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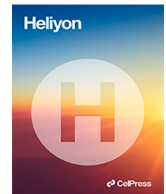

Effects of LINC00261 targeting miR-148a/WNT10b axis on the proliferation and apoptosis of colorectal cancer cells

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ABSTRACT

Objective: Colorectal cancer remains a significant challenge with high mortality rates. The aim of this study was to investigate the effect of targeting the microRNA-148a/WNT10b axis with the long non-coding RNA LINC00261 on the proliferation and apoptosis of colorectal cancer cells.
Methods: In vitro, small interfering RNA-LINC00261 and microRNA-148 inhibitor sequences were synthesized and transfected into SW480 cells. The study groups included a control group, small interfering RNA negative control group, small interfering RNA group, small interfering RNA negative control + microRNA -inhibitor group, small interfering RNA + microRNA -inhibitor group, and small interfering RNA + microRNA-negative control group. The transfection efficiency and expression levels of LINC00261 and miR-148a were evaluated by quantitative reverse transcription polymerase chain reaction. Cell proliferation, apoptosis, cell cycle distribution, and protein expression levels of WNT10b and β-catenin were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, flow cytometry, and Western blot, respectively.
Results: After small interfering RNA-LINC00261 transfection, a significant decrease in cell proliferation ($p < 0.05$) and an increase in apoptosis ($p < 0.05$) were observed, accompanied by cell cycle arrest in the G1 phase. Inhibition of LINC00261 by small interfering RNA resulted in increased microRNA-148a expression and decreased protein expression of WNT10b and β-catenin. However, the small interfering RNA + microRNA inhibitor group showed significantly increased levels of WNT10b and β-catenin protein expression.
Conclusions: These results suggest that silencing of long non-coding RNA LINC00261 could potentially affect the proliferation of SW480 cells by regulating the micro RNA -148a/WNT10b axis.

1. Introduction

Colorectal cancer (CRC), one of the most common and aggressive malignancies, is a leading cause of cancer-related deaths worldwide [1,2]. Despite advances in medical researches, treatment options for CRC primarily include surgery, radiotherapy, chemotherapy, targeted therapy, and other modalities [1]. Early detection of the disease and identification of distant metastases

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remain challenging. As a result, the prognosis for advanced CRC is typically unfavorable, resulting in unsatisfactory five-year survival rates. Drug resistance of tumor cells also contributes to this outcome [2], highlighting the critical need for new and effective targets for advanced CRC patients.

Among the various long non-coding RNAs (lncRNAs), LINC00261 has attracted attention as a tumor suppressor in several cancer types [3,4]. Researches have highlighted its potential as a prognostic biomarker in endometrial cancer [5], primarily due to its inhibition of cell proliferation and promotion of apoptosis [6]. In addition, LINC00261 has been implicated in reducing the invasiveness of tumor cells by suppressing epithelial-mesenchymal transition. In breast cancer, LINC00261 has been shown to reduce cell proliferation and migration [7,8]. How LINC00261 exerts its role in cancers remains under exploration.

Previous studies investigating cisplatin resistance have revealed the activation of the WNT/β-catenin signaling pathway in the progression of SW480 colon adenocarcinoma cells [9]. In addition, miR-148a can bind to the 3′UTR region of WNT10b, thereby inhibiting tumor proliferation, migration, and invasion [10]. With regards to LINC00261, its association with the WNT/β-catenin pathway has been reported, highlighting its potential as a suppressor of cancer progression [11,12]. LINC00261 has demonstrated prognostic implications in CRC, showing a positive correlation with overall survival (OS) [12]. However, the precise effects of LINC00261 on CRC proliferation and apoptosis, and its association with the miR-148a/WNT axis, remain unclear and require further investigation.

Therefore, the aim of our study was to evaluate the effect of LINC00261 on cell proliferation and apoptosis in CRC patients. In addition, we aimed to investigate the association between LINC00261 and the microRNA (miR)-148a/WNT10b axis in SW480 cells.

2. Methods and materials

2.1. Cells and materials

SW480 human colon adenocarcinoma cells were obtained from the Shanghai Cell Bank, Chinese Academy of Sciences. The cell culture medium was L15, which was purchased from Solarbio company (catalog number: LA9510). Fetal bovine serum (FBS) was purchased from Gibco company (catalog number: 10270-106). We used 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution from Solarbio company (catalog number: M1025) and dimethyl sulfoxide (DMSO) from SIGMA company (catalog number: D2650).

Annexin V-fluorescein isothiocyanate/propidium iodide (Annexin V-FITC/PI) apoptosis detection kit and propidium iodide/ribonuclease (PI/Rnase) staining buffer solution were both purchased from BD Company (catalog numbers: 556547 and 550825, respectively). Trizol reagent for RNA extraction was purchased from Ambion company (catalog number: 15596026), and the reverse transcription reagents were purchased from Takara company. SYBR Green dye for quantitative reverse transcription polymerase chain reaction (qRT-PCR) analysis was purchased from KAPA Biosystems company (catalog number: KM4101).

