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LEF-AS1 participates in occurrence of colorectal cancer through adsorbing miR-505 and promoting KIF3B expression

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Abstract. – OBJECTIVE: To explore the possible role and mechanism of long non-coding ribonucleic acid LEF-AS1 (lncRNA LEF-AS1) in the pathogenesis of colorectal cancer (CRC).

PATIENTS AND METHODS: The expression levels of LEF-AS1 in 54 CRC tissue samples and adjacent normal ones were examined by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). In the meantime, CRC cell lines were screened for subsequent *in vitro* experiments. LEF-AS1 siRNA was transfected into CRC cells using the liposome method. Then, cell counting kit-8 (CCK-8) and 5-Ethynyl-2'-deoxyuridine (EdU) assays were conducted to detect cell proliferation. Thereafter, transwell assay was performed to evaluate the cell migration capacity, as well as invasiveness, Caspase-3 activity was examined to estimate cell apoptosis, and flow cytometry was applied for cell cycle detection. Subsequently, bioinformatics analysis was carried out to predict the target genes of micro RNA (miR)-505 that were predicted to be able to bind to LEF-AS1, while the relative activity of luciferase between miR-505 and KIF3B or LEF-AS1 was examined *via* luciferase gene reporter assay. In addition, the interaction between KIF3B and miR-505, as well as LEF-AS1, was further verified by RNA knockdown assay and cell reversal experiments.

RESULTS: The expression of LEF-AS1 in CRC tissue specimens was found to be markedly higher than that in normal colon tissues. After transfection with LEF-AS1 siRNA, the cell viability, as well as cell migration and invasion capacities, were both attenuated. However, the cell apoptosis rate was conversely elevated. Dual-Luciferase reporter assay revealed that LEF-AS1 could combine with miR-505, which was capable of targeted binding to KIF3B. In addition, LEF-AS1 siRNA transfection attenuated cell proliferation, migration, and invasion capacities, which could be partially reversed by the overexpression of KIF3B.

CONCLUSIONS: In this research, LEF-AS1 is highly expressed in CRC tissues and cell lines. However, the down-regulation of LEF-AS1 reduces the proliferation rate and suppresses the invasiveness and metastasis of CRC cells through the LEF-AS1/miR-505/KIF3B axis.

Key Words:
Colorectal cancer, LEF-AS1, MiR-505, KIF3B.

Introduction

Colorectal cancer (CRC), one of the common malignant tumors, refers to the malignant lesions of colonic mucosal epithelium triggered by various carcinogenic factors such as environment or genetics[1]. According to the statistics, the annual incidence rate of CRC in the United States accounts for 8% of all new cancer patients. In 2016, there were 134,490 new patients and 49,190 deaths, ranking third among all cancers[2]. In China, the mortality rate of CRC also ranks first, and the disease endangers human health[3]. Since CRC has no evident symptoms in the early stage, it cannot be detected until it develops to the advanced stage, which is one of the reasons for its high mortality rate[4]. Therefore, its early diagnosis and treatment is a problem to be solved. In addition, the occurrence of CRC is a complex process induced by many factors, which is the result of the synergistic effect of many proto-carcinoma and tumor suppressor genes. Tumors may occur once the balance between these oncogenes is broken[5].

In recent years, several studies have recorded 70-90% transcripts of the human genome, but less than 2% of the genes in the total genome encode proteins, indicating that most human ribonucleic acid (RNA) transcripts are non-coding

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RNAs (ncRNAs). Huarte et al[6] have reported that lncRNAs play a significant regulatory role in many diseases, especially in tumors. Evidence[7,8] has shown that lncRNAs can serve as ceRNAs. For example, scholars have found that in bladder cancer cells, down-regulated lncRNA MEG3 enhances cell proliferation *via* activating autophagy[9], and in thyroid cancer cells, lncRNA BANCR can affect cell activity by regulating autophagy[10]. Besides, lncRNA MALAT1 is involved in the proliferation and metastasis of pancreatic cancer[11].

LncRNA LEF-AS1 has been reported to be engaged in the occurrence of non-small cell lung cancer through the miR-489/SOX4 axis, and to participate in the regulation of the proliferation and migration of vascular smooth muscle cells through miR-544a/PTEN. In this research, the expression of LEF-AS1 in CRC and its impact on the function of CRC cells were investigated to provide new insights and targets for the pathogenesis and treatment of CRC.

Patients and Methods

Sample Collection

A total of 54 pairs of CRC tissue specimens and corresponding adjacent ones were collected from patients with CRC in our hospital. None of the above subjects received radiotherapy before surgery. The collection of specimens received informed consent from all subjects and was approved by the Ethics Committee of the hospital. All specimens were confirmed by postoperative pathology. This investigation was conducted in accordance with the Declaration of Helsinki.

