Original Article
LncRNA TPTEP1 inhibited the proliferation and metastasis of non-small cell lung cancer cells by targeting miR-761/LATS2 axis

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Abstract: Objective: Non-small cell lung cancer (NSCLC) is highly metastatic that can lead to high fatality rate. This study aimed at investigating the possible role of LncRNA TPTEP1 (TPTEP1) in NSCLC progression. Methods: Cell proliferation was determined by MTT and colony formation assays. Transwell and scratch assays were adopted to assess cellular metastasis. RT-qPCR and western blot were used to detect TPTEP1 expression transcriptionally and translationally, respectively. The dual luciferase reporter assay and RNA immunoprecipitation assay were used to identify the specific target relationships. Results: Compared with the normal adjacent tissues, the expressions of TPTEP1 and LATS2 were significantly down-regulated in the NSCLC tissues, while the expression of miR-761 was significantly increased. Overexpression of TPTEP1 exhibited substantial antitumor effects on NSCLC, including inhibition of cell proliferation and metastasis, which was achieved by targeting miR-761 and subsequently attenuated the expression of LATS2. LATS2 was identified as a direct target of miR-761. Overexpression of miR-761 could significantly block the inhibitory effects of TPTEP1 on NSCLC, which clearly indicated that miR-761 played an oncogenic role in promoting proliferation and metastasis, while its downstream factor, LATS2, exerted opposite effects. Conclusion: The study showed that TPTEP1 played an inhibitory role in cancer progression of NSCLC cells by regulating miR-761/LATS2 cascade, thereby highlighting the potential therapeutic significance of TPTEP1/miR-761/LATS2 axis.

Keywords: Epithelial to mesenchymal transition, cell proliferation, LncRNA TPTEP1, miR-761, LATS2, NSCLC

Introduction

Lung cancer is a malignant disease and the leading cause of cancer related mortality worldwide. Non-small cell lung cancer (NSCLC) accounts for more than 80% of all lung cancers [1]. The current treatments of NSCLC include chemotherapy, radiotherapy and surgery. However, NSCLC is a type of tumor that is highly metastatic [2]. The most prominent feature of malignant tumors is their significant invasive and metastatic capacities. It has been found that even when the tumor has been completely removed by surgery after it was first diagnosed, and in spite of the administration of adjuvant chemotherapy and other treatments after surgery, some patients still experience recurrence or metastasis [2]. Therefore, it is necessary to explore the invasion and metastasis mechanisms that can possibly regulate the progression of NSCLC [3].

Recently, long non-coding RNAs (LncRNAs) have been shown to exert diverse biological functions in regulating several physiological processes (such as epigenetic regulation, transcriptional and post-transcriptional regulation). LncRNAs have the ability to regulate expression of genes, and can significantly affect several disease processes, including cancer [4]. It has been reported that many LncRNAs regulated the tumor progression in NSCLC. For instance, LncRNA PTAR promotes cell proliferation, migration and invasion of NSCLC, while LncRNA NBR2 suppresses the progression of epithelial to mesenchymal transition (EMT) [5, 6]. A num-

ber of previous studies have revealed that LncRNA TPTEP1 (herein referred to TPTEP1) plays a pivotal antitumor role in different kinds of cancers, such as hepatocellular carcinoma, and glioma [7, 8]. In NSCLC cells, TPTEP1 has been reported to inhibit cell proliferation [9]. However, the underlying mechanisms of TPTEP1 in NSCLC progression remain unclear.

It has been proved that LncRNAs can bind to microRNA (miRNAs) as competitive endogenous RNAs (ceRNAs), thus influencing the expression of various target genes [10]. The contribution of miRNAs in different chronic diseases including cancer had been widely described [11, 12]. In our study, we predicted that TPTEP1 could directly target miR-761, which has been reported to play an oncogenic role in various cancers. For example, in triple-negative breast cancer, miR-761 induced aggressive phenotypes, and enhanced the drug resistance in synovial sarcoma [13]. In NSCLC cells, miR-761 had also been reported to promote the tumor progression and metastasis [14]. The interaction between miR-761 and LATS2 had also been predicted. LATS2, a Dbf2-related kinase, acts as a potential tumor suppressor [15]. It has been reported that LATS2 can effectively act as a central regulator of cell fate by modulating the functions of various downstream effectors [15]. Moreover, LATS2 exhibited an inhibitory role against tumor metastasis, and is regulated by miRNAs, such as miR-302 and miR-372-3p [16-19]. The antitumor effects of LATS2 have also been implicated. The decreased expression of LATS2 indicated poor prognosis of NSCLC, and LATS2 can promote cell apoptosis, inhibit cell growth, as well as suppress migration and motility [20-22].

In NSCLC, although the respective roles of TPTEP1, miR-761 and LATS2 have been described earlier, their possible interactions have not been revealed. In this study, whether TPTEP1 inhibited the migration and invasion by regulating miR-761/LATS2 axis was investigated, in order to provide a theoretical basis for the therapy of NSCLC.

Materials and methods

Cell lines and clinical tissues

NSCLC cell lines (A549 and H1299) were purchased from American type culture collection (ATCC) and incubated in DMEM medium (Gibco, Grand Island, USA) containing 12% fetal bovine serum (FBS) (Gibco) at 37°C with 5% CO_2. The clinical tumor tissues of NSCLC patients who were recently diagnosed (n=32) and patient-matched normal samples were collected. This study was approved by the Ethics Committee of the First Affiliated Hospital of China Medical University and College of Basic Medical Sciences.

Cell transfection

For LATS2 and TPTEP1 knockdown experiments, LATS2 short-hairpin RNA (shRNA) and TPTEP1 short-hairpin RNA (shRNA) were used. For overexpression of TPTEP1, pcDNA3.1 TPTEP1 was constructed. pcDNA3.1 or shRNA of TPTEP1, and the mimics or inhibitor of miRNA were obtained from GenePharma company (Shanghai, China). pcDNA3.1-TPTEP1 (full length): F: 5'-GTGAATTCCTCGAGACTAGTTCT-GCCTCTCCCGGTACCTGCT-3', R: 5'-GGATCCG-CGGCCGCTCTAGCACTAGTTTTTGATGGAATTTTT-AGTTT-3', TPTEP1 shRNA: ACAGCAUCGAA-GUAAGAGAUCUCUUACUUCGAUGCUGU, LATS2 shRNA: CCGGCACTTGAAATTCTGCTCTTCACTC-GAGTGAAGAGCAGAATTTCAAGTGTTTTTTG, sh-RNA NC: 5'-TTCTCCGAACGTGTCACGTTTCTT-CAAGAGAGAAACGTGACACGTTCGGTGAA-3', mimic NC: 5'-ACUACUGAGUGACAGUAGA-3', inhibitors NC: 5'-UUCUCCGAACGUGUCACGU-TT-3', miR-761 mimic: 5'-UCAGCAGGCAGGCU-GGUGCAGCGGUAUUCGCACUGGAUACGACGA-ACTTT-3', miR-761 inhibitor: 5'-GCUCCUGGAG-CGUGUUAAUCCUGA-3'. 2-5×10^4 cells were added in 12-well plates containing FBS-free DMEM medium. The transfection solution was diluted with FBS-free DMEM medium and mixed with Lipofectamine™ 2000 reagent (Thermo Fisher Scientific, USA) carefully, and the plasmids were also diluted with FBS-free DMEM medium. The Lipofectamine™ 2000 reagent and plasmids were mixed and incubated for 20 min, and then added into 12-well plates containing the cells. After 48 h, the cells were used for the various experiments.

Bioinformatics analysis

DIANA TarBase (http://www.microrna.gr/tarbase) and Starbase 2.0 (http://starbase.sysu.edu.cn) were used to predict the binding sites of miR-761 with TPTEP1 or LATS2.

Dual-luciferase reporter assay

Dual-luciferase reporter assay was used to examine whether miR-761 could target LATS2 or TPTEP1 directly. The mutant sequences in TPTEP1 and LATS2 belonged to its predicted binding site to miR-761. TPTEP1 WT reporter plasmid, mutated type (mut) TPTEP1 reporter plasmid (mut TPTEP1), LATS2 WT reporter plasmid, and mutated LATS2 reporter plasmid (mut LATS2) were purchased from Promega (Madison, WI, USA). The assay was performed according to the procedures described previously [23].

