lncRNA LINC00963 downregulation regulates colorectal cancer tumorigenesis and progression via the miR-10b/FGF13 axis

YUJIN WU[1], LONGLING CONG[1], WENJIAN CHEN[1], XUECHUAN WANG[1] and FANGHUA QIU[2]

Departments of [1]Gastroenterology and [2]Hospital Infection Control, The Affiliated TCM Hospital of Guangzhou Medical University, Guangzhou, Guangdong 510130, P.R. China

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Abstract. Long non-coding RNAs (lncRNAs) serve a key role in different types of cancer, including colorectal cancer (CRC). The exact roles and mechanisms underlying lncRNA00963 [long intergenic non-protein coding RNA 963 (LINC00963)] in CRC are not completely understood. The present study aimed to identify the effects and mechanisms underlying LINC00963 in CRC. Firstly, the LINC00963 expression was detected using reverse transcription-quantitative PCR and the results demonstrated that LINC00963 expression levels were significantly increased in CRC tissues and cell lines compared with healthy tissues and HpoEpiC cells, respectively. Online database analysis indicated that high levels of LINC00963 were associated with low survival rates. The results of functional experiments, such as CCK-8 assay, colony formation assay, wound healing assay and Transwell invasion assay, indicated that LINC00963 knockdown significantly inhibited CRC cell proliferation, colony formation, migration and invasion compared with the small interfering RNA (si)-negative control (NC) group. Furthermore, the luciferase reporter indicated that LINC00963 competitively regulated microRNA (miR)-10b by targeting fibroblast growth factor 13 (FGF13). Compared with si-NC, LINC00963 knockdown decreased the expression levels of FGF13, vimentin and N-cadherin, and increased the expression of E-cadherin as detecting by western blotting. miR-10b inhibitors partly attenuated si-LINC00963-induced inhibition of CRC cell proliferation, migration and invasion. Collectively, the results of the present study suggested a potential role of the LINC00963/miR-10b/FGF13 axis in the tumorigenesis and progression of CRC, indicating a novel lncRNA-based diagnostic or therapeutic target for CRC.

Correspondence to: Professor Fanghua Qiu, Department of Hospital Infection Control, The Affiliated TCM Hospital of Guangzhou Medical University, 16 Zhuji Road, Guangzhou, Guangdong 510130, P.R. China
E-mail: fanghua_qiu@163.com

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Introduction

Colorectal cancer (CRC) is one of the most common types of malignant cancer worldwide (1,2). In Asia, a number of countries, including Japan, South Korea, Singapore and Taiwan, where CRC is highly prevalent, have developed population-based screening programs (3). A few potential reasons for the high incidence of CRC include the lack of early diagnosis (4) and the fact that malignant CRC often displays characteristics of rapid progression and invasion, which can result in a poor prognosis (5,6). Therefore, the identification of critical molecules involved in CRC tumorigenesis and progression may aid with the development of targets and biomarkers for antitumor drugs against malignant CRC.

Long non-coding RNAs (lncRNAs) are non-protein-coding RNA transcripts that are >200 nucleotides in length (7-9), with the number of lncRNA genes being much greater compared with protein-coding genes (10,11). The aberrant expression of lncRNAs is associated with a number of different types of cancer, such as breast and lung cancer (12,13), including CRC (14-16). Numerous studies have revealed that lncRNAs serve a key role in various aspects of cancer biology, including proliferation, migration and invasion (17-19). For example, lncRNA RBM5 antisense RNA 1 is involved in the self-renewal of CRC cells (20). Due to their tissue specificity, lncRNAs can be used as diagnostic biomarkers and therapeutic targets for certain types of cancer, such as colorectal and breast cancer (21,22). lncRNA00963 [long intergenic non-protein coding RNA 963 (LINC00963)] is encoded in chromosome 9 and has been reported to serve a key role in different types of cancer; for example, LINC00963 can promote hepatocellular carcinoma progression, and its upregulation can facilitate melanoma progression and invasion (23-26). Additionally, a previous study confirmed that LINC00963 served as a potential prognostic biomarker in melanoma (27). However, the potential effect and mechanism underlying LINC00963 in CRC is not completely understood. Therefore, the present study explored the potential function of LINC00963 in CRC.

Materials and methods

Tissue samples. A total of 28 patients with CRC were recruited at the Department of Gastroenterology, Guangzhou Hospital of Traditional Chinese Medicine (Guangzhou, China).

Samples were collected between February 2018 and October 2018 and the patients ranged in age from 46-78 and comprised 12 men and 16 women. All the samples were from primary colorectal cancer. All patients provided written informed consent. The present study was approved by the Shanghai Tenth People's Hospital Institutional Review Board (approval no. SHSY-IEC-KY-4.0/17-23/01). CRC tissues and corresponding adjacent healthy tissues (5 cm away from the tumor) were collected during surgery, All the histological diagnoses for CRC and normal tissues were reviewed and recognized by two pathologists independently. All tissue specimens were promptly frozen in liquid nitrogen and stored at -80˚C.

