

Long non-coding RNA LINC01503 promotes colorectal cancer cell proliferation and invasion by regulating miR-4492/FOXK1 signaling

SHUI-RONG LU^{1*}, QIN LI^{1*}, JIN-LAI LU¹, CHONGNI LIU², XIAOHONG XU¹ and JING-ZE LI¹

¹Department of Gastroenterology, Shanghai East Hospital, Tongji University, Shanghai 200123;

²Department of Gastroenterology, Xianyang Central Hospital, Xianyang, Shaanxi 712000, P.R. China

Received March 20, 2018; Accepted July 26, 2018

DOI: 10.3892/etm.2018.6775

Abstract. Increasing evidence indicates that long non-coding RNAs (lncRNAs) are closely associated with the progression of human cancer, including colorectal cancer (CRC). A previous study suggested that lncRNA LINC01503 promotes squamous cell carcinoma progression. However, the function of LINC01503 in CRC has remained elusive. The present study indicated that LINC01503 was significantly upregulated in CRC tissues compared with that in adjacent normal tissues as detected by reverse transcription-quantitative polymerase chain reaction. It was demonstrated that knockdown of long intergenic non-protein coding RNA (LINC)01503 markedly inhibited the proliferation and invasion of CRC cells, whereas overexpression of LINC01503 had the opposite effects, as indicated by Cell Counting kit-8 and Transwell assays. Mechanistically, it was revealed that LINC01503 serves as a sponge for microRNA (miR)-4492, which targets forkhead box K1 (FOXK1) in CRC cells. In addition, luciferase reporter assays demonstrated the direct binding of miR-4492 mimics to LINC01503 and to a sequence in the 3'-untranslated region of FOXK1. Furthermore, it was demonstrated that overexpression of LINC01503 reduced the availability of miR-4492 in CRC cells. Furthermore, miR-4492 mimics inhibited FOXK1 expression, while simultaneous overexpression of LINC01503 abolished this effect. Finally, it was demonstrated that restoration of FOXK1 abolished the inhibitory effect of LINC01503 knockdown on CRC cell proliferation and invasion. Taken together, the present results suggested that LINC01503 promotes CRC progression via acting as a competing endogenous RNA for miR-4492/FOXK1.

Introduction

Colorectal cancer (CRC) is one of the most common and aggressive cancer types in the digestive tract and poses a serious threat to human life and health (1). Among all cancer types, the incidence and mortality of CRC are the third and fourth highest, respectively (2). The development and progression of CRC are induced by a cascade of multiple events, including genetic and epigenetic deregulation, with changes at the genetic and protein expression level (3). To develop novel diagnostic biomarkers and effective therapeutic targets for CRC patients, it is urgently required to better understand the mechanisms involved in CRC progression.

Long non-coding RNAs (lncRNAs) are a class of non-coding RNAs with a length of >200 nucleotides and no protein coding ability (4,5). Accumulating studies have illustrated that certain lncRNAs are involved in tumorigenesis (6). For instance, Wei and Li (7) reported that lncRNA sex-determining region Y 21 antisense diverging transcript 1 (AS1) sponges microRNA (miRNA/miR)-145 to promote the tumorigenesis of CRC by targeting myosin, class VI. Wang *et al* (8) reported that lncRNA nuclear paraspeckle assembly transcript 1 regulates the proliferation, migration and invasion of gastric cancer cells via inhibiting the binding of miR-335-5p to Rho associated coiled-coil containing protein kinase 1. Fu *et al* (9) indicated that long intergenic non-protein coding RNA (LINC)00210 drives Wnt/ β -catenin signaling activation and liver tumor progression in a catenin β interacting protein 1-dependent manner. In addition, Xie *et al* (10) indicated that lncRNA zinc finger NFX1-type containing 1 antisense RNA 1 sponges miR-484 to promote cell proliferation and invasion in CRC. Therefore, it has been widely acknowledged that lncRNAs have essential roles in human cancer, including CRC.

A recent study implied that LINC01503 contributes to squamous cell carcinoma progression (11). However, the function of LINC01503 in CRC has remained to be elucidated. In the present study, it was identified that LINC01503 was highly expressed in CRC tissues. Furthermore, knockdown of LINC01503 significantly inhibited the proliferation and invasion of CRC cells, while ectopic expression of LINC01503 had the opposite effect. Regarding the mechanism of action, LINC01503 was indicated to promote the expression

Correspondence to: Professor Jing-Ze Li, Department of Gastroenterology, Shanghai East Hospital, Tongji University, 1800 Yuntai Road, Pudong New District, Shanghai 200123, P.R. China

E-mail: lijingze_8@163.com

*Contributed equally

Key words: LINC01503, proliferation, invasion, microRNA-4492, forkhead box K1, colorectal cancer

of forkhead box K1 (FOKK1) by sponging miR-4492. Through regulating the miR-4492/FOKK1 axis, LINC01503 contributes to CRC progression. Taken together, the present results demonstrated the pivotal role of LINC01503 in CRC progression and elucidated the underlying mechanisms, which suggested that LINC01503/miR-4492/FOKK1 axis may be a potential therapeutic target.

Materials and methods

Clinical specimens. All tissue samples (47 cases) and pair-matched non-cancerous tissues (28 cases; 1.5 cm from the lesions) from patients with CRC were collected at the Shanghai East Hospital, Shanghai Tongji University (Shanghai, China) between January 2014 and December 2016. None of the patients included in the present study had received any therapy prior to surgery. Informed consent was obtained from all patients prior to sample collection. All tissues obtained during surgery were confirmed by histopathological evaluation and then immediately frozen until use. The protocol of the present study was approved by the Ethics Committee of Shanghai Tongji University.

