Oncology Research, Vol. 22, pp. 75–84
Printed in the USA.

0965-0407/15 $90.00 + .00
DOI: http://dx.doi.org/10.3727/096504014X14024160459203
E-ISSN 1555-3906
www.cognizantcommunication.com

MicroRNA-181a Inhibits Tumor Proliferation, Invasiveness, and Metastasis and Is Downregulated in Gastric Cancer

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MicroRNAs (miRNAs) play crucial roles in the development and progression of human cancers, including gastric cancer. The discovery of miRNAs may provide a new and powerful tool for studying the mechanism, diagnosis, and treatment of gastric cancer. Here we show that miR-181a levels were significantly downregulated in gastric cancer tissues compared with the adjacent normal regions in 80 paired samples. Moreover, the lower levels of miR-181a were associated with the pM or pTNM stage in clinical gastric cancer patients. In addition, the ectopic expression of miR-181a in the gastric cancer cell line HGC-27 inhibited cell proliferation, cell migration, and invasion by directly interacting with the mRNA encoding the oncogenic factor Prox1. Taken together, our results indicate that miR-181a might act as a tumor suppressor in gastric cancer, which may provide a novel diagnostic and therapeutic option for human gastric cancer in the near future.

Key words: Gastric cancer; miR-181a; Tumor suppressor; Prox1

INTRODUCTION

Different strategies have been used to treat gastric cancer (GC), which is the fourth most prevalent cancer and the second leading cause of cancer fatalities in the world (1). Currently, patients with late-stage GC have an overall 5-year survival rate of approximately 20% (2). Like other cancers, GC is the result of a complex, multistep process associated with various genetic and epigenetic changes. Many factors are involved in the development of GC, including viral infection, chronic alcohol abuse, obesity, and factors inducing chronic gastric inflammation. Furthermore, the factors that lead to GC are becoming more widespread in the Western world (3,4). Although the molecular mechanisms underlying the pathogenesis of *Helicobacter pylori* infection have been examined in great detail, the pathophysiology and detailed mechanisms of the initiation and progression of GC are not completely understood.

Recently, the classical categories of oncogenes and tumor-suppressor genes have been expanded to include a new family of RNAs known as microRNAs (miRNAs), which may regulate a vast number of protein-coding genes, including tumor-related genes (5,6). MicroRNAs regulate gene expression mainly through effects on productive translation and mRNA stability. The dysregulation of miRNAs is assumed to play an important role in cancer development by regulating cell proliferation, differentiation, apoptosis, and carcinogenesis (7,8). Previous studies have shown that many miRNAs are aberrantly downregulated or overexpressed during GC progression, including miR-10b, miR-101, miR-200, and miR-34b (9–12). However, the role and function of miRNAs in GC is still emerging, and the roles of many other aberrantly expressed miRNAs in GC development as well as the mechanisms involved are still unknown.

miR-181a is derived from an overlapping gene locus that is highly conserved among mammalian species (13). These earlier studies found that miR-181a is enriched in the brain, and its aberrant expression has been associated with brain diseases (14,15). miR-181a expression is reduced in human gliomas and glioma cell lines, and expression is negatively correlated with tumor grade (14). Despite its abundance and functional importance, very few studies to date have evaluated the role of miR-181a in GC development or, in particular, the role of miR-181a in the regulation of cell proliferation and invasion. Our study analyzed the expression levels of miR-181a in 80 paired GC tissues and adjacent normal tissues, and we demonstrated that miR-181a levels were downregulated both in GC tissues compared with normal controls as well as in the GC cell lines HGC-27, MGC-803, MKN-45, and SGC-7901. Furthermore, restoring miR-181a expression

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by transfection with miRNA mimics inhibited cell proliferation and invasion in HGC-27 cells. The tumor-suppressor role of miR-181a in GC cells was mediated by the inhibition of Prox-1. These results suggest that miR-181a functions as a tumor suppressor in GC; targeting miRNA expression could be a potential therapeutic strategy for GC patients.

MATERIALS AND METHODS

Cell Culture and Transfection

A total of four human gastric cancer cell lines were purchased from the American Type Culture Collection for use in this study: MGC-803, HGC-27, MKN-45, and SGC-7901 (ATCC, Manassas, VA, USA). The MGC-803 cell line was propagated in Dulbecco's modified Eagle medium (Gibco; Invitrogen; Life Technologies, Darmstadt, Germany) supplemented with 10% fetal bovine serum (FBS; PAA, Pasching, Austria), streptomycin (100 μg/ml), and penicillin (100 U/ml). The HGC-27, MKN-45, and SGC-7901 cell lines were maintained in RPMI 1640 medium (PAA) supplemented with 10% FBS (PAA). HGC-27 cells were transfected with miRNA mimics (GenePharma, Shanghai, China) or negative control miRNA (Scramble; GenePharma; Shanghai, China) at a final concentration of 30 nmol/L using Dharmafect 1 (Dharmacon, Lafayette, CO, USA) in accordance with the manufacturer's instructions. The MKN-45 and SGC-7901 cell lines were maintained in RPMI 1640 medium (PAA) supplemented with 10% FBS (PAA).

