

LncRNA LINC01116 Promotes the Development of Colorectal Cancer by Targeting miR-9-5p/STMN1

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Purpose: The aim was to research the role of LINC01116 in the prognosis of colorectal cancer (CRC) patients and development of colorectal cancer cells.

Methods: In total 62 colorectal cancer patient tissues and human CRC cell lines (OUMS23, SW116, SW480 and LOVO) were obtained for this study. SiLINC01116, miR-9-5p mimic, LINC01116, oe-STMN1 and their controls were transfected. The qRT-PCR method and Western blot were used to detect the levels of LINC01116, miR-9-5p and STMN1 in tissues and cells. CCK8 assay and flow cytometry were processed for proliferation and apoptosis, respectively. Transwell assay was undertaken to verify invasion and migration. Luciferase assay and pull down assay were processed to confirm the binding relationship among LINC01116, miR-9-5p and *STMN1*. Immunohistochemistry assay also detected the expression of *STMN1*. Kaplan–Meier survival curve was used to analyze patient survival rate. Pearson correlation analysis was used to evaluate the regulatory relationship between LINC01116, miR-9-5p and *STMN1* in tissues.

Results: LINC01116 was expressed higher in CRC tissues and cells. Patients with higher expression of LINC01116 had worse prognosis. Knockdown of LINC01116 suppressed development of CRC cell. LINC01116 negatively regulated miR-9-5p, while miR-9-5p was negatively related to *STMN1*. miR-9-5p mimic could rescue the effect of LINC01116, inhibit migration and invasion, and improve apoptosis of CRC cells. Oe-STMN1 could also rescue the effect of miR-9-5p on the development of colorectal cancer.

Conclusion: LINC01116 promoted the development of colorectal cancer via modulating miR-9-5p/*STMN1* axis.

Keywords: colorectal neoplasms, LINC01116, miR-9-5p, STMN1, target

Introduction

Colorectal cancer (CRC) is one of the most frequent malignant tumors worldwide.¹ Globally, the mortality and incidence of colorectal cancer ranks third among all genders, which is increasing at a rate of more than 1 million new cases each year.² Typical symptoms are alternation of diarrhea, constipation, mucus and blood.³ Clinically, comprehensive treatment based on surgery should generally be adopted.⁴ The treatment measures are decided according to the patient's general condition and the function of each organ, the location of the tumor, the clinical stage of the tumor, the pathological type, and the biological behavior.⁵ At present, the treatment of colorectal cancer mainly includes surgery, radiation therapy, chemotherapy, and targeted therapy.⁶

In recent years, due to the development of molecular biology, the research on colorectal cancer development mechanism is focused on changes of the gene levels. Gene-targeted therapy is a future development trend. Compared with traditional therapy, gene therapy is more targeted and shorter, which can avoid the emergence

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of drug resistance and systemic toxic reactions.⁷ Although a large number of genetic mechanisms or protein changes involved in the evolution of colorectal cancer have been discovered and the molecular framework of colorectal cancer evolution has been elucidated, the current research results are far from enough to achieve effective molecular targeted therapy for colorectal cancer clinically.⁸ Therefore, finding new research strategies and molecular targets is an urgent problem in the field of basic tumor research and clinical treatment.

LncRNA is a non-coding RNA closely related to tumor genesis process, attracting investigator's attention. LINC01116 is more highly expressed in many tumors, which promotes proliferation and invasion of cells.⁹ For instance, lncRNA-LINC01116 could target Vascular Endothelial Growth Factor A (*VEGFA*), and then effect the cell proliferation, invasion, migration, cycle, and angiogenesis of brain glioma tumor.¹⁰ In addition, Zhang et al¹¹ confirmed that LINC01116 improves development of osteosarcoma cancer by regulating the expression of miR-520a-3p and Interleukin 6 Receptor (*IL6R*) and participating in the Jak-stat signaling pathway.¹¹ Hu et al¹² also referred that LINC01116 overexpression was a biomarker of poor prognosis for breast cancer patients. Moreover, LINC01116 was higher expressed in head and neck squamous cell carcinoma, which could promote cell invasion of this disease by involving the epithelial mesenchymal transition pathway.¹³ Thereby, LINC01116 is important for development of various cancers. However, the role and mechanism of LINC01116 in CRC has not been reported. The aim of this research was to investigate the expression of LINC01116 in colorectal cancer, its effect on patient prognosis, and the molecular mechanism of LINC01116 in CRC cells. Interestingly, based on the bioinformatics online prediction software (Starbase and targetscan), LINC01116 was closely related to miR-9-5p/*STMN1* expression. MiR-9-5p has been verified to participate in various cancers, such as non-small-cell lung cancer,¹⁴ thyroid cancer,¹⁵ and prostate cancer.¹⁶ Besides, *STMN1* protein is a highly conserved intracellular protein. During the mitotic interval, the active state of *STMN1* protein promotes microtubule depolymerization.¹⁷ The expression level of *STMN1* in tumors is directly related to the prognosis of patients, and the expression of stathmin protein in tumor cells affects the sensitivity of vinblastine and paclitaxel drugs.¹⁸

Based on the above evidence, LINC01116 plays a critical role in tumor development. However, the detail of the molecular mechanism of LINC01116 in CRC is still unclear. Thereby, exploring the role of LINC01116 and related factors

may lay the foundation for CRC treatment. In this study, the expression of LINC01116 in CRC tissues and cells was detected, and the relationship between LINC01116 level and patient survival rate was analyzed. Moreover, the effect of LINC01116 expression on migration, invasion, and apoptosis was also researched. Finally, the regulation of LINC01116, miR-9-5p and *STMN1* was explored, and these factors in CRC development were also confirmed.

