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Long non-coding RNA MALAT1 regulates proliferation, apoptosis, migration and invasion *via* miR-374b-5p/SRSF7 axis in non-small cell lung cancer

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Abstract. – OBJECTIVE: Non-small cell lung cancer (NSCLC) is a common type of lung cancer. Long noncoding RNA (lncRNA) metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) was reported to play a tumor-promoting role in NSCLC; however, the regulatory mechanism of MALAT1 in NSCLC progression remains largely unknown.

MATERIALS AND METHODS: The expression levels of MALAT1, miR-374b-5p and SRSF7 were measured by quantitative real-time polymerase chain reaction (qRT-PCR), and the protein level of SRSF7 was detected by Western blot analysis. Cell proliferation and apoptosis were determined by cell counting kit-8 (CCK-8) assay and flow cytometry, respectively. Cell migration and invasion were assessed by transwell assay. In addition, starBase3.0 software and dual-luciferase reporter assay were used to identify the correlations between miR-374b-5p and MALAT1 or SRSF7. Nude mouse xenograft assay was performed to explore the effects of MALAT1 on NSCLC *in vivo*.

RESULTS: We first observed that the levels of MALAT1 and SRSF7 were upregulated while miR-374b-5p was downregulated in NSCLC tissues; meanwhile, the expression level of MALAT1 was negatively correlated with miR-374b-5p and positively correlated with SRSF7. Both knockdown of MALAT1 and miR-374b-5p overexpression inhibited proliferation, migration and invasion and induced apoptosis in NSCLC cells. Then, we identified that miR-374b-5p was a target of MALAT1 and SRSF7 was the downstream of miR-374b-5p. In addition, overexpression of SRSF7 reversed the effects of MALAT1 knockdown on proliferation, apoptosis, migration and invasion in NSCLC cells. Finally, overexpression of MALAT1 suppressed NSCLC tumor growth *in vivo*.

CONCLUSIONS: Our results demonstrated that MALAT1 contributed to NSCLC progression through the MALAT1/miR-374b-5p/SRSF7 axis.

Key Words:
Non-small cell lung cancer, LncRNA MALAT1, MiR-374b-5p, SRSF7.

Introduction

Lung cancer is the most prevalent malignancy and the leading cause of cancer death around the world, accounting for 18.4% of the total cancer deaths[1]. Among lung cancer patients, about 85% are attacked by non-small-cell lung cancer (NSCLC)[2]. Compared with small cell lung cancer, NSCLC has a weaker ability of cell proliferation and metastasis, so it is usually diagnosed in the middle or advanced stages[3]. Therefore, identifying effective biomarkers is of great significance and urgency for the therapy and prognosis of NSCLC.

Approximately 75% of the human genome has no protein-coding function, encoding noncoding RNAs (ncRNAs)[4]. It has been shown that ncRNAs play crucial functions in diverse physiological processes[5]. Moreover, long noncoding RNAs (lncRNAs), a kind of ncRNAs with >200 nucleotides, have been reported to participate in the progression of various tumor. LncRNA metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is found to have high expression in multiple cancers[6,7], including NSCLC[8]. Researchers have proved that MALAT1 promotes NSCLC development, indicating the carcinogenesis roles of MALAT1 in NSCLC [9-11]. Also, MALAT1 has been suggested to be a molecular marker for the prediction of cancer[12]. Although there are many studies on MALAT1, the precise mechanism of MALAT1 on regulating NSCLC is still much unclear.

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MicroRNAs (miRNAs) are also a kind of ncRNAs, containing only 18-25 nucleotides. It is well known that miRNAs[13,14] participate in the development and progression of cancers through regulating the expression of target genes. Previous studies have revealed that miR-374b-5p is strongly related to the progression and the development of various malignancies[15]. MiR-374b-5p inhibits bladder cancer cells migration, invasion and epithelial-mesenchymal transition (EMT) in bladder cancer cells via targeting ZEB2[16]. MiR-374b also plays a tumor-suppressor activity in NSCLC via the p38/ERK pathway[17]. Furthermore, silencing miR-374b-5p enhances chemotherapeutic resistance in pancreatic cancer[18]. These findings suggest that miR-374b-5p acts as an anti-oncogene in many cancers.

Serine/arginine-rich splicing factor 7 (SRSF7) is a member of the serine/arginine-rich protein family which has a crucial role in regulating gene expression. Knockdown of SRSF7 impacts the expression of osteopontin splice variants and inhibits renal cancer cells proliferation[19]. It is also found silencing SRSF7 induces apoptosis of colon and lung cancer cells [20]. In addition to SRSF7, the other serine/arginine-rich protein family members are also associated with cancer progression. Among them, SRSF1 promotes cell cycle by stabilizing NEAT1 in glioma[21]; SRSF5 is upregulated in small-cell lung cancer and it is revealed to serve as a diagnostic marker for SCLC[22].

In the present study, we first measured the levels of MALAT1, miR-374b-5p and SRSF7 in NSCLC tissues. Then we investigated the functions of MALAT1 and miR-374b-5p in proliferation, apoptosis, migration and invasion in NSCLC cells via MALAT1 knockdown and miR-374b-5p overexpression, respectively. Additionally, the relationship between miR-374b-5p and MALAT1 or SRSF7 was identified, and the regulation mechanism was finally investigated.

Materials and Methods

Tissue Samples

After the approval from Ethics Committee of Yantai Yuhuangding Hospital, thirty paired NSCLC tissues and adjacent normal tissues were obtained from patients with NSCLC by surgical dissection at the Yantai Yuhuangding Hospital. All patients signed informed consent.