Tissue Samples

Gastric tumors and their morphologically normal tissue (located >3 cm away from the tumor) were obtained between November 2008 and November 2012 from 80 GC patients undergoing surgery at The Affiliated Jiangyin Hospital of Southeast University Medical College. Tissue samples were cut into two parts. One part was fixed with 10% formalin for histopathological diagnosis, and the other was immediately snap-frozen in liquid nitrogen and stored at −196°C in liquid nitrogen until RNA was extracted. The patient group consisted of 57 males and 23 females with a median age of 59 years (range, 31–82 years). The use of tissue samples for all experiments was approved by the patients and by the Ethics Committee of the institution. Patient characteristics are described in Table 1.

TaqMan RT-PCR for miRNA Expression

Total RNA was extracted from cells and tissues with TRIzol reagent (Invitrogen, Carlsbad, CA, USA). miRNAs were quantitated by real-time PCR using the TaqMan MicroRNA assay (Invitrogen, USA). First-strand complementary DNA (cDNA) synthesis was performed using 1 μg of total RNA in a final volume of 12 μl containing

Table 1. Patient Characteristics

ID	Sex	Age	Venous Invasion	Borrmann Typing	pT Stage	pN Stage	pM Stage	pTNM Stage
1	M	54	1	2	4a	1	0	IIIA
2	F	44	1	1	4a	2	1	IV
3	F	47	0	2	3	1	0	IIB
4	M	50	1	3	3	1	0	IIB
5	F	55	1	1	3	0	0	IIA
6	F	56	0	2	3	3a	1	IV
7	F	57	0	1	3	1	0	IIB
8	M	41	0	1	3	1	0	IIB
9	F	65	0	2	4a	2	1	IV
10	F	73	0	2	4a	1	0	IIIA
11	F	61	1	1	4a	1	1	IV
12	F	73	0	1	4b	3a	1	IV
13	F	51	0	1	1b	0	0	IA
14	F	64	0	1	4a	1	1	IV
15	M	36	0	3	3	1	0	IIB
16	M	51	0	2	3	3	1	IV
17	F	43	1	1	4a	1	1	IV
18	M	51	0	3	3	1	0	IIB
19	F	52	0	1	2	0	0	IA
20	M	50	0	1	4a	0	0	IIB
21	M	51	1	2	4a	1	1	IV
22	F	53	1	1	4a	1	1	IV
23	M	53	1	1	4a	1	0	IIIA
24	M	53	0	3	2	0	1	IV
25	M	54	1	1	3	2	1	IV
26	M	55	0	1	2	0	0	IA
27	M	55	1	3	3	1	0	IIB
28	M	55	0	2	3	3a	1	IV
29	F	58	0	1	2	2	0	IIA
30	M	57	1	2	2	1	1	IV
31	M	58	0	1	2	0	0	IA
32	M	58	0	1	2	0	0	IA
33	M	48	1	1	4a	1	1	IV
34	M	59	1	3	2	1	0	IIA
35	M	59	0	1	2	2	0	IIA
36	F	60	0	1	2	1	0	IIA
37	M	60	0	1	4a	1	0	IIIA
38	M	60	1	1	4b	2	1	IV
39	M	60	0	1	4b	2	1	IV
40	F	67	1	1	4a	0	1	IV
41	M	61	1	1	2	1	0	IIA
42	M	56	1	1	2	1	0	IIA
43	M	61	0	4	4a	1	0	IIIA
44	M	61	0	3	2	3a	1	IV
45	F	57	0	1	2	0	0	IA
46	M	62	1	1	2	0	0	IB
47	M	62	0	1	2	1	0	IIA
48	M	58	0	3	3	1	0	IIA
49	F	63	1	1	2	1	0	IIA
50	M	63	1	1	2	1	0	IIA
51	M	63	1	1	4a	1	0	IIIA
52	M	63	0	2	4a	2	0	IIIA
53	M	64	1	1	2	1	0	IIA

(*continued*)

Table 1. Patient Characteristics (*Continued*)

ID	Sex	Age	Venous Invasion	Borrmann Typing	pT Stage	pN Stage	pM Stage	pTNM Stage
54	M	64	0	1	4b	1	0	IIIA
55	M	53	0	2	4b	1	1	IIIA
56	M	64	0	1	4b	2	0	IV
57	F	52	0	1	4a	0	1	IIB
58	M	65	0	1	3	0	0	IIA
59	M	65	1	1	4a	1	0	IIIA
60	M	51	0	1	3	0	0	IA
61	M	66	0	1	3	0	1	IIA
62	M	66	0	1	2	2	0	IIA
63	M	53	0	1	4a	1	0	IIIA
64	M	67	1	2	3	1	0	IIA
65	M	67	1	1	4a	1	0	IIIA
66	M	57	1	1	4b	2	0	IIIA
67	M	68	0	1	4a	1	0	IIIA
68	M	68	1	2	4a	1	0	IIIA
69	M	69	0	1	2	0	0	IA
70	F	69	0	1	1b	0	0	IA
71	M	71	0	1	4a	1	0	IIIA
72	M	58	1	1	4a	1	0	IIIA
73	M	67	0	1	4a	2	0	IIIB
74	F	72	1	1	4a	1	0	IIIA
75	M	55	1	1	4a	1	0	IIIA
76	M	73	0	1	4a	1	0	IIIA
77	M	72	1	2	4a	1	0	IIIA
78	F	69	0	1	4b	1	0	IIIA
79	M	59	0	1	4a	1	0	IIIA
80	M	68	0	1	4b	1	0	IIIA