RNA Extraction and Quantitative Real Time Polymerase Chain Reaction (qRT-PCR)

After ground in liquid nitrogen, TRIzol (Invitrogen, Carlsbad, CA, USA) was added to the tissue samples, which were then homogenized using a homogenizer. After chloroform was added, the samples were centrifuged at 10,000 g for 15 min, the aqueous phase was transferred to a new tube, and the RNA was precipitated in the aqueous phase with isopropanol. Then, the RNA pellet was washed with 75 % ethanol, and the supernatant was discarded. After dried at room temperature, diethyl pyrocarbonate (DEPC) water (Beyotime, Shanghai, China) was added, and the extracted RNA was stored at –20°C. Subsequently, RNAs were reversely transcribed into cDNAs by PrimeScript RT Master Mix, RNA, RNase Free dH20 according to the instructions, followed by qRT-PCR using SYBR® Green Master Mix (TaKaRa, Otsu, Shiga, Japan). The primer sequences are as follows: glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (F: 5'-CACCCACTCCTCCACCTTTG-3', R: 5'-GCTCATTCAACGGATAAGTC-3'), LEF-AS1 (F: 5'-CCGTTTGCCCCAAAAGAAGG-3', R: 5'-CGACGCAAGTGGGTAGCTTT-3') and KIF3B (F: 5'-TGGATGTGGATGTTAAGCTGGG-3', R: 5'-TCGGAACGTCTCATCGTACAG-3'.

Cell Culture and Transfection

Human CRC cell lines (HCT116, HT29, SW480, and LoVo) and a normal colon cell line (NCM460) were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA), and cultured at 37°C in a 5% CO_2 incubator with Roswell Park Memorial Institute-1640 (RPMI-1640) medium (Gibco, Rockville, MD, USA) containing 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA), 100 U/mL penicillin and 100 μg/mL streptomycin. Cells in good growth state were seeded into cell culture plates and underwent transfection when the cell density reached 50-60%. The liposome Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) and the transfection reagent were mixed, the static mixture was added to the cells, which were incubated at 37°C for 6 h, and the complete medium was added for further culture. The transfection reagent is as follows: LEF-AS1 siRNA: 5'-GGA GAU UAA UGC AGA ACA A-3'. MiR-505 mimics, inhibitor, and KIF3B overexpression plasmids were synthesized by Shanghai Jikai (Shanghai, China).

Cell Counting Kit-8 (CCK-8) Assay

At 24 h after transfection, the cells were collected and plated into a 96-well plate at 2,000 cells per well. The cells were cultured for 24 h, 48 h, 72 h, and 96 h, respectively, and then added with CCK-8 solution (Dojindo Laboratories, Kumamoto, Japan). After incubation for 2 h, the optical density (OD) value of each well was measured in the microplate reader at 450 nm absorption wavelength.

5-Ethynyl-2'- Deoxyuridine (EdU) Assay

Cells in the logarithmic growth phase were seeded in a 96-well plate and subjected to various

treatments. After incubation with EdU solution (Invitrogen, Carlsbad, CA, USA) for 2 h, the medium was discarded, and the cells were immobilized by 4% paraformaldehyde in phosphate-buffered saline (PBS) and incubated with glycine and 0.5% TritonX-100, followed by Apollo staining. Finally, DNA staining and photographing were performed.

Cell Cycle Detection

The cells treated for 24 h were routinely digested to make a single cell suspension, washed with Hanks' solution, and centrifuged at 800 rpm for 5 min. Then, the supernatant was discarded, the cells were resuspended, and precooled 70% alcohol was added to incubate cells overnight. After washing with PBS, the cells were stained with propidium iodide (PI) staining solution for 30 min, and the test was repeated three times.

Transwell Assay

At one day before the experiment, a 24-well plate and several transwell chambers were prepared. The FN was diluted 10 times to a final concentration of 100 μg/mL, and Matrigel was diluted at 1:9 with serum-free medium. The bottom of each chamber was coated with 50 μL of FN and placed in a clean bench for 2 h for air dry. The inside of the chamber was coated with 100 μL of Matrigel and placed in a cell culture incubator overnight. On the day of the experiment, the cells were suspended in serum-free medium, and 100 μL of cell suspension was added into the upper layer of the transwell chamber. After that, 600 μL of 10% FBS medium was added to the outside of the chamber. After incubation for 24 h in the incubator, the transwell chamber was taken out, fixed with 95% methanol, and photographed after staining. Cell migration experiment was the same except for the Matrigel step.

Dual-Luciferase Reporter Gene Assay

Cells were divided into four groups according to the different transfection with plasmids: wild type plasmid + miRNA NC group, mutant plasmid + miRNA NC group, wild type plasmid + miRNA mimics group, and mutant plasmid + miRNA mimics group. The sequences of wild type and mutant plasmids were designed based on the sequence in the database that binds to the miRNA. After co-transfection with plasmids for 48 h, the medium was discarded, and the PLB cleavage was used to completely lyse the cells. The intensity of the luciferase reaction was measured. After the end of the assay, Stop & Glo Reagent was added to measure the intensity of the internal *Renilla* luciferase reaction.

Caspase-3 Activity Assay

The cells were collected and centrifuged at 16,000-20,000 g for 10-15 min at 4°C. Then, the supernatant was transferred to a pre-cooled centrifuge tube in an ice bath, and the enzyme activity of Caspase-3 was measured. Subsequently, an appropriate amount of Ac-DEVD-pNA (2 mM) was placed in an ice bath for later use. After c-DEVD-pNA (2 mM) was added, the cells were incubated at 37°C for 60-120 min. A405 can be determined when the color change was observed to be evident. If the color change was not evident, the incubation time could be extended appropriately.

RNA Knockdown

Biotinylated probes against LEF-AS1 were purchased from Gima (Shanghai, China) and incubated with cell lysates. The probe was then captured by M280 streptavidin magnetic beads (Invitrogen, Carlsbad, CA, USA), and the precipitant was eluted by probe followed by qPCR analysis.

