Acta Biochim Biophys Sin, 2021, 53(4), 454–462
doi:10.1093/abbs/gmab015
Advance Access Publication Date: 4 March 2021
Original Article

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Circ_002059 suppresses cell proliferation and migration of gastric cancer via miR-182/MTSS1 axis

Ting Li*, Xiaomin Zuo, and Xiangling Meng

Department of General Surgery, The First Affiliated Hospital of Anhui Medical University, Hefei 230022, China
*Correspondence address. Tel: +86-551-62922114; E-mail: drliting@yeah.net

Received 21 July 2020; Editorial Decision 2 December 2020

Abstract

Circular RNAs (circRNAs) play either oncogenic or tumor suppressive roles in gastric cancer (GC). A previous study demonstrated that circ_002059, a typical circRNA, was downregulated in GC tissues. However, the role and mechanism of circ_002059 in GC development are still unknown. In this study, the levels of circ_002059, miR-182, and metastasis suppressor-1 (MTSS1) were examined by real-time quantitative polymerase chain reaction and western blot analysis. Cell proliferation and migration were evaluated by MTT assay and Transwell migration assay, respectively. The interactions between miR-182 and circ_002059 or MTSS1 were analyzed by dual-luciferase reporter assay. A GC xenograft model was established to validate the role of circ_002059 in GC progression *in vivo*. Overexpression of circ_002059 significantly inhibited, whereas knockdown of circ_002059 notably facilitated, cell proliferation and migration in GC cells. MTSS1 was found to be a direct target of miR-182 and circ_002059 upregulated MTSS1 expression by competitively sponging miR-182. Transfection with miR-182 mimic and MTSS1 silencing abated the inhibitory effect of circ_002059 on GC progression. Circ_002059 inhibited GC cell xenograft tumor growth by regulating miR-182 and MTSS1 expression. Collectively, Circ_002059 inhibited GC cell proliferation and migration *in vitro* and xenograft tumor growth in mice, by regulating the miR-182/MTSS1 axis.

Key words: gastric cancer, circ_002059, miR-182, MTSS1

Introduction

Gastric cancer (GC) is a severe malignancy with an overall 5 year survival rate below 30% [1]. Despite significant progress in the diagnosis and treatment, the prognosis of advanced GC remains unsatisfactory. Metastasis is the main cause of cancer-related death in GC patients [2,3]. Proliferation and migration are indispensable for the process of tumor metastasis. Therefore, it is crucial and urgent to elucidate the molecular mechanism underlying GC metastasis for identifying new therapeutic targets and developing novel therapeutic strategies.

Circular RNAs (circRNAs) belong to a special kind of noncoding RNA molecules with closed-loop structures by covalent binding [4]. CircRNAs have neither 5′ to 3′ polarity nor polyadenylated tail and are steadier than linear RNAs with stronger resistances to the exonuclease RNase R [5]. Increasing evidence suggests that circRNAs are dysregulated and play either oncogenic or tumor-suppressive roles in various cancers, including GC [6,7]. For example, hsa_circ_0003159 plays a suppressive role in GC progression by inhibiting cell proliferation, migration, invasion, and xenograft tumor growth and promoting apoptosis [8]. Circ_0008035 facilitates cell proliferation and inhibits apoptosis and ferroptosis in GC [9]. A previous study by Li *et al*. [10] demonstrated that hsa_circ_002059, a typical circRNA, was downregulated in GC tissues and its lower expression was significantly associated with distal metastasis, TNM stage, gender, and age. However, the functional role and mechanism of circ_002059 in GC progression are still largely unknown.

Recently, the competing endogenous RNA (ceRNA) hypothesis proposed that circRNAs, acting as ceRNA sponges, interact with microRNAs (miRNAs) to segregate miRNAs away from target mRNAs and suppress the biological functions of miRNAs [8,9]. miR-182 is involved in regulating cell proliferation, migration, and invasion in multiple types of human cancers, such as bladder can-

cer [11], prostate cancer [12], and non-small cell lung cancer [13]. Importantly, it has been shown that miR-182 is highly expressed in GC tissues and promotes cell proliferation, mitosis, migration, and invasion of GC cell lines [14,15]. In addition, the metastasis suppressor-1 (MTSS1) gene plays a key role in inhibiting tumor cell proliferation and metastasis in several types of human cancers [16,17], including GC [18]. Interestingly, Circular RNA Interactome and Targetscan online predicted that the miR-182 binding sites between circ_002059 and MTSS1 are the same. Thus, we hypothesized that circ_002059 may upregulate MTSS1 expression by competitively sponging miR-182, thereby inhibiting GC progression.

In the present study, we investigated the effect of circ_002059 on GC cell proliferation and migration *in vitro* and xenograft tumor growth in mice. Furthermore, we assessed whether the action of circ_002059 is associated with the regulation of miR-182 and MTSS1.

Materials and Methods

Cell culture

Human GC cell lines (BGC-823 and HGC-27) were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). Cells were cultured in RPMI-1640 medium (Invitrogen, Carlsbad, USA) supplemented with 10% fetal bovine serum (FBS; Invitrogen), 2.5 g/l glucose, 0.11 g/l sodium pyruvate, and 1% penicillin-streptomycin (Invitrogen) at 37°C under 5% CO_2.

Cell transfection

The circ_002059 overexpression vector (pcDNA3.1-circ_002059) and empty pcDNA3.1 vector were purchased from Guangzhou Geneseed Biotech Co. (Guangzhou, China). The si-circ_002059 (5′-UGGAUUUGUACCAUUCUUCUG-3′), scrambled siRNA negative control (si-NC) (5′-AGUUUCAACCGUCUUAAUCAG-3′), miR-182 mimic (5′-UUUGGCAAUGGUAGAACUCACACU-3′), and mimic NC (5′-UCACAACCUCCUAGAAAGAGUAGA-3′) were purchased from GenePharma (Shanghai, China).

