miR-320a modulates cell growth and chemosensitivity via regulating ADAM10 in gastric cancer

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Abstract. MicroRNAs (miRNAs) may function as tumor suppressor or onco-miRNAs and have critical roles in the pathogenesis of gastric cancer (GC). The exact function and mechanism of miRNA (miR)-320a in GC remains to be elucidated. The present study performed gain- and loss-of-function analyses by transfecting cells with mimics or inhibitors and subsequently performing colony formation, proliferation and cisplatin-sensitivity assays. Additionally, *in vivo* xenograft models were also performed. Bioinformatics algorithms, luciferase reporter activity assay and western blotting were used to predict the potential target of miR-320a. Additionally, the effect of knockdown or overexpression of ADAM metallopeptidase domain 10 (*ADAM10*) on cell growth and chemosensitivity was examined. The expression of miR-320a and ADAM10 was also determined in primary tumors. The present study revealed that the expression of miR-320a was reduced in GC cells and ectopic miR-320a expression significantly inhibited cell growth *in vitro* and *in vivo* and enhanced the sensitivity of GC cells to cisplatin. ADAM10 was a direct target of miR-320a in GC. Knockdown of ADAM10 attenuated the proliferative ability of GC cells, and increased the sensitivity of GC cells to cisplatin. The upregulated ADAM10 accelerated cell growth rate and reduced the cisplatin-sensitivity of cells. Clinically, a significantly negative correlation was identified between the expression of miR-320a and mRNA levels of ADAM10 in tumors. The findings of the present study suggested that miR-320a may function as a tumor suppressor in GC progression and potential therapeutic strategies for GC may be based on the miR-320a/ADAM10 axis.

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Introduction

Gastric cancer (GC) is the second leading cause of cancer-associated mortality and the fourth highest incidence of human malignancy worldwide. It has been previously estimated that over 70% of novel cases and deaths occur in developing countries, including China (1). The 5-year survival rate of GC patients with successful surgery, with or without chemotherapy, is <40% (2).

MicroRNAs (miRNAs) are a group of endogenous, small, non-coding single-stranded RNAs (~18-25 nucleotides in length) that post-transcriptionally modulate target gene expression by binding to the 3'-untranslated region (UTR) of mRNAs (3). Previous studies have revealed that miRNA-regulated tumor initiation and progression occurs as they function as tumor suppressors or oncogenes (4-6). The deregulated miRNAs were closely associated with carcinogenesis and may also function as diagnostic or prognostic markers in human malignancies (7-9). Therefore, in order to explore the potential target and molecular mechanism responsible for the gastric carcinogenesis is critical for cancer treatment and prognosis prediction.

Previous studies have reported that miR-320a may significantly inhibit cell proliferation and invasion in colorectal cancer (CRC), nasopharyngeal carcinoma and breast cancer (10-14). Additionally, upregulated miR-320a was confirmed in paclitaxel-resistant formalin-fixed paraffin-embedded tissues from ovarian tumor samples (15). Restoration of miR-320a expression was suggested as a potential therapeutic approach for tamoxifen-resistant breast cancer (16). Previous studies determined that plasma miR-320a was significantly increased in osteosarcoma and hepatitis B-positive hepatocellular carcinoma patients (17,18), whereas its levels were reduced in patients with CRC (19). However, the function and clinical significance of miR-320a have been investigated in various types of cancer, the importance of miR-320a in the development of GC remains to be elucidated.

ADAM metallopeptidase domain 10 (ADAM10) belongs to the a disintegrin and metalloproteinase (ADAM) family,

which has been widely reported to be overexpressed in human malignancies and promote cancer development and metastasis (20,21). ADAM10 noticeably contributed to cell motility in pancreatic carcinoma and oral squamous cell carcinoma (22,23) and in pituitary adenoma and lung cancer via regulation of CD44 cleavage and Notch1 signaling (24,25). Knockdown of ADAM10 in hepatocellular carcinoma cells enhanced the level of doxorubicin-induced apoptosis (26). Several miRNAs, including miR-140-5p and miR-122-5p, mediated cell migration and the mRNA expression of ADAM10 by targeting its 3'-UTR in hypopharyngeal squamous cell carcinoma and breast cancer, respectively (27,28). Therefore, the present study examined the association of miR-320a and ADAM10 in gastric carcinogenesis.

