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Original Article

miR-27b-3p suppresses cell proliferation, migration and invasion by targeting LIMK1 in colorectal cancer

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Abstract: Accumulating evidence has indicated that microRNAs (miRNAs) can act as candidate tumor suppressors. However, the mechanism of miR-27b-3p in colorectal cancer (CRC) is still unknown. In this study, the relative expression levels of miR-27b-3p and LIMK1 were detected by quantitative real-time PCR (qRT-PCR) in CRC and adjacent normal tissues as well as in CRC cell lines. The proliferation, cell cycle and migratory and invasive abilities of SW620 cells were analyzed by CCK-8, flow cytometry, wound healing assays, and Transwell assays, respectively. The regulatory relationships between LIMK1 and miR-27b-3p were detected by qRT-PCR, luciferase reporter gene and Western blot assays. The results showed that the expression levels of miR-27b-3p were downregulated in CRC samples compared with those in adjacent nontumor tissues. Moreover, we found that miR-27b-3p suppressed CRC cell proliferation, cycle and migration and invasion. Additionally, there was an inverse correlation between LIMK1 expression and miR-27b-3p expression in CRC tissues. LIMK1 was identified as a direct target of miR-27b-3p. Our findings indicated that miR-27b-3p inhibited CRC cell proliferation, migration and invasion by targeting LIMK1.

Keywords: miR-27b-3p, LIMK1, colorectal cancer, proliferation, migration, invasion

Introduction

Colorectal cancer (CRC) is one of the most commonly diagnosed malignancies worldwide and is one of the most common leading causes of cancer-specific mortality [1]. In the past few years, the survival rates of patients with CRC have gradually increased because of earlier diagnosis and improved treatments; however, approximately 30-50% of patients who undergo excision surgery suffer from local tumor recurrence or metastasis [2]. According to available statistics, patients treated with chemotherapy in combination with monoclonal antibody have an average overall survival of 20 months, and the best response rates are 50% [3]. However, this treatment is expensive for patients with CRC, and chemotherapy may lead to overtreatment of patients with toxic drugs that generate severe adverse effects [4]. Therefore, new biomarkers and therapeutic targets should be found to obtain effective treatments for more aggressive stages of CRC.

miRNAs are a type of small, non-coding RNA 20-22 nucleotides in length that regulate the expression of hundreds of target genes by binding to the 3'-untranslated region (3'-UTR) of their target mRNAs [5]. miRNAs play critical roles in many kinds of physiological and pathological processes such as apoptosis, cell proliferation, differentiation, development, stress response and migration [6, 7]. A growing amount of evidence has revealed that miRNAs can act as either tumor suppressors or oncogenes in the progression of many kinds of tumors by regulating posttranscriptional gene expression [8-10]. A variety of miRNAs are aberrantly expressed in CRC [8].

miR-27 is a family of miRNA precursors found in humans. miRNAs are typically transcribed into precursors approximately 70 nucleotides in length and subsequently processed by the Dicer enzyme to yield a product approximately 22 nucleotides in length. miR-27b-3p is the mature miRNA of the miR-27 family. It has been reported that miR-27b-3p is differentially expressed in cervical cancer [11], colorectal neoplasia [12], and gastric cancer [13]. Furthermore, studies have shown that dysregulated miR-27b-3p plays a critical role in the pro-

liferation and invasion of many solid malignancies, which makes it a potential target for tumor treatment [13, 14]. However, the exact function of miR-27b-3p in CRC has not yet been studied.

LIMK1 is a member of the LIM kinase (LIMK) family, which contains key regulators of the actin cytoskeleton and are related to cell motility and invasion, and has been identified as a therapeutic target for metastatic disease [15]. A large study showed that LIMK1 as an oncogene participates in the process of various types of cancer [16-18]. For example, LIMK1 affected the invasion ability of prostate cancer by regulating membrane type matrix metalloproteinase 1 (MT1-MMP) [17]. LIMK1 was also verified recently to be involved in the development of CRC [19]. However, the regulatory mechanism and the role of LIMK1 in the progression of CRC are poorly understood.

In the current study, we aimed to detect the expression levels of miR-27b-3p in CRC tissues and cell lines and elucidate the roles of miR-27b-3p on CRC cell proliferation, cell cycle, migration and invasion. Furthermore, we confirmed that LIMK1 is a target gene of miR-27b-3p.

Materials and methods

Samples collection

CRC samples and adjacent normal tissues were collected from 83 patients who underwent tumor resection surgery at our hospital between 2012 and 2016. All patients signed informed consent. The tissues were separately frozen in liquid nitrogen for further study. Protocols were approved by the institutional ethics committee at our hospital and conducted in accordance with the guidelines for ethical management.

Cell lines

Human embryonic kidney 293T (HEK293T) cells, human-derived colonic epithelial NCM-460 cells and human CRC cell lines (SW480, DLD-1, HT29, HCT116, and SW620) were purchased from the Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China. SW620, HT29 and HEK293T cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen). NCM460, SW480, HCT116, and DLD-1 cells were cultured in RPMI 1640 medium (Invitrogen, Carlsbad, CA, USA). Each medium contained 10% fetal bovine serum (FBS, Invitrogen, Carlsbad, CA, USA) and 100 U/ml penicillin and streptomycin (Invitrogen). All the cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2.

