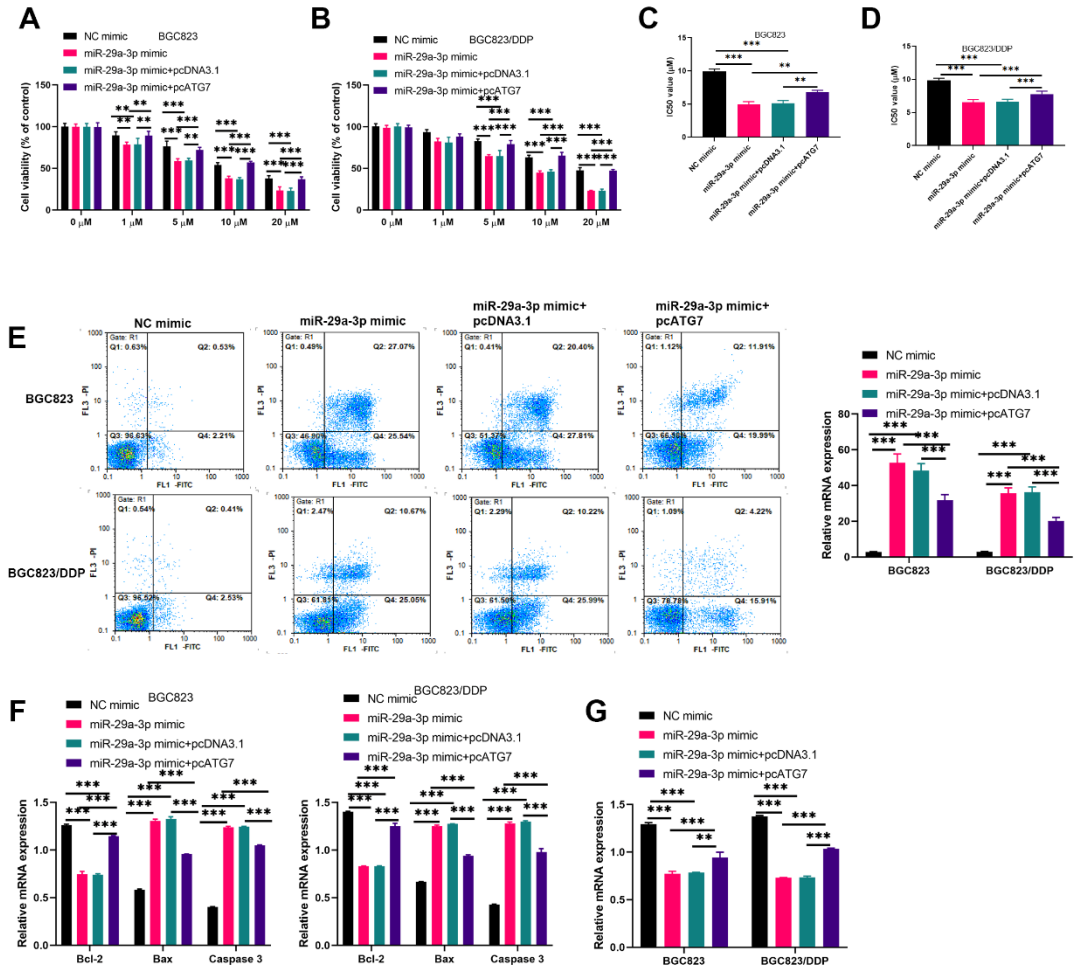

Figure 4 miR-29a-3p contributed to cisplatin sensitivity by inhibiting ATG7 expression.
After BGC823/DDP and BGC823 cells were transfected with miR-29a-3p mimic and its negative control, or transfected with ATG7 plasmid and its negative control, (A)-(B) CCK-8 assay was used to determine cell viability after stimulated with increasing does of cisplatin (0, 1, 5, 10, 20 μM). (C)-(D) IC50 values of BGC823/DDP and BGC823 cells were calculated according to the results of CCK-8 assay. (E) Annexin-V FITC/PI staining was used to detect the apoptotic cells. (F) QRT-PCR was used to measure the mRNA and proteins levels of Bcl-2, Bax, and caspase 3 both in BGC823/DDP and BGC823 cells. ***P*<0.01, ****P*<0.001.

