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Up-regulation of miRNA-105 inhibits the progression of gastric carcinoma by directly targeting SOX9

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Abstract. – OBJECTIVE: MicroRNAs (miRNAs) are involved in the tumorigenesis and progression of multiple tumor types and function as either tumor suppressor genes or oncogenes. This study was designed to investigate the functional behaviors and regulatory mechanisms of miR-105 in the progression of gastric carcinoma.

PATIENTS AND METHODS: 24 pairs of patients with gastric carcinoma were enrolled in this study. The levels of miR-105 in gastric carcinoma tissues and cells were determined using quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) assay. The biological functions of miR-105 in gastric carcinoma cell were detected by colony formation, transwell invasion and wound-healing assay. Luciferase activity assay and immunoblotting assay were applied to validate the direct target of miR-105. The expression of SRY-Box 9 (SOX9) was detected using immunofluorescence staining assay. Furthermore, the role of miR-105 on the growth of gastric carcinoma cell was examined in the established xenograft model. The role of miR-105 in the metastasis of gastric carcinoma cel*l *in vivo*, **an experimental metastasis assay was performed.

RESULTS: Herein, we proved that miR-105 was down-regulated in gastric carcinoma specimens as well as gastric cancer cells. Up-regulation of miR-105 suppressed the colony formation and aggressiveness traits of gastric carcinoma cell lines BGC823 and SGC7901 *in vitro*. Furthermore, over-expression of miR-105 inhibited the tumor growth as well as lung metastasis of gastric carcinoma cell in vivo. Further investigation identified SOX9 was the target gene of miR-105 in gastric cancer and its expression was negatively associated with the expression of miR-105 in gastric carcinoma tissues. Finally, overexpression of SOX9 partially reversed the influence of miR-105 on the growth and aggressiveness of gastric carcinoma cell.

CONCLUSIONS: These results revealed the crucial role of miR-105 in the progression and metastasis of gastric carcinoma, which indicated the potential application of miR-105 in the treatment of gastric carcinoma.

Key Words:
MiRNA-105, Gastric carcinoma, SOX9, Metastasis, Invasion.

Introduction

Gastric carcinoma, which belongs to a highly heterogeneous disease, includes a variety of clinical behaviors as well as a range of pathological entities[1,2]. These features are based on genetic alterations that result in altered cellular processes[3]. Therefore, the molecular mechanism of gastric carcinoma progression is still unclear, which brings enormous challenges for the diagnosis and treatment[4,5]. Recently, the hallmark of the tumor has become highly influential in the investigations about gastric carcinoma. The 6 markers of malignant cancer are unique and complementary to the ability to cause the infinite growth and metastatic spread of cancer cell[6]. Maintaining proliferative signaling is one of the necessary abilities during the metastasis of gastric carcinoma, which facilitates tumor growth. Meanwhile, cancer metastasis is proved to be the important prognostic indicator for prostate cancer, gastric carcinoma and a variety of tumor types[7]. MicroRNAs (miRNAs), which are

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endogenous short non-coding RNAs, exert crucial regulatory roles through binding to their target genes[8,9]. Therefore, dysregulation of miRNAs often involves many diseases, including multiple cancers. Previous studies[10,11] have clarified that miRNAs participate in various cellular processes of gastric carcinoma, such as growth, apoptosis, stem cell turnover, epithelial-mesenchymal transition (EMT) and metastasis. MiR-105 has been proved to be significantly dysregulated in many cancers and functions as an oncogene or a tumor suppressor[12]. MiR-105 inhibits cancer cell proliferation in hepatoma carcinoma, and promotes the metastasis of melanoma cell[13]. In addition, miR-105 was substantially down-regulated in glioma, and the low expression of miR-105 is related to the unfavourable prognosis of the patient with glioma[14]. Nevertheless, the potential function of miR-105 in gastric cancer progression, especially its actions in the growth, metastasis and progression of gastric carcinoma, have not been fully understood. In the current work, we verified that miR-105 was down-expressed in gastric carcinoma tissue and over-regulation of miR-105 inhibited gastric carcinoma cell growth both *in vitro* and *in vivo*. Further investigations indicated that miR-105 inhibited the proliferation and aggressiveness of gastric carcinoma cell *in vitro*, as well as tumorigenesis and metastasis *in vivo*. In addition, SRY-Box 9 (SOX9) had been shown to be the target regulator of miR-105. Therefore, our results are helpful for better understanding the molecular mechanisms underlying the development and progression of gastric carcinoma.

Materials and Methods

Cell Culture

Gastric cancer lines (MKN-28, MKN-45, BGC823 and SGC7901) and human gastric epithelial cell line, GES-1 was obtained from Nanjing Cobioer Biotechnology Co., Ltd (Nanjing, Jiangsu, China). Cells were cultured using Roswell Park Memorial Institute-1640 (RPMI-1640; HyClone, South Logan, UT, USA) media supplemented with 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA) and 1% penicillin/streptomycin (Wisent, Quebec, Canada). GES-1 cell was cultured in MCCOY'S 5A medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% FBS and 1% penicillin/streptomycin. All cells were cultured in a humidified atmosphere of 5% CO_2 and 95% air at 37°C.