Vector construction, lentivirus production and cell transfection

miR-27b-3p mimics were packaged into lentiviral vector (GenePharma) to overexpress miR-27b-3p in CRC cells, and miR-NC was used as a control. Cells were seeded into 6-well plates at a 30% density and cultured overnight before transfection. Cells were transfected with miR-27b-3p mimics or control mimics using Lipofectamine 3000 reagent (Invitrogen, Carlsbad, California, USA) according to the manufacturer's protocols.

Human LIMK1 was amplified from SW620 cells by RT-PCR. The PCR products were cloned into a pcDNA vector. SW620 cells (1×10^4 cells/well) were seeded in 24-well plates and transfected with either LIMK1 plasmid or negative control (NC) using Lipofectamine 3000 reagent (Invitrogen, Carlsbad, California, USA) according to the manufacturer's protocols.

Quantitative real-time PCR

Total RNA was extracted from the tissues or cells using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions and quantified by measuring the absorbencies at 260 and 280 nm (A260/280). Subsequently, the RNA was reverse-transcribed into cDNA using a reverse transcription system (Thermo Scientific, CA, USA). The expression levels of miR-27b-3p were detected by an Applied Biosystems 7500 Real-time PCR system (ABI) using TaqMan MicroRNA assay kits (Applied Biosystems, California, USA). U6 was used as the control for normalization. The gene expression of LIMK1 also analyzed using SYBR Green and was normalized to GAPDH expression. The primer sequences were as follows: GAPDH, 5'-TGT TCG TCA TGG GTG TGA AC-3' (forward) and 5'-ATG GCA TGG ACT GTG GTC AT-3' (reverse) (internal control); LIMK1, 5'-AAG CCA GAC ACT TCA GCT GCT GAT-3' (forward) and 5'-AAG ATC TGC TGG GAG TAG CTG

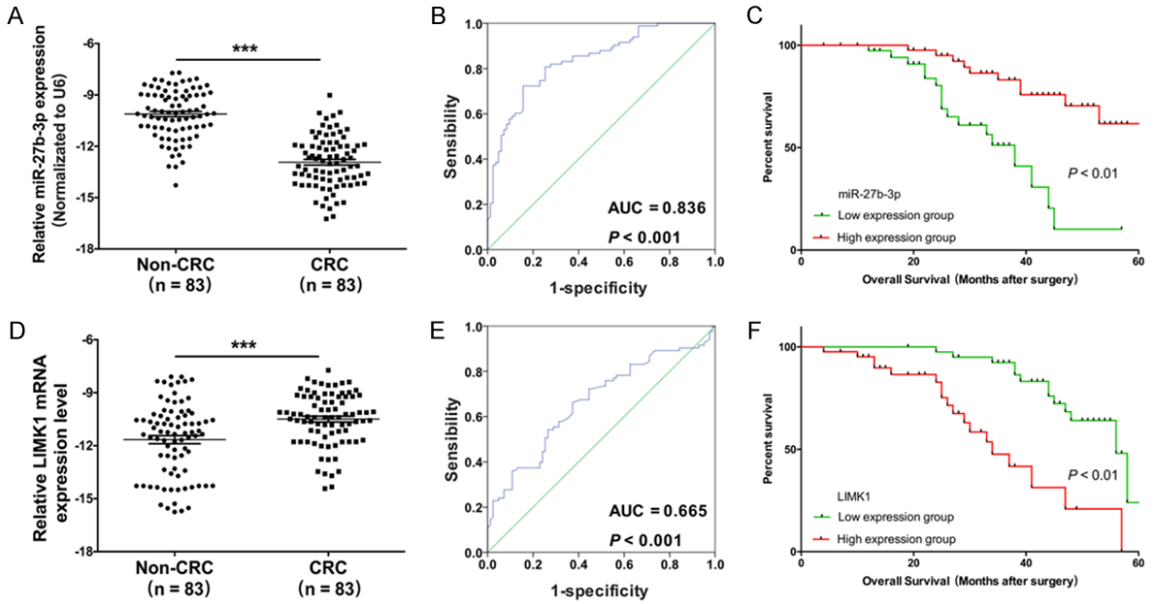

Figure 1. The expression and prognostic power of miR-27b-3p and LIMK1 in CRC tissues. A. The expression levels of miR-27b-were was measured by qRT-PCR in CRC tissues and corresponding noncancerous tissues (n = 83, $^{***}P < 0.001$). B. The prevalence rate was predicted by the receiver operating characteristic curve (ROC) of miR-27b-3p (AUC = 0.836, $P < 0.001$). C. Survival curve comparisons between high (n = 41) and low (n = 42, $^{***}P < 0.01$) expression of miR-27b-3p. D. LIMK1 expression was detected by qRT-PCR in CRC tissues (n = 83, $^{***}P < 0.001$). E. The prevalence rate was predicted by the ROC of LIMK1 (AUC = 0.665, $P < 0.001$). F. Survival curve comparisons between high (n = 41) and low (n = 42, $^{***}P < 0.01$) levels of LIMK1 expression.