Cell Culture

Human normal bronchial cell lineBEAS-2B, human embryonic kidney 293T cells were obtained from American Type Culture Collection (ATCC; Manassas, VA, USA) and four NSCLC cell lines (H460, A549, H661 and H358) were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). All cells were cultured in a humidified atmosphere using Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA, USA) with additional 10% fetal bovine serum (FBS; Invitrogen), maintaining the conditions at 37°C and 5% CO_2.

Cell Transfection

Small interfering RNAs (siRNAs) against MALAT1 (si-MALAT1#1, si-MALAT1#2), miRNA-374b-5p mimic, miRNA-374b-5p inhibitor and the control fragments (si-control, mimic-control) were synthesized from RiboBio (Guangzhou, China). For the overexpression of SRSF7, the full length of SRSF7 cDNA was cloned and inserted to vector pcDNA3.1. Lipofectamine 2000 was used for cell transfection following the manufacturer's protocol.

Quantitative Real Time Polymerase Chain Reaction (qRT-PCR)

TRIzol Reagent was used to obtain the total RNA from NSCLC or non-tumor tissues and cells. Then reverse transcription was conducted by Primescript reverse transcriptase (TaKaRa, Otsu, Shiga, Japan), and SYBR Premix Ex Taq (TaKaRa) was used for qPCR following the manufacturer's instructions. GAPDH or U6 was used to normalize the genes. The primers were as follow:

MALAT1-F:
5'-CGCAGCCTGCAGCCCGAGAC-3',
MALAT1-R:
5'-CCCAATCAAGATTTTTTTATTC-3';
miR-374b-5p-F:
5'-TCAGCGGATATAATACAACCTGC-3',
miR-374b-5p-R:
5'-TATCGTTGTTCTCCACTCCTTCAC-3';
SRSF7-F:3'-GCGGTACGGAGGAGAAAC-5'
SRSF7-R:
3'-TCGGGAGCCACAAATCAC-5';
GAPDH-F:
3'-GCACCGTCAAGGCTGAGAAC-5',
GAPDH-R:
3'-ATGGTGGTGAAGACGCCAGT-5';
U6-F: 3'-CTCGCTTCGGCAGCACA-5',
U6-R: 3'-AACGCTTCACGAATTTGCGT-5'.

Western Blot Analysis

Tissues and cells were collected for the extraction of proteins with lysis buffer. Equal amounts of proteins were added into each line of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to separate them. After that, the proteins were transferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). Next, the membrane was blocked by 5% nonfat milk with TBST buffer. Subsequently, the membrane was incubated with SRSF7 antibody (1:1000; Abcam, Cambridge, United Kingdom) or GAPDH antibody (1:5000; Abcam) overnight for 4°C. After washed with TBST for 3 times, the secondary antibody (1:5000; Abcam) was used to incubate the membrane for 1 h at room temperature. The protein levels were detected by an enhanced chemiluminescence (ECL) system (Millipore, Billerica, MA USA).

Cell Counting Kit-8 (CCK-8) Assay

For the detection of cell proliferation, CCK-8 assay was implemented. Cells were placed into a 96-well plate with a density of 2000 cells per well. At 24 h, 48 h and 72 h after the culture, the cells were added with Cell Counting Kit-8 (Sigma-Aldrich, St. Louis, MO, USA) and incubated for 2 h. Then, we measured the absorbance at 450 nm to evaluate cell proliferation.

Flow Cytometric Analysis

Annexin V-fluorescein isothiocyanate (V-FITC)/propidium iodide (PI) apoptosis kit (BD Bioscience, Franklin Lakes, NJ, USA) was used to measure apoptosis according to the manufacturers' manuals. Cells were collected at 48 h after transfection and resuspended with binding buffer. Subsequently, FITC and PI were added to stain the cells, and after that the cells were incubated in a dark condition for 20 min. Cell apoptosis was measured by flow cytometry (BD Bioscience, Franklin Lakes, NJ, USA).

Transwell Assay

For the detection of cell migration and invasion, transwell assay was performed. The upper chamber without or with Matrigel was used for the detection of migration or invasion, respectively. Cells with the serum-free medium were added into the upper chamber, and cell medium with additional 10% serum was added into the basolateral chamber. After 24 h, the cells through the membrane were fixed with 4% paraformaldehyde, and then 0.5% crystal violet solution was used to dye the cells. The dyed cells were observed with a microscope.

Dual-Luciferase Assay

After the binding site between miR-374b-5p and MALAT1 or SRSF7 was predicted by starBase3.0, the MALAT1 sequence containing the predicted binding site (WT-MALAT1), full-length 3'UTR of SRSF7 (WT-3'UTR-SRSF7) and their mutant sequence (MUT-MALAT1, MUT-3'UTR-SRSF7) were constructed into the pGL3 vector, respectively. Each vector and microRNA (miR-374b-5p or miR-control) were co-transfected into 293T cells. After transfection for 48 h, the activities of the Firefly and Renilla luciferases were detected with a Dual-Luciferase reporter system (Promega, Madison, WI, USA).

Nude Mouse Xenograft Assay

H460 cells were transfected with pcDNA3.1-MALAT1 or control pcDNA3.1 and then subcutaneously injected into nude mice. The length and width of tumors were measured once a week, and the tumor volume was computed using the formula length×width^2/2. After injection for four weeks, the tumor tissues were resected and weighed. All animal experimental procedures obtained the approval of the Animal Ethics Committee of the Yantai Yuhuangding Hospital.

Statistical Analysis

Every assay was carried out with at least three independent experiments. All data were showed as mean ± standard deviation and analyzed by SPSS19.0 (IBM Corp., Armonk, NY, USA). The Student's *t*-test or One-Way Analysis of Variance (ANOVA) followed by Tukey's test was used to assess significant differences for comparing two groups or multiple groups. p-value <0.05 was considered statistically significant.

