Original Article
miR-106 serves as a prognostic biomarker for gastric cancer and promotes cancer progression

Xuemei Zhang1, Wenqi Song2, Yanli Zhu1, Zhuoxin Cheng3, Shihua Zhang4, Qianzi Liu5

1Department of Gastroenterology, First Affiliated Hospital of Jiamusi University, Jiamusi 154002, Heilongjiang, China; 2Department of Pharmacy, Jiamusi Central Hospital, Jiamusi 154002, Heilongjiang, China; 3Department of General Surgery, First Affiliated Hospital of Jiamusi University, Jiamusi 154002, Heilongjiang, China; 4Department of Brain Surgery, First Affiliated Hospital of Jiamusi University, Jiamusi 154002, Heilongjiang, China; 5Department of Pharmacy, School Clinic of Jiamusi University, Jiamusi 154002, Heilongjiang, China

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Abstract: MicroRNAs (miRNAs) have been shown play crucial roles in tumorigenesis and tumor progression. The aim of this study was to investigate the expression pattern, clinical value, and functional roles of miR-106 in gastric cancer. Expression of miR-106 was detected by quantitative real-time PCR. Kaplan-Meier and Cox regression analyses were performed to estimate the potential prognostic value of miR-106 in gastric cancer. Cell experiments were carried out to explore the functional roles of miR-106. Expression of miR-106 was significantly increased in gastric cancer tissues and cell lines, compared with controls (all \( P < 0.05 \)). Increased miR-106 expression was significantly associated with lymph node metastasis and TNM stage. High miR-106 expression predicted shorter overall survival, compared with low expression levels (log-rank \( P = 0.028 \)). Multivariate Cox regression analysis results showed that miR-106 was an independent prognostic factor for patients. Cell experiments demonstrated that cell proliferation, migration, and invasion were enhanced by upregulation of miR-106 expression, but were suppressed by reduction of miR-106 expression (all \( P < 0.05 \)). Overexpression of miR-106 may serve as an independent prognostic biomarker of gastric cancer and promote tumor progression.

Keywords: microRNA-160, gastric cancer, prognosis, progression

Introduction
Gastric cancer is a leading cause of cancer-related mortality in the digestive system, worldwide. Its high mortality rate can be observed in Eastern Asian, Eastern Europe, and South America, likely due to both environmental and social factors [1, 2]. Although incidence of gastric cancer is now in a declining trend and the survival rates are rising, the mortality of gastric cancer patients remain high in China [3, 4]. Prognosis of gastric cancer at an advanced stage remain poor, with a 5-year survival rate below 40% [3]. Thus, identification of tumor biomarkers and therapeutic targets is essential for gastric cancer patients.

MicroRNAs (miRNAs) are a class of small non-coding RNAs that are approximately 22 nucleotides in length. They regulate target gene expression post-transcriptionally through targeting the 3′-untranslated region (3′-UTR) of target mRNAs [5]. An increasing number of studies have demonstrated that miRNAs function as a tumor suppressor or oncogenes in tumorigenesis, serving as key regulators in various biological processes, including cell proliferation, migration, invasion, and apoptosis [6, 7]. More importantly, some specific miRNAs and their targets have been reported to play important roles in the development and progression of various types of human cancer, including gastric cancer [8-10]. For instance, serum miR-106 was found to be upregulated in gastric cancer. It could be a novel non-invasive biomarker for detection of gastric cancer [11]. Another study showed that serum miR-106 was upregulated in gastric cancer [12]. However, expression patterns of miR-106 in gastric cancer tissues and its biological roles in gastric cancer require further investigation.

The current study investigated expression levels of miR-106 in gastric cancer tissues and adjacent non-tumor tissue samples and cell lines. The prognostic significance of miR-106 in
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**Materials and methods**

**Patients and specimens**

A total of 132 paired human gastric cancer tissues and adjacent non-tumor tissue specimens were collected from gastric cancer patients that underwent surgery at First Affiliated Hospital of Jiamusi University, between 2008 and 2012. All tissue specimens were verified by experienced pathologists. No patients received any preoperative therapy. Gastric cancer tissues and normal tissue specimens were snap frozen immediately in liquid nitrogen and stored at -80°C until total RNA was extracted. Clinicopathological characteristics of the gastric cancer patients were collected and are listed in **Table 1**. Written informed consent was obtained from each patient participating in this study. The present study was approved by the Ethics Committee of First Affiliated Hospital of Jiamusi University. Five-year follow-up information was collected by telephone for subsequent analysis.