CTA-3' (reverse); and U6, 5'-CTC GCT TCG GCA GCA CA-3' (forward) and 5'-AAC GCT TCA CGA ATT TGC GT-3' (reverse); The primer sequence for hsa-miR-27b-3p is 5'-TTC GGT TCA CAG TGG CTA AG-3'. The specificity of the primer sequences was analyzed by a dissociation curve, and $2^{-\Delta\Delta Ct}$ (cycle threshold) was used to calculate the relative gene expression levels.

Western blotting

Protein from tissues or cells was collected using RIPA buffer containing a protease inhibitor cocktail and phosphatase inhibitors (Sigma, St. Louis, MO, USA) according to the operating instructions and quantified using a bicinchoninic acid (BCA) kit (Thermo Scientific, CA, USA). Equal amounts of protein (30 μg per lane) were separated by SDS-PAGE and then transferred onto polyvinylidene difluoride (PVDF, Millipore, Bedford, MA, USA) membranes. Western blotting was performed using anti-GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA) and anti-LIMK1 (Abcam, Cambridge, MA, USA) antibodies. The protein levels were detected with an ECL kit (Thermo Scientific, CA, USA) following the manufacturer's instructions.

Dual luciferase reporter assay

TargetScan software (http://www.targetscan.org/vert_71/) was used to identify the potential target gene of miR-27b-3p. Either putative miR-27b-3p binding sites or mutated binding sites on the 3'UTR of LIMK1 were cloned into a vector to generate LIMK1 3'-UTR WT and LIMK1 3'-UTR Mut, respectively. Either LIMK1 3'-UTR WT or LIMK1 3'-UTR Mut was transfected into HEK-293T cells that stably over-expressed miR-27b-3p; cells transfected with negative control were used as control cells. A Dual-Luciferase Reporter Assay System (Promega, Madison, USA) was used to detect the luciferase activity 36 hrs. after transfection.

CCK-8 assay

The CCK-8 assay was performed to evaluate cell proliferation following the manufacturer's protocols. Experimental cells were seeded at a density of 5×10^3 cells per well in 96-well plates and were transfected with miR-NC, miR-27b-3p, miR-27b-3p and negative control (NC), or miR-27b-3p and LIMK1. The absorbance at 450 nm was measured using an electrolumi-

Table 1. Association between miR-27b-3p expression and the clinicopathological characteristics of enrolled colorectal cancer patients

Characteristics	No. of patients	Mean ± SD	*P* value
Total no. of patients	83		
Age (years)			
> 60	51 (61.4%)	12.38 ± 1.48	0.197
≤ 60	32 (38.6%)	12.87 ± 1.94	
Gender			
Male	42 (50.6%)	11.96 ± 1.64	0.279
Female	41 (49.4%)	12.32 ± 1.35	
Invasion			
T0-T2	56 (67.5%)	12.68 ± 1.29	0.085
T3-T4	27 (32.5%)	12.09 ± 1.73	
Lymphatic metastasis			
N0-N1	59 (71.1%)	11.84 ± 1.73	0.001**
N2-N3	24 (28.9%)	13.13 ± 1.06	
Distal metastasis			
M0	68 (81.9%)	11.47 ± 2.36	0.007**
M1	15 (18.1%)	13.26 ± 1.88	
TNM stage			
0 & I & II	72 (86.7%)	11.37 ± 1.94	0.003**
III & IV	11 (13.3%)	13.31 ± 2.16	

**Indicates statistical significance between the two groups ($P < 0.01$).

nescence immunosorbent assay reader (Thermo Fisher Scientific, Waltham, MA).

Cell cycle assay

Flow cytometry was used to analyze cell cycle distribution. First, cells were cultured in serum-free medium for 24 hours to induce cell cycle synchronization. Cells were then collected and washed twice with ice-cold phosphate-buffered saline (PBS) to remove floating cells. Cells were then fixed with 70% ethanol, rehydrated with PBS, treated with RNase A (10 mg/ml), and stained with propidium iodide (10 μg/ml). The cell cycle was evaluated using flow cytometry. Data were analyzed using FlowJo 7.6 software.

Wound healing assay

Cells were seeded into 6-well plates. Wounds were created in the confluent cell monolayer using a 200 μl sterile pipette tip, and any free-floating cells and debris were removed by washing the scratched monolayers with PBS three times. Medium was then added, and the culture plates were incubated at 37°C for another 48 hrs. Wound healing within the scraped wound line was measured at the 48-hour time point using a microscope (Nikon, Tokyo, Japan).