The present study determined that miR-320a was downregulated in GC cells and tissues and overexpression of miR-320a inhibited tumor growth *in vitro* and *in vivo*. It is of note that miR-320a increased the sensitivity of GC cells to cisplatin. ADAM10 was a direct and functional target of miR-320a. ADAM10 was inversely associated with miR-320a in primary GC tissues. The findings of the present study suggested that miR-320a had an important role in regulating cancer cell growth and chemoresistance of GC through regulating ADAM10 expression.

Materials and methods

Cell lines and tissues. A total of 4 GC cell lines, including SGC-7901, AGS, BGC-823 and MGC-803 were obtained from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China), maintained in our laboratory and used in the present study. A normal gastric mucosal epithelial cell line GES-1 (Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China) was also used. All cells were cultured in RPMI-1640 (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Invitrogen; Thermo Fisher Scientific, Inc.), 100 μg/ml streptomycin and 100 U/ml penicillin, in a culture incubator under the atmosphere of 5% CO_2 and 37˚C.

Tissue samples of GC and adjacent normal tissues were obtained from 40 patients (male, n=24; female, n=16) who were diagnosed at the 401 Hospital of People's Liberation Army between January 2009 and December 2012. Participants were between 42 and 76 years of age and had no history of tumors. Written informed consent was obtained from patients and the protocols were approved by the ethics committee of the hospital. Non-cancerous tissues from the macroscopic tumor margin were isolated simultaneously and used as control. All tissues were snap frozen and stored in -80˚C until use.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). miRNAs were extracted from cells and tissues and purified using a miRNA isolation system (Omega Bio-Tek, Inc., Norcross, GA, USA) and processed with the miScript II RT kit (Qiagen GmbH, Hilden, Germany) to generate cDNA. The template cDNA was used for the qPCR assay with the miScript SYBR-Green PCR kit (Qiagen GmbH) following the manufacturer's protocol. The PCR thermal parameters were: 15 min at 95˚C, 40 cycles of 15 sec at 94˚C followed by 30 sec at 55˚C, and 30 sec at 72˚C. The expression threshold cycle values of miRNAs were calculated by normalizing with internal reference U6 and the relative quantity of miRNAs were calculated using the $2^{-\Delta\Delta Cq}$ method (29). For mRNA analysis, total RNA was isolated using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) and followed by RT reaction using the Prime-script RT reagent kit (Takara Biotechnology Co., Ltd., Dalian, China) and the RT reaction was 37˚C for 60 min, followed by 85˚C for 5 sec. RT-qPCR was conducted using SYBR Premix Ex Taq (Qiagen GmbH) and β-actin was used as internal control. The PCR reaction was 95˚C for 5 min, followed by 45 cycles of 95˚C for 15 sec and 58˚C for 30 sec. The sequences of the PCR primers were as follows: miR-320a forward, 5'-AAAAGCTGGGTT GAGAGGGCG-3'; U6 forward, 5'-CTCGCTTCGGCAGCA CA-3' and U6 reverse, 5'-AACGCTTCACGAATTTGC GT-3'; ADAM10 forward, 5'-CTGCCCAGCATCTGACCC TAA-3' and reverse, 5'-TTGCCATCAGAACTGGCACAC-3'; GAPDH forward, 5'-GACCCAGATCATGTTTGAGACC-3', and reverse 5'-ATCTCCTTCTGCATCCTGTCG-3'. Relative quantification of ADAM10 expression was calculated using the $2^{-\Delta\Delta Cq}$ method (29). Each sample was analyzed in triplicate.

Transfection. miR-320a mimics, mimic control, inhibitor and inhibitor control were synthesized by GenePharma (Shanghai, China) and were transfected into cells ($1x10^5$) with a final concentration of 20 nmol/l using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). miR-320a mimics, 5'-AAAAGC UGGGUUGAGAGGGCGA-3'; mimics control, 5'-UUCUCC GAACGUGUCACGUTT-3'; inhibitor, 5'-UCGCCCUCUCAA CCCAGCUUUU3'; inhibitor control, 5'-CAGUACUUUUGU GUAGUACAA-3'. The specific anti-ADAM10 small interfering (si)RNA (siADAM10) and its non-specific scramble siRNA (control) were purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA), and then transfected into cells using Lipofectamine® 2000 according to the manufacturer's protocol. Briefly, cells were cultured in RPMI-1640 medium without antibiotics. Transfection procedure was performed once the cultured cells are at a confluency of 80%. SiRNA (5 μl) and 4.5 μl RNAi-MAX complexes (Thermo Fisher Scientific, Inc.) were diluted in 250 μl Opti-MEM medium and incubated for 20 min. The mixture was then added to the cells and incubated at 37˚C. The efficiency of siADAM10 was confirmed by western blot analysis. ADAM10 cDNA with 3'-UTR were inserted into pcDNA3.1(+) vector (Invitrogen; Thermo Fisher Scientific, Inc.) to build the plasmid pcDNA3.1(+)-ADAM10 (pcADAM10), and the empty vector (Invitrogen; Thermo Fisher Scientific, Inc.) was used as control. The samples were collected for gene and protein detection 48 h post-transfection.