Gastric Carcinoma Tissues

Gastric carcinoma tissues and corresponding normal tissues were collected from patients who received surgical resection in the Affiliated Hospital of Jining Medical University. Tissues were snap-frozen in liquid nitrogen immediately and were stored at -80°C for future studies. Informed consent was obtained from patients with gastric carcinoma and the study protocol was approved by the Ethics Committee of Affiliated Hospital of Jining Medical University.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

RNAs were extracted using the miRNeasy miRNA isolation mini kit (Qiagen, Hilden, Germany). RNA samples were reverse transcribed using the miRCURY LNA SYBR Green Master Mix kit (Exiqon Inc., Woburn, MA, USA). Following cDNA synthesis, quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) was conducted on the ABI Prism 7500 HT using the SYBR green qRT-PCR Supermix and microRNA LNA PCR primer sets for miRNA-105 (Exiqon Inc., Woburn, MA, USA). The primers used for PCR were as follows (sense and antisense, respectively): U6: CTCGCTTCGGCAGCACA and AACGCTTCACGAATTTGCGT; miR-NC: UUCUCCGAACGUGUCACGUTT and ACGUGACACGUUCGGAGAATT; miR-105: UCAAAUGCUCAGACUCCUGUGGU and CACAGGAGUCUGAGCAUUGAUU. U6 was to evaluate the level of miRNA-105 using the $2^{-\Delta\Delta CT}$ method.

Cell Transfection

100 nM miRNA-negative control (miR-NC) or miR-105 (Ambion, Grand Island, NY, USA) was transfected into the cell using Lipofectamine RNAiMAX Transfection Reagent (Invitrogen, Carlsbad, CA, USA) for 24 h. SOX9 was cloned into the pcDNA3.1 to increase the expression of SOX9. SiRNA control (siCon) or siRNA-Sox9 (siSox9; Santa Cruz Biotechnology, Santa Cruz, CA, USA) was transfected into cells using Lipofectamine RNAiMAX Reagent (Invitrogen, Carlsbad, CA, USA). MiR-105 was cloned into the pLVX-IRES-ZsGreen1 Vector (Clontech Laboratories, CA, USA) for stable overexpression of miR-105.

Luciferase Reporter Gene Assay

The wild type (wt) 3′-UTR of SOX9 containing miR-105 binding sequences was inserted into the downstream of the Renilla Luciferase gene of the psiCHECK-2 vector (Promega, Madison,

WI, USA). The mutant type (mut) 3′-UTR of SOX9 containing the mutated binding sites was synthesized using the Mut Express® MultiS Fast Mutagenesis Kit (Vazyme, Shanghai, China). HEK293T cells were cotransfected with 3′-UTR of SOX9 and miR-105 mimics for 24 h. Finally, the Luciferase activity was detected using the Dual-Luciferase® Reporter (DLR™) assay system (Promega, Madison, WI, USA).

Migration Assay

Gastric carcinoma cells were cultured in six well plates for overnight. Then, a scratch was made by scraping confluent cell monolayer using a 100 μL pipette tip. Next, cells were cultured using the serum-free medium for 24 h. The wounds in 6-well plates were captured under a microscope at 0 hour or 24 hours, respectively.

Invasion Assay

In transwell invasion analysis, the transwell membrane was coated with Matrigel (diluted 1:4, BD Biosciences, Franklin Lakes, NJ, USA) to form a matrix barrier. 200 μL cells (2×10^3) were placed into the upper chamber and 600 μL medium containing 20% FBS was added into the lower chamber. The invaded cells were fixed using 95% ethyl alcohol and stained using 1% crystal violet for 10 minutes. The invaded cells were photographed and the number of the invaded cell was counted.

Immunofluorescence Staining Assay

Cells were fixed by pre-cold acetone and permeabilized in 0.1% Triton X-100. Cells were incubated with 1% BSA/PBS and then with SOX9 antibody (Abcam, Cambridge, MA, USA) at 4°C for 24 h. After being washed with pre-cold PBS, cells were incubated with fluorescein isothiocyanate (FITC)-conjugated secondary antibody (Beyotime Biotechnology, Shanghai, China). Nuclei were counterstained with DAPI (Beyotime Biotechnology, Shanghai, China). Images were taken using a Zeiss invert microscope (CarlZeiss, Hallbergnoos, Germany).

Immunoblotting Assay

Total proteins were extracted from cells using lysis buffer and were separated using 10% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). After that, proteins were transferred to polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). PVDF membrane was incubated with the SOX9 antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) or antibody against glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Santa Cruz Biotechnology, Santa Cruz, CA, USA) followed by incubation with horseradish peroxidase (HRP)-conjugated IgGs (Bioworld Biotechnology, Nanjing, Jiangsu, China). The target proteins were detected using the enhanced chemiluminescence (ECL) detection kit (Millipore, Billerica, MA, USA).

Xenotransplanted Tumor Model and Lung Metastasis Model

In the xenotransplanted tumor model, 100 μl SGC7901 cells (1×10^5) were transplanted into BALB/c mice. The tumor volume was measured each week after inoculation. The tumor volume was calculated using the formula: (length × width^2)/2. The isolated tumor tissue was weighted. *In vivo* experimental lung metastasis assay, 200 μL cells (1×10^5) were injected into BALB/c mice via the lateral tail vein. Mice were sacrificed after four weeks and the lung tissue was fixed using 4% paraformaldehyde and used for hematoxylin-eosin (H&E) staining analysis. All protocols involving animals were approved by the Committee for Animal Research of the Affiliated Hospital of Jining Medical University and complied with the Guide for the Care and Use of Laboratory Animals (NIH publication No. 86-23, revised 1985).

Statistical Analysis

Statistical analyses were performed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Data were presented as Mean ± SD. The differences in the two groups were analyzed using either two-tailed Student's *t*-test or one-way ANOVA followed by post-hoc Dunnett's test. $p<0.05$ was considered statistically significant.