Results

MALAT1 and SRSF7 were Upregulated and miR-374b-5p was Downregulated in NSCLC

First, we detected the expression of MALAT1 and miR-374b-5p in NSCLC tissues by qRT-PCR. The results showed that the expression of MALAT1 was significantly higher while miR-374b-5p was lower in NSCLC tissues than in normal tissues (Figure 1A and B). Then the mRNA

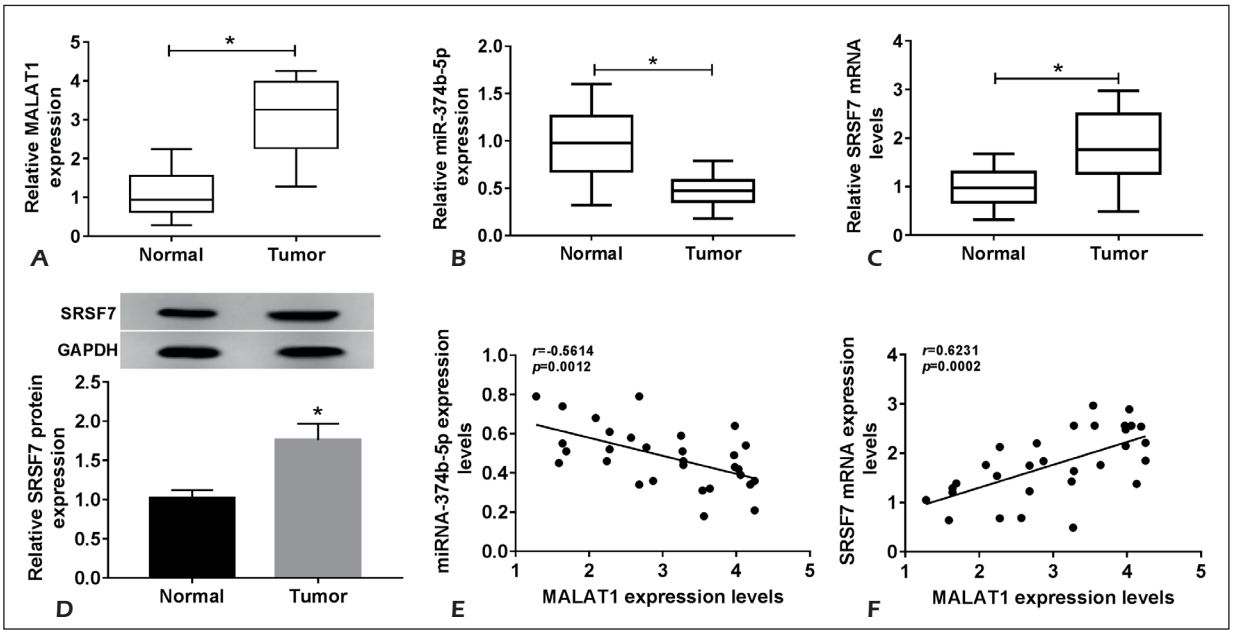

Figure 1. Expression levels of MALAT1, miR-374b-5p and SRSF7 in NSCLC tissues and adjacent normal tissues. **A**, the expression of MALAT1 in NSCLC tissues was detected by qRT-PCR. **B**, the expression of miR-374b-5p in NSCLC tissues was detected by qRT-PCR. **C** and **D**, the mRNA and protein levels were detected by qRT-PCR and Western blot. **E**, the correlation between MALAT1 and miR-374b-5p mRNA levels in NSCLC tissues was showed. **F**, the correlation between MALAT1 and SRSF7 mRNA levels in NSCLC tissues was showed. *$p<0.05$.H460 cells transfected with si-MALAT1 #2 or si-MALAT1 #2 + pcDNA3.1 SRSF7. **D** and **E**, CCK-8 assay was used to measure the proliferation of H661 and H460 cells transfected with si-MALAT1 #2 or si-MALAT1 #2 + pcDNA3.1 SRSF7. **F**, apoptosis of H661 and H460 cells transfected with si-MALAT1 #2 or si-MALAT1 #2 + pcDNA3.1 SRSF7 was detected by flow cytometry. **G** and **H**, migration and invasion were measured by transwell assay in H661 and H460 cells transfected with si-MALAT1 #2 or si-MALAT1 #2 + pcDNA3.1 SRSF7. *$p<0.05$.

and protein levels of SRSF7 were measured by qRT-PCR and Western blot, respectively, and the results revealed that SRSF7 was upregulated in NSCLC tissues (Figure 1C and D). In addition, we investigated the correlation between MALAT1 and miR-374b-5p or SRSF7. The results showed that the expression levels of MALAT1 and miR-374b-5p were negatively correlated (Figure 1E), while the levels of MALAT1 and SRSF7 mRNA were positively correlated (Figure 1F). These results suggested a possible regulatory relationship among MALAT1, miR-374b-5p and SRSF7.