Cell culture and transfection. The CRC cell lines (HT29, HCT8, LS513, SW620 and HCT116) and a non-cancerous colon epithelial FHC cell line were purchased from the American Type Culture Collection (Manassas, VA, USA). CRC cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) with 10% fetal bovine serum (FBS; HyClone; GE Healthcare, Logan, UT, USA), 100 units/ml penicillin and 100 mg/ml streptomycin at 37°C in a humidified atmosphere containing 5% CO₂. The small interfering (si)RNA against LINC01503 (5'-TCTGACAAGTGTGTACCTA-3'), siRNA negative control (siLINC; 5'-AATTCTCCGAACGTGTCA CGT-3'), miR-4492 mimics (5'-GGGGCUGGGCGCGCGC CC-3'), miR negative controls (NC mimics; 5'-UCACAACCU CCUAGAAAGAGUAGA-3') and pCDNA3-LINC01503 were synthesized and purchased from RiboBio Co. (Guangzhou, China). Transfection of SW620 and HCT8 cells was performed using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol.

Cell proliferation assay. For the CRC cell proliferation assay, SW620 and HCT8 cells were transfected with siLINC01503 or pCDNA3-LINC01503 for 0.5 h according to manufacturer's protocol. Subsequently, the cells were re-seeded in 96-well plates at 3x10³ cells/well. Following culture for 24, 48 or 72 h, the proliferation of CRC cells was measured using a Cell Counting kit-8 (Dojindo, Kumamoto, Japan) according to the manufacturer's protocol. The absorbance of CRC cells was measured at 450 nm to draw cell proliferation curves. The CCK-8 assay was performed independently three times.

Transwell assay. Cell invasion was determined by Transwell assays. The membrane of the Transwell chambers (pore size, 8 µm; cat. no. PSET010R5; EMD Millipore, Billerica, MA, USA) was coated with 30 mg/cm² Matrigel® (BD Biosciences, San Jose, CA, USA) for 1 h at 37°C to form a matrix barrier. The lower chambers were filled with 600 µl DMEM supplemented with 10% FBS. The SW620 or HCT8 cells were suspended in

DMEM and 1x10⁵ cells were loaded into each upper well in a volume of 200 µl. After the chambers cultured at 37°C with 5% CO₂ for 24 h, the cells at the upper side of the membrane were wiped off. The cells in the lower side of the membrane were fixed with methanol for 10 min at 25°C and stained with 0.1% crystal violet for 10 min at 25°C. Subsequently, the cells were washed with PBS. Counts were obtained from five random fields at a magnification of x200. Three replicates per condition were performed.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from 1x10⁷ cultured SW620 and HCT8 cells using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol and complementary (c)DNA was synthesized from total RNA with a PrimeScript RT Reagent kit (Takara Bio, Inc., Otsu, Japan). miRNA from total RNA was reverse transcribed using the PrimeScript miRNA cDNA Synthesis kit (Takara Bio, Inc.). Real-time PCR was performed with the SYBR-Green Premix Ex Taq II (Takara Bio, Inc.) on an Applied Biosystems Step One Plus Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The thermocycling conditions were as follows: Denaturation at 95°C for 10 min, followed by 40 cycles of denaturation at 95°C for 15 sec, 60°C for 20 sec and 72°C for 30 sec. GAPDH was used as the endogenous control for detection of mRNA and lncRNA expression levels, while U6 was used as an endogenous control for miRNA expression analysis. Relative gene expression levels were calculated using the 2^{-ΔΔC_q} method (12). Primer sequences were as follows: LINC01503 forward, 5'-GGG ACGGAGACAAATGACGG-3' and reverse, 5'-GCAGGC TCCCTGACACGTA-3'; miR-4492 forward, 5'-AACGAG ACGACGACAGAC-3' and reverse, 5'-GGGGCTGGGCGC GCGCC-3'; FOKK1 forward, 5'-ACGTTTGGTGACCAG AGGGA-3' and reverse, 5'-CGACAGAATTCAAGCCGC AC-3'; U6 forward, 5'-AACGAGACGACGACAGAC-3' and reverse, 5'-GCAAATTCGTGAAGCGTTCCATA-3' and GAPDH forward, 5'-ATGTTGCAACCGGGAAGGAA-3' and reverse, 5'-AGGAAAAGCATCACCCGGAG-3'.

Luciferase reporter assay. The putative interacting sites between LINC01503 and miR-4492, and between miR-4492 and FOKK1 were predicted using miRDB (<http://mirdb.org/miRDB/index.html>) and TargetScan7 (http://www.targetscan.org/vert_71/) tools. The targeting association between miR-4492 and LINC01503 or the 3'-untranslated region (3'-UTR) of FOKK1 was validated by luciferase reporter assays. The wild-type (WT) LINC01503 sequence or the WT 3'-UTR fragment from FOKK1 mRNA containing the predicted miR-4492 binding site was amplified and inserted into a pmirGLO dual-luciferase miRNA target expression vector (Promega Corp., Madison, WI, USA) to construct the reporter vector pmirGLO-LINC01503-WT or pmirGLO-FOKK1-WT as previously described (13). The putative binding site #2 of miR-4492 in the LINC01503 or FOKK1 3'-UTR was mutated using a GeneArt™ Site-Directed Mutagenesis PLUS System (cat. no. A14604; Thermo Fisher Scientific, Inc.). The mutant (mut) LINC01503 or FOKK1 3'-UTR was inserted into a pmirGLO vector to form the reporter vector pmirGLO-LINC01503-Mut or pmirGLO-FOKK1-Mut. The respective reporter vector and

