Circ_0007385 regulates cell proliferation, apoptosis and stemness via targeting miR-493-3p/RAB22A axis in non-small cell lung cancer

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Abstract
Background: Non-small cell lung cancer (NSCLC) is a common cancer in the United States. Previous studies have shown that circular RNAs (circRNAs) can affect NSCLC progression, but its regulatory mechanism is still indistinct. In this study, we unfold the roles of circular RNA_0007385 in NSCLC tissues and cells.

Methods: Expression levels of circ_0007385, microRNA-493-3p (miR-493-3p) and Ras-related protein Rab-22A (RAB22A) were detected by quantitative real-time polymerase chain reaction (qRT-PCR) in NSCLC tissues and cells. Cell proliferation, apoptosis and stemness were examined by cell counting kit 8 (CCK8) assay, 5-ethynyl-2'-deoxyuridine (EdU) assay, flow cytometry analysis and sphere-formation assay. The interaction between miR-493-3p and circ_0007385 or RAB22A was forecasted by bioinformatic analysis and detected by dual-luciferase reporter assay, RNA immunoprecipitation (RIP) and RNA pulldown assays. In vivo experiments were implemented to verify the effect of circ_0007385 in vivo.

Results: Expression of circ_0007385 and RAB22A increased, whereas miR-493-3p level was decreased in NSCLC tissues in contrast to that in normal tissues. For functional analysis, circ_0007385 deficiency inhibited cell proliferation and stemness, whereas it promoted cell apoptosis in NSCLC cells. Mechanically, circ_0007385 acted as a miR-493-3p sponge to modulate RAB22A expression. Moreover, circ_0007385 could regulate the development of NSCLC by sponging miR-493-3p to regulate the expression of RAB22A. In addition, circ_0007385 silence also attenuated tumor growth in vivo.

Conclusions: Circ_0007385 promoted NSCLC progression by sponging miR-493-3p to increase RAB22A expression, which also offered an underlying targeted therapy for NSCLC treatment.

KEYWORDS
circ_0007385, miR-493-3p, non-small cell lung cancer, RAB22A

INTRODUCTION
Lung cancer is one of the most common and fatal tumors. Non-small cell lung cancer (NSCLC) accounts for about 85% to 90% of lung cancer and has a very low 5-year overall survival (OS) of about 15%. The risk of lung cancer increases steadily with age. Studies have shown that nearly a third of lung cancer patients are older than 75 years old. At present, the main treatment methods in NSCLC are surgery and stereotactic body radiation therapy (SBRT), but the prognosis still needs to be further improved. Thus, the discovery of potential targets has always been an urgent problem in the treatment of NSCLC.

Circular RNAs (circRNAs) belong to a class of non-coding RNAs, which can stabilize existence in plentiful types of organisms. Studies have shown that circRNAs exert
important effects in the process of many cancers.\textsuperscript{7,8} For instance, circ\_0007385 has been found to suppress malignant behaviors and cisplatin resistance of NSCLC.\textsuperscript{9} CircSATB2 has been found to promote NSCLC cell progression.\textsuperscript{10} Circ0100146 has been reported to inhibit the proliferation of NSCLC cells,\textsuperscript{11} and CircFGFR1 to promote the progression of NSCLC cells.\textsuperscript{12} In addition, circ\_0078767 has been found to suppress NSCLC.\textsuperscript{13} However, the specific regulatory mechanism of circ\_007385 on NSCLC is still unclear, and needs further research.

MicroRNAs (miRNAs) belong to a type of small non-coding RNAs, which could affect target genes and ultimately regulate cellular processes.\textsuperscript{5,6} According to previous studies, some miRNAs have been reported in human cancers. For example, it has been reported that miR-493-3p could regulate ovarian cancer cell progression.\textsuperscript{14} In addition, miR-493-3p plays a part in the progression of laryngeal squamous cell carcinoma,\textsuperscript{15} and miRNA-148a serves as a prognostic factor in NSCLC.\textsuperscript{16} Moreover, miR-7-5p suppresses tumor metastasis of NSCLC.\textsuperscript{17} Nevertheless, the specific effects of miRNAs in NSCLC remain restricted and require further study.