Transwell assay

Cell migration and invasion assays were performed using 24-well Transwell plates (8.0-μm pores; BD BioCoat, USA). For the migration assay, transfected cells were plated onto the upper chamber of 24-well plate with a membrane insert. For the invasion assay, the upper chamber of the 24-well plate was pre-treated with Matrigel (100 μg per well; BD Biosciences, USA). In the lower portion of the chambers, fresh medium contained 10% FBS was added to each well. After the cells were incubated for 24 hrs at 37°C, the cells in the upper chamber were carefully removed. Invading cells were fixed with 4% formaldehyde, stained with 0.5% crystal violet, and counted under a microscope.

Mouse xenograft assay

Cells were suspended in serum-free medium and then subcutaneously injected into the flanks of mice at a concentration of 2×10^5 cells/ml. Three weeks later, the mice were treated with miR-27b-3p, control, miR-27b-3p+NC or miR-27b-3p+LIMK1. At 3-7 weeks after RNA injections, the mice were euthanized, and the tumor volumes were calculated.

Statistical analysis

All quantitative data are presented as the mean ± SD from at least three independent experiments. One-way ANOVA test or Student's t-test was used to analyze the differences between the groups. The statistical significance was set at $P < 0.05$. SPSS 18.0 and Graph Pad Prism 6.0 software were used for the data analysis.

Results

Expression and prognosis of miR-27b-3p and LIMK1 in CRC tissues

To confirm the underlying molecular mechanisms of CRC, we analyzed the expression levels of miR-27b-3p and LIMK1 in a series of

Table 2. Association between LIMK1 expression and clinicopathological characteristics in enrolled colorectal cancer patients

Characteristics	No. of patients	Mean ± SD	*P* value
Total no. of patients	83		
Age (years)			
> 60	51 (61.4%)	10.46 ± 1.63	0.080
≤ 60	32 (38.6%)	9.79 ± 1.75	
Gender			
Male	42 (50.6%)	10.53 ± 1.49	0.110
Female	41 (49.4%)	9.95 ± 1.77	
Invasion			
T0-T2	56 (67.5%)	9.86 ± 1.85	0.217
T3-T4	27 (32.5%)	10.39 ± 1.39	
Lymphatic metastasis			
N0-N1	59 (71.1%)	10.85 ± 2.28	0.011*
N2-N3	24 (28.9%)	9.47 ± 1.96	
Distal metastasis			
M0	68 (81.9%)	10.75 ± 2.05	0.009**
M1	15 (18.1%)	9.28 ± 1.27	
TNM stage			
0 & I & II	72 (86.7%)	11.02 ± 1.94	0.018*
III & IV	11 (13.3%)	9.48 ± 2.16	

*Indicates statistical significance between the two groups ($P < 0.05$);
**Indicates statistical significance between the two groups ($P < 0.01$).

clinical CRC tissues. The results showed that miR-27b-3p expression was markedly decreased in cancer samples compared with that in adjacent normal tissues (**Figure 1A**). In contrast, the mRNA expression levels of LIMK1 were significantly increased (**Figure 1D**). Subsequently, we predicted the prevalence rate of CRC using the receiver operating characteristic curves (ROC) of miR-27b-3p and LIMK1 and found that the area under the ROC curves of miR-27b-3p was 0.836 (**Figure 1B**) and that the area under the ROC curves of LIMK1 was 0.665 (**Figure 1E**). Furthermore, survival curves were analyzed between high miR-27b-3p expression (n = 41) and low miR-27b-3p expression (n = 42), and the results showed that miR-27b-3p increased the survival rate of CRC patients (**Figure 1C**) and that LIMK1 reduced the survival rate of CRC patients (**Figure 1F**). The relationships between miR-27b-3p and LIMK1 expression and the clinicopathological features of the enrolled patients with CRC are summarized in **Tables 1** and **2**.

LIMK1 is a direct target of miR-27b-3p

In vitro, we first measured the expression level of miR-27b-3p in human-derived colonic epithelial NCM460 cells and CRC cell lines (SW480, DLD-1, HT29, HCT116, and SW620) using qRT-PCR and found that the expression levels of miR-27b-3p in CRC cells were significantly lower than those in NCM460 cells (**Figure 2A**). Then, SW620 cells were transfected with either miR-NC or miR-27b-3p. qRT-PCR was used to detect the expression levels of miR-27b-3p and LIMK1. The results revealed that miR-27b-3p was highly expressed in SW620 cells transfected with miR-27b-3p compared with cells transfected with miR-NC (**Figure 2B**). miR-27b-3p inhibited the expression of LIMK1 (**Figure 2C**). To illustrate that LIMK1 is a direct target of miR-27b-3p, LIMK1 wild-type (WT) or mutant (Mut) 3'-UTRs were subcloned into a luciferase reporter vector and co-transfected with either miR-27b-3p mimics or negative control (NC) into HEK-293T cells. Then, luciferase activity was detected at 48 hrs after transfection. The results demonstrated that miR-27b-3p markedly inhibited luciferase activity in cells transfected with the LIMK1 WT 3'-UTR but had no influence on cells transfected with the mutant 3'UTR (**Figure 2D**). To verify whether miR-27b-3p negatively regulates LIMK1, we analyzed the correlation between LIMK1 and miR-27b-3p expression in CRC tissues and found that there was a negative correlation between LIMK1 and miR-27b-3p expression (**Figure 2E**).