Statistical Analysis

Data analysis was performed using Statistical Product and Service Solutions (SPSS) 22.0 statistical software (IBM, Armonk, NY, USA), and GraphPad (Version X; La Jolla, CA, USA) was used for image editing. Analysis was performed using Pearson correlation analysis. Differences between two groups were analyzed using the Student's *t*-test. Comparison among multiple groups was performed using one-way ANOVA test and Post-Hoc Test (Least Significant Difference). Data were represented as mean ± SD. The difference was statistically significant at $p<0.05$.

Results

LEF-AS1 Was Highly Expressed in CRC Tissues

The expression of LEF-AS1 in 54 CRC tissue samples and adjacent ones was detected by qPCR,

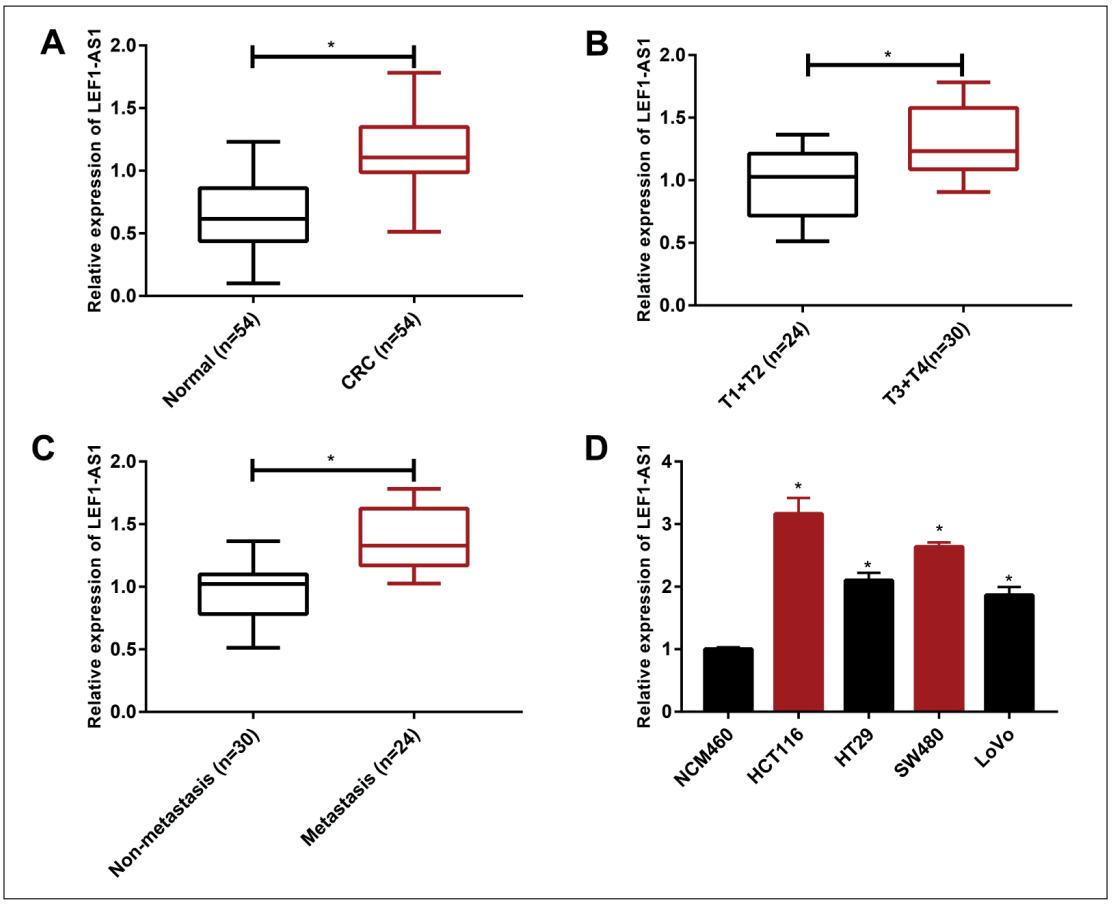

Figure 1. LEF1-AS1 is highly expressed in CRC tissues. **A,** Detection of LEF1-AS1 expression in 54 pairs of CRC tissues and adjacent normal tissues by qRT-PCR. **B,** Detection of LEF1-AS1 expression in different stages of CRC by qRT-PCR. **C,** Detection of LEF1-AS1 expression in metastatic and non-metastatic CRC tissues by qRT-PCR. **D,** Detection of LEF1-AS1 expression in NCM460 cells and CRC cell lines by qRT-PCR.

and the results indicated that LEF-AS1 level was markedly higher in the former than that in the latter, with a statistically significant difference ($p<0.05$; Figure 1A). The clinical information of patients with CRC was collected, and the expression of LEF-AS1 was further detected according to the different stages of patients. As a result, patients in T3+T4 stage or occurred lymph node metastasis showed a markedly higher expression of LEF-AS1 than those in T1+T2 or without lymph node metastasis (Figure 1B, 1C). Next, we examined the expression of LEF-AS1 in CRC cell lines (HCT116, HT29, SW480, LoVo) and normal colon cells NCM480 by qPCR, and found that its expression in CRC cells was markedly elevated, especially in HCT116 and SW480 cells (Figure 1D). The latter were selected for subsequent cell experiments.

