Objective: Worldwide, gastric cancer (GC) is one of primary reasons for cancer-related deaths. However, the pathogenic mechanism underlying GC remains to be fully understood. MicroRNAs are momentous regulators of diverse biological progression in cancer. Even though the ability of miR-219a-1-3p to inhibit malignant progression in pancreatic cancer have been previously reported, its role in GC remains to be elucidated.

Methods: Quantitative real-time PCR (qRT-PCR) was performed to measure miR-219a-1-3p expression levels in collected GC samples (n=98) and paired nearby non-tumor tissues. Cell proliferation, migration, and invasion assays were then conducted to explain the biological influences of miR-219a-1-3p in vitro. In vivo effects were confirmed by subcutaneously injecting miR-219a-1-3p overexpressing MGC-803 cells into nude mice. Methylation-specific PCR was employed to evaluate the CpG island upstream methylation condition of miR-219a-1-3p in collected clinical tissues (n=22), GC cell lines and GES-1 cells. GC cells were supplemented with 5-aza-2′-deoxycytidine to identify the miR-219a-1-3p expression changes using qRT-PCR.

Results: The miR-219a-1-3p expression was obviously suppressed in GC tissues relation to nearby non-tumor tissues, along with in GC cell lines in comparison to GES-1. Moreover, in vivo and in vitro functional evaluations indicated the function of miR-219a-1-3p in inhibiting the malignant characteristics of GC cells. Mechanistically, MiR-219a-1-3p expression was partly regulated utilizing DNA hypermethylation in GCs. In addition, overexpression of miR-219a-1-3p inhibited PI3K/AKT signaling.

Conclusions: MiR-219a-1-3p might function as a tumor suppressor in GC, and our investigation creates a foundation to diagnose of GC.

Keywords: DNA methylation; gastric cancer; MiR-219a-1-3p; tumor suppressor.

Introduction

Human health is severely threatened by gastric cancer (GC), a malignancy that arises from the stomach mucosal epithelium. In 2021, the latest statistics indicated that GC has no less than 1 million new cases and 768 thousand deaths, ranking fifth in incidence rate and third in death rate due to malignant tumors [1] Early stage of GC is often without symptoms or manifests with no evident symptoms, resulting in delayed identification and lost chances for radical operation. The individuals with survival rate of five years with early GC was 90 %, but it was less than 30 % for those with progressed GC [2]. Although recent breakthroughs in treatment strategies including surgery coupled with chemotherapy and radiation, have improved the prognosis for those who have advanced GC, the prognosis is still bad [3]. Hence, it is crucial to discover an efficient, innovative, and noninvasive diagnostic marker for earlier GC screening.

More recently, the possible importance of circulating microRNAs (miRNAs) as molecular markers of many cancers, including GC, has been gradually explored [4, 5]. microRNAs are single-chain, non-coding, small-molecule RNAs with an average length of 22 nucleotides [6]. Emerging data demonstrates that cancer cells express miRNAs differently than healthy tissues and cells [7, 8]. Numerous investigations have shown that microRNAs were crucial regulators of several biological progressions in cancer, such as cell growth, migration, and invasion [8, 9]. Moreover, miRNAs might flow in the blood with a stable extracellular type, and the identification of miRNAs that circulate might be utilized to
Various blood antigen biomarkers, such as carcinoembryonic antigen (CEA), carbohydrate antigen 125 (CA125), carbohydrate antigen 19-9 (CA19-9), carbohydrate antigen 72-4 (CA72-4) and pepsinogen (PG), were traditionally used to suggest cancer risk factors. However, these indicators were affected by many other factors and lack sufficient sensitivity and specificity [11–13]. At present, with the prevalence of liquid biopsy, the potential function of miRNAs as molecular markers of cancer has been gradually explored. MiR-219 family consists of six members: miR-219a-1, miR-219a-2, miR-219a-1-3p, miR-219a-2-3p, miR-219a-5p, and miR-219b. The miR-219 family has many biological functions, including anti-inflammatory effects, maintenance of neuronal activity, and anti-cancer effects [14–16]. In humans and mice, miR-219a precursors have two genomic loci (miR-219a-1 and miR-219a-2). MiR-219a-5p is located at the 5’ ends of both precursors, and miR-219a-1-3p and miR-219a-2-3p are located at the 3’ ends of pre-miR-219a-1 and pre-miR-219a-2, respectively [17]. In which, many studies focus on miR-219a-5p. It restrained malignant cell overgrowth, relocation, and invasion in, breast cancer [18, 19], osteosarcoma [20] and non-small cell lung cancer [21, 22]. MiR-219a-1 was primarily described as a light-regulated gene and a brain-specific miRNA clock, which has a vital function in modulating the extent of the circadian cycle [23]. MiR-219a-1, situated on chromosome 6 (NC_000006.12) [17, 19], was associated with the progression of different cancers in breast cancer [19], hepatocellular cancer [24], and colon cancer [25]. To date, even though the ability of miR-219a-1-3p to inhibit malignancy in pancreatic cancer have been previously reported [26], its role in GC remains to be clarified.