Materials and Methods

Clinical Samples

In total 62 patients who were diagnosed with colorectal cancer and treated with surgery from October 2018 to June 2019 in our hospital were included. This study was approved by the ethics committee of The Second Affiliated Hospital of Qingdao University, and was conducted in accordance with the Declaration of Helsinki. All patients' families were informed of the study and signed informed consent. CRC tissues and normal adjacent tissues (Less than 3 cm adjacent to cancer) were obtained, confirmed by biopsy and stored immediately at -80°C in a refrigerator. Clinical characteristics including ages, gender, tumor size, TMN stage, local invasion, lymphatic metastasis and overall survival were recorded and analyzed. Based on the median expression of LINC01116, all patients were divided into two groups. Ages, gender, local invasion and lymphatic metastasis between the two groups were not significantly different (Table 1). However, patients with LINC01116 high expression always had larger tumor size and more serious TMN stage.

Cell Culture and Transfection

CRC cell lines [OUMS23 (JCRB1022, Epithelial-like, chondrosarcoma), SW116 (ATCC[®] CCL-228, Colon adenocarcinoma at stage III), SW480 (ATCC[®] CCL-228, Dukes' B type, colon adenocarcinoma, established from primary colon adenocarcinoma) and LOVO (ATCC[®] CCL-229, Dukes' C type, stage IV, colorectal adenocarcinoma)] and human colon epithelial cell line (NCM460, Epithelial-like) were purchased from Beina Chuanglian Biotechnology Research Institute (Beijing, People's Republic of China). Based on these cell lines, the expression and mechanism of LncRNA LINC01116 in various colon cancer cell lines can be more completely presented and verified. The cell lines were cultured in DMEM medium with 10% FBS in a cell incubator (37°C , 5% CO_2 , Thermo Fisher Scientific, USA). The sequences of

Table 1 The Correlation Between LINC01116 Expression and COAD Clinical Pathology

Characteristics	Number of Patients	LINC01116 Low Expression (<Median)	LINC01116 High Expression (≥Median)	P-value
Number	62	30	32	
Age (years)				0.400
<60	31	16	15	
≥60	31	14	17	
Gender				0.503
Female	30	15	15	
Male	32	15	17	
Tumor size				0.011
≤5 cm	29	19	10	
>5 cm	33	11	22	
TMN stage				0.021
I-II	30	19	11	
III-IV	32	11	21	
Local invasion				0.400
T1+T2	31	14	17	
T3+T4	31	16	15	
Lymphatic metastasis				0.497
Yes	32	16	16	
No	30	14	16	

siLINC01116-1, siLINC01116-2, siLINC01116-3 and siCtrl were synthesized by Shanghai Biotech Bioengineering Co., Ltd. LOVO and SW116 cells in logarithmic growth phase were seeded in 6-well plates with 2×10^5 cells per well. All cells were randomly grouped into the siLINC01116 group and the negative control group, with three replicates in each group. For each group, cells were routinely incubated for 24 hours before adding transfectants. All operations were performed strictly according to the instructions of Lipofectamine 2000 (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). The cells of each group were cultured in a cell incubator at 37°C, 5% CO₂, and saturated humidity. Similarly, miR-9-5p mimic, LINC01116, oe-STMN1 and their controls were also transfected as above steps.

qRT-PCR

The level of LINC01116 in tissues and cells was detected by qRT-PCR. TRIzol reagent was added to tissues or cells. After sufficient lysis, chloroform was added, and the samples were centrifuged at 12,000 r/min for 5 minutes on ice. The supernatant was taken and added with isopropanol. After

precipitation, the precipitate was washed with 75% ethanol and the RNA was collected, and an appropriate amount of DEPC water was added to dissolve it. The NanoDrop 2000 ultra-micro spectrophotometer was used to detect the extracted total RNA concentration. The extracted total RNA was reverse transcript into cDNA using RR047A PrimeScript™ RT reagent Kit (TaKaRa). GADPH was used as an internal reference. RR420A SYBR Premix Ex Taq (TaKaRa) was used to perform qRT-PCR, and the relative expression of lncRNA LINC01116 was calculated using the 2-ΔΔCt method. GADPH was the reference for analysis of qRT-PCR results. The primer sequences were shown in Table 2. The primer sequences of lncRNA LINC01116 were as follows: (upstream) 5'-CTTCTTCCCTCCAAGTGAT-3', (downstream) 5'-TTAGCAAGTCAGCAAGTCCT-3'. Primer 5.0 was used for primer sequence design. Both design and synthesis processes of all primers were completed by GenScript Biotechnology Co., Ltd. (People's Republic of China).

CCK8 Assay for Proliferation

The CRC cells were inoculated into a 96-well plate with cell suspension (100 μL/well). Transfection was performed at

Table 2 Primer Sequences for qRT-PCR

	Upstream	Downstream
LINC01116	5'-CTTCTTCCCTCCAAGTGAT-3'	5'-TTAGCAAGTCAGCAAGTCCT-3'
STMN1	5'-CCAGGCTTTTGAGCTGATTC-3'	5'-TGTGCCTCTCGTTCTCGTTCTCTT-3'
miR-9-5p	5'-CAGAGAAGGGCAGTGGAGAC-3'	5'-ACGAGAGAGACCGAAAAAGG-3'
GADPH	5'-ACGGATTGGTCGTATTGGG-3'	5'-CGCTCCTGGAAGATGGTGAT-3'

a density of 50%. After 48 hours of transfection, the culture medium was removed from each well of the 96-well plate. Then, 10 μ L of Cell Counting Kit-8 (CCK8) solution (Beyotime Biotechnology, People's Republic of China) was supplied to each well, and the culture plate was then incubated for 2 hours. After termination of the culture, the absorbance was measured at 450 nm with a Microplate reader (Thermo Fisher Scientific, Shanghai, People's Republic of China) to determine cell viability.