Colony formation assay. Transfected cells were seeded into 6-well culture dishes (400 cells/well) and cultured for 14 days. The colonies were fixed with 75% ethanol and stained with 0.2% crystal violet (Sangon Biotech Co., Ltd., Shanghai, China) for 20 min at room temperature and counted under a microscope (SZ61-ILST; Olympus Corporation, Tokyo, Japan). The number of formed colonies was counted in 6 different fields (magnification, x100).

Viability assay. MMT was used for the viability assay identify the effect of miR-320a. The transfected GC cells were seeded

in 96-well plates at 2,000 cells/well and maintained at 37˚C and 5% CO_2 for 4 days. Then, 150 μl DMSO was added to block the reaction and the cell proliferation was determined by measuring the optical density at 570 nm using a spectrophotometric reader (Thermo Fisher Scientific, Inc.). All tests were performed in triplicate.

Luciferase reporter activity assay. The wild-type 3'-UTR and the mutated 3'-UTR sequences of ADAM10 were inserted into the *Spe*I and *Hind*III sites of the pMiR-REPORT (Ambion; Thermo Fisher Scientific, Inc.) to produce constructs of wild-type 3'-UTR segment of ADAM10 (ADAM10-WT) and mutated 3'-UTR segment of ADAM10 (ADAM10-Mut), respectively. The following primers were used: forward 5'-AGTCCT TATGTGGCATGCCCCCTATG-3' and reverse 5'-ATTGCG ATGATACAAGTCCATCGGATTATTTCA-3'. SGC-7901 cells were seeded in 24-well plates ($3x10^5$ cells/well). After 1 day, ADAM10-WT, ADAM10-Mut, and pMiR-REPORT control vectors were co-transfected with miR-320a and β-galactosidase into SGC-7901 cells. Following incubation at 37˚C for 48 h, the luciferase activity was quantified using Dual-Light Combined Reporter Gene Assay system (Thermo Fisher Scientific, Inc.) 48 h after transfection.

MTS assay. The sensitivity of cells to cisplatin was determined using the Cell Titer 96 AQueous One Solution Cell Proliferation Assay kit (Promega Corporation, Madison, WI, USA). Cells were cultured in 96-well plates seeded at 3,000 cells/well and different concentrations of cisplatin were added. The RPMI-1640 medium was replaced with fresh medium (containing cisplatin) every 24 h. After 3 days, MTS (0.02 ml/well) was added. The absorbance was recorded at 490 nm for each well on the BioTek Synergy 2 (BioTek, Winooski, VT, USA).

Western blot analysis. Cells were collected and treated with cell lysis buffer (Beyotime Institute of Biotechnology, Haimen, China), followed by centrifugation for 30 min at 4˚C and 13,400 x g, and the protein samples were collected. Protein quantity was determined using BCA Protein Assay kit (Beyotime Institute of Biotechnology) and then separated on a 10% SDS-PAGE gel and transferred onto PVDF membranes. The membranes were subsequently incubated at 4˚C overnight with primary antibody ADAM10 (sc-28358; 1:500; Santa Cruz Biotechnology, Inc.) or β-actin (sc-8432; 1:2,000; Santa Cruz Biotechnology, Inc.), followed by incubated with horseradish peroxidase-conjugated secondary goat anti-mouse antibodies (STAR117P, 1:1,000; Bio-Rad Laboratories, Hercules, CA, USA) at room temperature for 30 min. The western blots were visualized using an enhanced chemiluminescence system (Thermo Fisher Scientific, Inc.).