Cell culture. A normal colonic epithelial cell line (HcoEpiC) and five CRC cell lines (SW480, SW620, HT-29, HCT116 and LOVO) were obtained from Nanjing Kesheng Biotechnology Co., Ltd. HcoEpiC cells were incubated in Dulbecco's modified Eagle's medium (DMEM) (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin-streptomycin (Gibco; Thermo Fisher Scientific, Inc.). CRC cell lines were incubated in Roswell Park Memorial Institute 1640 (RPMI-1640; Gibco; Thermo Fisher Scientific, Inc.) medium supplemented with 10% FBS and 1% penicillin-streptomycin. All cells were maintained in an incubator at 37˚C with 5% CO_2 in a humidified atmosphere.

Cell transfections. siRNAs targeting LINC00963 (si-LINC00963#1, 5'-GCUCACUGACUUUCUGAATT-3'; si-LINC00963#2, 5'-CCAGACACTGAACTGCCTT-3'; and si-LINC00963#3, 5'-GGCAAGUGCUUUCAACUCUTT-3') and scrambled control (si-NC, 5'-UUCUCCGAACGUGUCACGUTT-3') were synthesized by Shanghai GenePharma Co., Ltd. miR-10b mimics (5'-UACCCUGUAGAACCGAAUUUGUG-3'), miRNA mimics NC (NC mimics; 5'-UCACAACCUCCUAGAAAGAGUAGA-3'), miR-10b inhibitors (5'-CACAAAUUCGGUUCUACAGGGUA-3') and miRNA inhibitors NC (5'-CAGUACUUUUGUGUAGUACAA-3') were purchased from Guangzhou RiboBio Co., Ltd. LINC00963 overexpression and control plasmids (empty vector) were constructed as previously described. HCT116 and LOVO were used for siRNAs and miR-10 mimics transfection. HCT116, LOVO, SW480 and SW620 were used for miR-10 inhibitors and overexpression plasmids transfection. For siRNAs and mimics transfection, cells were transfected with 50 nM siRNA or mimics with 50% cells confluence. For plasmids transfection, cells were transfected with 2 μg overexpression plasmid or control plasmid with 80% cells confluence. All transfections were performed using Lipofectamine® 2000 transfection reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. At 24 h post-transfection with siRNAs or mimics, cells were used for subsequent reverse transcription-quantitative PCR (RT-qPCR) experiments, CCK-8, colony formation assays, Wound healing assay and Transwell assay. At 48 h post-transfection, cells were used for subsequent western blotting experiments.

RT-qPCR. Total RNA was extracted from tissues and all cells (HCoEpiC, SW480, SW620, HT-29, HCT116 and LOVO) using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.). RNA samples were reverse transcribed into cDNA using a Superscript III reverse transcriptase kit (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Subsequently, qPCR was performed using an ABI 7500 Fast RT-PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.) and SYBR Premix Ex Taq (Takara Biotechnology Co., Ltd.). The following primers were used for qPCR: LINC00963 forward, 5'-GCCAAGGAGGGAGTTGTGGCTGC-3' and reverse, 5'-CTGTTGCCACACCATGCACCACTCC-3'; β-actin forward, 5'-GTGGACATCCGCAAAGAC-3' and reverse, 5'-AAAGGGTGTAACGCAACTA-3'; miR-10b forward, 5'-TACCCTGTAGAACCGAATTTG-3' and reverse, 5'-GTGCGTGTCGTGGAGTC-3'; and U6 forward, 5'-CGCTTCACGAATTTGCGT-3' and reverse, 5'-CTCGCTTCGCAGCACA-3'. The PCR conditions: 95˚C 10 min for initial denaturation, 40 cycles of denaturation 15 sec at 95˚C, annealing 30 sec at 60˚C, elongation 30 sec at 72˚C, and final extension for 5 min at 72˚C. The levels of LINC00963 were normalized to β-actin and the levels of miR-10b were normalized to U6. The $2^{-\Delta\Delta Cq}$ method (28) was used to calculate the relative expression levels.

Cell proliferation analysis. For Cell Counting Kit-8 (CCK-8) assays, HCT116 and LOVO cells were seeded into 96-well plates ($4x10^3$ cells/well) in 100 μl complete medium and cultured for 1, 2, 3, 4 and 5 days. At each time point, 10 μl CCK-8 solution (Dojindo Molecular Technologies, Inc.) was added into each well and incubated in 37˚C for 2 h according to the manufacturer's protocol. Subsequently, the absorbance was measured at a wavelength of 450 nm using a microplate reader.

For colony formation assays, cells were seeded into 12-well plates ($5x10^2$ cells/well) and cultured for 10-12 days. Subsequently, colonies were fixed with 4% paraformaldehyde (Sigma-Aldrich; Merck KGaA) for 10 min at room temperature and stained with 0.1% crystal violet (Sigma-Aldrich; Merck KGaA) at room temperature for 10 min, washed with water 3 times and the number of the colonies (>50 cells) were counted manually.

Wound healing assay. HCT116 and LOVO cells were seeded into 6-well plates ($1x10^5$ cells/well) and starved in FBS-free culture medium overnight prior to the assay. Subsequently, when the cell confluence reached ≥95%, a wound was made in the cell monolayer using a 200-μl pipette tip. Cells were incubated in 37˚C with medium supplemented with 2% FBS and representative images were captured under a light microscope (Olympus Corporation; magnification, x100) at 0 and 36 h. Area of the wound was measured at different time points. The newly healed area (wound area at 0 h-wound area at 36 h) was analyzed using ImageJ software (ImageJ bundled with 64-bit Java 1.8.0_172; National Institutes of Health) and the si-NC group was set as 1.

