miR-92a contributes to cell proliferation, apoptosis and doxorubicin chemosensitivity in gastric carcinoma cells

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Abstract. MicroRNAs (miRNAs) are a class of short noncoding RNAs that negatively regulate gene expression and act as oncogenes or tumor suppressors. Numerous miRNAs have been reported be associated with the occurrence and development of gastric carcinoma (GC). For instance, miR-92a has been observed to be overexpressed in GC; however, the precise mechanisms underlying the role of miR-92a in GC and its role in clinical therapy require further investigation. In the present study, it was reported that miR-92a expression was significantly upregulated in GC tissues compared with in adjacent tissues. Additionally, suppression of miR-92a significantly reduced SGC7901 cell viability as demonstrated by a Cell Counting Kit-8 and colony formation assays. Suppression of miR-92a inhibited SGC7901 cell proliferation as determined by Ki-67 immunofluorescence staining, and the expression levels of proliferating cell nuclear antigen, cyclin dependent kinase (CDK)4 and CDK6, and increased that of p53. In addition, we reported that suppression of miR-92a induced apoptosis in SGC7901 cells. Furthermore, bioinformatics analysis identified that ING2 as a potential target of miR-92a. Downregulation of miR-92a significantly increased ING2 expression at the mRNA and protein levels. A dual-luciferase reporter assay validated a direct binding site of miR-92a on ING2. In addition, SGC7901 cells with suppression of miR-92a were more sensitive to doxorubicin treatment. Knockdown of miR-92a reduced the half-maximal inhibitory concentration of doxorubicin from 147.6 nM to 82.1 nM in SGC7901 cells. Knockdown of miR-92a also reduced SGC7901 cell survival under doxorubicin stimulation. Furthermore, SGC7901 cells with suppression of miR-92a harbored a greater number of DNA damage foci upon doxorubicin treatment compared with in control cells. The findings of the present study revealed that miR-92a contributes to cell proliferation, apoptosis and doxorubicin chemosensitivity in GC cells, which suggests a potential therapeutic strategy for the treatment of GC.

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Introduction

Gastric carcinoma (GC) is one of the most common types of malignancy and the second leading cause of cancer-associated mortality worldwide (1,2). Despite the advances in diagnosis and cancer therapy in the past decade, the survival rate remains low (3). This may be attributed to a limited understanding of the exact causes and mechanisms underlying GC. Thus, it is an urgent to determine the potential molecular mechanisms of GC and develop novel therapeutic strategies.

MicroRNAs (miRNAs/miRs) are endogenous noncoding RNAs that are vital for cancer development by acting as tumor suppressors or oncogenes (4-6). In GC, numerous miRNAs exhibit aberrant expression and contribute to the progression of GC and chemoresistance (7-10). For example, miR-26a/b inhibits GC growth and angiogenesis by targeting the hepatocyte growth factor-vascular endothelial growth factor axis (11). In addition, miRNA-106a induces multidrug resistance in GC by targeting runt related transcription factor 3 (12). Circulating miR-18a contributes to the detection and monitoring of GC (13); however, the roles of numerous miRNAs in GC remain unknown.

In the present study, miR-92a was analyzed. It has been demonstrated that miR-92a is upregulated in GC (14); however, the role of miR-92a and its associated mechanisms are unknown. The present study reported that miR-92a is upregulated in GC tissues compared with in adjacent tissues. Functional analyses revealed that suppression of miR-92a significantly inhibited GC cell proliferation and induced apoptosis. Further investigation suggested that inhibitor of growth protein 2 (ING2) is a direct target of miR-92a. Additionally, suppression of miR-92a sensitized GC cells to doxorubicin treatment. Collectively, the results of the present study highlight the oncogenic role of miR-92a in GC and may provide a potential therapeutic basis for the treatment of GC.

Materials and methods

Clinical samples. A total of 10 GC tumor samples and their corresponding adjacent tissues were collected from patients

(45-60 years old) at The Third Affiliated Hospital of Harbin Medical University form February 2015 to August 2017 (Harbin, China). Informed consent was obtained from all patients and the present study was approved by the Ethics Committee of Harbin Medical University. All tissue samples were immediately frozen and preserved in liquid nitrogen until further use.

Cell culture. GC cell lines, including AGS, SGC7901, MGC803 and BGC823, and human gastric epithelial GES-1 cells were purchased from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). Cells were cultured in Dulbecco's Modified Eagle's medium (DMEM; Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) with 10% fetal bovine serum (Invitrogen; Thermo Fisher Scientific, Inc.), 50 U/ml penicillin and 50 μg/ml streptomycin (Invitrogen; Thermo Fisher Scientific, Inc.). All cells were maintained at 37˚C in a humidified incubator at 5% CO_2.

