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LncRNA ROR1-AS1 promotes colon cancer cell proliferation by suppressing the expression of DUSP5/CDKN1A

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Abstract. – OBJECTIVE: The purpose of this study was to explore the possible role of ROR1-AS1 in the pathogenesis of colon cancer and the underlying mechanism.

PATIENTS AND METHODS: The expression levels of ROR1-AS1 in 75 colon cancer tissue samples and adjacent ones, as well as in cell lines were examined by quantitative Polymerase Chain Reaction (qPCR). Then, ROR1-AS1 overexpression plasmid and siRNA were transfected into colon cancer cells using liposome method. After that, Cell Counting Kit-8 (CCK-8) and plate colony formation assays were conducted to analyze cell proliferation, while flow cytometry was applied for the analysis of cell cycle and apoptosis. At last, the mechanism of action of ROR1-AS1 was further explored by nuclear separation, RNA binding protein immunoprecipitation (RIP) and chromatin immunoprecipitation (CHIP) assays.

RESULTS: ROR1-AS1 level in colon cancer tissues was remarkably higher than that in normal tissues, and the expression in tumors of stage III and IV was remarkably higher than those of stage I and II. Meanwhile, tumors with diameters more than 5 cm had a higher ROR1-AS1 expression than those less than 5 cm. After transfection with ROR1-AS1 overexpression plasmid, the cell proliferation ability was enhanced, the G0/G1 phase time of cell cycle was shortened, and the apoptosis was suppressed. However, the opposite result was observed after ROR1-AS1 was downregulated. Furthermore, RIP showed that ROR1-AS1 can bind to enhancer of zeste homolog 2 (EZH2) and inhibit the expression of DUSP5, and thus be engaged in the proliferation and apoptosis of colon cancer cells.

CONCLUSIONS: ROR1-AS1 is highly expressed either in colon cancer tissues or in cell lines, which is able to enhance cell proliferation, accelerate cell cycle, and inhibit cell apoptosis. The mechanism of ROR1-AS1 to participate in the development of colon cancer may be the downregulation of DUSP5 via combination with EZH2.

Key Words:
Colon cancer, ROR1-AS1, EZH2, DUSP5.

Introduction

Due to poor diet and lifestyle, the incidence and mortality of colon cancer show a significant increasing trend, and colon cancer has become one of the most common gastrointestinal malignancies[1]. The classic treatment for colon cancer is surgery combined with chemotherapy. In recent years, with the development of the chemotherapy drugs, the survival rate in colon cancer has been significantly improved. The 5-year survival rate of patients with carcinoma *in situ* is more than 90% after treatment, but the 5-year survival rate of patients with lymph node metastasis or distant organ metastasis is less than 50%. Distant metastasis and recurrence are still the main causes of colon cancer death[2,3]. Therefore, exploring the molecular mechanism involved in the growth, invasion, and metastasis of colon cancer, and studying the relevant key molecules will contribute to the comprehensive prevention and treatment of colon cancer, thereby improving the survival rate and quality of life of colon cancer patients.

Long non-coding RNAs (lncRNAs) are a class of RNA molecules whose transcript length exceeds 200 nt, and they have no function of protein coding. LncRNAs are involved in regulatory processes, such as chromatin modification, transcriptional interference, transcriptional activation, intracellular transport, selective shearing, and regulation of proto-oncogene activation, thereby regulating gene expression at multiple levels including epigenetic, transcriptional, or post-transcriptional[4,5]. Abnormal

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LncRNA expression and/or function are involved in the occurrence and development of many diseases, especially malignant tumors[6,7]. Many tumor-related lncRNAs have been found using large-scale and high-throughput technologies, such as lncRNA chips and RNA sequencing[8]. Although some lncRNAs have similar expression changes and perform similar functions in different malignant tumors, some lncRNAs have different expressions and functions in different tumors[9]. Numerous studies have also been conducted on lncRNAs closely related to colon cancer. For example, lncRNA pvt-1 is upregulated in colon cancer tissues and positively correlated with vascular invasion and lymph node metastasis[10]. LncRNA MALAT1 promotes proliferation, invasion, and metastasis in colon cancer cells[11]. LncRNA ncRNA expression was downregulated in colon cancer tissues and correlated with 5-year survival rate of patients[12]. LncRNA PLAT 1 is upregulated in colon cancer tissues and can be used as an indicator of 5-year survival of patients[13]. LncRNA ccat1-l is upregulated in colon cancer and has the function of MYC gene enhancer[14]. Besides, LncRNA PRNCR1 mutation affects the occurrence and development of colon cancer[15].

This study reported for the first time that a novel lncRNA, ROR1-AS1, was remarkably upregulated in colon cancer tissues. In addition, ROR1-AS1 was inhibited to detect its effect on the proliferation, cycle and apoptosis of colon cancer cells, which will be helpful to reveal the new molecular mechanism of the occurrence and development of colon cancer and may also provide a new target for the treatment of colon cancer.