Transwell invasion assay. A Transwell chamber (pore size, 8 μm; Corning, Inc.) was used to perform the invasion assay. HCT116 and LOVO cells ($2x10^5$/well) were cultured in the upper chamber with Matrigel (BD Biosciences), the Matrigel was precoated at 37˚C for 30 min, and complete medium (RPMI-1640) containing 20% FBS was added to the lower

chamber. Following incubation for 36 h at 37˚C, cells adhering to the lower surface of the Transwell membrane were fixed in 20% methanol at room temperature for 15 min and stained with 0.1% crystal violet at room temperature for 10 min. The number of invaded cells was determined from five random fields under an Olympus light microscope (Olympus Corporation; magnification, x100) and analyzed using ImageJ software (ImageJ bundled with 64-bit Java 1.8.0_172; National Institutes of Health).

Luciferase reporter assay. The binding sequences of miR-10b and LINC00963 were predicted using the online databases miRcode (mircode.org) and RNA22 (https://cm.jefferson.edu/rna22/Interactive/). The 3'untranslated regions (UTRs) of LINC00963 [wild-type (WT)-1 and WT-2; mutant (Mut)-1 and Mut-2], containing the miR-10b-binding sequences were inserted into a pmirGL3-basic vector (Promega Corporation) to construct dual luciferase reporter plasmids. Subsequently, HCT116 and LOVO cells were spread on a 24-well plate at 80% confluence and then transfected using Lipofectamine® 2000 transfection reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol with WT-1, WT-2, Mut-1 or Mut-2 and NC mimics or miR-10b mimics (the concentration of plasmids were 0.5 μg and the mimics were 50 nM). At 48 h post-transfection, luciferase activity was detected using a Dual Luciferase Reporter Gene Assay kit (Beyotime Institute of Biotechnology) according to the manufacturer's protocol. The relative firefly luciferase activity was normalized to *Renilla* luciferase activity.

Western blotting. HCT116 and LOVO cells were lysed with RIPA lysis buffer (Beyotime Institute of Biotechnology) containing protease inhibitors and quantified via BCA protein assay (Beyotime Institute of Biotechnology). Total protein (25 μg/lane) was separated via 8-15% SDS-PAGE and transferred to PVDF membranes. Following blocking with 5% skimmed milk for 60 min at room temperature, membranes were incubated overnight with primary antibodies targeted against: FGF13 (cat. no. ab186300; Abcam; dilution 1:1,000), E-cadherin (cat. no. 14472; Cell Signaling Technology, Inc. dilution 1:1,000), vimentin (VIM; cat. no. 5741; Cell Signaling Technology, Inc.; dilution 1:1,000), N-cadherin (cat. no. ab18203; Abcam; dilution 1:1,000) and GAPDH (cat. no. 5174; Cell Signaling Technology, Inc.; dilution 1:5,000) at 4˚C. Following primary incubation, the membranes were incubated with fluorescence-conjugated secondary antibodies Alexa Fluor Plus 800 (cat. no. A32730; Thermo Fisher Scientific, Inc.; dilution 1:10,000) for 30 min at room temperature. Protein bands were detected using a two-color infrared laser imaging system (Odyssey; Li-Cor Biosciences). GAPDH was used as the loading control.

Flow cytometry analysis of apoptotic cells. HCT116 and LOVO cells were transfected with si-LINC00963 and si-NC. At 48 h post-transfection, cells were suspended ($1x10^6$ cells/ml) in incubation buffer. Cells were incubated in a reagent containing Annexin V-FITC and propidium iodide according to the manufacturer's protocol (FITC Annexin V Apoptosis Detection Kit; BD Biosciences) at room temperature for 15 min in the dark. Apoptotic cells (early + late apoptotic cells) were analyzed via flow cytometry software (BD FACSDiva Software v6.1.3; BD Biosciences) using a CYTOMICS FC 500 flow cytometer (Beckman Coulter, Inc.).

Online database analysis. The expression of LINC00963 was analyzed using an online database (gepia.cancer-pku.cn/index.html).

Statistical analysis. Each experiment was performed ≥3 times. Data are presented as the mean ± standard deviation. Statistical analyses were performed using SPSS software (version no. 22.0; IBM Corp.). Continuous variables were analyzed using paired Student's t-test or one-way ANOVA followed by Tukey's post hoc test. The correlation between the expression levels of LINC00963 and miR-10b were analyzed using Pearson's correlation coefficient. Overall survival rates were calculated using the Kaplan-Meier method with the log-rank test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