**Cell lines and transfection**

Human gastric cancer cell lines (MGC-803, AGC, SGC-7901, and BGC-823) and one normal gastric epithelium cell line (GES-1) were purchased from Cell Bank of Chinese Academy of Sciences (Shanghai, China). All cells were cultured in Gibco Dulbecco’s modified Eagle’s medium (DMEM, Thermo Fisher Scientific, Inc., Waltham, MA, USA), supplemented with heat-inactivated 10% fetal bovine serum (FBS) at 37°C in a humidified incubator with 5% CO₂. Cells were seeded at a density of 5 × 10⁴ cells per well in 6-well plates and incubated for 24 hours. The cells were then transfected with the miR-106 mimic, mimic negative control (NC), miR-106 inhibitor, or inhibitor NC using Lipofectamine™ 2000 (Invitrogen; Thermo Fisher Scientific, Inc.), following manufacturer instructions. Untreated cells were used as blank control.

**RNA extraction and reverse transcription-quantitative polymerase chain reaction (qRT-PCR)**

Total RNA was extracted from the cell lines and tissue specimens using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA), following manufacturer protocol. The purity of RNA was assessed by measuring absorbance at 260 nm and 280 nm. The ratio of OD was 260/280. Purified total RNA was then reverse transcribed to complementary DNA (cDNA) using the RevertAid First Strand cDNA Synthesis kit (Thermo Fisher Scientific, Inc.). Next, qRT-PCR was performed using SYBR Green Premix Ex Taq (Takara, Japan) on an Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems, USA). Relative miR-106 expression was calculated with the 2^ΔΔCt method and normalized to U6.

**Cell proliferation assay**

Cell proliferation was detected using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. SGC-7901 and MGC-803 cells were seeded in 96-well plates with a density of 5,000 cells per well. After incubation...
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overnight, cells were transfected with miR-106 mimic, inhibitor, or their negative controls. The cells were cultured at 37°C for 1-4 days before the addition of 10 µL MTT (Sigma-Aldrich, 5 mg/mL) to the culture medium in each well. After 4 hours, the medium was removed and the precipitate was dissolved using 100 µL DMSO (Sigma-Aldrich). Absorbance was measured at a wavelength of 490 nm for each well.

Cell migration and invasion assays

Transwell assays were used with a 24-well Transwell chamber (8-µm pore size, Multiskan MK3, Thermo, Waltham, MA) to determine the migration and invasion capacities of transfected cells. For the migration assay, cells were added in the top chamber at a concentration of 1 × 10⁴ per well. For the invasion assay, 5 × 10⁴ cells were plated in the top compartment with the Matrigel-coated membrane (Bedford, MA, USA) on the upper side. Both assays were then incubated in serum-free DMEM medium at 37°C for 16 hours. The lower chamber was filled with 600 mL DMEM containing 10% FBS as a chemo-attractant. After incubation for 16 hours, the non-migrated/non-invaded cells were removed using a cotton swab. Cells that migrated/invaded into the lower compartment were fixed in 3.7% formaldehyde for 5 minutes and stained with 0.1% crystal violet for 15 minutes. The number of cells was counted under a microscope (Olympus, Tokyo, Japan).

Statistical analysis

SPSS 21.0 software (SPSS, Inc., Chicago, IL, USA) and GraphPad Prism 5.0 software (GraphPad Software, Inc., Chicago, USA) was used to perform all statistical analyses. Measurement data are presented as the mean ± standard deviation (SD). Data for groups were analyzed with paired Student’s t-tests or one-way ANOVA. The relationship between clinicopathological characteristics of patients and miR-106 expression was analyzed by the χ² test. Kaplan-Meier and Cox regression analysis were used to analyze the clinical significance of miR-106 in gastric cancer. Statistical significance is indicated when P is less than 0.05. Each experiment in this study was repeated at least three times.