Patients and Methods

Specimen Collection

In this study, a total of 75 matched tissue specimens of colon cancer and adjacent tissues were obtained from surgically treated cases of the Affiliated Hospital of Jiangnan University. All specimen collections were approved by the Clinical Medical Ethics Committee of the Affiliated Hospital of the Jiangnan University. All patients had not received any radiation therapy or chemotherapy before surgery. The normal colon mucosa tissue was taken more than 10 cm away from the colon cancer tissue, and it was confirmed by pathology that there was no cancer cell infiltration in the mucosa tissue. After surgical removal of the specimen, the tissue was selected as quickly as possible, taken out, and rinsed, placed in liquid nitrogen for cryopreservation, and then, preserved in a -80°C freezer. All specimens were confirmed to be colon tumor by postoperative histopathological examination. All patients provided written informed consent. This study was conducted in accordance with the Declaration of Helsinki.

Cell Culture

Colorectal cancer cell lines (HCT116, DLD1, SW480, LOVO) and human normal colon cells (HcoEpic) were purchased from the Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences. All cell lines were cultured with Roswell Park Memorial Institute-1640 (RPMI-1640) medium containing 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA), penicillin (50 ug/mL) and streptomycin (100 μg/mL) in a 37°C, 5% CO_2 incubator.

RNA Extraction and Quantitative Polymerase Chain Reaction (qPCR)

RNA was extracted by TRIzol (Invitrogen, Carlsbad, CA, USA), chloroform, and isopropanol, and the extracted RNA was measured for concentration and stored at -80°C until use. Then, the cDNA was obtained by reverse transcription, and 1 uL cDNA sample was taken for qPCR detection. Finally, SYBR Green method was used for PCR detection. The primer sequences are as follows: ROR1-AS1-F 5'-CTGACGAAACACTGGAACTC-3' ROR1-AS1-R 5'-GTCTGATTTGGTAGCTTGGATG-3'; DUSP5-F 5'-TCCCTGACTTCTAGCCCTGT-3' DUSP5-R 5'-TTTAGCAGGATGTGGCCGTT-3'; CDKN1A-F 5'-AAGTCAGTTCCTTGTGGAGCC-3' CDKN1A-R 5'-GGTTCTGACGGACATCCCCA-3'; CDK2-F 5'-GACACGCTGCTGGATGTCA -3' CDK2-R 5'-GGAATGCCAGTGAGAGCAGA-3'; CDK6-F 5'-AGAGTGCTGGTAACTCCTTCC-3' CDK6-R 5'-GCGCGTCTCAGTCCAGAATC-3' U6-F 5'-CTCGCTTCGGCAGCACA-3'; U6-R 5'-AACGCTTCACGAATTTGCGT-3'. GAPDH-F 5'-AGGTCGGTGTGAACGGATTTG-3'; GAPDH-R 5'-TGTAGACCATGTAGTTGAGGTCA-3'.

Cell Transfection

Transient transfection was performed using liposome Lipofectamine 2000 according to the manufacturer's instructions. Cells were plated one day before transfection. After 24 hours, the plasmid and liposome were separately added to 2 tubes of culture medium containing no antibiotics and serum and allowed to stay at room temperature for 5 minutes. Then, the two tubes containing plasmid

or liposome were mixed and allowed to stay at room temperature for 20 minutes. Next, the mixture was evenly dropped into the cell culture supernatant and gently shaken for several times, and the cells were placed in the incubator to continue the culture. After 6 hours, the culture supernatant was replaced with a fresh complete medium. The transfection sequences are as follows: si-ROR1-AS1 1#: 5'-CUG AAG AGC UGG UGA GAA U-3'; si-ROR1-AS1 2#: 5'-GAG GAA GAC CAA AGC UUA A-3'; si-ROR1-AS1 3#: 5'-GAG GAA AAG AUU UGG AUC A-3'. The overexpression plasmid was synthesized by Shanghai Jima, China.

Cell Counting Kit (CCK-8) Assay

After 24 h of transfection, the cells were collected and plated into 96-well plates at 1´10^4 cells per well. The cells were cultured for 6 h, 24 h, 48 h and 72 h, respectively, and then, added with 5 mg/ml of CCK-8 reagent. After incubation for 4 h, the OD value of each well was measured in the microplate reader at 450 nm absorption wavelength.

Cell Cycle Analysis

The cells in the logarithmic growth phase were digested with 0.25% trypsin, centrifuged, washed twice with phosphate-buffered saline (PBS), and fixed with 70% ethanol overnight. The supernatant ethanol solution was discarded after centrifugation. Then, the cells were washed twice with PBS, and each tube was added with a concentration of 50 ug/ml of propidium iodide (PI) solution at room temperature for 30 minutes to stand for staining. Finally, cell cycle detection was performed using a flow cytometer.

Apoptosis Detection

The cells in the logarithmic growth phase were digested with 0.25% trypsin, centrifuged at 2000 rpm for 5 min, washed twice with PBS, and centrifuged at 2000 rpm for 5 minutes. Later, KeyGen's Annexin V-enhanced green fluorescent protein (EGFP)/PI Apoptosis Detection Kit was used for detection. The brief steps were as follows: cells were resuspended with 1´ binding buffer and the cell density was adjusted to 1´ 10^6/ml, then, 5 μl of Annexin V-EGFP and 5 μl of PI were added into cells for incubation for 30 minutes in the dark. At last, flow cytometry detection was performed to analyze cell apoptosis.

Colony Formation Assay

The cells in the logarithmic growth phase were digested and plated in a 6-well plate with 50 cells per well. After incubation for 3 weeks at 37°C in a 5% CO_2 incubator, the culture was terminated when macroscopic cell clones appeared in the culture plate. Next, the cells were rinsed 3 times with PBS, dried in air, and stained with 0.1% crystal violet stain for 20 minutes. Ultimately, the number of cell clones visible to the naked eye was counted.

