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LncRNA NEAT1 regulated cell proliferation, invasion, migration and apoptosis by targeting has-miR-376b-3p/SULF1 axis in non-small cell lung cancer

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Abstract. – OBJECTIVE: Recently, lncRNA has been determined to play an important role in cancer formation and development. However, the regulatory mechanism of lncRNA in NSCLC has not been fully explored.

PATIENTS AND METHODS: The expression of NEAT1, miR-376b-3p, and SULF1 was detected in each group *via* quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). The proteins expression of SULF1, p-MAPK, MAPK, p-Akt, Akt, and GAPDH were measured *via* Western blot. MTT assay was applied to detect cell proliferation in each group. Transwell assay was used to assess cell invasion and migration of each group. Cell apoptosis was assessed with flow cytometry. The relationship among NEAT1, miR-376b-3p, and SULF1 was determined using Luciferase reporter assay.

RESULTS: In this study, the expression of NEAT1 and SULF1 was upregulated in NSCLC tissues and cells. Of note, the knockdown of NEAT1 and SULF1 could inhibit cell proliferation, migration, and invasion and promote cell apoptosis in NSCLC. Moreover, NEAT1 regulated SULF1 expression *via* binding to miR-376b-3p in NSCLC cells. Otherwise, the effects of NEAT1 on cell growth and apoptosis were reversed by improving the SULF1 expression in NSCLC cells. Meanwhile, si-NEAT1 transfection inhibited MAPK and Akt signaling pathway by modulating SULF1 in NSCLC cells.

CONCLUSIONS: In this study, we found that lncRNA NEAT1 regulated cell proliferation, invasion, migration, and apoptosis by targeting has-miR-376b-3p/SULF1 axis in NSCLC. Moreover, the regulatory network of NEAT1 participated in the phosphorylation levels of MAPK and Akt to affect cell progression of NSCLC, providing a new regulatory pathway in the pathogenesis of lung cancer.

Key Words:
NSCLC, LncRNA NEAT1, MiR-376b-3p, SULF1, Proliferation, Migration, Invasion, Apoptosis.

Introduction

Lung cancer is the most common malignant tumor with the highest morbidity and mortality in the world, including small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC)[1,2]. NSCLC accounts for about 80% of lung cancer, which has a high incidence and a poor prognosis[3]. Moreover, the five-year survival rate of NSCLC is poor, thus the treatment of NSCLC is pivotal[4]. However, studies on the pathogenesis of NSCLC have not been fully elucidated.

Long non-coding RNA is a type of non-coding RNA that is more than 200 nts in length and plays multiple roles in the formation and development of cancer cells. LncRNA, as an important regulatory factor, was involved not only in various cell development processes, such as cell proliferation, migration, apoptosis, but also in chemoresistance of cancers[5-8]. Liu et al[9] reported that lncRNA HOTAIR promoted cell progression in cervical cancers. Otherwise, the similar function of lncRNA HORAIR has also verified in gastric cancer and

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colorectal cancer[9,10]. HORAIR also promoted gastric cancer cells cisplatin resistance[11].

LncRNA Nuclear Enriched Abundant Transcript 1 (NEAT1) has been reported to be highly expressed in a variety of cancers tissues and cells, including prostate cancer, breast cancer, renal cancer, gastric cancer, and NSCLC, which prompted cell growth, invasion, and proliferation[12-15]. Moreover, lncRNA NEAT1 could affect cell proliferation, migration, and inflammation *via* regulating Akt signaling pathway and MAPK signaling pathway[16-19]. In NSCLC, NEAT1 was upregulated in cancer tissues and cells and could participate in the regulation of lung cancer cell proliferation, apoptosis, migration, and invasion[19-21]. Therefore, NEAT1 was considered to be an important oncogene involved in NSCLC formation and metastasis. However, the regulatory mechanism of NEAT1 in NSCLC has not been fully elucidated.

In this study we found that NEAT1 is highly expressed in NSCLC tissues and cells and predicted that miR-376b-3p was a target miRNA of NEAT1 and SULF1 was a target mRNA of miR-376b-3p *via* StarBase v3.0. During a series of experiments, we finally determined that NEAT1 affected cell growth by sponging miR-376b-3p to regulate SULF1 in NSCLC.

Patients and Methods

Tissues and Patients

Forty NSCLC tissues and matched adjacent tissues were obtained from patients who were diagnosed at The First Affiliated Hospital of Shantou University Medical College. Informed consents were obtained from all patients. This study was approved by the Research Ethics Committee of The First Affiliated Hospital of Shantou University Medical College. All tissues were immediately frozen in liquid nitrogen and then stored in -80°C.

Cell Culture and Transfection

NSCLC cell lines (A549 and H292), normal human lung epithelial cell line (BEAS-2B), and 293T cells were purchased from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). Then, all cell lines were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA, USA), penicillin and streptomycin (Invitrogen, Carlsbad, CA, USA) at 37°C with 5% of CO_2.

The si-NEAT1, si-SULF1, PcDNA-SULF1, and negative control siRNA (si-NC and PcDNA-control) were obtained from Ribobio (Guangzhou, China). The miR-376b-3p inhibitor, miR-376b-3p mimic, and their negative control (inhibitor NC and miRNA NC) were purchased from Ribobio (Guangzhou, China). All oligonucleotides and vectors were transfected into NSCLC cell lines (A549 and H292) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