Therefore, in this study, we tested the biology impacts of miR-219a-1-3p in vitro and in vivo. Moreover, both the reason affecting the miR-219a-1-3p expression and the influence of its were discussed.

Materials and methods

Cells cultured and tissue samples collected

All of the cell lines (HGC-27, MGC-803, SGC7901, K562, MKN-45, and GES-1) were supplied by the American Type Culture Collection (ATCC, Manassas, VA, USA). MGC-803 and GES-1 cells were raised in Dulbecco’s modified Eagle’s medium, whereas HGC-27, SGC7901, K562, and MKN-45 cells were kept in RPMI 1640 (Invitrogen, Carlsbad, CA, USA). Every cell was preserved in complete medium containing 10 % fetal bovine serum (FBS; Gibco, USA), 100 U penicillin, and 100 g streptomycin, and was kept at 37 °C and 5 % CO2 according to ATCC proposals.

All clinical tissues were collected from the First People’s Hospital of Chengdu, China, and informed consent was obtained from all patients.

qRT-PCR reaction

Regarding the recommendations of manufacturer, total RNA was sorted from all cell lines and clinical tissues employing TRIzol Reagent (Invitrogen, Rockford, IL, USA) and opposite transcription was conducted utilizing the Hifair® III 1st Strand cDNA Synthesis Kit (gDNA digester plus) (Yesen, Shanghai, China). With the 2−ΔΔCt SYBR Green qPCR Master Mix (TaKaRa, Tokyo, Japan) and CFX Manager v2.1, RT-qPCR was applied Bio-Rad (Hercules, CA, USA). The relative target genes expression was assessed utilizing the 2−ΔΔCt, and all qRT-PCR primers are given in Supplemental Table S1 (Table S1).

Cell transfection with miRNA mimics

GenePharma (Shanghai, China) produced control and miR-219a-1-3p mimics (Table S1). GC cells were transfected with miR-219a-1-3p mimic (30 nM) and control mimic (30 nM) employing Lipofectamine 2000 (Invitrogen, Rockford, IL, USA) per the recommendations of manufacturer. Following 24 h of transfection, more studies were conducted. Employing RT-qPCR at 24 h, the overexpression of miR-219a-1-3p in indicated cells was verified.

Cell proliferation assay

GC cells were treated with control-mimic or miR-219a-1-3p mimics, and properly transfected cells were coated at a concentration of 5,000 cells for every well in 96-well plates. Employing Cell Counting Kit-8 (ABP Biosciences, Rockville, MD, USA), cell viability was detected at 0, one, two, three, and four days, and 10 L cck8 was supplemented to every well and preserved at 37 °C for 1 h. Using a microplate reader (Thermo scientific, Rockford, IL, USA) with the absorbance set at 450 nm, the optical density was measured.

Cell migration assay

GC cells were cultivated in 6-well plastic plates to up to 95 % confluence and then supplemented with control or miR-219-2-3p mimics. Utilizing a 200 µL pipe tip, linear scratch wounds were generated on syncratic cell monolayers 24 h following transfection. To prevent effect of cell growth on the experimental outcomes, serum-free media was employed to sustain the cells. At 0, 24, 36, and 48 h, the migrating cells and healing of wound were observed and photographs were captured (50×; Olympus, Tokyo, Japan).