Nuclear Separation

Cells were digested, collected into the Eppendorf tube (EP; Hamburg, Germany) and then centrifuged. Thereafter, the pellets were resuspended with the cell fractionation buffer and kept on ice. Then, the supernatant was collected as the cytoplasm lysis. Next, the nuclear component of the cell was located in the sediment, which was resuspended with the cell disruption buffer and kept on ice. After centrifugation, the supernatant was collected as the nuclear lysis. Subsequently, the cytoplasmic lysis and the nuclear lysis were sequentially added with lysis binding solution and anhydrous ethanol, and then, centrifuged using an adsorption column, respectively. After washing with washing solution 1, 2, 3, the relative fraction was finally eluted using the elution solution, and the RNA concentration was measured and then reverse transcribed.

RNA Binding Protein Immunoprecipitation (RIP) Test

After the cell lysate was obtained, the magnetic beads were prepared and finally resuspended in Wash Buffer and placed on ice, followed by RIP. RNA purification was carried out by phenol, chloroform, Salt Solution I, Salt Solution II, Precipitate Enhancer, absolute ethanol (no RNAse), dissolved in 20 ul of DEPC water, and stored at -80°C. Finally, qPCR was used to detect the expression of each gene in EZH2, SUZ12 protein, and IgG precipitate.

Chromatin Immunoprecipitation (CHIP)

The cells were taken for formaldehyde cross-linking and sonication. After the ultrasonication was completed, the insoluble matter was removed by centrifugation. 100 μl of the cell lysis was added with antibody as the experimental group, and 100 μl of the cells was used as the control group. Meanwhile, 100 μl of the lysis was added with 5 M NaCl, treated at 65°C for 3 h, and then, electrophoresis was performed to detect the effect of ultrasonic disruption. ChIP Dilution Buffer, PIC

and Protein A Agarose/Salmon Sperm DNA were added in the sonicated product. Next, the supernatant was centrifuged and 20 µl was used as input. 1 µl of antibody was added to each tube and igG was added to the other. After overnight incubation at 4°C, 60 µl of Protein A Agarose/Salmon Sperm DNA was added to each tube. The precipitated complex was washed, and the elution started. After the completion of the cross-linking overnight at 65°C, the DNA fragment was recovered and finally analyzed by qPCR.

Statistical Analysis

All experiments were repeated 3 times and analyzed by the Statistical Product and Service Solution 19.0 statistical software (SPSS IBM Corp., Armonk, NY USA). The measurement data were expressed as mean ± standard deviation, and *t*-test was used to compare data between groups. The difference was statistically significant at $p<0.05$.

Results

Increased Expression of ROR1-AS1 in Colon Cancer Tissues and Cells

ROR1-AS1 level in 75 colon cancer tissue samples and adjacent ones was examined using qPCR, and the results revealed that ROR1-AS1 level in colon cancer tissues was remarkably higher than that in paracancer tissues, and the difference was statistically significant ($p<0.05$) (Figure 1A). Subsequently, ROR1-AS1 level in colon cancer cell lines (HCT116, SW480, DLD1, LOVO) and normal colon cells (HcoEpic) was detected. As a result, it was found that the expression of ROR1-AS1 was higher in colon cancer cells than in normal colon cells (Figure 1B). In addition, the colon cancer patients of stage III and IV had an increased ROR1-AS1 expression when compared to those of stage I and II (Figure 1C). Meanwhile, patients with tumor diameter more than 5 cm had a higher ROR1-AS1 expression than that those less than 5 cm (Figure 1D). These observations suggest that ROR1-AS1 may be engaged in the progression of colon cancer.