Quantitative RT-PCR

Total RNA was extracted from NSCLC tissues and cells using TRIzol reagents (Invitrogen, Carlsbad, CA, USA). The cDNA for miR-376b-3p was reverse transcribed using TaqMan miRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). The cDNA of SULF1 and NEAT1 was reverse transcribed by using M-MLV Reverse Transcriptase (Promega, Madison, WI, USA). The quantitative Real Time-PCR was performed with SYBR Green PCR Master Mix (TaKaRa, Otsu, Shiga, Japan) on an ABI7500 system (Applied Biosystems, Foster City, CA, USA). The expression of miR-376b-3p, SULF1, and NEAT1 was calculated with the $2^{-\Delta\Delta CT}$ method with u6 and GAPDH as reference gene, respectively. The primer sequences are as follows: NEAT1-F: 5'-CTTCCTCCCTTTAACTTATCCATTCAC-3', NEAT1-R: 5'-CTCTTCCTCCACCATTACCAACAATAC-3', GAPDH-F: 5'-AGCCTCCCGCTTCGCTCTCT-3', GAPDH-R: 5'-GCGCCCAATACGACCAAATCCGT-3'. miR-376b-3p-F: 5'-TTGGTGGGTGGTGTATTTGAGAAGATAATCATTG-3' miR-376b-3p-R: 5'-CCAAAGCAAGAAATCATATGCTGTTCTCAGTGC-3', U6-F: 5'-CTCGCTTCGGCAGCACA-3' U6-R: 5'-AACGCTTCACGAATTTGCGT-3 SULF1-F: 5'-CCACCTTCATCAATGCCTT-3', SULF1-R: 5'-CCTTGACCAGTCCAAACTGCCC-3',

Western Blot

Tissues and cells were lysed in Tris-Buffered Saline (TBS) and added with the RIPA buffer (Thermo-Fisher Scientific, Inc., Waltham, MA, USA). The proteins were separated by using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). After blocking in non-fat milk, the membranes were incubated

with primary antibody against SULF1, p-MAPK, MAPK, p-Akt, Akt, and GAPDH (1:1500, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) overnight at 4°C. Then, the membranes were incubated with second antibody peroxidase-conjugated AffiniPure goat anti-mouse IgG (1:1000, Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA). Finally, the blot was detected using a Pierce enhanced chemiluminescence (ECL) Plus Substrate (Thermo-Fisher Scientific, Waltham, MA, USA).

5-diphenyltetrazolium Bromide (MTT) Assay

MTT assay was used to measure the capacity of cell proliferation. In this study, cell proliferation was detected using MTT assay kit (Abcam, Cambridge, MA, USA). Briefly, transfected cells ($2x10^3$ cells per well) were seeded into 96-well plates and each well was added into 50 ul MTT solution and incubated at room temperature for 4 hours. Then, they were added into 150 μL MTT solvent and incubated for 3 h. The optical density (OD) was measured with a spectrophotometric microplate reader (Beyotime Institute of Biotechnology, Haimen, China) at 450 nm.

Flow Cytometry

Flow cytometry was mainly applied to assess cell apoptosis rate. In this experiment, cell apoptosis was measured by using the Annexin V-fluorescein isothiocyanate/Propidium Iodide (FITC/PI) Apoptosis Detection Kit (BD Biosciences, Franklin Lakes, NJ, USA) according to the manufacturer's instructions. After transfected cells were washed with PBS, the binding buffer was added to resuspend the cells and then the Annexin V-FITC and PI was added. Lastly, cell apoptosis was measured using flow cytometry.

Transwell Migrated and Invasive Assay

Cell migration and invasion were assessed by using transwell migrated and invasive assay. For cell invasion, the transwell (Corning, Corning, NY, USA) and Matrigel (BD Biosciences) were applied. For cell migration, the transwell (Corning, NY, USA) was needed without Matrigel. The transfected cells were seeded in the upper chamber and incubated at 37°C with 5% of CO_2 for 24 h. Then, the cells were removed in the upper chamber, and the cells in the lower chamber were counted using a Leica DM3000 microscope (Wetzlar, Germany).

Luciferase Reporter Assay

To explore whether NEAT1 could directly target miR-376b-ap or miR-376b-3p could directly target SULF1, the Luciferase reporter assay was applied. The lncRNA NEAT1 containing wild type and mutant type was amplified, and then, inserted into the Luciferase reporter vector psiCHECK-2 (Promega, Madison, WI, USA) to construct the vector of WT-NEAT1 and MUT-NEAT1. The SULF1 containing wild type and mutant type was amplified and then inserted into the Luciferase reporter vector psiCHECK-2 (Promega, Madison, WI, USA) to construct the vector of WT-SULF1-3'UTR and MUT-SULF1-3'UTR. Then, the WT-NEAT1 or MUT-NEAT1 was co-transfected with miR-376b-3p or NC into the 293T cells using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, MA, USA). The WT-SULF1-3'UTR or MUT-SULF1-3'UTR was co-transfected with miR-376b-3p or NC into the 293T cells using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, MA, USA). The Luciferase activities were detected with a Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA).

Statistical Analysis

Statistical analysis was displayed with GraphPad Prism 7.0 (GraphPad Software, San Diego, CA, USA). All data were shown as mean ± standard deviation (SD). Student's *t*-tests were applied to analyze all comparisons. $*p < 0.05$ was considered as statistically significant.

Results

The Expression of NEAT1 and SULF1 Was Upregulated in NSCLC Tissues and Cells

To explore the function of NEAT1 and SULF1 in NSCLC progression, 40 NSCLC tissues and matched adjacent tissues were collected. As shown in Figure 1A-1B, the result of qRT-PCR showed that NEAT1 and SULF1 expression was significantly improved in NSCLC tissues. Otherwise, the protein level of SULF1 in NSCLC tissues was significantly higher than that in normal tissues (Figure 1C). Then, we also found that NEAT1 and SULF1 expression was remarkably upregulated in NSCLC cell lines (A549 and H292) compared with that in normal lines (BEAS-2B) (Figure 1D and 1E). In addition, the protein level of SULF1 was increased in NSCLC cells (Figure 1F). Therefore, the expression of