For cell transfection, BGC-823 and HGC-27 cells in the logarithmic phase of growth were seeded into 6-well plates at a density of 5×10^5 cells per well. After 24 h of incubation at 37°C under 5% CO_2, cells were transfected with these vectors, siRNAs, or mimics according to different experimental purposes. Transfection was performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions.

Cell proliferation assay

Following transfection, BGC-823 and HGC-27 cells in the logarithmic phase of growth were seeded into 6-well plates at a density of 5×10^5 cells per well. After 48 h of incubation at 37°C under 5% CO_2, Methyl Thiazolyl Tetrazolium (MTT) (20 μl; Sigma-Aldrich, St Louis, USA) was added to each well. After 4 h of incubation, the medium was replaced by 150 μl dimethylsulfoxide (DMSO) (Sigma-Aldrich) and incubated for 10 min. Cellular viability was examined by measuring the optical density (OD) values at 570 nm using the Fluoroskan Ascent Fluorometer (Thermo Fisher Scientific, Waltham, USA).

Transwell migration assay

At 24 h after transfection, BGC-823 and HGC-27C cells were resuspended in serum-free RPMI-1640 medium. The cell suspension (100 μl) was added to the upper chambers of Transwell inserts precoated without Matrigel. A total of 500 μl medium containing 10% FBS was added into the lower Transwell chambers. After incubation for 24 h at 37°C with 5% CO_2, migrated cells were fixed with 4% paraformaldehyde and stained with crystal violet. The stained cells were photographed and counted under an inverted microscope (Leica, Wetzlar, Germany).

Dual-luciferase reporter assay

The fragments of circ_002059 or MTSS1 3′-UTR containing the predicted wild-type or mutant binding sequences of miR-182 were cloned into the pmirGLO vector (Promega, Madison, USA) to generate luciferase reporter vectors (circ_002059 WT, circ_002059 Mut, MTSS1 WT, MTSS1 Mut1, MTSS1 Mut2). HEK293 cells were co-transfected with luciferase reporter vectors and mimic NC or miR-182 mimic. At 48 h after transfection, the activities of luciferase were analyzed using a Dual-Luciferase Reporter Assay Kit (Promega).

RNA pull-down assay

The biotin-labeled circ_002059 pull-down probe and random pull-down probe sequence as negative control (NC) were synthesized. Total RNA bound to circ_002059 from cell lysate was isolated using RNA pull-down assay. Briefly, HGC-27 cells were lysed in 1 ml 0.1% NP40 lysate containing protease inhibitor. The probes were incubated with M-280 streptavidin magnetic beads (Sigma) at 25°C for 2 h to generate probe-coated magnetic beads and then incubated with the cell lysates at 4°C for 30 min. The precipitated miR-182 level was detected by real-time quantitative polymerase chain reaction (qRT-PCR).

Animal experiments

Specific-pathogen-free conditioned female athymic BALB/c nude mice (age, 6−8 weeks old; weight, 17−19 g) were purchased from the Animal Center of Anhui Medical University (Hefei, China) and maintained in a controlled temperature (23 ± 2°C), in 45%–55% relative humidity, with a 12 h day/night cycle and free access to food and water. All animal experiments were approved by the Ethics Committee of the First Affiliated Hospital of Anhui Medical University (Hefei, China).

Animals were randomly assigned into three groups ($n = 8$ per group): control group, LV-NC (an NC lentiviral vector) group, and LV-circ_002059 (a lentiviral vector stably overexpressing circ_002059) group. The LV-NC and LV-circ_002059 were obtained from GeneChem (Shanghai, China). HGC-27 cells were infected with LV-NC or LV-circ_002059 and selected using 2 μg/ml puromycin (Invitrogen). Mice in the LV-NC and LV-circ_002059 groups received a subcutaneous injection of HGC-27 cells (1×10^6) which were infected with LV-NC and LV-circ_002059, respectively. The mice in the control group received a subcutaneous injection of untreated HGC-27 cells (1×10^6). Tumor volume (V, cm^3) was monitored every week after injection using a caliper and calculated using the following formula: $V = \pi/6 \times \text{minor axis}^2 \times \text{major axis}$. Six weeks post-injection, mice were sacrificed under anesthesia with an intraperitoneal injection of 0.72 mg (45 mg/kg) pentobarbital sodium. Tumor tissues were then exercised and weighed. Then,

total RNA and protein were extracted from the tumor tissues for qRT-PCR and western blot analysis.

qRT-PCR analysis

Total RNA was isolated from cells or tumor tissues using the Rneasy Mini kit (Qiagen, Hilden, Germany), and then reverse transcribed into cDNA using a reverse transcription kit (Takara, Dalian, China). After reverse transcription, the expression levels of circ_002059 and MTSS1 were detected using the PrimeScript™ RT reagent Kit (Takara), whereas the expression of miR-182 was measured using the miRNA qRT-PCR kit (GeneCopoeia, Rockville, USA). The expression levels of candidate genes were calculated using the $2^{-\Delta\Delta Ct}$ method and normalized to the internal control *U6* (for miR-182) or *GAPDH* (for circ_002059 and MTSS1). The specific primers were synthesized by Sangon Biotechnology (Shanghai, China). The sequence of primers is listed in Table 1.