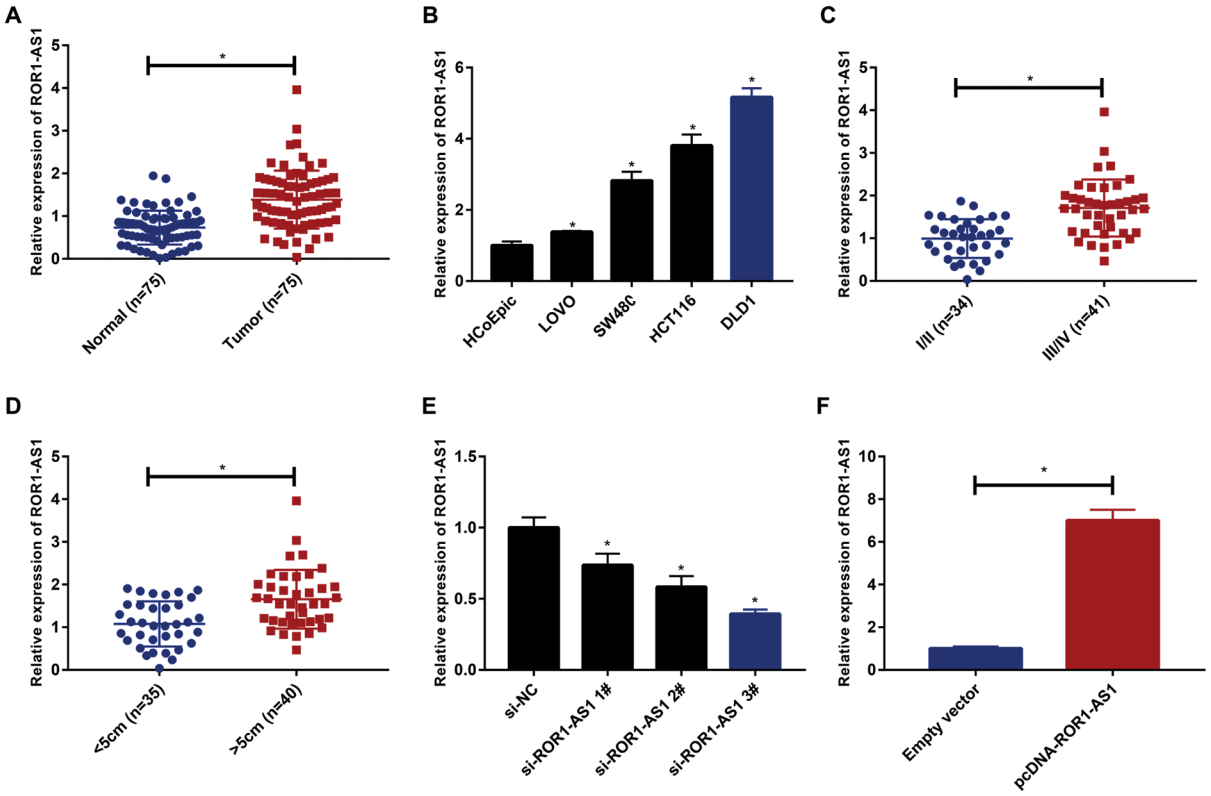

Figure 1. Increased expression of ROR1-AS1 in colon cancer tissues and cells. **A,** The expression of ROR1-AS1 in colorectal cancer tissues and corresponding non-tumor tissues is examined by qPCR. **B,** The expression of ROR1-AS1 in colorectal cancer cell lines (DLD1, HCT116, SW480, a d LOVO) and normal human colorectal epithelial cell lines (HCoEpiC) is assessed by qPCR. **C,** The expression of ROR1-AS1 in I/II and III/IV colorectal cancer tissues is examined by qPCR. **D,** The expression of ROR1-AS1 in colorectal cancer tissues with tumor diameter <5 cm and >5 cm is examined by qPCR. **E,** The knockout efficiency of si-ROR1-AS1 1# 2# 3# is examined by qPCR. **F,** The transfection efficiency of pcdna-ROR1-AS1 is examined by qPCR.

ROR1-AS1 Promotes Cell Proliferation and Inhibits Apoptosis

DLD1 cell line was selected for subsequent cell experiments for its high expression of ROR1-AS1. To explore the effect of ROR1-AS1 on colon cancer, ROR1-AS1 siRNA, and ROR1-AS1 overexpressing plasmid were transfected to achieve ROR1-AS1 knockdown and overexpression (Figure 1E, 1F). Then, cell proliferation at 24, 48, and 72 h was detected using CCK-8 method, and the results revealed that after ROR1-AS1 knockdown, the cell activity of colon cancer cells was remarkably attenuated (Figure 2A), and the result of cloning formation assay was consistent with that of CCK-8 assay (Figure 2B). However, after overexpression of ROR1-AS1, cell proliferation ability was enhanced (Figure 2C, 2D). After transfection, the expression levels of cell proliferation-related genes, including DUSP5 and CDKN1A, were detected. In the si-ROR1-AS1 group, DUSP5 and CDKN1A levels were remarkably increased, while the opposite result was observed in the overexpression group (Figure 2E, 2F). Meanwhile, the result of flow cytometry indicated that the ratio of S phase of cell cycle in the si-ROR1-AS1 group was reduced while that of G0/G1 was increased, and the opposite observation was found in the overexpression group (Figure 2G, 2H). Besides, the expressions of cycle-related genes, including CDK2 and CDK6 were also decreased, which was consistent with above results (Figure 2I, 2J). Moreover, cell apoptosis detection result showed that downregulation of ROR1-AS1 led to an increased apoptosis, while the overexpression of ROR1-AS1 inhibited the cell apoptosis (Figure 2K, 2L). These results suggest that low expression of ROR1-AS1 may inhibit proliferation and promote apoptosis in colon cancer cells.

ROR1-AS1 Regulates DUSP5 and CDKN1A Expression by Combining with EZH2

In order to further analyze the mechanism by which ROR1-AS1 acted in colon cancer, subcellular localization of cytoplasmic nucleus separation was conducted, and it was found that ROR1-AS1 was mainly located in the nucleus (Figure 3A), indicating that it was mainly involved in gene regulation at the transcription level. LncRNAs are usually involved in tumorigenesis by binding to specific RNA binding proteins. Therefore, RIP assay was performed to detect the interaction of ROR1-AS1 with potential RNA-binding proteins that regulate transcriptional level targets in colon cancer cells. The results revealed that ROR1-AS1 directly bound to EZH2 and SUZ12, but the binding to EZH2 was more evident (Figure 3B). Then, potential targets of EZH2 (DUSP5 and CDKN1A) were selected for further study, and when EZH2 was knocked out, DUSP5 and CDKN1A expressions were remarkably increased in colon cancer cells (Figure 3C). Further, CHIP analysis was performed. The results revealed that EZH2 could directly bind to DUSP5 and CDKN1A and mediate the methylation modification of H3K27me3 (Figure 3F). However, the low expression of ROR1-AS1 could reduce the binding ability of DUSP5 and CDKN1A to EZH2 and inhibit the methylation of H3K27me3 (Figure 3D, 3E). These results suggest that ROR1-AS1 regulated DUSP5 and CDKN1A expressions by combining to EZH2.