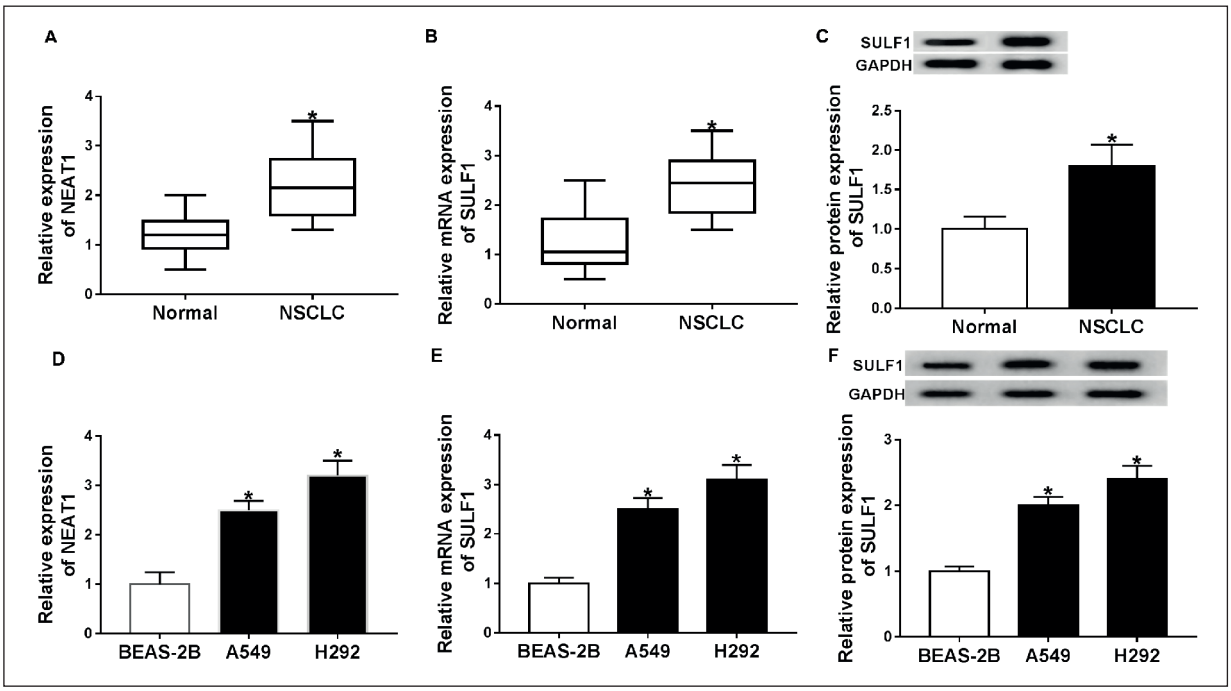

Figure 1. The expression of NEAT1 and SULF1 was upregulated in NSCLC tissues and cells. **A,** and **B,** The expression of NEAT1 and SULF1 in normal tissues and NSCLC tissues was detected by using qRT-PCR assay. **C,** The protein expression of SULF1 in normal tissues and NSCLC tissues was detected by using Western blot. **D,** and **E,** The expression of NEAT1 and SULF1 in normal human lung epithelial cell line (BEAS-2B) and NSCLC cell lines (A549 and H292) was detected by using qRT-PCR assay. **F,** The protein expression of SULF1 in normal human lung epithelial cell line (BEAS-2B) and NSCLC cell lines (A549 and H292) was detected using Western blot. $*p < 0.05$.

NEAT1 and SULF1 was upregulated in NSCLC tissues and cells, determining that NEAT1 and SULF1 play roles in NSCLC progression.

Knockdown of NEAT1 Could Inhibit Cell Proliferation, Migration, and nvasion and Promote Cell Apoptosis in NSCLC Cells

To further investigate the effects of NEAT on NSCLC cell growth and apoptosis, si-NC and si-NEAT1 were transfected into A549 and H292 cells and si-NEAT1 significantly inhibited NEAT1 expression (Figure 2A). MTT assay determined that si-NEAT1 transfection remarkably inhibited cell proliferation in A549 and H292 cells Figure 2B and 2C). Additionally, cell apoptosis of si-NEAT1 group was significantly higher than that of si-NC group in A549 and H292 cells (Figure 2D). Moreover, analysis of transwell assay showed that si-NEAT1 sharply inhibited cell migration and invasion in A549 and H292 cells (Figure 2E and 2F). Thus, the downregulation of NEAT1 could inhibit cell growth and induced cell apoptosis in NSCLC.

NEAT1 Modulated Cell Proliferation, Migration, Invasion and Apoptosis by Targeting miR-376b-3p in NSCLC Cells

Next, we used StarBase v3.0 to predict the target miRNA of NEAT1, and the results showed that NEAT1 has binding sites with miR-376b-3p (Figure 3A). The analysis of Luciferase reporter assay showed that when miR-376b-3p combined WT-NEAT1, rather than MUT-NEAT1, the Luciferase activity was significantly lowered (Figure 3B). Moreover, we found that miR-376b-3p inhibitor significantly suppressed the expression of miR-376b-3p (Figure 3C). Of note, as shown in Figure 3D and 3E, si-NEAT1 inhibited cell proliferation, which was impaired by decreasing miR-376b-3p expression in A549 and H292 cells. By performing flow cytometry, we found that NEAT1 down-expression could significantly promote cell apoptosis of A549 and H292 cells; miR-376b-3p inhibitor remarkably weakened its effects on cell apoptosis (Figure 3F). In addition, inhibition of NEAT1 could repress cell invasion and migration of A549 and H292 cells,