miR-27b-3p inhibits CRC cell proliferation via LIMK1

Next, to verify the role of miR-27b-3p on CRC cell proliferation via LIMK1, SW620 cells were transfected with miR-NC, miR-27b-3p, miR-27b-3p and negative control (NC), or miR-27b-3p and LIMK1, and then cells were collected for analysis. The expression levels of LIMK1 in transfected SW620 cells were measured by qRT-PCR and Western blot. The results indicated that the mRNA and protein expression levels of LIMK1 were dramatically suppressed by miR-27b-3p and that LIMK1 expression was

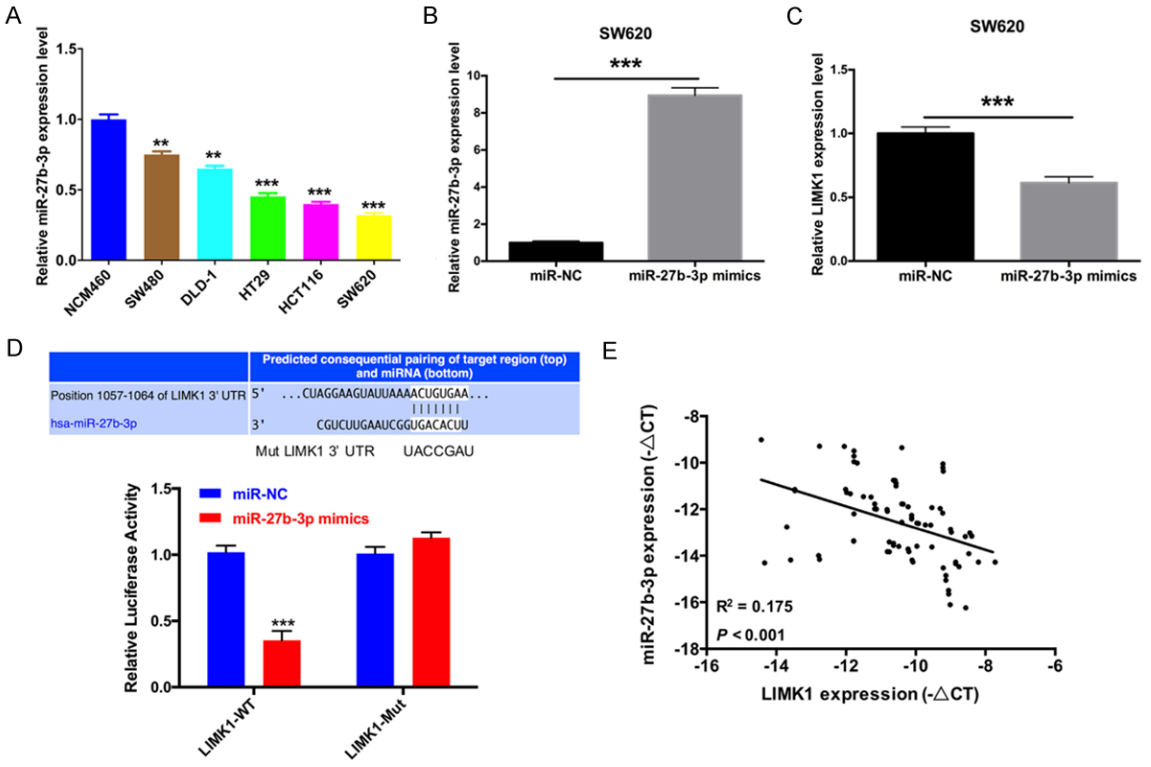

Figure 2. LIMK1 is a direct target of miR-27b-3p. A. The mRNA expression levels of miR-27b-3p were detected by qRT-PCR in human-derived colonic epithelial NCM460 cells and CRC cell lines (SW480, DLD-1, HT29, HCT116, and SW620, $^{**}P < 0.01$, $^{***}P < 0.001$). B. qRT-PCR was used to detect the expression levels of miR-27b-3p in SW620 cells transfected with miR-NC and miR-27b-3p ($^{***}P < 0.001$). C. LIMK1 expression in transfected SW620 cells was detected by qRT-PCR ($^{***}P < 0.001$). D. Sequence of miR-27b-3p and the LIMK1 3'-UTR, which contains a predicted miR-27b-3p binding site. The results of the luciferase assay in CRC cells co-transfected with miR-27b-3p mimics and miR-NC and containing either the LIMK1 3'-UTR (WT) or mutant (Mut) constructs ($^{***}P < 0.001$). E. The negative correlation between LIMK1 and miR-27b-3p expression in CRC tissues was assayed by qRT-PCR.