Results

MiR-105 is Down-Regulated in Gastric Carcinoma

To explore the roles of miR-105 in the progression of gastric carcinoma, we analyzed the levels of miR-105 in a panel of gastric carcinoma cell lines using qRT-PCR assay. As shown in Figure 1A, the levels of miR-105 in gastric carcinoma cell lines were significantly down-regulated compared to that in the human gastric epithelial cell line, GES-1. Subsequently, the levels of miR-105 in gastric carcinoma tissues and corresponding normal tissues were detected using qRT-PCR analysis. Consistently, miR-105 was also down-regulated

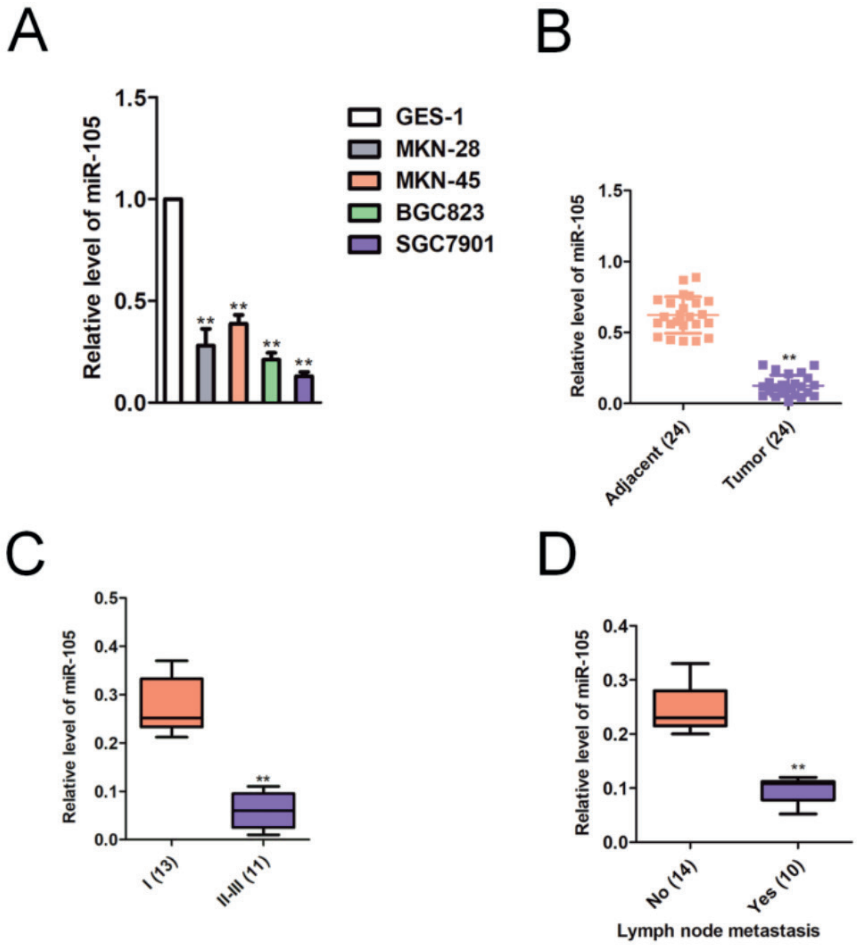

Figure 1. Expression of miR-105 in gastric carcinoma cell lines and tissues. ***A,*** qRT-PCR analysis of miR-105 level in GES-1 and gastric carcinoma cell lines. $^{**}p<0.01$ compared to GES-1. ***B,*** Comparison of miR-105 in 24 paired tumor and adjacent non-tumor tissues. The relative expression of miR-105 normalized to the internal control U6 is shown. $^{**}p<0.01$ compared to adjacent. ***C,*** The levels of miR-105 in patients of different stages were detected via qRT-PCR. $^{**}p<0.01$ compared to stage I-II. ***D,*** The levels of miR-105 in patients with or without lymph node metastasis were detected via qRT-PCR. $^{**}p<0.01$ compared to stage patient without lymph node metastasis.

in gastric cancer sample in comparison with the adjacent control tissue (Figure 1B). Importantly, the lower level of miR-105 tended to be related to an advanced stage and lymph node metastasis of gastric carcinoma (Figure 1C-1D). All these findings suggested that the miR-105 was down-regulated in gastric carcinoma sample and cell.

Up-Regulation of miR-105 Inhibits Metastasis-Relevant Traits of Gastric Carcinoma Cell

As BGC823 and SGC7901 cell lines have lower expressions of miR-105 among all tested gastric carcinoma cells, we performed the functional investigations mainly using SGC7901 and BGC823. Firstly, miR-NC or miR-105 mimics were transiently transfected into BGC823 and SGC7901 cell, and the level of miR-105 was assessed by qRT-PCR analysis (Figure 2A). As shown in Figure 2B, over-regulation of miR-105 markedly inhibited the proliferation of BGC823 and SGC7901 cell. Consistently, the clone formation of SGC7901 and BGC823 cells was significantly inhibited by miR-105 transfection (Figure 2C). Furthermore, we analyzed the impacts of miR-105 on the metastasis-relevant traits of gastric carcinoma cell *in vitro*. As shown in Figure 2D-2E, over-regulation of miR-105 markedly depressed the aggressiveness traits of BGC823 and SGC7901 cell compared to the miR-NC group as demonstrated by wound healing and transwell invasion assay. All these results indicated that miR-105 strikingly decreased the abilities of proliferation, clonogenicity and aggressiveness of BGC823 and SGC7901 cell *in vitro*.