In vivo xenograft models. The miR-320a precursor sequences were cloned into pCDH-CMV-MCS-EF1-Puro vector (System Biosciences, Palo Alto, CA, USA) to construct stably miR-320a-expressing cells used in xenograft models. A total of 5 male BALB/c nu/nu mice (5-weeks old; weight, 18-20 g) were provided by the Animal Center of the Chinese Academy of Science (Shanghai, China) and used for the *in vivo* xenograft tumor model. Animals were housed in a specific pathogen-free room with a 12-h light/dark cycle and 40-70% humidity at 26-28˚C. SGC-7901 cells ($3x10^6$) with miR-320a stable expression vector or empty vector (control) were subcutaneously injected into the left and right flank. Tumor volumes were calculated using the formula: Tumor volume=(length x $width^2$)/2. After 35 days, all mice were sacrificed following the standard procedure and harvested tumors were weighed.

Statistical analysis. Each experiment was repeated at least three times. Data are reported as mean ± standard deviation and statistical tests were performed using SPSS version 14.0 (SPSS, Inc., Chicago, IL, USA) and Prism version 5.0 (GraphPad, La Jolla, CA, USA). Statistical significance was determined using a two-sided Student's t-test. Paired-sample t-test was used to compare the expression levels of miR-320a and ADAM10 in clinical samples, and Pearson correlation was used to determine if there was a relationship between. Multiple group comparisons were analyzed using one-way analysis of variance. Tukey post hoc tests were used when comparing multiple parameters. $P<0.05$ was considered to indicate a statistically significant difference.

Results

miR-320a is downregulated in GC cell lines. In order to determine the endogenous miR-320a level in GC cells, the present study used RT-qPCR to detect miR-320a expression in four GC cell lines (AGS, SGC-7901, BGC-823 and MGC-803 cells) and compared them with miR-320a expression in a normal gastric mucosal epithelial cell line GES-1 cells. The data revealed that compared with GES-1, all GC cells had significantly reduced expression levels of miR-320a (Fig. 1A), suggesting miR-320a may contribute to GC development.

miR-320a inhibits cell growth and enhances sensitivity of GC cells to cisplatin. The present study identified the role of miR-320a in GC cells by gain- or loss-of-function analysis (Fig. 1A). SGC-7901 cells were selected as they have relatively low endogenous miR-320a expression and BGC-823 cells were selected due to their relatively high endogenous miR-320a and were transfected with mimics or inhibitor. The expression of miR-320a in transfected cells was confirmed by RT-qPCR (Fig. 1B).

Colony formation and MTT assays were used to identify the effect of miR-320a on GC cell growth. As presented in Fig. 1C, the proliferation rate of SGC-7901 cells was significantly inhibited by miR-320a mimics, whereas the proliferation of BGC-823 cells was promoted by miR-320a inhibitor, in comparison with their corresponding control cells. For colony formation, the number of formed colonies was markedly reduced in miR-320a overexpressing cells, whereas it was increased in cells where miR-320a was inhibited (Fig. 1D). These findings indicated that miR-320a suppressed cell growth *in vitro*.

The present study determined the effects of ectopic miR-320a expression on cell chemosensitivity. It was revealed that miR-320a mimics effectively sensitized SGC-7901 and BGC-823 cells to cisplatin, compared with control-transfected cells. By contrast, the sensitivity of SGC-7901 and BGC-823 cells to cisplatin was reduced by the miR-320a inhibitor, when