Radioimmunoprecipitation assay (RIPA) (strong) tissue cell rapid lysate and bicinchoninic acid (BCA) protein concentration assay kit were purchased from Solarbio company (catalog numbers: R0010 and PC0020, respectively). Antibodies against WNT10b, β-catenin, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were purchased from Bioswamp (catalog numbers: PAB44580, PAB30715, and PAB36269, respectively). A Leica DMIL LED inverted fluorescence microscope was used for imaging, and a NovoCyte flow cytometer (Essen, Germany) was used for cytometry analysis.

2.2. Cell culture

SW480 cells were cultured in L15 + 10 % FBS medium in a 37 °C, 100 % air incubator, and passaged at a ratio of 1:2. The final percentage of DMSO was 0.1 % for cell culture, and 10 % for cell freezing.

2.3. Transfection and identification of siRNA-LINC00261 and miR-148 inhibitor

Lipofectamine® RNAiMAX was used for siRNA transfection. The concentration of siRNAs used was 100 pmol for cell transfection in 6-well plates. We followed the instructions for transfection (Thermo Fisher Scientific) provided by the manufacturer. The siRNA sequence and siRNA-NC sequence were designed and synthesized according to the LINC00261 gene sequence (genInfo identifier (GI) number: 223278358). We prepared three siRNAs targeting LINC00261. Through PCR validation, the siRNA with the lowest expression of LINC00261 in the cells was selected for the following experiments. The inhibitor sequence and negative control (NC) sequence were designed and synthesized based on miR-148 gene sequence (GI number: 262206160). After 24 h of transfection, SW480 cells were harvested for total RNA extraction. The transfection efficiency was determined by qRT-PCR using non-transfected cells as a control

Table 1
Sequences of siRNAs and miR-148.

primer name	sequence
LINC00261-siRNA1	TGTTCAGAATCCTAATGAA
LINC00261-siRNA2	TGCCATAGATTACACCTAT
LINC00261-siRNA3	ACAGTCACATTAAGTTGAT
miR-148	UCAGUGCACUACAGAACUUUGU

group. The sequences of these siRNAs and miR-148 inhibitors are listed in Table 1.

2.4. Groups and procedures

A total of six groups were included in the study: the control group, the si-NC group, the siRNA group, the si-NC + miR-inhibitor group, the siRNA + miR-inhibitor group, and the siRNA + miR-NC group. The cells in the si-NC group were transfected with NC siRNAs, while the cells in the siRNA group were transfected with siRNA-LINC00261. The si-NC + miR-inhibitor group, siRNA + miR-inhibitor group, and siRNA + miR-NC group were transfected according to the design of their respective groups.

The MTT assay was performed to assess cell viability. Cells were seeded in a 96-well plate at a density of 3×10^3 cells per well. The following day, the respective treatments were added to the wells, and the cells were incubated for 48 h. After incubation, 10 μL MTT solution was added to each well, and the culture was continued for another 4 h. The supernatant was then discarded, and 150 μL DMSO was added to dissolve the formazan crystals. The absorbance of each well was measured at 490 nm using an enzyme-linked immunosorbent assay (ELISA) reader after 10 min of low-speed shaking. This measurement provides an indication of cell viability and proliferation.

2.5. Detection of apoptosis by flow cytometry

After 48 h of transfection, cells in each group were harvested at a density of 1×10^6 cells and resuspended. The cells were then centrifuged at 4 °C for 5 min, and the supernatant was discarded. Next, 1 mL of pre-cooled phosphate-buffered saline (PBS) was added to the cells and mixed gently before centrifugation. The cells were resuspended in 200 μL PBS.

To evaluate apoptosis, 10 μL Annexin V-FITC and 10 μL PI were added to the cell suspension. The mixture was gently mixed and incubated at 4 °C for 30 min in the dark. After incubation, 300 μL PBS was added and flow-through detection was performed using a flow cytometer named NovoCyte. The data obtained were analyzed using Novo Express analysis software, which allowed the assessment and quantification of apoptotic cells in the different groups.

2.6. Cell cycle detection by flow cytometry

After 48 h of transfection, cells from each group at a population of 1×10^7 were collected and resuspended. The cell suspension was centrifuged at 400 g for 5 min, and the supernatant was discarded. The resulting pellet was resuspended in 300 μL PBS.

Next, 700 μL absolute ethanol was added to the cell suspension, and the cells were fixed by placing them in a −20 °C refrigerator for 24 h. After the fixation period, the solution was centrifuged at 700 g for 5 min, and the supernatant was discarded. The cells were then washed with PBS to remove residual ethanol.

The cell pellet was then resuspended in 0.5 mL of PI/Rnase staining buffer solution and incubated at room temperature for 15 min. Flow cytometry was used to measure the DNA content of the cells, and to determine the proportion of cells in each phase of the cell cycle. The data were analyzed using Novo Express software, which facilitating the analysis and interpretation of flow cytometry results in terms of cell cycle distribution.

2.7. qRT-PCR detection of the expression of LINC00261 and miR-148a expression

After 48 h of treatment, cells from each group were harvested at a density of 1×10^6. To extract total RNA from the cells, 1 mL Trizol reagent was added. After that, cells were lysed and total RNA was isolated using the standard Trizol extraction protocol. The extracted RNA served as a template for complementary DNA (cDNA) synthesis, which was performed using the Takara Reverse Transcription Kit according to the instructions provided. Reverse transcription allows the conversion of RNA into cDNA, which can then be analyzed.