Cell transfection. The sequences of the miR-92a mimics (cat. no. miR10000092-1-5), mimics-negative control (NC; cat. no. miR01101-1-5), miR-92a inhibitor (cat. no. miR20000092-1-5) and inhibitor-NC (miR02201-1-5) were obtained from Guangzhou Ribobio Co., Ltd. (Guangzhou, China). miR-92a mimics or inhibitor (20 nM) was used for transfection with by Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) with serum-free medium according to experiments request.The sequences were as follows: mimics-NC, 5'-UUUGUACUACACAAA AGUACUG-3' and 3'-AAACAUGAUGUGUUUUCAU GAC-5'; miR-92a mimics, 5'-UAUUGCACUUGUCCCGGC CUGU-3' and 3'-AUAACGUGAACAGGGCCGGACA-5'; inhibitor-NC, 5'-CAGUACUUUUGUGUAGUACAAA-3' and miR-92a inhibitor, 5'-ACAGGCCGGGACAAGUGCAAUA-3'. SGC7901 cells ($8x10^3$) were seeded into 6 cm dishes 24 h prior to transfection. miR-92a, inhibitor or inhibitor-NC were transfected using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) with serum-free medium according to the conditions of the subsequent experiments. After 5 h, the cell medium was replaced with complete medium. The cell lysates were harvested 48 h post-transfection.

RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Cells and tissues were lysed and total RNA was extracted using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocols and transcribed into cDNA using Superscriptase II (Invitrogen; Thermo Fisher Scientific Inc.). qPCR was performed using Power SYBR Green PCR master Mix (Thermo Fisher Scientific, Inc.). The following primer sets were used for *ING2* detection. *ING2*, forward 5'-GCA GCAACTGTACTCGTCG-3', reverse, 5'-GACTCCACGCAC TCAAGGTA-3'; and β-actin, forward 5'-CATGTACGTTGC TATCCAGGC-3', reverse, 5'-CTCCTTAATGTCACGCAC GAT-3'.

miR-92a expression was measured using the TaqMan MicroRNA assay kit (Thermo Fisher Scientific, Inc.). In brief, 1 μg of RNA was reverse transcribed into cDNA using an miR-92a specific stem-loop primer at 25˚C for 10 min, 37˚C for 120 min, 85˚C for 5 min, and 4˚C for 5 min. qPCR with miR-92a specific primers and Taqman probes was performed on an ABI7500 real-time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.), over 35 cycles of 95˚C, 30 sec at 60˚C and 72˚C for 35 sec. The data were analyzed using $2^{-\Delta\Delta Cq}$ method (15). The sequence of miR-92a and U6 were as follows: miR-92a , forward, 5'-GCTGAGTATTGCACTTGT CCCG-3', reverse, 5'-GTGTCGTGGAGTCGGCAA-3' and U6, forward 5'-CTCGCTTCGGCAGCACA-3' and reverse, 5'-AACGCTTCACGAATTTGCGT-3'.

Acridine orange/ethidium bromide (AO/EB) fluorescence staining. SGC7901 cells were transfected with miR-92a inhibitor or inhibitor-NC. Cells ($5x10^3$) were incubated with AO/EB mixing solution for 5 min (Beijing Solarbio Science and Technology Co., Ltd., Beijing, China) at room temperature. A total of eight fields were randomly analyzed to examine the cellular morphology alterations under a fluorescence microscope (magnification, x200). The percentage of apoptotic cells was calculated by the following formula: Apoptotic rate (%) = number of apoptotic cells/number of all cells counted.

Dual-luciferase reporter assay. Wild-type *ING2* (ING2-WT) and mutant *ING2* (ING2-Mut) 3'-untranslated region (UTR) sequences were separately inserted into the *Spe*I and *Hin*dIII sites of pMIR-REPORT Luciferase vectors (Ambion; Thermo Fisher Scientific, Inc.). SGC7901 cells were seeded in 6-well plates and transfected with the specific vectors using Lipofectamine 2000 for 48 h. Luciferase activity was assessed using the Dual Luciferase-reporter 1000 assay system immediately after transfection (Promega Corporation, Madison, WI, USA). *Renilla* activity was used for normalization.

Antibodies and western blotting. Cells were lysed with radioimmunoprecipitation assay lysis buffer containing a protease inhibitor cocktail (Roche Diagnostics, Basel, Switzerland). Protein concentration was measured using a BCA Protein assay kit (Beyotime Institute of Biotechnology, Shanghai, China). Equal amounts of protein (40 μg) were separated by 12.5% SDS-PAGE and transferred to a polyvinylidene difluoride transfer membrane (Thermo Fisher Scientific, Inc.). Following blocking with 5% skim milk for 2 h at room temperature, the blots were probed with primary antibodies against β-actin (cat. no. 3700, dilution: 1:20,00), B-cell lymphoma 2(Bcl-2)-associated X (Bax; cat. no. 2772, dilution: 1:1,000), Bcl-2-associated death promoter (Bad; cat. no. 9292, dilution: 1:500), p53 (cat. no. 2524, dilution: 1:500) and cleaved caspase-3 (cat. no. 9953, dilution: 1:1,000) (Cell Signaling Technology, Inc., Dallas, TX, USA), proliferating cell nuclear antigen (PCNA) (cat. no. sc-25280, dilution: 1:1,000), cyclin dependent kinase (CDK)4 (Cat. sc-70832, dilution: 1:1,000) and CDK6 (cat. no. sc-7961, dilution: 1:1,000) (Santa Cruz Biotechnology, Inc., Dallas, TX, USA) and ING2 (cat. no. ab109504, dilution: 1:1,000) (Abcam, Cambridge, UK). Following washing with PBST and incubating with rabbit or mouse secondary antibodies (Cell Signaling Technology, Inc.), the blots were visualized by an enhanced chemiluminescence reagent (GE Healthcare, Chicago, IL, USA). Three individual experiments were performed.