Western blot analysis

Total protein was isolated from cells or tissues using radioimmunoprecipitation assay lysis buffer. Protein samples were qualified and subject to sodium dodecyl sulfate–polyacrylamide gel electrophoresis and then transferred onto polyvinylidene fluoride (PVDF) membranes. Subsequently, the membranes were blocked in phosphate buffered saline containing 5% skim milk and then incubated with primary antibodies against MTSS1 and GAPDH (1:1000; Santa Cruz Biotechnology, Dallas, USA) overnight at 4°C, followed by incubation with horseradish peroxidase-conjugated secondary antibodies for 2 h. Finally, protein bands were visualized using enhanced chemiluminescence kit (Beyotime, Haimen, China) and analyzed using QuantityOne software.

Statistical analysis

Data were presented as the mean ± standard deviation ($n = 3$). Statistical analysis was performed using SPSS 20.0. The student's *t*-test was used for comparison between two groups, and ANOVA was used for multiple comparisons. $P<0.05$ was considered statistically significant.

Results

Circ_002059 inhibits GC cell proliferation and migration

We initially examined circ_002059 expression in four GC cell lines (including HGC-27, MKN-28, MGC-803, and BGC-823) and a gastric epithelial cell line GES-1. Compared with the GES-1 cells, GC cells express lower circ_002059 expression (**Supplementary Fig. S1A**). To examine the functional role of circ_002059 in regulating GC cell proliferation and migration, we overexpressed or silenced circ_002059 in two GC cell lines (BGC-823 and HGC-27) and detected cell proliferation and migration. The results of the qRT-PCR analysis confirmed that circ_002059 expression was remarkably upregulated following transfection with the circ_002059 overexpression vector, whereas notably downregulated following transfection with si-circ_002059 in both cells (Fig. 1A). MTT assay demonstrated that circ_002059 overexpression led to a notable decrease in OD_{570} value, whereas circ_002059 silencing significantly increased it in both cells (Fig. 1B). Transwell migration assay showed that circ_002059 overexpression notably decreased the number of migrated cells. In contrast, circ_002059 silencing yielded the opposite results in both cells (Fig. 1C).

Circ_002059 directly binds with miR-182 which targets MTSS1

To investigate the molecular mechanism by which circ_002059 inhibits GC cell proliferation and migration, we used CircInteractome database (https://circinteractome.nia.nih.gov/bin/mirnasearch) to predict the putative miRNA targets of circ_002059 and found that there are putative binding sites between circ_002059 and miR-182. In addition, TargetScan analysis (http://www.targetscan.org/vert_72/) showed that miR-182 has the binding sites of the 3′-UTR of MTSS1. Therefore, the luciferase activity assays were performed to analyze the interaction between miR-182 and circ_002059 or MTSS1. Upon transfection with miR-182 mimic, a significant decrease in luciferase activity was observed in the circ_002059 WT group but not in the circ_002059 Mut group. In addition, miR-182 mimic notably decreased the luciferase activity in HEK293 cells when co-transfected with the MTSS1 WT reporter vector. However, mutation at any of the two sites partly abrogated the inhibitory effect (Fig. 2A). Furthermore, RNA pull-down assay showed significantly higher abundance of miR-182 in probe-circ_002059 group when compared with that in the NC-circ_002059 group (**Supplementary Fig. S2**). These data indicate that circ_002059 directly binds with miR-182 which targets MTSS1.

Circ_002059 upregulates MTSS1 expression by sponging miR-182

Next, we examined whether circ_002059 acts as a sponge of miR-182 to regulate MTSS1 expression. To this end, we co-transfected GC cells with circ_002059 overexpression vector and miR-182 mimic. Transfection with miR-182 mimic notably decreased MTSS1 expression, at both mRNA and protein levels, in both BGC-823 and HGC-27 cells. However, the suppressive effect of miR-182 on MTSS1 expression was compromised when circ_002059 was overexpressed in both cells (Fig. 2B,C). These observations suggest that circ_002059 upregulates MTSS1 expression by sponging miR-182.

Table 1. Sequence of primers used in qRT-PCR analysis

Gene	Forward primer (5′→3′)	Reverse primer (5′→3′)
miR-182	GGCITTGGCAATGGTAGAAC	GTGCGTGTCGTGGAGTCG
U6	GCTTCGGCAGCACATATACTAAAAT	CGCTTCACGAATTTGCGTGTCAT
circ_002059	CCCGATAACACAAGTGCAGC	CCTGGACCTTCCACCTTCTC
MTSS1	TGGGTCCACTGAGCCCCACACATTGTTG	GGTGGCCATTGTGGGGTGGAATGAA
GAPDH	TCGACAGTCAGCCGCATCTTCTTT	ACCAAATCCGTTGACTCCGACCTT

Circ_002059 inhibits GC cell proliferation and migration by inhibiting miR-182 and inducing MTSS1 expression

Next, we determined whether circ_002059 inhibits GC cell proliferation and migration by inhibiting miR-182 function and upregulating MTSS1 expression. MTT assay and Transwell migration assay showed that the inhibitory effect of circ_002059 overexpression on cell proliferation and migration was notably compromised when the miR-182 expression was upregulated by transfection with miR-182 mimic, in both BGC-823 and HGC-27 cells (Fig. 3A,B). Meanwhile, MTSS1 silencing significantly facilitated cell proliferation and migration and also abrogated the circ_002059 overexpression-mediated inhibition on cell proliferation and migration in both cells (Fig. 4A,B). These results manifest that circ_002059 inhibits GC cell proliferation and migration by regulating the miR-182/MTSS1 axis.