increased when we transfected LIMK1 plasmid into SW620 cells transfected with miR-27b-3p (**Figure 3A** and **3B**). The CCK-8 assay demonstrated that miR-27b-3p significantly suppressed SW620 cell proliferation, and the proliferative ability of SW620 cells was significantly restored via overexpression of LIMK1 compared with cells transfected with miR-27b-3p mimics (**Figure 3C**). Furthermore, to study the cell cycle, we used flow cytometry analysis to detect the cell cycle distribution of the transfected SW620 cells, which is shown in **Figure 3D**. Then, the cell number of the G1, S and G2 phases was counted, and we found that cell cycle arrest was characterized by a higher percentage of cells in G1/S phase of the cell cycle, and fewer cells were in G2 phase in SW620 cells transfected with miR-27b-3p compared to the cells transfected with miR-NC. Meanwhile, cell cycle arrest was characterized by fewer cells in G1/S phase of the cell cycle, and SW620 cells co-transfected with miR-27b-3p and LIMK1 presented more cells in G2 phase in than cells in the miR-27b-3p group. This suggested that G1 cell cycle progression in SW-620 cells was inhibited by miR-27b-3p and that overexpression of LIMK1 can restore this change.

miR-27b-3p suppresses CRC cell migration and invasion through LIMK1

Furthermore, we confirmed the role of miR-27b-3p on CRC migration and invasion through LIMK1. SW620 cells were transfected with miRNC, miR-27b-3p, miR-27b-3p and negative control (NC), or miR-27b-3p and LIMK1. The migratory ability of transfected SW620 cells was measured by wound healing and Transwell migration assays. The results showed that miR-27b-3p suppressed the migratory ability of SW620 cells and that restoration of LIMK1

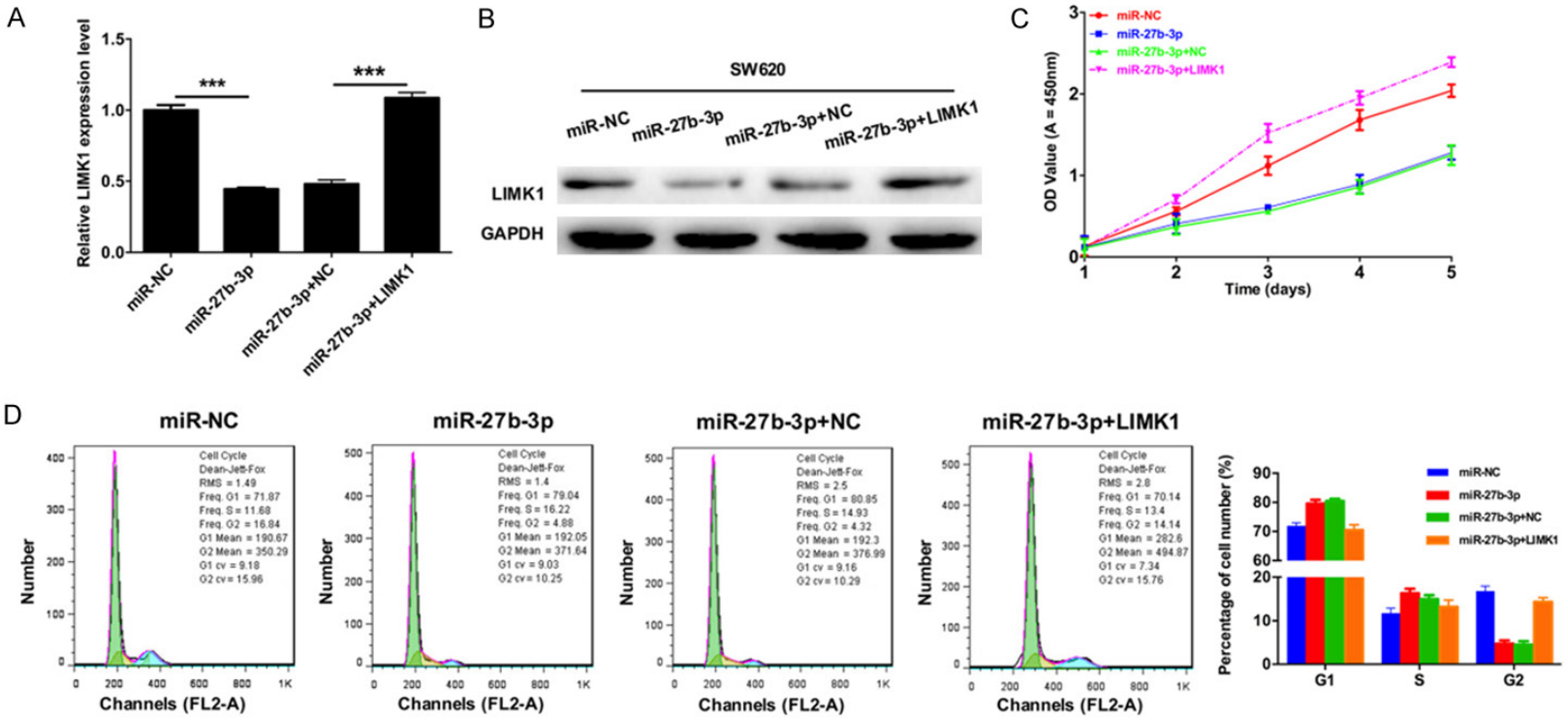

Figure 3. miR-27b-3p inhibits CRC cell proliferation via LIMK1. SW620 cells were transfected with miR-NC, miR-27b-3p, miR-27b-3p and negative control (NC), or miR-27b-3p and LIMK1. A. The mRNA expression levels of LIMK1 in transfected SW620 cells were measured by qRT-PCR (***$P < 0.001$). B. Western blotting was used to detect the protein expression levels of LIMK1 in transfected SW620 cells, and GAPDH was used as a protein-loading control. C. The proliferation of CRC cells was detected by the CCK-8 assay. D. Cell cycle distribution was analyzed by flow cytometry.

