Long non-coding RNA ATB is associated with metastases and promotes cell invasion in colorectal cancer via sponging miR-141-3p

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Received April 7, 2020; Accepted August 21, 2020

DOI: 10.3892/etm.2020.9391

Abstract. Long non-coding RNAs (lncRNAs) serve crucial roles in cancer development and progression. lncRNA-activated by transforming growth factor-β (lncRNA-ATB) mediates cell proliferation. However, the association between lncRNA-ATB and human colorectal cancer (CRC) is not completely understood. Therefore, the present study aimed to investigate the role of lncRNA-ATB in CRC, as well as the underlying mechanism. 50 pairs of tumor tissues and adjacent normal tissues from patients with primary CRC were collected. The expression of lncRNA-ATB and microRNA (miR)-141-3p in CRC tissues, adjacent normal tissues and cell lines was detected using reverse transcription-quantitative PCR. CCK-8, colony formation, Transwell, western blot, dual luciferase reporter gene, RNA immunoprecipitation and immunohistochemistry staining assays were conducted to assess the biological function of lncRNA-ATB and miR-141-3p in CRC progression. lncRNA-ATB was upregulated in CRC tissues and cell lines compared with healthy tissues and cells, respectively. Moreover, high expression of lncRNA-ATB was significantly associated with advanced TNM stage and metastasis in CRC. In addition, the results indicated that lncRNA-ATB expression predicted the prognosis and overall survival of patients with CRC. Compared with small interfering RNA-negative control, lncRNA-ATB knockdown inhibited CRC cell proliferation, migration and invasion, whereas, compared with vector, lncRNA-ATB overexpression promoted CRC cell proliferation, migration and invasion. Furthermore, the *in vivo* experiment suggested that lncRNA-ATB knockdown inhibited tumor growth. The results also indicated that lncRNA-ATB may contribute to CRC progression via binding to tumor suppressor microRNA-141-3p. Collectively, the present study suggested a crucial role of lncRNA-ATB in CRC tumorigenesis, suggesting that lncRNA-ATB may serve as an important marker for the diagnosis and development of CRC.

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Key words: long non-coding RNA, lncRNA-activated by transforming growth factor-β, colorectal cancer, microRNA-141-3p, prognosis

Introduction

Colorectal cancer (CRC) is the third most common malignancy worldwide (1). Despite advances in therapeutic strategies, the overall survival rate of patients (15-50%) with CRC remains poor (2,3). CRC is a complex process characterized by multiple genomic variations and an aberrant biological microenvironment (4). Although previous studies have reported that alterations in numerous oncogenes and cancer-suppressor genes are correlated with CRC, the biological molecular mechanism underlying CRC development and progression is not completely understood (5-8). Therefore, developing an effective strategy for the early diagnosis and treatment of CRC is important.

Only 2% of the human genome is protein encoding, whereas the remaining genome consists of non-coding RNA (9,10). Previous studies have identified the role of non-protein-coding genes in normal physiological processes and the etiopathogenesis of diseases, such as cancer (11-13). Long non-coding RNAs (lncRNAs), which are >200 nucleotides in length, lack protein coding abilities (14). Numerous lncRNAs are involved in regulating gene expression, cell differentiation, ontogenetical processes and epigenetic modification, as well as other cellular biological processes (15-18). lncRNAs have also been identified as diagnostic and prognostic biomarkers, and serve critical roles in cancer progression-related signaling pathways. For example, lncRNA-linc00152 inhibited by microRNA (miR)-376c-3p suppresses colorectal cancer cell proliferation and induces apoptosis (19).

lncRNA-activated by transforming growth factor β (lncRNA-ATB) has been identified as an oncogene (20). Abnormal expression of lncRNA-ATB is observed in various malignant tumors, such as breast, colon and lung cancer,

as well as hepatocellular carcinoma (20). For instance, Wei *et al* (21) demonstrated that lncRNA-ATB increased lung cancer cell proliferation and metastasis by stimulating the p38 signaling pathway. In colon cancer, lncRNA-ATB promotes cancer metastasis via inhibition of E-cadherin, and serves as a predictor of poor prognosis (22). Previous studies have reported the biological functions of lncRNA-ATB in CRC. For example, Gao *et al* (23) investigated the role of lncRNA-ATB in CRC cell proliferation and apoptosis, and indicated that lncRNA-ATB promotes CRC cell proliferation and suppresses apoptosis via downregulation of miR-200c. Moreover, Yang *et al* (24) reported that lncRNA-ATB maintained CRC cell stemness by suppressing the β-catenin signaling pathway. A further previous study identified that higher expression of lncRNA-ATB was correlated with CRC metastasis (25). However, to the best of our knowledge, the mechanism underlying lncRNA-ATB-mediated promotion of CRC metastasis is not completely understood.

The present study aimed to investigate the association between the expression of lncRNA-ATB and the clinical characteristics and prognosis of patients with CRC. The biological function of lncRNA-ATB in CRC *in vitro* and *in vivo* was also investigated. Therefore, the results of the present study might provide novel insight into the biological function of lncRNA-ATB in CRC.

Materials and methods

Tissue samples. A total of 50 tumor and adjacent non-cancerous tissues (~2 cm distance from the tumor margin) were obtained from patients (mean age, 58) who underwent tumor resection surgery at Shenzhen People's Hospital, The Second Clinical Medical College of Jinan University between February 2017 and September 2019. Patients did not receive any radiotherapy or chemotherapy prior to surgery, which was the inclusion criteria. The exclusion criteria were patients who received radiotherapy or chemotherapy prior to surgery. All tissues were pathologically confirmed via histopathology. Written informed consent was obtained from all patients. The present study was approved by Shenzhen People's Hospital Medical Ethics Committee (approval no. HH-TD-2017388).

Cell lines. A total of five CRC cell lines (CaCO-2, HT-29, SW480, LoVo and HCT116 cells) and a normal intestinal mucous cell line (CCC-HIE-2) were purchased from The Cell Bank of Type Culture Collection of the Chinese Academy of Sciences. The HT-29 cell line was authenticated as a colorectal cancer cell line by STR profiling, which was performed by the supplier. Cells were cultured in DMEM (Sigma-Aldrich; Merck KGaA) supplemented with 10% FBS (Thermo Fisher Scientific, Inc.), and 1% penicillin and streptomycin (Sigma-Aldrich; Merck KGaA) at 37˚C with 5% CO_2.