Low Expression of LEF-AS1 Inhibits Cell Proliferation and Migration

To verify the influence of LEF-AS1 on cell functions, LEF-AS1 siRNA was transfected into cells, and the expression of LEF-AS1 was detected. As shown in Figure 2A, LEF-AS1 was successfully transfected and its expression was markedly reduced. Subsequently, CCK-8 and EdU assays revealed that cell viability and proliferation were markedly reduced after transfection of LEF-AS1 siRNA (Figure 2B, 2C). In addition, detection of Caspase-3 activity verified that down-regulation of LEF-AS1 accelerated cell apoptosis (Figure 2D), while flow cytometry indicated a shortened S phase and a prolonged G0/G1 phase (Figure 2E). Furthermore, transwell assay demonstrated a suppressed cell migration and invasiveness capac-

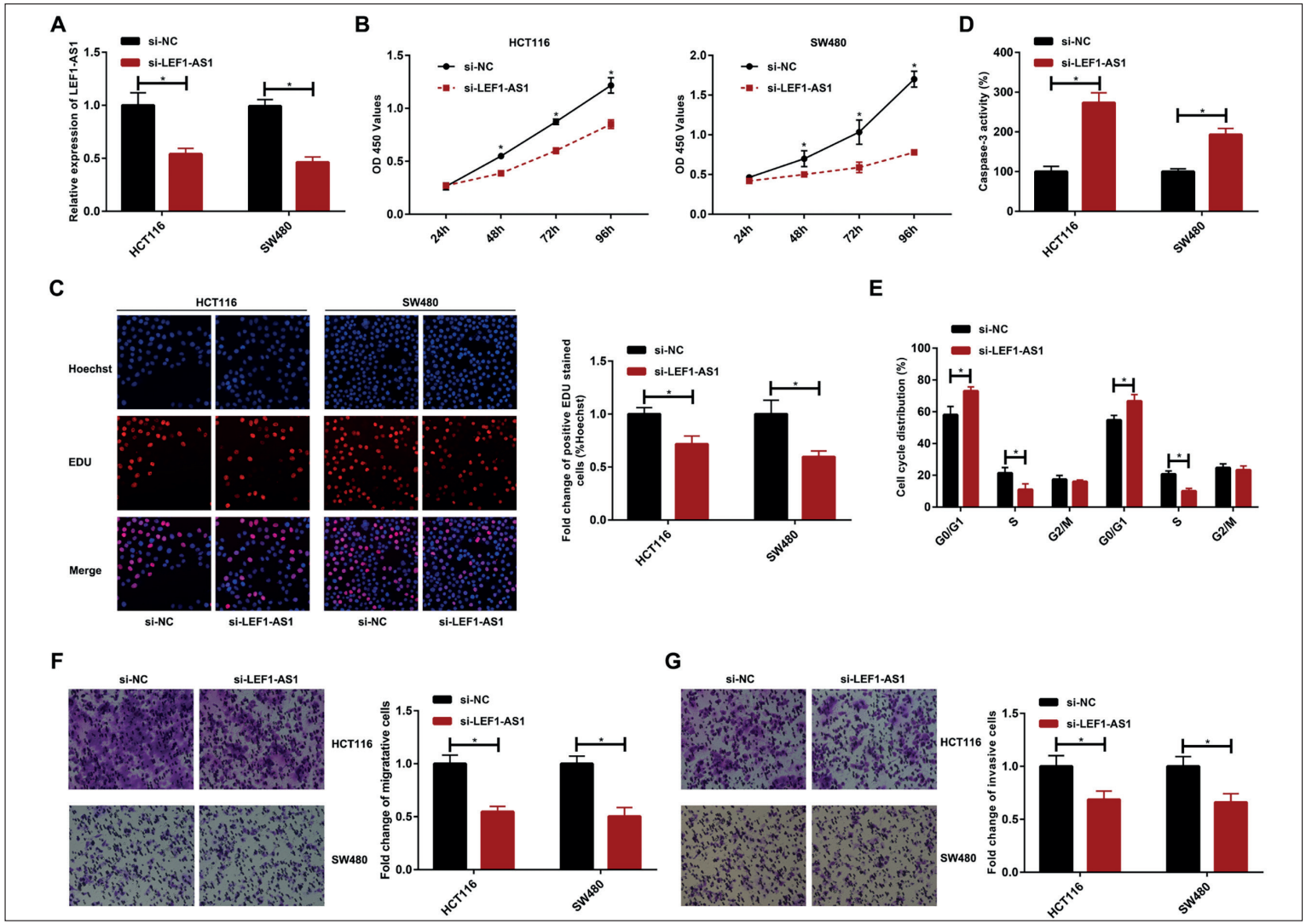

Figure 2. Down-regulation of LEF1-AS1 inhibits cell proliferation and migration. ***A,*** Detection of transfection efficiency of si-LEF1-AS1 by qRT-PCR. **B,** Detection of HCT116 and SW480 cell proliferation after down-regulation of LEF1-AS1 by CCK-8. **C,** Detection of HCT116 and SW480 cell proliferation after down-regulation of LEF1-AS1 by EDU (40x). **D,** Detection of Caspase-3 activity in HCT116 and SW480 cells after down-regulation of LEF1-AS1. **E,** Detection of cell cycle distribution of HCT116 and SW480 cells after down-regulation of LEF1-AS1. **F,** Detection of HCT116 and SW480 cell migration after down-regulation of LEF1-AS1 by transwell assay (40x). ***G,*** Detection of HCT116 and SW480 cell invasion after down-regulation of LEF1-AS1 by transwell assay (40×).

ity (Figure 2F, 2G), indicating that knockdown of LEF-AS1 can inhibit cell proliferation and migration but promote cell apoptosis.