LINC00963 is upregulated in CRC tissues and cells, and its upregulation is associated with a low survival rate. To investigate the role of LINC00963 in CRC, the expression levels of LINC00963 were analyzed in 28 pairs of CRC tissues and adjacent healthy tissues via RT-qPCR. The results indicated that the expression of LINC00963 was significantly upregulated in CRC tissues compared with healthy tissues (Fig. 1A). Additionally, by analyzing an online database (gepia.cancer-pku.cn/index.html), the results suggested that high LINC00963 expression was associated with a low survival rate and low LINC00963 expression was associated with a high survival rate (Fig. 1B). Subsequently, the expression of LINC00963 in the CRC cell lines (SW480, SW620, HT-29, HCT116 and LOVO) compared with the normal colonic epithelial cell line HcoEpiC was analyzed. As presented in Fig. 1C, the expression of LINC00963 in all CRC cell lines was significantly higher compared with HcoEpiC cells. HCT116 and LOVO cells were selected for subsequent experiments as these cell lines displayed the highest LINC00963 expression levels among the CRC cell lines. Overall, the results indicated that LINC00963 was upregulated in human CRC.

LINC00963 knockdown inhibits cell proliferation, colony formation, migration and invasion in vitro. To explore the regulatory effect of LINC00963 on CRC cell progression, loss-of-function experiments were performed by knocking down LINC00963 expression levels. Compared with transfection with si-NC, a significant decrease in LINC00963 expression was observed following transfection with si-LINC00963#1, si-LINC00963#2 and si-LINC00963#3 (Fig. 2A). Cells transfected with si-LINC00963#3 were used for subsequent experiments due to the optimal transfection efficiency of the siRNA. Subsequently, the effect of LINC00963 knockdown on cell proliferation was examined by performing CCK-8 and colony formation assays. The results indicated that LINC00963 knockdown significantly inhibited cell proliferation compared with the si-NC group (Fig. 2B-D). Furthermore, the effect of LINC00963 on cell migration and invasion was

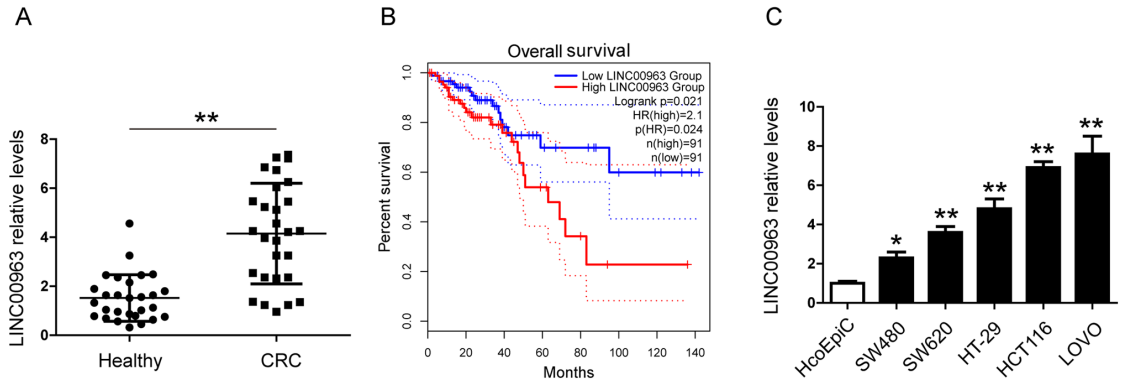

Figure 1. LINC00963 is upregulated in human CRC tissues and cell lines. (A) LINC00963 expression levels in 28 pairs of CRC tissues and adjacent healthy tissues. **P<0.01. (B) Survival curve analysis was performed using an online database, with low and high risks indicated in blue and red, respectively. (C) LINC00963 expression levels in a normal colonic epithelial cell line (HcoEpiC) and five CRC cell lines. Data are presented as the mean ± standard deviation. *P<0.05 and **P<0.01. LINC00963, long intergenic non-protein coding RNA 963; CRC, colorectal cancer.