2-M stem-loop primer and 10 mM dNTP Mix (Invitrogen, USA). The mix was incubated at 65°C for 5 min, and then, 5× RT buffer, 0.1 M DTT, 200 U/μl MultiScribe reverse transcriptase, and 40 U/μl RNase inhibitor were added (Invitrogen, USA). The mix was then incubated at 37°C for 55 min, followed by 70°C for 15 min, and then held at −20°C. Real-time PCR was performed using a standard TaqMan PCR protocol. The 20-μl PCR reactions included 1 μl of RT product, 1× Universal TaqMan Master Mix, and 1× TaqMan probe/primer mix (Invitrogen, USA). All RT reactions, including the no-template controls, were run in triplicate. All mRNA quantification data were normalized to U6. The relative amount of transcript was calculated using the comparative Ct method.

Cell Proliferation

Cells were incubated in 10% CCK-8 (DOJINDO, Kumamoto, Japan) diluted in normal culture media at 37°C until a visual color conversion occurred. Proliferation rates were determined at 0, 24, 48, 72, and 96 h after transfection. The absorbance of each well was measured with a microplate reader set at 450 nM and 630 nM. All experiments were performed in quadruplicate.

Cell Migration and Invasion Assays

HGC-27 cells were grown to confluence in 12-well plastic dishes and treated with miRNA mimics or scramble. Then, 24 h after transfection, linear scratch wounds (in triplicate) were created on the confluent cell monolayers using a 200-μl pipette tip. To remove cells from the cell cycle prior to wounding, cells were maintained in serum-free media. To visualize migrated cells and wound healing, images were taken at 0, 24, and 48 h. A total of 10 areas were selected randomly from each well, and the cells in three wells from each group were quantified.

For the invasion assays, 24 h after transfection, 1×10^5 cells in serum-free media were seeded in Transwell migration chambers (8 μm pore size; Millipore, Zürich, Switzerland). The upper chamber of the Transwell inserts was coated with Matrigel (Sigma-Aldrich, St. Louis, MO, USA). Medium containing 20% FBS was added to the lower chamber. After 24 h, the noninvading cells were removed with cotton wool. Invasive cells located on the lower surface of the chamber were stained with May–Grunwald–Giemsa stain (Sigma-Aldrich) and counted using a microscope (Olympus, Tokyo, Japan). Experiments were independently repeated three times.

Dual Luciferase Assays

Cells were cotransfected with 0.4 μg of the reporter construct, 0.2 μg of pGL-3 control vector, and/or negative controls. Cells were harvested 24 h posttransfection and assayed using the Dual Luciferase Assay (Promega, WI, USA) according to the manufacturer's instructions. Firefly luciferase values were normalized to Renilla, and the ratio of Firefly/Renilla values was reported. All transfection assays were performed in triplicate.

Western Blotting Analysis

Western blotting was performed using standard methods. Proteins were separated by 10% SDS-PAGE and then transferred to PVDF membranes (Amersham, Buckinghamshire, UK). The membranes were blocked overnight with 5% nonfat dried milk and incubated for 2 h with anti-Prox-1 antibody (Abcam, Cambridge, UK) at 1:1,000 or anti-GAPDH antibody at 1:50,000 (Proteintech, Chicago, IL, USA). After washing with TBST (10 mM Tris, pH 8.0, 150 mM NaCl, and 0.1% Tween 20), the membranes were incubated for 2 h with goat anti-rabbit antibody (ZSGB-BIO, Beijing, China) at a 1:5,000 dilution or a 1:50,000 dilution.

Rescue Assays of Prox1 Gene Expression

The full-length Prox1 ORF was PCR amplified and cloned into pCDNA3.1 to generate pcCDNA-Prox1 constructs, which were used in the rescue assays. HGC-27 cells in six-well plates were first transfected with mimic

or scramble (30 nM). After 24 h in culture, these HGC-27 cells were then cotransfected with either mimic (30 nM) and 2.0 μg pCDNA-Prox1 constructs or pCDNA empty vectors. Cells were harvested at the indicated times and assayed, as required.

RESULTS

miR-181a Is Downregulated in GC Cells

The expression of miR-181a was examined in four human GC cell lines (HGC-27, MGC-803, SGC-7901, and MKN-45) and four GC tissues and their adjacent non-neoplastic tissues (Fig. 1A). The GC cell lines exhibited extraordinarily low expression of miR-181a compared with the four pairs of primary cancer tissues and adjacent tissues (Fig. 1A).