LEF-AS1 Adsorbed MiR-505 to Regulate KIF3B Expression

Bioinformatics analysis predicted that LEF-AS1 might bind to miR-505 (Figure 3A). To further verify this prediction, miR-505 expression in CRC tissues was examined firstly (Figure 3B), and it was found to be markedly reduced, which was negatively correlated with LEF-AS1 level (Figure 3C). Subsequently, the Dual-Luciferase reporter gene assay based on the binding sequence confirmed that LEF-AS1 could bind to miR-505 (Figure 3D). RNA-knockdown using biotin-LEF-AS1 revealed a remarkable increase in miR-505 expression (Figure 3E). Meanwhile, subsequent knockdown of LEF-AS1 was found to significantly enhance miR-505 expression (Figure 3F).

LEF-AS1/MiR-505/KIF3B Axis Promoted CRC Development

The target gene of miR-505 was predicted by bioinformatics, and KIF3B was selected through functional analysis, which was further verified by luciferase reporting assay. First, KIF3B wild type sequence KIF3B WT 3'UTR and mutant sequence KIF3B MUT 3'UTR were successfully constructed (Figure 4A). Transfection with miR-505 mimics attenuated the luciferase in KIF3B WT 3'UTR group, while transfection with miR-505 inhibitor enhanced it. However, no significant difference was observed in KIF3B MUT 3'UTR group, suggesting that KIF3B is the target gene of miR-505 (Figure 4B). Moreover, transfection with si-LEF-AS1 reduced the fluorescence value in KIF3B WT 3'UTR group, which was reversed by co-transfection with si-LEF-AS1 and m-505 inhibitor. At the same time, KIF3B MUT group showed no significant difference (Figure 4C). To further demonstrate that LEF-AS1 can act as lncRNA to regulate KIF3B and participate in the development of CRC, cells were co-transfected

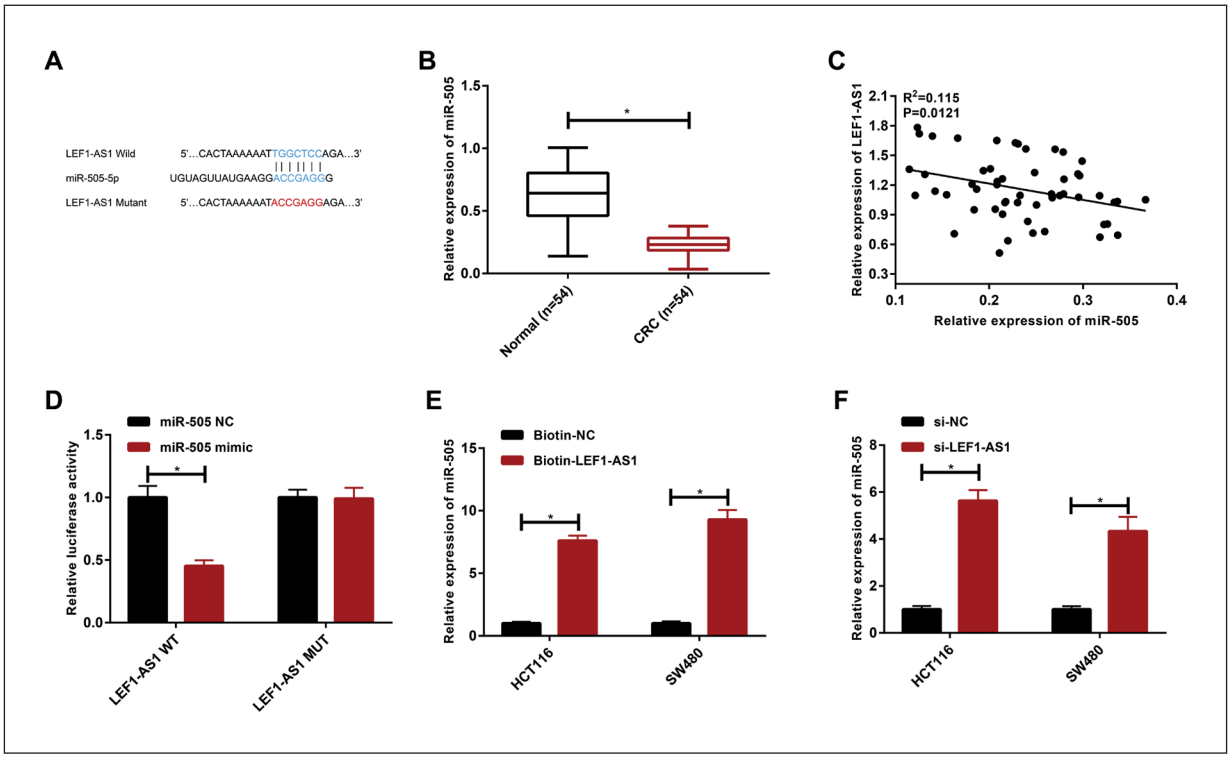

Figure 3. LEF1-AS1 adsorbs miR-505 to regulate KIF3B expression. **A,** Binding site between LEF1-AS1 and miR-505. **B,** Detection of miR-505 expression in 54 CRC tissues and adjacent normal tissues by qRT-PCR. **C,** Correlation analysis of the expressions of LEF1-AS1 and miR-505 in CRC. **D,** Luciferase reporter gene assay shows that LEF1-AS1 can bind to miR-505. **E,** Relative enrichment of miR-505 is analyzed by qRT-PCR after knockdown by biotin-labeled KEF1-AS1 probe. **F,** Down-regulation of LEF1-AS1 promotes the expression of miR-505.