Circ_002059 overexpression inhibits GC cell tumorigenesis *in vivo*

Finally, we explored the role of circ_002059 in regulating GC cell tumorigenesis *in vivo*. To address this issue, HGC-27 cells that were infected with LV-NC or LV-circ_002059 were subcutaneously injected into nude mice. Xenograft tumors were resected from mice at 6 weeks post-injection. The tumor volume and weight in the LV-circ_002059 group were decreased when compared with those in the LV-NC group (Fig. 5A), suggestive of the anti-cancer role of circ_002059 in GC. Expectedly, circ_002059 expression was significantly upregulated, whereas miR-182 expression was downregulated in the tumors formed by LV-circ_002059-infected HGC-27 cells (Fig. 5B). In addition, the MTSS1 mRNA and protein levels in tumors were significantly upregulated after injection with LV-circ_002059-infected HGC-27 cells (Fig. 5C,D). There were no statistically significant differences between the control and LV-NC group. These results suggest that circ_002059 overexpression inhibits GC cell tumorigenesis *in vivo* by regulating miR-182 and MTSS1 expression.

Discussion

GC is a severe malignancy with a poor prognosis. CircRNAs play an important role in carcinogenesis and cancer metastasis and can function as potential therapeutic targets for various cancers, including GC [6–8]. Existing work indicates that the downregulation of circ_002059 expression in GC tissues is associated with distal metastasis, TNM stage, gender, and age [10]. The TNM stage and distal

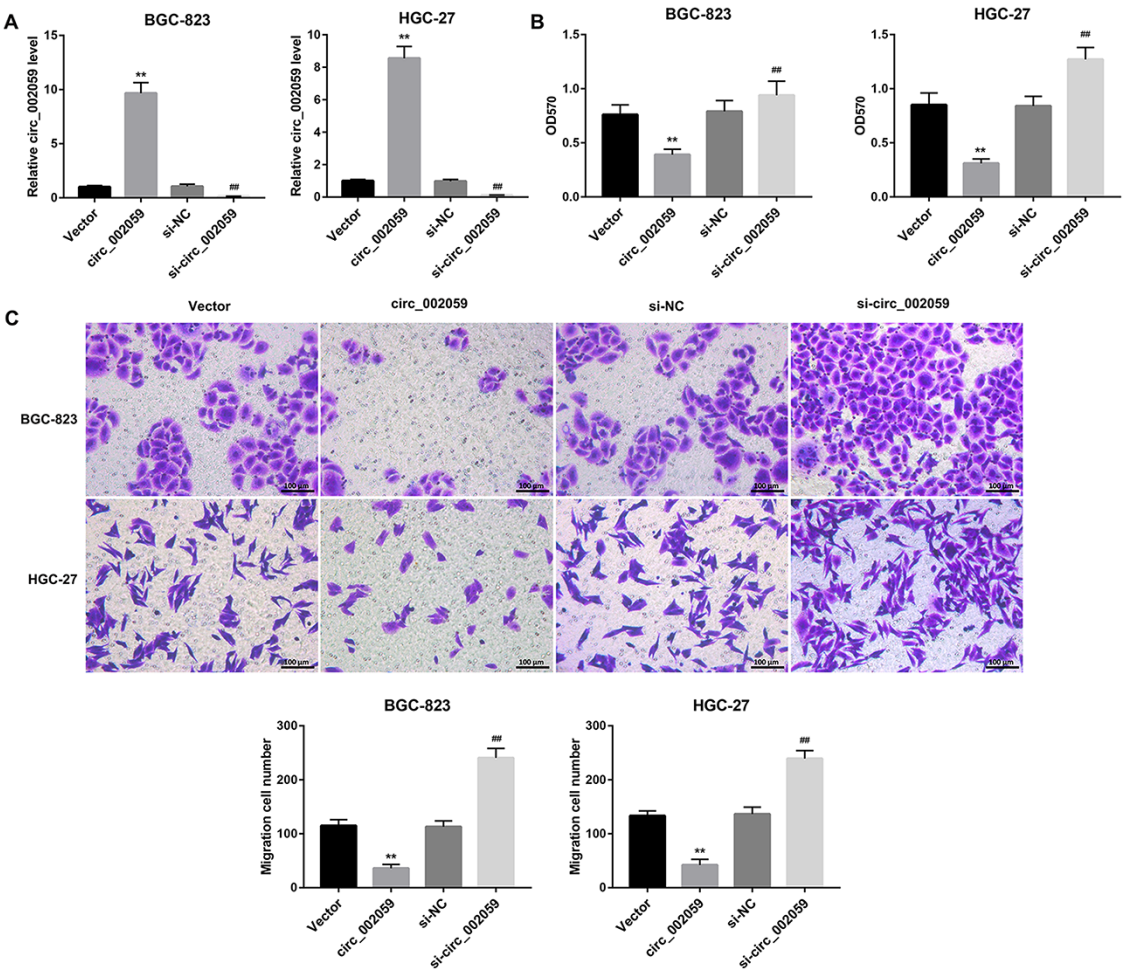

Figure 1. Effect of circ_002059 overexpression/silencing on GC cell proliferation and migration BGC-823 and HGC-27 cells were transfected with empty vector, circ_002059 overexpression vector, si-NC, or si-circ_002059. (A) Relative circ_002059 expression was determined by qRT-PCR analysis. (B) Cell viability was quantified by MTT assay. (C) Cell migration was determined by Transwell migration assay. $^{**}P<0.01$ vs Vector; $^{\#\#}P<0.01$ vs si-NC.

metastasis are crucial factors in the evaluation of the prognosis of GC. Thus, lower expression levels of hsa_circ_002059 may be associated with poor prognosis. Nevertheless, the role of circ_002059 in regulating GC progression is still largely unknown. In this study, we performed gain/loss of function studies to explore the effect of circ_002059 on GC cell proliferation and migration *in vitro* and established xenograft tumor models using HGC-27 cells to examine its effect on tumor growth *in vivo*. Our results showed for the first time that circ_002059 inhibited GC cell proliferation and migration *in vitro* and xenograft tumor growth in mice, suggesting that circ_002059 could act as a tumor suppressor in GC. Together with results from the literature, our data suggest that circ_002059 may be a potential therapeutic target in GC patients.