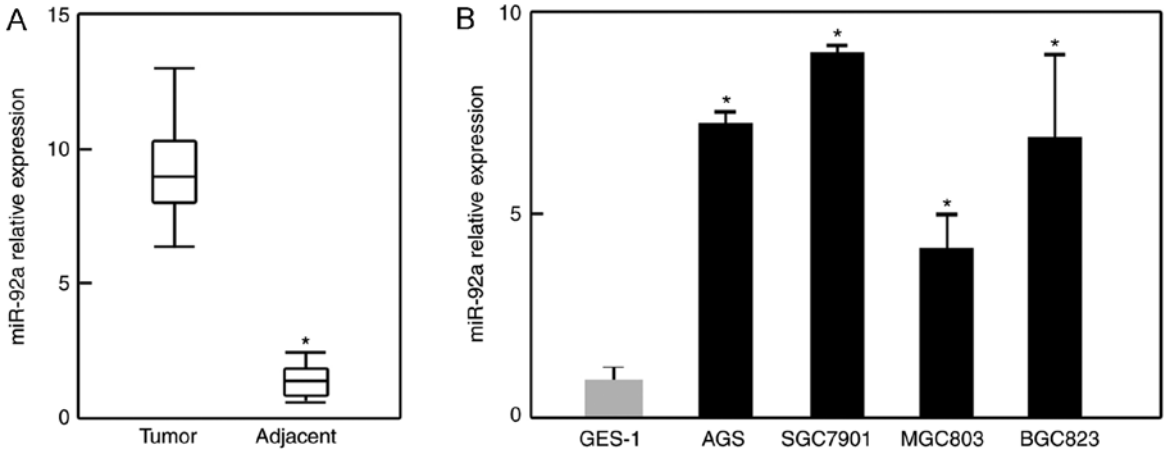

Figure 1. miR-92a is downregulated in gastric carcinoma tissues. (A) miR-92a expression was normalized with U6. (B) The expression of miR-92a was analyzed in several GC cell lines. miR, microRNA. *P<0.05 vs. tumor group or GES-1.

Cell Counting Kit-8 (CCK-8) viability assay. SGC7901 cells transfected with miR-92a inhibitor or inhibitor-NC were seeded into 96-well plates with a density of $5x10^3$ per well and cultured for 4 days. Cell viability was assessed with a CCK-8 kit (Dojindo Molecular Technologies, Inc., Kumamoto, Japan) at days 0, 2 and 4 at 450 nm with a microplate reader (Thermo Fisher Scientific, Inc.). To assess the cell viability upon doxorubicin treatment, the cells transfected with miR-92a inhibitor or inhibitor-NC were seeded into 96-well plates with a density of $5x10^3$ per well and treated with various concentrations 5, 10, 20, 50, 100, 200, 300 and 500 nM) of doxorubicin for 24 h. Cell viability was measured at 450 nm with a microplate reader.

Colony formation assay. A total of $8x10^2$ SGC7901 cells transfected with miR-92a inhibitor or inhibitor-NC were counted and seeded into 6 cm dishes. After 10 days of culturing, the colonies were stained with 0.1% crystal violet in 20% methanol for 15 min at room temperature. The samples were imaged and the numbers of visible colonies were counted.

Ki-67and γH2AX immunofluorescence staining. SGC7901 cells were seeded on cover slips and transfected with miR-92a inhibitor or inhibitor-NC. After 48 h, the cells were fixed with 4% PFA at room temperature for 10 min and then incubated with Ki-67 antibody (cat. no. 9129, Cell Signaling Technology, Inc.) or γH2AX (cat. no. 7631, Cell Signaling Technology, Inc.) for 1 h and then incubated with Alexa-488 conjugated anti-rabbit IgG (cat. no. 4412, Cell Signaling Technology, Inc.) at room temperature for 20 min. H2AX expression was analyzed following treatment with 80 nM doxorubicin for 24 h at 37˚C. The cells were then counterstained with 1 μg/ml DAPI at room temperature for 10 min to stain the cell nuclei. All cover slips were mounted using Prolong Diamond Antifade Mountant (Applied Biosystems; Thermo Fisher Scientific, Inc.). Nine random fields per slips were captured for analysis using a fluorescence microscope (magnification, x200).

Target gene prediction. Potential miRNA-target gene interactions were predicted using www.Targetscan.org; release 7.2) and microrna.org.

