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miR-17-5p promotes proliferation by targeting SOCS6 in gastric cancer cells

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ABSTRACT

This study aimed to test the exact functions and potential mechanisms of miR-17-5p in gastric cancer. Using real-time PCR, miR-17-5p was found to be expressed more highly in gastric cancer compared with-normal tissues. Gain- and loss-of-function assays demonstrated that miR-17-5p increased the proliferation and growth of gastric cancer cells in vitro and in vivo. Through reporter gene and western blot assays, SOCS6 was shown to be a direct target of miR-17-5p, and proliferative assays confirmed that SOCS6 exerted opposing function to that of miR-17-5p in gastric cancer. In short, miR-17-5p might function as a pro-proliferative factor by repressing SOCS6 in gastric cancer.

1. Introduction

MicroRNAs (miRNAs) are non-coding RNAs that are as small as 19–22 nucleotides in length. miRNAs can work in association with the RNA-induced silencing complex (RISC) to silence the expression of various genes by either inducing translational repression or causing degradation of the mRNAs of the targeted genes [1,2]. In the last decade, it has been widely confirmed that miRNAs can modulate multiple key cellular processes in normal cells, including cell growth, cell differentiation, cell cycle, and cell death. While in cancers, miRNA can act as oncogenes or tumor suppressors to regulate multiple malignant phenotypes such as cancer drug resistance, cancer cell proliferation, and cancer cell metastasis.

miR-17-5p is one of the members of the miRNA cluster of miR-17-92 which contains seven members (miR-17-5p, miR-17-3p, miR-20a, miR-19a, miR-19b, miR-92a and miR-18a) and was previously identified as an oncomiR-1 [3]. miR-17-5p is also one of the most studied miRNAs related to cancers in this cluster. Compared with normal tissues, higher expression of miR-17-5p has been found in various cancers such as colorectal cancer, gastric cancer, hepatocellular carcinoma, glioma, basal cell carcinoma, and pancreatic cancer [4–9]. miR-17-5p is also functionally involved in the regulation of the malignancies of multiple cancers. For example, in colorectal cancer, miR-17-5p regulates tumorigenesis, proliferation, invasiveness, and cell cycle progression by targeting various tumor suppressors [10–12]. In pancreatic cancer, cells transfected with miR-17-5p precursors showed significantly higher cell growth ratios than the corresponding control cells [13].

Although the miR-17-92 cluster has been established as oncomiRs and miR-17-5p was identified to have oncogenic abilities in several cancers, the oncogenic contribution of miR-17-5p in gastric cancer has not been assigned. The present study first found that miR-17-5p was highly expressed in gastric cancer tissues compared with the adjacent normal tissues. To test the function of the highly expressed miR-17-5p in gastric cancer, stable and transient transfection were used, and the in vitro and in vivo malignant phenotypes of gastric cancer cells were tested. Furthermore, SOCS6 was confirmed as a direct target of miR-17-5p. Our findings provide evidence for miR-17-5p's function in gastric cancer and have important implications for understanding the mechanisms of gastroenterological disorders resulting from altered modulation of miRNA pathways.

2. Materials and methods

2.1. Ethics statement

For the analyzed tissue specimens, all patients were given consent to inform the use of excessive pathological specimens for lab-

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oratory research purposes. The protocols employed in the present study were approved by the Protection of Human Subjects Committee of Xijing Hospital. The use of the human pathological specimens was approved by the Fourth Military Medical University's institutional review board and conformed to the Helsinki Declaration and the local legislation. Informed consent forms were signed by patients to offer FFPE (Formalin-fixed, paraffin-embedded) samples from gastrectomies to use in this study. For animal experiments, all procedures were performed in accordance with the guidelines of Institutional Animal Care and Use Committee of the Experimental Animal Center of the Fourth Military Medical University.

2.2. Cell culture and reagents

The cell line SGC7901 for human gastric adenocarcinoma was obtained from the Academy of Military Medical Science, and the human gastric adenocarcinoma cell line MKN28 and human embryonic kidney 293 cells were obtained from the Shanghai Cell Bank (Shanghai, China). These cell lines were preserved in our institute. The firefly luciferase stable expressing SGC7901-Luc cells were generated and preserved in our lab. All of the cells were grown in RPMI1640 (Invitrogen, Carlsbad, CA, USA) and were supplemented with 10% heat-inactivated fetal calf serum (FCS) at 37 °C with 5% CO_2 in a humidified incubator (Forma Scientific, Marietta, OH, USA). The precursors (pre-miRs) and inhibitors (miR-inhs) of miR-17-5p were obtained from Applied Biosystems (Invitrogen, Carlsbad, CA, USA).

2.3. Lentivirus miR-17-5p infection and stable cells

The lenti-viral system with EGFP expressing miR-17-5p (lenti-miR-17-5p) and the negative control lenti-vector (lenti-NC) were purchased from Genechem (Shanghai Genechem Co., Ltd., Shanghai, China). The gastric cancer cell lines SGC7901, SGC7901-Luc and MKN28 were infected with lenti-miR-17-5p or lenti-NC, according to the manufacturer's instructions, and stable cells were isolated by flow cytometry to sort EGFP-positive cells.

