MicroRNA-27a Promotes the Proliferation and Invasiveness of Colon Cancer Cells by Targeting SFRP1 through the Wnt/β-Catenin Signaling Pathway

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Key Words
Colon cancer • MicroRNA-27a • Secreted Frizzled-related protein 1 • Wnt/β-catenin signaling pathway • Proliferation • Invasion

Abstract

Objective: This study aims to explore the effects of microRNA-27a (miR-27a) on the proliferation and invasiveness of colon cancer cells through the Secreted Frizzled-related protein 1 (SFRP1) and the Wnt/β-catenin signaling pathway. Methods: Colon cancer tissues and adjacent normal tissues from 125 colon cancer patients, together with the HCEpic, HCT-116, HT-29, SW480 and SW620 cell lines, were prepared for this study. The transfected HCT-116 cells were divided into the miR-27a mimics, miR-27a-NC, anti-miR-27a, blank, Lv-SFRP1, Lv-NC, and miR-27a mimics + Lv-SFRP1 groups. RT-qPCR was performed to detect the expressions of miR-27a and SFRP1 mRNA. A dual-luciferase reporter assay was conducted to examine the effect of miR-27a on SFRP1. Western blotting was used to measure the expressions of the SFRP1, β-catenin, GSK-3β, p-β-catenin, p-GSK-3β, c-Myc and cyclin D1 proteins. MTT, soft agar clone formation and Transwell chamber assays were performed to detect cell proliferation and invasion. Results: Compared with normal tissues and cells, colon cancer tissues and cells demonstrated significantly higher expression of miR-27a, but lower expressions of SFRP1 mRNA and protein. MiR-27a negatively regulated the expression of SFRP1 mRNA. SFRP1 was also found to be a target gene of miR-27a. In the miR-27a mimic group, the proliferation and invasiveness of colon cancer cells were significantly increased, while the expressions of GSK-3β and p-β-catenin were remarkably down-regulated; in contrast, the expressions of p-GSK-3β, β-catenin, c-Myc and cyclin D1 were up-regulated. While the proliferation and invasiveness of colon cancer cells in the anti-miR-27a and Lv-SFRP1 groups were decreased, the expressions of GSK-3β and p-β-catenin were elevated, and the expressions of p-GSK-3β, β-catenin, c-Myc and cyclin D1 were decreased. Conclusion: These findings indicated that miR-27a could promote the proliferation and invasiveness of colon cancer cells by targeting SFRP1 through the Wnt/β-catenin signaling pathway.

S. Ba and Y. Xuan are regarded as co-first authors.
Introduction

Colon cancer is one of the most common cancers worldwide, and together with lung and prostate cancer, is one of the major causes of cancer mortality [1]. Colon cancer is a common malignant tumor of the digestive tract, whose incidence has increased in recent years in China [2]. It is estimated that 2 ~ 5% of colon cancers occur in the setting of well-defined inherited syndromes, while a large proportion arises due to unhealthy lifestyles [3, 4]. Due to its established efficacy, postoperative adjuvant chemotherapy is considered the international standard treatment for patients with stage III colon cancer [5, 6]. The five-year survival rates of patients with stage II, III and IV colon cancer are 78.5%, 54% and 8.1%, respectively [7]. Therefore, it is of great significance to identify and understand changes in the cancer genome to develop targeted therapeutics [8]. MicroRNAs (miRNAs) are widely accepted to have a profound effect on gene expression, cellular homeostasis, and expressions of multiple oncogenes and tumor suppressor genes in cancer cells [9].

MiRNAs are short (18~25 nucleotides), endogenous non-coding molecules that cause translational repression or mRNA cleavage by binding to the 3'-untranslated regions (3'-UTRs) of target mRNAs [7, 10]. MiRNAs have been demonstrated to regulate many cellular processes, including differentiation, proliferation and apoptosis [11-13]. Aberrant expressions of miRNAs have been observed in most tumors, which suggests their important role in carcinogenesis and in the prognosis of cancers [14, 15]. Approaches to the modulation of functions of miRNAs may be effective in the development of novel adjuvant therapeutics [7]. MiRNA-27a (miR-27a), which is located on chromosome 19, is a target of anticancer drugs [16]. It has been reported that miR-27a functions as a tumor promoter in many human cancers, including breast cancer, gastric adenocarcinoma, hepatocellular carcinoma and pancreatic cancer [17-20].