Establishment of doxorubicin-resistant SGC7901 cell lines. The SGC7901 cell line was continuously exposed to increasing doses of doxorubicin (5, 10 and 15 μM) at 37˚C in a humidified incubator at 5% CO_2 (Aladdin Biochemical Technology Co. Shanghai, China) for ~9 weeks. The starting dose was 5 μM and this was increased to 10 μM after 4 weeks, to 15 μM after another 4 weeks and continued at 15 μM for the last 4 weeks. The established resistant SGC7901 cell line was then maintained in DMEM medium with 10% (v/v) FBS and 10 μM doxorubicin.

Statistical analysis. Data were obtained from at least 3 independent experiments and are presented as the mean ± standard deviation. For the clinical tissue test, the data was evaluated by a paired Student's t test, and data from the other experiments were evaluated by unpaired Student's t-test or analysis of variance followed by a Tukey's post-hoc test to compare multiple groups. P<0.05 was considered to indicate a statistically significant difference. Statistical values were calculated using SPSS 19.0 software (IBM Corp., Armonk, NY, USA) and illustrated using the GraphPad Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA).

Results

miR-92a is overexpressed in GC. To determine the expression of miR-92a in GC, 10 GC tissues and their corresponding adjacent tissues were collected. Via RT-qPCR, miR-92a was observed to be upregulated by ~6.1-fold in GC tissues than in adjacent tissues (Fig. 1A). In addition, the expression of miR-92a was analyzed in several GC cell lines. As presented in Fig. 1B, the expression levels of miR-92a were significantly increased in all GC cell lines compared with in human gastric epithelial GES-1 cells. These observations suggest a potential role of miR-92a in GC. As SGC7901 cells expressed the highest levels of miR-92a, further experiments were conducted using this cell line.

miR-92a contributes to the proliferative ability of SGC7901 cells. To investigate the function of miR-92a in GC, SGC7901 cells were transiently transfected with an miR-92a inhibitor or