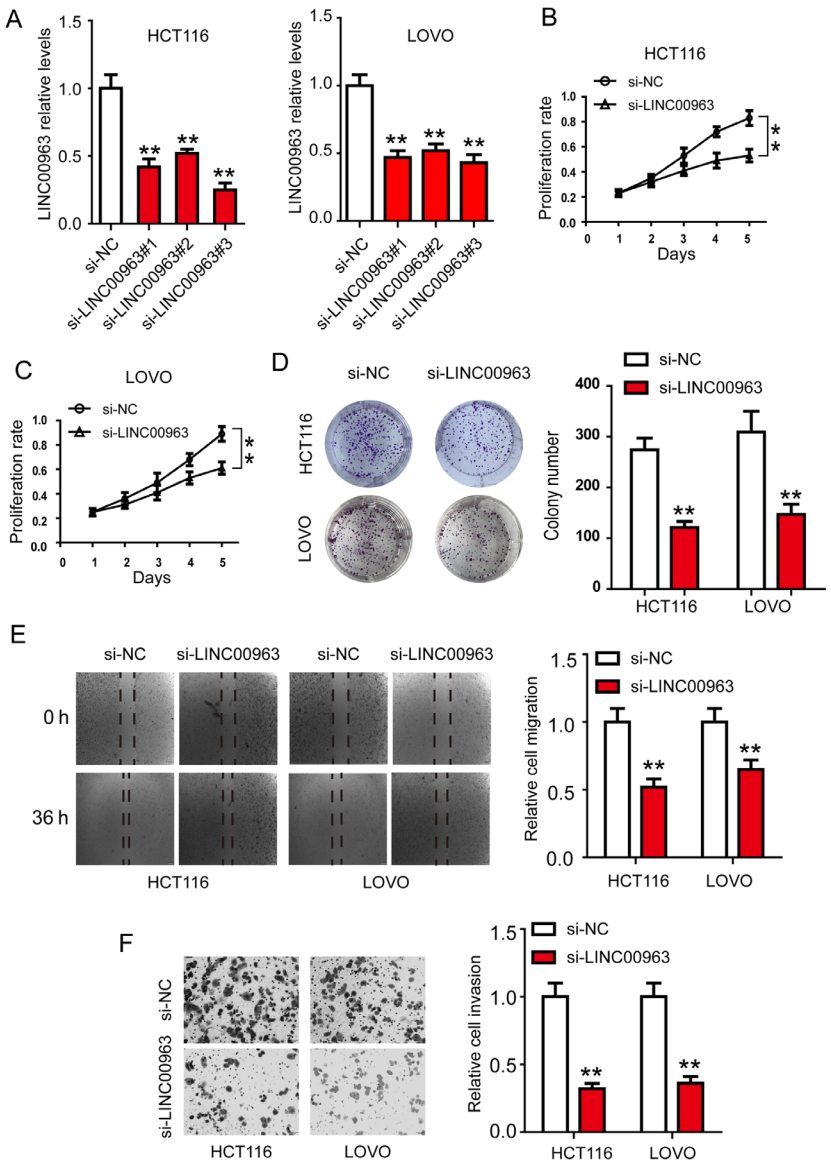

Figure 2. LINC00963 knockdown inhibits proliferation, colony formation, migration and invasion in CRC cell lines. HCT116 and LOVO cells were transfected with si-LINC00963 (si-LINC00963#1, si-LINC00963#2 and si-LINC00963#3) and si-NC. (A) LINC00963 expression levels were measured via reverse transcription-quantitative PCR. Cell Counting Kit-8 assays were performed to assess (B) HCT116 and (C) LOVO cell proliferation. (D) Colony formation assays were performed to assess HCT116 and LOVO cell proliferation, magnification, x1. (E) Wound healing and (F) Transwell assays were performed to evaluate the effect of LINC00963 on CRC cell migration and invasion, magnification, x100. Data are presented as mean ± standard deviation. **P<0.01. LINC00963, long intergenic non-protein coding RNA 963; CRC, colorectal cancer; si, small interfering RNA; NC, negative control.