RT-PCR was performed to evaluate the expression levels of LINC00261 and miR-148a. Specific PCR primers, synthesized by Wuhan Tianyi Huayu Gene Technology Company, Limited, were used for amplification. The sequences of the PCR primers are listed in Table 2.

Table 2
Primer sequences.

primer name	sequence
LINC00261-F	TGCTGAACCAATAGACCAAC
LINC00261-R	CCATCTTTCACCCCAAAC
miR-148a-F	CTGGTGTCGTGGAGTCGG
miR-148a-R	GGGGAAAGTTCTGAGACACT
U6–F	CTGCTTCGGCAGCACA
U6-R	AACGCTTCACGAATTTGCGT
GAPDH-F	GGGAAACTGTGGCGTGAT
GAPDH-R	GAGTGGGTGTCGCTGTTGA

2.8. Western blot method to detect the protein expression levels of WNT10b and β-catenin

After 48 h of transfection, cells from each group were collected and lysed with 200 μL lysis buffer, which contained protease and phosphatase inhibitors. The lysis process was performed at 4 °C to ensure protein stability. The lysed cells were then centrifuged at 12,000 g for 10 min, and the resulting supernatant containing the protein extracts was collected.

The protein concentration in the supernatant was determined using a protein quantification assay. After that, 20 μg of protein was loaded onto a gel for separation by gel electrophoresis. The separated proteins were then transferred to a polyvinylidene fluoride (PVDF) membrane to immobilize them for subsequent analysis.

To prevent non-specific binding, the PVDF membrane was blocked overnight at 4 °C with a blocking agent. This step ensures that the antibodies used for protein detection specifically bind to their target proteins during subsequent incubation steps. After blocking, primary antibodies against WNT10b, β-catenin, and GAPDH were added to the membrane at a 1:1000 dilution, followed by incubation at room temperature for 1 h. Then, a secondary antibody was added to the membrane and incubated for another 1 h at room temperature.

To visualize the protein bands, a chemiluminescent reagent was added to the membrane. The gray value of the protein bands was then quantified using TANON GIS software or similar image analysis software. This quantification allows for assessment of the relative protein expression levels of WNT10b, β-catenin, and GAPDH in the different groups.

2.9. Statistical analysis

SPSS 19.0 software and GraphPad Prism 8 software were used for statistical analysis of the data. The experimental data were expressed as mean ± standard deviation ($\bar{x}\pm s$) of three independent experiments, the comparison among multiple groups was performed by analysis of variance (ANOVA), the pairwise comparison between groups was performed by least significant difference (LSD) test, and the comparison between unpaired groups was performed by the *t*-test. $P < 0.05$ indicated that the difference was statistically significant.

2.10. Survival analysis

GEPIA (http://gepia.cancer-pku.cn/) [13] was used to evaluate the prognostic value of LINC00261 expression in patients in the high and low expression groups based on expression values and validated by K-M survival curves. The log-rank test was used for analysis, presented as hazard ratio (HR) with its 95 % confidence interval (CI), and $P < 0.05$ was considered significant.

2.11. LncACTdb 3.0 database

LncACTdb (http://bio-bigdata.hrbmu.edu.cn/LncACTdb/) is an easy-to-use, comprehensive, and interactive web resource for lincRNA data analysis [14]. In this study, we used LncACTdb 3.0 to investigate the associations of LINC00261/miR-148a/WNT10b and

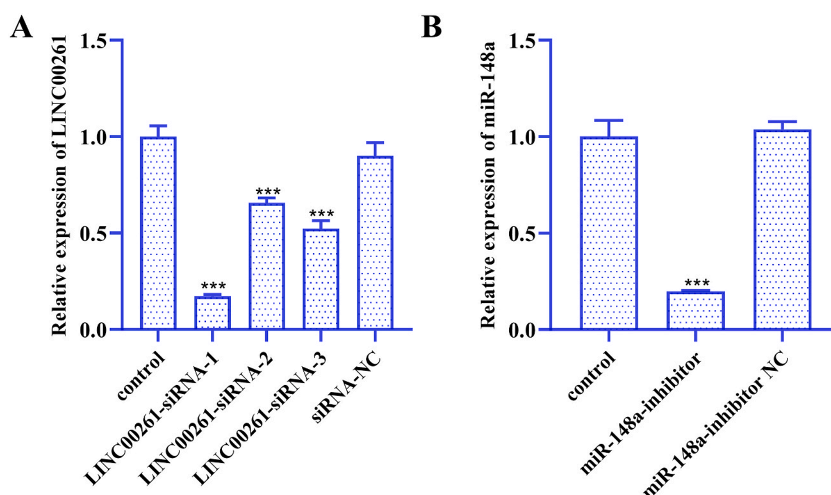

Fig. 1. Expression of LINC00261 and micro RNA-148a in SW480 cells after transfections (A) Expression levels of LINC00261 in different groups after treated with different small interfering RNA. The LINC00261-small interfering RNA-1 shows the most significant inhibiting effect on LINC00261 expression as detected by reverse transcription polymerase chain reaction (F = 160.9; n = 3, mean ± standard deviation). (B) Expression of LINC00261 in SW480 cells treated with micro RNA-148a inhibitor or control. Bars represent the relative expression of genes after transfection. Significant differences were assessed by analysis of variance (F = 243.0; n = 3, mean ± standard deviation). n. s., not significant; *p < 0.05, **p < 0.01, ***p < 0.001. miR, micro RNA; siRNA, small interfering RNA; NC, negative control.

survival of CRC patients. Cox proportional hazards regression analysis was used to generate P value.