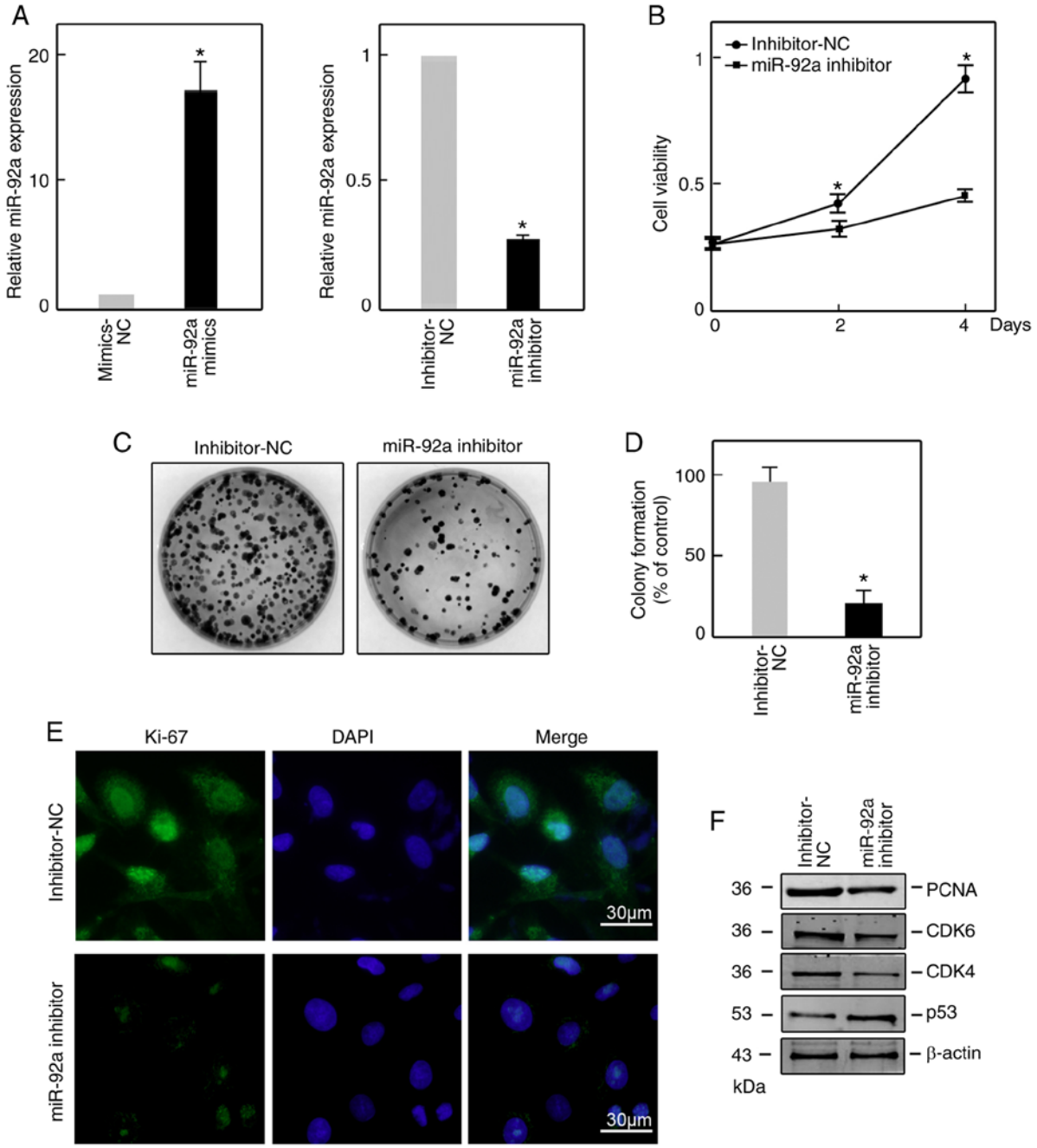

Figure 2. miR-92a contributes to the proliferative ability of SGC7901 cells. (A) miR-92a mimics and inhibitor successfully upregulated and reduced miR-92a expression in SGC7901 cells, respectively. (B) Downregulation of miR-92a suppressed SGC7901 cell viability. (C) Suppression of miR-92a decreased the colony formation ability of SGC7901 cells. (D) Number of colonies were counted and quantified from (C). (E) miR-92a knockdown significantly decreased SGC7901 cell proliferation as assessed by Ki-67 immunofluorescence staining. Scale bar, 30 μm. (F) Knockdown of miR-92a led to decreased expression of PCNA, CDK4 and CDK6, and increased p53 expression. *P<0.05 vs. mimics-NC or inhibitor-NC. CDK, cyclin dependent kinase; miR, microRNA; NC, negative control; PCNA, proliferating cell nuclear antigen.

mimics to suppress or upregulate the expression of miR-92a, respectively. The suppression and overexpression efficiencies were confirmed by RT-qPCR at 48 h post-transfection (Fig. 2A). A CCK-8 assay was performed to analyze viable cells at day 0, 2 and 4 post-transfection. The data revealed that suppression of miR-92a significantly suppressed cell viability compared with in the inhibitor-NC-transfected group by a CCK-8 assay (Fig. 2B). The colony formation assay revealed significantly fewer visible colonies upon miR-92a knockdown (Fig. 2C and D). Furthermore, Ki-67 immunofluorescence staining exhibited notably lower signals in miR-92a inhibitor-transfected SGC7901 cells compared with in inhibitor-NC-transfected cells (Fig. 2E). Consistently, western blotting demonstrated that the protein expression levels of PCNA, CDK4 and CDK6 were notably reduced, while p53 was upregulated (Fig. 2F). All these results indicate that miR-92a contributes to the maintenance of proliferation in SGC7901 cells.

Suppression of miR-92a induces the apoptosis of SGC7901 cells. To investigate the role of miR-92a in cell