MTT assay

MTT assay was used to detect the cell viability and reflect cell proliferation. Briefly, 5,000 cells were diluted in a 100 μL medium, and were then seeded and incubated in 96-well plate. 20 μL 5 mg/mL MTT solution (Dojindo, Shanghai, China) was added in each well for the reaction and the plate was incubated under culture conditions for 3-4 h. 100 μL DMSO was added after the supernatant was removed. The optical density (OD) value was determined by a Microtiter plate reader (BioTek, Winooski, VT, USA) at 570 nm wavelength.

Colony formation assay

0.25% trypsin (Gibco) was used to digest cells. Thereafter, the cells were inoculated into a culture plate. The cells were incubated for 2-3 weeks. After that colony formation was observed visually, and 4% paraformaldehyde (Sigma-Aldrich) was used to fix the cells for 15 min. The 0.1% crystalline violet solution (Abcam) was then applied by adding an appropriate amount of the dye solution to the fixative solution for 10 to 30 min. The dye solution was then slowly washed away with the tap water, and the plates were allowed to air dry. The petri dishes were inverted and covered with a transparent grid. The number of the colonies formed (n>10) were then counted under a light microscope (low magnification).

RNA immunoprecipitation (RIP) assay

RIP experiments were carried out by using an EZ-Magna RIP kit (Millipore, USA). First, lysis buffer was used to lyse the collected cells, and then the magnetic beads with antibody targeting Ago2 or IgG as well as RIP buffer were added into the lysate. After this, proteinase K was employed to culture with these magnetic beads, and the precipitated RNA was subsequently eluted and purified by using PCR. The primers used for RIP were as follows: TPTEP1 F: 5'-CTGGGAGAAGTGCCCTTGC-3', TPTEP1 R: 5'-CACCTCATCAGTCATTTGCTCA-3'.

Transwell assay

The upper chambers were precoated with Matrigel (BD Biosciences, USA). Thereafter, the cells were diluted to a density of 2×10^5/well and suspended into an upper chamber. DMEM medium (with 12% FBS) was added to the lower chamber. After the cells have passed through the membrane, paraformaldehyde was used to fix them and the cells were stained with 0.1% crystal violet. The cells were then observed under microscope and counted.

Scratch assay

The cells were seeded in 6-well plates at a density of 6×10^5/well for 12 h. Then, a sterile pipette tip was used to make a scratch in the surface of these wells, and each well was washed with PBS to remove the debris. The pictures of these cells were taken immediately after the scratch was made and 48 h later. The relative migration rate of cells was obtained by counting the percentage of wound healing area.

Quantitative reverse transcription PCR (RT-qPCR)

RNA extraction (TRIzol reagent, Takara, Otsu, Japan), cDNA reversion (RevertAid cDNA Synthesis Kit, ThermoFisher Scientific) and amplification (ABI Prism 7900HT platform, Applied Biosystems, Foster City, CA, USA) were performed according to the procedures described in a previous study [24]. Data were calculated relative to U6 and GAPDH expression, respectively, using the $2^{-\Delta\Delta Cq}$ method.

The primers sequences used are: GAPDH-F: 5'-TCCGTGGTCCACGAGAACT-3'; GAPDH-R: 5'-GAAGCATTTGCGGTGGACGAT-3'; miR-76-F: 5'-ACAGCAGGCACAGAC-3'; miR-76-R: 5'-GAGCAG-GCTGGAGAA-3'; LATS2-F: 5'-ATGAGCTCCACTC-TGCTCAATGTCACGG-3'; LATS2-R: 5'-GCAAG-CTTCTCTA CCAAGAATGAAAGAGCAT3'; TPTEP1-F: 5'-CTGGGAGAAGTGCCCTTGC-3'; TPTEP1-R:
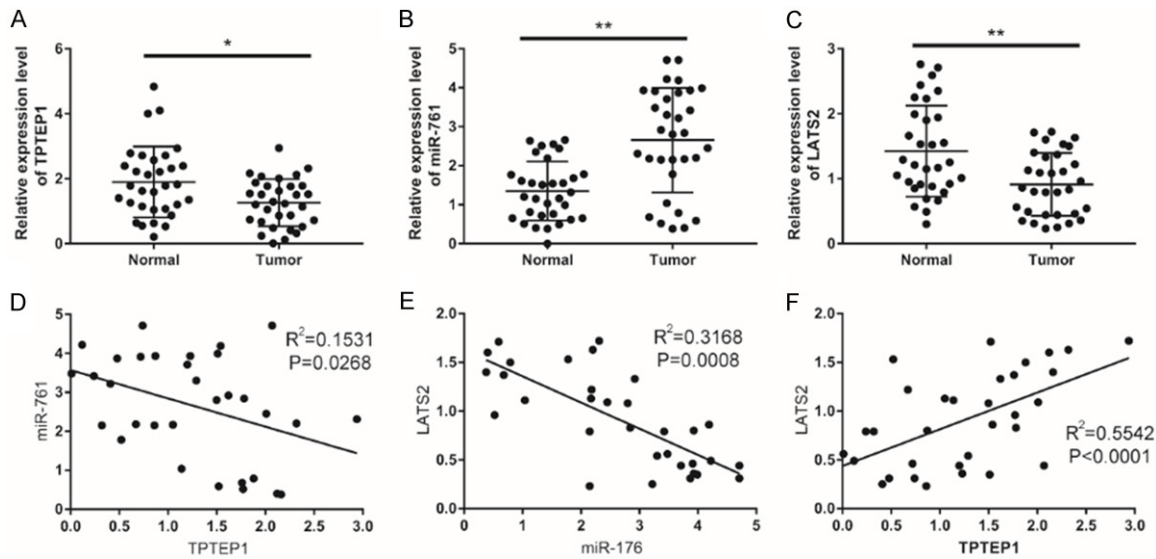
Figure 1. The expression of TPTEP1, miR-761 and LATS2 was decreased in tumor tissues of NSCLC. A-C: The expression of TPTEP1, miR-761 and LATS2 in the tumor tissues and normal adjacent tissues of NSCLC (n=32); D-F: Correlation analysis by Pearson method between TPTEP1 and miR-761, miR-761 and LATS2, TPTEP1 and LATS2. *P<0.05, **P<0.01.

5'-CACCTCATCAGTCATTTGCTCA-3'; U6-F: 5'-CT-CGCTTCGGCAGCACA-3'; U6-R: 5'-AACGCTTCAC-GAATTTGCGT-3'.

Western blot

The protein extraction (RIPA buffer, Thermo-Fisher Scientific, Waltham, MA, USA), determination of the protein concentration (BCA protein assay kit, ThermoFisher Scientific), separation and transferring to the nitrocellulose filter membrane (Millipore, Boston, USA), and incubation with BSA and antibodies were carried out as described previously [16]. The primary antibodies against E-cadherin (1:2,000; sc-8426), Twist (1:2,000; sc-81417), N-cadherin (1:2,000; sc-8424), β-actin (1:2,000; sc-8432), and Vimentin (1:2,000; sc-6260) were obtained from Santa Cruz Biotechnology (CA, USA). Antibody against LATS2 (1:2,000; ab111054) was obtained from Abcam (UK). The secondary antibodies were purchased from Proteintech (USA). The protein expression was detected by ECL system (Amersham Pharmacia, Piscataway, NJ, USA). The protein band intensity relative to the loading control was determined by carrying out densitometric analysis using Image J software.

Statistical analysis

SPSS 18.0 software was used for statistical analyses. Measurement data were presented as the mean ± standard deviation ($\bar{x}$ ± sd) from at least three independent experiments. The differences between the groups were analyzed by Student's t-test or one-way ANOVA followed by Bonferroni's post hoc test. Significant differences were indicated by *P<0.05. The graphic software used for image analysis was Adobe Photoshop CS6 and Adobe illustrator CS6.