3. Results

3.1. Selecting the optimal siRNA targeting LINC00261

The transfection efficiency of LINC00261 siRNA was determined by qRT-PCR. As shown in Fig. 1A, compared with the control group, the expression level of LINC00261 in the siRNA-NC group had no significant change, but the expression level of LINC00261 in the siRNA groups decreased significantly ($P < 0.01$). Among these guides, LINC00261 siRNA-1 showed highest decrease in LINC00261 levels, so it was selected for the following experiments. As shown in Fig. 1B, after treatment with miR-148a inhibitor, the expression of miR-148a was significantly reduced than that of the control group ($P < 0.01$).

3.2. Targeting LINC00261 or miR-148 affects the proliferation of SW480 cells

Compared with the control group, there was no significant change in cell viability in the si-NC group, while the proliferation rate of cells in the siRNA group was significantly decreased, indicating that targeting LINC00261 could affect the cell proliferation (Fig. 2). Compared with the si-NC group, the cell proliferation of the si-NC + miR-inhibitor group was significantly increased, indicating that inhibition of miR-148 could promote cell proliferation. Compared with the siRNA group, the cell proliferation of the siRNA + miR-148 inhibitor group was significantly increased ($P < 0.01$).

3.3. Targeting LINC00261 or miR-148 shows different effects on apoptosis and cell cycle in SW480 cells

Compared with the control group, the apoptosis rate of cells in the si-NC group did not change significantly, but the apoptosis rate of cells in the siRNA group increased significantly ($P < 0.05$). Compared with the siRNA group, the apoptosis rate of cells in the siRNA + miR-inhibitor group was significantly reduced ($P < 0.05$). The results are shown in Fig. 3.

Compared with the control group, the proportion of G1 phase cells in the si-NC group did not change significantly, while it increased significantly in the siRNA group. In addition, the proportion of G2 phase cells in the siRNA group decreased. These indicated that the cells were blocked in G1 phase. Compared with siRNA group, the proportion of cells in G1 phase decreased and G2 phase

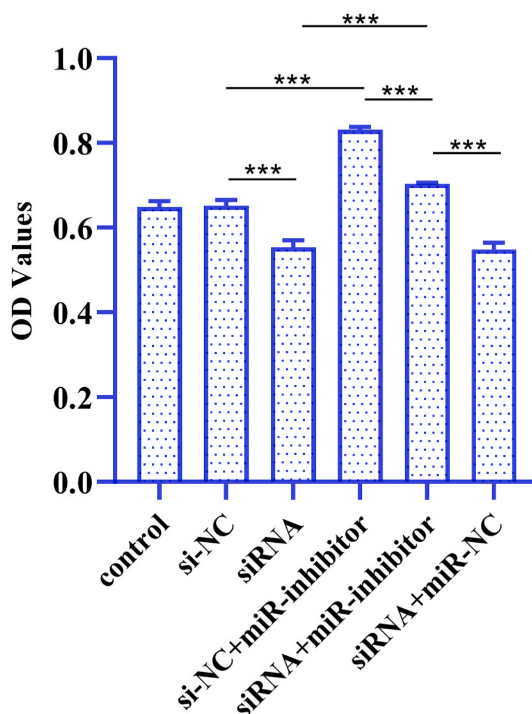

Fig. 2. The effect of small interfering RNA-LINC00261 on SW480 cell viability Cell proliferation levels were determined in negative control or small interfering RNA-transfected SW480 cells by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Bars represent the proliferation rates of cells after transfection. Significant differences were assessed by analysis of variance ($F = 215$; $n = 3$, mean ± standard deviation). n. s., not significant; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. miR, micro RNA; siRNA, small interfering RNA; si-NC, small interfering RNA-negative control; miR-NC, micro RNA-negative control; OD, optical density.

increased in the siRNA + miR inhibitor group. The results are shown in Fig. 4.

3.4. Determining the expression of LINC00261 and miR-148a after treatment by RT-PCR

Compared with the control group, the expressions of LINC00261 (Fig. 5A) and miR-148a (Fig. 5B) were not significantly changed in the si-NC group, while the expression of LINC00261 was significantly decreased and the expression of miR-148a was significantly increased in cells of the siRNA group ($P < 0.01$). Compared with the siRNA group, the expression of LINC00261 in the siRNA + miR-inhibitor group was significantly increased (Fig. 5A), and the expression of miR-148a (Fig. 5B) was significantly decreased ($P < 0.01$). The results are shown in Fig. 5.