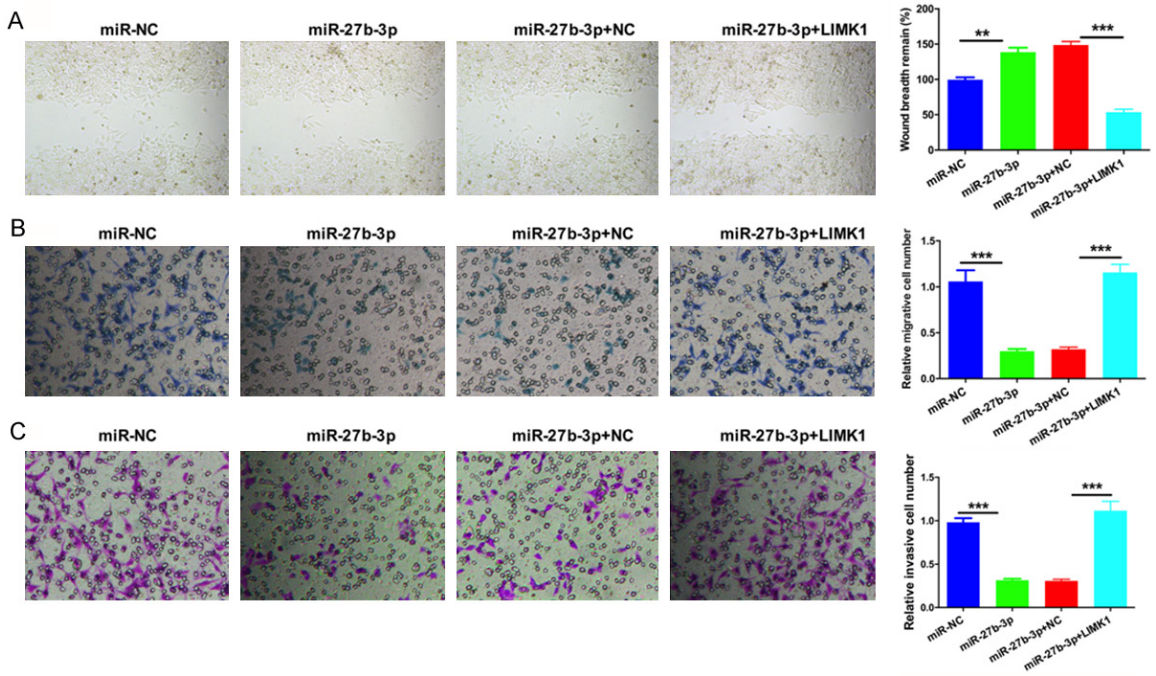

Figure 4. miR-27b-3p suppresses CRC cell migration and invasion via LIMK1. SW620 cells were transfected with miR-NC, miR-27b-3p, miR-27b-3p and negative control (NC), or miR-27b-3p and LIMK1. A. The migratory ability of transfected SW620 cells was measured by the wound healing assay (***P* < 0.01, ****P* < 0.001). B. The migratory ability of transfected SW620 cells was measured by the Transwell assay (****P* < 0.001). C. The invasive ability of transfected SW620 cells was measured by Transwell assays (****P* < 0.001).

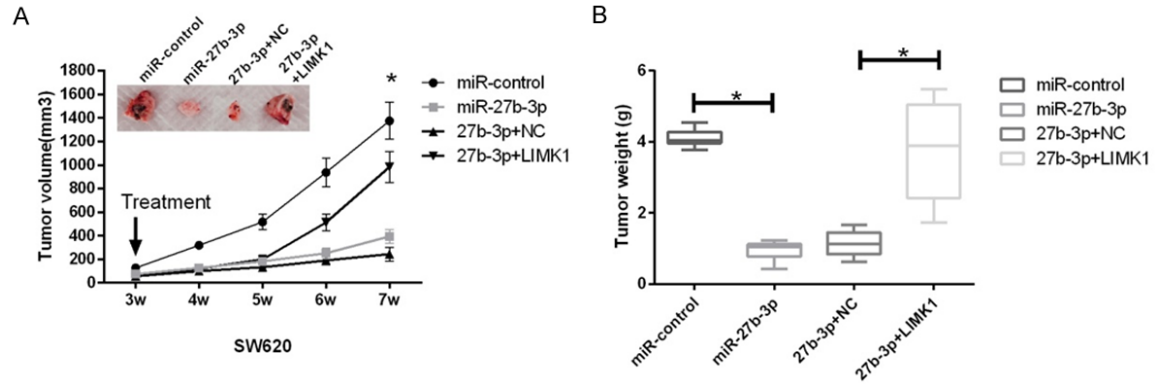

Figure 5. Effects of miR-27b-3p and LIMK1 on tumor growth in an in vivo mouse xenograft model. A and B. SW480 cells were subcutaneously injected into the nude mouse. Three weeks later, the tumors were treated with miR-NC, miR-27b-3p, miR-27b-3p and negative control (NC), or miR-27b-3p and LIMK1. Then, the animals were euthanized, and the volume and weight of the tumors were measured (**P* < 0.05).