RNA isolation and reverse transcription-quantitative PCR (RT-qPCR). Total RNA was isolated from samples using TRIzol® (Sigma-Aldrich; Merck KGaA). Total RNA was reversed transcribed into cDNA using PrimeScript™ RT Master Mix (Takara Biotechnology Co., Ltd.) according to the manufacturer's instructions. The temperature protocol using for RT was as follows: 37˚C for 15 min and 85˚C for 5 sec. Subsequently, qPCR was performed using SYBR-Green Supermix (Bio-Rad Laboratories, Inc.) and the Bio-Rad CFX 96 RT PCR system (Bio-Rad Laboratories, Inc.). The thermocycling conditions used for qPCR were as follows: Step 1: 95˚C for 30 sec; step 2: 95˚C for 5 sec, 60˚C for 30 sec (repeated for 39 cycles); step 3: 95˚C for 10 sec followed by 65˚C to 95˚C in 0.5˚C/5 sec increments. The following primers were used for qPCR: miR-141-3p (26) forward, 5'-CGTCGCTAACACTGTCTGGTAA-3' and reverse, 5'-GTGCAGGGTCCGAGGTATTC-3'; U6 forward, 5'-GCTTCGGCAGCACATATACTAAAAT-3' and reverse, 5'-CGCTTCACGAATTTGCGTGTCAT-3'; lncRNA-ATB (27) forward, 5'-ACAAGCTGTGCAGTCTCAGG-3' and reverse, 5'-CTAGGCCCAAAGACAATGGA-3'; and GAPDH forward, 5'-AGCAAGAGCACAAGAGGAAG-3' and reverse, 5'-GGTTGAGCACAGGGTACTTT-3'. mRNA and miRNA expression levels were normalized to the internal reference genes GAPDH and U6, respectively. Quantification of mRNA and miRNA expression was performed by using the $2^{-\Delta\Delta Cq}$ method (28).

Transfection. Cells were seeded ($2x10^5$ cells/well) into 6-well plates and cultured for 24 h at 37˚C with 5% CO_2. HT-29 cells were transfected with 100 nM of lncRNA-ATB small interfering (si)RNA (si-ATB; 5'-ATAAGAGCCCTTGGTCCTTAA-3') or negative control (NC; si-NC; 5'-GATTTACCAGAGAATAATCTA-3'; both provided by Sigma-Aldrich; Merck KGaA) using Lipofectamine® 3000 (Thermo Fisher Scientific, Inc.). HCT116 cells were transfected with 0.5 μg/μl of lncRNA-ATB overexpression plasmid or pcDNA3.1 vector (Both overexpression plasmid and empty vector were purchased from Shanghai GenePharma Co., Ltd.). HCT116 cells ($2x10^5$ cells/well in 6-well plate) were transfected with 50 nM miR-141-3p mimics (5'-UAACACUGUCUGGUAAAGAUGG-3') or mimics NC (5'-AGCCGCACUGUACGAUGCUAUGA-3'; both obtained from Guangzhou RiboBio Co., Ltd.) using Lipofectamine 3000. At 48 h post-transfection, cells were used for subsequent experiments.

Cell proliferation assay. Transfected cells were seeded ($4x10^3$ cells/well) into 96-well plates. To assess cell proliferation, a Cell Counting Kit-8 (CCK-8) assay (Beyotime Institute of Biotechnology) was performed according to the manufacturer's protocol.

For the colony formation assay, transfected cells were seeded ($4x10^2$ cells/well) into 6-well plates and cultured at 37˚C with 5% CO_2 for 2 weeks. Following staining with 0.2% crystal violet for 30 min at room temperature, visible colonies (>50 cells) were counted under a light microscope (x40 magnification).

Migration and invasion assay. Transwell assays were performed to assess CRC cell migration and invasion. In the Transwell chamber (8-μm pore size; EMD Millipore), cells ($1x10^4$) in 100 μl serum-free medium were plated in the upper chambers, which were pre-coated with Matrigel at 37˚C with 5% CO_2 for 30 min for the invasion assay. In the lower chamber, 600 μl culture medium supplemented with 10% FBS was plated. Following incubation for 24 h at 37˚C with 5% CO_2, cells on the upper surface of the membrane were removed with a cotton swab. Invading and migratory cells were fixed with 4% formaldehyde for 15 min at room temperature and stained with 0.2% crystal violet for 30 min at

room temperature. Stained cells were visualized using a light microscope (x200 magnification).

Western blotting. Total protein was extracted from cells using RIPA buffer (Thermo Fisher Scientific, Inc.) and quantified using a Bicinchoninic Acid Protein Assay kit (Thermo Fisher Scientific, Inc.). A total of 50 μg of protein was loaded per lane. Proteins were separated via 10% SDS-PAGE and transferred to PVDF membranes (EMD Millipore), which were blocked with 5% skim milk at room temperature for 1 h. Subsequently, the membranes were incubated overnight at 4˚C with primary antibodies targeted against: E-Cadherin (cat. no. 14472; 1:1,000; Cell Signaling Technology, Inc.), Vimentin (cat. no. 5741; 1:1,000; Cell Signaling Technology, Inc.) and β-Actin (cat. no. 4970; 1:1,000; Cell Signaling Technology, Inc.). Following primary incubation, the membranes were incubated for 1 h at room temperature with HRP-conjugated anti-mouse IgG (cat. no. 7076; 1:2,000; Cell Signaling Technology, Inc.) and anti-rabbit IgG (cat. no. 7074; 1:2,000; Cell Signaling Technology, Inc.) secondary antibodies. Protein bands were visualized using an enhanced chemiluminescence reagent (Bio-Rad Laboratories, Inc.) with ChemiDoc MP imaging system (Bio-Rad Laboratories, Inc.). Densitometry was measured using Image Lab Touch Software, version 2.4 (Bio-Rad Laboratories, Inc.). β-actin was used as the loading control.

Tumor formation in nude mice. A total of 12 BALB/c-nu/nu nude mice (male; age, 4-6 weeks; weight, ~20 g) were supplied by SLAC National Accelerator Laboratory. Mice were housed in climate-controlled (18-23˚C) research laboratory with 40-60% humidity and 12-h light/dark cycle. Food and water were accessible at all times. The animal experiments were approved by Shenzhen People's Hospital Medical Ethics Committee (approval no. SZRMYY20190211). All animal experiments were performed under the Guidelines for the Care and Use of Laboratory Animals of Shenzhen People's Hospital, The Second Clinical Medical College of Jinan University. The mice were randomly divided into two groups (n=5 per group), an si-ATB group injected with si-lncRNA-ATB-transfected HT-29 cells and the other group was negative control (NC) group, injected with si-lncRNA-NC-transfected HT-29 cells. si-lncRNA-ATB- or si-NC-transfected HT-29 cells ($5x10^6$) were mixed with 0.2 ml Matrigel Matrix (BD Biosciences) and injected into the subcutaneous tissue of the back near the right forelimb of the mouse. After 12 days, the tumors were palpable (~100 mm^3). Body weight and tumor size were measured every 3 days using an electronic balance and caliper, respectively. Tumor volume was calculated according to the following formula: $length x width^2/2$. On day 33, mice were anesthetized with an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg), and sacrificed by cervical dislocation. Death was verified by the absence of vital signs. The tumors were excised for further analysis.