Ras-related protein Rab-22A (RAB22A) is a member of the RAS oncogene family, and its expression is upregulated in many tumor cells.\textsuperscript{18} For example, several studies have reported that RAB22A is upregulated in hepatocellular carcinoma and osteosarcoma tissues.\textsuperscript{19,20} RAB22A has also been reported to be involved in autophagy and tumor development.\textsuperscript{18} Moreover, RAB22A has been found to promote the invasion and migration rate of osteosarcoma cells.\textsuperscript{20} However, the underlying mechanisms of RAB22A in NSCLC progression are still unclear.

In this study, we discovered the function of circ\_0007385 in NSCLC cells and revealed that circ\_0007385 may facilitate tumor development by sponging miR-493-3p to increase RAB22A expression. Our findings might therefore provide a novel insight into the evolution of targeted therapies for NSCLC.

**METHODS**

**Clinical samples**

This study was approved by the Ethics Committee of People’s Hospital of Beilun district. Sixty pairs of NSCLC tissues and paracancerous tissues were collected from the People’s Hospital of Beilun district. All patients provided their written informed consent and all samples were preserved at \(-80^\circ\text{C}\) for later use.

**Cell lines and cell culture**

NSCLC cell lines (A549 and H1299) were purchased from American type culture collection (ATCC). In addition the human bronchial epithelial cell line 16HBE was purchased from Cell Bank, Chinese Academy of Sciences. All cells were cultured in Roswell Park Memorial Institute 1640 (RPMI-1640) culture medium (Gibco) with 10% fetal bovine serum (FBS, Gibco) at 37°C in an incubator with 5% CO\textsubscript{2}.

**Quantitative real-time polymerase chain reaction (qRT-PCR)**

RNAs were isolated from 60 paired samples and colorectal cancer cells using a trizol reagent (Sigma-Aldrich). Next, the total RNA was reverse transcribed into complementary DNA using M-MLV reverse transcriptase (Invitrogen) or miScript RT kit (Takara), and the SYBR Green kit (Takara) was used to conduct the qRT-PCR assay. GAPDH and RNU6 (U6) were employed as the internal controls for the normalization of circRNA and miRNA expressions. The primer sequences were shown as follow: circ\_0007385, F: 5‘-ATGTCTGACTCTCTTCG-3’ and R: 5‘-CAGGCTTATCCATAGGATCC-3’; miR-493-3p, F: 5‘-AGTGAAGGTCTACTGTGT-3’ and R: 5‘-GGATAC-3’. Relative expressions were calculated with the 2\(^{-\Delta\Delta Ct}\) method.

**Western blot assay**

Total protein was extracted using the RIPA lysis buffer (Beyotime), and was separated by SDS-PAGE gels and then transferred onto the PVDF membrane (Merck Millipore). The membranes were blocked with 5% nonfat milk, and then incubated with primary antibodies overnight at 4°C and goat anti-rabbit secondary antibody (ab6721; 1:5 000; Abcam) at room temperature for 1 h. Finally, images were developed with the Immuno Star LD (Wako Pure Chemical). The antibodies were listed as follows: anti-RAB22A (ab137093; 1:1 000; Abcam), anti-Bax (ab32503; 1:1000; Abcam), anti-Bcl-2 (ab32124; 1:500; Abcam), OCT4 (ab200834; 1:10000; Abcam), NANO (ab109250; 1:2 000; Abcam), CD133 (ab222782; 1:2000; Abcam) and anti-β-actin (ab8227; 1:1 000; Abcam).