MiR-105 Inhibits the Growth of Gastric Cancer Cell in vivo

Given that miR-105 suppresses the growth of gastric carcinoma cell *in vitro*, we constructed a subcutaneous transplanted tumor model using SGC7901 cell stably expressing miR-105. SGC7901 cell stably overexpressing miR-105 was generated by lentiviral transfection with a vector expressing miR-105. The qRT-PCR assay suggested that the level of miR-105 was effectively

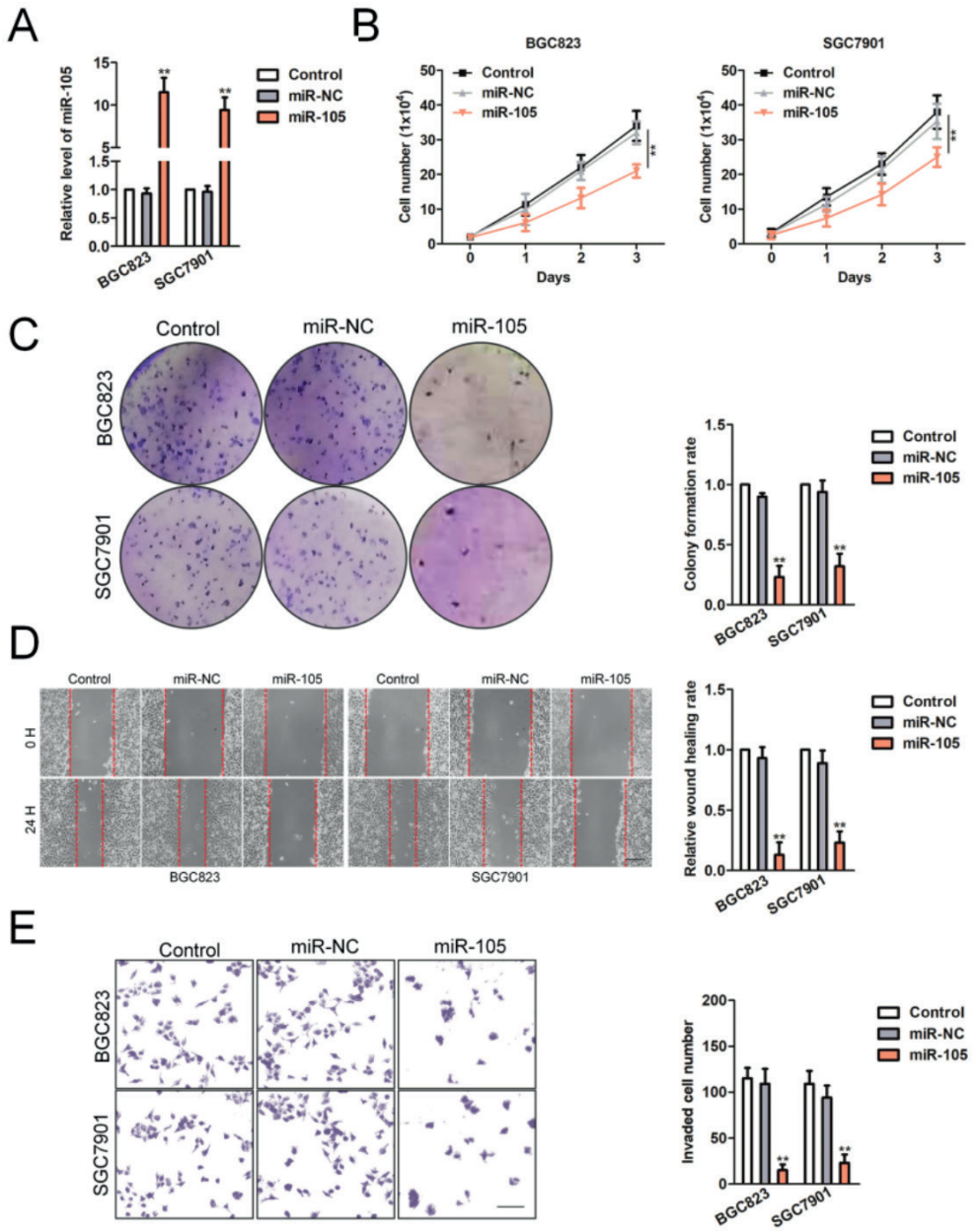

Figure 2. Overexpression of miR-105 suppresses proliferation, colony-forming, migration and invasion of gastric carcinoma cells *in vitro*. ***A,*** The level of miR-105 in BGC823 and SGC7901 cells after transfection of miR-NC or miR-105 mimics were detected using qRT-PCR assay. ***B,*** Growth curves of BGC823 and SGC7901 cells after transfection of miR-NC or miR-105 mimics for 48 h. ***C,*** Representative images of colony-forming ability in BGC823 and SGC7901 cells after transiently transfection. ***D,*** Wound healing assay of BGC823 and SGC7901 after transfection of miR-NC or miR-105 mimics. Representative images depicting the beginning ($t = 0$ h) and the end ($t = 24$ h) of the recording are shown. ***E,*** Invasion ability of BGC823 and SGC7901 transfected with miR-NC or miR-105 mimics. Cell invasion was analyzed 18 h after seeding in transwell. Data are representative of three independent experiments. $^{**}p < 0.01$ compared to stage control.