CircRNAs can exert their functions via acting as miRNA sponges [8,9]. Therefore, we investigated the ceRNA network mediated by circ_002059 in GC cells. miR-182 plays a critical role in the development of human cancers by regulating cancer cell proliferation, migration, and invasion [11–13]. In GC, existing studies suggested that miR-182 is highly expressed in GC tissues [15] and higher expression of miR-182 in peripheral blood of GC patients is closely associated with the clinicopathological features of GC, including tumor size, TNM stage, lymphatic metastasis, recurrence, and shorter survival time of GC patients [14]. Here, we first confirmed the direct interaction between miR-182 and circ_002059 using bioinformatics analysis and luciferase reporter assay. Furthermore, our results showed that miR-182 upregulation by miR-182 mimic transfection facilitated GC cell proliferation and migration, which is consistent with previous studies demonstrating the promoting effect of miR-182 on cell proliferation, mitosis, migration, and invasion of GC cell lines [14,15]. Importantly, our results also showed that the upregu-

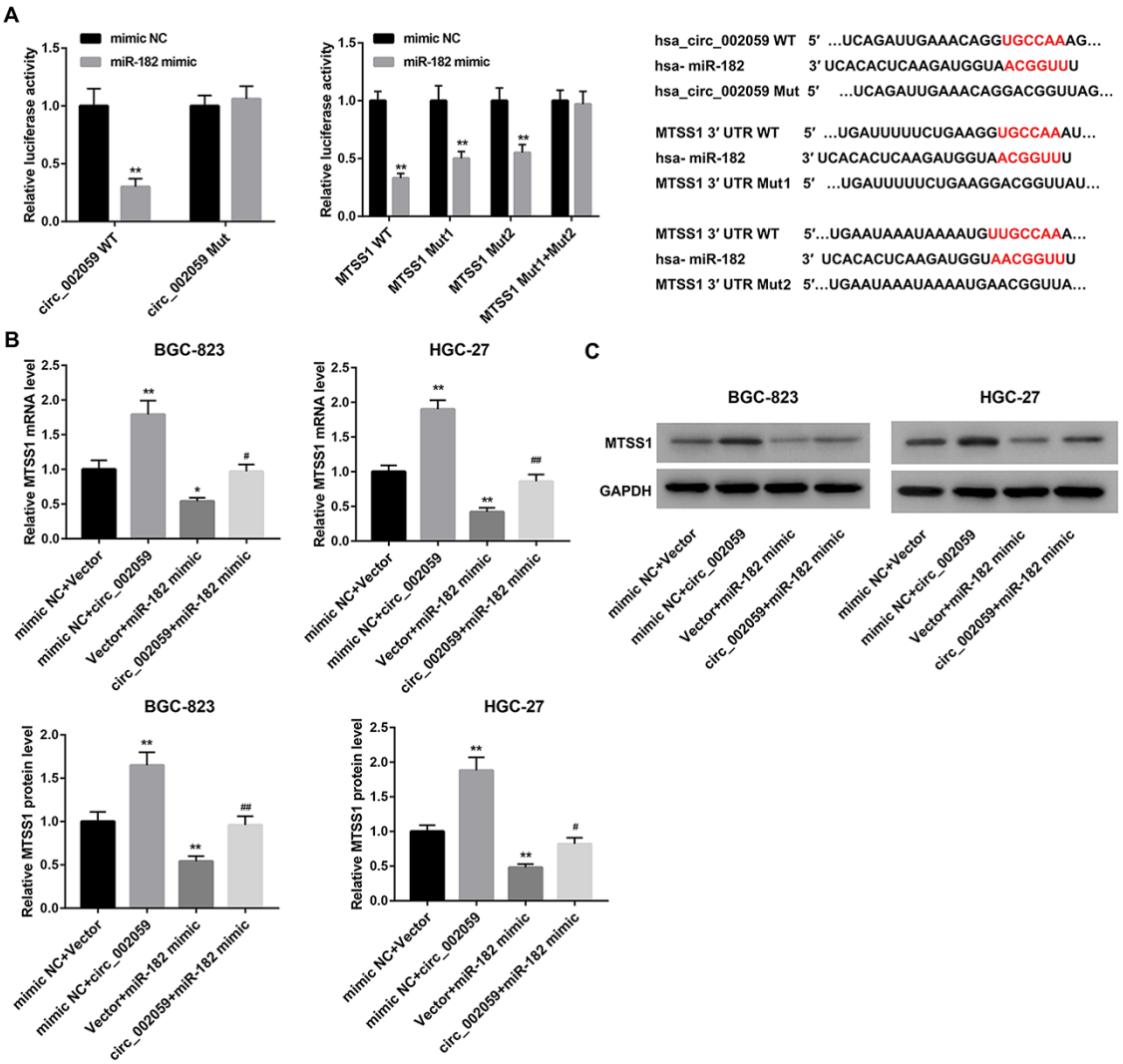

Figure 2. Circ_002059 upregulates MTSS1 expression by sponging miR-182 (A) The interaction between miR-182 and circ_002059 or MTSS1 was analyzed by luciferase activity assay. Diagrammatic sketch of the binding sites for miR-182 and circ_002059 or MTSS1 was shown (right). MTSS1 mRNA level (B) and protein level (C) in BGC-823 and HGC-27 cells co-transfected with empty vector/circ_002059 overexpression vector and mimic NC/miR-182 mimic were examined by qRT-PCR and western blot analysis, respectively. $^{*}P<0.05$, $^{**}P<0.01$ vs mimic NC or mimic NC + Vector; $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs mimic NC + circ_002059 or Vector + miR-182 mimic.

lation of miR-182 abolished the suppressive effect of circ_002059 on GC progression by its pro-cancer function. Together, these data indicate that circ_002059 inhibits GC progression by sponging miR-182 *in vitro*.