2.4. In vitro cell proliferation assay

The in vitro cell proliferation assay was performed as described previously [14]. A total of 10^3 cells were used for the assays in 200 μl of complete medium. The cultures were assayed each day and read at a 490 nm absorbance (A490) on a micro-plate reader (168-1000 Model 680, Bio-Rad Laboratories, Inc., Hercules, California, USA). Each experiment was performed in triplicate and repeated 3 times.

2.5. Animals and in vivo tumorigenecity assays

Four- to six-week-old BALB/C nude mice were feed in the Experimental Animal Center of the Fourth Military Medical University (Xi'an, Shaanxi Province, China) and were handled using the best humane practices and cared for in accordance with the NIH Animal Care Institutional Guidelines. Firefly luciferase labeled SGC7901 cells, —SGC7901-Luc, were infected with lenti-miR-17-5p or lenti-NC, as described above, and stable clones were isolated. To detect the tumorigenecity of the cells in nude mice, 5×10^6 cells containing lenti-miRs were injected subcutaneously into the back of each mouse; and each group contained six mice. After injection, bioluminescent signals were detected twice a week using the IVIS 100 Imaging System (Xenogen, Hopkinton, MA, USA). Five minutes before imaging, the mice were injected intraperitoneally with 100 mg/kg D-luciferin. The mice were killed four weeks after injection of gastric cancer cells. For histological analysis, the tumors generated from the nude mice were harvested and fixed in 10% formalin before paraffin embedding and were then sectioned and stained using H&E staining.

2.6. Reporter gene assay

For the reporter gene assay, cells were plated in 12-well plates and transfected with 2 μg of SOCS6 3′-UTR luciferase reporter plasmids (S) or the SOCS6 3′-UTR Anti-sense (AS) and the empty pGL3-Control vector (Promega Biotech Co., Ltd., Beijing, China) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). The cells were also co-transfected with the pre-miRNAs (150 nM, Applied Biosystems, Invitrogen, Carlsbad, CA, USA). Assays were performed 24 h after transfection using the Dual Luciferase Reporter Assay system (Promega Biotech Co., Ltd., Beijing, China), and firefly luciferase activities were normalized to Renilla luciferase activities. A microRNA precursor molecule control from Applied Biosystems (Invitrogen, Carlsbad, CA, USA) was used as one of the negative miRNA controls and was referred to as pre-miR-NC, and the miR-150 precursor molecule was used as a negative control. All experiments were performed in triplicate.

2.7. Western blot analysis

To determine the expressed protein levels, log-phase cells were harvested from 90-mm culture plates, lysed in RIPA lysis buffer (150 mM NaCl, 50 mM Tris–HCl (pH 8.0), 0.1% SDS, 2 mM EDTA, 1 mM PMSF, 1% NP40, 5 μg/ml aprotinin, and 1 μg/ml leupeptin) on ice, and then were centrifuged at 12 000 rpm for 10 min. Total proteins were resolved using 12% SDS–PAGE (Bio-Rad Laboratories, Inc, Hercules, CA, USA) and blotted onto nitrocellulose membranes (Amersham Biosciences Corp., Pittsburgh, PA, USA). The nitrocellulose membranes were then blocked with 10% non-fat powdered milk at room temperature for 2 h and incubated overnight with primary antibody: anti- SOCS6 (1:1000; Abcam plc. Cambridge, MA, USA) or anti-β-actin antibody (1:2000; Sigma–Aldrich Co., Louis, MO, USA). After three 5-min washes in Triethanolamine-Buffered Saline Solution with Tween (TBS-T), the membranes were incubated with horseradish peroxidase (HRP) – conjugated secondary antibodies (1:2000; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) for 4 h at room temperature and then washed again in TBS-T and visualized with an enhanced chemi-luminescence kit (ECL-kit, Santa Cruz Biotechnology, Inc., Dallas, TX, USA). All experiments were performed in triplicate.

2.8. RNA extraction and quantitative real-time PCR (qRT-PCR)

Total RNA from cancer tissues was extracted from FFPE-impeded tissue sections according to the manufacturer's instructions (AM1975, Applied Biosystems, Invitrogen, Carlsbad, CA, USA). Total RNA from cells or tumors from mice was extracted using TRIZOL (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions, with RNase-free DNase. Reverse transcription was performed according to the manufacturer's instructions (D350A, TaKaRa Biotechnology (DALIAN), Co., Ltd., DaLian, Liaoning Province, China). qRT-PCR was performed to determine the expression levels of each miRNA using the exact sequences (U to T) of the miRNAs as the forward primers and a unique q-PCR primer from the cDNA Synthesis Kit as the reverse primer. U6 was used as the internal control for the miRNAs, and beta-actin was used as the internal control for SOCS6. Each plate contained one cDNA sample for each primer as a calibration sample. In the experiments with cell lines, the calibration sample was cDNAs from a certain SGC7901 cell line. All experiments were performed in triplicate. Gene expression was calculated as previously reported [15].

2.9. Statistical analysis

Continuous variables were compared by Student's *t* test or the ANOVA test. If the test result of the variance homogeneity between the groups was significant, the Mann–Whitney test was appropriately adopted. In samples with small size ($n < 30$) and with non-normal distribution and/or elevated dispersion, we also used non-parametric statistics. The correlations among various genes were analyzed with Spearman's correlation analysis. Two-tailed *P*-values < 0.05 were considered statistically significant ($^{*}P < 0.05$; $^{**}P < 0.01$). All statistical analyses were conducted using the SPSS software, version 14.0 (Chicago, Illinois, USA).