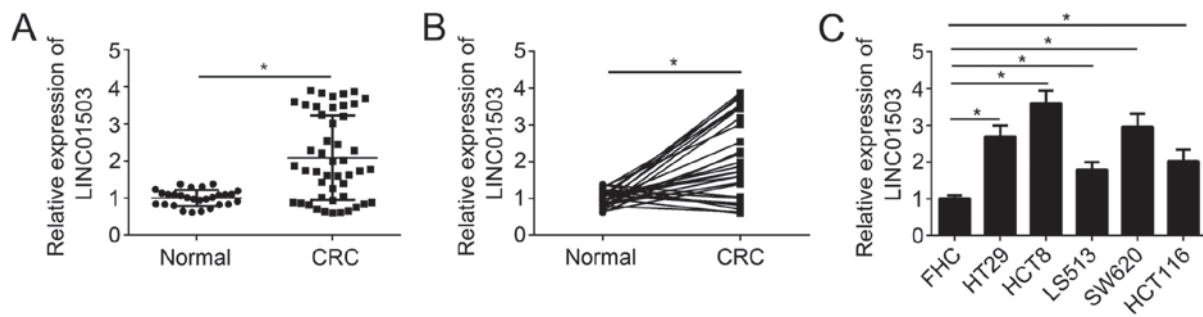


Figure 1. LINC01503 is highly expressed in CRC tissues. (A) Relative expression levels of LINC01503 in CRC tissues (n=47) and adjacent normal tissues (n=28) were determined by RT-qPCR. (B) Relative expression levels of LINC01503 in 28 pairs of CRC tissues and adjacent normal tissues were analyzed by RT-qPCR. (C) The expression levels of LINC01503 in CRC cell lines and the non-cancerous cell line FHC were examined by RT-qPCR. Values are expressed as the mean \pm standard deviation. * P <0.05. RT-qPCR, reverse transcription-quantitative polymerase chain reaction; CRC, colorectal cancer.

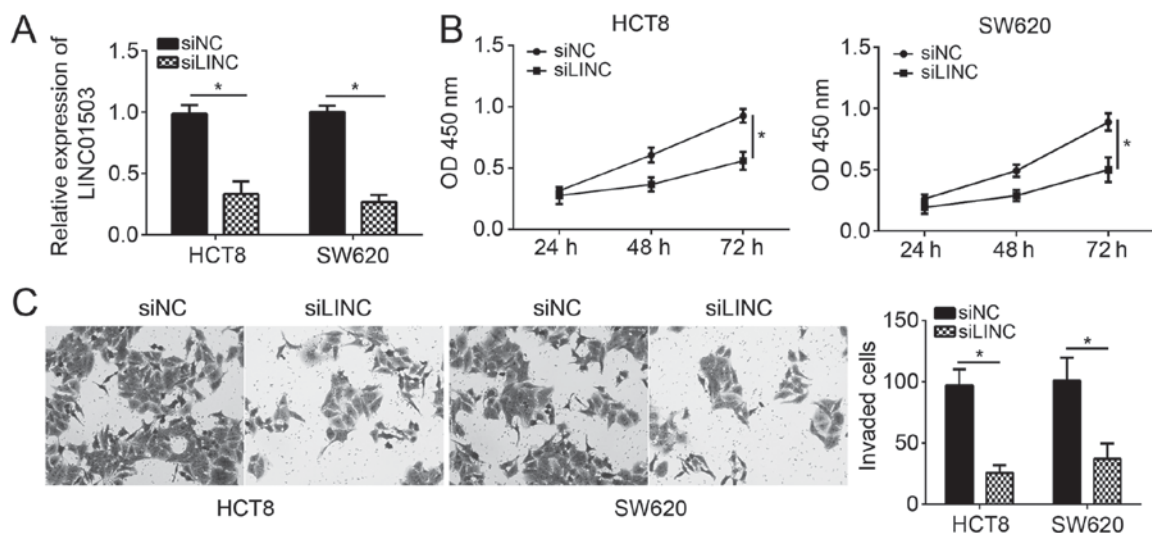


Figure 2. Silencing of LINC01503 inhibits CRC cell proliferation and invasion. (A) Relative expression levels of LINC01503 in HCT8 and SW620 cells transfected with siLINC01503 or siNC were measured by reverse transcription-quantitative polymerase chain reaction. (B) A Cell Counting kit-8 assay was used to determine CRC cell proliferation. (C) A Transwell assay was utilized to measure CRC cell invasion (magnification, $\times 100$). Values are expressed as the mean \pm standard deviation. * P <0.05. CRC, colorectal cancer; siLINC01503/siLINC, small interfering RNA selective for LINC01503; siNC, control siRNA; OD, optical density.

miR-4492 mimics or NC mimics were co-transfected into 2×10^4 SW620 and HCT8 cells, followed by incubation for 48 h. Subsequently, luciferase activities were measured using a Dual-Luciferase Reporter Assay System (Promega Corp.). The values were normalized to those of the Renilla luciferase transfection control using the Dual-Luciferase Reporter Gene Assay kit (Promega Corp.) according to the manufacturer's protocol.