lncRNA target prediction and dual luciferase reporter gene assay. lncRNA-ATB target prediction was performed using StarBase v2.0 (starbase.sysu.edu.cn) (29,30). pmirGLO-ATB-wild-type (WT) and pmirGLO-ATB-mutant (Mut) were constructed by Guangzhou RiboBio Co., Ltd. 293 cells were purchased from the Chinese Academy of Sciences Cell Bank. Cells were seeded into 96-well plate ($1x10^4$ cells/well) and co-transfected with 200 ng of pmirGLO-ATB-WT or pmirGLO-ATB-Mut and 50 nM of miR-141-3p mimics (5'-UAACACUGUCUGGUAAAGAUGG-3') or miR-NC (5'-AGCCGCACUGUACGAUGCUAUGA-3'; both obtained from Guangzhou RiboBio Co., Ltd.) using Lipofectamine® 3000. At 48 h post-transfection, relative luciferase activity was measured using the Dual Luciferase Reporter Assay system (Promega Corporation). *Renilla* luciferase activity was used to normalize firefly luciferase activity.

RNA immunoprecipitation assays (RIP). The Ago antibody (cat. no. ab186733, 1:30) and the normal mouse IgG antibody (cat. no. ab188776; 1:30) were purchased from Abcam. The RIP experiment was performed using a Magna RIP RNA-Binding Protein Immunoprecipitation kit (EMD Millipore) according to the manufacturer's protocol. The normal mouse IgG was used as the NC.

Immunohistochemistry. Tumors were fixed by using 4% paraformaldehyde at room temperature for 12 h. An ascending alcohol series was used for dehydration. Subsequently, tumors were embedded with paraffin. Xenograft tumors were sliced into ~5 μm thick sections. Sections were blocked with Immunol staining blocking buffer (cat. no. P0102; Beyotime Institute of Biotechnology) at room temperature for 1 h and then incubated with an anti-Ki67 primary antibody (cat. no. 12202; 1:400; Cell Signaling Technology, Inc.) at 4˚C overnight. Subsequently, sections were incubated with a secondary antibody (cat. no. 31822; 1:1,000; Invitrogen; Thermo Fisher Scientific, Inc.) at room temperature for 1 h. A DAB kit (cat. no. P0203; Beyotime Institute of Biotechnology) was used to identify the positive rate of Ki67. Images were obtained under a light microscope (x100 magnification) and analyzed using Image Pro-Plus software (version 6.0; Media Cybernetics, Inc.).

Statistical analysis. Statistical analyses were performed using GraphPad Prism software (version 7.0; GraphPad Software, Inc.). Data are presented as the mean ± SD (n=3). Comparisons between tumor and adjacent non-cancerous tissues were analyzed using a paired Student's t-test. Comparisons between two groups were analyzed using an unpaired Student's t-test. Comparisons among multiple groups were analyzed using one-way ANOVA followed by Tukey's post hoc test. Patient characteristics were analyzed using the χ^2 test. The relationship between lncRNA-ATB expression and overall survival was assessed via Kaplan Meier analysis, and survival curves were compared using the log-rank test. The correlation between lncRNA-ATB and miR-141-3p expression in CRC tissues was analyzed using Spearman's correlation analysis. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Upregulation of lncRNA-ATB in CRC tissues and its implication in cancer progression. RT-qPCR was performed to determine lncRNA-ATB expression levels in 50 paired CRC tissues and corresponding healthy tissues. lncRNA-ATB expression was significantly increased in CRC tissues compared with healthy tissues ($P<0.05$; Fig. 1A). Subsequently, patients were divided into high ATB expression (n=25) and

Table I. Association between lncRNA-ATB expression and clinicopathological features.

Variable	Relative lncRNA-ATB expression level		P-value
	High (n=25)	Low (n=25)	
Gender			0.0801
Male	15	11	
Female	10	14	
Age			0.5920
≤60	13	14	
>60	12	11	
TNM stage			0.0235
I, II	8	16	
III, IV	17	9	
Metastasis			0.0227
No	7	15	
Yes	18	10	
Histological type-differentiation			0.2575
Well	10	14	
Moderate or poor	15	11	

lncRNA-ATB, long non-coding RNA-activated by transforming growth factor β.