3. Results

3.1. miR-17-5p was over-expressed in gastric cancer tissues compared with normal tissues

miR-17-5p was previously regarded as a member of the oncomiR-1 cluster and was over-expressed in various cancers. Therefore, we wondered whether it is also over-expressed in gastric cancer when compared with normal tissues. Real-time PCR was used to test the miR-17-5p expression in gastric cancer tissues and the adjacent normal tissues using RNAs from 28 patients who underwent gastrectomy and were followed up at Xijing Hospital during 2004–2008. We used $2^{-\triangle\triangle Ct}$ analysis to statistically describe the expression levels of miR-17-5p. As shown in Fig. 1A, the results verified that the expression of miR-17-5p was higher in 18 of 28 randomly selected human gastric cancer tissues compared with the adjacent normal tissues. The data was also shown in a simplest plot indicated in Supplementary Fig. 3.

To test if miR-17-5p expression is associated with the survival of gastric cancer patients, we also compared the miRNA expression data with the overall survival data of the gastric cancer patients'. The results showed that miR-17-5p expression was not associated with the overall survival of gastric cancer patients, with a *P* value of 0.264 and HR (95% CI) = 1.005 (0.996–1.013). To test the relationship between miR-17-5p expression and the clinic pathological statistics in gastric cancer patients, we performed a Mann–Whitney test on the statistics on the clinic-pathology and expression of miR-17-5p. However, the expression of miR-17-5p was neither associated with the overall survival of gastric cancer patients nor associated with clinic-pathological statistics such as age, gender, TNM stage, lymph node metastasis, or remote metastasis, as shown