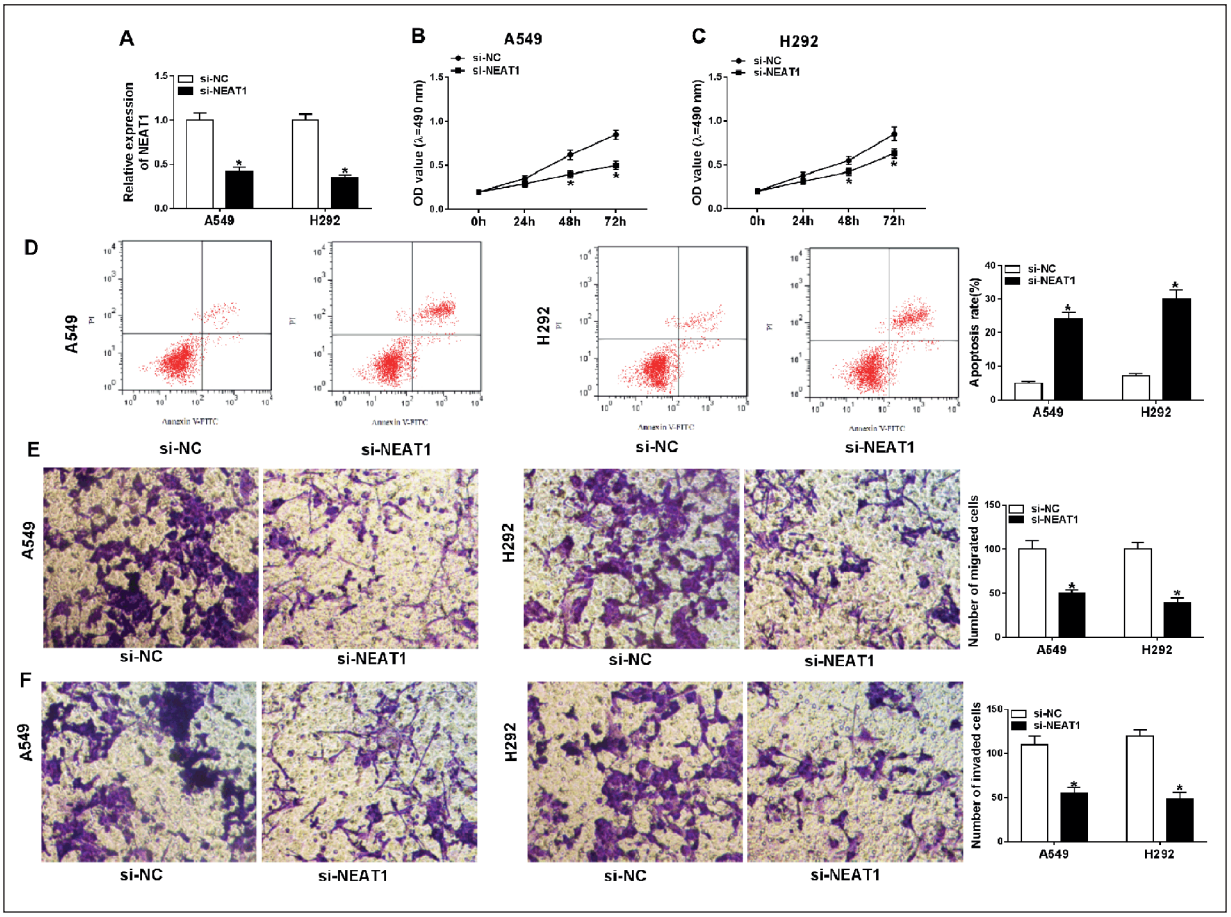

Figure 2. Knockdown of NEAT1 could inhibit cell proliferation, migration and invasion and promote cell apoptosis in NSCLC cells. **A,** qRT-PCR was applied to measure the expression of NEAT1 in si-NC and si-NEAT1 groups of A549 and H292 cells. **B,** and **C,** MTT assay was performed to measure cell proliferation in si-NC and si-NEAT1 groups of A549 and H292 cells. **D,** Flow cytometry was used to detect cell apoptosis in si-NC and si-NEAT1 groups of A549 and H292 cells. **E,** and **F,** Transwell assay was used to assess cell migration and invasion in si-NC and si-NEAT1 groups of A549 and H292 cells (100×). *$p < 0.05$.

which was reversed by miR-376b-3p inhibitor transfection (Figure 3G and 3H). Therefore, NEAT1 affected cell growth and apoptosis *via* targeting miR-376b-3p in NSCLC cells.

NEAT1 Regulated SULF1 Expression Via Binding to miR-376b-3p in NSCLC Cells

To further explore the regulatory mechanism of NEAT1 and miR-376b-3p, we found that SULF1 was a potential target mRNA of miR-376b-3p using StarBase v3.0, which has binding sites of miR-376b-3p in its 3'UTR (Figure 4A). Luciferase reporter assay showed that when the miR-376b-3p bound WT-SULF1-3'UTR, rather than MUT-SULF1-3'UTR, the Luciferase activity was significantly decreased (Figure 4B). Notably, overexpressed miR-376b-3p significantly inhibited the expression of SULF1 mRNA and protein by qRT-PCR and Western blot in A549 and H292 cells (Figure 4C and 4D). Furthermore, SULF1 mRNA and protein expression were reduced by si-NEAT1 transfection, which was reversed by downregulation of miR-376b-3p in A549 and H292 cells (Figure 4E and 4F). All results indicated that NEAT1 regulated SULF1 expression *via* binding to miR-376b-3p.

Knockdown of SULF1 Could Suppress Cell Growth and Promote Cell Apoptosis in NSCLC Cells

To further clarify the function of SULF1 in NSCLC, cells transfected with si-SULF1 were