3.5. Inhibition of LINC00261 and/or miR-148a on the expression of WNT10b and β-catenin

The Western blots showed that the levels of WNT10b and β-catenin were altered in different treatment groups (Fig. 6A). Compared with the control group, the protein levels of WNT10b and β-catenin in the si-NC group did not change significantly (Fig. 6B and C), whereas the protein levels of WNT10b and β-catenin in the siRNA group were significantly decreased ($P < 0.01$). Compared with the siRNA group, the expression levels of WNT10b and β-catenin were significantly increased in the siRNA + miR-inhibitor group ($P < 0.01$). The results are shown in Fig. 6.

3.6. The prognostic role of LINC00261/miR-148a/WNT10b axis in colon cancer

We then explored the prognostic value of LINC00261 in colon cancer patients using public databases. First, we explored the relationship between lncRNA LINC00261 and the prognosis of colon cancer patients using the TCGA database [13,15], and found an increased trend (although not significant) in the OS of patients with high LINC00261 expression compared with that of colon cancer patients in the low expression group (Fig. 7, $p = 0.09$). We further investigated the correlation between LINC00261/miR-148a/WNT10b axis and the prognosis of colon cancer patients using LncACTdb 3.0 [14]. The result showed that the expression of WNT10b was more likely to be a significant predictor of survival. And the ceRNA showed a clear significance in predicting the overall survival (Table 3), indicating that the LINC00261/miR-148a/WNT10b axis may be a potential prognostic marker in colon cancer.

4. Discussion

CRC, a common malignancy of the digestive system, has experienced an increase in incidence in recent years [16]. Aberrant expression of lncRNAs has been observed in various cancers, highlighting their critical involvement in the regulation of cellular

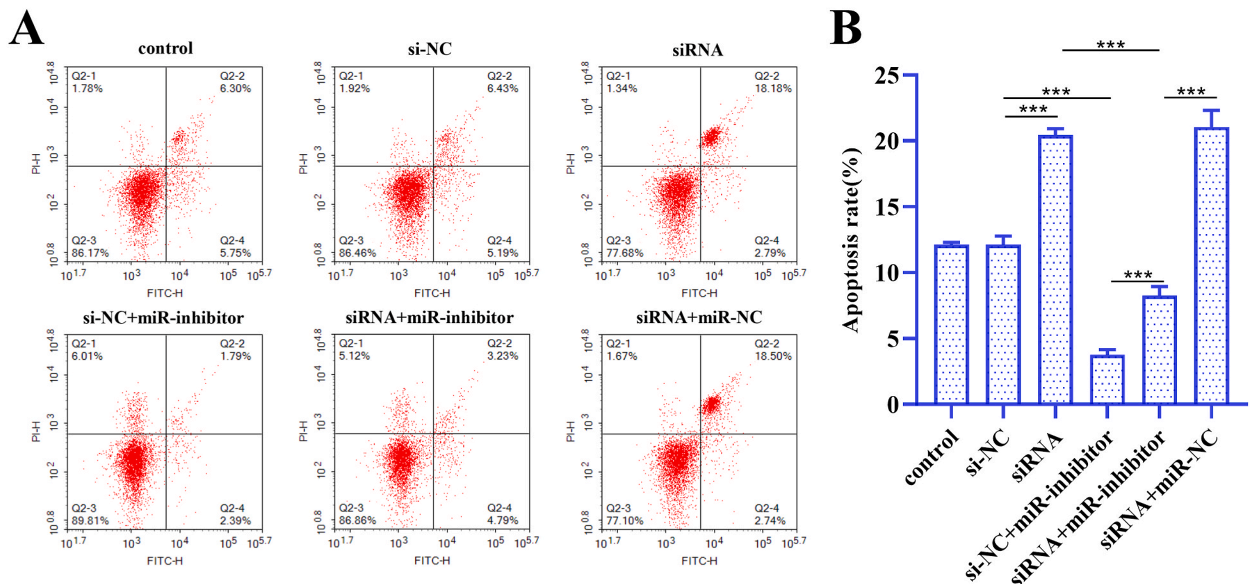

Fig. 3. The effect of small interfering RNA-LINC00261 on apoptosis of SW480 cells (A) Apoptosis analysis by flow cytometry are used to determine the role of LINC00261 small interfering RNA in SW480 cells apoptosis. (B) Bars represent the apoptosis rates of cells after transfection (F = 278.9). All data are expressed as mean ± standard deviation of three independent experiments (n = 3). Significant differences were assessed by analysis of variance. n. s., not significant; *p < 0.05, **p < 0.01, ***p < 0.001. miR, micro RNA; siRNA, small interfering RNA; si-NC, small interfering RNA-negative control; miR-NC, micro RNA-negative control.