It has been reported that miR-182 promotes cell invasion and proliferation of human prostate cancer cell lines [19] and increases the invasive potential of hepatocellular carcinoma cell lines [20], via targeted suppression of MTSS1. However, it remains unclear whether MTSS1 can be targeted by miR-182 to regulate GC progression. Accordingly, we further investigated whether MTSS1 is involved in the ceRNA network mediated by circ_002059. We confirmed MTSS1 as a direct target of miR-182 and validated that circ_002059 could upregulate MTSS1 expression by competitively binding to miR-182. The *MTSS1* gene has been considered as a tumor metastasis suppressor in several cancers [16,17]. In GC, Liu *et al.* [21] reported that MTSS1 was downregulated in GC tissues and its lower expression was associated with nodal metastasis and poor outcome in Chinese patients with GC. Here, we found that MTSS1 silencing promoted GC cell proliferation and migration, indicating the suppressive role of MTSS1 in GC development. This is also in agreement with a previous study showing that MTSS1 suppresses proliferation, migration, and invasion of GC cells [18,22]. Of note, our rescue experiments showed that the inhibitory effect of circ_002059 overexpression on GC cell proliferation and migration was abrogated partially by MTSS1 silencing, indicating that circ_002059 inhibited GC progression

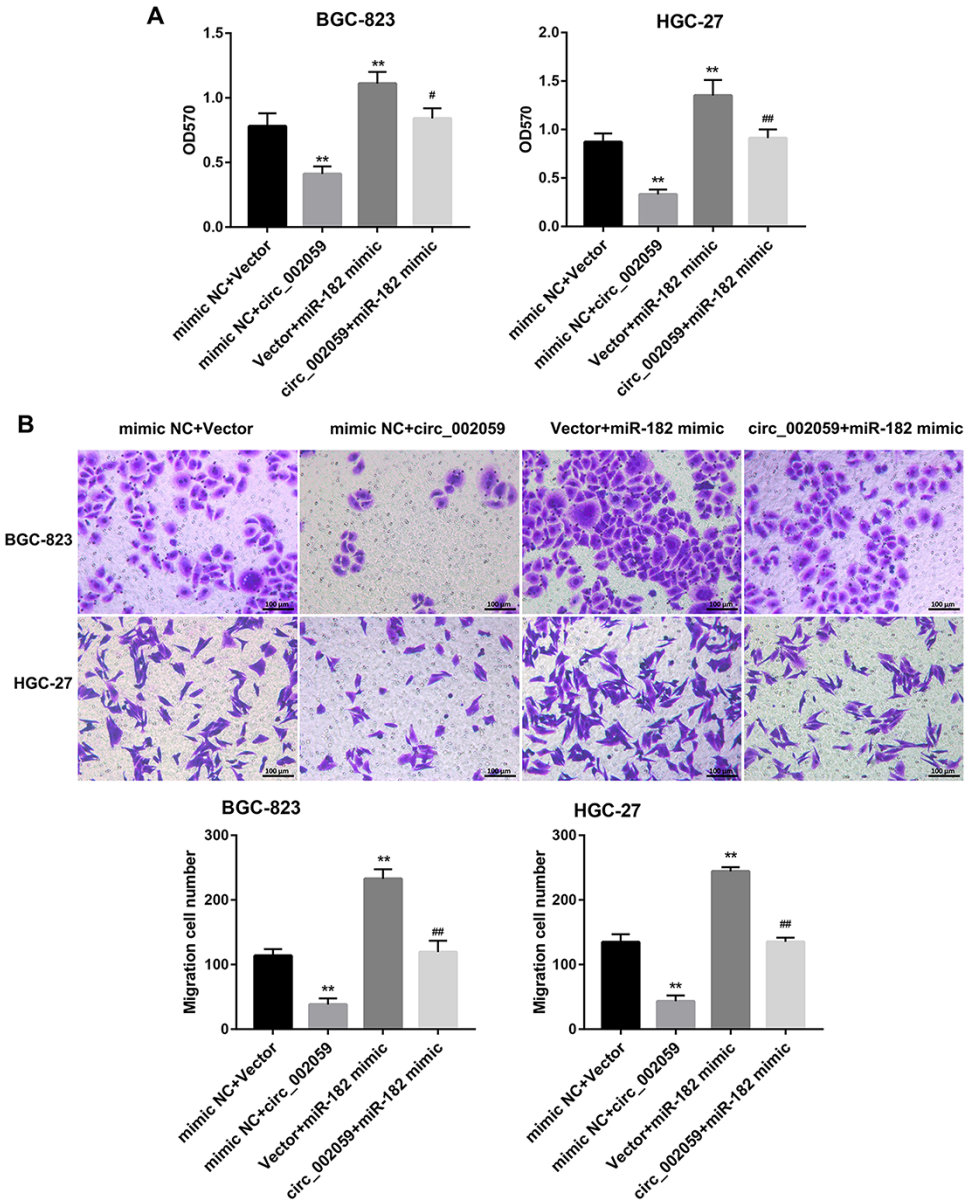

Figure 3. Circ_002059 inhibits GC cell proliferation and migration by inhibiting miR-182 expression BGC-823 and HGC-27 cells were co-transfected with empty vector/circ_002059 overexpression vector and mimic NC/miR-182 mimic. (A) Cell viability was quantified by MTT assay. (B) Cell migration was determined by Transwell migration assay. $^{**}P<0.01$ vs mimic NC + Vector; $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs mimic NC + circ_002059 or Vector + miR-182 mimic.

by targeting MTSS1. The murine xenograft models also validated the anti-GC role of circ_002059 and the regulatory network of circ_002059/miR-182/MTSS1 *in vivo*.