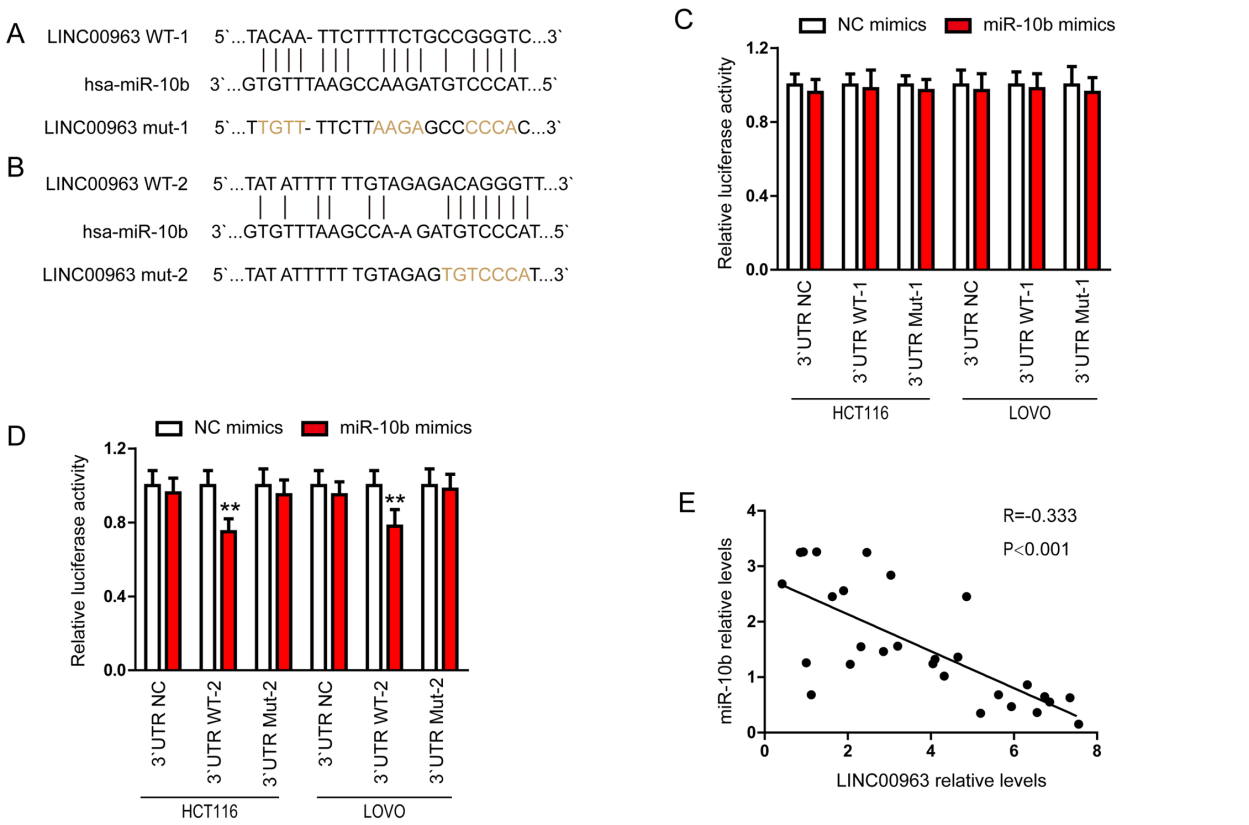

Figure 3. LINC00963 directly binds to miR-10b in CRC. The potential binding sites of miR-10b within the (A) WT-1, Mut-1, (B) WT-2 and Mut-2 sequences in LINC00963. Dual luciferase reporter assays were performed to assess the luciferase activity of HCT116 and LOVO cells transfected with (C) WT-1, Mut-1, (D) WT-2 and Mut-2. (E) Spearman's correlation analysis of the expression levels of LINC00963 and miR-10b in CRC tissues. Data are presented as mean ± standard deviation. **P<0.01. LINC00963, long intergenic non-protein coding RNA 963; CRC, colorectal cancer; miR, microRNA; WT, wild-type; Mut, mutant.

assessed by performing wound healing and Transwell assays, respectively. Compared with the si-NC group, cell migration and invasion were significantly decreased following LINC00963 knockdown (Fig. 2E and F). Additionally, cell apoptosis following LINC00963 knockdown was assessed; however, the results indicated that LINC00963 knockdown did not markedly alter cell apoptosis compared with the si-NC group (Fig. S1). Collectively, the results indicated that LINC00963 knockdown inhibited CRC cell proliferation, colony formation, migration and invasion, indicating that LINC00963 may serve as an oncogene in CRC.

LINC00963 is a sponge of miR-10b. To further explore the molecular mechanism underlying LINC00963 in CRC, online databases, including miRcode and RNA22, were analyzed to predict potential miRNAs that directly bound to LINC00963. miR-10b was identified as a promising target of LINC00963 and two potential binding sequences between miR-10b and LINC00963 were reported (Fig. 3A and B). Subsequently, luciferase reporter vectors containing WT-1 and WT-2 or Mut-1 and Mut-2 miR-10b-binding sequences in LINC00963 were co-transfected into CRC cell lines with miR-10b mimics or NC mimics. The transfection efficiency of miR-10b mimics and miR-10b inhibitors was detected (Fig. S2). Luciferase activity was significantly decreased in both cell lines co-transfected with LINC00963 WT-2 and miR-10b mimics compared with cells co-transfected with LINC00963 WT-2 and NC mimics, which verified the interaction between LINC00963 and miR-10b (Fig. 3C and D). Additionally, a negative linear correlation was identified between LINC00963 and miR-10b expression levels in CRC tissues using Pearson's correlation analysis (Fig. 3E). Furthermore, the effects of miR-10b overexpression, LINC00963 overexpression and LINC00963 knockdown on LINC00963 and miR-10b expression levels were determined (Fig. S3A-D). Compared with NC group, the expression of LINC00963 was downregulated in HCT116 and LOVO cells with miR-10b overexpression. The expression of miR-10b was downregulated in the LINC00963 overexpression group, but upregulated in the si-LINC00963 group, compared with their own control group. Therefore, the results suggested that LINC00963 served as a sponge of miR-10b in CRC.

LINC00963 knockdown inhibits CRC progression via the miR-10b/FGF13 axis. Rescue experiments were performed following the confirmation of the direct interaction between miR-10b and LINC00963. Subsequently, si-LINC00963 and miR-10b inhibitors were co-transfected into HCT116 and LOVO cell lines, and cell proliferation, migration and invasion were assessed by performing CCK-8, wound healing and Transwell assays. The results indicated that miR-10b inhibitors partly reversed the suppressive effect of si-LINC00963 on cell proliferation, migration and invasion (Fig. 4A-D). It has been previously reported that miR-10b suppresses CRC cell proliferation and metastasis by targeting FGF13 (29). Therefore, the