Statistical analysis. All statistical analyses were performed using SPSS 20.0 (IBM, Corp., Armonk, NY, USA) and GraphPad Prism (version 6; GraphPad Inc., La Jolla, CA, USA). Student's t-test and one-way analysis of variance followed by Tukey's post-hoc test was used to assess differences between 2 or multiple groups, respectively. P <0.05 was considered to indicate a statistically significant difference.

Results

LINC01503 is overexpressed in CRC tissues. A total of 47 CRC samples and 28 adjacent normal tissues were collected and the expression levels of LINC01503 were determined

in these tissue samples by RT-qPCR. The results indicated that LINC01503 expression was significantly upregulated in CRC tissues compared with that in adjacent normal tissues (Fig. 1A). Furthermore, the expression of LINC01503 was higher in most CRC tissues compared with that in paired adjacent normal tissues (Fig. 1B). Similarly, the expression pattern of LINC01503 was measured in CRC cell lines and the normal colon epithelial FHC cell line. The results indicated that LINC01503 expression was significantly upregulated in CRC cell lines compared with that in the non-cancerous reference cell line (Fig. 1C). These results implied that LINC01503 may be involved in the genesis of CRC.

Silencing of LINC01503 inhibits CRC cell proliferation and invasion. To determine the function of LINC01503 in CRC, HCT8 and SW620 cells were selected for the subsequent experiments. After transfection with siLINC01503, the expression of LINC01503 was significantly downregulated in HCT8 and SW620 cells (Fig. 2A). A CCK-8 and Transwell assays were then performed to detect the effects of LINC01503 on CRC cell proliferation and invasion. The results suggested that

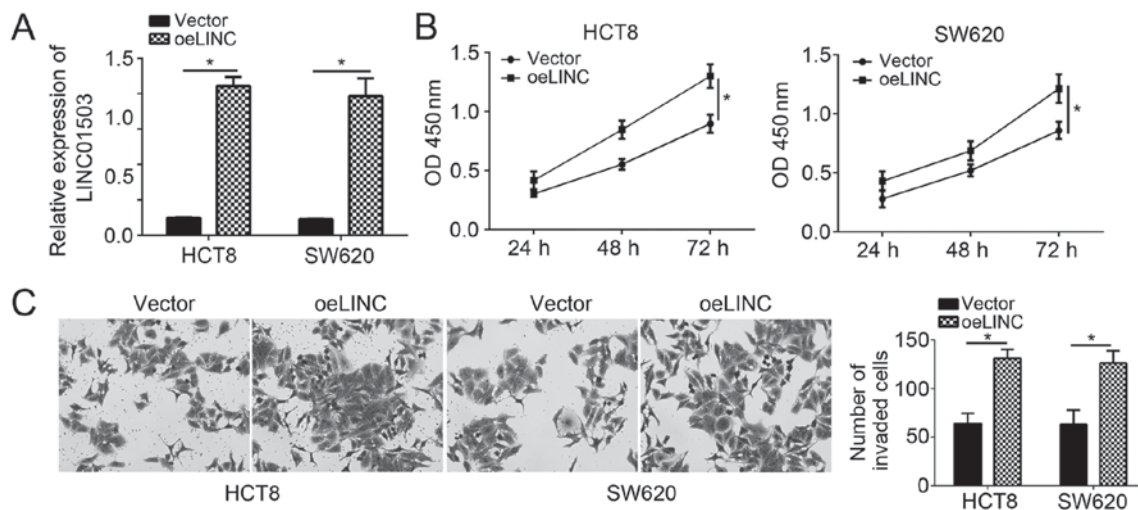


Figure 3. LINC01503 overexpression promotes colorectal cancer cell proliferation and invasion. (A) Relative expression levels of LINC01503 in HCT8 and SW620 cells transfected with pCDNA3-LINC01503 or empty vector were analyzed by reverse transcription-quantitative polymerase chain reaction. (B) A Cell Counting kit-8 assay was used to detect the proliferation in HCT8 and SW620 cells transfected with pCDNA3-LINC01503 or empty vector. (C) A Transwell assay was used to assess the invasion of HCT8 and SW620 cells transfected with pCDNA3-LINC01503 or empty vector (magnification, x100). Values are expressed as the mean \pm standard deviation. * $P < 0.05$. OD, optical density; oeLINC, LINC01503 overexpression vector.

LINC01503 knockdown significantly impaired the proliferation of HCT8 and SW480 cells (Fig. 2B). Consistently, HCT8 and SW480 cells transfected with siLINC01503 displayed an attenuated potential of invasion (Fig. 2C). These results indicated that LINC01503 contributes to malignant behavior of CRC cells that is associated with cancer progression.

LINC01503 overexpression promotes CRC cell proliferation and invasion. To further confirm the effects of LINC01503 on CRC cells, LINC01503 was overexpressed in HCT8 and SW620 cells by transfection with pCDNA3-LINC01503. RT-qPCR analysis indicated that LINC01503 expression was significantly upregulated in HCT8 and SW620 cells after transfection (Fig. 3A). CCK8 and Transwell assays were then performed. Conversely to the observations made following silencing of LINC01503, overexpression of LINC01503 markedly promoted the proliferation of HCT8 and SW480 cells (Fig. 3B). Furthermore, the invasion ability of HCT8 and SW480 cells was also enhanced following transfection with LINC01503 ectopic expression plasmid (Fig. 3C). These results demonstrated that LINC01503 has an oncogenic role in CRC cells.