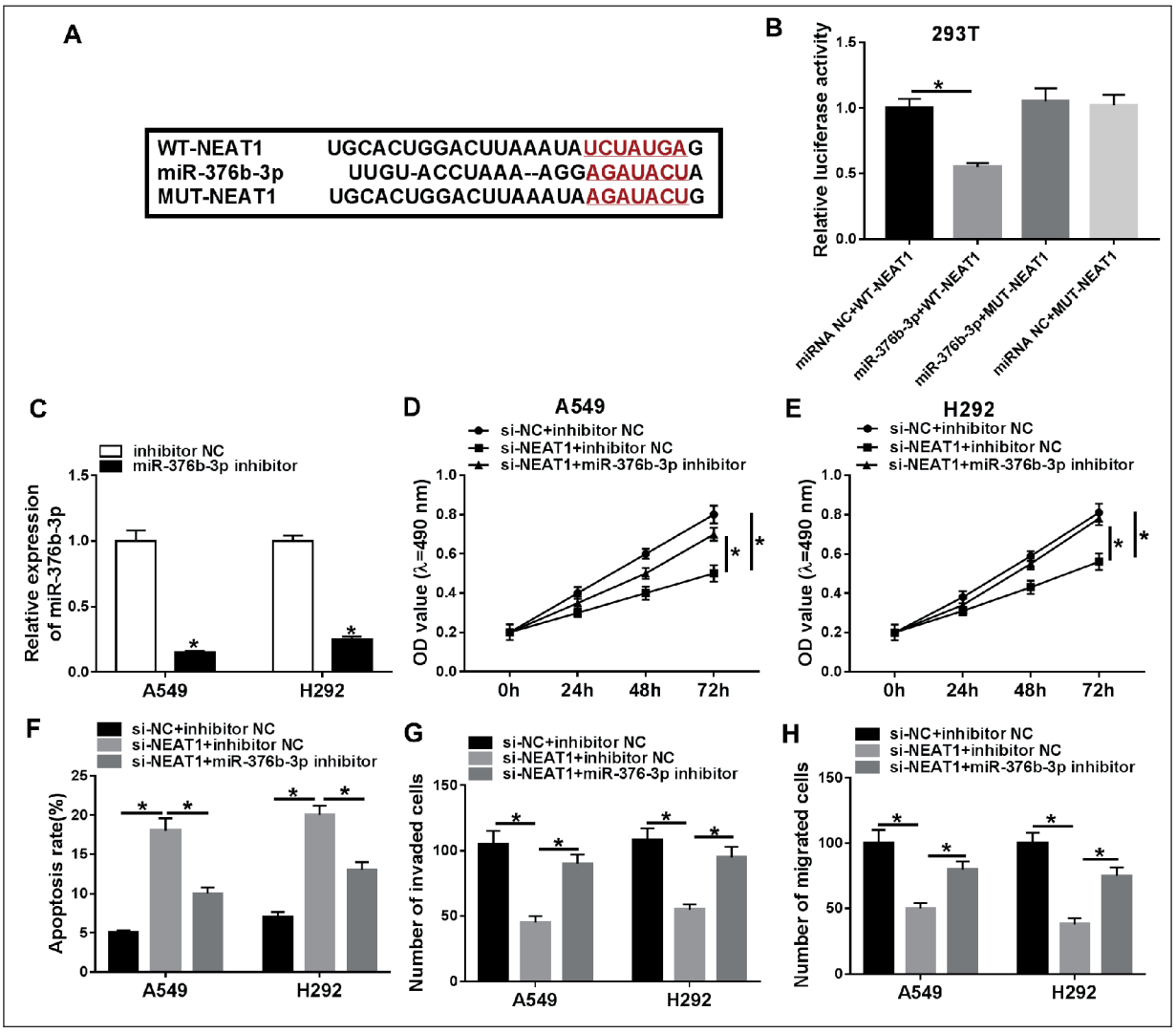

Figure 3. NEAT1 modulated cell proliferation, migration, invasion and apoptosis through targeting miR-376b-3p in NSCLC cells. **A,** Predicted binding sites for miR-376b-3p on the NEAT1 transcript. **B,** Luciferase activity was measured in 293T cells co-transfected with miR-376b-3p and miRNA NC or WT-NEAT1 and MUT-NEAT1. **C,** qRT-PCR was applied to measure the expression of miR-376b-3p in inhibitor NC and miR-376b-2p inhibitor groups of A549 and H292 cells. **D,** and **E,** MTT assay was performed to measure cell proliferation in si-NC + inhibitor NC, si-NEAT1 +inhibitor NCand si-NEAT1 + miR-376b-2p inhibitor groups of A549 and H292 cells. **F,** Flow cytometry was used to detect cell apoptosis in si-NC + inhibitor NC, si-NEAT1 + inhibitor NC and si-NEAT1 + miR-376b-2p inhibitor groups of A549 and H292 cells. **G,** and **H,** Transwell assay was used to assess cell migration and invasion in si-NC + inhibitor NC, si-NEAT1 + inhibitor NC and si-NEAT1 + miR-376b-2p inhibitor groups of A549 and H292 cells. *$p < 0.05$.

used for this experiment, which significantly decreased the mRNA and protein expression of SULF1 in A549 and H292 cells (Figure 5A and 5B). Also, the inhibitor of SULF1 remarkably attenuated the proliferation, migration, and invasion of A549 and H292 cells and enhanced apoptosis (Figure 5C-5G). In total, the knockdown of SULF1 could suppress cell growth and promote cell apoptosis in NSCLC cells.

The Effects of NEAT1 on Cell Growth and Apoptosis Were Reversed by Improving the SULF1 Expression in NSCLC Cells

To further clarify whether SULF1 is involved in the regulatory network of NEAT1, pcDNA-SULF1 and si-NEAT1 were co-transfected into A549 and H292 cells. The results of qRT-PCR and Western blot showed that a high expres-