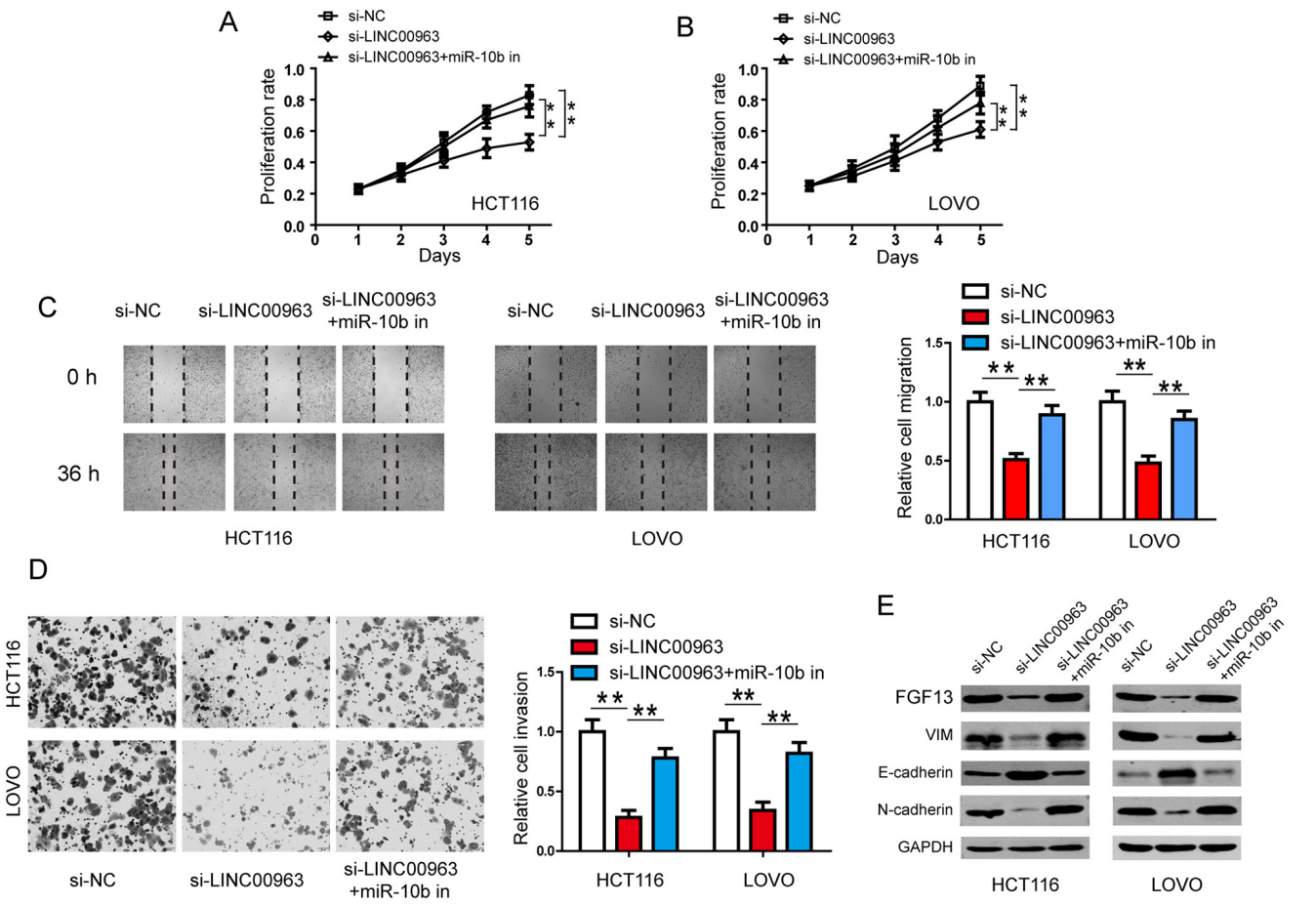

Figure 4. LINC00963-mediated effects are associated with the miR-10b/FGF13 axis. Cell Counting Kit-8 assays were performed to assess (A) HCT116 and (B) LOVO cell proliferation following transfection with si-NC, si-LINC00963 or si-LINC00963 + miR-10b. (C) Wound healing and (D) Transwell assays were conducted to assess HCT116 and LOVO cell migration and invasion, respectively, following transfection with si-NC, si-LINC00963 or si-LINC00963 + miR-10b-magnification, x100. (E) FGF13, VIM, E-cadherin and N-cadherin protein expression levels were measured via western blotting. Data are presented as mean ± standard deviation. **P<0.01. LINC00963, long intergenic non-protein coding RNA 963; miR, microRNA; FGF13, fibroblast growth factor 13; si, small interfering RNA; NC, negative control; miR-10b in, miR-10b inhibitors; VIM, vimentin.

expression levels of FGF13 and three epithelial-mesenchymal transition markers (VIM, E-cadherin and N-cadherin) were detected via western blotting in HCT116 and LOVO cell lines transfected with si-LINC00963 or co-transfected with si-LINC00963 and miR-10b inhibitors. The results indicated that FGF13 and VIM and N-cadherin expression levels were decreased, whereas the expression levels of E-cadherin were upregulated following LINC00963 knockdown compared with the si-NC group; however, si-LINC0093-mediated effects were reversed by co-transfection with miR-10b inhibitors (Fig. 4E). Furthermore, the expression of FGF13 following miR-10b overexpression was examined (Fig. S3E). Compared with the NC group, the expression of FGF13 was downregulated in the miR-10b overexpression group. In summary, the results indicated that LINC00963 served as an oncogene via inhibition of miR-10b and by targeting FGF13.

Discussion

As next-generation sequencing has improved, research has revealed numerous non-coding RNAs (ncRNAs) in diverse organisms and diseases (30,31), including cancer (32). Regulatory ncRNAs can be broadly classified into two major classes: Short ncRNAs and lncRNAs (33). lncRNAs are one of the most important ncRNAs that were initially considered as 'junk genes' (34); however, lncRNAs have been demonstrated to be involved in the regulation of tumorigenesis and progression as tumor suppressor genes or oncogenes (35-37).