Results

Expression of miR-106 in tissue specimens and cells

To investigate the potential significance of miR-106 in the development of gastric cancer, expression levels of miR-106 in 132 paired gastric cancer tissues and adjacent normal tissue specimens were detected. Moreover, qRT-PCR results showed that miR-106 was indeed increased in tumor tissues, compared with that in adjacent normal tissue specimens (P < 0.001, Figure 1A). Expression of miR-106 was also detected in gastric cancer cell lines and normal cell lines. As shown in Figure 1B, expression of miR-106 was found markedly increased in all gastric cancer cell lines, compared with that in normal gastric epithelium cell line GES-1 (all P < 0.001).

Expression of miR-106 correlated with clinical characteristics of gastric cancer patients

This study further investigated the relationship between miR-106 and clinical features of ga-
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Increased expression of miR-106 in tumor specimens and the correlation with clinical characteristics of patients led to an investigation of the functional roles of miR-106 in gastric cancer, as well as its effects on tumor cell proliferation, migration, and invasion. These were examined using miR-106 mimics or miR-106 inhibitors. Gastric cancer cell lines MGC-803 and SGC-7901 were used in the cell experiments, as they had rather high miR-106 expression. Moreover, qRT-PCR results indicated that expression of miR-106 in gastric cancer cells transfected with the miR-106 mimics was significantly higher, while in cells transfected with miR-106 inhibitors it was reduced, compared to controls (all \( P < 0.05 \), Figure 3A). MTT assay was used to measure cell proliferation. Results showed that upregulation of miR-106 by miR-106 mimics promoted gastric cancer cell proliferation, while downregulation of miR-106 by miR-106 inhibitors inhibited cell proliferation, compared to controls (all \( P < 0.05 \), Figure 3B).

In addition to proliferation, cell migration and invasion were assessed using Transwell assays. Assay results indicated that gastric cancer cells transfected with miR-106 mimics promoted the capacities of migration and invasion, but cells transfected with miR-106 inhibitors decreased migration and invasion capacities, compared to controls (all \( P < 0.05 \), Figure 4).

**Discussion**

Surgical resection treatment remains the main therapy for gastric cancer. Even though a vari-

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**Table 2. Multivariate Cox analysis for miR-106 in gastric cancer patients**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Multivariate analysis</th>
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<tbody>
<tr>
<td></td>
<td>HR</td>
</tr>
<tr>
<td>miR-106</td>
<td>1.910</td>
</tr>
<tr>
<td>Gender</td>
<td>0.737</td>
</tr>
<tr>
<td>Age</td>
<td>1.382</td>
</tr>
<tr>
<td>Tumor size</td>
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<tr>
<td>Differentiation</td>
<td>0.682</td>
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<tr>
<td>Local invasion</td>
<td>0.871</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td>0.977</td>
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<tr>
<td>TNM stage</td>
<td>0.989</td>
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</tbody>
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**Effects of miR-106 on cell proliferation, migration, and invasion**

Kaplan-Meier analysis showed that survival times of patients with high miR-106 expression were shorter than in patients with low miR-106 expression (log-rank \( P = 0.028 \), Figure 2). Moreover, miR-106 expression and all clinical parameters were included in the Cox regression analysis to explore factors that had independent prognostic value on overall survival of gastric cancer patients. As shown in Table 2, miR-106 (HR = 1.190, 95% CI = 1.139-3.203, \( P = 0.014 \)) was an independent prognostic factor for gastric cancer patients.

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A. In the two cell lines, expression of miR-106 was markedly increased by miR-106 mimic but was decreased by the miR-106 inhibitor, compared with controls. (**P < 0.001). B. Proliferation ability was enhanced by overexpression of miR-106 but was suppressed by downregulation of miR-106. (*P < 0.05).

Figure 3. Effects of miR-106 on cell proliferation in MGC-803 and SGC-7901 cells. A. In the two cell lines, expression of miR-106 was markedly increased by miR-106 mimic but was decreased by the miR-106 inhibitor, compared with controls. (**P < 0.001). B. Proliferation ability was enhanced by overexpression of miR-106 but was suppressed by downregulation of miR-106. (*P < 0.05).

of comprehensive treatments, including surgery, chemotherapy, and radiotherapy, have been adopted, the 5-year survival rate of gastric cancer is still unsatisfactory. Many patients will have recurrence and metastasis after surgery [13, 14]. Numerous studies have indicated that biomarkers used for diagnosis and/or prognosis demonstrate high potential to improve outcomes of cancer patients [15-18]. In gastric cancer, some diagnostic or prognostic biomarkers have also been investigated. For instance, the NOB1 gene was found to be correlated with clinical outcomes and prognosis of gastric cancer patients, suggesting that it was an independent risk factor for 5-year mortality of patients. This provides more clinical evidence for it as a new target for diagnosis and treatment for gastric cancer [19]. A study by Yörüker EE et al. identified that circulating IncRNA H19 was overexpressed in gastric cancer and had potential diagnostic value [20]. These studies suggest that the development of cancer-related biomarkers may help predict patient prognosis and treatment strategies.