Knockdown of MALAT1 Suppressed Proliferation, Migration and Invasion and Promoted Apoptosis in NSCLC Cells

To investigate the role of MALAT1 in NSCLC cells, we first detected the expression level of MALAT1. A higher MALAT1 expression was observably in four NSCLC cell lines compared with BEAS-2B cells, especially in NSCLC cell lines H661 and H460 (Figure 2A). Afterwords, we silenced MALAT1 with si-MALAT1 #1 or si-MALAT1 #2 in H661 and H460 cells. The qRT-PCR results showed that the expression of MALAT1 was downregulated by si-MALAT1 #1 or si-MALAT1 #2 and was lower in both H661 and H460 cells transfected with si-MALAT1 #2 (Figure 2B), so the si-MALAT1 #2 was used for following experiments. CCK-8 assay indicated that MALAT1 knockdown inhibited proliferation of H661 and H460 cells (Figure 2C and D). Meanwhile, knockdown of MALAT1 promoted apoptosis of H661 and H460 cells (Figure 2E). Moreover, the migration and invasion were also measured, and the results showed that the migration and invasion were suppressed in both H661 and H460 cells transfected with si-MALAT1 #2 (Figure 2F and G). These data clarified that MALAT1 knockdown inhibited proliferation, migration and invasion and promoted apoptosis in NSCLC cells *in vitro.*

Overexpression of miR-374b-5p Inhibited Proliferation, Migration and Invasion and Induced Apoptosis in NSCLC Cells

To explore the functions of miR-374b-5p in NSCLC, we overexpressed miR-374b-5p in NS-

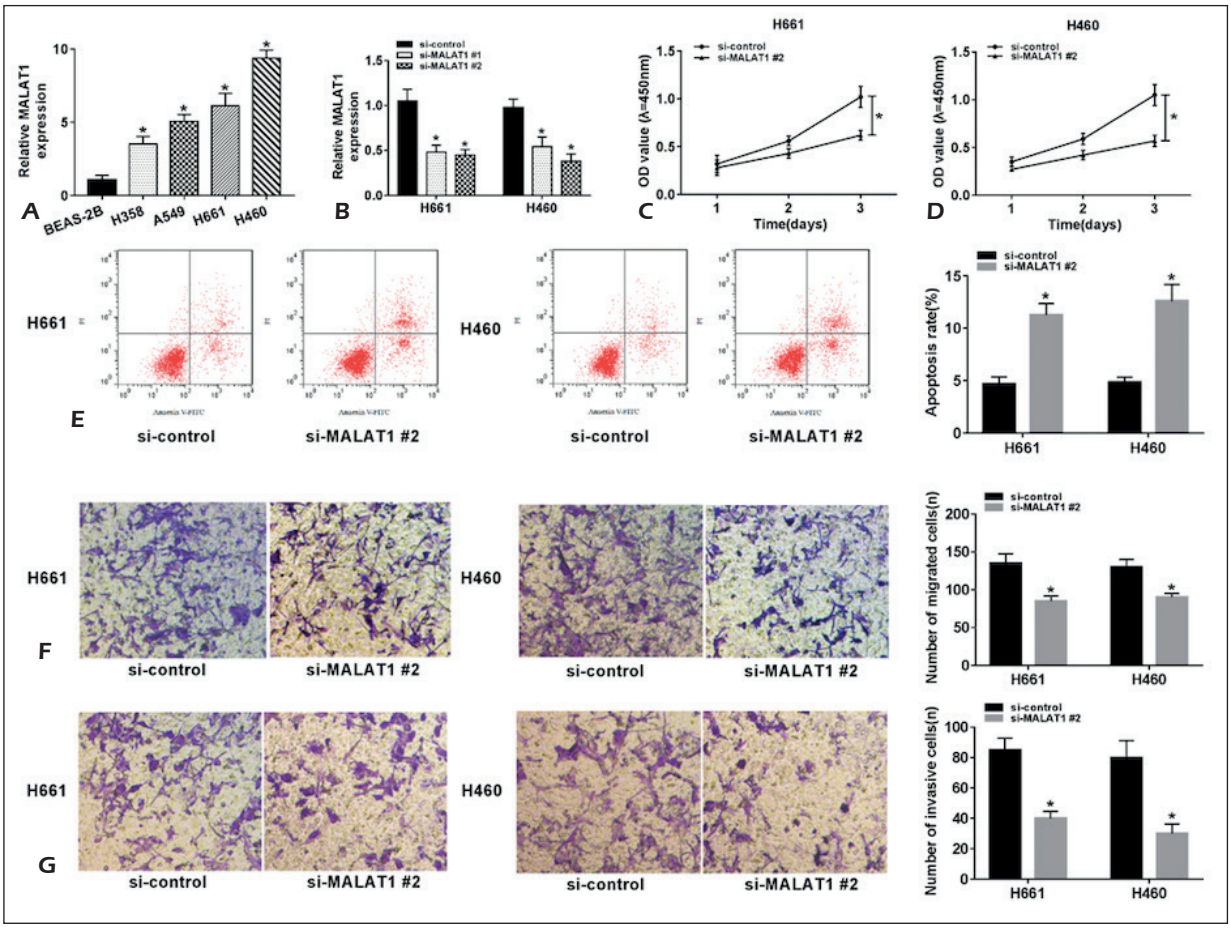

Figure 2. Knockdown of MALAT1 inhibited proliferation, migration and invasion and promoted apoptosis in NSCLC cells. **A**, the expression of MALAT1 in BEAS-2B and four NSCLC cells was detected by qRT-PCR. **B**, the expression of MALAT1 in H661 and H460 cells transfected with si-MALAT1 #1 or si-MALAT1 #2 was detected by qRT-PCR. **C** and **D**, proliferation of H661 and H460 cells was measured by CCK-8 assay after knockdown of MALAT1. **E**, apoptosis was detected by flow cytometry in H661 and H460 cells transfected with si-MALAT1 #2. **F** and **G**, migration and invasion were measured by transwell assay in H661 and H460 cells transfected with si-MALAT1 #2 (magnification ×100). *$p<0.05$.