LINC01503 facilitates FOXK1 expression via sponging of miR-4492. To further explore the downstream mechanisms of the effects of LINC01503 in CRC cells, the target miRNAs were predicted by a bioinformatics analysis. It was identified that miR-4492 was the most probable target miRNA of LINC01503, as two potential binding sites for miR-4492 were identified in LINC01503 (Fig. 4A). Furthermore, bioinformatics analysis also suggested FOXK1 as a potential downstream target of miR-4492, as five potential binding sites of miR-4492 were identified in the 3'-UTR of FOXK1 mRNA (Fig. 4A). To verify the predicted binding interactions between miR-4492 and LINC01503 or FOXK1, luciferase reporter assays were performed. First, the efficiency of the transfection of miR-4492 mimics into HCT8 and SW620 cells was

confirmed by RT-qPCR (Fig. 4B). Subsequently, HCT8 and SW620 cells were co-transfected with the constructed WT or mut LINC01503 reporter plasmid as well as miR-4492 mimics or controls, and the luciferase activity was then measured. The results indicated that overexpression of miR-4492 significantly repressed the luciferase activity of pmirGLO-LINC01503-WT but not pmirGLO-LINC01503-Mut in HCT8 and SW620 cells (Fig. 4C). Similarly, miR-4492 mimics also inhibited the luciferase activity of pmirGLO-FOXK1-WT but not pmirGLO-FOXK1-Mut in HCT8 and SW620 cells (Fig. 4D). These results demonstrated that miR-4492 interacted with LINC01503 and FOXK1 mRNA in CRC cells. To further prove this, RT-qPCR analysis was performed, which demonstrated that overexpression of LINC01503 significantly reduced the levels of miR-4492 in HCT8 and SW620 cells (Fig. 4E). Furthermore, miR-4492 mimics caused a decrease in the mRNA levels of FOXK1 in HCT8 and SW620 cells, while simultaneous overexpression of LINC01503 abrogated this effect (Fig. 4F). In addition, miR-4492 expression was significantly downregulated, while FOXK1 expression was significantly increased in CRC tissues compared with that in adjacent normal tissues (Fig. 4G and H). Of note, the expression levels of miR-4492 and FOXK1 were negatively correlated in CRC tissues (Fig. 4I). Taken together, these results indicated that LINC01503 promotes FOXK1 expression via inhibition of miR-4492 in CRC cells.

Restoration of FOXK1 expression abrogates the effects of LINC01503 knockdown. The present study then sought to determine whether FOXK1 expression is responsible for the effects of LINC01503 on CRC cells. LINC01503-silenced CRC cells were subjected to ectopic overexpression of FOXK1. RT-qPCR analysis indicated that FOXK1 expression, which was markedly declined after silencing of LINC01503, was significantly rescued after simultaneous transfection of FOXK1 expression plasmid in CRC cells (Fig. 5A). CCK8 and Transwell assays were then performed. The results indicated