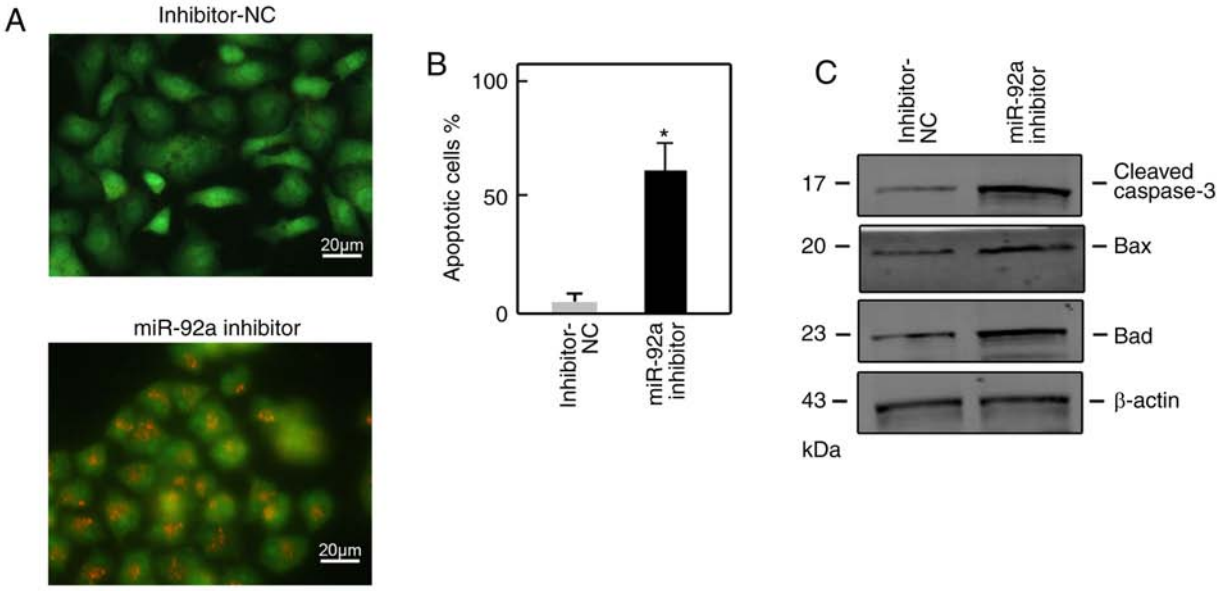

Figure 3. Knockdown of miR-92a induces the apoptosis of SGC7901 cells. (A) SGC7901 cells transfected with miR-92a inhibitor or inhibitor-NC were subjected to acridine orange/ethidium bromide staining to detect alterations in the nucleus. The orange regions indicated initiation of apoptosis. Scale bar, 20 μm. (B) Statistical analysis of apoptotic cells from (A) (C) Knockdown of miR-92a increased the levels of cleaved caspase-3, Bax and Bad. *P<0.05 vs. inhibitor-NC. Bax, B-cell lymphoma 2-associated X protein; Bad, B-cell lymphoma-associated death promoter; miR, microRNA; NC, negative control.

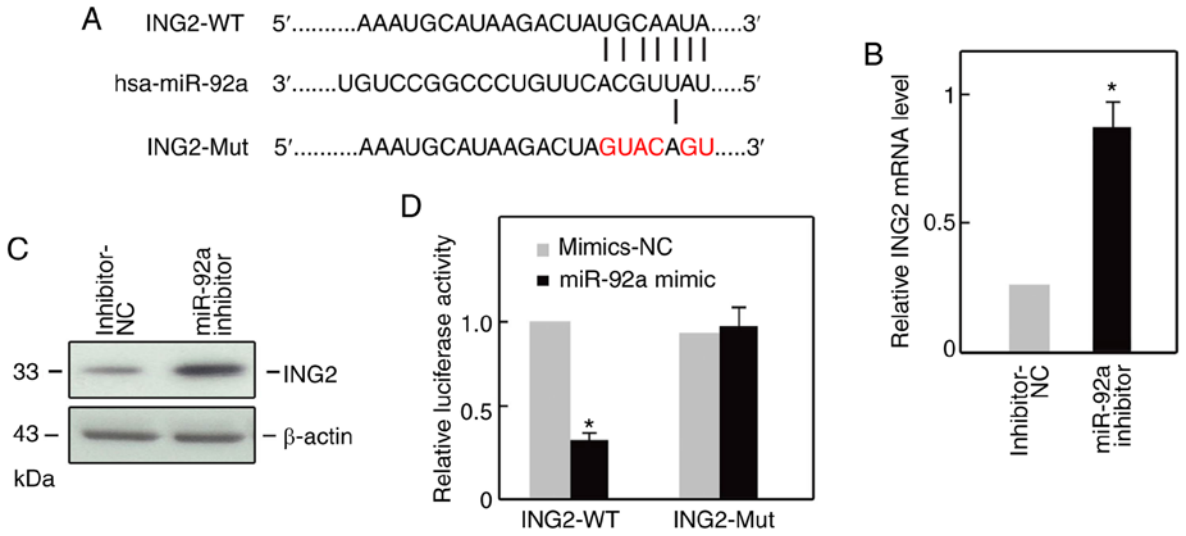

Figure 4. ING2 is a direct target of miR-92a. (A) Schematic diagram of the miR-92a binding site in ING2 mRNA. (B) Downregulation of miR-92a upregulated ING2 mRNA expression. (C) Downregulation of miR-92a upregulated ING2 protein expression. (D) Luciferase reporter assay. Upregulation of miR-92a reduced luciferase activity in ING2-WT group rather than ING2-Mut group. *P<0.05 vs. mimics-NC or inhibitor-NC. ING2, inhibitor of growth protein 2; miR, microRNA; Mut, mutant; WT, wild type.

apoptosis, we subjected miR-92a inhibitor-transfected SGC7901 cells to AO/EB staining. The results revealed that >50% of apoptotic cells were counted upon miR-92a inhibitor transfection; however, <5% of apoptotic cells were counted upon inhibitor-NC transfection (Fig. 3A and B). In addition, proapoptotic proteins, including Bax, Bad and cleaved caspase-3, were determined to be increased via the suppression of miR-92a compared with in control cells (Fig. 3C).

ING2 is a direct target of miR-92a. Conventionally, miRNAs suppress gene expression by binding to the 3'-UTR sequence of target mRNA (16). By employing bioinformatics databases (TargetScan, microrna.org), we observed that *ING2* was a potential target of miR-92a (Fig. 4A). Additionally, ING2 expression at the mRNA and protein levels was notably increased following miR-92 inhibitor transfection compared with the control (Fig. 4B and C). To investigate whether ING2 was a direct target of miR-92a, a luciferase reporter construct containing the ING2-WT or ING2-Mut of *ING2*. Co-transfection of the miR-92a mimic and ING2-WT resulted in increased luciferase activity (Fig. 4D); however, miR-92a inhibitor did not markedly alter the luciferase activities in the ING2-Mut group (Fig. 4D). Thus, these findings support the result that ING2 is a direct target of miR-92a.