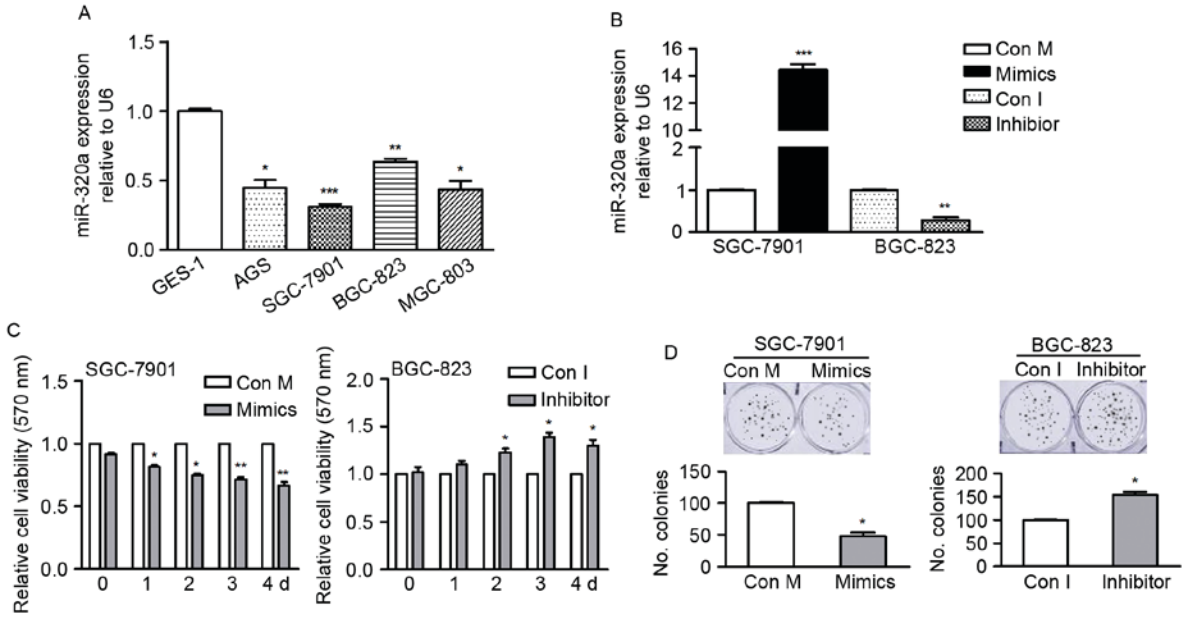

Figure 1. Low expression and suppressive role of miR-320a in GC cells. (A) RT-qPCR analysis, the expression of miR-320a was downregulated in AGS, SGC-7901, BGC-823 and MGC-803 cells compared with GES-1 cells. U6 was used as an internal reference gene. Data are presented as the mean ± standard error of the mean. (B) RT-qPCR analysis was used to confirm the efficacy of transfection with mimics or inhibitor. After transfection, the growth ability of GC cells was determined using (C) MTT and (D) colony formation assays. *P<0.05 vs. control group, **P<0.01 vs. control group, ***P<0.001 vs. control group. Con M, mimic control; Con I, inhibitor control; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

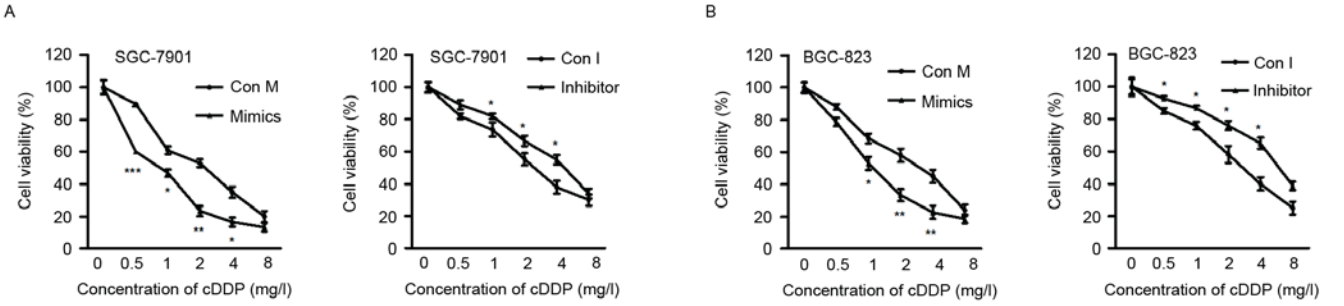

Figure 2. Effect of up- or downregulation of miR-320a on cisplatin-sensitivity of GC cells. Cisplatin-sensitivity of (A) SGC-7901 and (B) BGC-823 cells was measured using MTS assay. *P<0.05; **P<0.01; ***P<0.001. Con M, mimics control; Con I, inhibitor control; cDDP, cisplatin.