Expression of miR-181a in Clinical GC Patients and Correlation Analysis With Clinicopathological Characteristics

Of the 80 GC samples, miR-181a was downregulated in 65 (65/80, 81.3%) when compared with adjacent tissues when the cutoff was set at 2.0 (Fig. 1B). Overall, the expression of miR-181a in GC tissues was significantly lower than in adjacent tissues ($p < 0.01$) (Fig. 1C). To evaluate the correlation between miR-181a expression and clinicopathologic characteristics, patients were divided into groups with high and low expression. To further study the relationship between miR-181a expression and clinicopathological factors, the levels of miR-181a in GC tissues (in patients with full clinical information) were statistically analyzed (nonparametric test). Low levels of miR-181a expression were associated with pTNM stage ($p \leq 0.01$, stage II vs. III; $p \leq 0.01$, stage II vs. IV) (Fig. 1D), and even lower levels of miR-181a were associated with pM stage ($p \leq 0.01$, metastasis vs. no metastasis) in GC patients (Fig. 1E). These data suggested that alterations of miR-181a could be involved in GC progression.

miR-181a Inhibits GC Cell Proliferation and Cyclin D1 Expression

GC cells were transfected with scrambled control oligo or miR-181a mimics, which exhibited a high transfection efficiency (Fig. 2A). CCK-8 proliferation assay results showed that cell proliferation was inhibited in miR-181a mimic-transfected GC cells compared with scrambled oligo-transfected cells or untreated cells (Fig. 2B). The proliferative effect of miR-181a was further confirmed by evaluating cyclin D1 expression. As shown in Figure 2C and D, there was a significant decrease in the protein and mRNA levels of cyclin D1 in the group transfected with miR-181a mimics when compared with the control or untreated group.

miR-181a Inhibits Cell Migration and Invasion In Vitro

To analyze the role of miR-181a in cell migration and invasion, which are the key determinants of malignant progression and metastasis, wound healing and Transwell assays were performed in HGC-27 cells. Cells treated with miR-181a mimics were distinctively less migratory than scramble control-treated or untreated cells at 24 and 48 h after scratching (Fig. 3A). Furthermore, we conducted cell invasion assays using Matrigel and stained the invading cells to measure the directional invasion ability following the ectopic expression of miR-181a in HGC-27 cells. The invasiveness of cells transfected with miR-181a mimics was dramatically decreased compared with the scramble control and untreated cells (Fig. 3B).

miR-181a Suppresses the Expression of Prox1 in GC Cells

miRNAs function by negatively regulating their target genes. As a potential miR-181a target, Prox1 was selected for further assessment because PicTar predicted complementarity between miR-181a and the Prox1 3′-UTR (Fig. 4A). The overexpression of miR-181a reduced the protein expression but not the mRNA level of Prox1 in NP cells (Fig. 4B and D). Next, the effect of miR-181a on the translation of Prox1 mRNA into protein was assessed by a luciferase reporter assay (Fig. 4C). The overexpression of miR-181a remarkably reduced luciferase activity in the Rac wild-type reporter gene but not the mutant Rac 3′-UTR, indicating that miR-181a directly targeted the Rac 3′-UTR. Because previous studies have noted the oncogenic role of Prox1 in various cancers, we reasoned that interfering with Prox1 expression in GC cells might have an inhibitory effect on cell growth. As expected, the ectopic expression of Prox1 induced marked increases in cell proliferation and cell migration (Fig. 4F and G; see group pcDNA-Prox1 + scramble and group pcDNA + scramble). Next, we adapted a "rescue" strategy to examine the functional relevance of the Prox1 interaction in GC cells. The level of Prox1 was reduced when miR-181a mimics were transfected with pcDNA-Prox1 after 24 h (Fig. 4E). In agreement with the reduced expression of target proteins, decreased cell proliferation (Fig. 4F) was accompanied by decreased cell invasion (Fig. 4G). These results were also observed in HGC-27 cells transfected with miR-181a mimics following the insertion of the pcDNA-Prox1 construct. Thus, we conclude that the repression of cell growth by miR-181a is a consequence of decreased Prox1 expression in GC.

DISCUSSION

Although miRNA signatures for GC have been established, elucidation of the role of deregulated miRNAs in gastric carcinogenesis remains in the early stage. The data