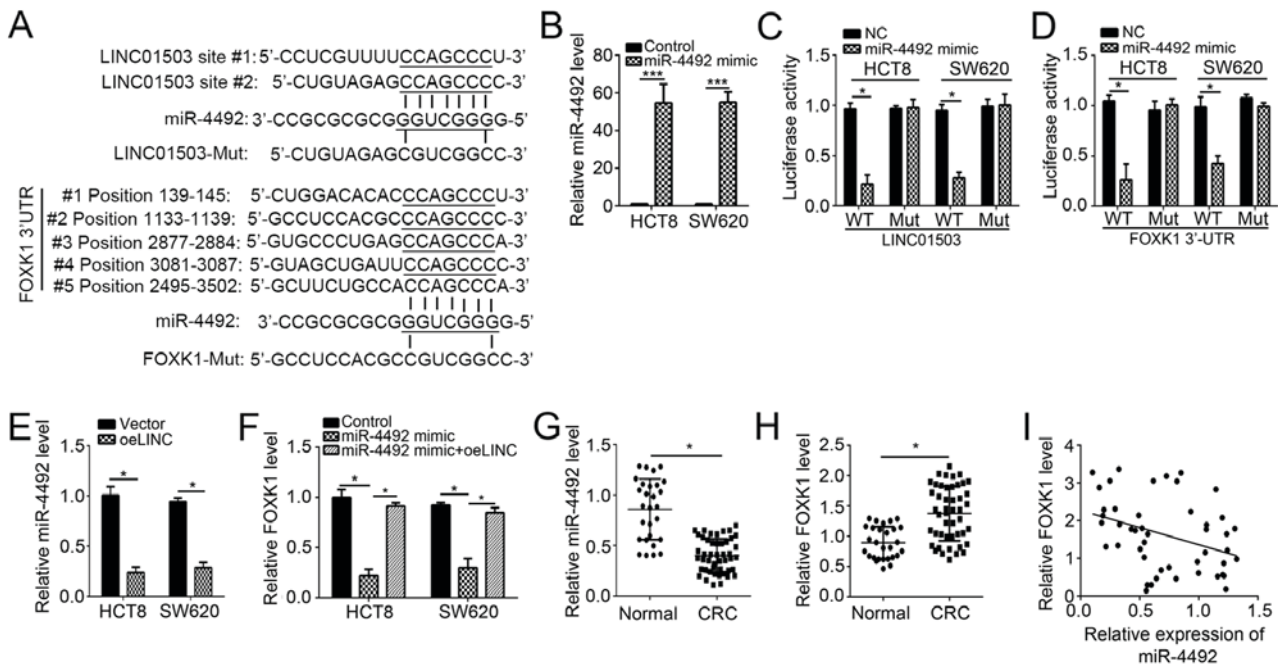


Figure 4. LINC01503 facilitates FOXK1 expression via sponging miR-4492. (A) Predicted binding sites of miR-4492 in LINC01503 and the 3'-UTR of FOXK1. (B) Relative expression of miR-4492 in HCT8 and SW620 cells transfected with miR-4492 mimics or controls. (C and D) Luciferase reporter assays were used to verify the interaction between miR-4492 with LINC01503 or the 3'-UTR of FOXK1 in HCT8 and SW620 cells. (E) Overexpression of LINC01503 inhibited the expression of miR-4492 available in HCT8 and SW620 cells. (F) miR-4492 mimics inhibited the mRNA expression of FOXK1 in HCT8 and SW620 cells, while ectopic expression LINC01503 abrogated this effect. (G) Relative expression of miR-4492 in CRC tissues and adjacent normal tissues. (H) Relative expression of FOXK1 in CRC tissues and adjacent normal tissues. (I) Assessment of the correlation between miR-4492 and FOXK1 in CRC tissues by reverse transcription-quantitative polymerase chain reaction. Values are expressed as the mean \pm standard deviation. * P <0.05. CRC, colorectal cancer; UTR, untranslated region; miR, microRNA; FOXK1, forkhead box K1; oeLINC, LINC01503 overexpression vector; NC, negative control; mut, mutated; WT, wild-type.

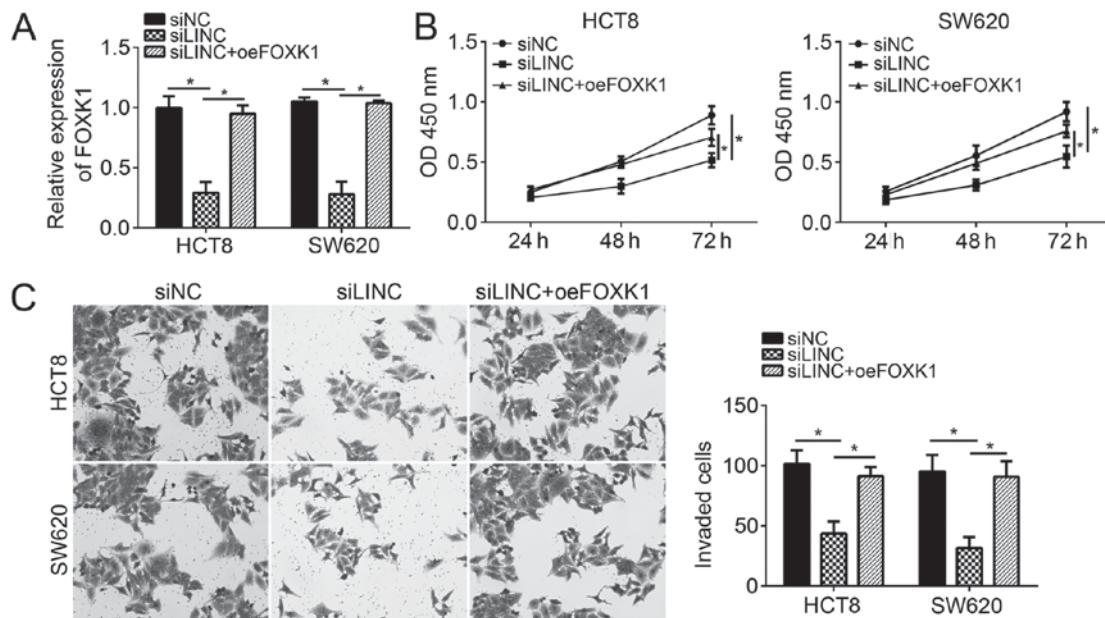


Figure 5. Restoration of FOXK1 abrogates the effects of LINC01503 knockdown. HCT8 and SW620 cells subjected to LINC01503 knockdown and optional transfection with FOXK1 overexpression vector. (A) The relative expression levels of FOXK1 were measured by reverse transcription-quantitative polymerase chain reaction. (B) A Cell Counting kit-8 assay was used to determine the proliferation of the cells. (C) Transwell assays were utilized to analyze the invasion of the cells (magnification, $\times 100$). Values are expressed as the mean \pm standard deviation. * P <0.05. FOXK1, forkhead box K1; siLINC01503/siLINC, small interfering RNA selective for LINC01503; siNC, control siRNA; oeLINC, LINC01503 overexpression vector; OD, optical density.

that LINC01503 knockdown suppressed the proliferation and invasion of CRC cells, whereas restoration of FOXK1 significantly reversed the effects of LINC01503 on CRC

cells (Fig. 5B and C). Taken together, the present results demonstrated that LINC01503 promoted the proliferation and invasion of CRC cells via enhancing FOXK1 expression.

Discussion

CRC is one of the most prevalent cancer types worldwide and the fourth leading cause of cancer-associated death (2). Most patients are diagnosed with CRC at the advanced stage, which is usually accompanied with metastasis, leading to poor outcomes for CRC patients. Therefore, the discovery of novel diagnostic biomarkers and development of therapeutic targets for CRC is an urgent priority. In the present study, it was identified that LINC01503 is overexpressed in CRC tissues and cell lines compared with normal adjacent tissues and a reference cell line, respectively. Furthermore, it was demonstrated that overexpression of LINC01503 enhanced the proliferation and invasion of CRC cells, while knockdown of LINC01503 had the opposite effect. It was also demonstrated that LINC01503 exerts its effects by promoting FOXK1 expression through acting as a sponge for miR-4492 in CRC cells.