Secreted frizzled-related protein-1 (SFRP1), which is located within the 8p11-12 region, is homologous to the extra-cellular cysteine-rich domain of the wingless and integration site growth factor (WNT) receptor Frizzled, but lacks the intracellular and trans-membrane domains [21]. It has been reported that the down-regulation of SFRP1 can lead to the over-activation of Wnt signaling pathways, which promotes tumorigenesis in human mammary tissues [22, 23]. Wnts are a family of 19 secreted glycoproteins that primarily send signals via seven-pass trans-membrane receptors of the frizzled family [24]. The β-catenin is the major component of the canonical Wnt signaling pathway (Wnt/β-catenin pathway) and functions as a transcription factor that regulates cellular activities such as the cell cycle, communication, apoptosis and cell differentiation [25]. A previous study has reported that Wnt signals may not only accelerate tissue expansion and cell proliferation but that they also regulate fate determination and terminal differentiation of post-mitotic cells [26]. Therefore, this study was performed with the aim to discover the potential role of miR-27a in the proliferation and invasiveness of colon cancer cells by the targeting of SFRP1 through the Wnt/β-catenin signaling pathway, to understand the mechanisms of miR-27a in colon cancer progression.

Materials and Methods

Ethics statement

This study was approved by the Ethics Committee of Fudan University Shanghai Cancer Center. All samples were collected based on the informed consent of the study subjects.

Study subjects

From February 2013 to April 2015, 125 patients diagnosed with colon cancer at Fudan University Shanghai Cancer Center were recruited for this study. The tumor tissues from these patients were obtained and included in the experimental group. Among all patients, 54 were males and 71 were females, with median age of 48 years (range from 24 to 84 years). The diagnosis was made based on the classification
criteria for colon cancer released by the World Health Organization [27]. According to tumor differentiation, 37 cases had a high degree of tumor differentiation, 46 cases had a medium degree and 42 had a low degree of differentiation. According to the depth of invasion, 48 cases were in the T1 + T2 stages and 77 cases were in the T3 + T4 stages. In terms of metastasis, 75 cases had lymphatic metastasis and 66 cases had distal metastasis. Fifty-three cases had tumors with diameters larger than 2 cm. The adjacent tissues that were 2 cm away from the lesions were selected as the control group. This study excluded patients (1) who received preoperative radiotherapy or chemotherapy; (2) who were diagnosed with gastrointestinal stromal tumors or lymphomas; (3) who had tumors outside the colon; (4) who did not give their permission to be involved in the study. All the tissues were preserved in liquid nitrogen within 10 min after resection.

Cell culture

Normal human colonic epithelial HCEpiC cells and human colon cancer cells lines (HCT-116, HT-29, SW480 and SW620) were purchased from the Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). The five cell lines were cultured in an incubator (37°C, 5% CO2 and saturated humidity) with RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS). The culture medium was replaced every two days. When the cells reached 90% confluency, they were passaged at a ratio of 1:2.

Dual-luciferase reporter assay

According to the biological information online software TargetScan, miR-27a was predicted to be able to bind to the 3'UTR of SFRP1 mRNA. The target sequence and mutation sequence were designed based on the binding sequence of the SFRP1 3'UTR and miR-27a. Then, the target sequence was chemically synthesized, and sites cleaved by the XhoI and NotI enzymes were added to both ends of the sequence. The synthesized fragment was cloned into a PUC57 vector. After the positive clones were identified, the recombinant plasmids were identified by DNA sequencing, subcloned into a psiCHECK-2 vector, and then transformed into Escherichia coli DH5α cells for amplification. Plasmid extraction was performed in accordance with the instructions of the Omega Plasmid Extraction Kit (Omega Bio-tek Inc, Norcross, GA, USA). The cells were inoculated into a 6-well plate (2 × 10^5 cells/well), and after the cells adhered to the wells, transfection was performed according to the aforementioned method. Subsequently, the cells were cultured for 48 h and then collected. The experimental cells were divided into the miR-27a-NC + psi-CHECK2-SFRP1-3'UTR group, the miR-27a-NC + psi-CHECK2-SFRP1-3'UTR mut group, the miR-27a mimics + psi-CHECK2-SFRP1-3'UTR group and the miR-27a mimics + psi-CHECK2-SFRP1-3'UTR mut group. The effect of miR-27a on the luciferase activity of the SFRP1 3'-UTR was detected using a dual-luciferase reporter assay kit (Genecopoeia, Rockville, MD, USA). A fluorescence detector (type: Glomax20/20; Promega Corporation, Madison, WI, USA) was then used to determine the fluorescence intensity. This experiment was repeated 3 times for each group.