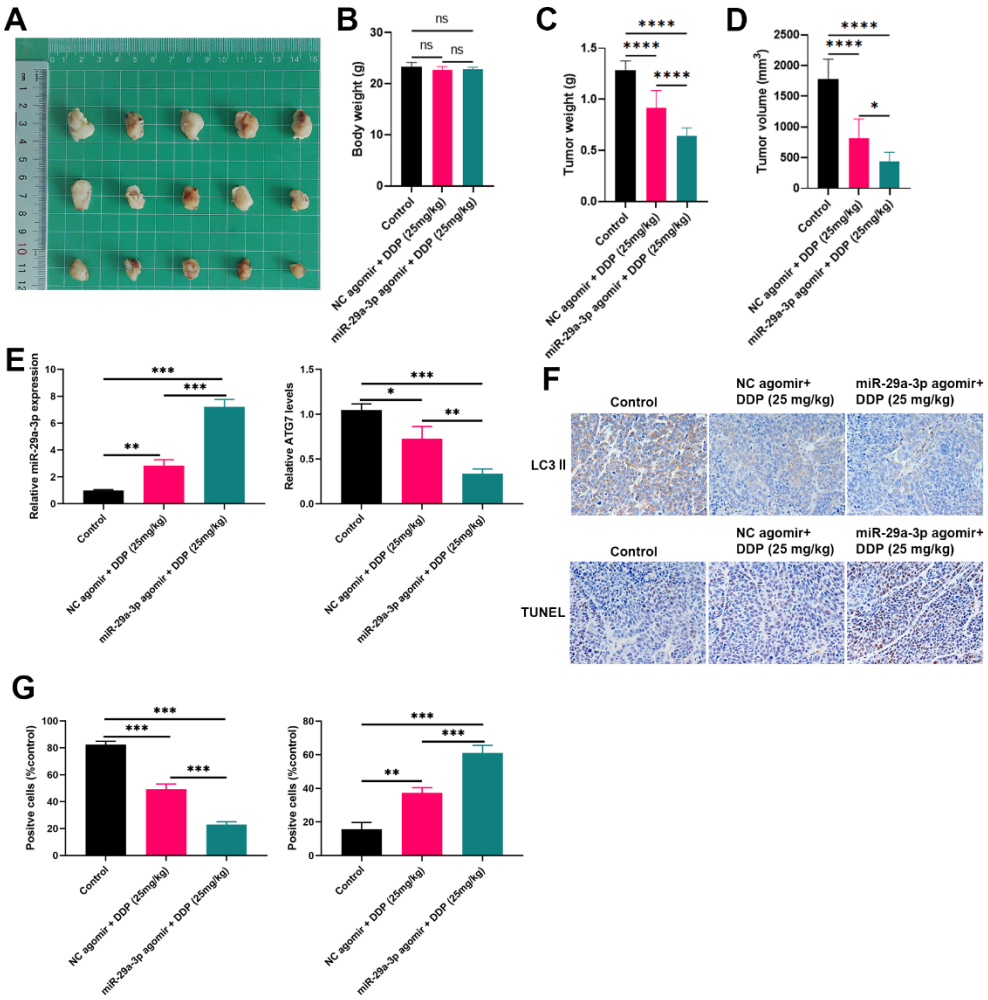

Figure 5 Overexpression of miR-29a-3p inhibited tumor growth and enhances cisplatin-sensitivity *in vivo*.

BGC823/DDP cells were stably transfected with miR-29a-3p angomir and its negative control, then transplanted into mice and accepted 25 mg/kg DDP treatment, then, (A)-(D) Tumors were separated from mice, the body weight, tumor weight, and tumor volume were calculated.

(E) QRT-PCR was used to measure the mRNA expression of miR-29a-3p and ATG7. (F)-(G) IHC and TUNEL staining were used to detect LC3II levels and cell apoptosis in tumor tissues. **P*<0.05, ***P*<0.01, ****P*<0.001.