Besides miRNAs, lncRNAs have also been reported to be essential post-transcriptional regulators of gene expression that regulate various biological processes, including cell proliferation, survival and mobility (14). For instance, Fu *et al* (15) indicated that lncRNA LINC00978 promotes cancer growth and acts as a diagnostic biomarker in gastric cancer. Furthermore, Chen *et al* (16) reported that lncRNA FOXD2-AS1 promotes the genesis of nasopharyngeal carcinoma by modulating the miR-363-5p/S100 calcium binding protein A1 pathway. In addition, Xie *et al* (17) indicated that hepatocellular carcinoma (HCC)-associated lncRNA facilitates the growth and metastasis of HCC by acting as a competing endogenous (ce)RNA of lysosomal-associated transmembrane protein 4B. The abovementioned studies evidenced that dysregulation of lncRNA expression is associated with various types of human cancer. Another study suggested that LINC01503 was upregulated in squamous cell carcinoma and contributed to its progression (11). However, the function of LINC01503 in CRC has remained largely elusive. In the present study, LINC01503 was identified to have an oncogenic role in CRC and to be associated with its progression.

An increasing number of studies demonstrate that lncRNAs serve as ceRNAs to act as a sponge on miRNAs to consequently regulate gene expression (18). For instance, Chang *et al* (19) reported that lncRNA for Pvt1 oncogene promotes epithelial to mesenchymal transition via mediating the targeting of twist family basic helix-loop-helix transcription factor 1 by miR-186 in prostate cancer. The present study assessed whether LINC01503 may also serve as a ceRNA in CRC. To investigate this, the target miRNAs of LINC01503 were predicted by a bioinformatics analysis. miR-4492 was suggested as a direct target of LINC01503 in CRC cells, which was then experimentally verified. It was demonstrated that miR-4492 mimics repressed the luciferase activity of a LINC01503 reporter vector in CRC cells. Furthermore, ectopic overexpression of LINC01503 suppressed the levels of miR-4492 in HCT8 and SW620 cells. These results demonstrated that LINC01503 acted as a sponge for miR-4492 and reduced its availability. To the best of our knowledge, the function of miR-4492 has not been explored in cancer cells previously. The present study was the first to suggest that miR-4492 acts as a tumor suppressor in CRC. However, the expression patterns and functions of miR-4492 require further exploration.

In addition, the present study further identified FOXK1 as a direct target gene of miR-4492. A luciferase reporter assay confirmed the interaction between miR-4492 and FOXK1 mRNA. Furthermore, it was indicated that miR-4492 mimics significantly repressed the mRNA expression of FOXK1 in CRC cells. However, simultaneous overexpression of LINC01503 rescued the expression of FOXK1 in CRC cells transfected with miR-4492 mimics. These results demonstrated that LINC01503 promotes the expression of FOXK1 by acting as a sponge for miR-4492. Previous studies have indicated that FOXK1 has an oncogenic role in various cancer types, including esophageal cancer (20), ovarian cancer (21), gastric cancer (22), CRC (23) and prostate cancer (24). To determine whether LINC01503 exerts its oncogenic effects by regulating FOXK1 expression, loss- and gain-of-function assays were performed. CCK-8 and Transwell assays, demonstrated that restoration of FOXK1 significantly abrogated the effects of LINC01503 knockdown on CRC cell proliferation and invasion, which suggested that FOXK1 has an oncogenic role in CRC. This result was consistent with that of a previous study on FOXK1 (25). Therefore, these results demonstrated that LINC01503 promotes CRC progression via enhancing the expression of FOXK1.

In conclusion, the present study was the first, to the best of our knowledge, to demonstrate that LINC01503 promotes CRC cell proliferation and invasion by reducing the formation of the miR-4492/FOXK1 complex through acting as a ceRNA. These results suggested that the LINC01503/miR-4492/FOXK1 interaction may be a promising therapeutic target for CRC.

Acknowledgements

Not applicable.