CLC cells. The expression of miR-374b-5p was significantly increased in H661 and H460 cells transfected with miRNA-374b-5p mimic compared with the control group (Figure 3A). The proliferation of H661 cells was reduced when miR-374b-5p was overexpressed (Figure 3B), and there was a similar result in H460 cells (Figure 3C). Otherwise, apoptosis results revealed that the overexpression of miR-374b-5p resulted in increased apoptosis rates of both H661 and H460 cells (Figure 3D). Further, transwell assay results showed that migration and invasion abilities were enhanced in H661 and H460 cells transfected with miRNA-374b-5p mimic (Figure 3E and F). These results suggested that miR-374b-5p overexpression suppressed proliferation, migration and invasion and promoted apoptosis in NSCLC cells *in vitro*.

MALAT1 Regulated SRSF7 Expression by Acting as a ceRNA of miR-374b-5p

It is known that lncRNAs can function as competing endogenous RNAs (ceRNAs) to regulate gene expression. Online software was used to predict the targeted relationships between MALAT1 and miR-374b-5p as well as miR-374b-5p and SRSF7, and the potential binding sites were also predicted (Figure 4A). And then the dual-luciferase assay was performed. The result indicated that the luciferase activity was significantly reduced in 293T cells co-transfected with miR-374b-5p and WT-MALAT1 rather than MUT-MALAT1 (Figure 4B). Also, the luciferase activity was significantly reduced in 293T cells co-transfected with miR-374b-5p and WT-3'UTR-SRSF7 and had no change when the 293T cells were co-transfected with miR-374b-5p and

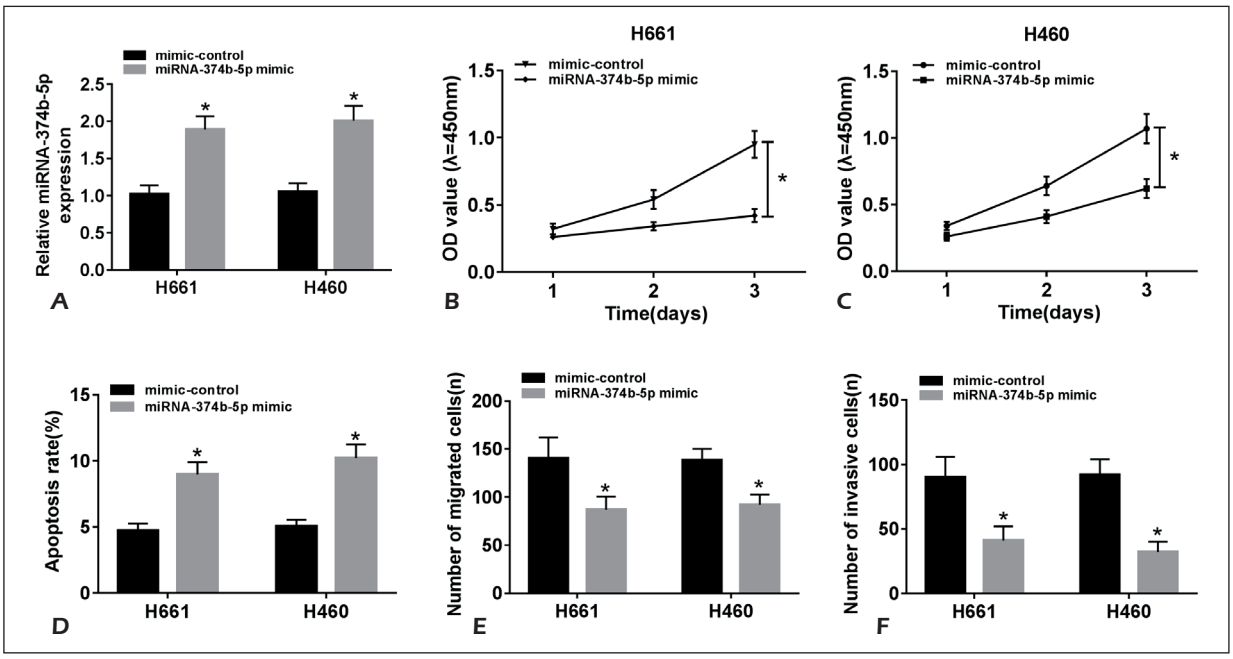

Figure 3. Overexpression of miR-374b-5p inhibited proliferation, migration and invasion and contributed to apoptosis in NSCLC cells. **A**, the expression of miR-374b-5p in H661 and H460 cells transfected with miRNA-374b-5p mimic was detected by qRT-PCR. **B** and **C**, proliferation of H661 and H460 cells was measured after overexpression of miR-374b-5p. **D**, apoptosis of H661 and H460 cells transfected with miRNA-374b-5p mimic was detected by flow cytometry. **E** and **F**, migration and invasion were measured by transwell assay in H661 and H460 cells transfected with miRNA-374b-5p mimic. *p<0.05.