LINC00963, an intergenic lncRNA, was first reported as a novel non-coding RNA involved in the transition of prostate cancer from androgen dependence to androgen independence (38). Yu *et al* (39) revealed that LINC00963 facilitates lung cancer metastasis via the phosphoglycerate kinase 1-activated AKT/mTOR signaling pathway (39). Additionally, LINC00963 has been demonstrated to promote hepatocellular carcinoma progression by activating the PI3K/AKT signaling pathway (23). LINC00963 has been reported to promote tumorigenesis and radioresistance in breast cancer by sponging miR-323-3p and inducing activated cell division cycle 42-associated kinase 1 expression (24). Furthermore, LINC00963 has been demonstrated to facilitate proliferation and invasion in osteosarcoma by suppressing the miR-204-3p/fibronectin 1 axis (26). Jiao *et al* (27) reported that LINC00963 upregulation predicted poor prognosis in melanoma and facilitated progression via the miR-608/nucleus accumbens-associated protein 1 signaling pathway. A recent

study reported that LINC00963 inhibited cutaneous squamous cell carcinoma progression by targeting SOX4 (25). However, the exact functions and mechanism underlying LINC00963 in CRC are not completely understood. To the best of our knowledge, the present study reported for the first time that LINC00963 was upregulated in human CRC tissues and cell lines compared with healthy tissues and HCoEpiC cells, respectively, via RT-qPCR. Moreover, high LINC00963 expression levels were associated with low survival rates in patients with CRC, which was consistent with a previous study investigating melanoma (27). The results indicated that LINC00963 knockdown significantly inhibited CRC cell proliferation, colony formation, migration and invasion compared with si-NC. The function of LINC00963 in CRC identified in the present study was similar to that identified in other tumors, such as osteosarcoma (26). Furthermore, the effect of LINC00963 knockdown on CRC cell apoptosis was assessed; however, there was no notable difference between the si-LINC00963 and si-NC groups.

Numerous studies have revealed various potential biological mechanisms underlying lncRNAs and the theory of competing endogenous RNAs (ceRNAs) is currently one of the generally accepted mechanisms (21,22,40). ceRNAs bind directly to miRNAs and influence the expression of target mRNAs, which affect cellular processes, such as cell proliferation, migration and invasion (41-43). LINC00963 has been previously identified as a ceRNA in several types of cancer. For instance, in breast cancer and melanoma, LINC00963 has been reported to function as a ceRNA for miR-324-3p and miR-608 (24,27). In the present study, it was hypothesized that LINC00963 regulated CRC progression by binding to miRNAs. In a previous study, bioinformatics analysis predicted that miR-10b, which has been reported to suppress the growth and metastasis of CRC, was a potential gene on binding to LINC00963 on the online databases (29). Interestingly, the present study identified two miR-10b binding sites within LINC00963; however, only one binding site was verified by the luciferase reporter assay results.

Generally, miRNAs regulate the expression of target genes by binding to their 3'UTRs (44). miR-10b has been reported to directly target FGF13, leading to suppression of the growth and metastasis of CRC (29). FGF13 is overexpressed in several types of cancer, such as melanomas and pancreatic endocrine tumors (45,46). Moreover, FGF13 was significantly associated with the occurrence of liver metastasis and serves as a novel prognostic marker for the prediction of poorer outcomes in neuroendocrine tumors (46). Bublik *et al* (47) indicated that the role of FGF13 allowed tumor cells to proliferate under stress-inducing conditions. Furthermore, Song and Li (29) revealed that FGF13 overexpression rescued the suppressive effects of miR-10b on CRC cell proliferation, migration and invasion. The results of the present study demonstrated that LINC00963 knockdown significantly decreased the expression levels of FGF13, VIM and N-cadherin, but increased E-cadherin expression levels compared with the si-NC group. Additionally, miR-10b inhibitors attenuated si-LINC00963-mediated effects on CRC cell proliferation, migration and invasion. The *in vitro* experiments indicated that the miR-10b/FGF13 axis may be involved in the anti-CRC effects of si-LINC00963. However, further investigations using *in vivo* models should be conducted to verify the results of the present study.

In summary, to the best of our knowledge, the present study suggested for the first time that LINC00963 was upregulated in CRC tissues and cell lines compared with healthy tissues and HcoEpiC cells, respectively, and high LINC00963 expression levels were associated with low survival rates. The results indicated that LINC00963 knockdown was associated with the regulation of tumorigenesis and progression in CRC. Additionally, miR-10b was identified as a novel target of LINC00963 and the results demonstrated that si-LINC00963 significantly decreased the expression levels of FGF13 compared with NC mimics. In conclusion, the present study indicated that the LINC00963/miR-10/FGF13 axis served an important role in CRC, indicating a novel diagnostic and therapeutic target for CRC.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

FQ and YW designed the present study, YW and LC collected the tissue samples, YW, WC and XW performed the cell experiments, LC and XW performed the molecular experiments and FQ analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All patients provided written informed consent. This study was approved by the Shanghai Tenth People's Hospital Institutional Review Board (approval no. SHSY-IEC-KY-4.0/17-23/01).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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