DUSP5 Inhibits Cell Proliferation and Promotes Apoptosis

To determine whether DUSP5 was involved in the regulation of cell function, the effects of DUSP5 on colon cancer cells were examined. After DUSP5 was overexpressed with pcdna-DUSP5 (Figure 4A), cell proliferation was detected, and the high expression of DUSP5 remarkably inhibited cell proliferation (Figure 4B). Colony formation revealed that high expression of DUSP5 overexpression inhibited colony formation (Figure 4C). Meanwhile, flow cytometry analysis revealed that the apoptosis rate of DUSP5 was remarkably increased compared with the control cells (Figure 4D). Subsequently, in order to determine whether ROR1-AS1 regulated cell proliferation by inhibiting the expression of DUSP5, a reversal experiment was conducted to co-transfect colon cancer cells with pcDNA-ROR1-AS1 and pcDNA-DUSP5. The results of the proliferation test revealed that co-transfection could partially reverse the enhanced proliferation caused by pcdna-ROR1-AS1 transfection (Figure 4E). These results suggest that ROR1-AS1 can regulate the proliferation of colon cancer cells by downregulating DUSP5 expression.

Discussion

One of the important reasons for the high mortality and poor prognosis of colon cancer is that the molecular mechanism of the occurrence and development of colon cancer is still unclear[16]. With the rapid development of modern cell and molecular biology, people begin to study the gen-