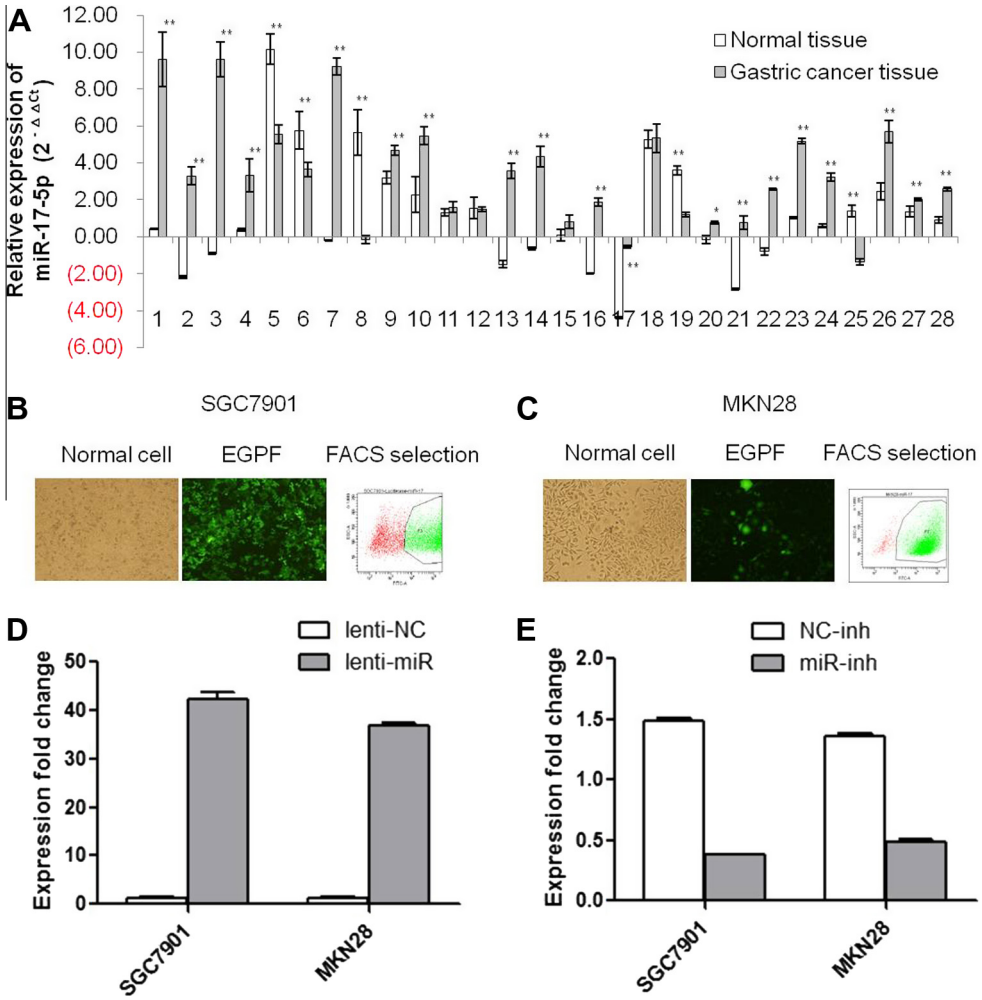

Fig. 1. miR-17-5p is over-expressed in gastric cancer tissues compared with normal tissues. (A) Real-time PCR shows the expression of miR-17-5p in 28 randomly selected gastric cancer samples compared with adjacent normal tissues ($^{**}P \leqslant 0.01$, $^{*}P \leqslant 0.05$). Each experiment was independently repeated at least 3 times. Error bars correspond to the mean ± S.D. (B and C) Infection of lenti-miR-17-5p in SGC7901 (B) and MKN28 (C) cells was confirmed using GFP under a fluorescence microscope and sorted using flow cytometry for GFP-positive cells to enrich the miR-17-5p overexpressing cells. (D) Successful stable over-expression of lenti-miR-17-5p in the gastric cancer cell lines SGC7901 and MKN28 was confirmed using real-time PCR. The fold change was used to calculate each of the values of miR-17-5p relative to the negative control (lenti-NC). Each experiment was repeated at least 3 times. Error bars correspond to the mean ± S.D. (E) Successful transient knock down of miR-17-5p inhibitors (miR-inh) in the gastric cancer cell lines SGC7901 and MKN28 was confirmed using real-time PCR. The fold change was used to calculate each of the values of miR-17-5p relative to the negative control (NC-inh). Each experiment was repeated at least 3 times. Error bars correspond to the mean ± S.D.

in Supplementary Table 1. However, the expression of miR-17-5p was associated with the differentiation degree of gastric cancer ($P = 0.003$).

3.2. Over-expression of miR-17-5p promoted gastric cancer growth and proliferation in vitro

To investigate whether miR-17-5p could have a functional effect on cell growth and proliferation, we stably infected the gastric cancer cell lines SGC7901 and MKN28 with lentivirus containing pre-miR-17-5p as shown in Fig. 1B and C. The successful over-expression of the mature miR-17-5p was confirmed via real-time PCR (Fig. 1D). The basic expression of miR-17-5p in SGC7901 and MKN28 cells was analyzed using real-time PCR and shown in Supplementary Fig. 1. Using the MTT (Fig. 2A and B) and colony formation assays (Fig. 2C and D), we found that cells stably over-expressing lenti-miR-17-5p proliferated faster than lenti-NC-infected cells. To test the knock down function of these miRNAs, we transiently transfected miR-17-5p inhibitors into the same gastric cancer cell lines SGC7901 and MKN28 (Fig. 1E) and tested their function using the above-mentioned proliferation assays. Conversely, using the MTT assay (Fig. 2A and B) and colony formation assay (Fig. 2C and D), we found miR-17-5p inhibitors caused cancer cells to proliferate slower than the negative control .To avoid the cellular changes caused by experimental artifacts, we also performed sense-transient transfections using miR-17-5p precursors and tested the cell proliferative functions. Consistent with stably expressing cells, the transient transfection of miR-17-5p promoted proliferation, as shown by the MTT and colony formation assays and indicated in Fig. 3A and B.

3.3. miR-17-5p exerted its function via targeting SOCS6

To identify direct targets of miR-17-5p for modulating growth and proliferation in gastric cancer, bioinformatics analysis was used. In silico analysis using the MiRanda software (http://www.microrna.org/microrna/home.do) showed that the 3′-UTR of SOCS6 contains conserved putative target sites for miR-17-5p as shown in Fig. 3C. To further validate this target site, the 3′-UTR of human SOCS6, in the sense (S) or antisense (AS) orientation, was inserted downstream of the luciferase gene in the pGL3-Control vector, providing Luc-Sense and Luc-Anti-sense respectively, which are referred to as Luc-S and Luc-AS, respectively in Fig. 3D.

Reporter gene assays showed that transfecting HEK-293 cells with miR-17-5p significantly decreased the expression of Luc-S, but caused no effect on Luc-AS expression (Fig. 3D). In contrast, miR-150 did not exhibit any effect on the expression of Luc-S, (Fig. 3D), in accordance with the fact that the 3′-UTR contains no miR-150 target sites. To further prove the targeting of miR-17 on SOCS6 professionally, we performed reporter gene assay using the site-mutation of 3′ UTR of SOCS6. As shown in Supplementary Fig. 2, miR-17 miRNAs could bind the wt sequence of SOCS6 3′ UTR, but could not bind to the mutated 3′ UTR of SOCS6. In this case, miR-150 miRNA also did not have effect on the expression of SOCS6 3′ UTR.

To evaluate the down-regulating effect of miR-17-5p on SOCS6, we performed Western blot analysis using anti-SOCS6 antibody. As expected, transfecting SGC7901 cells with pre-miR-17-5p decreased SOCS6 levels (Fig. 3E), whereas transfecting cells with miR-17-5p inhibitory RNAs had the opposite effect. These findings suggest that the miR-17-5p miRNA regulates SOCS6 expression in vivo at the post-transcriptional level.