Nevertheless, this study still has several limitations. First, the association between expression of circ_002059/miR-182/MTSS1 and pathological parameters of the patients with GC needs to be further explored. Second, the expression of circ_002059/miRNA-182/MTSS1 in GC tissues requires determination to verify whether they are correlated with each other. Third, the *in vivo* effects of circ_002059 on the metastasis of GC cells also need further investigation. Furthermore, research shows that one circRNA can sponge different miRNAs from various protein-coding genes. Therefore, it remains to be elucidated whether circ_002059 exerts its role by acting as a sponge for various miRNAs to regulate the expressions of other key regulators in the development of GC. Other pathways or targets might exist for the role of circ_002059 in GC. Furthermore, to exactly evaluate the proliferation of GC cells, BrdU/EDU incorporation assay and cell cycle test should be performed in future experiments.

To sum up, this study demonstrated that circ_002059 acts as a tumor suppressor in GC by suppressing GC cell proliferation, migration, and xenograft tumor growth. Mechanistically, the anti-GC

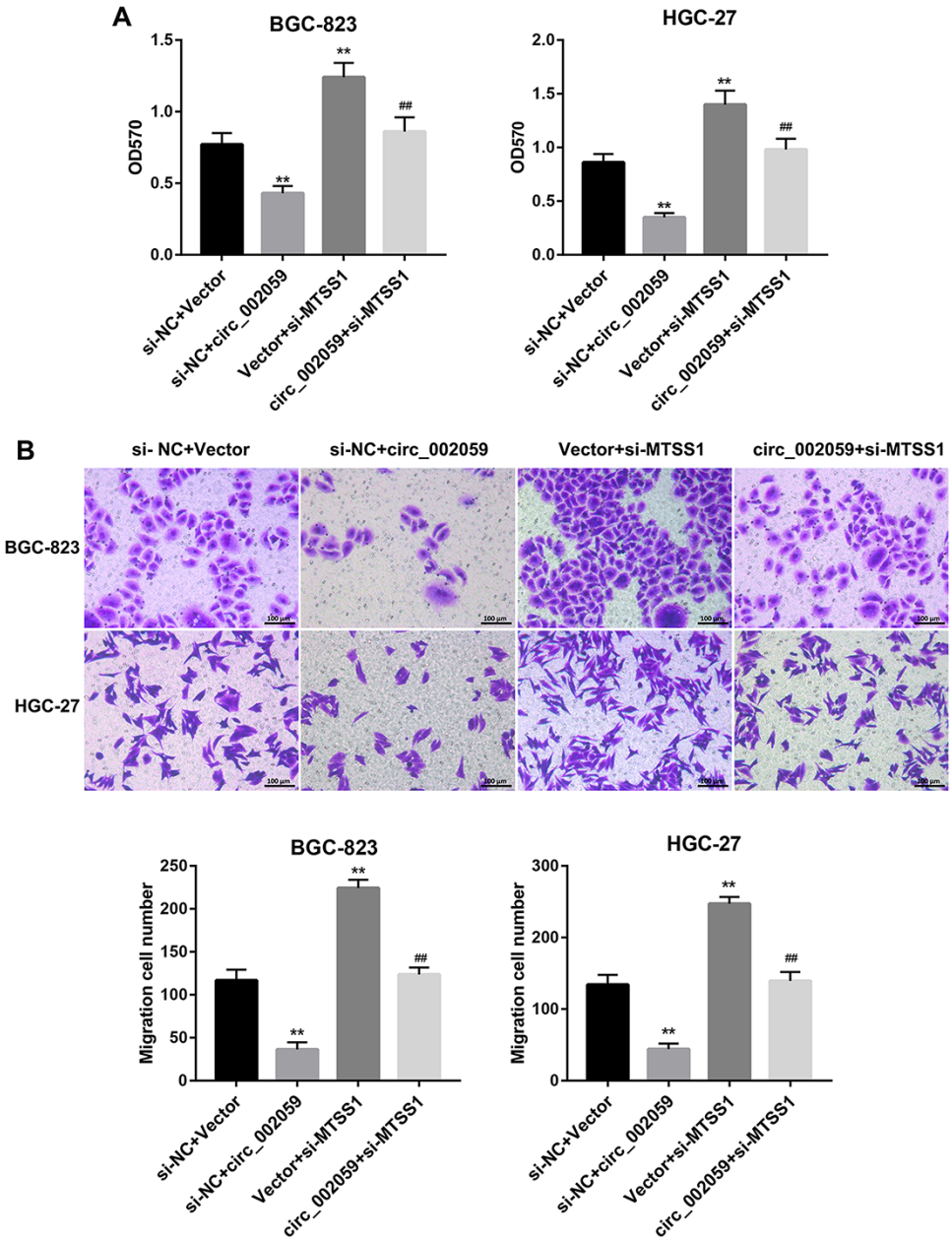

Figure 4. Circ_002059 inhibits GC cell proliferation and migration by promoting MTSS1 expression BGC-823 and HGC-27 cells were co-transfected with empty vector/circ_002059 overexpression vector and si-NC/si-MTSS1. (A) Cell viability was quantified MTT assay. (B) Cell migration was determined by Transwell migration assay. $^{**}P<0.01$ vs si-NC + Vector; $^{\#\#}P<0.01$ vs si-NC + circ_002059 or Vector + si-MTSS1 mimic.