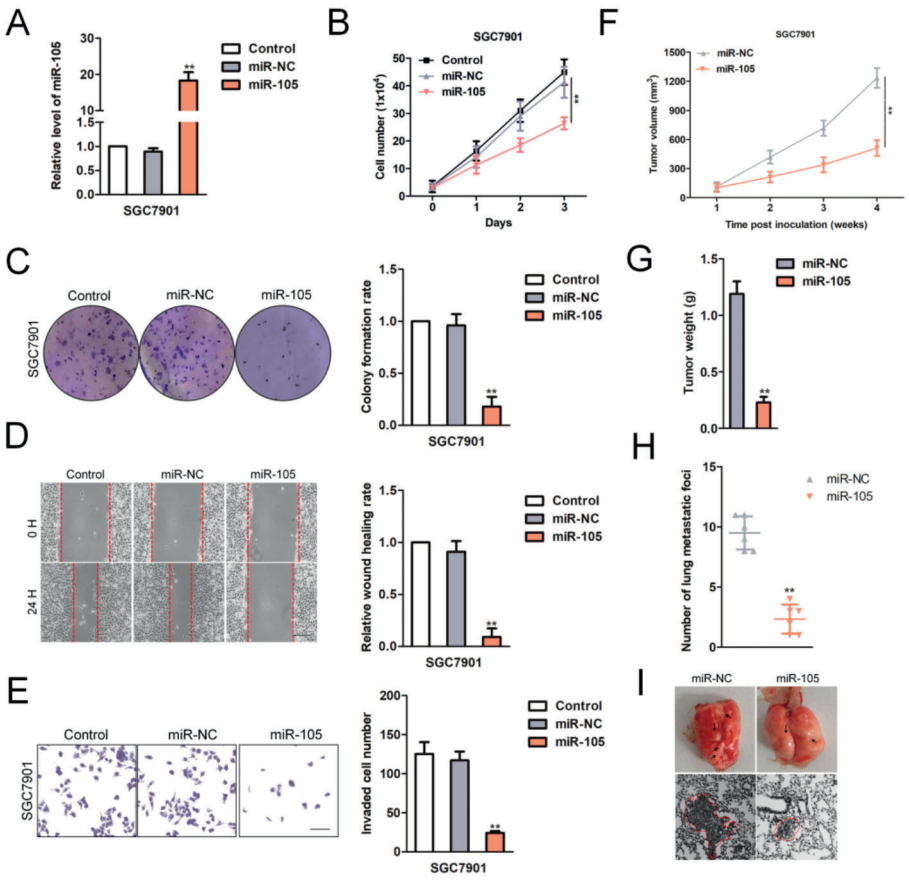

Figure 3. MiR-105 suppresses tumorigenesis and metastasis *in vivo*. ***A,*** The level of miR-105 was detected using qRT-PCR assay. ***B,*** Growth curves of SGC7901 cells. ***C,*** Colony formation assay. ***D,*** Wound healing migration analysis of miR-NC or miR-105 transfected cell. ***E,*** Transwell invasion assay. ***F,*** Tumors are separated from the indicated mice and weighted after four weeks post injection. ***G,*** Tumor weight. ***H,*** Representative lung metastasis burden of xenografted animals. ***I,*** H&E staining and H&E staining of lung tissue in miRNC and miR-105 transfected group. $^{**}p<0.01$ compared to control or miR-NC.

increased in the cell transfected with a lentiviral vector containing miR-105 (Figure 3A). As expected, the stable expression of miR-105 inhibited SGC7901 cell growth, colony formation, migration and invasion (Figure 3B-3E). Then, SGC7901 cells stably expressing miR-105 were injected into nude mice. As expected, stable expression of miR-105 significantly restrained the growth of SGC7901 cells *in vivo* (Figure 3F). In addition, the weight of the tumor that was formed by miR-105 expressing SGC7901 was markedly decreased (Figure 3G). Besides detecting the tumorigenesis *in vivo*, we also explored the influence of miR-105 in the metastasis of SGC7901 cell *in vivo*. MiR-105 or miR-NC transfected SGC7901 cell was injected into nude mice via the tail vein. As expected, miR-105 overexpression decreased lung metastasis of SGC7901 cell (Figures 3H). Meanwhile, the hematoxylin-eosin (H&E) staining using lung tissue suggested that the metastatic foci were markedly inhibited in mice that were injected with miR-105-expressing SGC7901 cell than that in the control group (Figure 3I). All these findings indicated that over-expression of miR-105 suppressed tumorigenesis as well as metastasis of gastric carcinoma cell *in vivo*.

MiR-105 Directly Targets SOX9

To investigate the target of miR-105, bioinformatics prediction software TargetScan (http://targetscan.org) was selected and we identified the 3′-untranslated regions (3′-UTR) of Sox9 contain putative miR-105 binding sequences (Figure 4A). Next, wild-type (wt) or mutant type (mut) 3′-UTR of SOX9 was inserted into the downstream of the Renilla Luciferase gene, respectively in the psiCHECK vector (Figure 4B). Then, HEK293T cells were transfected with these constructs com-