Flow Cytometry for Apoptosis

Two groups of single cell suspensions were prepared and adjusted to a cell concentration of 10^6 /mL. Then, 0.5 mL of cell suspension was taken, centrifuged at 1000 r/min for 4 minutes, and precipitation was obtained. Next, 0.5 mL of Binding Buffer was used to resuspend the cells, and 1 μ L of fluorescently labeled annexin V reagent was added. After mixing, the samples were continually kept for 20 minutes in the dark at room temperature before 5 μ L of PI reagent was added and cultured at 4° C for 5 minutes avoiding the light. Finally, 0.5 mL of Binding Buffer was added to the sample and analyzed by flow cytometry.

Transwell Assay for Migration and Invasion

Matrigel was melted, diluted, added to the Transwell upper chamber (Corning, USA), and equilibrated at 37° C for 5 hours. However, matrigel was not used for the cell migration experiment. In total 100 μ L of fetal bovine serum-free cell suspension (5.0×10^5 cells/mL) was aspirated and added to the upper chamber, while 600 μ L of medium containing 10% fetal bovine serum was added to the lower chamber. After culturing for 24 hours, the remaining liquid was discarded and matrigel and upper chamber cells were removed with a swab. Samples in the submembranous compartment were fixed by paraformaldehyde for 30 minutes, air-dried, and stained with 0.1% crystal violet. Cells of five regions were selected, photographed and calculated

under an inverted microscope (100 \times , Olympus, Japan), and the average value of cell numbers was taken.

Luciferase Assay

Fragments containing the wild-type 3' end noncoding region (3'UTR) or mutant 3'UTR of the LINC01116 were constructed. Enzyme cutting sites were designed at both ends, and a luciferase reporter gene plasmid (pGL3-promoter, Promega, USA) was inserted to construct pGL3-3'UTR wt, pGL3-3'UTR mut. HEK293 cells with good growth status were taken. After plasmid transfection for 48 hours, samples were lysed, luciferase substrate was added, and luciferase activity was detected according to instructions of the manufacturer.

Pull-Down Assays

Based on the transcript sequence of lncRNA LINC01116, primers were designed at the 5' and 3' ends, respectively. In order to obtain the lncRNAGAS5 transcript for the positive and negative strand templates, a T7 promoter sequence was added to the 5' end of the primer. Using pcDNA3.1 LINC01116 plasmid DNA as a template, DNA fragments were amplified using positive strand template primers and negative strand templates, respectively. Then, transcription products were randomly labeled with biotin and biotin RNA transcribed in vitro was purified. RNA pull-down was undertaken as reported previously.¹⁹ The pull-down products were detected by Western blotting for specific antibodies. The experiment was also undertaken to confirm the binding between miR-9-5p and *STMN1*.

Western Blot Assay

The cells of each group were collected, and the total protein was extracted after routine lysis, and the adjusted protein concentration was consistent. Then, 40 μ g of total protein was aspirated, transferred to PVDF after SDS-PAGE electrophoresis, and blocked with 5% skimmed milk powder. After incubation with the primary antibody (Cellular Signaling Technology, USA), the secondary antibody (Beijing Zhongshan Jinqiao Biotechnology Co., Ltd., People's Republic

of China) was added the next day, and the sample was cultured at 37°C for 2 hours. The ECL working solution was added to the sample, the gel imager was used to photo, and the gray value was analyzed by Quantity One. GAPDH was the internal reference.

Immunohistochemistry Assay

All specimens were fixed with formalin at room temperature, dehydrated with alcohol, xylene transparentized, embedded with paraffin immersion wax, and sectioned with paraffin microtome. The samples were placed in an oven at 65°C. Sections were dewaxed with conventional xylene and dehydrated with gradient alcohol. After the antigen was repaired, the sections were naturally cooled in the buffer for 60 minutes before 3% H₂O₂ was supplied dropwise to the sections and incubated, and left at 37°C for 10 minutes to block endogenous peroxidase. After the serum was blocked, a primary antibody (1: 100) was added dropwise to the sample and placed in a wet box at 4°C refrigerator for a whole night. The biotinylated secondary antibody was supplied dropwise and cultured at 37°C for 20 minutes. After the addition of horseradish-labeled streptavidin working solution, DAB coloration and hematoxylin counterstaining were performed. After the sample was dehydrated and transparent, the excess xylene was wiped off. Finally, the sample was sealed, observed and photographed.

Statistical Analysis

The software package SPSS 20.0 was used to analyze the data. Measurement data are shown as $M \pm SD$ ($\bar{x} \pm s$). Comparisons between groups were undertaken by *t*-test. Kaplan-Meier survival curve is used to analyze patient survival rate and survival time. Pearson correlation analysis was used to evaluate the regulatory expression among LINC01116, miR-9-5p and STMN1 in tissues. $P < 0.05$ was the threshold of statistically significant difference.

Results

LINC01116 is Highly Expressed in Colorectal Cancer Tissues and Cells

The expression of LINC01116 in CRC tissues and cells was detected by qRT-PCR. It is worth noting that LINC01116 had higher levels in tumor tissues than normal ($P < 0.0001$, Figure 1A). Moreover, LINC01116 had higher levels in tumor tissues at stage I–II than that of patients at stage III–IV ($P < 0.01$, Figure 1B). Survival curve analysis indicated that patients with highly expressed LINC01116 have poor

prognosis (Figure 1C). Similarly results were also obtained in vitro. LINC01116 expression was higher in human CRC cell lines (OUMS23, SW116, SW480 and LOVO) than that in human normal colon epithelial cell line (NCM460) (Figure 1D). It was worth noting that LINC01116 is expressed highest in SW116 and LOVO cell lines. Thereby, the two cell lines were used for further experiments.