Results

The expression of TPTEP1 and LATS2 was decreased, while miR-761 was increased in NSCLC tissues

The expression of TPTEP1 in NSCLC tissues was detected by RT-qPCR. Compared with the matched normal adjacent tissues, the expression level of TPTEP1 was found to be decreased in 32 pairs of the tumor tissues (**Figure 1A**). According to bioinformatics analysis, it was predicted that TPTEP1 could directly bind to miR-761. Besides, there existed a putative binding site for miR-761 in LATS2. Therefore, the transcriptional expression levels of both miR-761 and LATS2 were also measured. Compared with the matched normal adjacent tissues, the result showed that miR-761 expression was significantly up-regulated (**Figure 1B**), while that of LATS2 was inhibited in NSCLC tissues (**Figure 1C**). The possible correlation among TPTEP1, miR-761 and LATS2 was also analyzed. A negative correlation between expression of miR-761

and TPTEP1 (**Figure 1D**), as well as between expression of miR-761 and LATS2 (**Figure 1E**), and a positive correlation between expression of TPTEP1 and LATS2, were observed (**Figure 1F**).

Increased expression of TPTEP1 inhibited cell proliferation and metastasis of NSCLC cells

A549 and H1299 cell lines were used for *in vitro* experiments. Cells were transfected with TPTEP1 pcDNA3.1 to overexpress TPTEP1 (**Figure 2A**). Cell viability was decreased significantly after overexpression of TPTEP1 (**Figure 2B**). The colony formation assay also indicated that TPTEP1 overexpression inhibited cell proliferation significantly (**Figure 2C**). Next, the metastatic abilities of NSCLC cells were detected by Scratch and Transwell assays respectively. It was observed that overexpression of TPTEP1 inhibited the migratory (**Figure 2D**) and invasive abilities (**Figure 2E**) of NSCLC cells. These results suggested that TPTEP1 could exhibit significant inhibitory effects on both cell proliferation and metastasis.

Low expression of TPTEP1 promoted proliferation and metastasis of NSCLC cells

Next, we further explored the function of *TPTEP1*. Cells were transfected with shRNA targeting *TPTEP1* to knock down *TPTEP1* (**Figure 3A**). When *TPTEP1* was expressed at lower levels, cell viability increased significantly (**Figure 3B**). Colony formation assay also showed that *TPTEP1* knockdown significantly promoted cell proliferation (**Figure 3C**). Scratch and Transwell assays were used to detect the metastatic ability of NSCLC cells. The low expression of *TPTEP1* enhanced the migration and invasion potential of NSCLC cells (**Figure 3D**, **3E**). These findings further indicated that *TPTEP1* knockdown could significantly inhibit cell proliferation and metastasis.

miR-761 could be a direct target of TPTEP1

Here, DIANA was used to predicted the binding site of TPTEP1 to miR-761 (**Figure 4A**). As shown in **Figure 4B**, after transfection with TPTEP1 pcDNA3.1, overexpression of TPTEP1 reduced expression of miR-761 significantly. In order to assess the interaction between miR-761 and TPTEP1, the RIP and dual luciferase reporter assays were performed. It was found

that after being transfected with miR-761 mimics, the expression of miR-761 was enhanced. In contrast, miR-761 inhibitor reduced the expression of miR-761 (**Figure 4C**). Moreover, RIP assay further confirmed that there was an interaction between TPTEP1 and miR-761 in both A549 and H1299 cells (**Figure 4D**). In addition, miR-761 suppressed the luciferase activity notably in the cells transfected with wide type TPTEP1, while miR-761 inhibitor enhanced the luciferase activity (**Figure 4E**). However, luciferase activity was not affected in the cells transfected with mutated TPTEP1. Therefore, miR-761 may be directly targeted by TPTEP1.
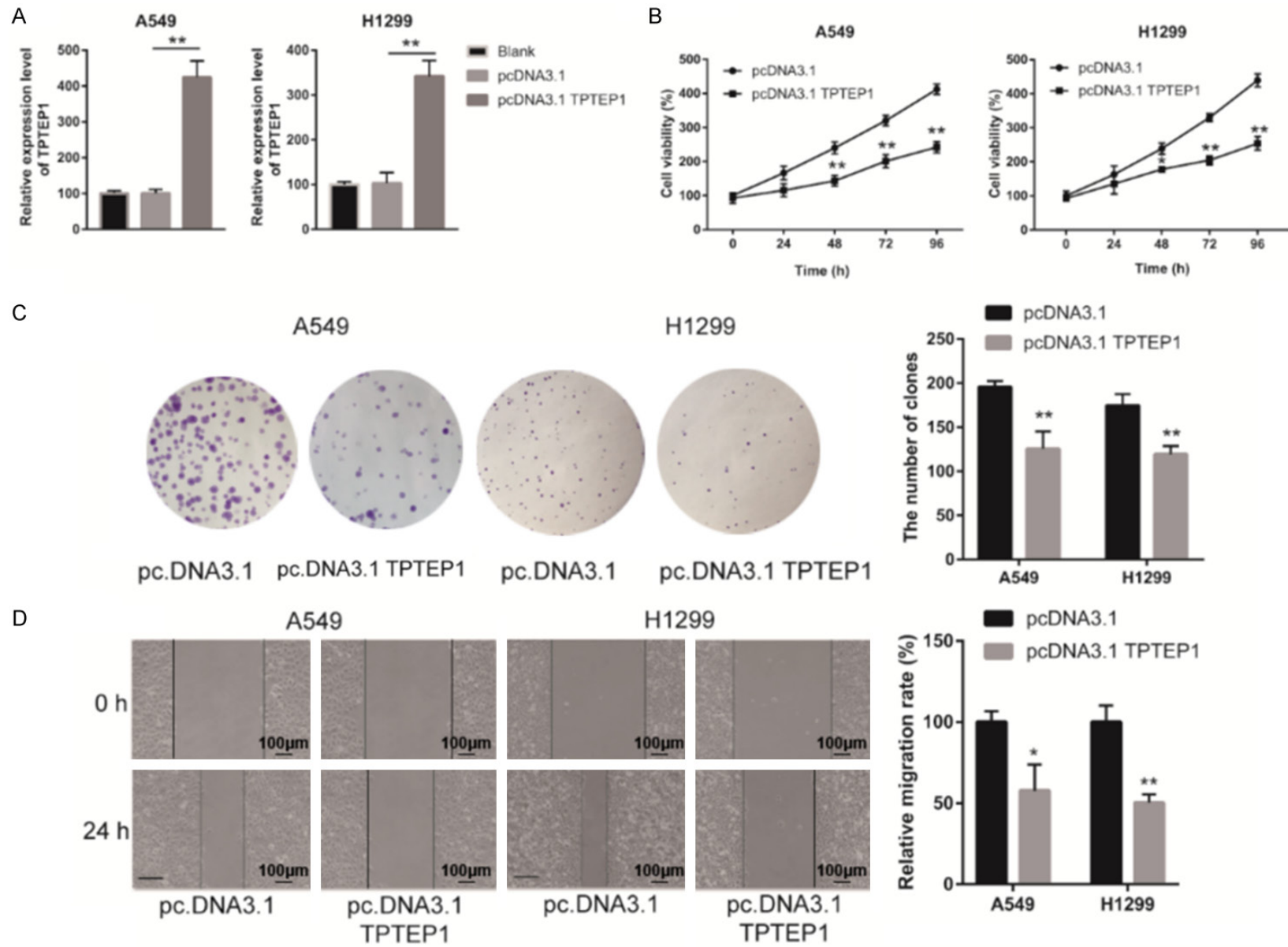
TPTEP1 inhibited the progression of NSCLC via suppressing miR-761

Next, the effects of miR-761 on the metastatic abilities of *NSCLC* cells were investigated. It was observed that overexpression of TPTEP1 inhibited cell viability notably, which could be blocked by miR-761 mimics (**Figure 5A**). This result was also verified by colony formation assay. As shown in **Figure 5B**, after co-overexpression of miR-761 and TPTEP1, miR-761 could effectively reverse the inhibitory effects of TPTEP1 overexpression on cell proliferation. In addition, overexpression of TPTEP1 suppressed the metastatic potential of NSCLC cells, which was also blocked by the overexpression of miR-761 (**Figure 5C**, **5D**). Hence, the inhibitory effects of TPTEP1 on cell proliferation and metastasis could also be achieved by suppression of miR-761.