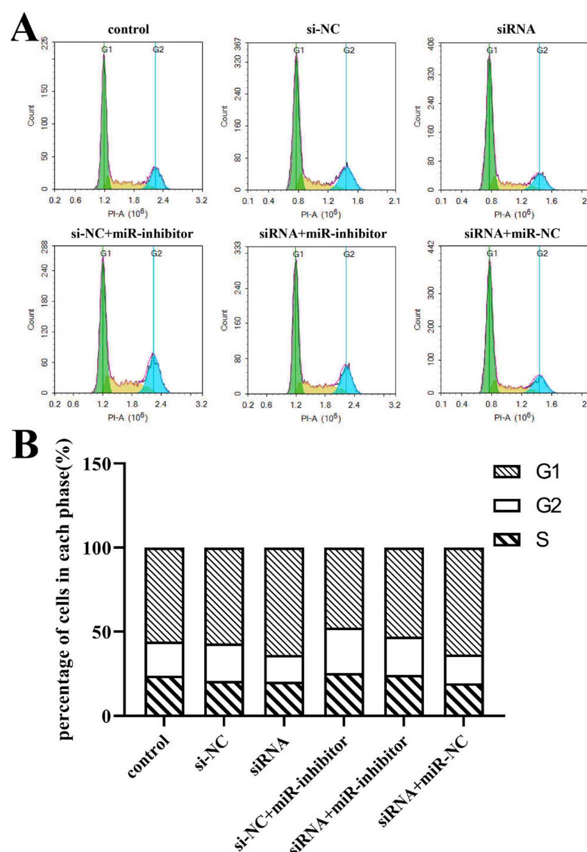

Fig. 4. small interfering RNA-LINC00261 repress the cell proliferation cycle of SW480 cells (A) Cell cycle analysis by flow cytometry was used to assess the role of LINC00261 small interfering RNA in SW480 cell proliferation. (B) The percentage of G1 phase cells in small interfering RNA + micro RNA-negative control group is significantly higher than those of the control and si-NC groups ($p < 0.01$). However, the number of G1 phase cells in small interfering RNA-negative control + micro RNA inhibitor group is significantly lower than all other groups ($p < 0.01$). miR, micro RNA; siRNA, small interfering RNA; si-NC, small interfering RNA-negative control; miR-NC, micro RNA-negative control.

processes [17]. Similarly, miRNAs exert regulatory control over various transcripts, and influence crucial cellular activities such as proliferation, apoptosis, differentiation, and migration. Consequently, miRNAs can function as either potential oncogenes or tumor suppressors during cancer development [18]. In our study, we transfected siRNA-LINC00261 and miR-148a inhibitor into SW480 cells and demonstrated the effect of LINC00261 on WNT pathway activation through the regulation of miR-148a expression. This, in turn, inhibited CRC progression.

In recent years, emerging evidence has highlighted the important role of LINC00261 in various types of cancer. In gastric cancer, LINC00261 acts as a tumor suppressor by facilitating the degradation of Slug and inhibiting epithelial-mesenchymal transition [19]. Conversely, LINC00261 expression is down-regulated in pancreatic cancer tissues and serum, and its silencing promotes the proliferation of pancreatic cancer cells, making it a potential prognostic marker for pancreatic cancer [20]. In addition, recent research has shown that LINC00261 can attenuate cisplatin resistance in colon cancer, resulting in reduced tumor volume and weight and enhancing the efficacy of cisplatin as an anticancer agent [21]. miR-148a, on the other hand, functions as an indirect tumor suppressor by regulating colitis and colitis-related tumorigenesis. It does so by inhibiting the expression of nuclear factor kappa B (NF-κB) and signal transducer and activator of transcription 3 (STAT3) signaling and their associated inflammatory responses [22]. In our study, transfection of siRNA-LINC00261 resulted in a significant decrease in cell viability, an increase in apoptosis rate, and cell cycle arrest in the G1 phase. These effects may be potentially mediated by the miR-148a/WNT10b axis. However, it is important to note that the diverse roles of LINC00261 in different cancer types may be due to variations in the characteristics, aberrant pathways, and developmental patterns specific to each cancer. Therefore, further investigation is required to better understand the underlying mechanisms, as other axes may also be involved in this process.

The wingless (Wnt)/integrated-1 β-catenin signaling pathway plays a critical role in the initiation and progression of cancer cells. It regulates cellular processes such as proliferation, growth, and survival, and is involved in key cellular events such as cell division cycles, immune cycles and circadian rhythms. Dysregulated activation of the Wnt/β-catenin pathway has been observed in various cancers, including breast, lung, and CRC, where it strongly promotes tumor recurrence [23]. Specifically, in colon cancer, Wnt/β-catenin signaling has been implicated in both the early stages of tumor progression and the later stages of invasion and