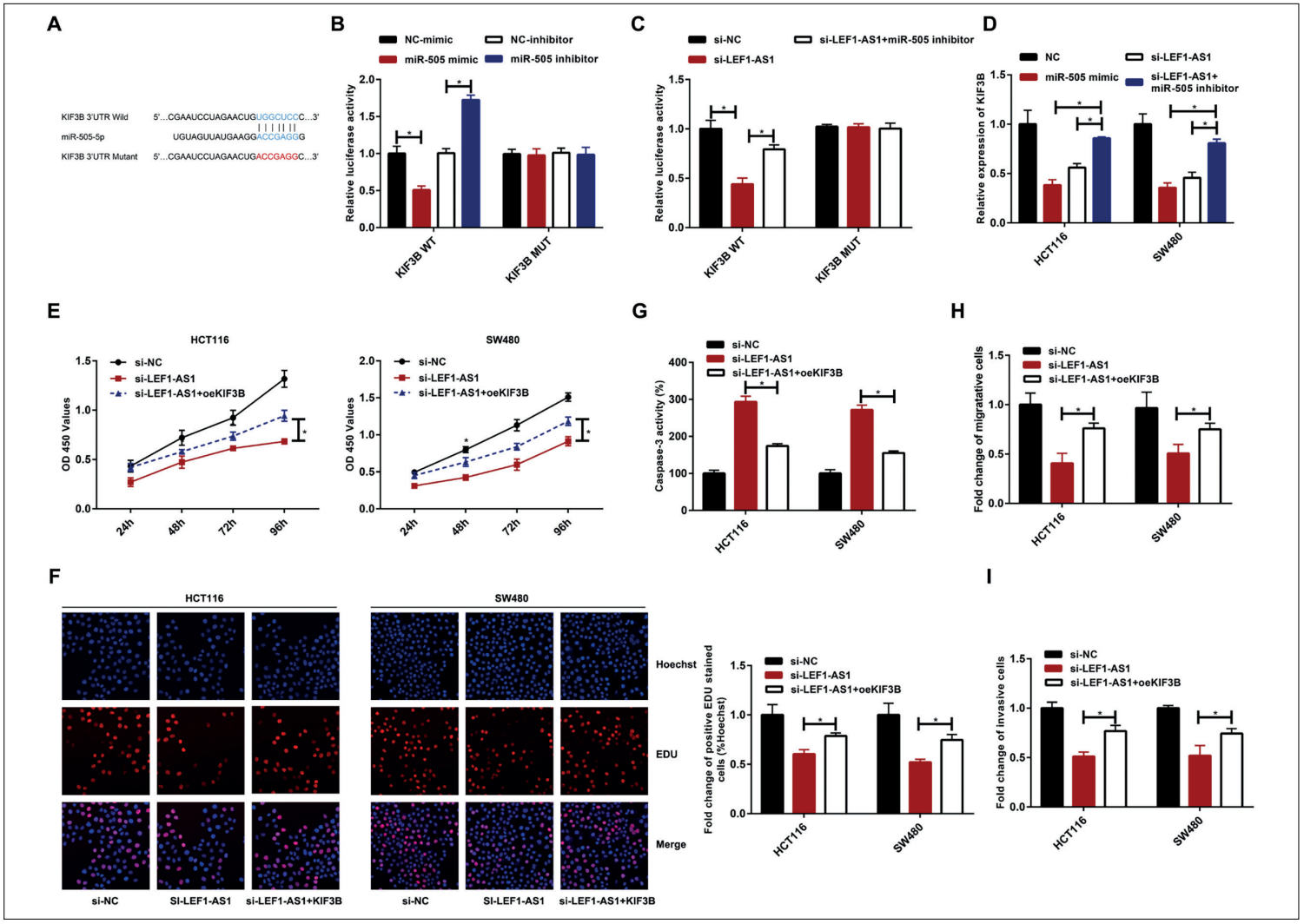

Figure 4. LEF1-AS1/miR-505/KIF3B axis promotes CRC development. **A,** Binding site between KIF3B and miR-505. **B,** Luciferase reporter gene assay reveals that KIF3B can bind to miR-505. **C,** Luciferase reporter gene assay indicates LEF1-AS1 can regulate the binding of KIF3B and miR-505. **D,** Detection of KIF3B expression after co-transfection of miR-505 and LEF1-AS1 by qRT-PCR. **E,** Detection of the proliferation of HCT116 and SW480 cells by LEF1-AS1/miR-505/KIF3B axis *via* CCK-8. **F,** Detection of the proliferation of HCT116 and SW480 cells by LEF1-AS1/miR-505/KIF3B axis by EDU assay (40x). **G,** Detection of the regulation of LEF1-AS1/miR-505/KIF3B axis on Caspase-3 activity in HCT116 and SW480 cells. **H,** Detection of the regulation of LEF1-AS1/miR-505/KIF3B axis on the migration of HCT116 and SW480 cells by transwell assay. **I,** Detection of the regulation of LEF1-AS1/miR-505/KIF3B axis on the invasion of HCT116 and SW480 cells by transwell assay.