miR-761 could directly target LATS2

The downstream mechanism of TPTEP1/miR-761 was also studied in A549 and H1299 cell lines. It was predicted by Starbase 2.0 software that LATS2 might be a direct target of miR-761 (**Figure 6A**), and it was noted that overexpression of TPTEP1 significantly enhanced the expression of LATS2 (**Figure 6B**). In addition, the expression of LATS2 was decreased in the cells with miR-761 overexpression, while it was enhanced significantly in the cells with miR-761 knockdown (**Figure 6C**). The western blot analysis data also revealed that expression of LATS2 protein was increased with overexpression or decreased with knockdown of miR-761 (**Figure 6D**). To identify the potential interaction
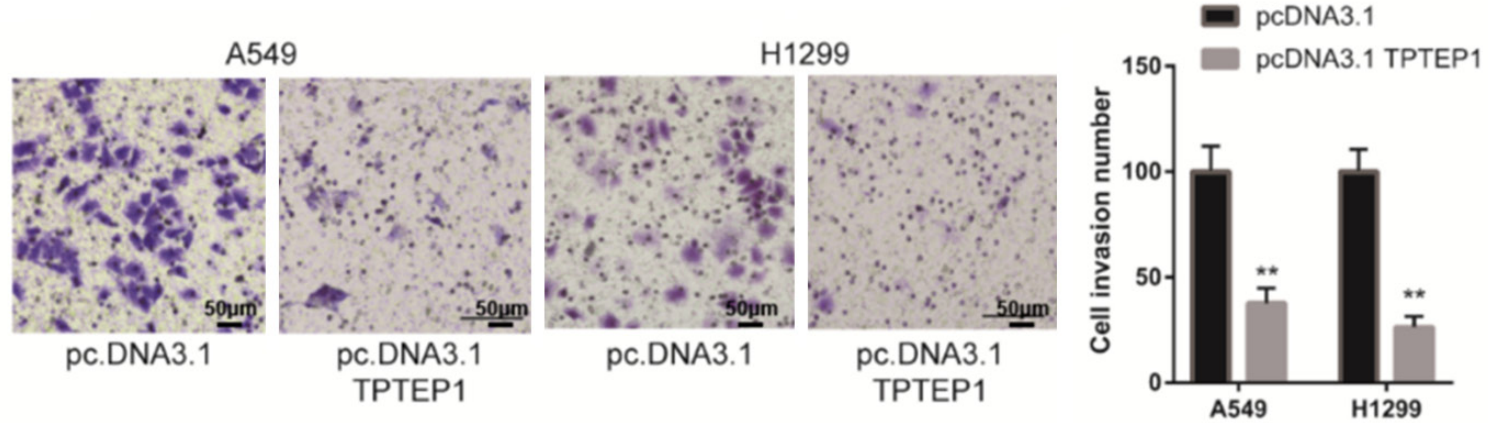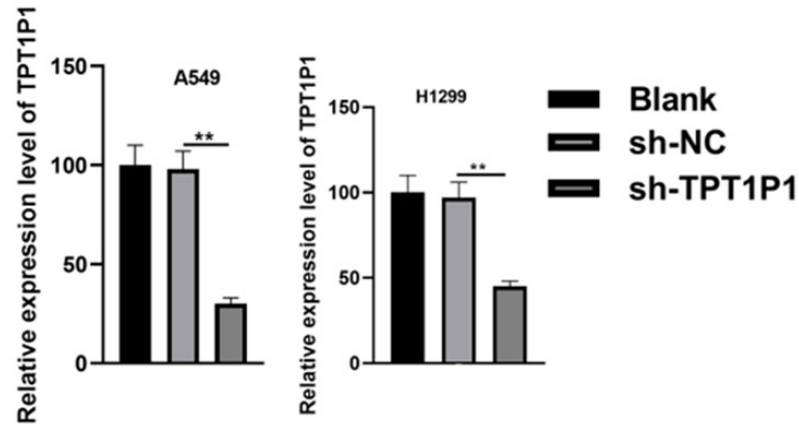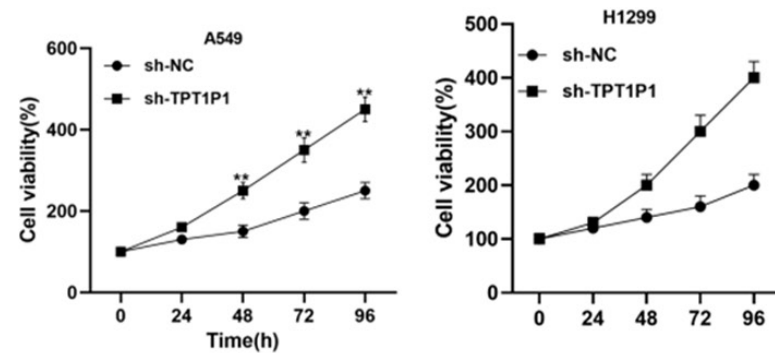
Figure 2. TPTEP1 inhibited the proliferation and metastasis in cell lines of NSCLC. The cell lines, including A549 and H1299 cells, were transfected with pcDNA3.1 NC and pcDNA3.1 TPTEP1. A: The expression of TPTEP1 in cell lines of NSCLC, including A549 and H1299 cells, transfected with TPTEP1 pcDNA3.1, was detected by RT-Qpcr; B: The cell viability was determined by MTT assay in cell lines of NSCLC transfected with TPTEP1 pcDNA3.1; C: The cell proliferation was determined by colony formation assay in cell lines of NSCLC transfected with TPTEP1 pcDNA3.1; D, E: The cell migration and invasion were detected by scratch assay (100×) and transwell assay (200×) in cell lines of NSCLC transfected with TPTEP1 pcDNA3.1, respectively. $^*P<0.05$, $^{**}P<0.01$.
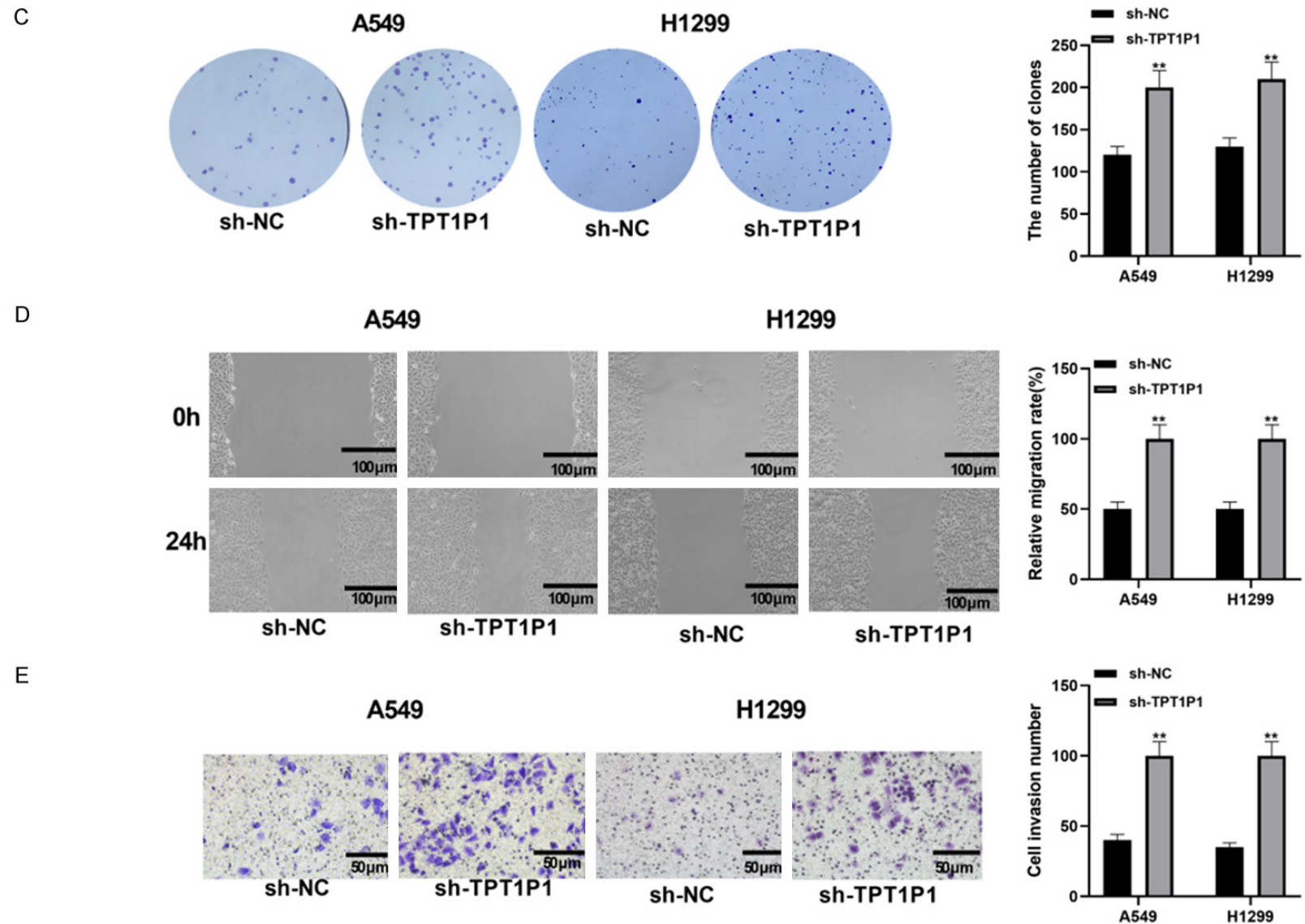