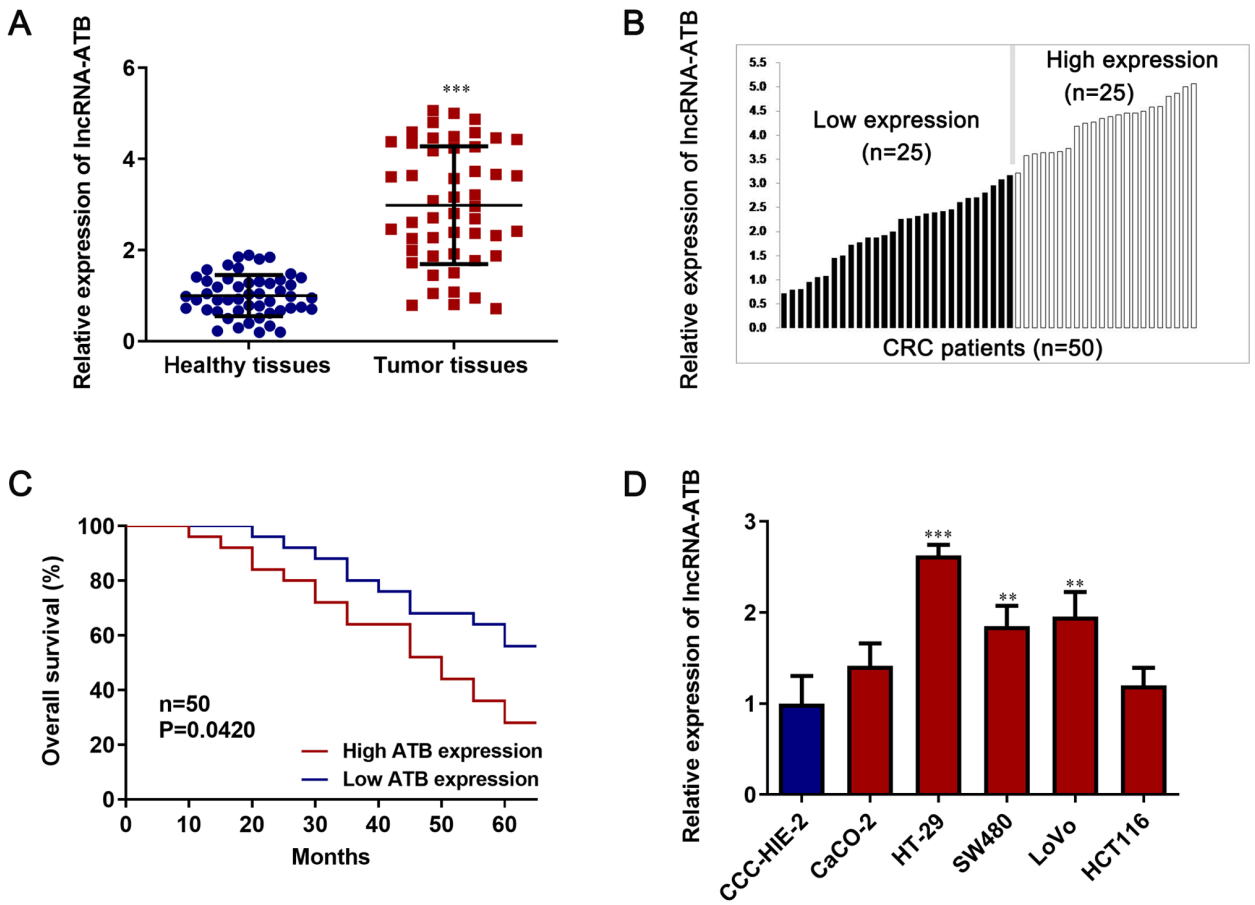

Figure 1. lncRNA-ATB expression is upregulated in CRC tissues and cell lines. (A) lncRNA-ATB expression in CRC tissues and paired healthy tissues. (B) Based on the median value of lncRNA-ATB expression in CRC tissues, patients were divided into low expression and high expression groups. (C) The relationship between lncRNA-ATB expression and overall survival. (D) lncRNA-ATB expression in CRC cell lines (CaCO-2, SW480, HT-29, HCT116 and LoVo cells) and a normal intestinal mucous cell line (CCC-HIE-2). **P<0.01 and ***P<0.001 vs. the corresponding control. lncRNA-ATB, long non-coding RNA-activated by transforming growth factor-β; CRC, colorectal cancer.

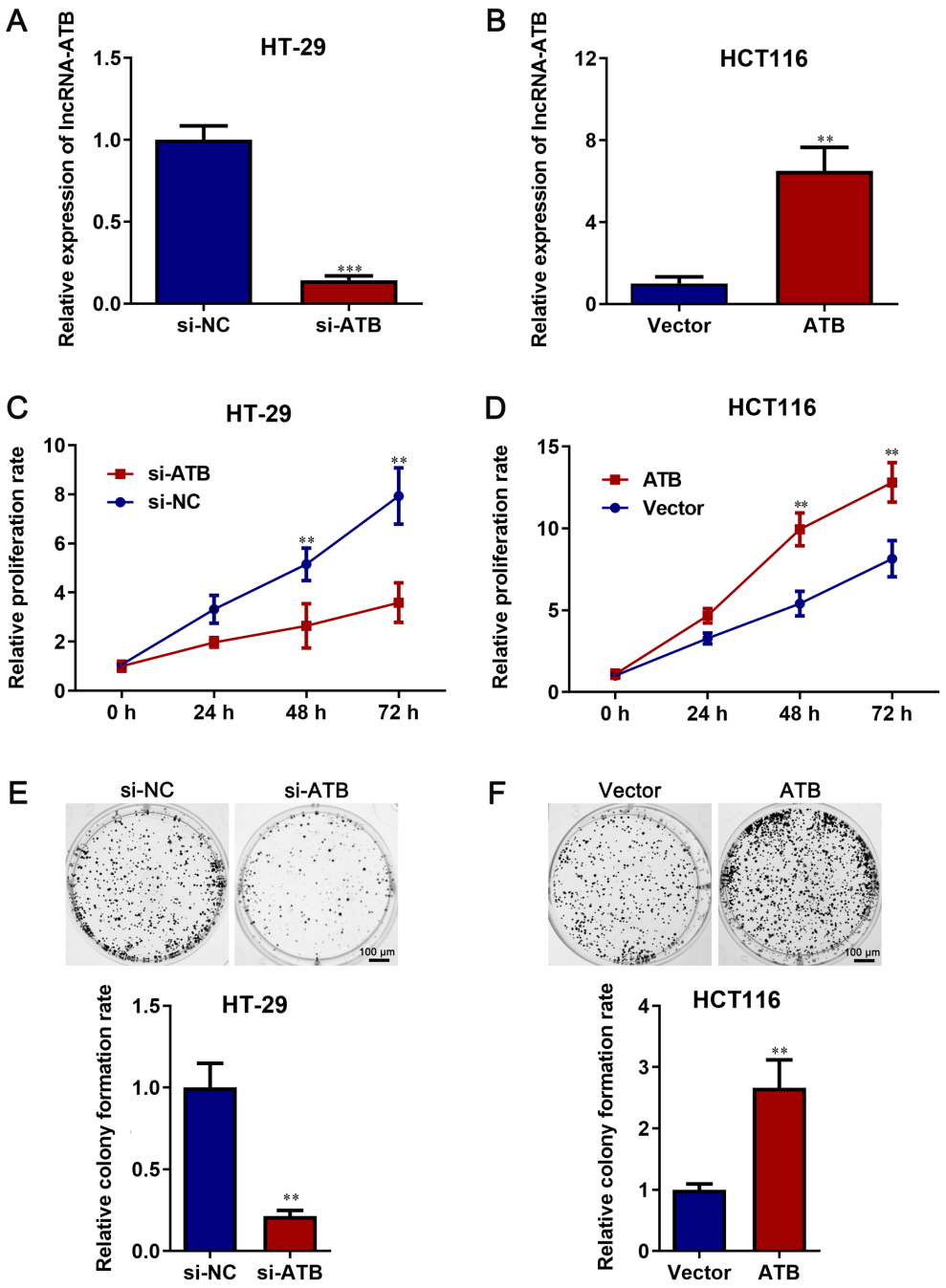

Figure 2. lncRNA-ATB promotes CRC cell proliferation. (A) Transfection efficiency of si-ATB in HT-29 cells. (B) Transfection efficiency of pcDNA3.1-lncRNA-ATB in HCT116 cells. Effect of lncRNA-ATB (C) knockdown and (D) overexpression on cell proliferation. Effect of lncRNA-ATB (E) knockdown and (F) overexpression on colony formation. **P<0.01 and ***P<0.001 vs. si-NC or vector. lncRNA-ATB, long non-coding RNA-activated by transforming growth factor-β; CRC, colorectal cancer; si, small interfering RNA; NC, negative control.

low ATB expression (n=25) groups, according to the median value (equal to 2.88) of lncRNA-ATB expression (Fig. 1B). The association between lncRNA-ATB and several clinical factors of CRC was analyzed (Table I). Higher expression of lncRNA-ATB was observed in patients with advanced TNM stages (P=0.0235) and metastasis (P=0.0235). However, there was no significant association between lncRNA-ATB and other clinical parameters, including sex, age and histological type-differentiation (P>0.05). In addition, patients with higher lncRNA-ATB expression displayed shorter overall survival, whereas patients with lower lncRNA-ATB expression displayed longer overall survival (P<0.05; Fig. 1C).