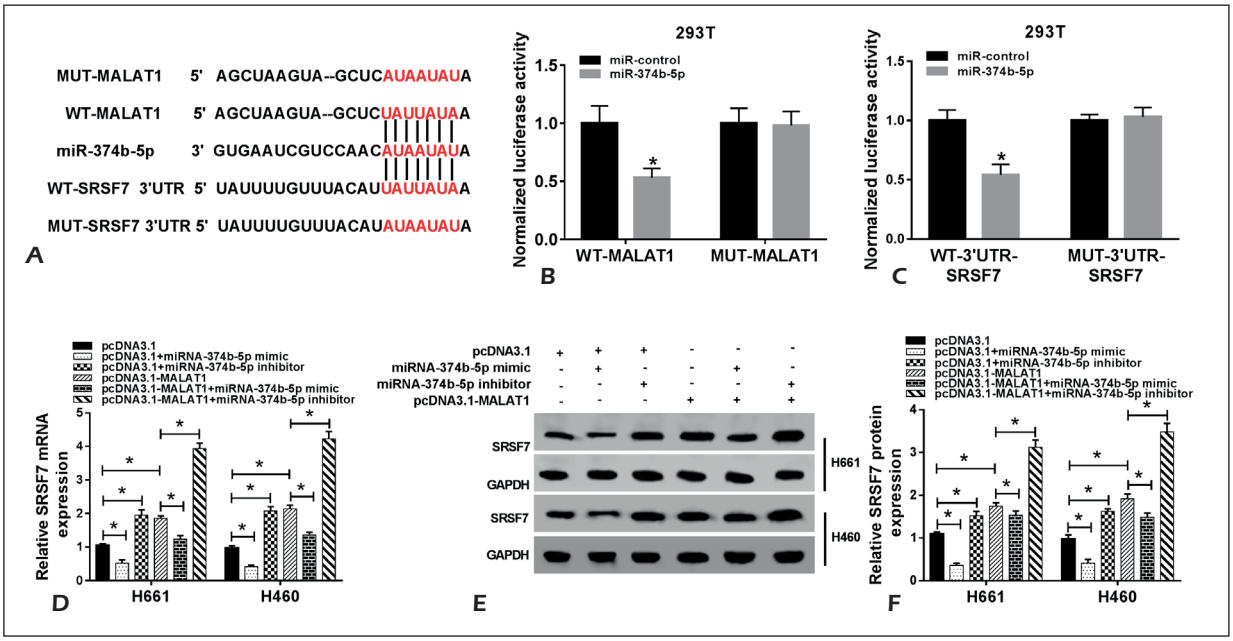

Figure 4. MALAT1 targeted miR-374b-5p to regulate SRSF7 expression. **A**, the potential binding sites between miR-374b-5p and MALAT1 or SRSF7 were predicated. **B**, the luciferase activity was measured in 293T cells transfected with miR-374b-5p and WT-MALAT1 or MUT-MALAT1. **C**, the luciferase activity was measured in 293T cells transfected with miR-374b-5p and WT-3'UTR-SRSF7 or MUT-3'UTR-SRSF7. **D-F**, the mRNA and protein levels of SRSF7 were detected in H661 and H460 cells transfected with miRNA-374b-5p mimic, miRNA-374b-5p inhibitor, pcDNA3.1-MALAT1, pcDNA3.1-MALAT1 + miRNA-374b-5p mimic or pcDNA3.1-MALAT1 +miRNA-374b-5p inhibitor. *p<0.05.

MUT-3'UTR-SRSF7 (Figure 4C). In addition, we measured the mRNA and protein levels of SRSF7 by qRT-PCR and Western blot. The result showed that the mRNA and protein levels of SRSF7 were downregulated by miRNA-374b-5p mimic and upregulated by miRNA-374-5p inhibitor or MALAT1 in both H661 and H460 cells; meanwhile, when compared with MALAT1 overexpression group, the level of SRSF7 was decreased when MALAT1 and miR-374b-5p both overexpressed, but increased in H661 and H460 cells transfected with pcDNA3.1-MALAT1 + miRNA-374b-5p inhibitor (Figure 4D-F). These data suggested that miR-374b-5p downregulated SRSF7 expression by directly targeting and MALAT1 upregulated SRSF7 expression through targeting miR-374b-5p.

SRSF7 Overexpression Reversed Effects of MALAT1 Knockdown on Proliferation, Apoptosis, Migration and Invasion of NSCLC Cells

To further investigate the relationship between MALAT1 and SRSF7, the H661 and H460 cells were transfected with si-MALAT1 #2 or si-MALAT1 #2 + pcDNA3.1 SRSF7. The mRNA and protein levels of SRSF7 were downregulated by MALAT1 knockdown but re-