Figure 3. The effect of low TPTEP1 expression on the proliferation and metastasis of NSCLC cell lines. Sh-NC and sh-TPTEP1 were transfected into A549 and H1299 cells. A: RT-qPCR was used to detect the expression of TPTEP1 in A549 and H1299 NSCLC cell lines transfected with sh-TPTEP1; B: MTT assay was used to detect the viability of NSCLC cell lines transfected with sh-TPTEP1; C: Proliferation of NSCLC cell lines transfected with sh-TPTEP1; D, E: Scratch test was used to detect the cell migration and invasion (100×). *P<0.05, **P<0.01
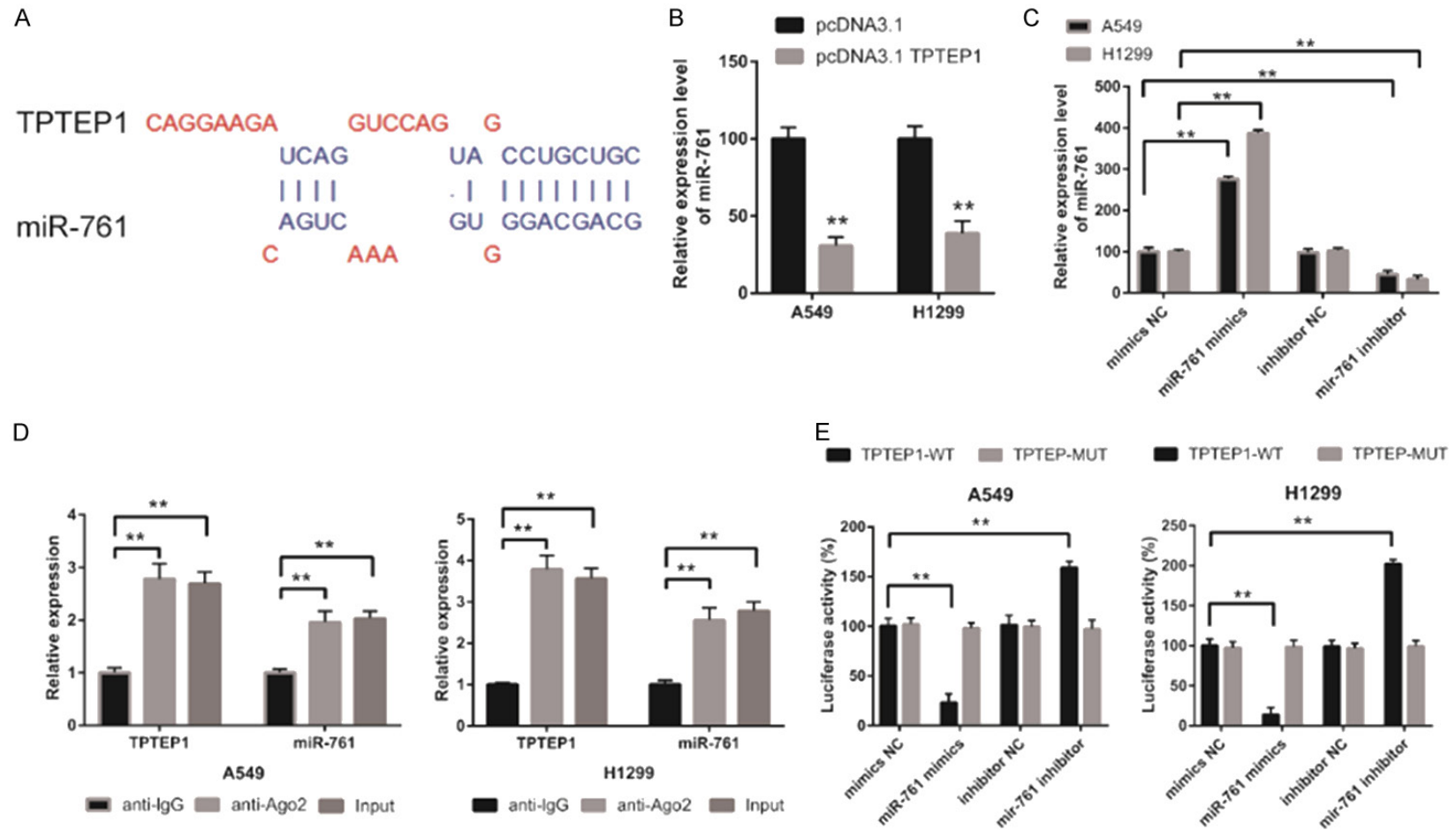
Figure 4. miR-761 was directly targeted by TPTEP1. A: The binding sites of TPTEP1 and miR-761 was predicted by DIANA; B: The expression of miR-761 was determined by RT-qPCR; C: The expression of miR-761 was determined by RT-qPCR in cells transfected with TPTEP1 pcDNA3.1; D, E: RIP assay and dual luciferase reporter assay was used to determine the interaction between miR-761 and TPTEP1 in cell lines of NSCLC. The miR-761 binding site of TPTEP1 was mutated from CCUGCUG to AAGCCCU. **P<0.01.
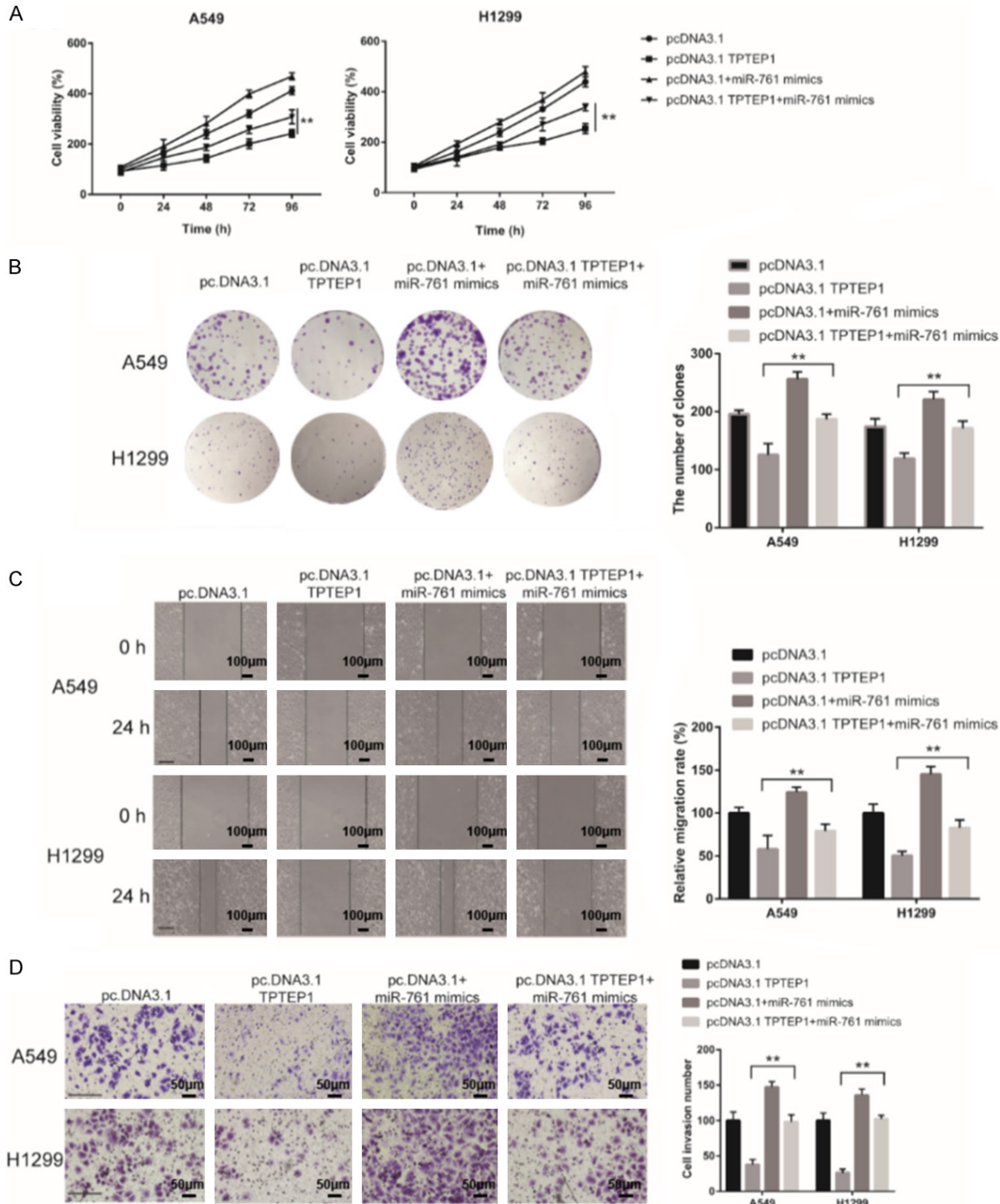
Figure 5. TPTEP1 inhibited the proliferation and metastasis in cell lines of NSCLC via suppressing miR-761. The cell lines, including A549 and H1299 cells, were transfected with pc DNA3.1 NC, pcDNA3.1 TPTEP1, and miR-761 mimics. A: The cell viability was determined by MTT assay in cell lines of NSCLC. B: The cell proliferation was determined by colony formation assay in cell lines of NSCLC; C, D: The cell migration and invasion were detected by scratch assay (100×) and transwell assay (200×) in cell lines of NSCLC, respectively. **P<0.01.