LINC01116 Knockdown Inhibited Development of CRC Cells

Knockdown of LINC01116 was performed in LOVO, SW116 cell lines, and the knockdown efficiency was detected. All three knock-down sequences could reduce the expression of LINC01116, indicating that the transfections were successful (Figure 2A). According to the transfection efficiency, siLINC01116-1 was selected for subsequent experiments. After transfection, the proliferation was significantly decreased (Figure 2B), while cell apoptosis was improved in both LOVO and SW116 cell lines (Figure 2C). In addition, migration, and invasion of CRC cells were also suppressed by siLINC01116 (Figure 2D). Thereby, we inferred that LINC01116 knockdown inhibited development of CRC cells.

LINC01116 Targeted miR-9-5p

Based on the bioinformatics online prediction software (Starbase and targetscan), the binding site of LINC01116 and miR-9-5p is presented in Figure 3A. Binding sites were detected by a double luciferase reporter assay. After miR-9-5p is overexpressed, the luciferase activity of wild-type lncRNA LINC01116 is significantly inhibited, but the luciferase activity of mutant lncRNA LINC01116 is not significantly affected (Figure 3B). The data show that miR-9-5p can directly bind to lncRNA LINC01116 binding site and affect luciferase activity. To determine the possible direct interaction between LINC01116 and miR-9-5p, a T7-LINC01116 nucleic acid probe was designed and constructed for RNA pull-down experiments in this study. It was found that LINC01116 could significantly pull-down miR-9-5p (Figure 3C). siLINC01116 decreased the expression of miR-9-5p in both SW116 and LOVO cell lines (Figure 3D). Rescue assay was processed to verify the effect of LINC01116 and miR-9-5p on colorectal cancer cells. Based on the transfection, cells were divided into Ctrl, LINC01116+miR-NC and LINC01116+miR-9-5p mimic groups. As the results show, LINC01116 increased the proliferation, migration, and invasion, but inhibited cell

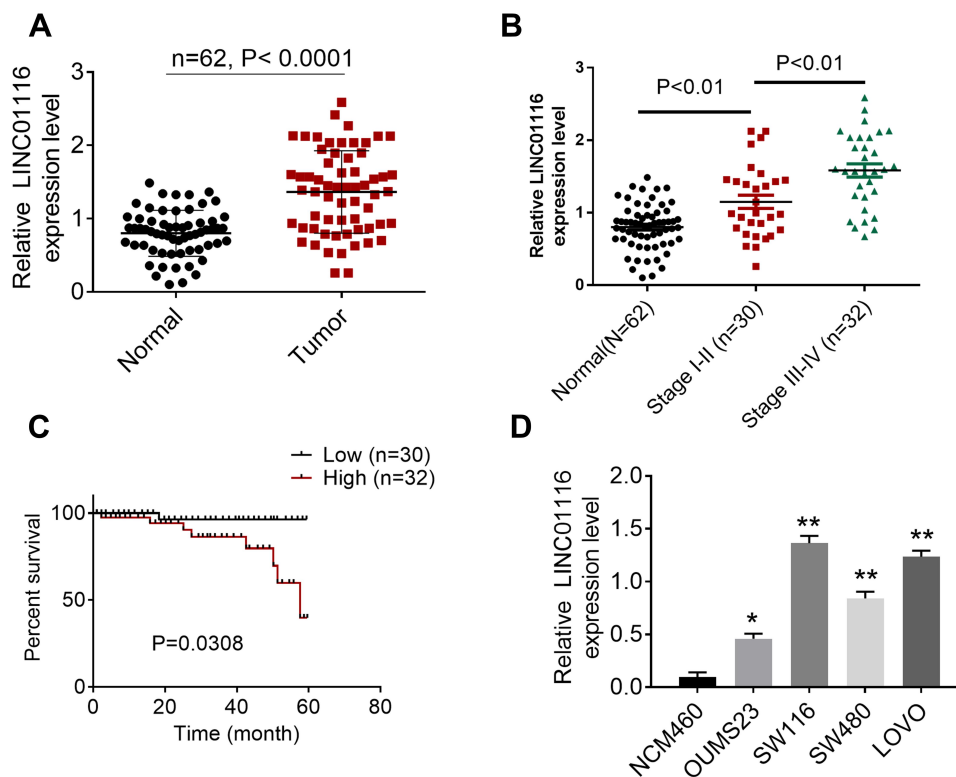


Figure 1 LINC01116 levels in colorectal cancer (CRC) and its effect on patients' prognosis. **(A)** The level of LINC01116 in CRC tissues. **(B)** The level of LINC01116 in CRC tissues at different stages. **(C)** Survival curve analysis for patients with colorectal cancer. **(D)** The expression of LINC01116 in colorectal cancer cells. * $P < 0.05$, ** $P < 0.01$.

apoptosis. Additionally, miR-9-5p rescued the effect of LINC01116 on the above progress (Figure 3E-G).

STMNI Was the Target of miR-9-5p

Starbase has an online prediction database for the binding site of *STMNI* and miR-9-5p, and the binding site is shown in Figure 4A. The result of luciferase assay showed that miR-9-5p could bind to *STMNI* binding site and affect luciferase activity (Figure 4B). QRT-PCR and Western blot was processed to detect the *STMNI* level after siLINC01116 or miR-9-5p mimic transfection. In both LOVO and SW116 cell lines, siLINC01116 and miR-9-5p could suppress the expression of *STMNI* (Figure 4C and D). The rescue assay also verified that up-regulated miR-9-5p could promote apoptosis and inhibit proliferation, migration and invasion of CRC cells, while oe-*STMNI* rescued the function of miR-9-5p mimic (Figure 4E-G).