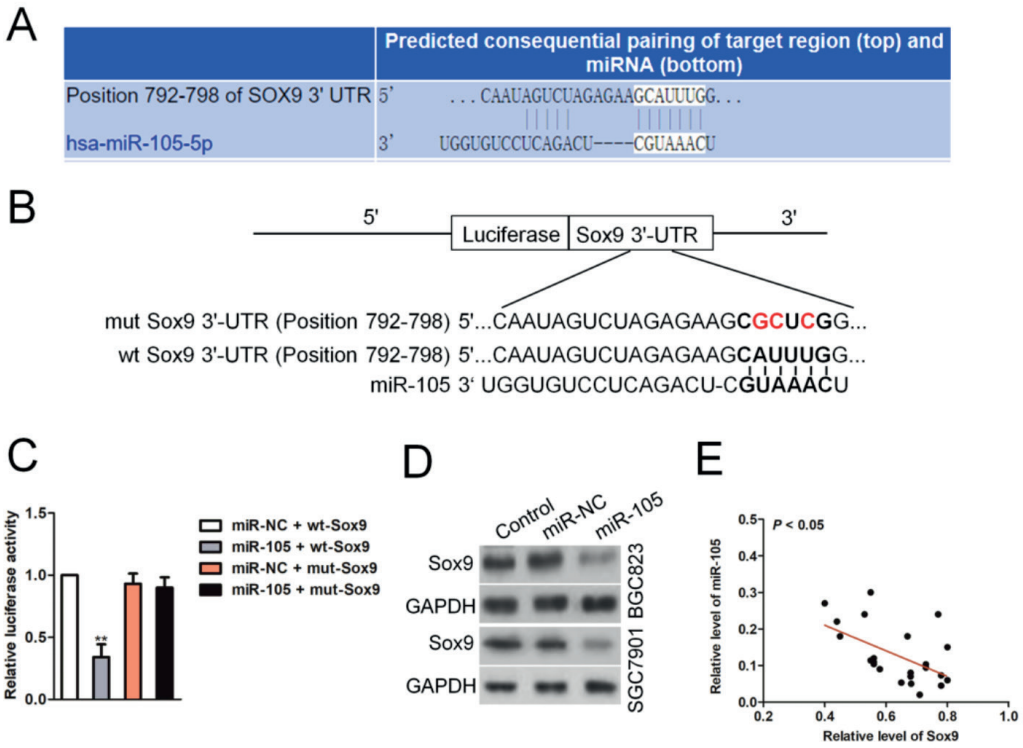

Figure 4. MiR-105 directly targets SOX9 in gastric carcinoma. ***A,*** Schematic of predicted miR-105 binding sites in human SOX9-3′UTR. ***B,*** Schematic diagram illustrating the mutant site of SOX9-3′UTR. ***C,*** The psiCHECK-2 reported plasmids are transiently transfected into HEK293T cells. Luciferase activities are measured after 24 h. $^{**}p<0.01$ compared to miR-NC + wt-Sox9. ***D,*** Western blot assays of SOX9 expression in gastric carcinoma cell lines SGC7901 and BGC823 transfected with miR-NC or miR-105, GAPDH serves as loading control. ***E,*** The correlation of miR-105 and SOX9 in clinical gastric carcinoma samples.

bination with miR-NC or miR-105. As shown in Figure 4C, miR-105 decreased the Luciferase activity of the reporter gene containing wt 3′-UTR of SOX9. Nevertheless, after being transfected with the mut 3′-UTR of SOX9, the Luciferase activity was compromised, but no significance inhibited by miR-105 transfection. Consistently, over-expression of miR-105 reduced the protein expressions of SOX9 in both SGC7901 and BGC823 cell (Figure 4D). Finally, the level of miR-105 was inversely related to the expression of SOX9 in gastric carcinoma (Figures 4E). Collectively, these findings suggested that SOX9 was the target of miR-105 in gastric cancer and its expression was negatively regulated by miR-105 in gastric carcinoma cell.

Down-Regulation of SOX9 Inhibits the Progression of Gastric Carcinoma Cell

To further explore the function of SOX9 in gastric cancer cell growth, migration and invasion, we designed siRNA against SOX9 and the protein expression of SOX9 was assessed in SGC7901 cells that were transfected with siSOX9 (Figure 5A). Then, we found that down-regulation of SOX9 also suppressed the proliferation, clone formation and aggressiveness of SGC7901 cell (Figure 5B-5E).

MiR-105 inhibits the Biological Behaviors of Gastric Carcinoma Cell Via targeting SOX9

To prove that miR-105-induced gastric carcinoma cell phenotype change dependent on SOX9, SGC7901 cell was cotransfected with SOX9 miR-105. The protein expression of SOX9 was detected using immunofluorescence staining (Figure 6A). Meanwhile, up-regulation of SOX9 rescued the growth and colony formation of SGC7901 that was inhibited by miR-105 when compared to miR-105 transfection alone (Figure 6B-6C). Consistently, the aggressiveness abilities of SGC7901 cell inhibited by miR-105 were partly rescued by the over-regulation of SOX9 (Figure 6D-6E). These findings suggested that SOX9 partially rescued the growth, migration and invasion of gastric