The relative expression of lncRNA-ATB was examined among the five CRC cell lines (CaCO-2, HT-29, SW480, LoVo and HCT116 cells) and the normal intestinal mucous cell line (CCC-HIE-2). CRC cell lines displayed markedly higher lncRNA-ATB expression levels compared with the CCC-HIE-2 normal cell line (P<0.05; Fig. 1D). Moreover, among the five CRC cell lines, HT-29 cells displayed the highest expression levels of lncRNA-ATB and HCT116 cells displayed the lowest lncRNA-ATB expression levels. Therefore, lncRNA-ATB knockdown was performed in HT-29 cells and lncRNA-ATB overexpression was established in HCT116 cells.

lncRNA-ATB facilitates CRC cell proliferation. Compared with si-NC, si-lncRNA-ATB significantly decreased lncRNA-ATB expression in HT-29 cells (P<0.05; Fig. 2A). Compared with vector, lncRNA-ATB overexpression plasmid significantly increased lncRNA-ATB expression in HCT116 cells (P<0.05; Fig. 2B).

A CCK-8 assay was performed to assess the role of lncRNA-ATB in cell proliferation. The results demonstrated that lncRNA-ATB knockdown significantly decreased HT-29 cell proliferation compared with si-NC (P<0.05; Fig. 2C). However, lncRNA-ATB overexpression significantly increased cell proliferation compared with vector (P<0.05; Fig. 2D). Moreover, the colony formation rate was significantly reduced by lncRNA-ATB knockdown in HT-29 cells compared with si-NC, but the colony formation rate was significantly increased by lncRNA-ATB overexpression in HCT116 cells compared with vector (P<0.05; Fig. 2E and F).

lncRNA-ATB increases CRC cell migration and invasion. Transwell assays were conducted to assess the role of lncRNA-ATB in CRC cell metastasis. lncRNA-ATB knockdown significantly reduced HT-29 cell migration and invasion compared with si-NC (P<0.05; Fig. 3A and B). However, lncRNA-ATB overexpression significantly increased HCT116 cell migration and invasion compared with vector (P<0.05; Fig. 3C and D). Furthermore, in HT-29 cells, si-ATB significantly increased the expression of the epithelial protein E-cadherin, but significantly decreased the expression of the mesenchymal protein Vimentin compared with si-NC (P<0.05; Fig. 3E and F). An opposite result was observed in HCT116 cells transfected with pcDNA3.1-lncRNA-ATB, as lncRNA-ATB overexpression significantly decreased E-cadherin expression but significantly increased Vimentin expression compared with vector (P<0.05; Fig. 3G and H). The results suggested that lncRNA-ATB had a positive effect on CRC cell migration, invasion and epithelial-mesenchymal transition.

lncRNA-ATB facilitates xenograft tumor growth. An *in vivo* study was conducted to assess the tumorigenic effect of lncRNA-ATB. lncRNA-ATB-knockdown HT-29 cells significantly decreased tumor diameter, volume and weight compared with the si-NC group (P<0.05; Figs. 4A, B and S1). However, there was no significant difference in body weight between the si-lncRNA-ATB and si-NC groups (P>0.05; Fig. 4C). In addition, the expression of lncRNA-ATB was measured in the xenograft tumors via RT-qPCR. lncRNA-ATB expression in the si-ATB group was significantly decreased compared with the si-NC group (P<0.05; Fig. 4D). Moreover, the immunohistochemistry results indicated that the positive rate of Ki67 was significantly lower in the si-ATB group compared with the si-NC group, indicating that lncRNA-ATB knockdown inhibited tumor growth *in vivo* (P<0.05; Fig. 4E and F). Therefore, the results suggested that lncRNA-ATB may serve as an oncogene in CRC.

lncRNA-ATB directly binds to miR-141-3p. To identify the molecular mechanism underlying lncRNA-ATB in promoting CRC cell proliferation and metastasis, a miRNA-lncRNA interaction module was selected. The comprehensive score of miR-141-3p was higher compared with other miRNAs, and miR-141-3p has been reported to regulate cancer cell proliferation and invasion (31-35). Therefore, miR-141-3p was selected for further study. lncRNA-ATB was predicted to bind with miR-141-3p (Fig. 5A). The dual luciferase reporter gene and RIP assays were performed to examine the interaction between lncRNA-ATB and miR-141-3p. Compared with miR-NC, miR-141-3p overexpression significantly downregulated the luciferase activity of lncRNA-ATB-WT (P<0.05), but had no significant effect on the luciferase activity of lncRNA-ATB-Mut vector (P>0.05; Fig. 5B). The RIP assay results indicated that the enrichment of lncRNA-ATB and miR-141-3p by the Ago2 antibody was significantly higher compared with the IgG antibody (P<0.05; Fig. 5C).

miR-141-3p was significantly downregulated in CRC tissues compared with paired healthy tissues (P<0.05; Fig. 5D). Moreover, miR-141-3p expression was significantly lower in CRC cell lines (CaCO-2, HT-29, SW480, LoVo and HCT116 cells) compared with the normal cell line CCC-HIE-2 (P<0.05; Fig. 5E). The correlation between lncRNA-ATB and miR-141-3p expression was assessed in CRC tissues, and the results indicated that lncRNA-ATB expression was negatively correlated with miR-141-3p expression (P<0.05; Fig. 5F). The results indicated that lncRNA-ATB directly bound to miR-141-3p, and miR-141-3p may serve a vital role in lncRNA-ATB-mediated CRC cell proliferation and metastasis.

miR-141-3p overexpression reverses the oncogenic effect of lncRNA-ATB. To further examine the association between lncRNA-ATB and miR-141-3p, rescue experiments were performed by transfecting lncRNA-ATB-overexpression HCT116 cells with miR-141-3p mimics. We confirmed miR-141-3p mimics can significantly increase the expression of miR-141-3p in HCT116 cells compared with mimics NC (Fig.S2). The CCK-8 and colony formation assay results demonstrated that miR-141-3p overexpression in lncRNA-ATB-overexpression HCT116 cells significantly decreased cell proliferation compared with lncRNA-ATB-overexpression HCT116 cells (P<0.05; Fig. 6A and B). In addition, miR-141-3p overexpression significantly inhibited the migration and invasion of lncRNA-ATB-overexpression HCT116 cells (P<0.05; Fig. 6C and D). Moreover, miR-141-3p mimics significantly increased E-cadherin expression and decreased Vimentin expression in lncRNA-ATB-overexpression HCT116 cells (P<0.05; Fig. 6E and F). Collectively, the results suggested that miR-141-3p may serve as an anticancer gene against the oncogenic effect of lncRNA-ATB in CRC cells.