Correlation Between LINC01116 and miR-9-5p/*STMNI* Expression in Colorectal Cancer Tissues

miR-9-5p and *STMNI* expression was detected by qRT-PCR in CRC tissues and normal tissues. As represented in Figure 5A,

miR-9-5p was significantly lower expressed in CRC tissues. However, *STMNI* was expressed higher in tumor tissues (Figure 5B). The results of IHC assay were similar to that of qRT-PCR (Figure 5C). Moreover, Pearson correlation analysis was used to evaluate the expression relationship between LINC01116, miR-9-5p and *STMNI* in tissues. LINC01116 negatively modulated the miR-9-5p level (Figure 5D). LINC01116 was negatively related to *STMNI*, while miR-9-5p was negatively related to *STMNI* expression (Figure 5E and F).

Discussion

LINC01116 is mainly used in various cancers for early diagnosis, targeted therapy, and observation of prognosis. In this study, LINC01116 was highly expressed in CRC tissues and cells. Patients with higher LINC01116 level always had poor prognosis. Knockdown of LINC01116 inhibited proliferation, migration, and invasion of CRC cells. LINC01116 negatively regulated miR-9-5p, while miR-9-5p was negatively related to *STMNI* expression.

LINC01116 (GC id: GC02M176629) has been reported to regulate numbers of miRNAs and genes in various cancers. In breast cancer, it could directly combine with miR-145, then

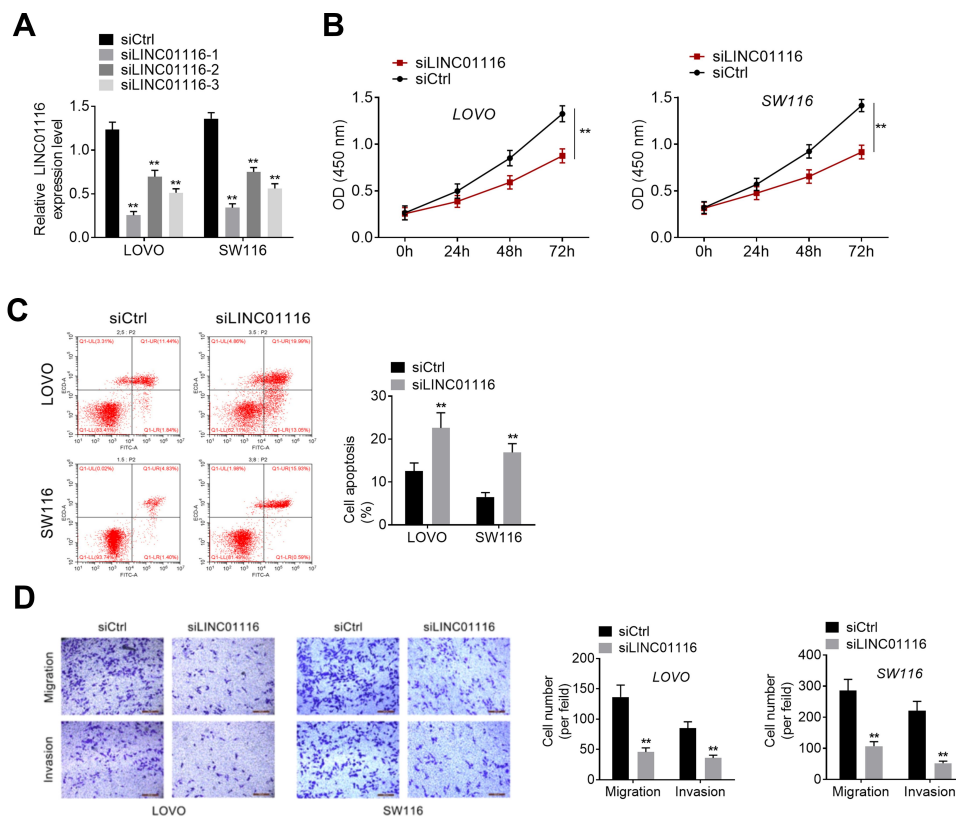


Figure 2 LINC01116 knockdown inhibited development of CRC cells. **(A)** LINC01116 was knocked-down by three siLINC01116. **(B)** After transfection, the proliferation was detected by CCK8 assay. **(C)** Cell apoptosis was verified by flow cytometry assay. **(D)** Transwell assay for migration and invasion of colorectal cancer cells. ** $P < 0.01$.

improve the expression of *ESR1* (GC id: GC06P151656), and promote the development of breast cancer.²⁰ Interestingly, Paun et al²¹ analyzed a comprehensive profile of epigenetic changes in colorectal cancer and concluded that methylation of *ESR1* was the biomarker of colorectal cancer. Furthermore, LINC01116 could regulate the expression of miR-520a-3p/*IL6R* axis, and then improve migration and proliferation of osteosarcoma cells.²² It worth noting that miR-520a-3p has been confirmed to inhibit invasion, promote apoptosis and decelerate tumor growth by regulating the expression of *EGFR* (GC id: GC07P055019).²³ Moreover, down-regulated *IL6R* (GC id: GC01P154405) could inhibit migration and invasion by interfering with the expression of *IL-6*.²⁴ Interestingly, LINC01116 was confirmed to contribute to gefitinib resistance in non-small cell lung cancer through regulating IFI44.²⁵ Thereby, this lncRNA may have a role in drug resistance as well. In this study, CRC patients with higher LINC01116 levels had poor prognosis. Knockdown of LINC01116 was confirmed to inhibit development of CRC cells. Based on the above evidence, we speculated that LINC01116 played a critical role in CRC development.