Cell transfection

For the cell transfection experiments, the cells were divided into the following groups: (1) miR-27a mimics group (transfected with artificially synthesized miR-27a mimics); (2) miR-27a-NC group (transfected with a scrambled sequence); (3) anti-miR-27a (transfected with miR-27a inhibitors); (4) blank group (no sequence transfected); (5) Lv-SFRP1 group (transfected with SFRP1 over-expression lentiviral vector); (6) Lv-NC group (transfected with empty plasmid vector); and (7) miR-27a mimics + Lv-SFRP1 group (transfected with miR-27a mimics and SFRP1 over-expression lentiviral vector). The transfection sequences for each group are shown in Table 1. The chemically synthesized miR-27a mimics, miR-27a inhibitors and negative control sequences were all purchased from Shanghai Gene Pharma Co., Ltd., Shanghai, China. The cells were inoculated into a 6-well plate 24 h before transfection. When the cells reached approximately 50% confluency, the human colon cancer HCT-116 cell line was transfected using Lipofectamine2000 (Invitrogen Inc., Carlsbad, CA, USA). After 6 hours, the culture medium was replaced. After 48 h in culture, the cells were then collected for further experiments.

Table 1. Gene sequences of miR-27a mimics, miR-27a-NC and anti-miR-27a. Note: NC, negative control

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence (5'-3')</th>
</tr>
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<tbody>
<tr>
<td>miR-27a mimics</td>
<td>UUCACAGUGGCUAAGUUCGG</td>
</tr>
<tr>
<td>miR-27a-NC</td>
<td>GGAACUUGCCACUGUGAAIUU</td>
</tr>
<tr>
<td>anti-miR-27a</td>
<td>CUAUGCACUGUGAA</td>
</tr>
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</table>
Lentiviral vector packaging and establishment of stable cell lines

The SFRP1 over-expression lentiviral vector (Lv-SFRP1) and empty plasmid vector (Lv-NC) used for colon cancer cell transfection were provided by Invitrogen Inc. (Carlsbad, CA, USA). Cells in the logarithmic growth phase were collected. After adjustment to the appropriate density, the cell suspension was inoculated into a 6-well plate (2 × 10^5 cells/well). After they adhered to the wells, the cells were transfected with lentivirus and screened using puromycin 3 days later. Selection of lentivirus-transfected cells was deemed successful if all the non-transfected cells died. After selection, the cells were expanded for use in downstream experiments.

Real-time quantitative polymerase chain reaction (RT-qPCR)

The RNA of colon cancer tissues, adjacent tissues and cells of each group was extracted according to the instructions of the TRIzol reagent kit (Invitrogen, USA). After reverse transfection, RT-qPCR was performed in an ABI7500 quantitative PCR instrument (Applied Biosystems, Inc., CA, USA) to determine the expressions of miR-27a and SFRP1 mRNA; the of U6 snRNA (U6) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used as internal references. The primer sequences are shown in Table 2. The PCR reaction system included forward and reverse primers (concentration: 10 μmol/L; each 1 μL), cDNA (1 μL), 2 × SYBR Green qPCR Mix (1 μL) and ddH₂O (7 μL). The reaction conditions were as follows: 95°C for 3 min, 95°C for 12 s and 62°C for 40 s for a total of 40 cycles. The relative expressions of genes were represented using the 2^−ΔΔCt method. The formulas were: 

\[ \Delta \Delta C_t = \Delta C_t_{\text{tumor}} - \Delta C_t_{\text{normal}} \]

\[ \Delta C_t = C_t_{RNA} - C_t_{\text{inter-referred}} \]

The experiment was repeated 3 times for each group.