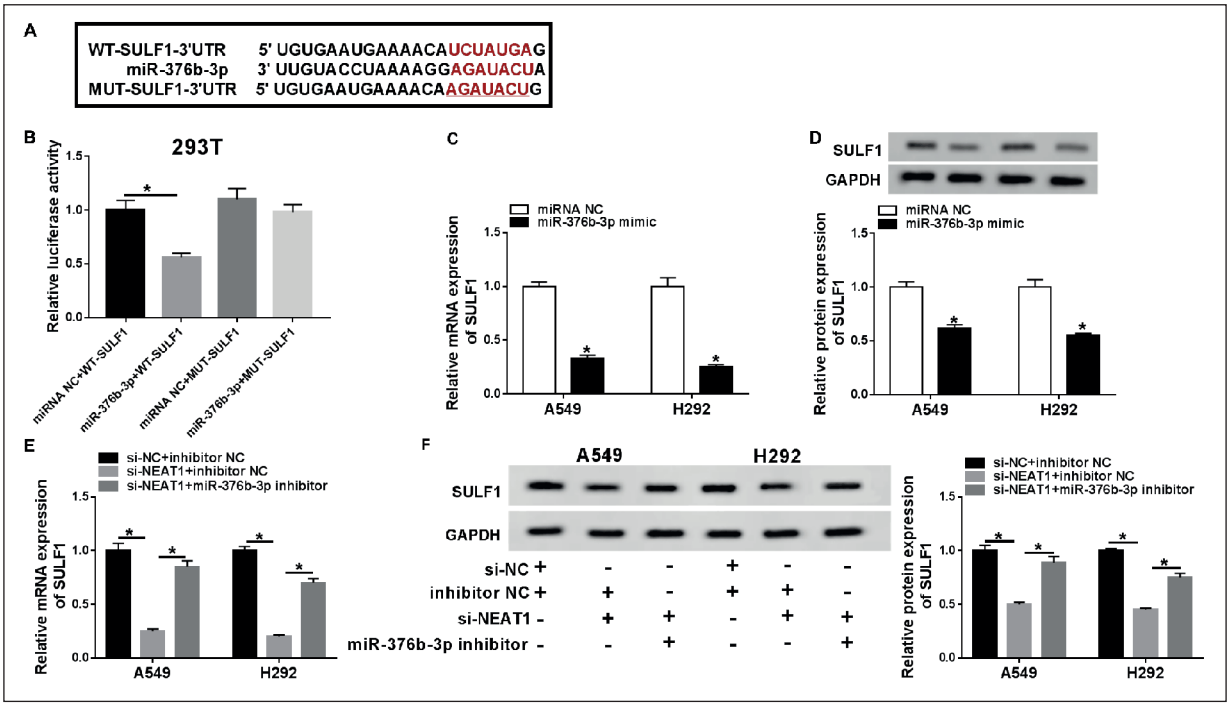

Figure 4. NEAT1 regulated SULF1 expression *via* binding to miR-376b-3p in NSCLC cells. **A,** Predicted binding sites for miR-376b-3p on the SULF1 transcript. **B,** Luciferase activity was measured in 293T cells co-transfected with miR-376b-3p and miRNA NC or WT-SULF1-3'UTR and MUT-SULF1-3'UTR. **C,** and **D,** qRT-PCR and Western blot were applied to detect the mRNA and protein expression of SULF1 in miRNA NC and miR-376b-3p mimic groups of A549 and H292 cells. **E,** and **F,** qRT-PCR and Western blot were applied to detect the mRNA and protein expression of SULF1 in si-NC + inhibitor NC, si-NEAT1 + inhibitor NCand si-NEAT1 + miR-376b-2p inhibitor groups of A549 and H292 cells. *$p < 0.05$.

sion of SULF1 attenuated the inhibitory effect of si-NEAT1 on SULF1 mRNA and protein expression in A549 and H292 cells (Figure 6A and 6B). Moreover, si-NEAT1 transfection inhibited cell proliferation, invasion, and migration and promoted apoptosis in A549 and H292 cells, which was reversed by the accumulation of SULF1 (Figure 6C-6H). All data indicated that SULF1 was involved in the regulatory network of NEAT1 in NSCLC cells.

Si-NEAT1 Transfection Inhibited MAPK and Akt Signaling Pathway by Modulating SULF1 in NSCLC Cells

In the experiment, we also found that low expression of NEAT1 significantly reduced the relative protein expression of p-MAPK/MAPK and p-Akt/Akt with Western blot, which was reversed by improving SULF1 expression in A549 and H292 cells (Figure 7A-7C), indicating that NEAT1 participated in the phosphorylation levels of MAPK and Akt to affect cell progression of NSCLC.

Discussion

As an important regulator of cell progression, lncRNA is widely involved in the metabolic progression of cancer cells[22,23]. In the diagnosis and treatment of cancer, the regulation mechanism of lncRNA is an indispensable important regulatory pathway[7,24,25]. In NSCLC, in addition to affecting cell progression, lncRNA is also closely related to autophagy and signal conduction[26-30]. For example, lncRNA GASS regulated cell cisplatin sensitivity *via* modulating cell autophagy in NSCLC[31].

The regulation of NEAT1 has been demonstrated in a variety of cancers[32,33]. Especially in NSCLC, NEAT1 is involved in cell growth and promotes tumor formation and development. Studies have found that lncRNA NEAT1 was highly expressed in NSCLC tissues and could promote tumorigenesis through the regulation of Oct4[34]. In our study, the effect of NEAT1 on NSCLC cells was consistent with previous studies. NEAT1 was highly expressed in NSCLC tissues and cells. The reduction of NEAT1