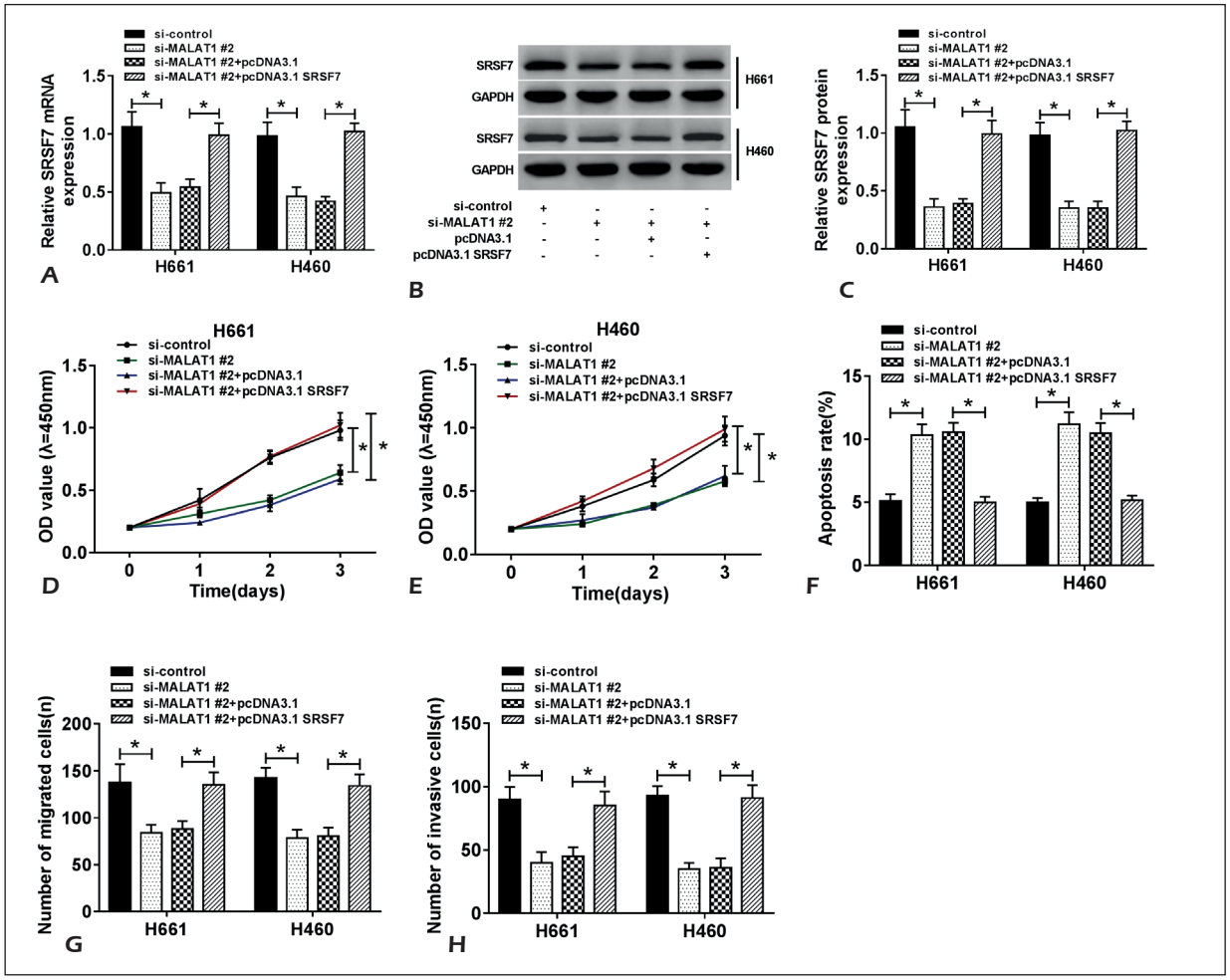

Figure 5. SRSF7 overexpression reversed the effects of MALAT1 knockdown on proliferation, apoptosis, migration and invasion of NSCLC cells. **A**, the mRNA expression level of SRSF7 was detected in H661 and H460 cells transfected with si-MALAT1 #2 or si-MALAT1 #2 + pcDNA3.1 SRSF7. **B** and **C**, Western blot analysis was used to measure the protein level of SRSF7 in H661 and H460 cells transfected with si-MALAT1 #2 or si-MALAT1 #2 + pcDNA3.1 SRSF7. **D** and **E**, CCK-8 assay was used to measure the proliferation of H661 and H460 cells transfected with si-MALAT1 #2 or si-MALAT1 #2 + pcDNA3.1 SRSF7. **F**, apoptosis of H661 and H460 cells transfected with si-MALAT1 #2 or si-MALAT1 #2 + pcDNA3.1 SRSF7 was detected by flow cytometry. **G** and **H**, migration and invasion were measured by transwell assay in H661 and H460 cells transfected with si-MALAT1 #2 or si-MALAT1 #2 + pcDNA3.1 SRSF7. *p<0.05.

versed in cells transfected with si-MALAT1 #2 + pcDNA3.1 SRSF7 (Figure 5A-C). Subsequently, CCK-8 assay indicated that the proliferation ability of H661 and H460 cells transfected with si-MALAT1 #2 was reduced and was rescued when co-transfected with pcDNA3.1 SRSF7 (Figure 5D and E). On the contrary, the apoptosis rate of H661 and H460 cells was significantly increased by MALAT1 knockdown and also reversed in cells transfected with si-MALAT1 #2 + pcDNA3.1 SRSF7 (Figure 5F). Additionally, the inhibiting effects of MALAT1 knockdown on H661 and H460 cells migration and invasion were abolished by ectopic expression of SRSF7 (Figure 5G and H).

Overexpression of MALAT1 Promoted NSCLC Tumor Growth

To investigate the role of MALAT1 in NSCLC growth *in vivo*, we measured the tumor growth in a xenograft mouse model. H460 cells with stably MALAT1 overexpression or control cells were subcutaneously injected into nude mice, and the result showed that the tumor volume and weight were markedly higher in MALAT1 overexpression group than in control group (Figure 6A and B). The expression level of MALAT1 was detected by qRT-PCR, verifying the overexpression efficiency (Figure 6C). In addition, our data indicated that overexpression of MALAT1 downregulated miR-374b-5p expression in resected tumors (Figure 6D); inversely, the mRNA and protein levels of SRSF7 were upregulated by MALAT1 overexpression *in vivo* (Figure 6E and F).

Discussion

Increasing evidence has demonstrated that long noncoding RNA (lncRNA) plays a crucial role in human tumorigenesis and progression. MALAT1 is a highly conserved lncRNA, and it is found to be overexpressed in various cancers, including colorectal cancer, hepatocellular carcinoma, gastric cancer and so on [23-25]. Meanwhile, the tumor-promoting action of MALAT1 was proved in these cancers. In our study, we found the level of lncRNA MALAT1 was upregulated in NSCLC