Western blotting

The total protein of cells and tissues was extracted with RIPA lysis solution (Gibco Company, Grand Island, NY, USA) containing phenylmethylsulfonyl fluoride (PMSF); the protein concentration was detected with the Bradford method. After 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), the protein was transferred onto a polyvinylidene fluoride (PVDF) membrane and then incubated for 1 h with Tris-buffered saline and Tween (TBST) containing 5% skim milk. Using GAPDH as an internal reference, the membrane was subsequently incubated at 4°C overnight with primary antibodies against SFRP1, β-catenin, GSK-3β, p-β-catenin, p-GSK-3β, c-Myc and cyclin D1 diluted 1:1000 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) and GAPDH diluted 1:5000 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA). Next, the membranes were incubated with horseradish peroxidase (HRP)-labeled secondary antibodies at room temperature for 2 h, followed by auto-radiography, imaging and recording. The films were scanned and the image analysis software Gel-Pro Analyzer 4.0 was used to analyze the gray scale images. The relative ratio was calculated by a comparison with the expression of the internal reference gene. The experiment was conducted 3 times for each group.

MTT assay

Colon cancer cells in the logarithmic growth phase were collected and inoculated into a 96-well plate after the cell concentration was adjusted to 5 × 10^5 cells/mL. After the cells adhered to the bottom of the plate, the cells were transfected and 10 μL MTT was added to each well after 12, 24 and 48 h of culture. Subsequently, the cells were cultured for another 4 h in the incubator. After the culture solution was discarded, 150 μL dimethyl sulfoxide (DMSO) was added, and the plates were allowed to shake for 10 min in the dark. The absorbance value at a wavelength of 570 nm was detected.

Soft agar colony formation assay

Forty-eight hours after transfection, RPMI 1640 medium supplemented with 10% FBS and 0.6% agar was added to the 6-well plate, which was kept at room temperature for 10 min. After solidification, a

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Table 2. Primer sequences of miR-27a, SFRP1, GAPDH and U6 for RT-qPCR.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence (5’-3’)</th>
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<tbody>
<tr>
<td>miR-27a</td>
<td>F: TTCCAGGGGCTCAAAG&lt;br&gt;R: GTGCCAGGTCCCAGGT</td>
</tr>
<tr>
<td>SFRP1</td>
<td>F: CAGAGGCTCATTCAGGT&lt;br&gt;R: ATGACCTATCGTCGAGGTC</td>
</tr>
<tr>
<td>GAPDH</td>
<td>F: ACAGTCAGGGCATCATCTTT&lt;br&gt;R: GACAAGTTCCGTCGTCAG</td>
</tr>
<tr>
<td>U6</td>
<td>F: CGGTCGCGACATATATCATAAA&lt;br&gt;R: GCTTCGGCAGCAGCATATCATAAA</td>
</tr>
</tbody>
</table>

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0.5-mL cell suspension (concentration: \(2 \times 10^5\) cells/mL) in RPMI 1640 medium supplemented with 10% FBS and 0.3% agar was added. The colony formation of the cells was observed after 21 d in culture at 37°C. Using cell numbers ≥ 50% as the colony formation standard, the number of colonies was counted by randomly selecting 5 horizons in each group. The experiment was repeated 3 times for each group.

**Transwell chamber assay**

Forty-eight hours after transfection, the cells were collected for serum starvation, where the cells were cultured for 24 h in FBS-free RPMI 1640 cell culture medium. The collected cells were suspended in FBS-free RPMI 1640 cell culture medium, which was followed by adjustment of the cell concentration to \(10^4\) cells/mL. The serum-free cell suspension (300 μL) was added to the upper chamber of a Transwell invasion chamber, and RPMI 1640 culture medium containing 10% FBS (500 μL) was added to the lower chamber; the cells were then cultured for 48 h at 37°C with 5% CO\(_2\). After the liquid in the upper chamber was absorbed, the cells that did not pass through the micropores on the membrane were removed with cotton swabs, which was followed by staining with 0.1% crystal violet for 10 min. Then, cells that had passed through the membrane and infiltrated the lower chamber were then imaged under a microscope. After the dye on the membrane was dissolved with acetic acid, the cells were transferred to a 96-well plate. The absorbance value of each well at a wavelength of 570 nm was detected. The experiment was repeated 3 times for each group.