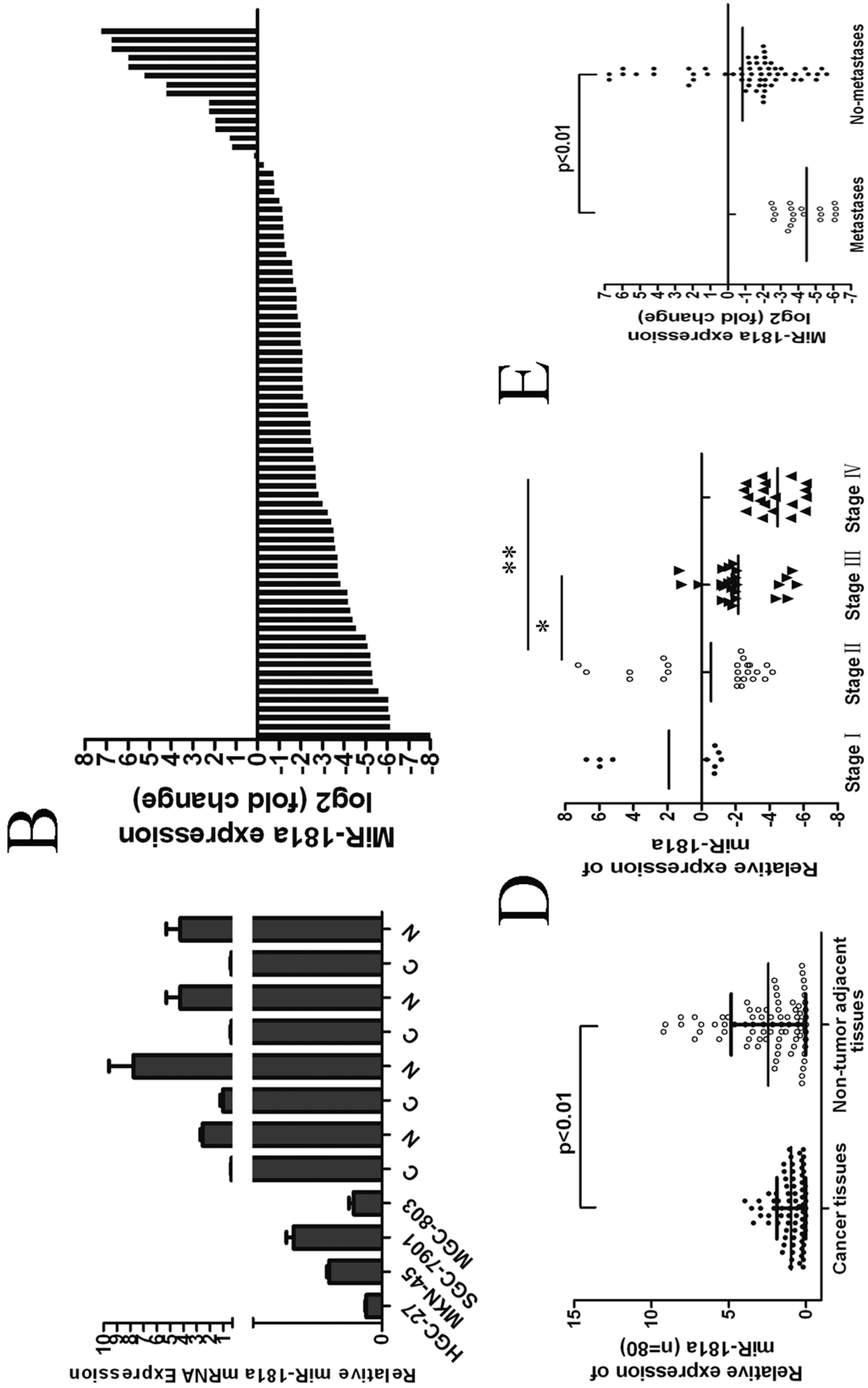

Figure 1. The expression of miR-181a in gastric cancer (GC) tissues and cell lines. (A) The relative level of miR-181a in GC cell lines (HGC-27, MGC-803, MKN-45, and SGC-7901) relative to four paired samples. (B) miR-181a levels were detected in 80 pairs of GC tissues and adjacent normal controls by quantitative RT-PCR. Data are presented as the log2 fold change of GC tissues relative to adjacent normal regions. (C) Relative miR-181a expression levels in GC tissues and adjacent normal regions. (D) and (E) Statistical analysis of the association between miRNA level, pTNM stage (I, II, III, and IV), and pM stage (No metastasis and Metastasis, respectively); $^{*}p<0.05$, and $^{**}p<0.01$.