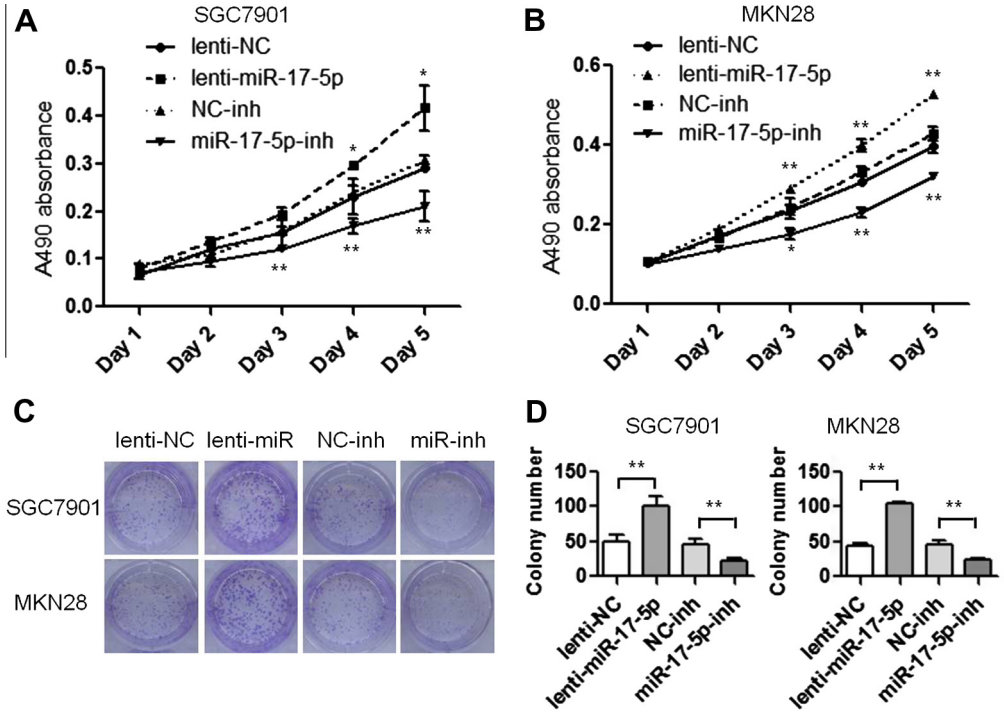

Fig. 2. Over-expression of miR-17-5p promoted gastric cancer growth and proliferation in vitro. (A and B) Cell proliferation was measured using the MTT assay with lenti-miR-17-5p and miR-17-5p inhibitors in SGC7901 (A) and MKN28 (B) gastric cancer cells and compared with that of the negative controls respectively (lenti-NC or NC-inh $^{**}P \leqslant 0.01$, $^{*}P \leqslant 0.05$). Each experiment was independently repeated at least 3 times. Error bars correspond to the mean ± S.D. (C and D) Colony formation assays were performed to evaluate the proliferative functions of lenti-miR-17-5p-infected cells or miR-17-5p inhibitor-transfected cells in SGC7901 (A) and MKN28 (B) gastric cancer cells and compared with the negative controls respectively (lenti-NC or NC-inh). Representative images show the colony intensities of cells using 150 cells/well in 24-well plates (C) and the obtained colony numbers (D). ($^{**}P \leqslant 0.01$). Each experiment was independently repeated at least 3 times. Error bars correspond to the mean ± S.D.