**Statistical analysis**

All data were analyzed using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). The measurement data were represented as \(\bar{x} \pm s\). The Kolmogorov-Smirnov method was used to test the normal distribution. The comparison of normally distributed measurement data between two groups was analyzed using a \(t\)-test, the expressions of miR-27a and SFRP1 in colon cancer tissues and adjacent tissues were compared using a paired \(t\)-test, and the relationship between the expressions of miR-27a and SFRP1 and different pathological features of colon cancer patients was analyzed using an independent samples \(t\)-test. The following were compared by one-way analysis of variance (ANOVA): expressions of miR-27a and SFRP1 in 5 cell lines, the luciferase activity in the different groups, the expressions of miR-27a and related proteins in multiple groups after transfection, the number of cell colonies formed and the number of invasive cells. Comparisons between two groups were determined by the least significant difference (LSD) method. A repeated measures ANOVA was used to compare the cell proliferation in each group after transfection, a non-parametric rank sum test was adopted to analyze measurement data that was not normally distributed, and the Pearson correlation method was used to test the correlation between miR-27a and SFRP1 expressions in cells and tissues. \(P<0.05\) was considered significantly different.

**Results**

*Expressions of miR-27a and SFRP1 was associated with colon cancer*

According to RT-qPCR, the expression of miR-27a was significantly higher in colon cancer tissues than in adjacent normal tissues \((P<0.05)\), while the expressions of SFRP1 mRNA and protein in colon cancer tissues were significantly lower than those in adjacent normal tissues (Fig. 1A-D). Moreover, it was also revealed that the expressions of miR-27a and SFRP1 mRNA were closely correlated with the degree of tumor differentiation, lymphatic metastasis, distal metastasis and depth of tumor invasion in patients with colon cancer (all \(P<0.05)\). However, the expressions of miR-27a and SFRP1 mRNA were not associated with gender, age or tumor size (all \(P>0.05)\) (Table 3). Compared with normal human colonic epithelial cells, the human colon cancer cell lines (HCT-116, HT-29, SW480 and SW620) demonstrated significantly up-regulated miR-27a expression but down-regulated SFRP1 mRNA and protein expressions (all \(P<0.05\)). In contrast, no significant difference was observed in the expressions of miR-27a and SFRP1 mRNA and protein among the four colon cancer cell lines (all \(P>0.05\)). Since the expressions of miR-27a and SFRP1 was lowest in HCT-116 cells (Fig. 1E-H), the HCT-116 cell line was used in further experiments. A correlation analysis showed that the expressions of miR-27a and SFRP1 mRNA was negatively correlated in both colon cancer tissues (Fig. 1I) and cells (Fig. 1J) (all \(P<0.05\)).
SFRP1 was the potential target gene of miR-27a

According to the biological information online analysis software (TargetScan, miRDB, microrna, PicTar and starBase), SFRP1 was confirmed to be a target gene of miR-27a. The luciferase reporter vector, which contained SFRP1 3’UTR fragments with wild-type (psi-CHECK2- SFRP1-3’UTR) or mutant (psi-CHECK2-SFRP1-3’UTR mut) miR-27a complementary sites, is shown in Fig. 2A. The dual-luciferase reporter assay (Fig. 2B) demonstrated a significant difference in the luciferase activity among the miR-27a-NC + psi-CHECK2-SFRP1-3’UTR, miR-27a-NC + psi-CHECK2-SFRP1-3’UTR mut, miR-27a mimics + psi-CHECK2-SFRP1-3’UTR and miR-27a mimics + psi-CHECK2-SFRP1-3’UTR mut groups (F = 91.72, P < 0.001). Compared with the miR-27a-NC + psi-CHECK2-SFRP1-3’UTR, miR-27a-NC + psi-CHECK2-SFRP1-3’UTR mut and miR-27a mimics + psi-CHECK2-SFRP1-3’UTR mut groups, the luciferase activity was significantly decreased in the miR-27a mimics + psi-CHECK2-SFRP1-3’UTR group (P < 0.05). RT-qPCR revealed a significant difference in miR-27a expression among the four groups (F = 35.64, P < 0.001). The expression of miR-27a was significantly elevated in the miR-27a mimics + psi-CHECK2-SFRP1-3’UTR and miR-27a
Table 3. Associations of miR-27a and SFRP1 mRNA expressions with clinicopathological features of colon cancer patients. Note: SFRP1, secreted frizzled-related proteins 1