Funding

No funding received.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

J-ZL, S-RL and QL conceived of and designed the current study, and performed some of the experiments. J-LL, CL and XX collected the samples and performed some of the experiments. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Regarding the use of human samples, the protocol of the present study was approved by the Institutional Ethics Committee of Shanghai East Hospital, Shanghai Tongji University, and all enrolled patients signed a written informed consent document.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Siegel RL, Miller KD and Jemal A: Cancer statistics, 2015. *CA Cancer J Clin* 65: 5-29, 2015.
2. Almasi Z, Rafiemanesh H and Salehiniya H: Epidemiology characteristics and trends of incidence and morphology of stomach cancer in Iran. *Asian Pac J Cancer Prev* 16: 2757-2761, 2015.
3. Krah NM and Murtaugh LC: Differentiation and inflammation: 'Best enemies' in gastrointestinal carcinogenesis. *Trends Cancer* 2: 723-735, 2016.
4. Mercer TR, Dinger ME and Mattick JS: Long non-coding RNAs: Insights into functions. *Nat Rev Genet* 10: 155-159, 2009.
5. Liu B, Ye B, Yang L, Zhu X, Huang G, Zhu P, Du Y, Wu J, Qin X, Chen R, *et al*: Long noncoding RNA lncKdm2b is required for ILC3 maintenance by initiation of Zfp292 expression. *Nat Immunol* 18: 499-508, 2017.
6. Ma Y, Ouyang J, Wei J, Maarouf M and Chen JL: Involvement of host non-coding RNAs in the pathogenesis of the influenza virus. *Int J Mol Sci* 18: E39, 2016.
7. Wei AW and Li LF: Long non-coding RNA SOX21-AS1 sponges miR-145 to promote the tumorigenesis of colorectal cancer by targeting MYO6. *Biomed Pharmacother* 96: 953-959, 2017.
8. Wang H, Zhang M and Sun G: Long non-coding RNA NEAT1 regulates the proliferation, migration and invasion of gastric cancer cells via targeting miR-335-5p/ROCK1 axis. *Pharmazie* 73: 150-155, 2018.
9. Fu X, Zhu X, Qin F, Zhang Y, Lin J, Ding Y, Yang Z, Shang Y, Wang L, Zhang Q and Gao Q: Linc00210 drives Wnt/ β -catenin signaling activation and liver tumor progression through CTNNBIP1-dependent manner. *Mol Cancer* 17: 73, 2018.
10. Xie S, Ge Q, Wang X, Sun X and Kang Y: Long non-coding RNA ZFAS1 sponges miR-484 to promote cell proliferation and invasion in colorectal cancer. *Cell Cycle* 17: 154-161, 2018.
11. Xie JJ, Jiang YY, Jiang Y, *et al*: Increased expression of the long non-coding RNA LINC01503, regulated by TP63, in squamous cell carcinoma and effects on oncogenic activities of cancer cell lines. *Gastroenterology*: 154, 2137-2151, 2018.
12. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2⁻(Delta Delta C(T)) method. *Methods* 25: 402-408, 2001.
13. Han P, Li JW, Zhang BM, Lv JC, Li YM, Gu XY, Yu ZW, Jia YH, Bai XF, Li L, *et al*: The lncRNA CRNDE promotes colorectal cancer cell proliferation and chemoresistance via miR-181a-5p-mediated regulation of Wnt/beta-catenin signaling. *Mol. Cancer* 16: 9, 2017.
14. Wang L, Wang F, Na L, Yu J, Huang L, Meng ZQ, Chen Z, Chen H, Ming LL and Hua YQ: LncRNA AB209630 inhibits gemcitabine resistance cell proliferation by regulating PI3K/AKT signaling in pancreatic ductal adenocarcinoma. *Cancer Biomark* 22: 169-174, 2018.
15. Fu M, Huang Z, Zang X, Pan L, Liang W, Chen J, Qian H, Xu W, Jiang P and Zhang X: Long noncoding RNA LINC00978 promotes cancer growth and acts as a diagnostic biomarker in gastric cancer. *Cell Prolif* 51, Feb 2018.
16. Chen G, Sun W, Hua X, Zeng W and Yang L: Long non-coding RNA FOXD2-AS1 aggravates nasopharyngeal carcinoma carcinogenesis by modulating miR-363-5p/S100A1 pathway. *Gene* 645: 76-84, 2018.
17. Xie CR, Wang F, Zhang S, Wang FQ, Zheng S, Li Z, Lv J, Qi HQ, Fang QL, Wang XM and Yin ZY: Long noncoding RNA HCAL facilitates the growth and metastasis of hepatocellular carcinoma by acting as a ceRNA of LAPT4B. *Mol Ther Nucleic Acids* 9: 440-451, 2017.
18. Shan Z, An N, Qin J, Yang J, Sun H and Yang W: Long non-coding RNA linc00675 suppresses cell proliferation and metastasis in colorectal cancer via acting on miR-942 and Wnt/ β -catenin signaling. *Biomed Pharmacother* 101: 769-776, 2018.
19. Chang Z, Cui J and Song Y: Long noncoding RNA PVT1 promotes EMT via mediating microRNA-186 targeting of Twist1 in prostate cancer. *Gene* 654: 36-42, 2018.
20. Chen D, Wang K, Li X, Jiang M, Ni L, Xu B, Chu Y, Wang W, Wang H, Kang H, *et al*: FOXP1 plays an oncogenic role in the development of esophageal cancer. *Biochem Biophys Res Commun* 494: 88-94, 2017.
21. Li L, Gong M, Zhao Y, Zhao X and Li Q: FOXP1 facilitates cell proliferation through regulating the expression of p21, and promotes metastasis in ovarian cancer. *Oncotarget* 8: 70441-70451, 2017.
22. Zhang P, Tang WM, Zhang H, Li YQ, Peng Y, Wang J, Liu GN, Huang XT, Zhao JJ, Li G, *et al*: MiR-646 inhibited cell proliferation and EMT-induced metastasis by targeting FOXP1 in gastric cancer. *Br J Cancer* 117: 525-534, 2017.
23. Xie R, Wang J, Liu X, Wu L, Zhang H, Tang W, Li Y, Xiang L, Peng Y, Huang X, *et al*: RUFY3 interaction with FOXP1 promotes invasion and metastasis in colorectal cancer. *Sci Rep* 7: 3709, 2017.
24. Chen F, Xiong W, Dou K and Ran Q: Knockdown of FOXP1 suppresses proliferation, migration, and invasion in prostate cancer cells. *Oncol Res* 25: 1261-1267, 2017.
25. Wu M, Wang J, Tang W, Zhan X, Li Y, Peng Y, Huang X, Bai Y, Zhao J, Li A, *et al*: FOXP1 interaction with FHL2 promotes proliferation, invasion and metastasis in colorectal cancer. *Oncogenesis* 5: e271, 2016.