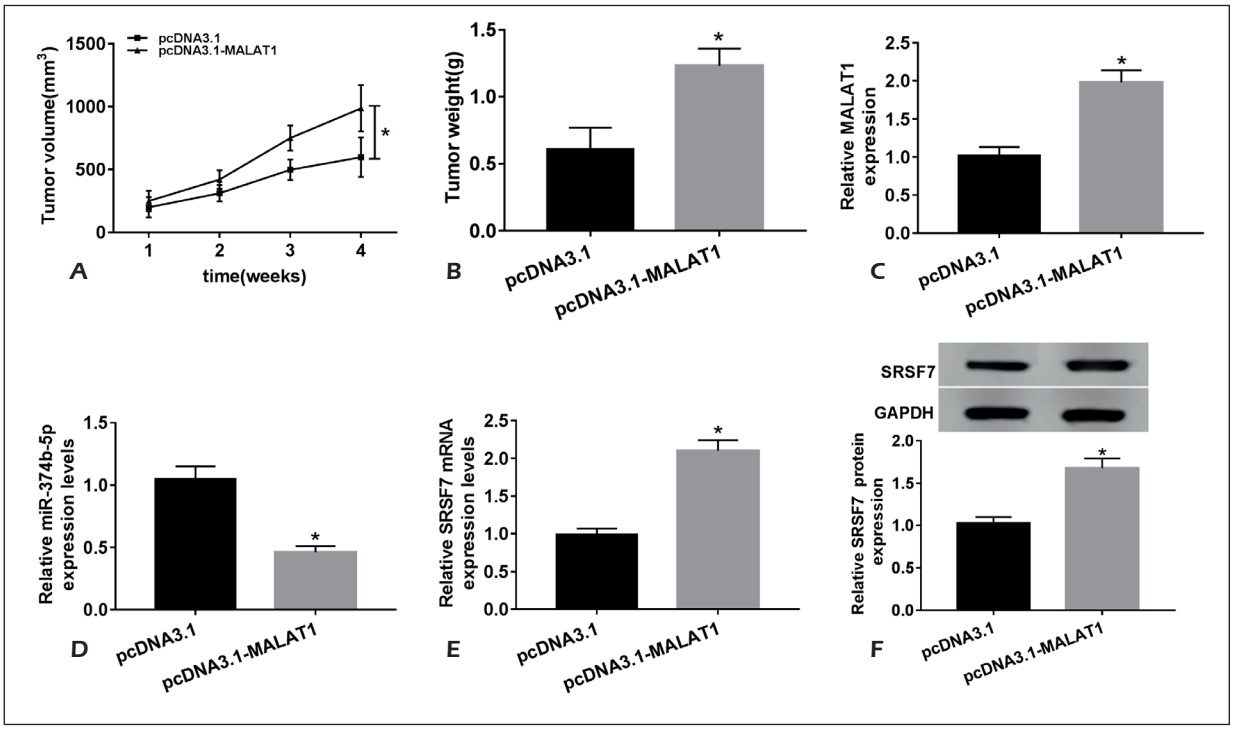

Figure 6. Overexpression of MALAT1 promoted NSCLC tumor growth *in vivo*. H460 cells transfected with pcDNA3.1-MALAT1 or pcDNA3.1 were subcutaneously injected into nude mice. **A**, tumor volume was measured at 1, 2, 3 and 4 weeks after implantation of H460 cells. **B**, tumor weight was measured at 4 weeks after implantation of H460 cells. **C** and **D**, the expression of MALAT1 and miR-374b-5p was detected by qRT-PCR at 4 weeks after implantation of H460 cells. E and F, the mRNA and protein levels were measured by qRT-PCR and Western blot at 4 weeks after implantation of H460 cells. *$p<0.05$.

tissues, silencing MALAT1 inhibited proliferation, migration and invasion and induced apoptosis in NSCLC cells, implying that MALAT1 contributed to NSCLC progression. Previous studies have reported that MALAT1 in NSCLC. MALAT1 regulated ERK/MAPK signaling pathway via targeting CXCL5, thereby promoting proliferation, migration and invasion in NSCLC cells[8]. In patients with NSCLC, high expression of MALAT1 was associated with the vessel invasion, recurrence and poor prognosis[26,27]. Besides, MALAT1 declined chemo-sensitivity by miR-197-3p/p120 axis in NSCLC cells[28]. It was also suggested that MALAT1 had a potential application for detecting early-stage NSCLC[29].

It has been discovered that lncRNAs might function as ceRNAs and regulate miRNAs expression in various physiological processes, including cancer progression[30]. Hence, lncRNA SBF2-AS1 upregulated FOXA1 expression by sponging microRNA-30a, thereby promoting osteosarcoma cells proliferation and metastasis[31]. Moreover, linc00460 promoted osteosarcoma progression via acting as a ceRNA of miR-1224-5p to upregulate FADS1 expression[32]. We predicted miR-374b-5p as a target of MALAT1 through bioinformatics analysis, and a dual-luciferase reporter assay confirmed this relationship. MiR-374b-5p was shown to have a tumor-suppressing capability in bladder cancer, pancreatic cancer and ovarian cancer[16,18,33]. However, the functions of miR-374b-5p in NSCLC and the potential molecular mechanism were largely unknown up to now. We found that the expression of miR-374b-5p was downregulated in NSCLC tissues, and the functional experiments showed that overexpression of miR-374b-5p suppressed proliferation, migration and invasion and contributed to apoptosis in NSCLC cells.

Further study indicated that SRSF7 was a direct target of miR-374b-5p. It was reported that SRSF7 could specifically bind to RNA and participate in the alternative splicing of various pre-mRNAs[34-37]. Additionally, SRSF7 was indicated to play a positive role in colon and lung cancer cells[20,37]. We showed that SRSF7 level was also upregulated in NSCLC tissues, and the mRNA level of SRSF7 in NSCLC tissues showed a positive correlation with the MALAT1 expression. Besides, overexpression of SRSF7 rescued the effect of MALAT1 on NSCLC cells. Therefore, these results suggested MALAT1 plays a tumor-promoting role in NSCLC by regulating SRSF7 expression.

Conclusions

Our study first established the relationship between miR-374b-5p and MALAT1 or SRSF7 and demonstrated that MALAT1 promoted NSCLC progression via miR-374b-5p/SRSF7 axis *in vitro* and *in vivo*. This study might provide a theoretical basis for the development of diagnosis and cure strategies in NSCLC.

Conflict of Interests

The authors declare that they have no financial conflicts of interest.

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