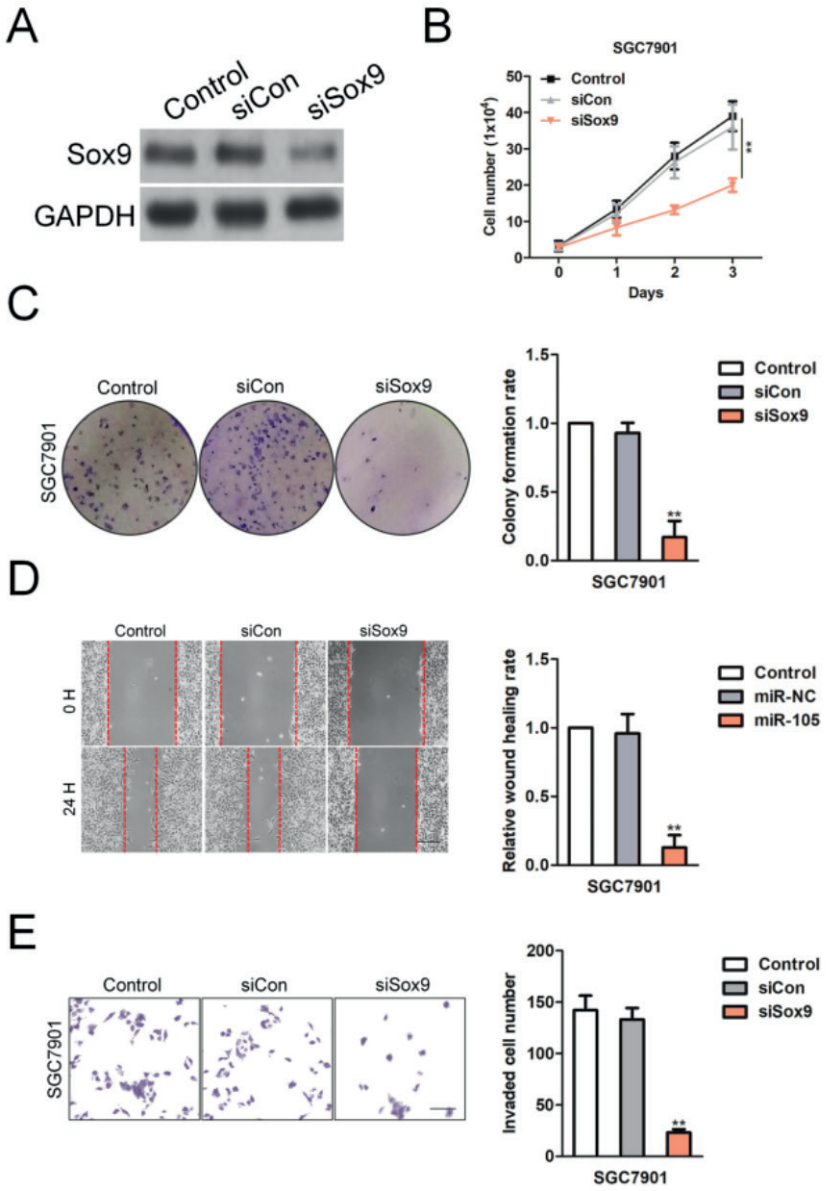

Figure 5. Dysfunction of SOX9 simulates miR-105 mediated biological behaviors in SGC7901 cells. ***A,*** Western blot assay shows the efficiency of RNAi against SOX9 in SGC7901 cells, with GAPDH as loading control. ***B,*** Effect of siSOX9 on the proliferation of SGC7901 cells. ***C,*** Representative images of colony formation in SGC7901 cells with the transfection of siSOX9. ***D,*** Representative images of wound healing migration assay of SGC7901 cells with the transfection of siSOX9. Data are representative of three independent experiments. ***E,*** Representative images of transwell invasion assay of SGC7901 cells with the transfection of siSOX9. $^{**}p<0.01$ compared to control.

carcinoma cell that were inhibited by miR-105, which indicated that SOX9 was the functional target of miR-105 in gastric carcinoma.

Discussion

MiRNAs have become significant regulators of the progression of several cancers by targeting tumor suppressor genes and/or oncogenes[15]. In the current study, we demonstrated that miR-105 was down-expressed in clinical gastric carcinoma tissues and cells. Furthermore, ectopic expression of miR-105 inhibited proliferation and metastasis-associated traits of *in vitro* as well as the growth and lung metastasis of gastric carcinoma cell *in vivo*. Mechanistic investigation indicated that SOX9 was the target gene of miR-105

in gastric carcinoma. These results confirmed that miR-105 played a vital role in the progression of gastric carcinoma. MiRNAs are highly cancer type specific, which suggests that miRNAs have different functional roles in different cancers[16]. Previous investigations[17-19] have proven that the level and action of miR-105 is different among various types of cancer. In triple negative breast carcinoma, miR-105 promotes the chemoresistance of cancer cell and circulating miR-105 functions as a diagnostic biomarker[17]. Another report[13] suggests that miR-105-1 is closely associated with the prognosis of patients with hepatocellular carcinoma (HCC) by regulating nuclear receptor coactivator 1 (NCOA1). In addition, miR-105 inhibits the progression of human glioma cell progression by targeting SOX9[14]. In this study, we found that miR-105 was down-ex-