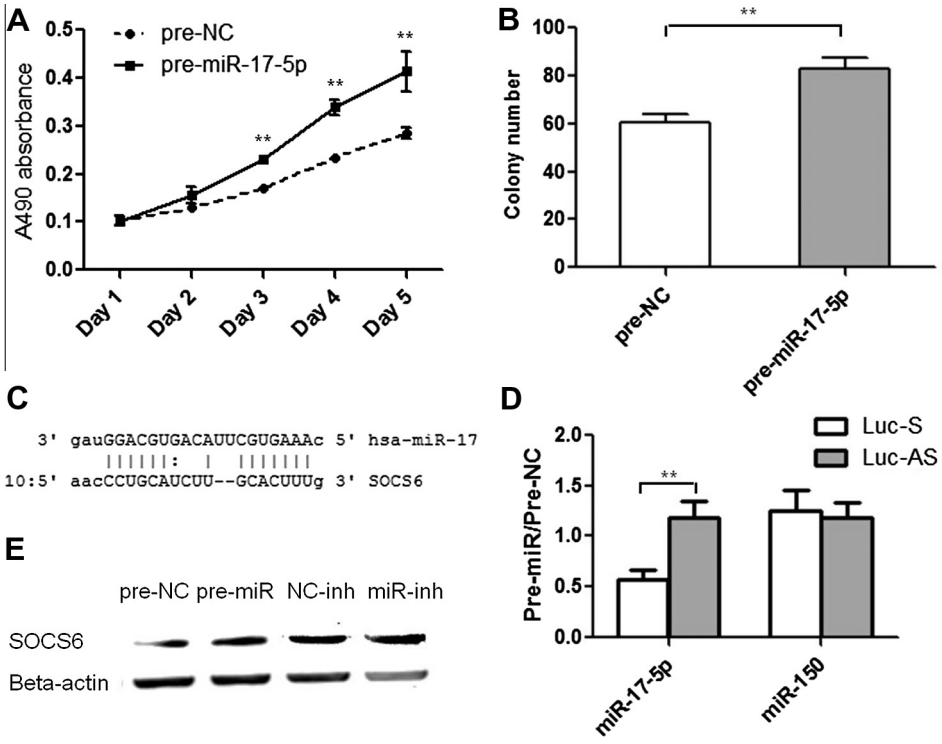

Fig. 3. miR-17-5p exert its function via targeting SOCS6. (A) Cell proliferation of pre-miR-17-5p-transfected SGC7901 gastric cancer cells was measured using MTT assay and compared with the negative control (pre-NC) (**$P \leqslant 0.01$). Each experiment was independently repeated at least 3 times. Error bars correspond to the mean ± S.D. (B) Colony formation assays were performed to evaluate the proliferative functions of pre-miR-17-5p-transfected cells compared with the negative control (pre-NC) in SGC7901 cells. The obtained colony numbers are shown (**$P \leqslant 0.01$). Each experiment was independently repeated at least 3 times. Error bars correspond to the mean ± S.D. (C) Schematic representation of the SOCS6 3′-UTR constructs. The conserved putative target sites for miR-17-5p miRNAs are indicated. (D) Luciferase assays were performed for SOCS6 in the sense (S) and antisense (AS) orientations, as indicated. Bars indicate the ratios of the firefly luciferase (normalized to Renilla luciferase) activity that was measured following transfection with miR-17-5p pre-miRNAs to that obtained following transfection with the pre-miR control (pre-NC) for the same construct (**$P \leqslant 0.01$, compared with the AS group, *t* test). Each experiment was repeated at least 3 times. Error bars correspond to the mean ± S.D. (E) Western blot showing the changes in SOCS6 protein levels after transient transfection of miR-17-5p precursors or inhibitors compared with the negative controls (pre-NC or NC-inh, respectively).

3.4. Over-expression of miR-17-5p increased tumorigenecity in nude mice

To validate the results of the in vitro cell proliferation assays, we performed in vivo assays to evaluate the tumorigenic effect of miR-17-5p in BALB/C nude mice using a luciferase-labeled lenti-miR-17-5p-infected SGC7901-Luc cell line. The expression efficiency of miR-17-5p was confirmed by real-time PCR, as shown in Fig. 4A The results showed that lenti-miR-17-5p-infected cells showed a pro-proliferative tendency in nude mice: stronger luciferase signals on tumors generated from lenti-miR-17-5p-infected cells were observed compared with the lenti-NC infected group as indicated in Fig. 4B and C.

The histological analyses of the tumors were confirmed using H&E staining (Fig. 4D). Immunohistochemistry showed that the expression of the miR-17-5p target gene SOCS6 was decreased in the lenti-miR-17-5p – treated group compared with the NC-treated group, as indicated in Fig. 4D. Real-time PCR was further used to determine the expression of miR-17-5p in these tumors. The results showed that tumors injected with lenti-miR-17-5p-infected SGC7901-Luc cells exhibited increased expression of miR-17-5p compared with the NC-treated group, as shown in Fig. 4E.

3.5. Over-expression of SOCS6 without its 3′ UTR impaired the miR-17-5p effects

To validate that SOCS6 is a target of miR-17-5p, we performed real-time PCR using RNAs from 28 paired malignant and normal tissues. As shown in Fig. 5A, the expression of SOCS6 adversely correlated with the expression of miR-17-5p ($P = 0.01$).

To further prove that SOCS6 is a downstream target of miR-17-5p, we performed cell proliferation assays using SGC7901 cells transfected with pre-NC, pre-miR-17-5p alone, or pre-miR-17-5p with SOCS6 without its 3′UTR. The results from MTT (Fig. 5B) and colony formation assays (Fig. 5C and D) showed that co-transfection of SOCS6 without its 3′UTR restricted the effects of pre-miR-17-5p transfection alone. In another word, the over-expression of miR-17-5p was rescued by over-expression of SOCS6 without its 3′UTR.

4. Discussion

miRNAs are non-coding RNAs that are 19–24nt in size [1]. The small molecular size of these RNAs makes it easier to transfer them into mammal bodies. In recent years, miRNAs have emerged as important regulators in the development of cancers [16]. Gastric cancer is the second largest cancer worldwide. However, the underlying mechanisms in the development of gastric cancer have not been fully elucidated. Understanding the molecules and pathways involved in the progression of gastric cancer will help in developing new biomarkers for diagnosis and new targets for therapeutic strategies. In the present study, we provided evidence that miR-17-5p might play significant roles in the growth and proliferation of gastric cancer.

Previous studies have found that miR-17-5p was over-expressed in various cancers [4–9]. The potential function of miR-17-5p was also partly explored in several studies. For example, Ma et al. found that miR-17-5p is a key factor in regulating colorectal cancer progression through regulating tumorigenesis