Cell invasion assays

Roughly 4×105 cells in media free from serum were planted into transwell migration chambers (Millipore, Switzerland) that were covered with Matrigel (Sigma-Aldrich, St. Louis, MO, USA) in the top one 24 h following transfection. Typically, 10 % FBS-containing media was administered to the bottom chamber. Following one day, non-invading cells were excluded employing cotton wool, and invasive cells were subjected to crystal violet stain (Sigma Diagnostics, St. Louis, MO, USA) and quantified with an Olympus microscope (200×; Olympus, Tokyo, Japan).
Flow cytometry (FCM)

Before examining the cell cycle, the indicated cells treated with the control mimic or miR-219a-1-3p mimic were preserved overnight with 70% alcohol. The cell cycle was then studied employing FCM after the cells were treated with Cell Cycle Kit stain (Yesen, Shanghai, China) in agreement with the recommendations of the manufacturer.

Histological section staining

Xenograft tumor tissues were fixed overnight in 4% paraformaldehyde, then embedded in paraffin and separated. As previously mentioned [27], H&E and IHC staining were performed. In IHC, Ki-67 (1:400 dilution, ab15580, Abcam, Cambridge, UK) was used, while control rabbit IgG (1:200; cat. no.011-000-003; Jackson Immunoresearch Labs, Inc., West Grove, PA, USA) served as a negative control. Utilizing microscope, specimens were seen and photographed (400x magnification).

Statistical analysis

The data are illustrated as mean ± standard deviation (SD). All data were examined for normality and variance equivalency to detect the most suitable statistical test. Employing a one-way analysis of variance, variations between different groups that were statistically significant were examined (ANOVA). The two cohorts were examined utilizing Student’s t-test with two tails. p<0.05 was judged as a statistically significant. All quantitative tests were conducted in duplicate and were performed three times.

Results

Western blot assay

Western blot was conducted as mentioned [29]. PI3K (ab151549, Abcam, Cambridge, UK), p-PI3K(Tyr607) (Catalog #PA5-104853, Invitrogen, Rockford, IL, USA), AKT (#4691, CST, MA, USA), p-AKT (Ser473) (#4060, CST, MA, USA) and β-actin (ab8226, Abcam, Cambridge, UK) were used in this study. p-AKT was diluted as 1:2000, and others were diluted as 1:1000.

Bioinformatics analysis

The CPG island were anticipated utilizing MethPrimer (http://www.urogene.org/methprimer/index1.html). The miRNA targets anticipated from miRDB (http://mirdb.org/miRDB/) and targetscan7.2 (http://www.targetscan.org). In both website, “Human” was selected as species setting, input “miR-219a-1-3p”, and submit for searching. The KEGG pathway analysis of miR-219a-1-3p potential target genes was conducted employing DAVID bioinformatics resources (https://david.ncifcrf.gov/tools.jsp). Overlap of target genes predicted by the two sites were selected to KEGG pathway analysis.

Subcutaneous transplanted tumor model in nude mice

For the xenograft trials, ten immunocompromised male nude rats aged 4 weeks were obtained from Yuanmin Biological Company (Shanghai, China). Single-cell suspensions of MGC-803 cells treated with the control mimic or miR-219a-1-3p mimic were preserved overnight with 5 × 10⁶ cells in 100 µL of PBS. After measuring cancer growth every three days for 31 days, beginning seven days following injection, all rats were euthanized. The cancer volume (mm³) was computed utilizing formula volume=length × width² × π/6.

The model of the liver metastasis of gastric cancer cells in nude mice

To generate single-cell suspensions, MGC-803 cells treated with the control mimic or the miR-219a-1-3p mimic were obtained by digesting the cells. Each rat was administered subcutaneously with 5 × 10⁷ cells. After measuring cancer growth every three days for 31 days, beginning seven days following injection, all rats were euthanized. The cancer volume (mm³) was computed utilizing formula volume=length × width² × π/6.

Methylation specific PCR (MSP)

MSP was employed to evaluate the methylation of the miR-219a-1-3p upstream region in GC and paired nontumor tissues. MethPrimer software toll (http://www.urogene.org/methprimer/index1.html) was employed to design MSP primers (Table S1). The tissue samples were subjected to MSP as previously described [28].

5-Aza-2′-Deoxycytidine treatment

The indicated cells were supplemented with last densities of 5, 15, and 30 µM 5-aza-2’-deoxycytidine (5-Aza-CdR, Sigma-Aldrich, Steinheim, Germany), which is a DNA methyltransferase suppressor. Following 24 h, RNA was obtained for analysis.