between miR-761 and LATS2, we performed dual luciferase reporter assay and found that LATS2 could directly target miR-761 (**Figure** **6E**). These results suggested that LATS2 could function as a downstream effector of TPTEP1/miR-761 and targeted by miR-761.
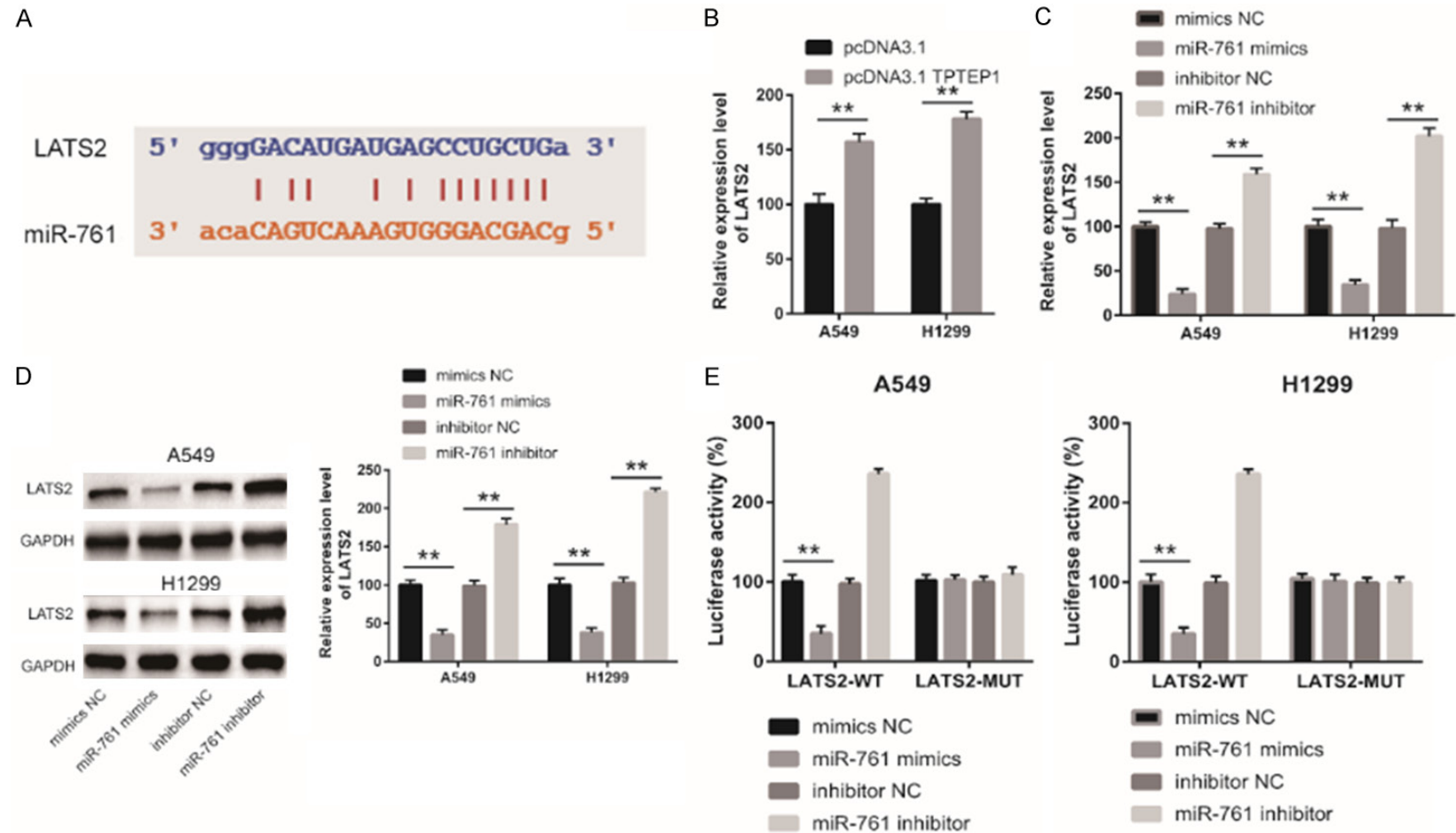
Figure 6. LATS2 was a direct target of miR-761. A: The binding sites of miR-761 and LATS2 was predicted by Starbase 2.0 software; B, C: The expression of LATS2 was determined by RT-qPCR in the cells with overexpression of TPTEP1 or miR-761; D: The expression of LATS2 was determined by western blot in cell lines of NSCLC; E: Dual luciferase reporter assay was used to determine the interaction between miR-761 and LATS2 in cell lines of NSCLC. The miR-761 binding site of LATS2 was mutated from CCUGCUG to AAGCCCU. **P<0.01.

miR-761 enhanced proliferation and metasta-sis of NSCLC cells via suppressing LATS2

We also explored the potential role of LATS2 on the proliferation and metastasis of NSCLC cells. It was observed that LATS2 shRNA reduced the expression of LATS2 significantly (**Figure 7A**). After transfection with miR-761 inhibitor and LATS2 shRNA, it was noted that knockdown of miR-761 suppressed the cell via-bility, while knockdown of LATS2 increased the cell viability, and LATS2 shRNA reversed the inhibitory effects of miR-761 inhibitor on cell proliferation (**Figure 7B**). The similar effects were also verified by colony-formation assay (**Figure 7C**). The inhibitory role of miR-761 inhib-itor on cell proliferation could be effectively blocked by LATS2 shRNA. As for metastasis, it was found that miR-761 inhibitor suppressed the ability of cellular migration and invasion, respectively (**Figure 7D**, **7E**). However, after knockdown of LATS2, the migratory and inva-sive abilities were increased significantly (**Figure 7D**, **7E**). Therefore, miR-761 could pos-sibly exert an antitumor role on NSCLC cells via negative regulation of LATS2.

TPTEP1 regulated EMT-related proteins by af-fecting miR-761/LATS2 axis

The possible role of TPTEP1/miR-761/LATS2 on EMT was finally analyzed. NSCLC cell lines were incubated with TPTEP1 pcDNA3.1, miR-761 mimics, miR-761 inhibitor or LATS2 shRNA. Thereafter, the EMT-related protein expres-sions, such as N-cadherin, Twist, Vimentin, and E-cadherin were detected. It was found that overexpression of TPTEP1 promoted the ex-pression of E-cadherin, while reduced the expression of N-cadherin, Twist, and Vimentin (**Figure 8A**). The effects of TPTEP1 could be reversed by overexpression of miR-761 (**Figure 8A**). Furthermore, miR-761 knockdown sub-stantially increased the expression of E-cadherin, while decreased the expression of N-cadherin, Twist, and Vimentin (**Figure 8B**). Additionally, these changes were reversed by the knockdown of LATS2 (**Figure 8A**). The sche-matic diagram of our research was summari-zed in **Figure 9**. Our research indicated that in NSCLC cells, TPTEP1/miR-761/LATS2 axis could actively participate in the regulation of EMT.