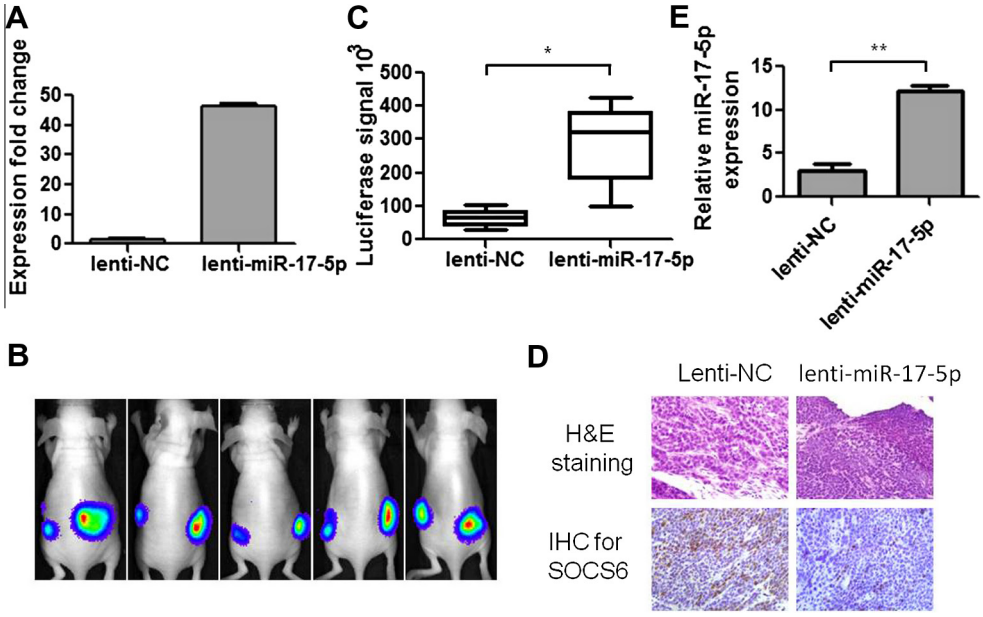

Fig. 4. Over-expression of miR-17-5p increased tumorigenicity in nude mice. (A) Successful over-expression of mature miR-17-5p in SGC7901-Luc cells was confirmed by real-time PCR. The relative fold change was used to calculate each of the values of miR-17-5p relative to the negative control. Each experiment was repeated at least 3 times. Error bars correspond to the mean ± S.D. (B and C) Representative images of mice four weeks after injection using the IVIS 100 system (B) and the detected luciferase signals (C). The left flanks of the mice were treated with lenti-NC, and the right flanks were treated with lenti-miR-17-5p. ($^{*}P \leqslant 0.05$ compared with lenti-NC infected cells, *t* test). Error bars correspond to the mean ± S.D. (D) Hematoxylin and eosin staining (H&E staining) and immunohistochemistry staining for SOCS6 of tumors generated from mice injected with lenti-miR-17-5p over-expressing cells or negative-control cells (lenti-NC). (E) The relative expression of miR-17-5p in tumors generated from mice injected with lenti-miR-17-5p over-expressing cells or the negative control cells (lenti-NC) were measured using real-time PCR. ($^{**}P \leqslant 0.01$, compared with lenti-NC-infected cells, *t* test) Each experiment was repeated at least 3 times. Error bars correspond to the mean ± S.D.

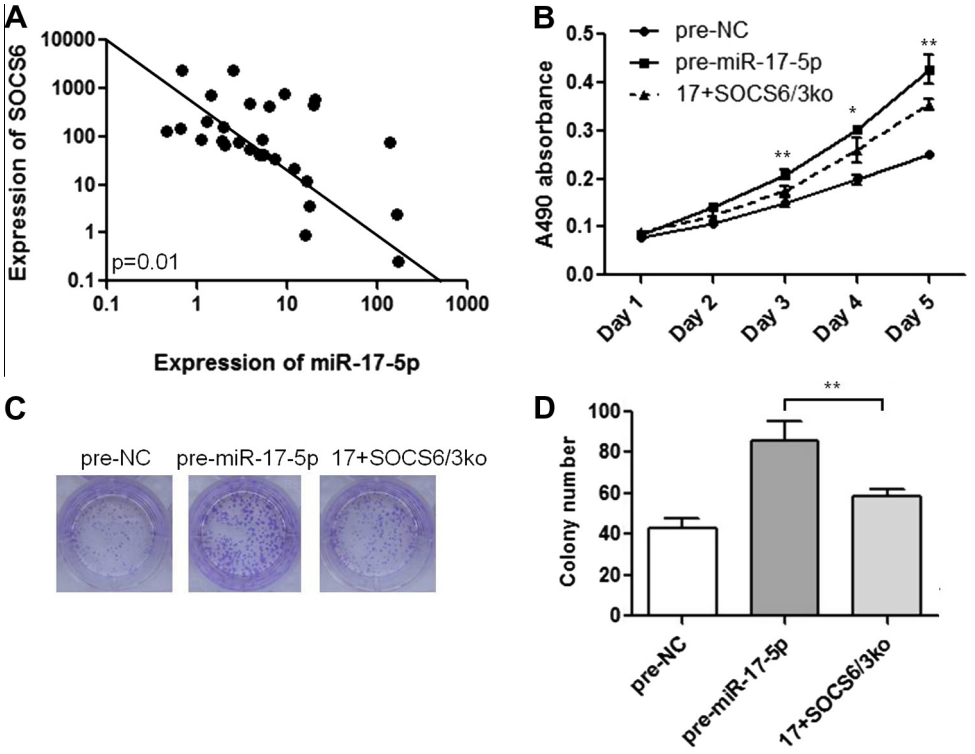

Fig. 5. Over-expression of SOCS6 without its3′ UTR impaired the miR-17-5p effects. (A) Correlation of SOCS6 expression with miR-17-5p expression in the 28 paired gastric cancer samples. The SOCS6 expression was reversely correlated with miR-17-5p expression. (B) MTT assays were used to measure the proliferative abilities of cells co-transfected with pre-miR-17-5p, pre-miR-17-5p with SOCS6 without its 3′UTR (17 + SOCS6/3ko), or the negative control (pre-NC) in SGC7901 gastric cancer cells. ($^{**}P \leqslant 0.01$, $^{*}P \leqslant 0.05$). Each experiment was independently repeated at least 3 times. Error bars correspond to the mean ± S.D. (C and D) Colony formation assays were performed to evaluate the proliferative functions of pre-miR-17-5p, pre-miR-17-5p with SOCS6 without its 3′UTR (17 + SOCS6/3ko), and the negative control (pre-NC) in SGC7901 gastric cancer cells. Representative images show the colony intensities of cells using 150 cells/well in 24-well plates (C) and the obtained colony numbers (D) ($^{**}P \leqslant 0.01$). Each experiment was independently repeated at least 3 times. Error bars correspond to the mean ± S.D.