Western blot was conducted as mentioned [29]. PI3K (ab151549, Abcam, Cambridge, UK), p-PI3K(Tyr607) (Catalog #PA5-104853, Invitrogen, Rockford, IL, USA), AKT (#4691, CST, MA, USA), p-AKT (Ser473) (#4060, CST, MA, USA) and β-actin (ab8226, Abcam, Cambridge, UK) were used in this study. p-AKT was diluted as 1:2000, and others were diluted as 1:1000.

Bioinformatics analysis

The CPG island were anticipated utilizing MethPrimer (http://www.urogene.org/methprimer/index1.html). The miRNA targets anticipated from miRDB (http://mirdb.org/miRDB/) and targetscan7.2 (http://www.targetscan.org). In both website, “Human” was selected as species setting, input “miR-219a-1-3p”, and submit for searching. The KEGG pathway analysis of miR-219a-1-3p potential target genes was conducted employing DAVID bioinformatics resources (https://david.ncifcrf.gov/tools.jsp). Overlap of target genes predicted by the two sites were selected to KEGG pathway analysis.

Statistical analysis

The data are illustrated as mean ± standard deviation (SD). All data were examined for normality and variance equivalency to detect the most suitable statistical test. Employing a one-way analysis of variance, variations between different groups that were statistically significant were examined (ANOVA). The two cohorts were examined utilizing Student’s t-test with two tails. p<0.05 was judged as a statistically significant. All quantitative tests were conducted in duplicate and were performed three times.

Results

MiR-219a-1-3p expression was downregulated in a GC tissues

To investigate the difference of miR-219a-1-3p expression in collected clinical GC samples and paracancerous tissues, the miR-219a-1-3p expression levels in 98 clinical GC specimens and corresponding nearby non-tumor tissues were distinguished employing qRT-PCR. We discovered that miR-219a-1-3p expression was decreased in 54 out of 98 (55.10%) GC tissues compared to that in paracancerous samples (p<0.05; Figure 1A, B). Furthermore, lower miR-219a-1-3p expression in GC tissues was positively linked with metastasis of lymph node, distant metastasis, and higher medical phase (refer to TNM stage) (p<0.05). Whereas, the miR-219a-1-3p expression has no significance in T stage (p=0.717) (Table 1). In addition, paired GC specimens and neighboring non-tumor samples were evaluated by H&E staining, which showed that peri-tumor tissues had an obvious
gastric mucosal structure compared to GC tissues (Figure 1C). Collectively, this data indicated that the miR-219a-1-3p expression was reduced in GC tissues compared to that in paracancerous tissues, and the decreased miR-219a-1-3p level was linked to unfavorable prognosis.

**Overexpression of miR-219a-1-3p inhibited the cell proliferation, migration, and invasion of GC cells in vitro**

To discover miR-219a-1-3p actions in GC cells, we initially measured the miR-219a-1-3p expression in five cell lines of GC (HGC-27, MGC-803, SGC7901, K562 and MKN-45) and gastric mucosa GES-1 cells with qRT-PCR. The outcomes revealed that the miR-219a-1-3p expression was substantially downregulated in all five GC cell lines contrasted with that in GES-1 cells (p<0.01) (Figure 2A). The HGC-27 and MGC-803 cell lines were randomly chosen for further experiments. Next, we engineered a miRNA mimic (miR-219a-1-3p) to over-express miR-219a-1-3p in the two chosen GC cell lines. The miR-219a-1-3p expression levels were confirmed in HGC-27 and MGC-803 cells by qRT-PCR (p<0.01; Figure 2B). The CCK8 test revealed that the miR-219a-1-3p overexpression observably suppressed cell growth in both HGC-27 and MGC-803 cells in comparison to that in the untreated cells and the control mimic cells (HGC-27 at 72, 96 h and MGC-803 cells at 48, 72, 96 h, p<0.05; Figure 2C, D). Moreover, the wound healing evaluation indicated that miR-219a-1-3p overexpression diminished the cell migration ability in comparison to that in the untreated cells and the control mimic cells (p<0.05; Figure 2E–H). Additionally, the transwell tests revealed that the miR-219a-1-3p overexpression noticeably weakened the cell invasion ability in vitro in comparison to that in the untreated cells and the control mimic cells (p<0.05) (Figure 3). These outcomes illustrated that the overexpression of miR-219a-1-3p suppressed the aggressiveness of GC cells in vitro.