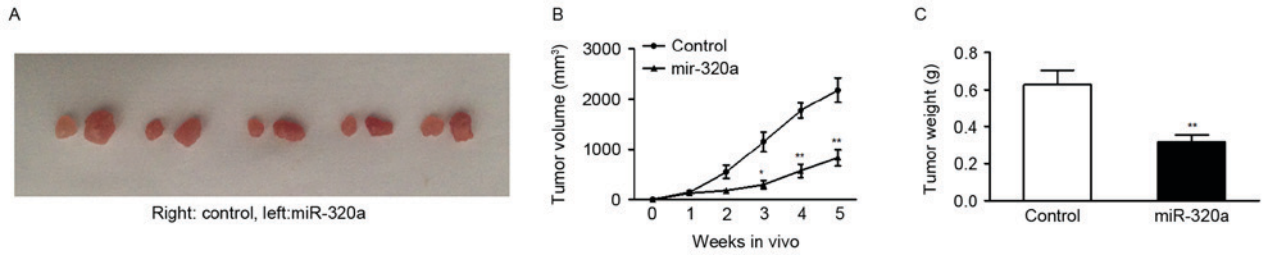

Figure 3. Overexpression of miR-320a inhibited tumor growth *in vivo*. SGC-7901 cells were transfected with a stably overexpressing miR-320a vector or control vector. Cells were then subcutaneously injected into 5 nude mice. (A) Tumors were harvested 5 weeks after injection. (B) Tumor sizes were calculated using the following formula: (lengthxwidth^2)/2. (C) Weight of xenograft tumors. *P<0.05; **P<0.01.

compared with the control cells (Fig. 2A and B). These findings collectively indicated that miR-320a sensitized GC cells to cisplatin.

miR-320a suppresses tumor growth in vivo. As it was evident that up or downregulating miR-320a expression had a functional role in regulating GC cell growth *in vitro*, the present study investigated whether overexpressing miR-320a may have a similar antitumor role in inhibition of tumor growth *in vivo*. SGC-7901 cells were transfected with stably expressing miR-320a vector or control vector. Subsequently, cells were subcutaneously injected into five null mice. The

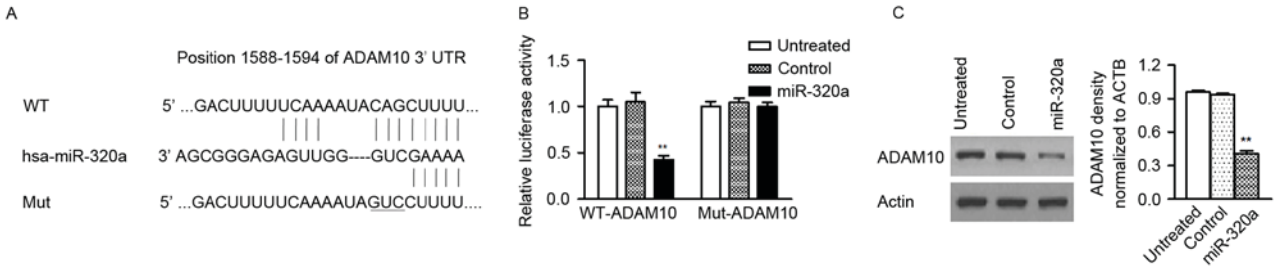

Figure 4. miR-320a directly targets the 3'-UTR of ADAM10 in GC cells. (A) The WT 3'-UTR of ADAM10 mRNA contains a putative miR-320a binding site. The mutant form is also presented. (B) Luciferase reporter assays. Cells were transfected with reporters containing the WT or Mut form after transfection with miR-320a mimics. (C) Western blotting analysis of ADAM10 protein level in SGC-7901 cells transfected with control or miR-320a mimics. β-actin was used as internal control. **P<0.01. 3'-UTR, 3'-untranslated region; ADAM10, ADAM metallopeptidase domain 10; WT, wild type; Mut, mutant.