and progression. The gene encoding P130 was found to be a target of miR-17-5p, and the Wnt/β-catenin pathway was subsequently activated. By grouping the colorectal cancer patients in two large cohorts and analysing the specimens, they also found that miR-17 expression was higher in patients who had shorter overall survival rate [10,11]. Zhang et al. also found that the miRNAs miR-21, miR-17-5p and miR-19a were elevated and STAT3 was activated in colon cancer cells when introducing the overexpression of PRL-3into the cancer cells. Subsequently, the miRNAs miR-21, miR-17-5p and miR-19a were found to contribute to the proliferative and invasive progression of colon cancer cells [11]. Up-regulated miR-17-5p promotes cell proliferative ability, tumor progression and cell cycle in colorectal carcinoma by targeting the tumor suppressive RND3 gene [12]. In pancreatic cancer, miR-17-5p was found to be overexpressed in cancer tissues compared with the normal tissues, the expression of miR-17-5p was associated with poor survival rate of pancreatic cancer patients. Moreover, in vitro experiments showed that introducing miR-17-5p precursor into SUIT-2 and KP-2 pancreatic cancer cells had significantly higher cell growth rates and higher invading cells than the control group, [13]. miR-17-5p [17] also was reported to promote human breast cancer cell migrative and invasive abilities through down-regulating of HBP1, and increases the migrative ability of hepatocellular carcinoma by regulating the p38 mitogen-activated protein kinase-heat shock protein 27 pathway [17]. In gastric cancer, previous studies have found that expression of miR-17-5p can be used as biomarkers in gastric cancer patients [18] however, the exact roles of miR-17-5p in gastric cancer cells are not fully understood. In the present study, we found that miR-17-5p is highly regulated in gastric cancer tissues and has a pro-proliferative function in the development of cancer cells.

miR-17-5p has several reported targets that are associated with cancer cell growth or cell cycle, such as E2F1, PKD2 and p21 [19–21]. The present study found that the pro-proliferative gene SOCS6 is also a potential target of miR-17-5p. SOCS6 is a member of the suppressors of cytokine signalling (SOCS) protein family which consist of negative feedback regulators of cytokine-mediated signaling in various tissues and have been demonstrated to play critical roles in tumorigenesis and tumor development in different cancers. Previous studies have found that SOCS6 was down-regulated in gastric cancer, liver cancer, and prostate cancer [22–25]; however, it was over expressed in breast cancer [26]. The differential expression of SOCS6 in various cancers might be due to the different features of cancers that are generated from different organs. In prostate cancer, the down-regulation of SOCS6 tended to be found in prostate cancer tissues with a higher Gleason score, advanced pathological stage, positive metastasis, and positive PSA failure; thus the dys-regulation of SOCS2 and SOCS6 may be related with the aggressive progression of prostate cancer [25]. In hepatocellular carcinoma, patients with low expression of SOCS2 and SOCS6 had poor prognosis. Moreover, multivariate analysis showed that SOCS2 and SOCS6 down-regulation were independent prognostic factors of overall and disease-free survival in hepatocellular carcinoma, indicating that the reduced expression of SOCS2 and SOCS6 might become potential prognostic markers in hepatocellular carcinoma [24].

Functionally, SOCS6 was reported to be targeted by the oncogenic miRNA miR-424-5p which can functionally increase the proliferation, migration and invasion in pancreatic cancer cells, indicating an anti-proliferative effect of SOCS6 in pancreatic cancer [27]. The absence of SOCS6 promotes the Ba/F3 and UT-7 cell proliferation that is induced by oncogenic internal tandem duplications of Flt3, which indicated that SOCS6 inhibited cell proliferation through Flt3 activation and the Erk signaling pathway [28]. In gastric cancer, SOCS6 ectopic expression suppressed cell proliferation and colony formation through inducing the intrinsic apoptotic pathway [23]. The present study consistently showed that, in gastric cancer, SOCS6 is an anti-proliferative factor that has the opposite effect of miR-17-5p. Additionally, the fact that co-transfection of the miR-17-5p precursor and SOCS6 without its 3′UTR impaired miR-17-5p functions in gastric cancer proliferation further indicated the mutual interaction of miR-17-5p with the 3′UTR of SOCS6.

As a last remark, miRNAs have been prevalently recognized to be a better choice for diagnostic or therapeutic targets because of their small size, which makes delivery easier, and because one miRNA has various targets and might modulate different pathways. Thus, targeting one miRNA might provide multiple effects that other molecules cannot reach. However, the exact role of each of the miRNAs in various cancers has not been specified. Only when we can understand the functions, targets, and the side effects of one miRNA in a certain cancer can we obtain better knowledge on that certain miRNA and apply it in cancer therapy. The present study provided evidence on the potential pro-proliferative function of miR-17-5p in gastric cancer and the potential mechanisms regarding this proliferation ability, which shed light on the understanding of the mechanisms of cancerous disorders resulting from altered expression of miR-17-5p miRNAs.

Conflict of interest statement

The authors have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2014.04.036.

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