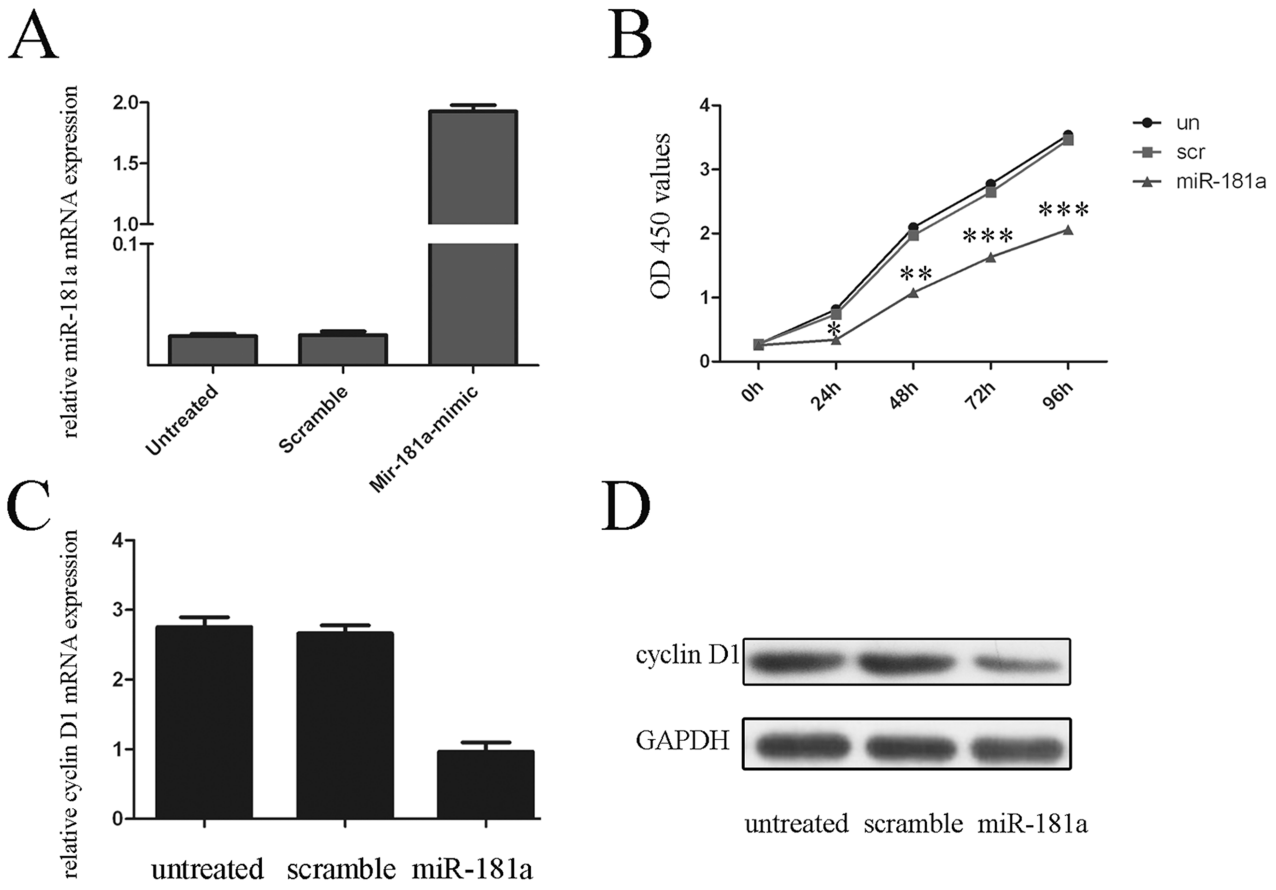

Figure 2. Overexpression of miR-181 inhibits GC cell growth. (A) Expression levels of miR-181a were examined by real-time PCR after the transfection of 30 nmol/L of miR-181a mimics or scramble or no transfection. (B) Growth of HGC-27 cells is shown after transfection with 50 nmol/L of miR-181a mimics or scramble or no transfection. The growth index was measured at 0, 24, 48, 72, and 96 h. (C) miR-181a inhibited cyclin D1 mRNA expression. HGC-27 cells were transfected with 50 nmol/L of miR-181a mimics or scramble or no transfection. Cyclin D1 levels were detected by real-time PCR. (D) miR-181a inhibited cyclin D1 protein expression. HGC-27 cells were transfected with 50 nmol/L of miR-21 mimics or scramble or no transfection. Cyclin D1 levels were detected by Western blot. GAPDH was also detected as a loading control. Values are presented as the mean ± SD. Compared with control, $^{**}p < 0.01$, $^{*}p < 0.05$, and $^{***}p < 0.001$.

presented here show a role for miR-181a in GC. In this study, we analyzed the expression of miR-181a in 80 GC patients and found that miRNAs were much lower in GC tissues compared with adjacent controls. Furthermore, we show that the restored expression of miR-181a in the GC cell line HGC-27 resulted in reduced cell proliferation. These results allow us to speculate that the silencing of miR-181a may confer a survival advantage to GC cells. On the other hand, metastasis is the major cause of morbidity and mortality in GC patients; therefore, we investigated the effects of miR-181a on cell migration and invasion. The introduction of miR-181a inhibited HGC-27 cell migration in wound healing assays and suppressed cell invasion in Transwell assays, further confirming their association with the degree of GC malignancy. Moreover, we identified Prox-1 as a direct target of miR-181a. Our findings, together with those from other groups, suggest that miR-181a plays a fundamental role in GC tumorigenesis and cell invasion.

Although miR-181a has been reported to be involved in colorectal cancer, breast cancer, lung adenocarcinoma, and esophageal squamous cell carcinoma, its expression and function in GC remains to be determined (13,16–18). Previously, miR-181a has been shown to mediate its oncogenic effect through downregulating KLF6 (19). However, miR-181a did not show a remarkable regulation of KLF6 in GC cells (data not shown), indicating that the oncogenic or tumor-suppressive effects of miR-181a might be tissue specific. The study of Zhang et al. also reported that miR-181a was overexpressed in nine human gastric tumor species. In addition, miR-181a is also known to play an important role in the progression of diverse types of cancers. The expression of miR-181a is downregulated in many types of cancer cells, and miR-181a is usually