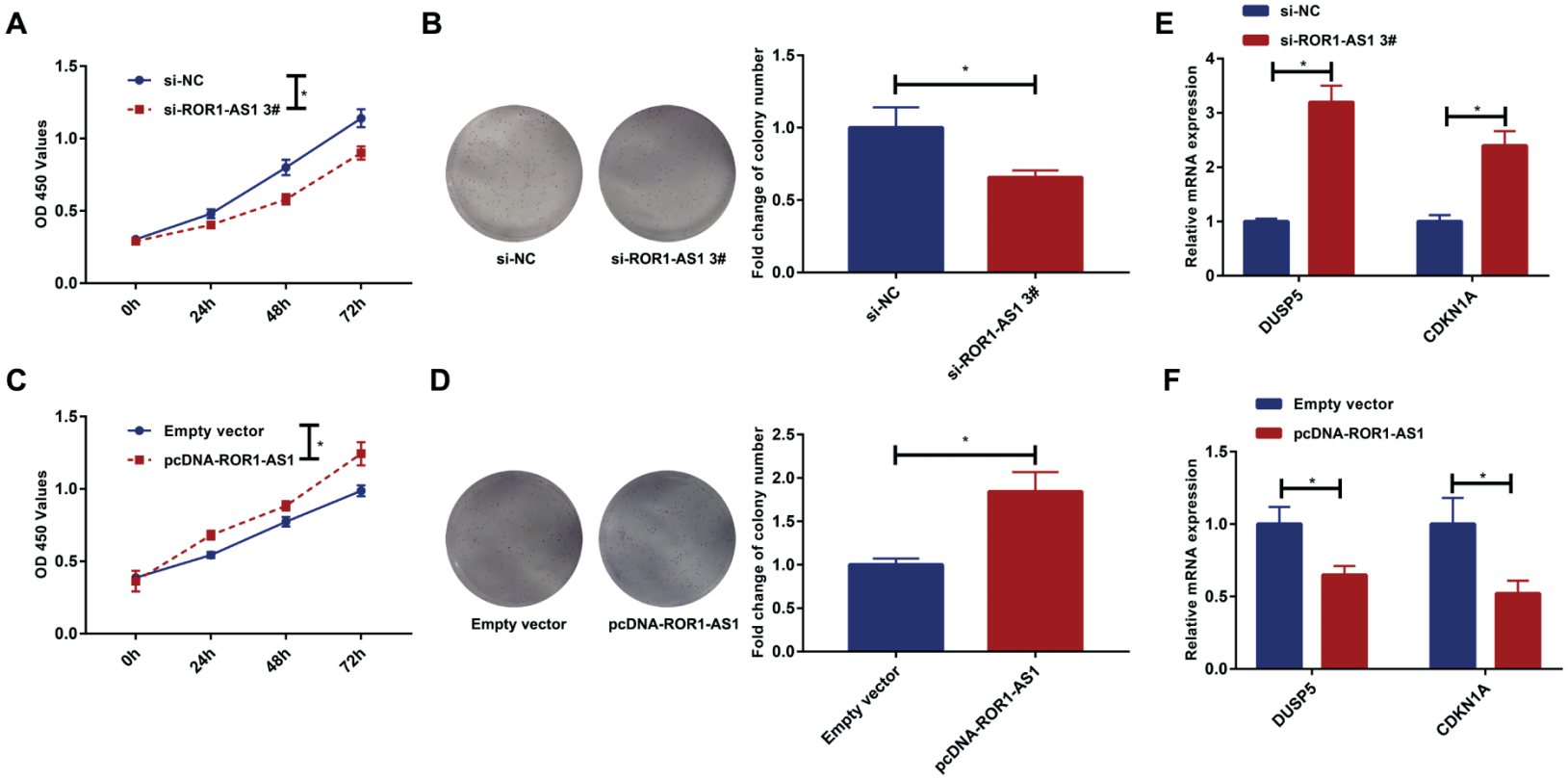

Figure 2. ROR1-AS1 promotes cell proliferation and inhibits cell apoptosis. **A-B,** After knockdown of ROR1-AS1 expression, cell proliferation is assessed by CCK-8 (**A**) and colony formation experiment (x10) (**B**). **C-D,** After overexpression of ROR1-AS1, cell proliferation is assessed by CCK-8 (**C**) and colony formation experiment (x10) (**D**). **E-F,** After knockdown (**E**) or overexpression (**F**) of ROR1-AS1 expression, the expression levels of proliferation-related genes DUSP5 and CDKN1A are examined by qPCR.

(Figure continued)

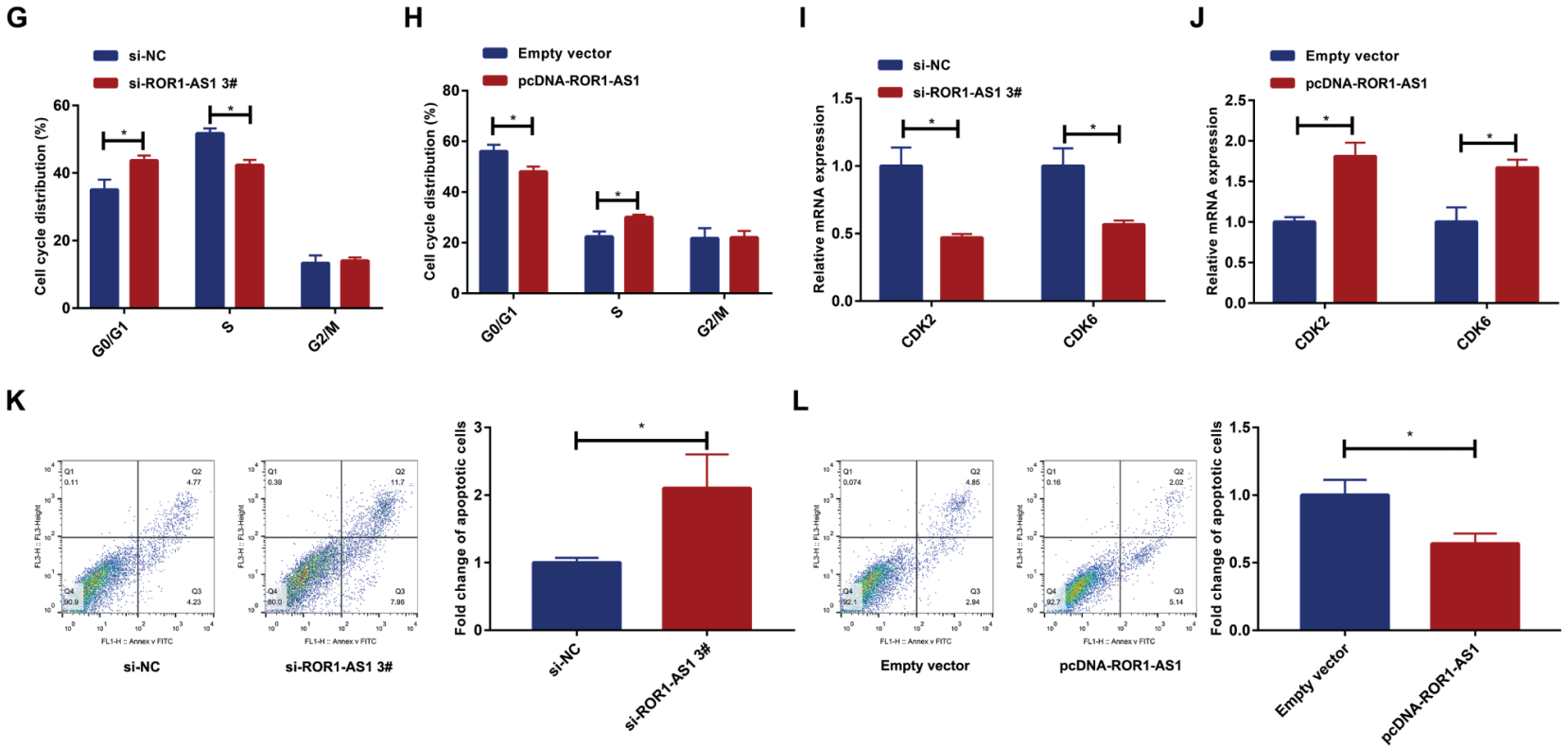

Figure 2. *(Continued).* **G-H,** Cell cycle distribution is detected by flow cytometry after knockdown (**G**) or overexpression (**H**) of ROR1-AS1 expression. **I-J,** After knockdown (**I**) or overexpression (**J**) of ROR1-AS1 expression, the expression levels of cycle-related genes CDK2 and CDK6 are examined by qPCR. **K-L,** Apoptosis is detected by flow cytometry after knockdown (**K**) or overexpression (**L**) of ROR1-AS1 expression.