**Overexpression of miR-219a-1-3p inhibited cell growth and liver metastasis in vivo**

To additional validate the impact of miR-219a-1-3p over-expression on cell growth in vivo, MGC-803 cells infected with the miR-219a-1-3p mimic were supplemented subcutaneously into athymic nude mice. After one week, the size of tumors was evaluated once every 3 days. We observed that the freshly retrieved tumor samples from the miR-219a-1-3p overexpression group inhibited cell growth

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**Figure 1:** The miR-219a-1-3p was downregulated in gastric cancer (GC) tissues compared to that in paired adjacent non-tumor tissues, and its downregulated expression was correlated with a poor clinical prognosis. (A) The mRNA expression of miR-219a-1-3p was detected in 98 GC tissues (C) and paired non-tumor tissues (N). (B) The expression of miR-219a-1-3p is presented as normalized data, *p<0.05. (C) The paraffin sections of adjacent non-tumor tissues and tumor tissues in GC patients were subjected to H&E staining and observed using a bright field microscope (100×). Scale bar: 50 μm.
miR-219a-1-3p methylation status of 22 GC samples and nearby healthy tissues was evaluated employing MSP. Compared with neighboring normal tissues, upstream region hypermethylation was found in seven out of 22 (31.82 %) GC tissues (Figure 5B). Additionally, the methylation levels of miR-219a-1-3p in GC cells MGC-803 and HGC-27 were also evaluated using MSP, and the outcomes illustrated that the methylation levels of miR-219a-1-3p in these cells were higher than those in GES-1 cells (Figure 5C). To further confirm that DNA methylation was among the reasons of miR-219a-1-3p suppression, HGC-27 and MGC-803 cells were supplemented with 5-aza-2-deoxycytidine (5′ AZa-CDR, a methyltransferase inhibitor). The current outcomes indicated that the miR-219a-1-3p expression was markedly raised in HGC-27 and MGC-803 cells following supplementation with 5′ AZa-CDR (p<0.05; Figure 5D). Collectively, the present outcomes indicated that miR-219a-1-3p was inhibited in GC and that this is partly due to upstream region hypermethylation.

**Overexpression of miR-219a-1-3p inhibits the proliferation of GC cells in vitro but has no connection with cell cycle arrest**

Cell cycle arrest is one of the reasons for the suppression of cancer cell development [30]. To define whether miR-219a-1-3p overexpression inhibits the malignant progression of GC cells owing to cell cycle arrest, we additional investigated the influence of miR-219a-1-3p on the cell cycle. FCM investigation revealed that miR-219a-1-3p overexpression did not considerably alter the cell cycle in GC cells (Figure S1). This result suggested that the overexpression of miR-219a-1-3p, which inhibited cell overgrowth in vitro, and was not associated with cell cycle arrest.

**Inhibition of the malignant progression of GC as a result of miR-219a-1-3p overexpression was linked with the PI3K/AKT pathway**

For more understand the pathway of miR-219a-1-3p, we analyzed the potential target genes of miR-219a-1-3p utilizing the online prediction software Targetscan7.2 (Table S2), and miRDB (Table S3). The overlap after crosslinking contained 262 genes (Figure 6A). KEGG pathway analysis of crosslinking genes was performed using DAVID, and the results predicted pathways that might be related to the miR-219a-1-3p mechanism (Figure 6B). In addition, we selected the predicted downstream PI3K/AKT pathway for preliminary

### Table 1: Correlation between the expression of miR-219a-1-3p and clinical features in 98 GC patients.

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*Mean of Log1.5(C/N); **Standard definition of Log1.5(C/N). C, normalized expression of cancer tissues; N, normalized expression of non-cancer tissues, *p<0.05.