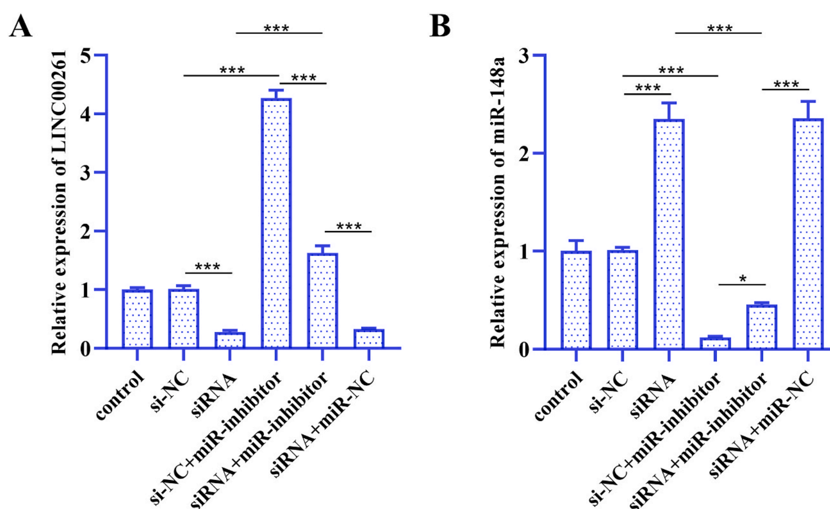

Fig. 5. The effects of small interfering RNA-LINC00261 on expressions of LINC00261 and micro RNA-148a in different groups (A) The relative expression of LINC00261 in SW480 cells (F = 1052). (B) The relative expression of micro RNA-148a in SW480 cells. Bars represent the relative expression of genes after transfection (F = 232.2). Significant differences were assessed by analysis of variance (n = 3, mean ± standard deviation). n. s., not significant; *p < 0.05, **p < 0.01, ***p < 0.001. miR, micro RNA; siRNA, small interfering RNA; si-NC, small interfering RNA-negative control; miR-NC, micro RNA-negative control.

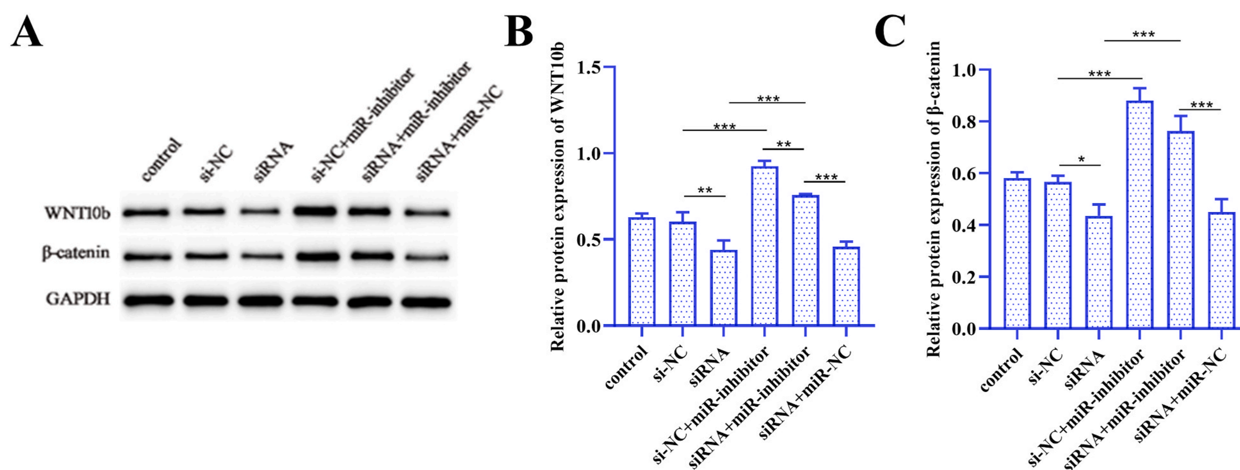

Fig. 6. The effects of small interfering RNA-LINC00261 on expressions of WNT10b and β-catenin
The levels of WNT10b and β-catenin are determined in negative control or transfected SW480 cells by Western blots. (A) Western blots of WNT10b, β-catenin, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The original versions of Fig. 6A are provided as Supplement fig. 1, and they are placed exactly the same order as in Fig. 6A. (B) The relative expression of WNT10b after various small interfering RNA transfection (F = 73.7). (C) The relative expression of β-catenin after different small interfering RNA transfection (F = 49.4). Significant differences were assessed by analysis of variance (n = 3, mean ± standard deviation). n. s., not significant; *p < 0.05, **p < 0.01, ***p < 0.001. miR, micro RNA; siRNA, small interfering RNA; si-NC, small interfering RNA-negative control; miR-NC, micro RNA-negative control.

metastasis [24]. Activation of the Wnt/β-catenin pathway leads to translocation of β-catenin from the cytoplasm to the nucleus, which further promotes cell proliferation and apoptosis [25]. In colon cancer, miR-590-3p has been shown to activate the Wnt/β-catenin signaling pathway, driving cell proliferation and influencing disease progression [26]. Furthermore, studies have shown that LINC00261 overexpression inhibits colon cancer progression by modulating miR-324-3p and Wnt signaling pathways [27]. In our experiment, transfection of siRNA-LINC00261 resulted in a significant decrease in LINC00261 expression, a significant increase in miR-148a expression, and a significant decrease in the protein expression levels of WNT10b and β-catenin. These results suggest that LINC00261 may affect colon cancer progression by modulating the miR-148a/WNT10b axis and interfering with Wnt/β-catenin signaling.

The inconsistent results between the public database data analysis and our results may be attributed to several factors. First, the limited sample size of patients included in the analysis may lead to an underestimation of the true role of LINC00261 in CRC patients.