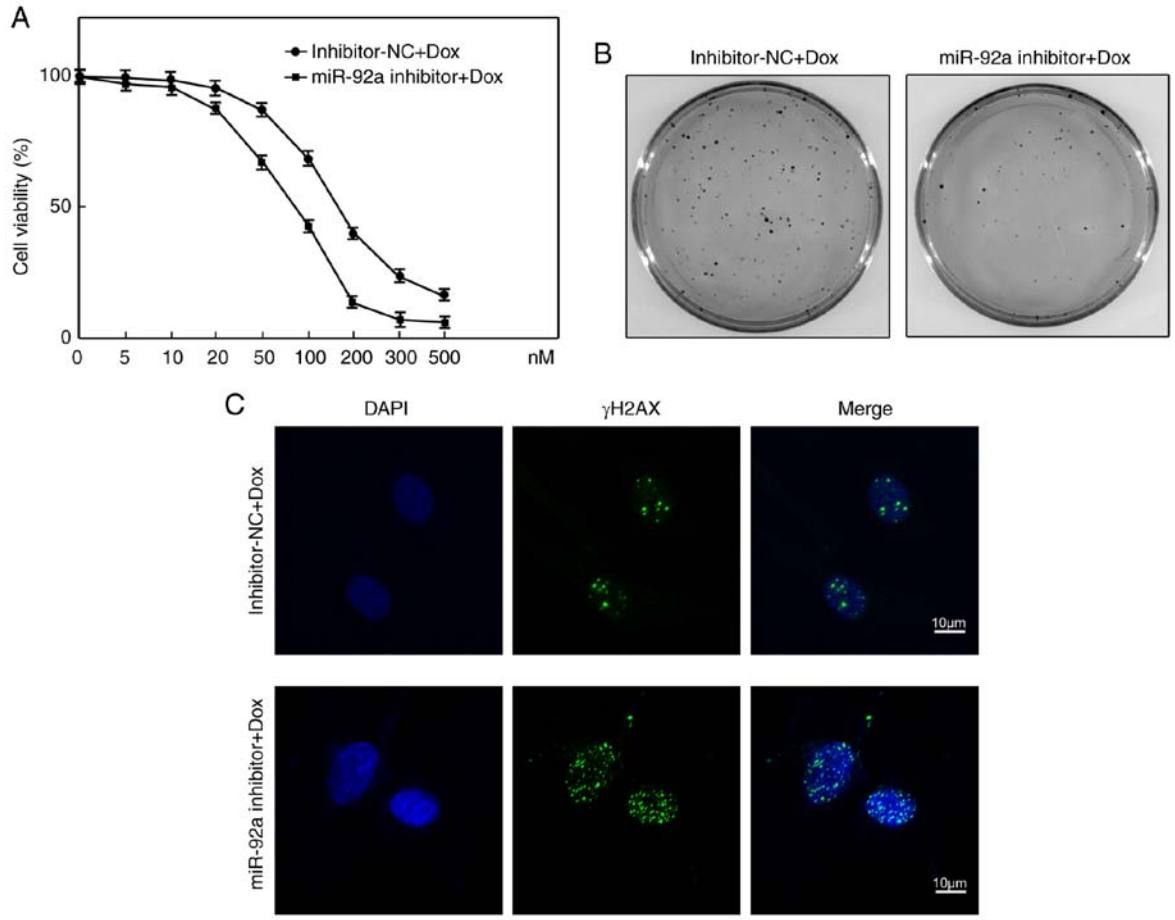

Figure 5. Suppression of miR-92a sensitizes SGC7901 cells to Dox treatment. (A) SGC7901 cells were transfected with miR-92a inhibitor or inhibitor-NC. All cells were treated with various concentrations of Dox for 48 h. Cell viability was analyzed by a Cell Counting Kit-8 assay. (B) SGC7901 cells transfected with miR-92a inhibitor or inhibitor-NC were treated with 80 nM Dox in a 3.5 cm dish. Cells were fixed and stained with 0.1% crystal violet. (C) 80 nM Dox induced more DNA damage foci in SGC7901 cells transfected with miR-92a inhibitor compared to that in inhibitor-NC transfected cells. Scale bar, 10 μm. Dox, doxorubicin; miR, microRNA; NC, negative control.