reversed the anti-migratory activity of miR-27b-3p (**Figure 4A** and **4B**). The invasion abilities of transfected SW620 cells were measured by a Transwell invasion assay. The results showed that miR-27b-3p inhibited the invasive ability of SW620 cells and that the invasion capability of SW620 cells was significantly restored via up-regulation of LIMK1 when compared with cells transfected with miR-27b-3p mimics (**Figure 4C**).

miR-27b-3p inhibited tumor growth in a xenograft nude mouse model

To explore the effects of miR-27b-3p in vivo, a xenograft nude mouse model of SW620 cells

was established. SW620 cells were subcutaneously inoculated into the nude mice, which were then treated with miR-27b-3p, control, miR-27b-3p+NC or miR-27b-3p+LIMK1. The results indicated that miR-27b-3p overexpression significantly decreased the volume and weight of the tumor; meanwhile, restoration of LIMK1 reversed the anti-proliferative effects of miR-27b-3p in vivo (**Figure 5A** and **5B**).

Discussion

CRC is the most frequently diagnosed malignancy, and the morbidity and mortality due to CRC are the fourth leading cause of malignant tumors in the digestive system after gastric cancer, esophageal cancer and primary liver cancer [20]. Therefore, the early diagnosis of CRC is critical to allow successful treatment. Currently, the main methods for CRC diagnosis are X-ray, endoscopic examination, and serum carcinoembryonic antigen (CEA). However, the therapeutic approaches are relatively limited.

miRNAs are a series of small non-coding RNAs that are widely involved in both physiological and pathological processes [21-23]. For example, miR-135a and miR-21 separately contribute to the cancer pathological process and insulin resistance [24, 25]. It has been widely speculated that dysregulating miRNAs may cause cell dysfunction and further promote oncogenesis [26]. Therefore, studying miRNAs may illustrate the understanding of CRC development and be a great help for recognizing potential biomarkers. At present, many studies have reported that miR-27b plays important roles in cancer progression; however, there is much to be confirmed before including miR-27 in the network of cancer progression pathways, and a series of studies have shown that miR-27b serves as a tumor suppressor in numerous malignant tumors [27-29]. However, limited information is certified regarding the clinical potential and underlying mechanisms of miR-27b-3p in CRC.

MiR-27b-3p is one of the most important miRNAs that has been studied in many types of cancers, including breast cancer [14], gastric cancer [13], glioma [30], and esophageal cancer [31]. In our study, we first investigated the expression levels of miR-27b-3p in CRC patients and found that it was markedly decreased in tissues from CRC patients. The expression levels of miR-27b-3p were also decreased in CRC cell lines compared to NCM460 cells. More importantly, miR-27b-3p inhibited CRC cell proliferation, migration and invasion. These results suggested that miR-27b-3p as a tumor suppressor plays an important role in the pathogenesis of CRC.

Increasing evidence has recognized that LIMK1 acts as a novel well-known biomarker for tumors while its effects on CRC oncogenesis have not been defined. There are also studies demonstrating that the expression of LIMK1 is dysregulated in many human cancers and that inhibiting LIMK1 expression can suppress the development of lung cancer [32], gastric cancer [33], breast cancer, and prostate cancer [34]. In the current study, we found that the 3'UTR of LIMK1 includes a highly conserved miR-27b-3p binding site, and direct interaction between these sites an miR-27b-3p downregulated the protein levels of LIMK1. Therefore, the purpose of this study is to demonstrate the antitumor effect of miR-27b-3p and its correlation with LIMK1 in CRC cells. We demonstrated the expression of LIMK1 and miR-27b-3p in CRC cells, analyzed the relationship between LIMK1 and miR-27b-3p, and studied the role of the miR-27b-3p-LIMK1 axis in CRC. Our study is the first report describing the role of miR-27b-3p in CRC cells by regulating LIMK1 expression. Therefore, we suggested that LIMK1 is a direct target gene of miR-27b-3p in CRC cells.

Based on the abovementioned data, we demonstrated that the levels of miR-27b-3p are downregulated in CRC patients and the cell lines. miR-27b-3p markedly suppressed CRC cell proliferation, cell cycle progression and migration and invasion by targeting LIMK1. Our findings introduced a better understanding of miR-27b-3p in CRC progression, which may also provide more precise targets for diagnosing and treating CRC.

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Disclosure of conflict of interest

None.

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