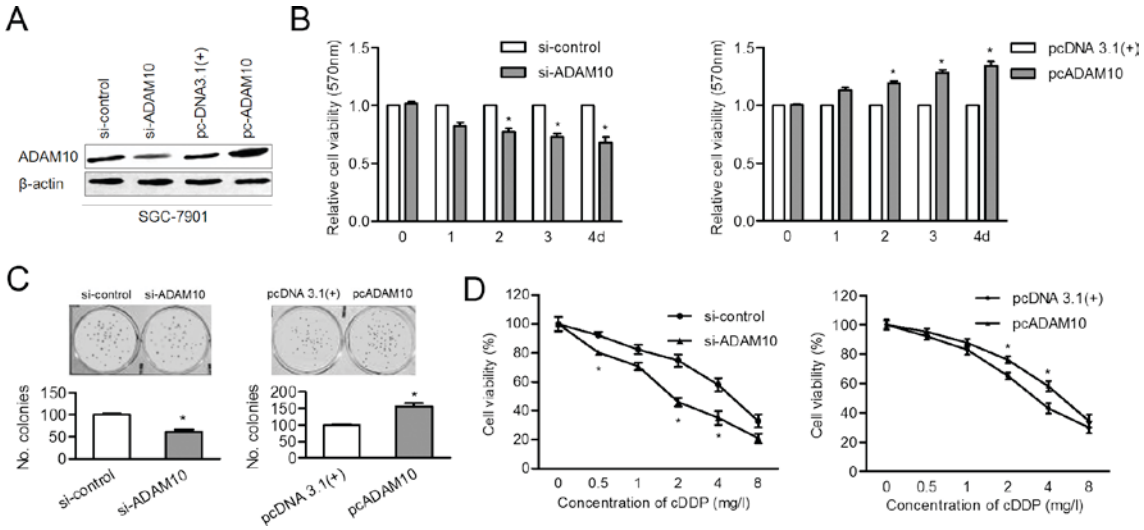

Figure 5. Effects of silencing or upregulating ADAM10 on growth and chemosensitivity of SGC-7901 cells. (A) Protein expression level was confirmed by western blotting analysis. Cell proliferation was determined by (B) MTT and (C) colony formation assays. (D) Cisplatin-sensitivity was determined using MTS assay. *P<0.05. ADAM10, ADAM metallopeptidase domain 10; si, small interfering; cDDP, cisplatin.

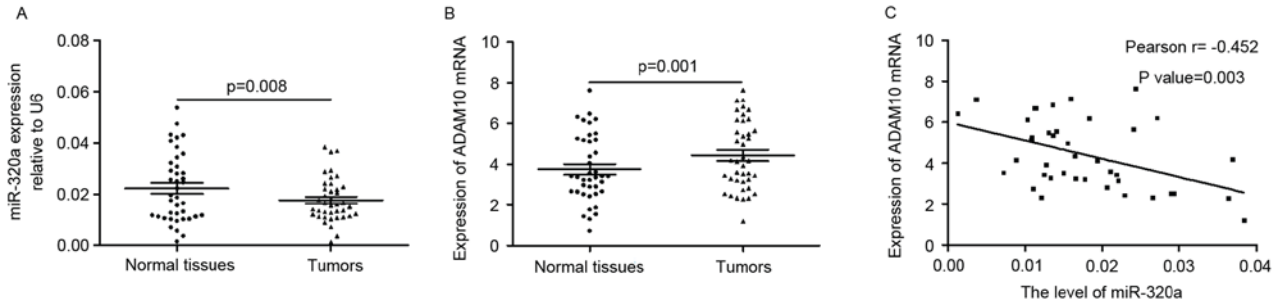

Figure 6. miR-320a expression was negatively correlated with ADAM10 expression in tumor samples. (A) Expression of miR-320a was significantly upregulated in tumors. Data are presented as the mean ± standard error of the mean. (B) ADAM10 mRNA expression level was reduced in primary tumors. (C) Expression of miR-320a was inversely associated with ADAM10 mRNA levels in tumors. ADAM10, ADAM metallopeptidase domain 10.

tumors were harvested 5 weeks after injection. The findings revealed that tumors were significantly smaller when miR-320a expression was upregulated (Fig. 3A and B). Quantification of tumor weight confirmed that miR-320a markedly suppressed the ability of SGC-7901 cells to form tumors *in vivo* (Fig. 3C).

miR-320a directly targets ADAM10 in GC cells. Three publically available databases (miRDB, microRNA, TargetScan) were used to predict the potential targets of miR-320a in GC cells and ADAM10 with conserved binding site in its 3'-UTR was selected for further analysis (Fig. 4A). To assess whether ADAM10 is a direct target of miR-320a, the luciferase reporter vectors with the putative ADAM10 3'-UTR target site for miR-320a (ADAM10-WT) and mutant type (ADAM10-Mut) were constructed. The present study confirmed that miR-320a significantly decreased luciferase activity of the ADAM10-WT plasmid, but not the ADAM10-Mut plasmid (Fig. 4B). Additionally, western blot assay also revealed that

the protein level of ADAM10 was reduced in GC cells with miR-320a mimic, compared with control-transfected cells (Fig. 4C). These findings indicated that miR-320a directly targets to the 3'-UTR of ADAM10 and then suppresses its protein expression.