Discussion

As CRC remains prevalent in China (36), identifying the molecular mechanism underlying CRC development and progression is an important clinical challenge. lncRNAs have gained increased research attention worldwide (37,38), and there is increasing evidence that lncRNAs are related to cancer development and progression (39,40). However, the role of lncRNAs in tumors is different, as some serve as tumor suppressor genes, whilst others serve as oncogenes (41). A variety of lncRNAs have been identified to serve a crucial role in CRC (25,42,43), thus, identifying novel lncRNAs in CRC is necessary.

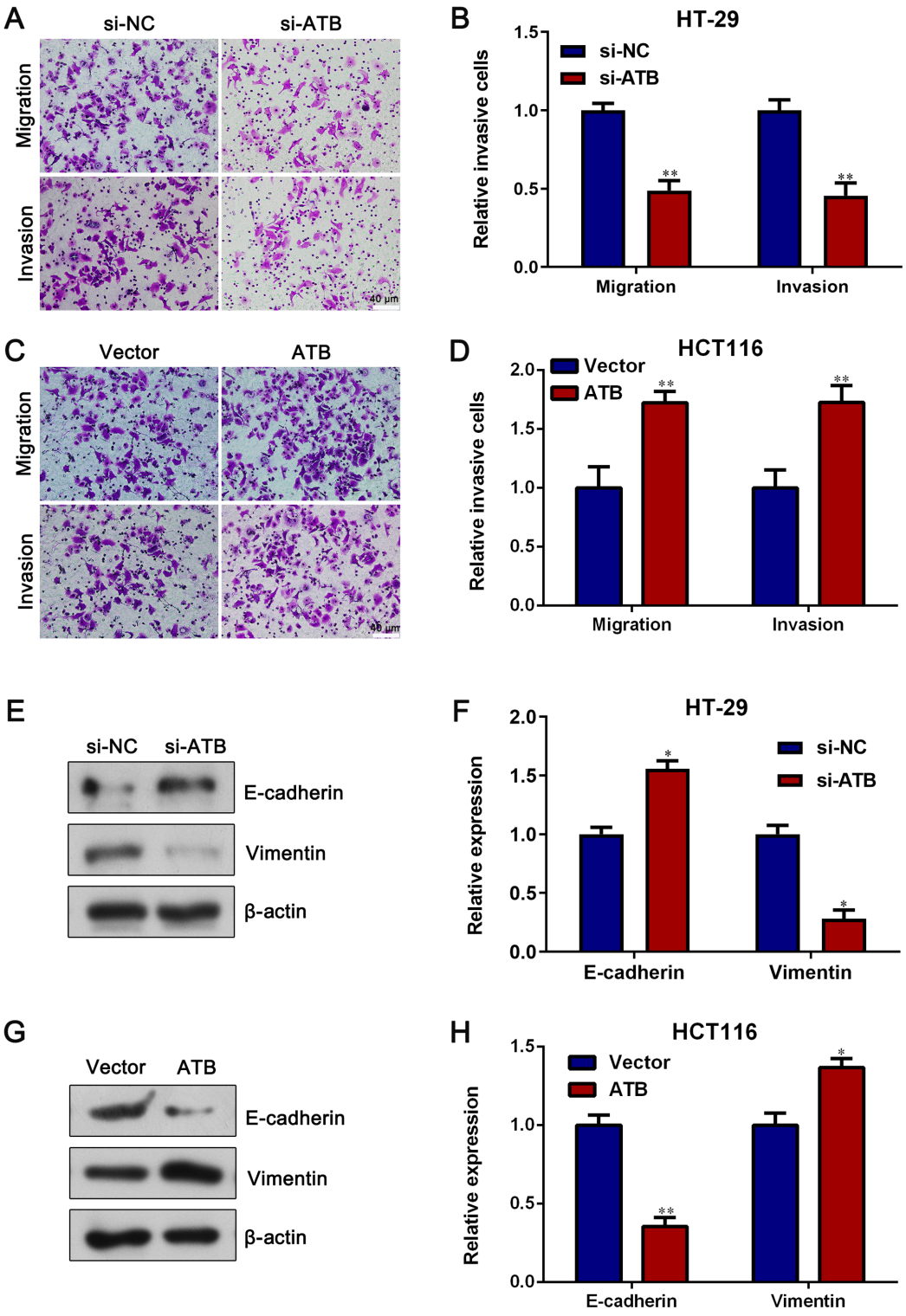

Figure 3. lncRNA-ATB promotes CRC cell migration. (A) Representative images of the Transwell assay in HT-29 cells. (B) Quantification of the effect of lncRNA-ATB knockdown on HT-29 cell migration and invasion. (C) Representative images of the Transwell assay in HCT116 cells. (D) Quantification of the effect of lncRNA-ATB overexpression on HCT116 cell migration and invasion assay. E-cadherin and Vimentin expression levels were (E) determined via western blotting and (F) semi-quantified in lncRNA-ATB-knockdown HT-29 cells. E-cadherin and Vimentin expression levels were (G) determined via western blotting and (H) semi-quantified in lncRNA-ATB-overexpression HCT116 cells. *P<0.05 and **P<0.01 vs. si-NC or Vector. lncRNA-ATB, long non-coding RNA-activated by transforming growth factor-β; CRC, colorectal cancer; NC, negative control; si, small interfering RNA.

A previous study reported that the expression of lncRNA-ATB was upregulated in ovarian cancer tissues and cell lines compared with adjacent tissue and normal cell lines, and a higher expression of lncRNA-ATB predicted poorer prognosis of ovarian cancer (44). Cai *et al* (45) demonstrated that lncRNA-ATB expression in breast cancer tissues was higher compared with corresponding adjacent healthy tissues. Furthermore, the underlying molecular mechanism study indicated that lncRNA-ATB competitively binds miR-98 with E2F transcription factor 5 and facilitates breast cancer cell