<table>
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<th>Clinicopathological feature</th>
<th>Cases</th>
<th>miR-27a</th>
<th>t</th>
<th>P</th>
<th>SFRP1 mRNA expression</th>
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<td>Male</td>
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<td>0.811</td>
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<td>71</td>
<td>2.59 ± 0.54</td>
<td>0.24</td>
<td>0.811</td>
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<td>Age (years)</td>
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<tr>
<td>&gt; 63</td>
<td>42</td>
<td>2.59 ± 0.50</td>
<td>0.107</td>
<td>0.915</td>
<td>0.46 ± 0.15</td>
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<tr>
<td>≤ 63</td>
<td>83</td>
<td>2.58 ± 0.49</td>
<td>0.107</td>
<td>0.915</td>
<td>0.41 ± 0.16</td>
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<td>0.811</td>
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<td>75</td>
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<td>T1 + T2</td>
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<td>T3 + T4</td>
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<td>0.24</td>
<td>0.811</td>
<td>0.42 ± 0.15</td>
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</table>

Fig. 2. The targeting of SFRP1 by miR-27a was confirmed by biological information prediction and dual-luciferase reporter assay. Note: (A) construction of the psi-CHECK2-SFRP1-3'UTR and psi-CHECK2-SFRP1-3'UTR mut vectors and the active sequence of miR-27a; (B) luciferase activity of HCT-116 cells in each group, in which * indicated P < 0.05 compared with the miR-27a-NC + psi-CHECK2-SFRP1-3'UTR, miR-27a-NC + psi-CHECK2-SFRP1-3'UTR mut and miR-27a mimics + psi-CHECK2-SFRP1-3'UTR mut groups; (C) miR-27a expression in each group, in which * indicated P < 0.05 compared with the miR-27a-NC + psi-CHECK2-SFRP1-3'UTR and miR-27a-NC + psi-CHECK2-SFRP1-3'UTR mut groups; SFRP1, secreted frizzled-related protein 1.

mimics + psi-CHECK2-SFRP1-3' UTR mut groups compared with the miR-27a-NC + psi-CHECK2-SFRP1-3'UTR and miR-27a-NC + psi-CHECK2-SFRP1-3' UTR mut groups (all P < 0.05).
Comparisons of miR-27a and SFRP1 expressions among the seven groups after transfection

After transfection, the expressions of miR-27a and SFRP1 mRNA and protein in the seven groups were significantly different (miR-27a: $F = 69.29, P = 0.001$; SFRP1 mRNA: $F = 124.7, P < 0.001$; SFRP1 protein: $F = 17.96, P = 0.006$) (Fig. 3). No significant difference was observed in the expressions of miR-27a and SFRP1 mRNA and protein among the blank, miR-27a NC and LV-NC groups (all $P > 0.05$). Compared with the blank, miR-27a NC and LV-NC groups, the miR-27a mimics group demonstrated significantly up-regulated miR-27a expression but down-regulated SFRP1 mRNA and protein expressions (all $P < 0.05$). By contrast, the anti-miR-27a group showed significantly decreased miR-27a expression but increased SFRP1 mRNA and protein expressions (all $P < 0.05$). In the LV-SFRP1 and miR-27a mimics + LV-SFRP1 groups, the expressions of SFRP1 mRNA and protein were both significantly increased (all $P < 0.05$), but no significant difference was found between these two groups (all $P > 0.05$). In addition, miR-27a expression was not significantly different between the miR-27a mimics group and the miR-27a mimics + LV-SFRP1 group (all $P > 0.05$).

MiR-27a promoted the proliferation of colon cancer cells by targeting SFRP1

An MTT assay (Fig. 4A) showed that the main effects of the different groups, different time points/interaction between groups and time on cell proliferation were statistically significant ($F = 194.1, 69.95$ and $4.034$, respectively, all $P < 0.001$). Compared with the blank, miR-27a NC, LV-NC and miR-27a mimics + LV-SFRP1 groups, cell proliferation in the miR-27a mimics group was significantly elevated after 12 h, 24 h and 48 h of transfection, while that in the anti-miR-27a and LV-SFRP1 groups was significantly reduced (all $P < 0.05$). No significant difference was noted in cell proliferation among the blank, miR-27a NC, LV-NC and miR-27a mimics + LV-SFRP1 groups at 12 h, 24 h or 48 h after transfection (all $P > 0.05$).
with the blank, miR-27a NC, LV-NC and miR-27a mimics + LV-SFRP1 groups, the number of colonies formed was significantly increased and the expressions of c-Myc and cyclin D1 were up-regulated in the miR-27a mimics group. In addition, the number of colonies formed was significantly decreased and the expressions of c-Myc and cyclin D1 were down-regulated in the anti-miR-27a and LV-SFRP1 groups (all \( P < 0.05 \)). No significant difference was found in the number of colonies formed and in the expressions of c-Myc and cyclin D1 among the blank, miR-27a NC, LV-NC and miR-27a mimics + LV-SFRP1 groups (all \( P > 0.05 \)).