ADAM10 contributes to proliferation and desensitization of GC cells to cisplatin. To clarify whether miR-320a regulates cell growth and drug sensitivity by targeting ADAM10, the current study induced knockdown or overexpression of ADAM10 in SGC-7901 cells. As presented in Fig. 5A, the protein level of ADAM10 was inhibited after siADAM10 transfection and ADAM 10 level was upregulated following cDNA transfection. The proliferation ability of SGC-7901 cells was impaired by knockdown of ADAM10 and promoted by overexpression of ADAM10 (Fig. 5B and C). Finally, the cisplatin-sensitivity of SGC-7901 cells was enhanced by siADAM10, whereas it was attenuated by overexpression of ADAM10 (Fig. 5D). These findings suggested that ADAM10 is a functional target of miR-320a in GC development and chemotherapy.

miR-320a is negatively correlated with ADAM10 in tumors. The present study determined the expression level of miR-320a in primary GC tissues and corresponding normal tissues. As presented in Fig. 6A, the expression of miR-320a was significantly downregulated in tumors when compared with adjacent normal tissues (0.1766±0.0085 vs. 0.2230±0.0134; P=0.008). The mRNA level of ADAM10 was higher in tumors compared with normal tissues (4.4278±1.6846 vs. 3.5373±1.6943; P=0.00; Fig. 6B). Pearson correlation coefficient analysis revealed that the expression of miR-320a was negatively correlated with the mRNA levels of ADAM10 (r=-0.452; P=0.003; Fig. 6C) in tumor tissues. These findings verified the negative correlation between miR-320a and ADAM10 in tumors.

Discussion

Previous studies reported that miR-320a has a functional role in proliferation, invasion and drug resistance of various tumors (10-13,16). However, to the best of our knowledge no studies examining the role of miR-320a in GC development have been previously conducted. The findings of the current study indicated that miR-320a inhibited GC cell growth *in vitro* and *in vivo*. The sensitivity of GC cells to cisplatin was increased by miR-320a overexpression, whereas it was decreased in cells where miR-320a was downregulated. ADAM10 was a direct target of miR-320a and involved in miR-320a-regulated cell proliferation and cisplatin-sensitivity.

RT-qPCR analysis was used in order to determine the endogenous miR320a levels in GC cell lines and the data revealed that miR-320a was reduced in all GC cells, suggesting the potential functional role of miR-320a in gastric tumorigenesis. Subsequently, by transfecting cells with mimics or inhibitor, the expression of miR-320a was effectively upregulated or downregulated. The effect of downregulated miR-320a on proliferative ability of GC cells was assessed by colony formation and MTT assays *in vitro*, as well as xenograft models *in vivo*. The presents study determined the role of miR-320a in GC and suggested that it may contribute to growth inhibition in gastric tumorigenesis. Similar results were found in CRC, glioma, and breast cancer (12,14,30). These findings indicated that miR-320a has an important role in the inhibition of tumor growth in various types of tumor.

Considering miR-320a was also reported as a chemotherapy-associated gene in tumors, the present study tested whether miR-320a modulated cisplatin-sensitivity of GC cells. It was determined that ectopic miR-320a expression significantly enhanced cisplatin-sensitivity of GC cells. miR-320a was identified to be significantly correlated with sensitivity to preoperative chemoradiotherapy (31). Low expression of miR-320a was correlated with shortened time to imatinib resistance (32). The function of miR-320a in chemoresistance revealed that restoration of miR-320a may a provide novel therapeutic strategy for GC treatment.

ADAM10, is a typical member of the ADAMs family, which has been previously reported to be upregulated in various types of cancers and contribute to cancer progression (33). The present study identified ADAM10 as a target of miR-320a. Knockdown of ADAM10 markedly inhibited cell proliferation and colony formation. On the contrary, overexpression of ADAM10 accelerated cell growth rate, which was in consistent with a previous study (20). A previous clinical study revealed that upregulated ADAM10 is associated with GC progression (34). The present study determined that ADMA10 was significantly upregulated in tumors, suggesting ADAM10 involvement in gastric tumorigenesis. Direct evidence was provided by the current study indicating that ADAM10 has an oncogene role in GC by stimulating cell growth. It is of note that silencing of ADAM10 impaired the cisplatin-sensitivity of GC cells, suggesting ADAM10 may be a promising target for the improvement chemotherapeutic efficacy in GC. The negative correlation between miR-320a and ADMA10 in tumors also suggested the miR-320a/ADAM10 axis may have an important role in GC development.

In conclusion, to the best of our knowledge, the present study demonstrated for the first time that downregulation of miR-320a contributed to GC progression and chemoresistance by targeting ADAM10. These findings collectively identified the miR-320a/ADAM10 axis as a promising therapeutic tool for further GC therapy.

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