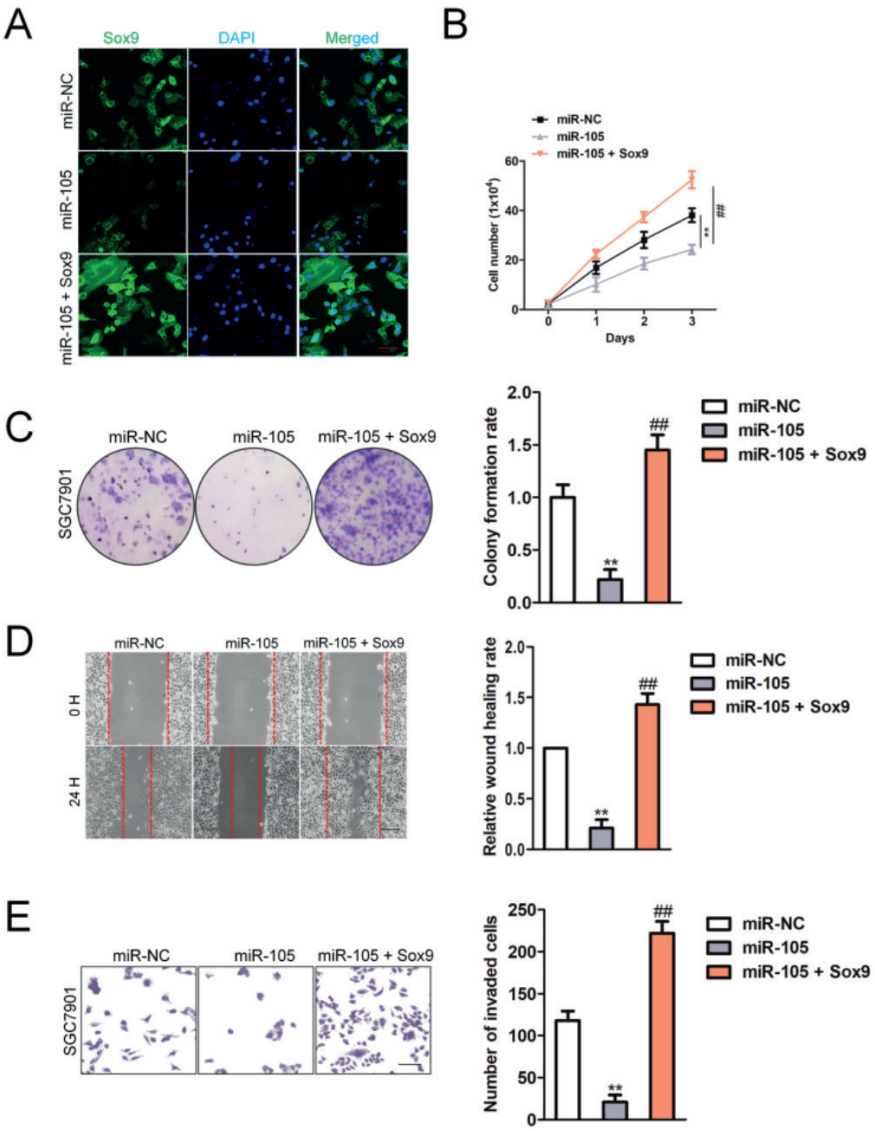

Figure 6. SOX9 re-expression attenuates miR-105 mediated inhibition of cell proliferation, colony formation, migration and invasion in gastric carcinoma cells. ***A,*** Immunofluorescence staining analyzing the expression of SOX9 in SGC7901 cells with the co-transfection of miR-NC or miR-105 together with either pcDNA3.1-EV or pcDNA3.1-SOX9. ***B,*** Growth curves of SGC7901 cells with the co-transfection of miR-NC or miR-105 together with either pcDNA3.1-EV or pcDNA3.1-SOX9. ***C,*** Colony formation ability assays of the SGC7901 cells with the co-transfection of miR-NC or miR-105 together with either pcDNA3.1-EV or pcDNA3.1-SOX9. ***D,*** Wound healing migration assays of the SGC7901 cells with the co-transfection of miR-NC or miR-105 together with either pcDNA3.1-EV or pcDNA3.1-SOX9. ***E,*** Transwell invasion assays of the SGC7901 cells with the co-transfection of miR-NC or miR-105 together with either pcDNA3.1-EV or pcDNA3.1-SOX9. $^{**}p<0.01$ compared to miR-NC, $^{\#\#}p<0.01$ compared to miR-105.

pressed in gastric carcinoma tissue compared to the corresponding normal tissue and investigated the role of miR-105 in the biological behaviors of gastric carcinoma. Our data suggested that up-regulation of miR-105 remarkably decreased gastric cancer cell growth and colony formation. Consistently, up-regulation of miR-105 markedly suppressed the invasion and migration of gastric cancer cell, suggesting the inhibitory effects of miR-105 on gastric growth and metastasis. MiRNAs acting as either tumor suppressors or oncogenes are depending on their target genes in particular tissues[20]. In our research, the bioinformatics prediction tool, TargetScan (http://www.targetscan.org/vert_72/) was selected to find the target gene of miR-105, and SRY-Box 9 (SOX9) was identified as a target of miR-105. SOX9 has been shown to play important roles in the regulation of normal embryogenesis, neural crest development and differentiation, and chondrogenesis, as well as the progression of cancer[21-23]. SOX9 is over-regulated in many different types of cancers, including chondrosarcoma, prostate cancer and breast carcinoma, and facilitates cancer cell proliferation, invasion and metastasis[24-26]. Thus, SOX9 acts as an oncogene and may be an anti-cancer target. In addition, our work proved that SOX9 dysfunction contributed to miR-105 biological functions, especially in the growth, migration as well as the invasion of gastric cancer cell. *In vivo* function assay, we found that overexpression of miR-105 decreased the tumor growth of gastric carcinoma cell in the xenograft model. To reveal the role of miR-105 on the metastasis of SGC7901 cell *in vivo*, miR-NC or miR-105 transfected SGC7901 cells were injected into nude mice via the tail vein. H&E staining analysis using lung tissue indicated that up-regulation of miR-105 significantly reduced the lung metastasis of gastric carcinoma cell *in vivo*.

Conclusions

We proved that miR-105 functions as a suppressor in gastric carcinoma. MiR-105 inhibits the growth and metastasis of gastric carcinoma cell by regulating the expression of SOX9, which indicates that miR-105 may be a potential therapeutic target in gastric cancer.

Conflict of interest

The authors declare no conflicts of interest.

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