**MiR-27a promoted the invasion ability of colon cancer cells by targeting SFRP1**

As shown by the Transwell invasion assay (Fig. 5), remarkable significant differences were found in the invasion ability of colon cancer cells among the different groups 48 h after transfection (\( F = 22.55, P < 0.001 \)). Compared with the blank, miR-27a NC, LV-NC and miR-27a mimics + LV-SFRP1 groups, the invasion ability of cells in the miR-27a mimics group was significantly increased, while that in the anti-miR-27a and LV-SFRP1 groups was significantly decreased (all \( P < 0.05 \)). No significant difference was seen in the invasion ability of cells among the blank, miR-27a NC, LV-NC and miR-27a mimics + LV-SFRP1 groups (all \( P > 0.05 \)).

**MiR-27a activated the Wnt/β-catenin signaling pathway by targeting SFRP1**

As shown in Fig. 6, statistically significant differences were found in the expressions of GSK-3β, p-GSK-3β, p-β-catenin and β-catenin among the different groups 48 h after transfection (\( F_{\text{GSK-3β}} = 68.59, F_{\text{p-GSK-3β}} = 117.1, F_{\text{p-β-catenin}} = 33.10, F_{\text{β-catenin}} = 37.26, \) all \( P < 0.001 \)). Compared with the blank, miR-27a NC, LV-NC and miR-27a mimics + LV-SFRP1 groups, the anti-miR-27a and LV-SFRP1 groups had significantly elevated GSK-3β and p-β-catenin expressions but decreased p-GSK-3β and β-catenin expressions (all \( P < 0.05 \)). In the miR-27a mimics group, the expressions of GSK-3β and p-β-catenin were significantly reduced,
whereas the expressions of p-GSK-3β and β-catenin were significantly increased (all \( P < 0.05 \)). No significant difference was observed in the expressions of GSK-3β, p-GSK-3β, p-β-catenin and β-catenin among the blank, miR-27a NC, LV-NC and miR-27a mimics + LV-SFRP1 groups (all \( P > 0.05 \)).

**Discussion**

As the third most common cancer, colon cancer is responsible for the second most cancer-related deaths in the western world, and thus new predictive and prognostic biomarkers are needed so that clinicians can determine the most effective treatment [28, 29]. Since aberrant miRNA expression is presently reported to be correlated with various human cancers [16],
this study aimed to reveal the role of miR-27a in the proliferation and invasiveness of colon cancer cells. MiR-27a affects proliferation and invasiveness by targeting SFRP1, which regulates the Wnt/β-catenin signaling pathway. This is one mechanism through which miR-27a affects colon cancer progression.