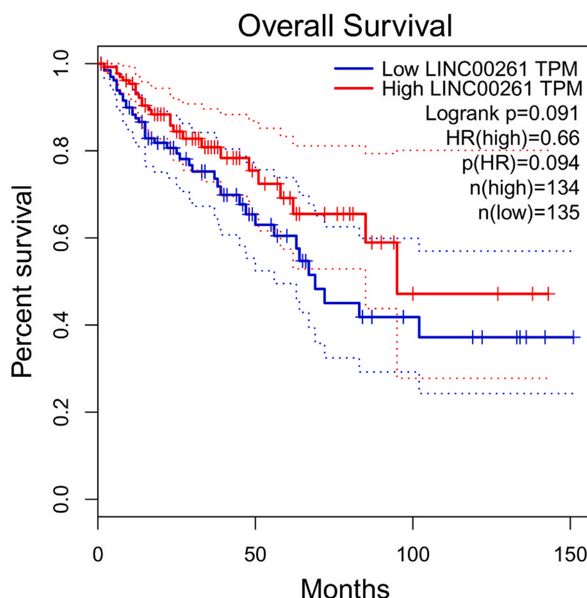

Fig. 7. Kaplan–Meier curves of overall survival (OS) in LINC00261-high and LINC00261-low groups. Using the colon adenocarcinoma data from The Cancer Genome Atlas website, the survival analysis based on expression of LINC00261 shows that the overall survival are not statistically different between patients with high versus low LINC00261 (hazard ratio = 0.66, $P = 0.09$, n = 269). TPM, transcripts per million.

Table 3
The prognostic role of LINC00261/micro RNA-148a/WNT10b axis in colon cancer patients.

Factors	Hazard ratio	95 % confidence interval	Z value	P value
LINC00261	1.02	0.91-1.14	0.27	0.79
WNT10B	1.24	1.08-1.43	3.04	<0.01
miR-148-a	0.96	0.70-1.31	−0.24	0.81
competing endogenous RNA	2.70	1.46-4.99	3.18	<0.01

In addition, variations in cancer stage, disease burden, treatment history and other factors among patients may contribute to the discrepancies. Second, the difference in experimental design between our in vitro study and the in vivo studies in the public database may account for the inconsistencies. In vitro studies provide valuable insights but may not fully reflect the complexity of the in vivo environment. Third, the definition of high versus low expression of LINC00261 may vary between studies and may depend on individual sample levels. This variation in the definition of expression levels may lead to different interpretations and conclusions. Fourth, our study used a single cell line, which provides limited evidence regarding the role of LINC00261. Inclusion of multiple cell lines in future studies would increase the generalizability of the results.

In addition, the role of LINC00261 may depend on other molecules, particularly competing endogenous RNAs (ceRNAs). Survival analysis based on ceRNA interactions showed that low level of LINC00261-miR-148a-WNT10b was associated with significantly longer OS compared to the high expression group. This highlights the complex nature of LINC00261 in cancer and the need for further investigation.

To explore the specific mechanisms of LINC00261 in colorectal cancer, bioinformatics analysis was used. However, it is important to note that the role of LINC00261 in CRC is multifaceted, and further studies are needed to validate its prognostic significance and elucidate the underlying ceRNA mechanisms. Future research should consider the inclusion of other cell lines, such as LINC00261 overexpression or reporter assays. Nevertheless, our results indicate a positive association between LINC00261 and OS in CRC patients.

5. Conclusion

In conclusion, our study shows that LINC00261 may be associated with the prognosis of colorectal cancer patients, suggesting that LINC00261 may serve as a potential biomarker for colon cancer patients (Fig. 8).

Data availability statement

Data associated with this study did not deposite into a publicly available repository. All necessary data are included in article, supplemental material and referenced in article. Further information could be accessed upon reasonable requests by contacting

corresponding authors.

Ethics statement

Ethics approval was not required for this research.

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CRediT authorship contribution statement

Juanli Xi: Writing – original draft, Methodology, Formal analysis, Data curation. **Lei Shi:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization. **DingJie Zhou:** Writing – original draft, Visualization, Software, Methodology, Formal analysis. **Dedong Cao:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Software, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Bo Peng:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations

lncRNAs long non-coding RNAs
OS overall survival
miR micro RNA
FBS fetal bovine serum
MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
DMSO dimethyl sulfoxide
V-FITC/PI V-fluorescein isothiocyanate/propidium iodide
PI/Rnase propidium iodide/ribonuclease
qRT-PCR quantitative reverse transcription polymerase chain reaction
RIPA radioimmunoprecipitation assay
BCA bicinchoninic acid
GAPDH glyceraldehyde 3-phosphate dehydrogenase
GI genInfo identifier
NC negative control
ELISA enzyme-linked immunosorbent assay
PBS phosphate-buffered saline
cDNA complementary DNA
PVDF polyvinylidene fluoride
ANOVA analysis of variance
LSD feast significant difference
HR hazard ratio
CI confidence intervals
NF-κB nuclear factor kappa B
STAT3 signal transducer and activator of transcription 3
Wnt wingless
ceRNAs competing endogenous RNAs
OD optical density
TPM transcripts per million

mRNA messenger RNA

Appendix A. Supplementary data

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