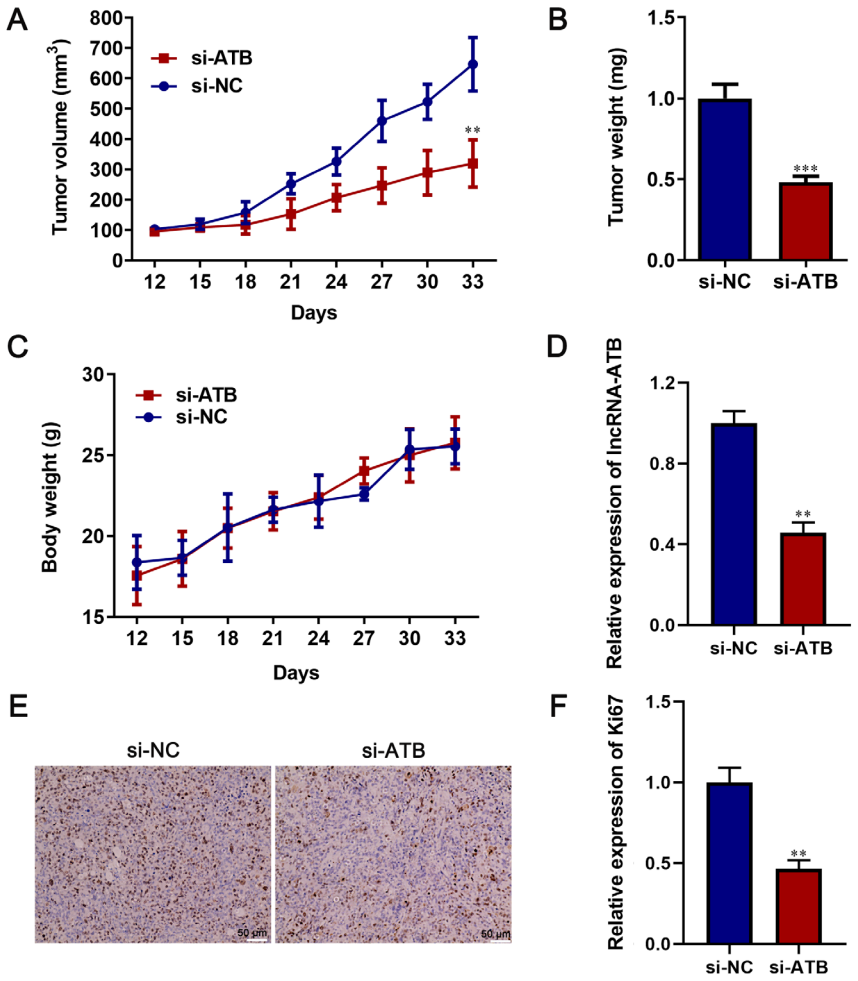

Figure 4. lncRNA-ATB knockdown inhibits tumor growth *in vivo*. (A) Tumor size was evaluated every 3 days. (B) Tumors were obtained on day 33 and weighed. (C) Body weight was measured every 3 days. (D) lncRNA-ATB expression in xenograft tumors. Ki67 expression in xenograft tumors was (E) assessed via immunohistochemistry and (F) quantified. **P<0.01 and ***P<0.001 vs. si-NC. lncRNA-ATB, long non-coding RNA-activated by transforming growth factor-β; si, small interfering RNA; NC, negative control.

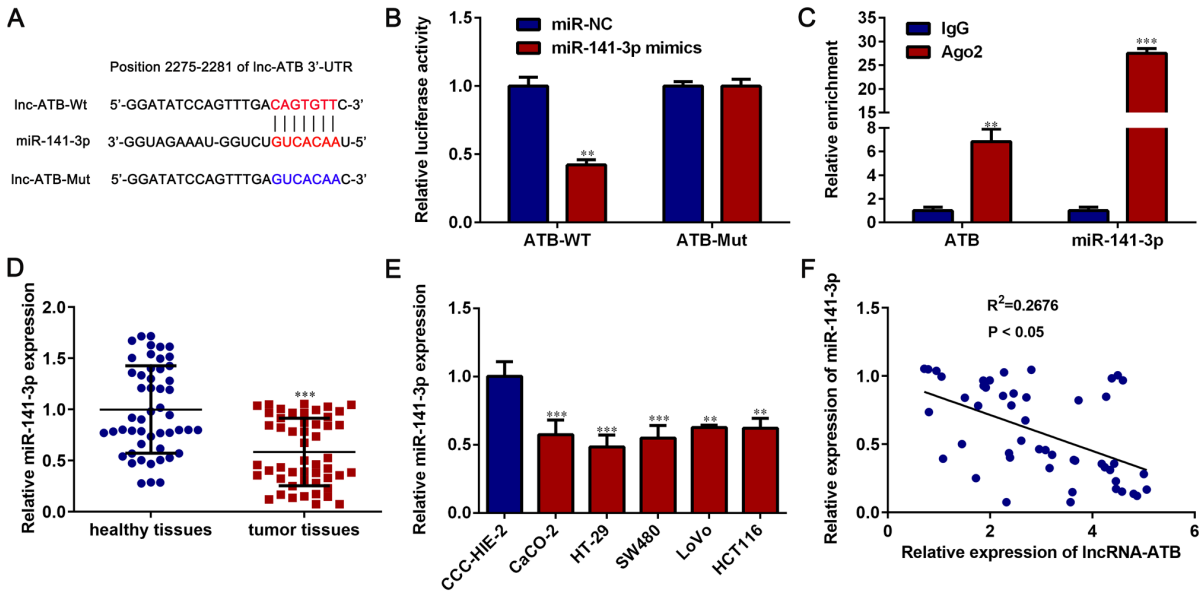

Figure 5. lncRNA-ATB is a target of miR-141-3p. (A) Putative binding site between lncRNA-ATB and miR-141-3p. (B) Relative luciferase activity of ATB-WT and ATB-Mut. (C) The endogenous interaction between lncRNA-ATB and miR-141-3p in HT-29 cells. (D) miR-141-3p expression in CRC tissues and healthy tissues. (E) miR-141-3p expression in CRC cell lines and a normal intestinal mucous cell line (CCC-HIE-2). (F) The correlation between lncRNA-ATB and miR-141-3p expression in CRC tissues. **P<0.01 and ***P<0.001 vs. the corresponding control. lncRNA-ATB, long non-coding RNA-activated by transforming growth factor-β; miR, microRNA; WT, wild-type; Mut, mutant; CRC, colorectal cancer; NC, negative control; UTR, untranslated region.