This study found a higher level of miR-27a and a lower level of SFRP1 in colon cancer tissues and cells compared with normal tissues and cells. Additionally, a correlation analysis further confirmed that miR-27a and SFRP1 were negatively correlated. A growing body of data has shown that miR-27a is an oncogene that is abnormally up-regulated in a large variety of human cancers, where it promotes the growth of cancer cells by targeting mRNAs [20, 30]. Moreover, miR-27a is involved in the regulation of cancer development and progression. Cell proliferation and anchorage-independent growth can be inhibited if miR-27a is suppressed, which demonstrates that miR-27a exerts effects on cell proliferation [30]. The tumor suppressor gene SFRP1 functions as a negative regulator of Wnt signaling via its ability to form a heterodimer with Frizzled; this then prevents the formation of non-functional receptor complexes by Wnt proteins [31]. As Wnt antagonists, proteins in the SFRP family are endogenous modulators of Wnt signaling that compete with Wnt ligands for binding to the Frizzled receptors. This binding attenuates Wnt signaling in colon cancer cells even when β-catenin mutations are present and probably exhibits synergy with downstream mutations during the process of colorectal cancer progression [32]. Promoter methylation or mutations in the coding region can inactivate SFRP1, which probably enables preneoplastic cells to escape from stress-induced senescence and accumulate mutations so that they can advance into full-blown tumors [33]. Hypermethylation of the SFRP1 promoter and its loss of expression, which allow for the development of aberrant Wnt signaling, have already been found in colorectal, kidney, lung, breast, ovarian and hepatocellular cancers [31]. Therefore, those data are in agreement with our finding that the expression of SFRP1 is decreased in colon cancer. The over-expression of miR-27a can activate Wnt/β-catenin signaling, and as SFRP1 inhibits this signaling pathway, it is not difficult to conclude that miR-27a and SFRP1 are negatively correlated [34]. Moreover, it was also shown that miR-27a regulates the expressions of SFRP1 mRNA and protein in an inverse manner, which indicates that miR-27a likely suppresses SFRP1 expression by the degradation of its mRNA rather than by the inhibition of protein translation. Guo et al. have reported that SFRP1, as the preferred target gene, is inhibited by miR-27a, which further confirms our result [34]. We also found that compared with the blank, miR-27a NC, LV-NC and miR-27a mimics + LV- SFRP1 groups, the proliferation and invasiveness of colon cancer cells were significantly increased in the miR-27a mimics group. This was accompanied by considerable down-regulation of GSK-3β and p-β-catenin and the up-regulation of p-GSK-3β, β-catenin, c-Myc and cyclin D1. By contrast, the anti-miR-27a and Lv-SFRP1 groups showed the opposite expression patterns. The results indicated that miR-27a can activate the Wnt/β-catenin signaling pathway to promote the proliferation and invasiveness of colon cancer cells. Glycogen synthase kinase-3β (GSK-3β), a widespread cellular serine/threonine kinase, plays a key role in the regulation of various signaling pathways, including Wnt/β-catenin signaling [35, 36]. In the Wnt pathway, GSK-3β is found in multimeric composites composed of the adenomatous polyposis coli (APC) protein, axin and β-catenin [37], where it causes phosphorylation of the N-terminal Ser/Thr in β-catenin [38]. It was revealed that the suppression of GSK-3β can activate Wnt signaling and β-catenin through dephosphorylation, where GSK-3β functions as an inhibitor of the Wnt pathway [38]. Wnts are secreted proteins that bind to a Frizzled receptor and lipoprotein receptor-related protein 5 (LRP5) or LRP6 co-receptor, which results in an increased level of β-catenin because its degradation via the proteasome is prevented [39]. Wnt signaling plays an essential role in cell survival, proliferation, and migration, whereby its aberrant activation acts as a major driving force of cell proliferation [40, 41]. Wnt signaling is currently regarded as a significant pathway in carcinogenesis, especially in colorectal cancer; by binding to Wnt molecules, SFRP1 can also act as an antagonist that inhibits Wnt signaling. MiR-27a can regulate the Wnt/β-catenin signaling pathway via the targeting of SFRP1, and after the activation of this signaling pathway, the cell cycle, viability, apoptosis, as well as invasion and
migration of cancer cells are affected [32, 42]. Wang K et al have also reported that SFRP1 is a direct target gene of miR-27a and that it can inhibit Wnt signaling through binding to Wnt molecules [42]. Since the identification of the core molecules that are important for genetic perturbations of canonical Wnt signaling is crucial to fully comprehend the complex process of malignancy in colorectal cancer [41], we studied miR-27a as a key molecule in the mechanism of the development and progression of colon cancer. MiR-27a is an antineoplastic factor that regulates the Wnt/β-catenin signaling pathway by binding to the SFRP1 3’-UTR, and thus it strongly enhances and reinforces colon cancer cell proliferation and invasion [42, 43].

In conclusion, our data illustrated that miR-27a promoted the proliferation and invasion of colon cancer cells via the inhibition of SFRP1 through the Wnt/β-catenin signaling pathway. The innate mechanism presented in this paper may be beneficial for the diagnosis and optimal treatment of patients with colon cancer. However, the correlation between miR-27a and SFRP1 mRNA was demonstrated to be weak due to the small sample size selected for this study. Therefore, further studies are still required to confirm our results.

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The authors have declared that no competing interests exist.

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