In recent years, miRNAs have obtained more and more attention for high diagnostic and/or prognostic significance in different human tumors, including gastric cancer [21-23]. Zhang et al. identified multiple miRNAs expression profiles and clinical information of gastric cancer patients, suggesting that a three-miRNA signature could play a role in predicting the prognosis of gastric cancer patients [24]. A study by Zheng et al. revealed that expression of miR-203 was downregulated in gastric cancer and that it might be a potential prognostic factor for gastric cancer [25]. The present study investigated expression of miR-106 in gastric cancer tissues and cell lines. Moreover, qRT-PCR results showed that expression of miR-106 was remarkably upregulated in gastric cancer tumor tissues and cell lines, indicating that miR-106 may be an oncogene in gastric cancer. Expression of miR-106 in gastric can-
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Increased evidence has shown that miR-106 plays a crucial role in the progression of some malignancies [26-28]. A study by Yen et al. showed that miR-106b and its cluster, miR-93 and miR-25, are upregulated in HCC patients and that miR-106b promotes cancer progression in hepatitis B virus-associated HCC [26]. In colorectal cancer, miR-106 was identified to be upregulated in metastatic colorectal cancer, promoting cell migration and invasion by directly targeting DLC1 [27]. To explore the functional roles of miR-106 in the progression of gastric cancer, the effects of miR-106 on gastric cancer cell proliferation, migration, and invasion were investigated. In two gastric cancer cell lines, MGC-803 and SGC-7901, it was found that expression of miR-106 was significantly correlated with prognosis of gastric cancer, suggesting that it might be an independent prognostic marker in gastric cancer.

Further clinical-based analysis found that expression of miR-106 was significantly correlated with lymph node metastasis and TNM stage, suggesting that miR-106 is involved in the development of gastric cancer. Present results suggest the potential relationship between high miR-106 expression and unfavorable prognosis of tumors. To further evaluate the prognostic significance of miR-106 in gastric cancer, this study analyzed the relationship between miR-106 expression and overall survival of patients using Kaplan-Meier and Cox analyses. Kaplan-Meier analysis results showed that patients in the high miR-106 expression group had a shorter survival duration than those in the low miR-106 expression group. According to multivariate Cox regression analysis, miR-106 expression was significantly correlated with prognosis of gastric cancer, suggesting that it might be an independent prognostic marker in gastric cancer.

Figure 4. Results of migration and invasion analysis for MGC-803 and SGC-7901 cells. A, B. Overexpression of miR-106 by miR-106 mimic could promote the cell migration and invasion, but downregulated miR-106 expression could inhibit cell migration and invasion. (*P < 0.05, **P < 0.01, ***P < 0.001).
that overexpression of miR-106 by miR-106 mimics could promote cell proliferation, migration, and invasion, while downregulation of miR-106 by miR-106 inhibitors inhibited cell proliferation, migration, and invasion. Taken together, results suggest that miR-106 could promote the progression of gastric cancer. In a study of bladder cancer, miR-106 overexpression was found to significantly reverse the effects of BAI on proliferation, migration, and apoptosis of T24 cells, which suppressed proliferation and migration and induced apoptosis of T24 cells through downregulating miR-106, along with inhibition of the JNK and MEK/ERK pathways [29]. However, the detailed molecular mechanisms of miR-106 in gastric cancer require further investigation.

In conclusion, the current study demonstrates that miR-106 functions as an oncogene in gastric cancer that promotes cancer progression. Upregulated expression of miR-106 may serve as a candidate prognostic biomarker and a therapeutic target for gastric cancer patients.

Disclosure of conflict of interest

None.

Address correspondence to: Shihua Zhang, Department of Brain surgery, First Affiliated Hospital of Jiamusi University, No. 348, Dexiong Street, Jiamusi 154002, Heilongjiang, China. Tel: 0454-8623330; Fax: 0454-8623330; Email: fu26578249yi@163.com

References