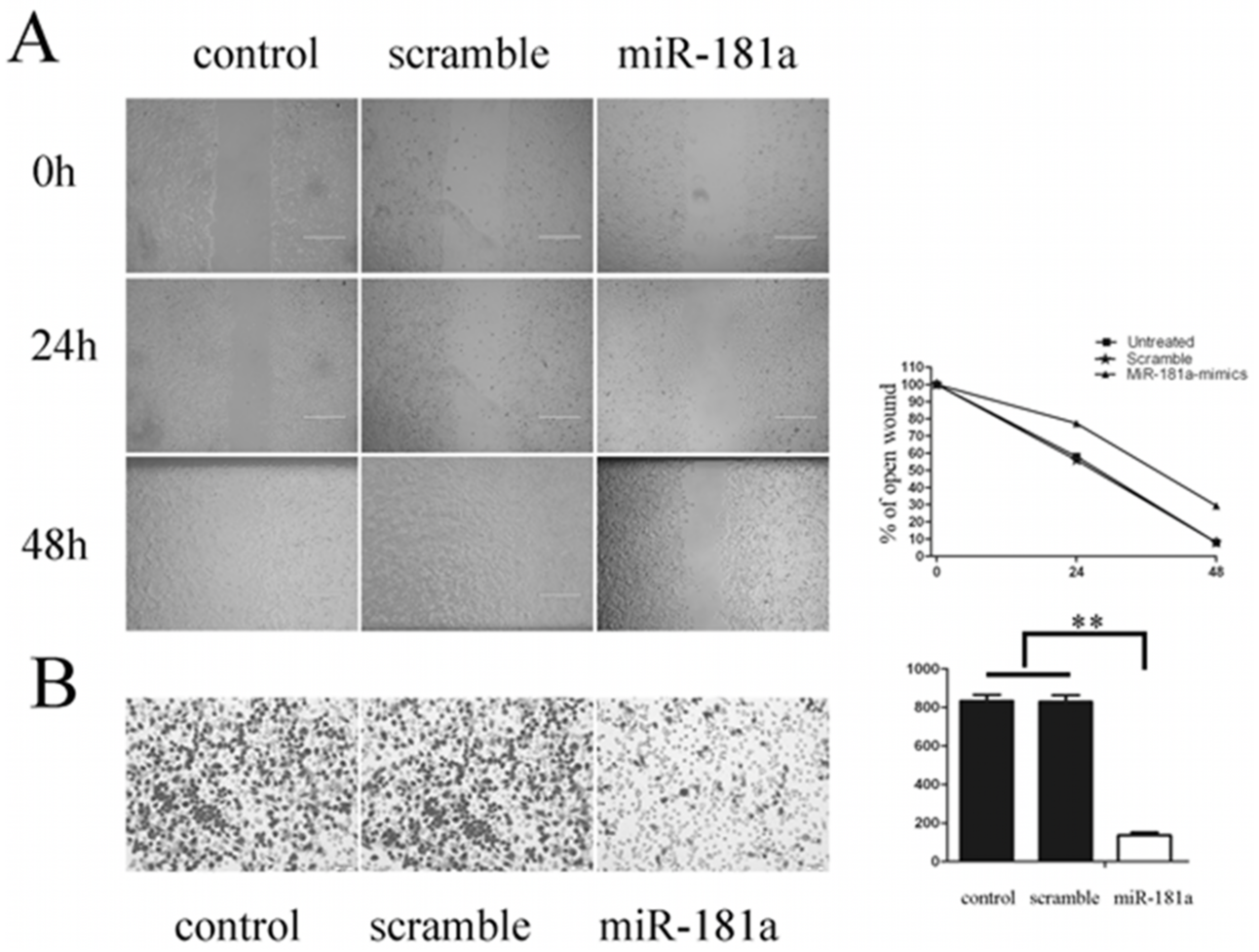

Figure 3. The influence of miR-181a on GC cell migration and invasion. (A) Wound healing assays of HGC-27 cells after treatment with miRNA mimics or scramble; the relative ratio of wound closure per field is shown. (B) Transwell analysis of HGC-27 cells after treatment with miRNA mimic or scramble; the relative ratio of invasive cells per field is shown. $^{**}p < 0.01$.

considered a tumor suppressant in both solid tumors and hematologic malignancies (17,20,21). Our results show that miR-181a was downregulated in 65 (65/80, 81.3%) GC tissues compared with the normal adjacent tissues, and the expression of miR-181a in GC tissues was significantly lower than in adjacent tissues.

To further clarify the role of miR-181a in the development of GC, cell transfection was performed. Overexpression of miR-181a significantly inhibited cell proliferation, migration, and invasion, indicating that the repression of miR-181a might promote tumor progression in GC carcinogenesis. These results suggest that miR-181a acts as a tumor suppressor and that its downregulation may contribute to the progression and metastasis of GC.

To explore the molecular mechanism by which miR-181a suppresses GC cell line growth, migration, and invasion, we identified Prox-1 as a direct target of miR-181a in GC cells (22). This conclusion is supported by the following: a complementary sequence of miR-181a was identified in the 3′-UTR of Prox-1 mRNA; the overexpression of miR-181a led to a significant reduction in Prox-1 at the protein level; the overexpression of miR-181a suppressed Prox-1 3′-UTR luciferase reporter activity, and this effect was abolished by mutating the miR-181a seed-binding site. These results indicate that miR-181a may function as a tumor suppressor that is partly mediated by repressing Prox-1 expression in GC development.

The homeobox gene Prox1 is a transcription factor related to the *Drosophila* gene prospero, which plays a major role in the development of the liver, central nervous system, and pancreas (23–25). Importantly, Prox1 is a master gene controlling the normal embryonic development and differentiation of the lymphatic vasculature (26). Recently, Prox1 has received much attention because new data demonstrate that Prox1 plays a more important role in the progression of various carcinomas than previously