**The expression of miR-219a-1-3p was partly regulated by DNA methylation**

To elucidate the reasons for the downregulated expression of miR-219a-1-3p in GCs, we first conducted bioinformatics analyses, and the CpG island of the miR-219a-1-3p upstream region was predicted using Meth Primer software (Figure 5A). The

### Additionally, the methylation levels of miR-219a-1-3p...
Figure 2: Overexpression of miR-219a-1-3p inhibited the proliferation, migration, and invasion of GC cells in vitro. (A) The expression of miR-219a-1-3p in GES-1 cells and five GC cell lines was measured using qRT-PCR. Data is presented as mean ± standard deviation (SD), n=3, **p<0.01. (B) The expression of miR-219a-1-3p in HGC-27 and MGC-803 cells treated with miR-219a-1-3p mimic or control mimic was confirmed using qRT-PCR. Data is presented as...
verification. The expression of p-PI3K and p-AKT were downregulated in the miR-219a-1-3p overexpression group by western blotting (Figure 6C). Altogether, these outcomes indicated that miR-219a-1-3p overexpression of reduced the malignant progression of GC and this effect was related to the PI3K/AKT pathway.

Discussion

In our investigation, we discovered that miR-219a-1-3p could serve as a novel suppressor of GC cells. A sequence of in vivo and in vitro functional evaluations elucidated miR-219a-1-3p function in suppressing the aggressiveness of GC cells. Mechanistically, we demonstrated that miR-219a-1-3p downregulation was partly modulated by DNA hypermethylation. Moreover, miR-219a-1-3p suppression of the malignant process of GC may be relative to inhibition of the PI3K/AKT signaling.

First, we discovered that the miR-219a-1-3p expression was reduced in 54/98 (55.10 %) GC samples in comparison to nearby healthy tissues, and the lower miR-219a-1-3p expression in malignancy tissues was positively connected to metastasis of lymph node, distant metastasis, and progressive clinical phase. Above outcomes suggested that miR-219a-1-3p might sever as a biomarker for GC clinical prognosis. In addition, as miR-219a-1-3p expression has no significance in T stage, miR-219a-1-3p also acted as a resultful diagnostic biomarker for early GC. Moreover, in biology functions, we demonstrated that miR-219-1-3p could significantly suppressed cell growth, migration, and invasion in both HGC-27 and MGC-803 cells in vitro. In vivo, miR-219a-1-3p remarkably inhibited cell growth and liver metastasis. Similarly, miR-219-1-3p could

Figure 3: Overexpression of miR-219a-1-3p suppressed cell invasion of GC cells in vitro. (A, B) Images of the invasion of cells in untreated, control-mimic, and miR-219a-1-3p overexpressing HGC-27 and MGC-803 cells. (C, D) Statistical chart of the transwell assay in untreated, control-mimic, and miR-219a-1-3p overexpressing HGC-27 and MGC-803 cells. Scale bar: 50 μm. Untreated group vs. Control mimic group, control mimic group vs. miR-219a-1-3p group. Data is presented as mean ± SD, n=3, *p<0.05, ns, No significance.

mean ± SD, n=3, **p<0.01. (C, D) The inhibitory effect of miR-219a-1-3p on HGC-27 and MGC-803 was measured by CCK8 at 0, 24, 48, 72, and 96 h, respectively. Untreated group vs. Control mimic group, control mimic group vs. miR-219a-1-3p group. Data is presented as mean ± SD, n=4, **p<0.05. (E–H) Images and statistical chart of the wound healing assay in untreated, control-mimic, and miR-219a-1-3p overexpressing HGC-27 and MGC-803 cells at 0, 24, 36, and 48 h. Scale bar: 500 μm. Untreated group vs. Control mimic group, control mimic group vs. miR-219a-1-3p group. Data is presented as mean ± SD, n=3, *p<0.05, **p<0.01.
inhibit cell growth and relocation in pancreatic cancer [26]. Furthermore, a new investigation indicated that miR-219a-1 could noticeably suppress the overgrowth and invasion of colon cancer cells [25]. In brief, our outcomes reveal that miR-219a-1-3p could as a tumor suppressor in GC.

Some miRNAs are normally expressed in the stomach. However, aberrant levels of these miRNAs can promote the growth and progression of GC [8, 31]. Epigenetic modulation of gene expression is before, during, and after transcription, and many studies have suggested that abnormal DNA methylation was a common phenomenon in human tumors [32, 33]. As epigenetic modulators, miRNAs complicate epigenetic regulation [34, 35]. A previous study reported that hypermethylated miR-219a-1 may be involved in glioblastoma multiforme pathogenesis [36]. Strikingly, epigenetic alterations were identified in both early and advanced stages of GC [37]. A previous finding discovered that miR-219a-2-3p is regulated by DNA methylation in GC [28].