Discussion

Metastasis is the main cause of high mortality observed in NSCLC patients, and no effective therapies are available to treat metastatic lung cancer [22]. Here, we analyzed the functions of TPTEP1 in regulating the metastasis of NSCLC cells and revealed the underlying mechanisms. It was found that TPTEP1 was downregulated in the NSCLC tissues, and its expression played an antitumor role in NSCLC cell lines. The anti-tumor effects of TETEP1 were primarily achieved by targeting miR-761, which effective-ly targeted and downregulated the expression of LATS2. Thus, TETEP1 potentially increased the expression of LATS2 via sponging miR-761 and then significantly inhibited metastasis of NSCLC.

The tumor suppressor roles of TPTEP1 have also been verified in other types of cancers. For example, in hepatocellular carcinoma, TPTEP1 suppressed the tumor progression via reducing phosphorylation of STAT3 [7]. In glioma, TETEP1 can target miR-106a-5p and regulate p38 MAPK signaling pathway to inhibit cell stem-ness and radio-resistance [8]. TPTEP1 has also been implicated as an independent prognostic biomarker in pediatric rhabdoid tumor of the kidney [25]. Our research verified the inhibitory role of TPTEP1 in regulating both metastasis and cell proliferation in NSCLC cells. A previous study has also illustrated that TPTEP1 can sponge miR-328-5p to inhibit proliferation of NSCLC cells [9]. As another downstream factor of TPTEP1, miR-328-5p can also target Src kinase signaling inhibitor 1 (SRCIN1) and cause an inhibition of proliferation and apoptosis. Since the downstream target of TPTEP1 in NSCLC may not only be limited to miR-761, an overexpression of miR-761 could not complete-ly reverse the inhibition of proliferation by over-expression of TPTEP1 in our study. Therefore, the potential role of miRNAs downstream of TPTEP1 in NSCLC and their contributions to the anti-tumor effect of TPTEP1 needs to be further explored. On the contrary, TPTEP1 has also been found to be associated with poor progno-sis in some tumors, including pediatric rhab-doid tumor of the kidney (RTK) [25]. In RTK, TPTEP1 expression was related to high risk of death and could promote the tumor develop-ment, which might result from the variations found in the tumors [25]. Therefore, the expres-sion and the possible effects of TPTEP1 in dif-ferent tumors need to be further confirmed.

We also reported the interaction between TPTEP1 and miR-761 for the first time, and
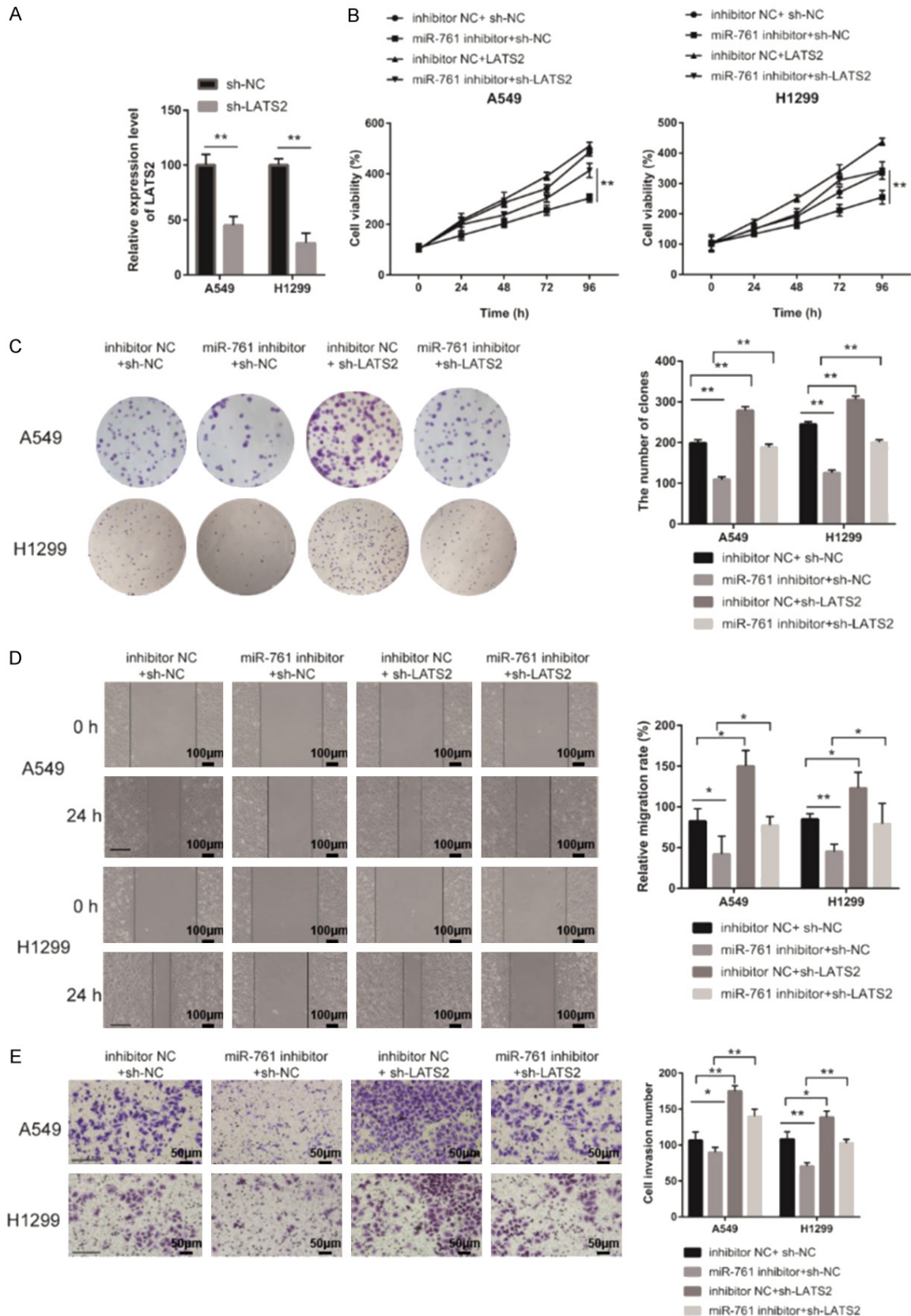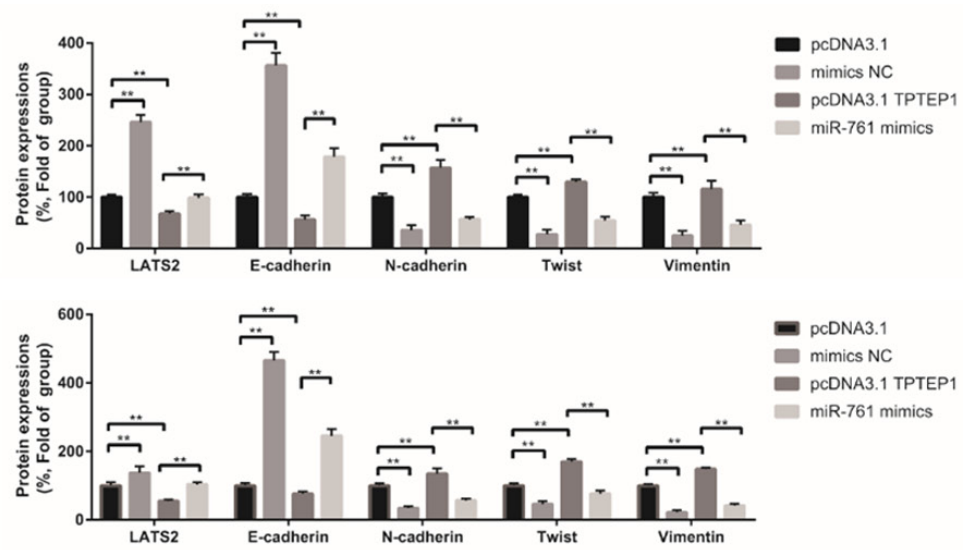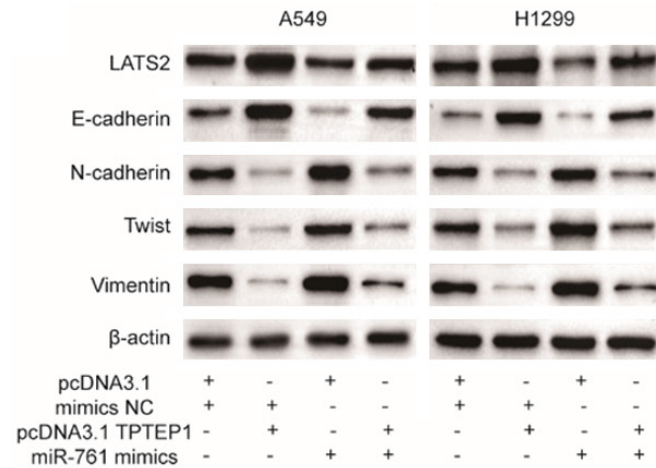
Figure 7. miR-761 promoted the tumor progression in cell lines of NSCLC via suppressing LATS2. The cell lines, including A549 and H1299, were transfected with inhibitor NC, shRNA NC, miR-761 inhibitor and LATS2 shRNA. A:
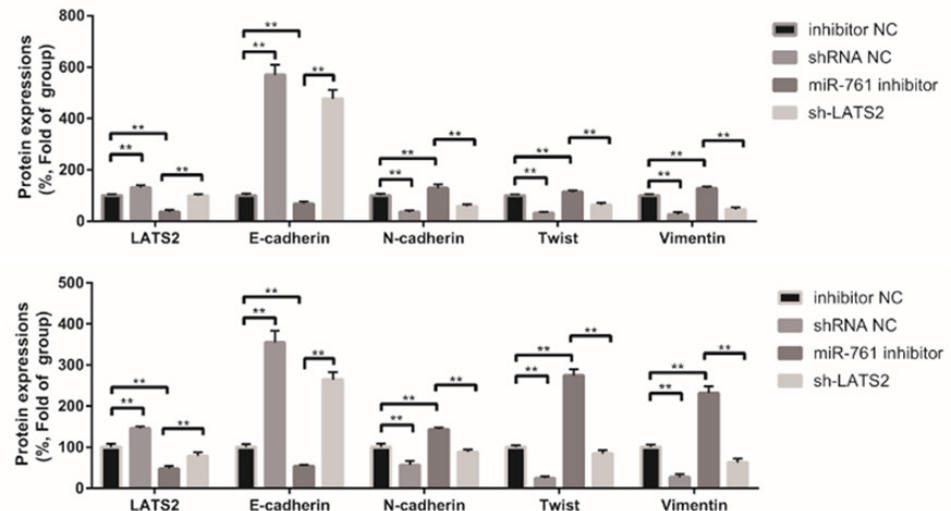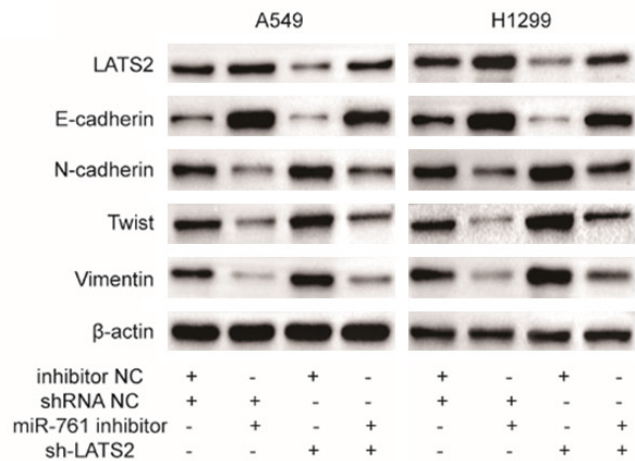
The transcriptional expression of LATS2 was detected by RT-qPCR in cell lines of NSCLC; B: The cell viability was determined by MTT assay in cell lines of NSCLC; C: The cell proliferation was determined by colony formation assay in cell lines of NSCLC; D, E: The cell migration and invasion abilities were detected by scratch assay (100×) and transwell assay (200×) in cell lines of NSCLC, respectively. *P<0.05, **P<0.01.
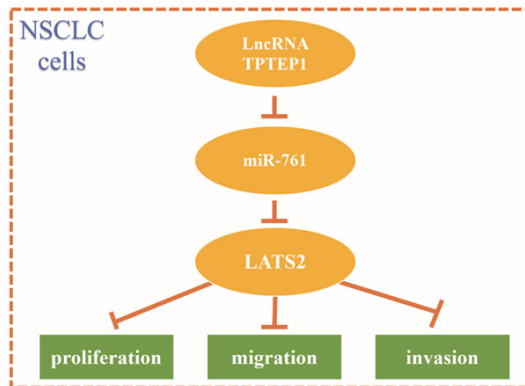
Figure 9. The mechanism of TPTEP1/miR-761/LATS2 axis in NSCLC cells.