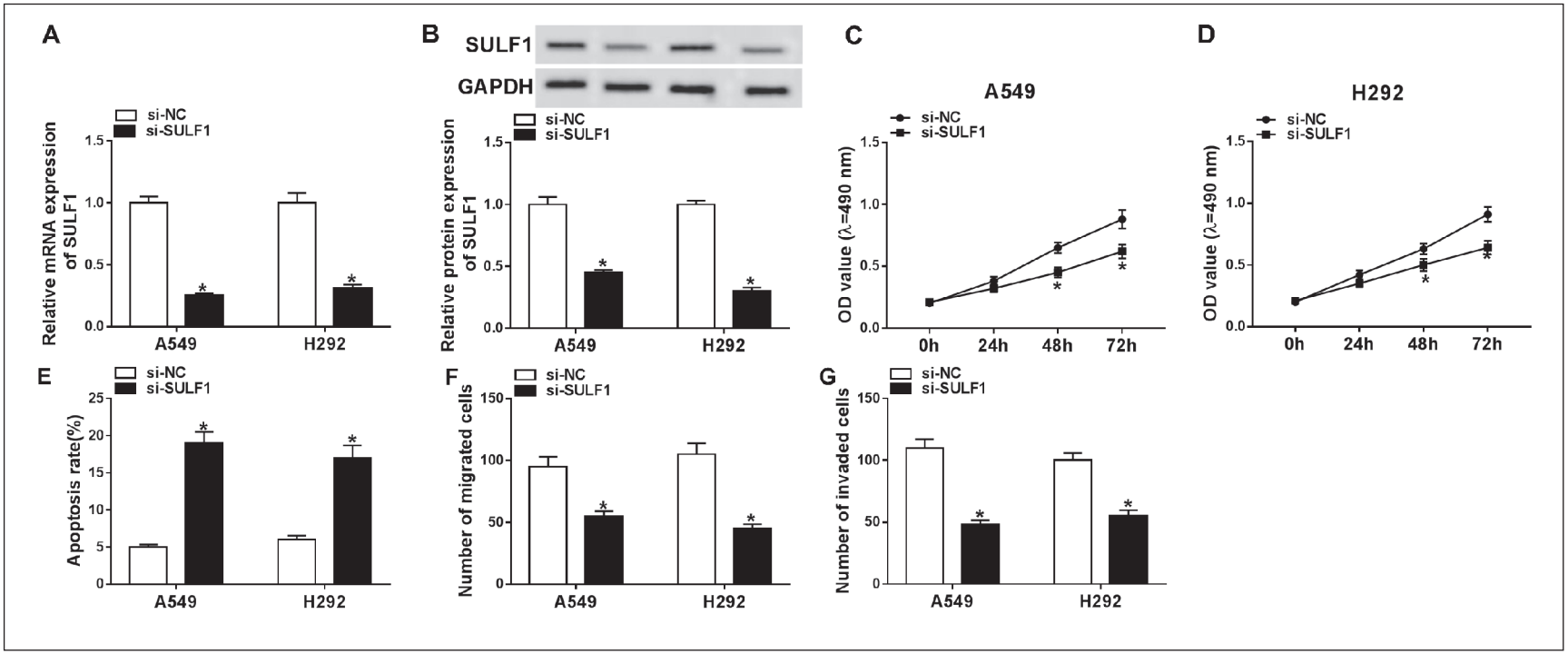

Figure 5. Knockdown of SULF1 could suppress cell growth and promote cell apoptosis in NSCLC cells. **A,** and **B,** qRT-PCR and Western blot were applied to detect the mRNA and protein expression of SULF1 in si-NC and si-SULF1 groups of A549 and H292 cells. **C,** and **D,** MTT assay was performed to measure cell proliferation in si-NC and si-SULF1 groups of A549 and H292 cells. **E,** Flow cytometry was used to detect cell apoptosis in si-NC and si-SULF1 groups of A549 and H292 cells. **F,** and **G,** Transwell assay was used to assess cell migration and invasion in si-NC and si-SULF1 groups of A549 and H292 cells. $*p < 0.05$.