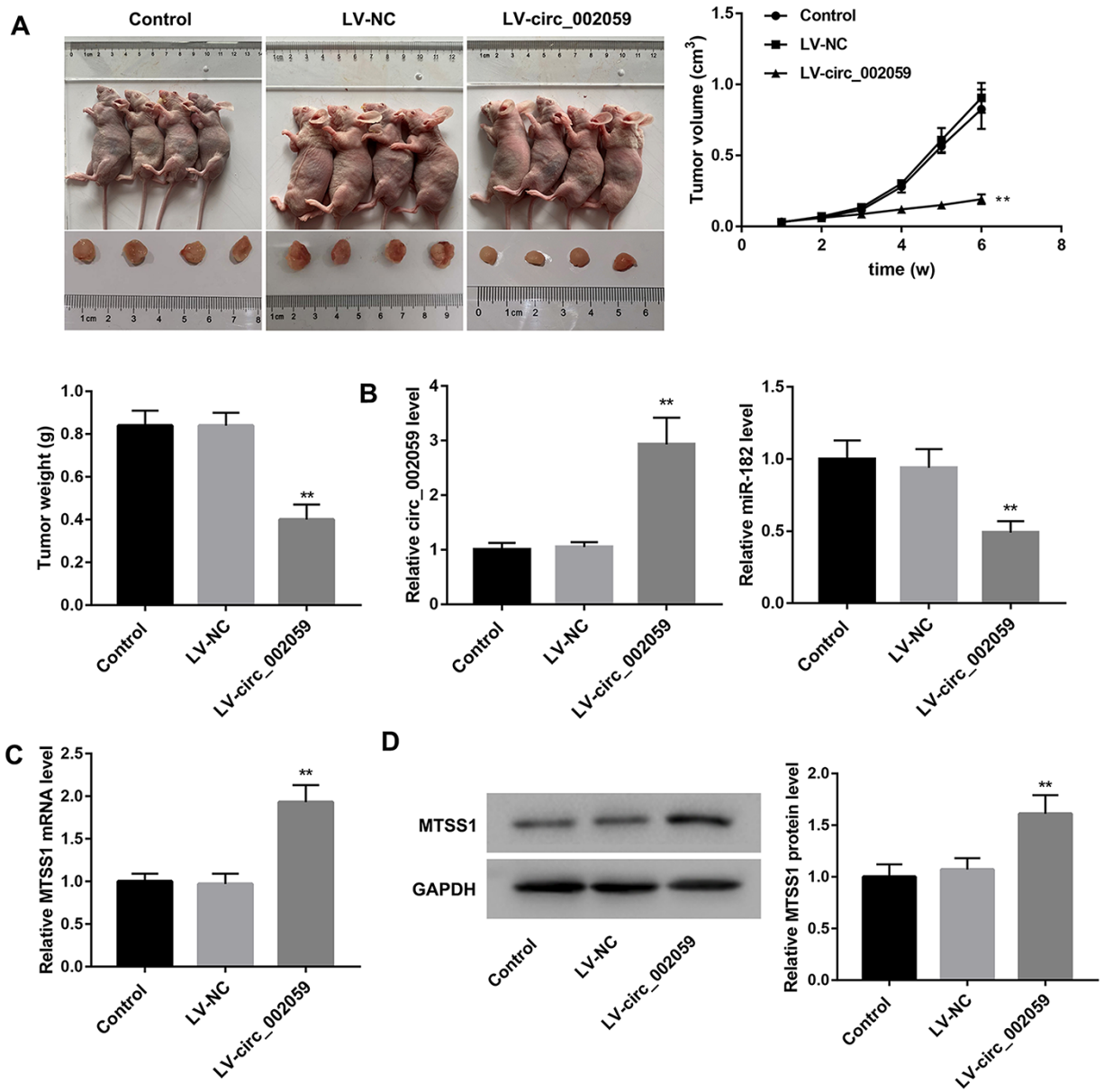

Figure 5. Circ_002059 overexpression inhibits gastric cancer cell tumorigenesis *in vivo* Six weeks after subcutaneous injection of HGC-27 cells infected with LV-NC or LV-circ_002059, the tumors were excised from mice and subjected to the following experiments. (A) Images of representative tumors excised from mice and tumor growth curve reflected by tumor volume. The tumor weight was recorded. (B) Relative circ_002059 and miR-182 expression and (C) MTSS1 mRNA level were determined by qRT-PCR analysis. (D) MTSS1 protein level was determined by western blot analysis in tumors from mice in the groups of Control, LV-NC, and LV-circ_002059. $n = 8$ per group. $^{**}P<0.01$ vs LV-NC.

role of circ_002059 is possibly mediated by regulation of the miR-182/MTSS1 axis. Our findings suggest that circ_002059 may be a novel target for the therapy of patients with GC.

Supplementary Data

Supplementary data is available at *Acta Biochimica et Biophysica Sinica* online.

Funding

This work was supported by the grant from 2019 Anhui University Natural Science Research Project (No. KJ2019A0262).

Conflict of Interest

The authors declare that they have no conflict of interest.

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