In addition, lncRNA LINC01116 was confirmed to target miR-9-5p (GC id: GC01M156420) in this study. It has been

found that miR-9-5p may play a protooncogenic or tumor suppressive effect in tumors, and its functions have not been fully elucidated. As reported in previous studies, miR-9-5p targeted *StarD13* (GC id: GC13M033103), and then improved the development of prostate cancer.²⁶ Similarly, miR-9-5p was also more highly expressed in hepatocellular carcinoma, which could regulate *SOX11* (GC id: GC02P005704) expression and then improve hepatocellular carcinoma growth.⁹ However, low expressed miR-9-5p was also verified and researched in different cancers. For instance, Zhang et al²⁷ verified that miR-9-5p inhibited the expression of *FOXP2* (GC id: GC07P114086) and further suppressed proliferation of glioma cells. Also, forced expression of miR-9-5p could protect liver from ischemia reperfusion-induced hepatic injury.²⁸ More importantly, Wang et al²⁹ found that up-regulated miR-9-5p inhibit proliferation and improve apoptosis in colorectal cancer by targeting *PAK4* (GC id: GC19P039125). Moreover, miR-9-5p was confirmed to regulate some other targets, such as POU2F,³⁰ FOX4, TGFBR2,³¹ and BCL2L1 in previous studies. Thereinto, POU2F1 could promote growth and metastasis of hepatocellular carcinoma through the FAT1 signaling pathway.³² FAT1 signaling pathway was also a critical

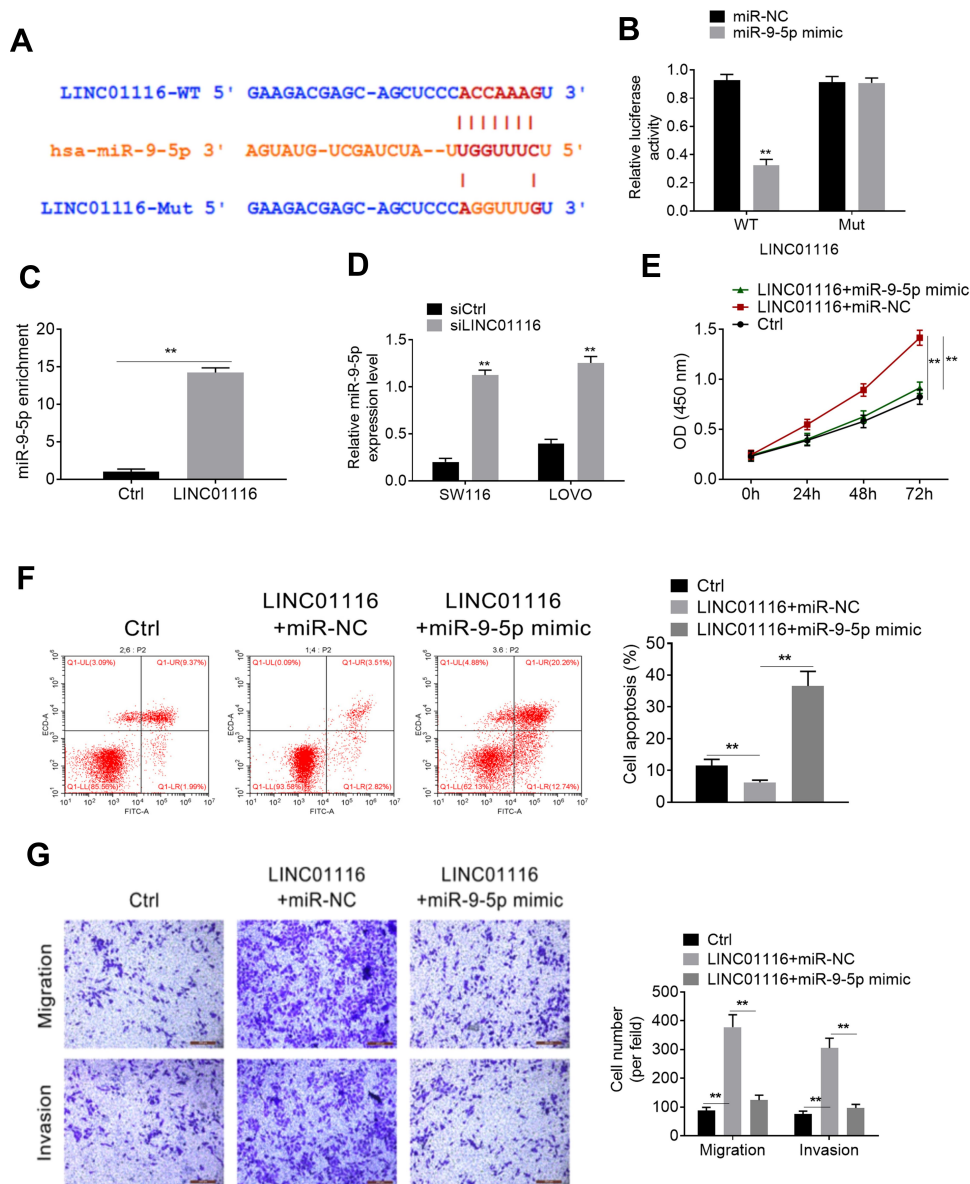


Figure 3 LINC01116 targeted miR-9-5p. **(A)** The binding site of LINC01116 and miR-9-5p. **(B)** The binding site of miR-9-5p and LINC01116 was verified by luciferase reporter assay. **(C)** LINC01116 significantly pull-down miR-9-5p. **(D)** RT-PCR detected the expression of miR-9-5p. **(E)** After transfection, the proliferation was detected by CCK8 assay. **(F)** cell apoptosis was verified by flow cytometry assay. **(G)** Transwell assay for migration and invasion. ** $P < 0.01$.

pathway in colorectal cancer development.³³ Moreover, Ogino et al³⁴ confirmed that TGFBR2 mutation was closely related with CpG island methylator phenotype in microsatellite instability-high colorectal cancer. Importantly, BCL2L1 was regarded as an apoptosis-associated gene.³⁵ Our findings are consistent with previous studies. miR-9-5p mimic could rescue the effect of LINC01116, inhibit migration and invasion, and improve apoptosis of colorectal cancer cells. Thereby, LINC01116 improves the progression of colorectal cancer by inhibiting the expression of miR-9-5p.