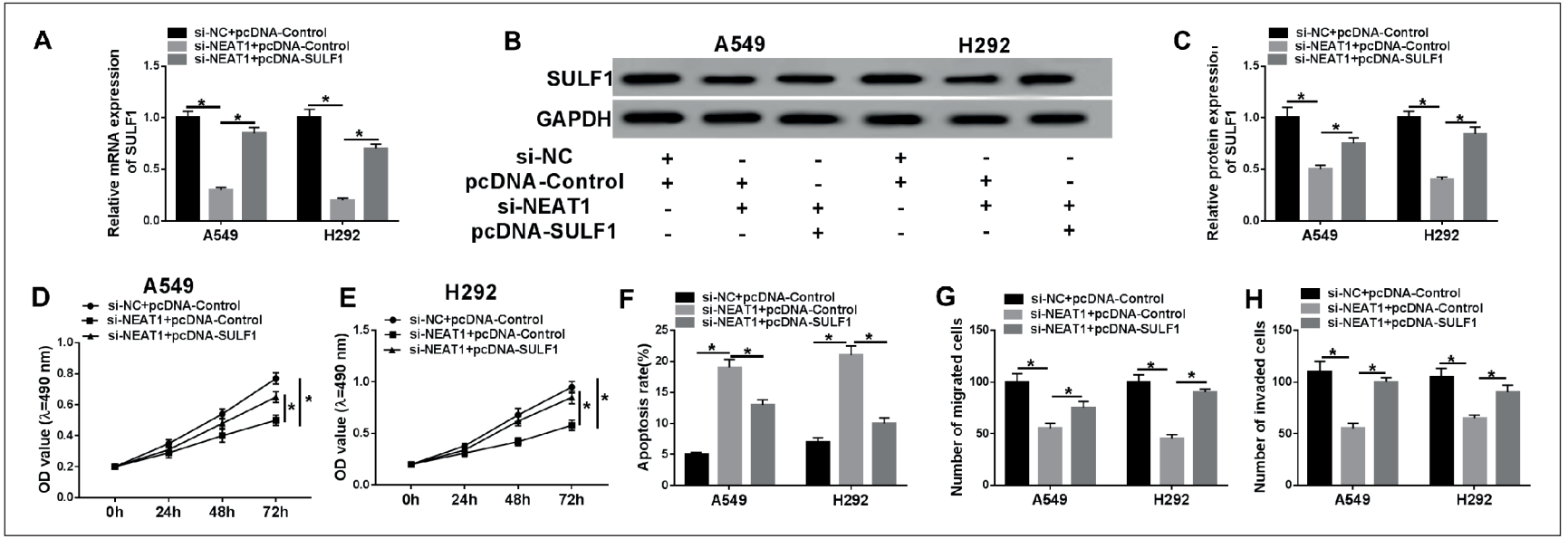

Figure 6. The effects of NEAT1 on cell growth and apoptosis were reversed by improving the SULF1 expression in NSCLC cells. **A,** qRT-PCR was applied to measure the expression of SULF1 in si-NC + pcDNA-Control, si-NEAT1 + pcDNA-Control and si-NEAT1 + pcDNA-SULF1 groups of A549 and H292 cells. **B,** and **C,** Western blot was used to measure the protein expression of SULF1 in si-NC + pcDNA-Control, si-NEAT1 + pcDNA-Control and si-NEAT1 + pcDNA-SULF1 groups of A549 and H292 cells. **D,** and **E,** MTT assay was performed to measure cell proliferation in si-NC + pcDNA-Control, si-NEAT1 + pcDNA-Control and si-NEAT1 + pcDNA-SULF1 groups of A549 and H292 cells. **F,** Flow cytometry was used to detect cell apoptosis in si-NC + pcDNA-Control, si-NEAT1 + pcDNA-Control and si-NEAT1 + pcDNA-SULF1 groups of A549 and H292 cells. **G,** and **H,** Transwell assay was used to assess cell migration and invasion in si-NC + pcDNA-Control, si-NEAT1 + pcDNA-Control and si-NEAT1 + pcDNA-SULF1 groups of A549 and H292 cells. *$p < 0.05$.

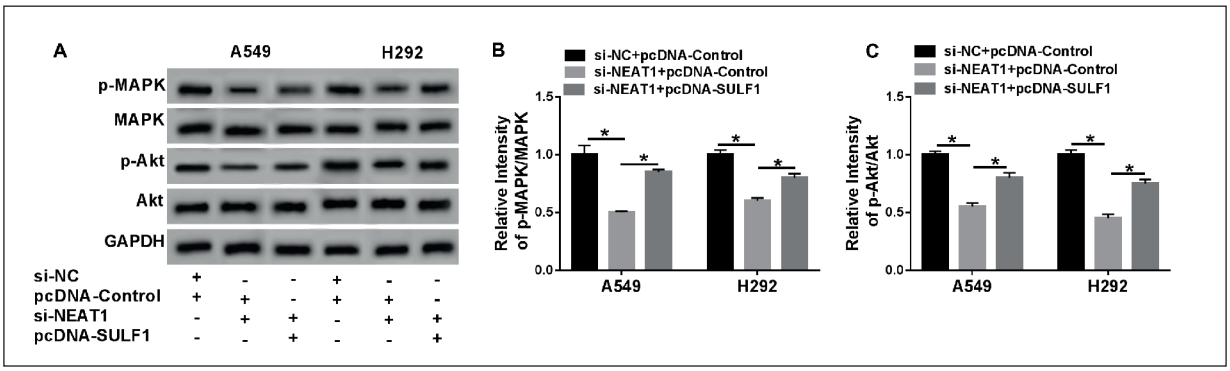

Figure 7. Si-NEAT1 transfection inhibited MAPK and Akt signaling pathway by modulating SULF1 in NSCLC cells. **A-C,** Western blot was used to measure the relative protein expression of p-MAPK/MAPK and p-Akt/Akt in si-NC + pcDNA-Control, si-NEAT1 + pcDNA-Control and si-NEAT1 + pcDNA-SULF1 groups of A549 and H292 cells. *$p < 0.05$.

effectively inhibits cell proliferation, invasion, and migration and induced cell apoptosis in NSCLC.

It is well known that lncRNA acts as a ceRNA and regulates the expression of mRNA at the transcriptional and translational levels by binding to miRNA[35,36]. Of note, lncRNA SNHG7 contributed to the cell proliferation through ID4 *via* sponging miR-342-3p in pancreatic cancer[37]. Moreover, NEAT1 has been reported to promote cell proliferation, metastasis, and invasion *via* targeting let-7a and miR-181a-5p in NSCLC[38,39]. However, there are few studies on the mechanism by which NEAT1 regulated the proliferation, migration, and invasion of NSCLC cells by targeting miRNA/mRNA. Herein, we found that miR-376b-3p was a target miRNA of NEAT1 by Luciferase reporter assay. Recently, miR-376b-3p has been determined to participate in cell differentiation in osteogenic cells and attenuate mitochondrial fission and cardiac hypertrophy[40,41]. In addition, hsa-miR-376b-3p was reduced in NSCLC cells and related to cell apoptosis and autophagy[42,43]. However, the specific regulatory mechanisms are still unclear. In this paper, the rescue experiment further demonstrates that miR-376b-3p was involved in the regulation of NEAT1 on NSCLC cells. The results indicated that NEAT1 regulated cell proliferation, invasion, migration, and apoptosis by binding to miR-376-3p.

Furthermore, SULF1 was predicted to be a target mRNA of miR-376b-3p. Sulfate 1 (SULF1) was a new member of the family of sulfatase enzymes discovered in recent years, which could participate in cell signaling by regulating the sulfation state of heparan sulfate proteoglycan on the cell surface, thereby producing a variety of physiological and pathological processes[44-46]. Meanwhile, the study found that SULF1 is upregulated in NSCLC cells relative to normal cells[47]. In this study, SULF1 knockdown reduced NSCLC cell growth and promoted apoptosis, similar to the function of lncRNA NEAT1. Further experiments showed that si-NEAT1 transfection inhibits cell proliferation, invasion, migration and furthered apoptosis through reduction of the expression of SULF1. Finally, we concluded that LncRNA NEAT1 regulates proliferation, migration, and invasion of non-small cell lung cancer by targeting hsa-miR-376b-3p/SULF1 axis.

The MAPK and Akt signaling pathway have been shown to be an important signaling pathway during cell development[48-50]. Studies[18,19] have shown that NEAT1 is also involved in the signal transduction of MAPK and Akt to affect cell metabolism and growth. In this study, we found that NEAT1 also participated in the phosphorylation levels of MAPK and Akt, and further affected cell progression of NSCLC. However, the specific regulatory path still needs further exploration and proof.

Conclusions

In this study, we found that lncRNA NEAT1 regulated cell proliferation, invasion, migration, and apoptosis by targeting has-miR-376b-3p/SULF1 axis in NSCLC. Moreover, the regulatory network of NEAT1 participated in the phosphorylation levels of MAPK and Akt to affect cell progression of NSCLC, providing a new regulatory pathway in the pathogenesis of lung cancer.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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