with LEF-AS1 siRNA+microRNA-505 inhibitor and miR-505 mimics on the basis of transfection of LEF-AS1 siRNA. As a result, miR-505 mimics and LEF-AS1 siRNA markedly reduced KIF3B expression, which could be reversed by miR-505 inhibitor (Figure 4D). In addition, CCK-8 results revealed that LEF-AS1 siRNA markedly inhibited cell proliferation, which was partially reversed after co-transfection with si-LEF-AS1 and oeKIF3B plasmid (Figure 4E), and the EDU results were consistent with CCK-8 results (Figure 4F). Further detection of apoptosis by flow cytometry indicated that low expression of LEF-AS1 promoted apoptosis, while the opposite result was observed after overexpression of KIF3B (Figure 4G). Furthermore, cell migration and invasion were found to be suppressed by LEF-AS1 siRNA treatment, which also could be reversed by KIF3B overexpression (Figure 4H, 4I). The above results demonstrate that LEF-AS1 accelerates the progression of CRC through the LEF-AS1/miR-505/KIF3B axis.

Discussion

As one of the common malignant tumors, the incidence of CRC has been on the rise. With the year-by-year increasing mortality rate, the disease seriously endangers human health[3]. Since CRC has no evident symptoms in the early stage, it cannot be detected until it develops to the advanced stage, which is one of the reasons for its high mortality rate[4]. Therefore, its early diagnosis and treatment is a problem to be solved.

Studies[12-14] over the years have shown that epigenetic modifications such as gene methylation, p53, APC, and other tumor susceptibility genes, and regulation of non-coding RNA are all engaged in the occurrence and development of CRC. Kogo et al[15] have found that lncRNA HOTAIR is associated with poor prognosis of CRC by regulating multi-comb-dependent chromatin modification. LncRNA H19 acts as a precursor of miR-675 in CRC as an anticancer gene[16]. Yang et al[17] have shown that lncRNA H19 promotes the proliferation of CRC cells by competitively binding to miR-200a and down-regulating the expression of β-catenin. LncRNA MALT1 and HULC are all related to the occurrence and development of CRC[18]. So far, whether LEF-AS1 affects the proliferation and apoptosis of CRC cells still remains elusive. Therefore, this study mainly explored the effect of LEF-AS1 on CRC. LEF-AS1 was found to be markedly increased in CRC tissues and cell lines, and the down-regulation of LEF-AS1 inhibited cell proliferation, migration, and invasion, but promoted cell apoptosis.

KIFS was discovered in 1985, which is a kind of conservative molecular motor protein dependent on microtubules and responsible for intracellular cargo transport by utilizing the energy of ATP hydrolysis[19,20]. KIFS concerns a variety of cellular processes, including cell mitosis, meiosis, and transport of macromolecules. Among them, KIF3 is one of the most widely expressed KIFS[21]. This complex is involved in intracellular transport of organelles next to the membrane and protein complexes in various tissues, such as neurons, melanosomes, and epithelial cells[22-24]. KIF3B is a member of KIF3 subfamily, which is composed of molecular motor family and plays the role of vesicle transport and membrane expansion by targeting other molecules during mitosis[25,26]. Adenomatous polyposis proteins (APC) are transported through the KIF complex and gathered at the membrane protrusion tips to regulate cell migration. In this study, LEF-AS1 promoted the development of CRC through the LEF-AS1/miR-505/KIF3B axis.

There are still deficiencies in this study. For example, LEF-AS1's effects have not been thoroughly studied in animal models, which will be carried out in the subsequent studies.

Conclusions

In this work, LEF-AS1 was detected to be highly expressed in CRC for the first time. However, knockdown of LEF-AS1 may inhibit the proliferation, migration, and invasion of CRC cells and promote apoptosis through the LEF-AS1/miR-505/KIF3B axis.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Gilardi L, Vadrucci M. Isolated metachronous splenic metastasis from colon cancer found by 18F-FDG PET/CT. Clin Nucl Med 2017; 42: 79-80.

2) Naves T, Battu S, Jauberteau MO, Cardot PJ, Ratinaud MH, Verdier M. Autophagic subpopulation sorting by sedimentation field-flow fractionation. Anal Chem 2012; 84: 8748-8755.

3) Kyo K, Maema A, Shirakawa M, Nakamura T, Koda K, Yokoyama H. Pseudo-Meigs' syndrome secondary to metachronous ovarian metastases from transverse colon cancer. World J Gastroenterol 2016; 22: 4604-4609.

4) Negoi I, Runcanu A, Paun S, Beuran M. Right hemihepatectomy for CRC metachronous liver metastasis in a patient with crohn\'s disease: case report and review of the literature. Chirurgia (Bucur) 2016; 111: 522-526.