Furthermore, *STMN1* (GC id: GC01M025884) was the target of miR-9-5p in this study. Interestingly, oe-*STMN1* could rescue the effect of miR-9-5p on the development of colorectal cancer cells. In previous studies, *STMN1* has been verified to promote biological behavior and invasion in various cancers, such as endometrial carcinoma,³⁶ cutaneous squamous cell carcinoma³⁷ and gastric cancer.³⁸ Moreover, high expression of *STMN1* was also regarded as a predictor of progression and poor survival of colorectal cancer patients.³⁹ In 2009, Ogino et al⁴⁰ processed

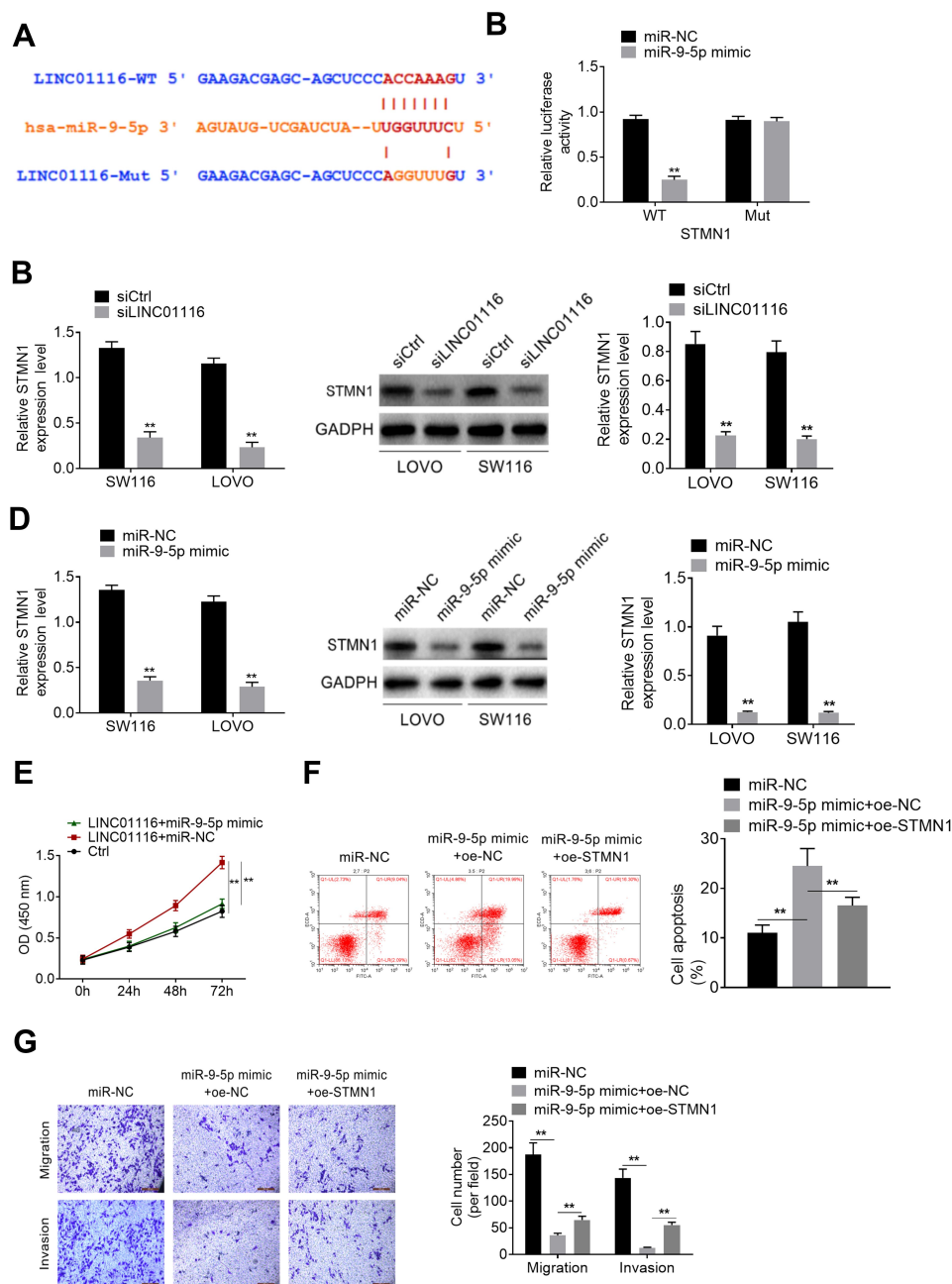


Figure 4 STMN1 was the target of miR-9-5p. **(A)** The binding site of STMN1 and miR-9-5p. **(B)** The binding site of STMN1 and miR-9-5p was verified by luciferase assay. **(C)** siLINC01116 suppressed the expression of STMN1. **(D)** MiR-9-5p suppressed the expression of STMN1. **(E)** After transfection, the proliferation was detected by CCK8 assay. **(F)** Cell apoptosis in rescue assay. **(G)** Migration and invasion of rescue assay. ** $P < 0.01$.

a cohort study and found that the expression of *STMN1* was closely related to survival time of colorectal cancer patients. Tan et al⁴¹ undertook a proteomic analysis, and revealed that *STMN1* was more highly expressed in both colorectal tumors and metastatic tissues. Additionally, overexpressed *STMN1* improved invasion and proliferation of colorectal cancer cells and also decreased overall survival of patients.⁴² It was worth mentioning that knock-down of *STMN1* could inhibit cell adherence and

metastasis and also improve chemoresponse to therapeutic medicine, such as 5-Fu.⁴³ More importantly, our results in vivo also confirmed the correlation between LINC01116 and miR-9-5p/*STMN1* expression in colorectal cancer.