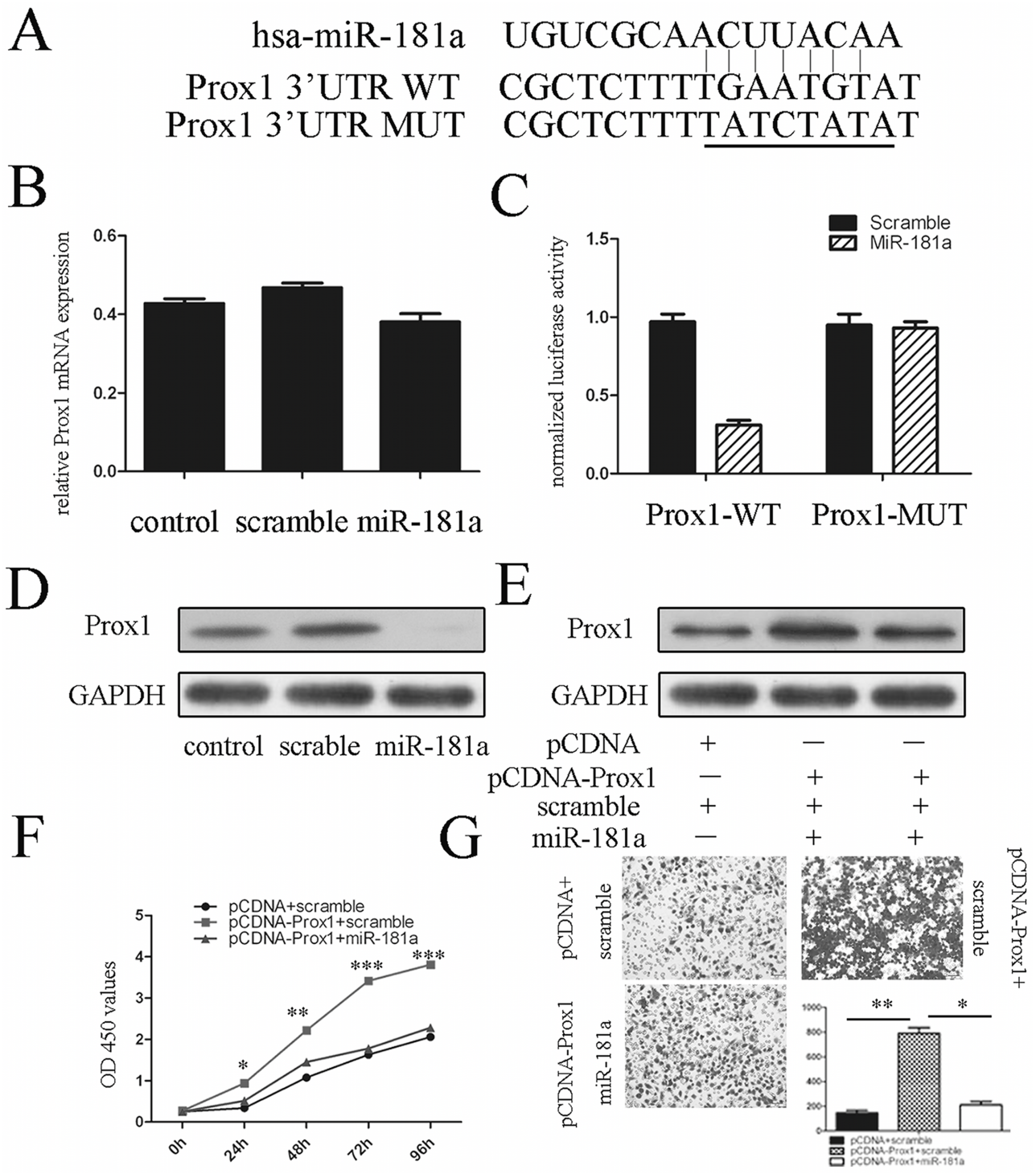

Figure 4. miR-181a targets Prox1 in GC cells. (A) Schematic representation of Prox1 3′-UTRs showing the putative miRNA target site. (B) miR-181a cannot alter the mRNA level of Prox1, according to real-time PCR. (C) Dual-luciferase analysis of reporter gene carrying the Prox1 3′-UTR, cotransfected with miRNA mimic or scramble. (D) Western blot analysis of Prox1 expression in HGC-27 cells transfected with miRNA mimics or scramble. (E) Western blot analysis of Prox1 in HGC-27 cells cotransfected with either miR-181a mimics (30 nM) or scramble (30 nM) and 2.0 μg pCDNA-Prox1 or pCDNA empty vector. (F) Cell proliferation assay of HGC-27 cells treated as described in (E) using CCK-8. (G) Transwell analysis of HGC-27 cells treated as described in (E). The relative ratio of invasive cells per field is shown. $^{**}p<0.01$, $p<0.05$.

thought. Earlier data revealed that Prox1 was silenced by hypermethylation in hematologic malignancies and breast cancers, deleted in biliary and hepatocellular carcinomas, or point mutated from base A to G in esophageal cancer (27,28). However, recent studies suggest that Prox1 is more likely to facilitate tumor formation and accelerate tumor progression and invasion (29–31). Our study confirmed that overexpression of Prox1 increases HGC-27 cell invasion. However, the underlying mechanisms are unclear. Our data show that the ability of miR-181a to target Prox1 may be one mechanism underlying the post-transcriptional control of Prox1.

In conclusion, the current study provides new evidence that miR-181a may function as a tumor suppressor in GC through the repression of Prox1 expression. Furthermore, the lower expression of miR-181a in GC specimens was correlated with metastasis. Our findings regarding miR-181a are encouraging and suggest that this miRNA could be a potential target for the treatment of GC in the future.

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