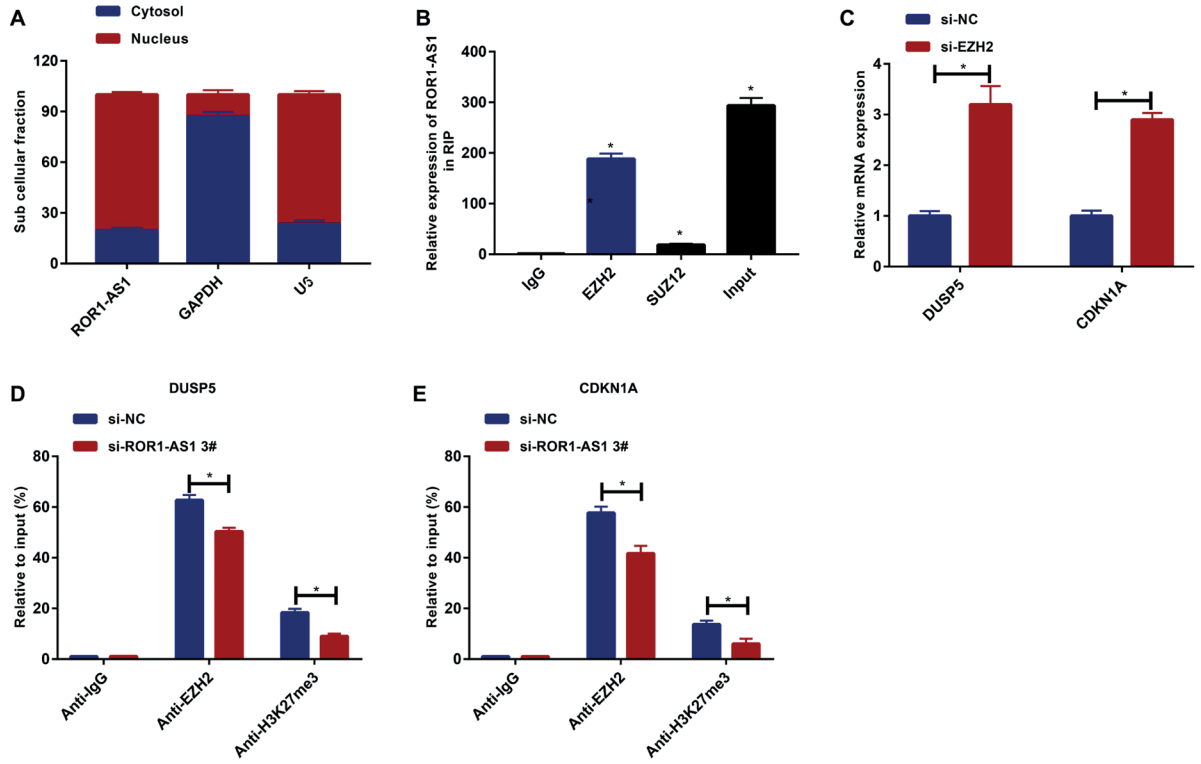

Figure 3. ROR1-AS1 regulates DUSP5 and CDKN1A expression by combining EZH2. **A,** Expression levels of ROR1-AS1 in the nucleus and cytoplasm are analyzed by qPCR. U6 is used as nuclear control and GAPDH as cytoplasmic control. **B,** RIP experiment is conducted, and the expression of ROR1-AS1 is detected by qPCR for the co-precipitated RNA. **C,** DUSP5 and CDKN1A expressions are examined by qPCR after EZH2 expression is silenced. **D-E,** The binding of EZH2 and H3K27me3 in DUSP5 (**D**) and CDKN1A (**E**) promoters in transfected cells is detected by chip-qPCR.