5) Maser EA, Sachar DB, Kruse D, Harpaz N, Ullman T, Bauer JJ. High rates of metachronous colon cancer or dysplasia after segmental resection or subtotal colectomy in Crohn's colitis. Inflamm Bowel Dis 2013; 19: 1827-1832.

6) Huarte M, Guttman M, Feldser D, Garber M, Koziol MJ, Kenzelmann-Broz D, Khalil AM, Zuk O, Amit I, Rabani M, Attardi LD, Regev A, Lander ES, Jacks T, Rinn JL. A large intergenic noncoding RNA induced by p53 mediates global gene repression in the p53 response. Cell 2010; 142: 409-419.

7) Tay Y, Kats L, Salmena L, Weiss D, Tan SM, Ala U, Karreth F, Poliseno L, Provero P, Di Cunto F, Lieberman J, Rigoutsos I, Pandolfi PP. Coding-independent regulation of the tumor suppressor PTEN by competing endogenous mRNAs. Cell 2011; 147: 344-357.

8) Tay Y, Rinn J, Pandolfi PP. The multilayered complexity of ceRNA crosstalk and competition. Nature 2014; 505: 344-352.

9) Ying L, Huang Y, Chen H, Wang Y, Xia L, Chen Y, Liu Y, Qiu F. Downregulated MEG3 activates autophagy and increases cell proliferation in bladder cancer. Mol Biosyst 2013; 9: 407-411.

10) Wang Y, Guo Q, Zhao Y, Chen J, Wang S, Hu J, Sun Y. BRAF-activated long non-coding RNA contributes to cell proliferation and activates autophagy in papillary thyroid carcinoma. Oncol Lett 2014; 8: 1947-1952.

11) Yin J, Ni B, Tian ZQ, Yang F, Liao WG, Gao YQ. Regulatory effects of autophagy on spermatogenesis. Biol Reprod 2017; 96: 525-530.

12) Ashktorab H, Brim H. DNA methylation and colorectal cancer. Curr Colorectal Cancer Rep 2014; 10: 425-430.

13) Su HX, Zhou HH, Wang MY, Cheng J, Zhang SC, Hui F, Chen XZ, Liu SH, Liu QJ, Zhu ZJ, Hu QR, Wu Y, Ji SR. Mutations of C-reactive protein (CRP) -286 SNP, APC and p53 in colorectal cancer: implication for a CRP-Wnt crosstalk. PLoS One 2014; 9: e102418.

14) El-Murr N, Abidi Z, Wanherdrick K, Svrcek M, Gaub MP, Flejou JF, Hamelin R, Duval A, Lesuffleur T. MiRNA genes constitute new targets for microsatellite instability in colorectal cancer. PLoS One 2012; 7: e31862.

15) Kogo R, Shimamura T, Mimori K, Kawahara K, Imoto S, Sudo T, Tanaka F, Shibata K, Suzuki A, Komune S, Miyano S, Mori M. Long noncoding RNA HOTAIR regulates polycomb-dependent chromatin modification and is associated with poor prognosis in colorectal cancers. Cancer Res 2011; 71: 6320-6326.

16) Tsang WP, Ng EK, Ng SS, Jin H, Yu J, Sung JJ, Kwok TT. Oncofetal H19-derived miR-675 regulates tumor suppressor RB in human colorectal cancer. Carcinogenesis 2010; 31: 350-358.

17) Yang W, Ning N, Jin X. The lncRNA H19 promotes cell proliferation by competitively binding to miR-200a and derepressing beta-catenin expression in colorectal cancer. Biomed Res Int 2017; 2017: 2767484.

18) Yu X, Zheng H, Chan MT, Wu WK. HULC: an oncogenic long non-coding RNA in human cancer. J Cell Mol Med 2017; 21: 410-417.

19) Vale RD, Reese TS, Sheetz MP. Identification of a novel force-generating protein, kinesin, involved in microtubule-based motility. Cell 1985; 42: 39-50.

20) Miki H, Okada Y, Hirokawa N. Analysis of the kinesin superfamily: insights into structure and function. Trends Cell Biol 2005; 15: 467-476.

21) Yu X, Wen H, Cao J, Sun B, Ding T, Li M, Wu H, Long L, Cheng X, Xu G, Zhang F. Temporal and spatial expression of KIF3B after acute spinal cord injury in adult rats. J Mol Neurosci 2013; 49: 387-394.

22) Tuma MC, Zill A, Le Bot N, Vernos I, Gelfand V. Heterotrimeric kinesin II is the microtubule motor protein responsible for pigment dispersion in Xenopus melanophores. J Cell Biol 1998; 143: 1547-1558.

23) Takeda S, Yamazaki H, Seog DH, Kanai Y, Terada S, Hirokawa N. Kinesin superfamily protein 3 (KIF3) motor transports fodrin-associating vesicles important for neurite building. J Cell Biol 2000; 148: 1255-1265.

24) Jimbo T, Kawasaki Y, Koyama R, Sato R, Takada S, Haraguchi K, Akiyama T. Identification of a link between the tumour suppressor APC and the kinesin superfamily. Nat Cell Biol 2002; 4: 323-327.

25) Fan J, Beck KA. A role for the spectrin superfamily member Syne-1 and kinesin II in cytokinesis. J Cell Sci 2004; 117: 619-629.

26) Keil R, Kiessling C, Hatzfeld M. Targeting of p0071 to the midbody depends on KIF3. J Cell Sci 2009; 122: 1174-1183.