showed the mechanism of miR-761 in NSCLC cells. A number of studies have reported the oncogenic roles of miR-761 in breast cancer, synovial sarcoma, and in NSCLC cells [13, 14, 26]. In NSCLC cells, previous studies have indicated that miR-761 can be negatively regulated by LncRNA FENDRR, which promotes the tumor progression, cell growth and aggressiveness [27, 28]. It has also been suggested that the oncogenic role of miR-761 could be achieved by targeting TIMP2 (tissue inhibitor of metalloproteinase 2) or ING4 (inhibitor of growth family, member 4), which has been reported to attenuate metastasis and act as tumor suppressors in A549 cells [14, 27, 29]. Our results indicated that miR-761 effectively promoted metastasis and cell proliferation, facilitated expression of various EMT-related genes and revealed that LATS2, one of the targets of miR-761, could play a significant antitumor role in NSCLC. miR-761 has also been reported to act as a tumor suppressor. For example, miR-761 can inhibit osteosarcoma by targeting CXCR1 [30]. In colorectal cancer, miR-761 reduces cell growth and motility via targeting HDAC1 [31]. In ovarian carcinoma, miR-761 can also attenuate tumor by targeting MSI1 [32]. These findings are consistent with the previous studies of miR-761 in NSCLC [14, 27, 28].

Moreover, it was found that miR-761 promoted EMT in A549 and H1299 cells, by targeting

LATS2 regulation. LATS2 is a tumor suppressor, whose low expression was associated with poor prognosis in NSCLC [20]. We found that the knockdown of LATS2 significantly reversed the cell proliferation and metastasis inhibition induced by miR-761 inhibitor, revealing that LATS2 could possibly regulate the downstream of miR-761 and suppress the progression of NSCLC. In addition to NSCLC, LATS2 has also been reported to inhibit malignant behaviors of glioma, promote cell death in liver cancer, and reduce therapeutic resistance [33-35]. EMT is a key process that drives tumor invasion and metastasis, and was originally defined as morphological transformation during embryogenesis. The concept of EMT is characterized by loss of epithelial markers, increased expression of interstitial markers, and enhanced migration and invasion in tumor cells [36]. LATS2 has been reported to play an important role in regulating cell metastasis and EMT. Thus, inhibition of LATS2 can effectively promote EMT progression [17, 24]. Therefore, over-expression of LATS2 may be a therapeutic strategy to target EMT and metastasis [24]. Although the inhibitory ability of LATS2 on invasion and migration of NSCLC has been verified in this study, the effects of LATS2 expression on EMT and its related proteins need to be further investigated.

In conclusion, our present study clearly showed that LncRNA TPTEP1 cascade played an inhibitory role in the metastasis of NSCLC cells by regulating miR-761/LATS2 expression, thereby highlighting the potential therapeutic value of TPTEP1/miR-761/LATS2 axis in NSCLC.

Disclosure of conflict of interest

None.

Abbreviations

NSCLC, Non-small cell lung cancer; RIP, RNA immunoprecipitation; LncRNAs, long non-coding RNAs; EMT, epithelial to mesenchymal transition; miRNAs, microRNA; ceRNAs, competitive endogenous RNAs; ATCC, American type culture collection; FBS, fetal bovine serum.

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