Furthermore, LINC01116 was found to be involved in different pathways, including the hippo signaling pathway and Jak-stat signaling pathway.^{44,45} It is worth noting that inactivating the hippo signaling pathway could promote stemness and chemotherapeutic resistance of colorectal

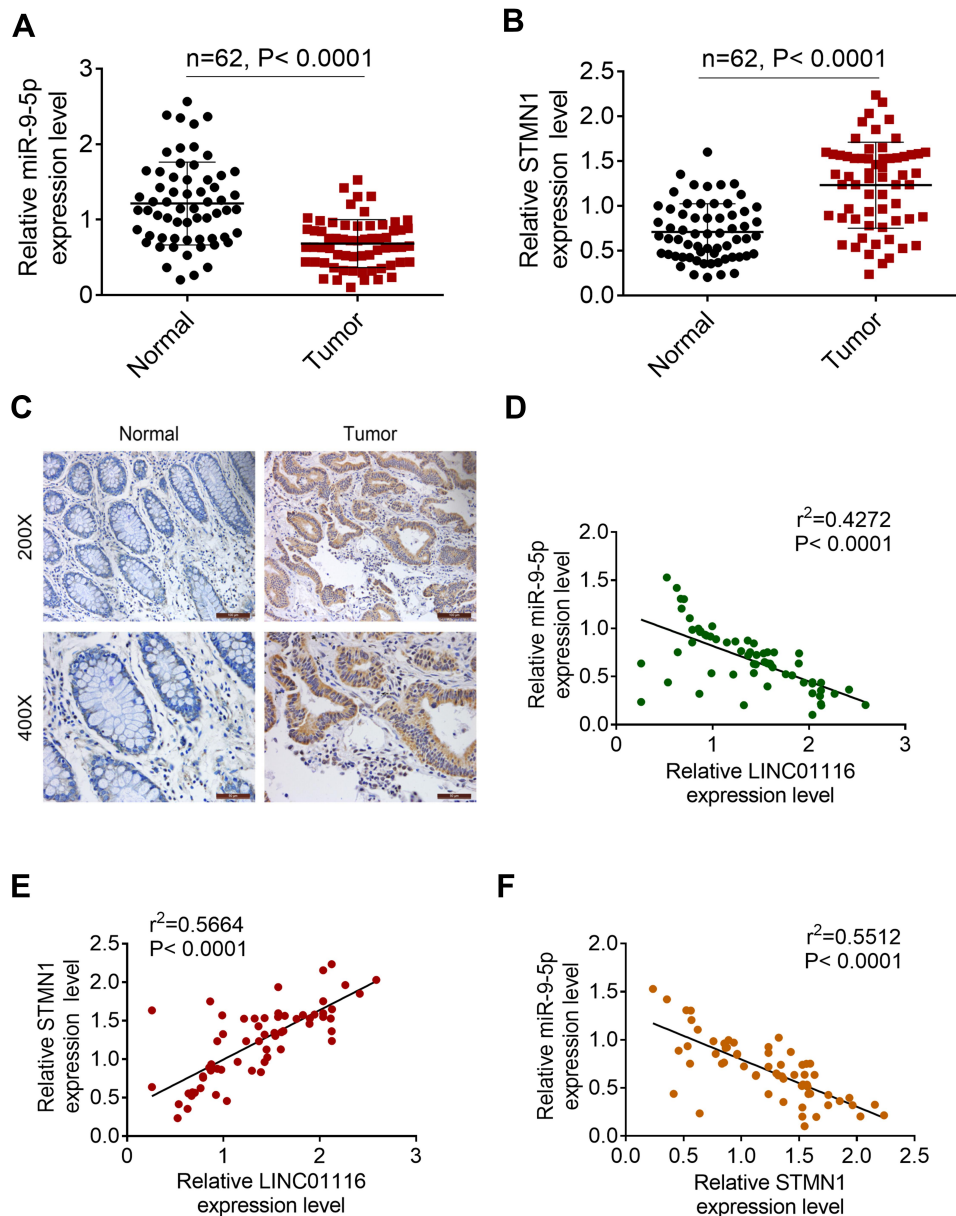


Figure 5 Correlation between LINC01116 and miR-9-5p/STMN1 expression in colorectal cancer tissues. **(A)** MiR-9-5p expression in colorectal cancer tissues and normal tissues. **(B)** STMN1 was expressed higher in tumor tissues. **(C)** IHC assay for STMN1 expression. **(D)** LINC01116 negatively regulated miR-9-5p expression. **(E)** LINC01116 was negatively related to STMN1. **(F)** MiR-9-5p was negatively related to STMN1 expression.

cancer.⁴⁶ Moreover, it could also promote proliferation and tumorigenicity of colorectal cancer.⁴⁷ Besides, the JAK-STAT signaling pathway was closely related with proliferation and apoptosis of colorectal cancer.⁴⁸ Thereby, LINC01116 was important for colorectal cancer development. However, there were limitations in this study. LINC01116 was related with different signaling pathways in this disease. In addition, the related pathways were complex and interacted with each other. However, a figure as a systematic view needed more research foundation, which will be the focus of our further research.

In conclusion, LINC01116 promoted the development of colorectal cancer via regulating miR-9-5p/STMN1 expression. It could be applied for treatment of colorectal cancer as a novel target.

Highlights

1. LINC01116 was expressed more highly in colorectal cancer cells and tissues.
2. Patients with more highly expressed LINC01116 always showed poor prognosis.

3. Knockdown of LINC01116 suppressed progression of colorectal cancer cells.
4. LINC01116 was negatively regulated by miR-9-5p.
5. MiR-9-5p was negatively related to *STMN1* expression.

Disclosure

The authors report no conflicts of interest in this work.

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