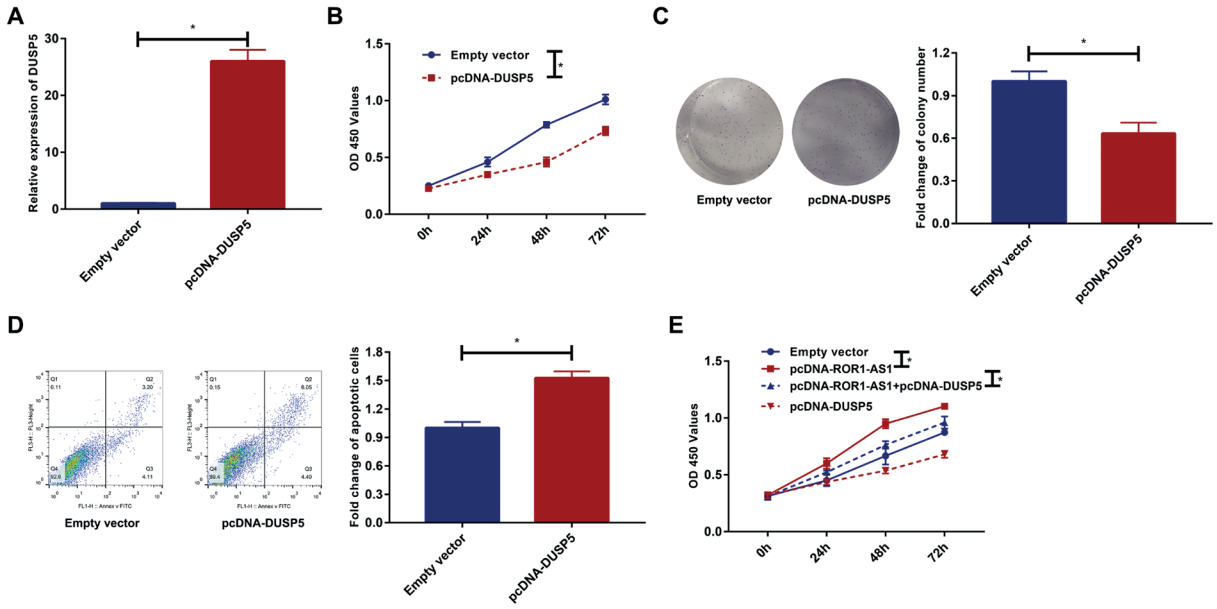

Figure 4. DUSP5 inhibits cell proliferation and promotes apoptosis. **A,** The transfection efficiency of pcdna-dusp5 is examined by qPCR. **B-C,** After overexpression of DUSP5, cell proliferation is assessed by CCK-8 (**B**) and colony formation assay (x10) (**C**). **D,** Apoptosis is assessed by flow cytometry after the overexpression of DUSP5. **E,** Cell proliferation is determined by CCK-8 after ROR1-AS1 and DUSP5 are overexpressed.

esis and development mechanism of tumor in many aspects from the molecular level. Therefore, in-depth exploration of the molecular mechanism of the occurrence and development of colon cancer, and the characterization of molecules as anti-tumor targets, can open up a new way for the clinical prevention and treatment of colon cancer.

Abbas et al[17] have found that only about 1% of the genes in the human genome can be transcribed into RNA with biological functions, while most of them are non-coding RNAs without the function of coding proteins. Among them, long non-coding RNA (lncRNA) transcripts are more than 200 nucleotides in length, which are widely transcribed in eukaryotic cells and have important biological functions[18,19]. LncRNAs are competing endogenous RNAs (ceRNAs) containing miRNA response elements (MERs), which can inhibit the regulation of miRNAs on downstream genes by competitively binding miRNA sites. Overexpression of ROR1-AS1 promotes the growth of b-cell lymphoma cells and decreases the sensitivity to treatment with ibrutinib and dexamethasone[20]. In this study, ROR1-AS1 level in colon cancer tissues was remarkably higher than that in adjacent tissues. ROR1-AS1 level in stage III and IV colon cancer patients was remarkably higher than that in stage I and II colon cancer patients. Meanwhile, ROR1-AS1 level in colon cancer patients with tumor diameter of > 5 cm was remarkably higher than that in patients with tumor diameter of > 5 cm. Therefore, ROR1-AS1 was closely related with colon cancer.

Enhancer of zeste homolog 2 (EZH2) belongs to the group of multi-comb complexes, which is the catalytic subunit of the multi-comb inhibiting complexes and induces trimethylation of lysine at histone H3 position 27, thereby promoting epigenetic gene silencing[21,22]. Currently, research[23] on EZH2, cell cycle progression, pluripotency of stem cells, and cancer biology has been at the forefront of scientific research. EZH2 abnormal expression will cause abnormal cell proliferation and tumor occurrence. Of note, EZH2 is often highly expressed in the tumor cells compared to normal cells[24]. EZH2 is currently recognized as new biomarker of cancer treatment goals. In this study, EZH2 can bind to ROR1-AS1 and regulate the expression of DUSP5. DUSPs (dual specificity phosphatases) are members of the tyrosine phosphatase family proteins, which are responsible for inactivating dephosphokinase phosphoserine/threonine and phosphorylated tyrosine residues[25]. DUSPs can inhibit downstream kinases that are associated with tumor proliferation, differentiation, metastasis and apoptosis, including MAPK/ERK, SAPK/JNK, and p38[26]. Nevertheless, the clinical significance of DUSP5 (dual specificity phosphatase 5) remains largely unknown. As a negative regulator of ERK, DUSP5 is usually upregulated in tumor samples with activated RAS/MAPK signaling. It is reported that overexpression of DUSP5 and inactivation of nuclear ERK1/2 signaling contribute to alleviate cardiac hypertrophy in diabetic OVE26 mice administrated with HDAC3 inhibitor[27]. Cardiac fibrosis is related to DUSP5 downregulation and MeCP2 overexpression. The silence of MeCP2 blocks proliferation of mouse cardiac fibroblasts and upregulates DUSP5 in the activated cardiac fibroblasts. It is suggested that MeCP2 is a vital regulator in pathological cardiac fibrosis, which accelerates proliferation and fibrosis in fibroblasts by downregulating DUSP5[28]. In this paper, our findings showed that abnormally expressed DUSP5 was associated with the development of colon cancer.

However, there are still some shortcomings in this report. For example, only one cell line was used in all functional researches in this study, and no *in vivo* experiments were carried out. Therefore, further investigations can be carried out in subsequent experiments.

Conclusions

This study indicated the high expression of ROR1-AS1 in colon cancer for the first time and found that the increased expression of ROR1-AS1 can promote the proliferation, cycle, and inhibit apoptosis of colon cancer cells by downregulating DUSP5 in combination with EZH2.

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Conflict of Interests

The Authors declare that they have no conflict of interests.

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