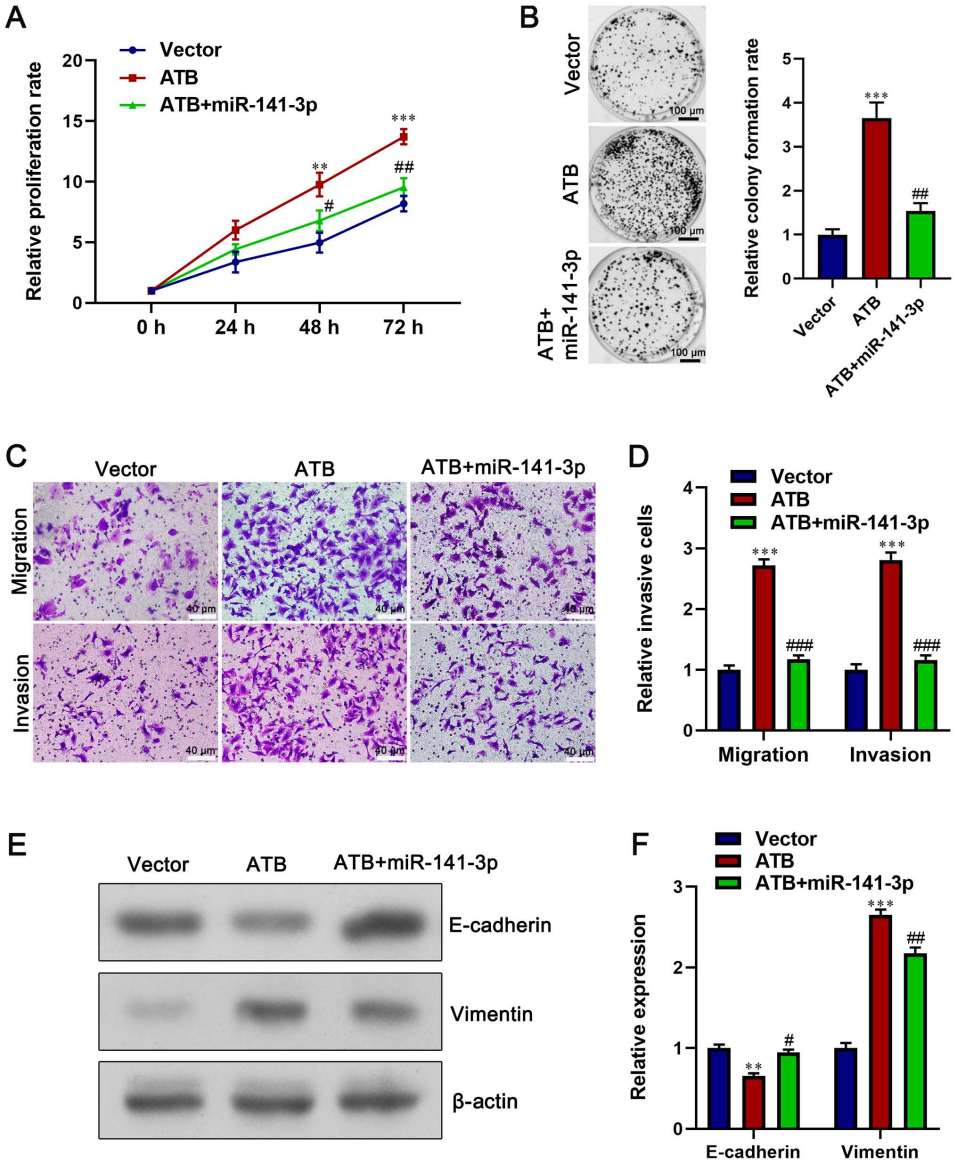

Figure 6. miR-141-3p overexpression antagonizes the oncogenic effect of lncRNA-ATB. HCT116 cells were co-transfected with miR-141-3p mimics and lncRNA-ATB overexpression plasmid. Cell proliferation was assessed by performing (A) Cell Counting Kit-8 and (B) colony formation assays, scale bars represent 100 μm. (C) Representative images of the Transwell assay in co-transfected HCT116 cells, scale bars represent 40 μm. (D) Quantification of cell migration and invasion in co-transfected HCT116 cells. E-cadherin and Vimentin protein expression levels were (E) measured via western blotting and (F) semi-quantified. (E) **P<0.01 and ***P<0.001 vs. Vector; $^{\#}$P<0.05, $^{\#\#}$P<0.01 and $^{\#\#\#}$P<0.001 vs. ATB. miR, microRNA; lncRNA-ATB, long non-coding RNA-activated by transforming growth factor-β; NC, negative control.

migration. However, the value of lncRNA-ATB in the clinical diagnosis of CRC is not completely understood.

The present study aimed to evaluate the biological role of lncRNA-ATB in CRC. Firstly, the expression of lncRNA-ATB was examined in clinical specimens of CRC. The clinical data demonstrated that lncRNA-ATB was significantly upregulated in CRC tissues compared with healthy tissues, and higher lncRNA-ATB expression indicated shorter overall survival in patients with CRC. In addition, high lncRNA-ATB expression was significantly associated with advanced TNM stages and tumor metastasis in patients with CRC.

To further investigate the carcinogenesis role of lncRNA-ATB in CRC, lncRNA-ATB knockdown and overexpression experiments were conducted. lncRNA-ATB knockdown inhibited HT-29 cell proliferation, colony formation, migration and invasion compared with si-NC. However, lncRNA-ATB overexpression facilitated HCT116 cell proliferation, colony formation, migration and invasion compared with vector. Moreover, the results indicated that aberrant E-cadherin and Vimentin expression levels facilitated tumor metastasis. Therefore, the results of the present study suggested that lncRNA-ATB suppressed E-cadherin expression and elevated Vimentin expression, indicating that lncRNA-ATB may serve as an oncogene in CRC.

Based on the increasing evidence of the competitive endogenous RNA regulatory network and the role of lncRNAs in regulatory loops (25-27), it was hypothesized that lncRNA-ATB may function as a competing endogenous RNA to modulate cell-associated biological process. Therefore, bioinformatics analysis and dual luciferase reporter gene assays were performed to predict the possible target of

lncRNA-ATB. The results indicated that miR-141-3p bound to the 3'-untranslated region of lncRNA-ATB. In addition, the RIP assay results identified the endogenous interaction between lncRNA-ATB and miR-141-3p.

miR-141-3p downregulation has been reported in a variety of tumors, such as prostate cancer and non-small cell lung cancer, and facilitates cancer development and progression via various mechanisms (31-35). For example, Huang *et al* (31) revealed that miR-141-3p expression was lower in prostate cancer with bone metastasis compared with prostate cancer with non-bone metastasis, and restoration of miR-141-3p reduced prostate cancer metastasis by inactivating the NF-κB signaling pathway. Moreover, Sun and Zhang (46) reported that lncRNA-X inactive specific transcript could facilitate pancreatic cancer cell proliferation, migration and invasion via targeting miR-141-3p. It has also been reported that miR-141-3p suppresses CRC cell proliferation, mobility and invasion by inhibiting TNF receptor associated factor-5 (47). In the present study, miR-141-3p was significantly downregulated in CRC tissues and cell lines compared with healthy tissues and cells, respectively. In addition, a negative correlation between miR-141-3p and lncRNA-ATB expression was identified in CRC tissues, suggesting that miR-141-3p may serve as an anticancer gene in CRC.

In conclusion, the results of the present study indicated that lncRNA-ATB was upregulated in CRC tissues and cell lines compared with healthy tissues and cells, respectively. The results also suggested that lncRNA-ATB promoted cancer cell proliferation, migration and invasion. Therefore, the present study suggested a critical role of lncRNA-ATB in CRC development and progression, and provided novel insights into the potential development of lncRNA-based targeted therapeutic strategies for CRC therapy.

Acknowledgements

Not applicable.

Funding

No funding was received.

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

XL and CW wrote the manuscript, performed the experiments, collected clinical samples and analyzed the data. CW designed the study and revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All patients provided written informed consent. The present study was approved by the Institutional Review Boards of the Shenzhen People's Hospital, the Second Clinical Medical College of Jinan University (approval nos. HH-TD-2017388 and SZRMYY20190211).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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