Figure 4: Overexpression of miR-219a-1-3p inhibited cell growth and liver metastasis in vivo. (A, B) control-mimic and miR-219a-1-3p-expressing MGC-803 cells were subcutaneously injected into four-week-old male nude mice (5/Group). Seven days after injection, the tumor size was measured every three days. MiR-219a-1-3p inhibited tumor volume (p<0.001). Scale bar: 10 mm. (C) The retrieved tumor samples were subjected to H&E and Ki-67 of IHC staining. Scale bar: 20 μm. (D) A statistical chart of the IHC of Ki-67 in control mimic and miR-219a-1-3p-overexpressing xenografts is presented. Control mimic group vs. miR-219a-1-3p group. Data is presented as mean ± SD, n=3, *p<0.05. (E) The number of liver nodules was measured 8 weeks after injection. Scale bar: 10 mm.
Based on the result, we hypothesized that miR-219a-1-3p is modulated by DNA methylation in GC. In recent investigation, we discovered that the upstream region of the genome spanned by miR-219a-1-3p was hypermethylated and that miR-219a-1-3p mRNA levels were decreased in tumor samples and cancer cell lines. Furthermore, the miR-219a-1-3p expression in GC cells was substantially upregulated after supplemented with 5-AZA-CdR, indicating that miR-219a-1-3p...
hypermethylation resulted in the reduction of the miR-219a-1-3p expression in GC cells. These data suggest that the upstream region of the genome spanned by miR-219a-1-3p results in a decrease of miR-219a-1-3p expression in GC.

MiRNA could affect many signal pathways and target genes, such as, miR-219a-5p represses the malignancy of GC cells via Wnt signaling [38]. Another study reported that miR219a-2-3p dramatically inhibited the overgrowth, relocation, and invasion of GC cells by ERK pathway [28]. Most importantly, in pancreatic cancer, miR-219-1-3p inhibiting malignant progression was corelative to AKT and ERK pathway [26]. In our study, the signaling pathways that miR219a-2-3p may affect were predicted. We further selected the predicted downstream PI3K/AKT pathway for

![Venn diagram of the number of genes identified as potential targets of miR219a-1-3p by TargetScan and miRanda.](image)

![KEGG pathway analysis of miR-219a-1-3p potential target genes by DAVID bioinformatics resources.](image)

![Effect of miR-219a-1-3p overexpression on the expression of the components of the PI3K/AKT pathway.](image)

Figure 6: Inhibition of the malignant progression of GC as a result of miR-219a-1-3p overexpression was associated with the PI3K/AKT signaling pathway. (A) Venn diagram of the number of genes identified as potential targets of miR219a-1-3p by TargetScan and miRanda. (B) KEGG pathway analysis of miR-219a-1-3p potential target genes by DAVID bioinformatics resources. (C) The effect of miR-219a-1-3p overexpression on the expression of the components of the PI3K/AKT pathway was detected using western blotting analysis. The cell lysate was collected and subjected to western blotting analysis with antibodies against members of the PI3K/AKT pathway. Levels of β-actin were used as a loading control.
preliminary validation. The expression of p-PI3K and p-AKT were downregulated in the miR-219a-1-3p overexpression group by western blotting. These outcomes indicated that miR-219a-1-3p overexpression suppressed the malignant progression of GC and this effect is related to the PI3K/AKT pathway. The specific regulatory mechanism underlying this effect requires further investigation.

Conclusions

Our investigation indicated that miR-219a-1-3p expression was suppressed in GC tissues contrasted with that in nearby healthy tissues. The miR-219a-1-3p expression was correspondingly reduced in GC cell lines contrasted with that in GES-1 cells. Additionally, the miR-219a-1-3p overexpression substantially repressed the aggressiveness of GC cells in vitro. MiR-219a-1-3p overexpression notably inhibited the growth of subcutaneous cancers and liver metastasis in nude mice. Mechanistically, we discovered that miR-219a-1-3p expression was partly modulated by DNA hypermethylation in GCs. In addition, miR-219a-1-3p overexpression inhibited the PI3K/AKT signaling pathway. Our outcomes demonstrate that miR-219a-1-3p may have a predominant function in regulating GC-associated biological mechanisms. This study suggests the possibility of developing novel therapeutics to target miR-219a-1-3p in GC.

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Data availability: The datasets used to support the findings of this study are available upon reasonable request from the corresponding author.

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References


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