Suppression of miR-92a sensitizes SGC7901 cells to doxorubicin treatment. It was proposed that suppression of miR-92a led to reduced proliferation and the induction of apoptosis in SGC7901 cells via upregulation of ING2; thus, whether suppression of miR-92a sensitizes SGC7901 cells to doxorubicin treatment was investigated. Compared with in inhibitor-NC-transfected cells, miR-92a inhibitor-transfected cells were more sensitive to doxorubicin-induced growth inhibition as determined by the CCK-8 assay (Fig. 5A). According to Fig. 5A, the half-maximal inhibitory concentration (IC_{50}) of inhibitor-NC transfected SGC7901 cells was ~147.6 nM; however, a notably lower IC_{50} (~82.1 nM) was calculated in SGC7901 cells following knockdown of miR-92a. The colony formation assay also demonstrated that suppression of miR-92a markedly reduced SGC7901 cell survival upon doxorubicin treatment, compared with control cells (Fig. 5B). The cytotoxic effect of doxorubicin is known to occur via the induction of DNA damage (16). Bioinformatics analysis revealed that *ING2* was a potential target of miR-92a (Fig. 4A). Additionally, we reported that ING2 at the mRNA and protein levels was increased by miR-92 inhibitor transfection (Fig. 4B and C). To validate whether ING2 was a direct target of miR-92a, a luciferase reporter construct containing ING2-WT or ING2-Mut of the *ING2* was generated. Co-transfection of the miR-92a mimics and ING2-WT resulted in increased luciferase activity (Fig. 4D). However, the miR-92a inhibitor did not change the luciferase activities in the ING2-Mut group (Fig. 4D). Thus, these findings support ING2 as a direct target of miR-92a. Thus, DNA damage foci formation was examined by γH2AX immunofluorescence staining. We observed that miR-92a downregulation was associated with numerous DNA damage foci than in control cells (Fig. 5C). These data suggest that suppression of miR-92a sensitizes SGC7901 cells to doxorubicin treatment.

Discussion

GC poses great challenge in clinical therapy and is associated with poor prognosis (17); however, increasing evidence has demonstrated that numerous genes are involved in GC (18,19). Therefore, understanding the potential molecular mechanisms of GC oncogenesis is required.

miRNAs have been predicted to regulate >60% of human protein-coding genes (20). Numerous studies have reported that miRNAs serve key roles in GC via downregulation of a variety of genes (21,22). Mir-92a has been identified to be involved in the regulation of the cell cycle and cell signaling,

which is critical for cancer progression (23,24). miRNA-92a functions as an oncogene in colorectal cancer by targeting phosphatase and tensin homolog (25). miR-92a also promotes lymph node metastasis of human esophageal squamous cell carcinoma via E-cadherin (26). A high level of circulating miR-92a expression correlates with poor prognosis in patients with non-small cell lung cancer (27). A study using miRNA-locked nucleic acid *in situ* hybridization reported that miR-92a is upregulated expressed in GC (14). Our study aimed to investigate the potential role of miR-92a in GC. Consistent with previous studies, we observed that miR-92a is upregulated in GC tissues compared with adjacent tissues by RT-qPCR. Suppression of miR-92a inhibited cell proliferation and induced cell apoptosis in SGC7901 cells. High expression of miR-92a is associated with poor prognosis and shorter overall survival of patients with GC (14). The results of the present study provides a key role for miR-92a in GC tumorigenesis, and targeting miR-92a may have potential for clinical application. Furthermore, previous studies revealed that higher expression of miR-92a is closely associated with poorer outcome of initial chemotherapy (28); upregulated tumor miR-92a expression is associated with decreased survival in GC patients (28). Similarly, our results indicated that knockdown of miR-92a promoted GC cells to doxorubicin-induced cell death. The present study proposed a key role for miR-92a in GC tumorigenesis, and targeting miR-92a may have potential for clinical application.

ING2 is a member of the ING family and is characterized as a type-II tumor suppressor gene (29). ING2 has important functions in cell apoptosis, cell cycle arrest, cell senescence, chromatin modification and the DNA damage response (30-34). *ING2* expression has been reported to be reduced in various types of cancer (35). ING2 can be sumoylated by small ubiquitin-related modifier 1 (Sin3a) on lysine 195; this sumoylation is required for the interaction between ING2 and SIN3 transcription regulator family member A to mediate the expression of gens downstream p53 (36). Additionally, ING2 also binds to Ski novel via its PHD domain to suppress cell proliferation (37). The present study reported ING2 as a direct target of miR-92a; knockdown of miR-92a increased ING2 expression, as well as p53 expression. These finding suggests that the oncogenic effects of miR-92a in GC occur at least partially via the suppression of ING2 expression.

ING2 is also involved in the DNA damage response (34). In response to doxorubicin treatment, ING2 stabilizes the mSin3a-histone deacetylase 1 complex at the promoter regions of proliferation-associated genes by its PHD domain and further inhibits the expression of these genes (33). As ING2 was determined to be a direct target of miR-92a, the effects of miR-92a following doxorubicin treatment were investigated. It was reported that suppression of miR-92a induced more DNA damage foci and sensitized SGC7901 cells to doxorubicin treatment.

Collectively, the present stud provided novel insight into the biological function of miR-92a in GC and its potential molecular mechanism. Our findings indicate that miR-92a contributed to cell proliferation, apoptosis and doxorubicin chemosensitivity in GC by inhibiting ING2 expression. Targeting miR-92a may be a potential therapeutic strategy to enhance the effects of doxorubicin in GC.

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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

XCT, XYZ, SBS and DQW made substantial contributions to conception and design, and acquisition of data, or analysis and interpretation of data. All authors were involved in drafting the manuscript or revising it critically for important intellectual content, and have given final approval of the version to be published.

Ethics approval and consent to participate

The present stud was approved by the Ethics Committee of Harbin Medical University (Harbin, China) and informed consent was obtained from all patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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