**Cell transfection**

RAB22A overexpression vector (pcDNA-RAB22A) and empty pcDNA3.1 vector (pcDNA) were produced by Ribobio. The small interfering RNAs (siRNAs) against circ\_0007385 (si-circ\_0007385\#1 and si-circ\_0007385\#2), short hairpin RNA (shRNA) against circ\_0007385 (sh-circ\_0007385), miR-493-3p mimics, miR-493-3p inhibitors, and their corresponding controls (si-NC, sh-NC, miR-NC
and anti-miR-NC) were acquired from Ribobio. For transfection, 20 nM siRNA, 50 nM miRNA mimic/inhibitor or 2 μg plasmid were transfected respectively into A549 and H1299 cells through lipofectamine 2000 (Sigma) in line with the manufacturer’s instructions.

### Cell counting kit 8 (CCK8) assay

A549 and H1299 cells (2.0 × 10^3/well) with diverse plasmid transfection were planted into 96-well plates. 0, 24, 48 or 72 h upon transfection, 20 μl of CCK8 (Beyotime) solution (5 mg/ml) was added to each well and allowed to incubate for 4 h. Then, the absorbance at 450 nm was measured.

### Flow cytometry assay

At 24 h post-transfection, A549 and H1299 (1 × 10^6) cells were cultured in 6-well plates and harvested. Subsequently, Annexin V-FITC/propidium iodide (PI) kit (Sigma) was used to stain the cells at 37°C for 20 min in the dark. Apoptotic cells were measured using a BD FACSCalibur flow cytometer (BD Biosciences). Meanwhile, the PI Flow Cytometry Kit (Abcam) was applied to detect the cell cycle distribution of A549 and H1299 cells. In brief, transfected A549 and H1299 cells were collected and resuspended in binding buffer solution. Finally, the cells were stained with PI solution and examined using a flow cytometer. The different DNA content represents the diverse cell cycle phase.

### Cell proliferation assay

5-ethynyl-2’-deoxyuridine (EdU) was conducted by using EdU Apollo in vitro Imaging Kit (RiboBio) to detect cell proliferation. Transfected A549 and H1299 cells were plated in 96-well plates for 24 h. Then, the cells were incubated with 50 μM EdU solution for 2 h. After being fixed with 4% paraformaldehyde for 30 min, the fluorescent DNA stain DAPI was used as a nuclear counterstain for 30 min in darkness. Cell proliferation was evaluated with a fluorescence microscope (Leica).

### Tumor sphere-formation assay

We then seeded 200 cells/well in 200 μl of nonserum medium to the ultra-low attachment 96-well plate (Corning) for 10–14 days. Spheres were established in serum-free medium supplemented with 2% B27, 20 ng/ml epidermal growth factor (EGF) and 20 ng/ml basic fibroblast growth factor (bFGF) (all from Sigma-Aldrich). Finally, a fluorescence microscope (Leica) was used to observe the cells, and the sphere formation efficiency (SFE) was calculated.

### Dual-luciferase reporter assay

The targeting sequence between miR-493-3p and circ_0007385 or RAB22A was forecasted using starBase (http://starbase.sysu.edu.cn/). Circ_0007385 or RAB22A 3’UTR sequences contained the wild-type (WT) or mutant type (MUT) miR-493-3p binding sites were synthesized and cloned into the pmirGLO vector to obtain the dual luciferase reporter vectors (circ_0007385-WT, RAB22A 3’UTR-WT or circ_0007385-MUT, RAB22A 3’UTR-MUT), which were constructed by Ribobio. A549 and H1299 cells were seeded in a 24-well plates and cultured for 24 h, and then cotransfected with wild-type or mutant vectors (300 ng) with miR-493-3p or miR-NC per well using lipofectamine 2000 reagent. Then, 48 h upon incubation, Renilla and firefly luciferase activities were examined using the dual-luciferase reporter assay system (Promega). Relative firefly reporter activity was normalized using the activity of Renilla luciferase encoded on the same plasmid.

### RNA immunoprecipitation assay (RIP) assay

RIP assay was applied using the RIP kit (Geneseed) to reveal the relationship between miR-493-3p and circ_0007385 or RAB22A. Briefly, NSCLC cell lysates were incubated with magnetic beads coated with anti-Argonaute2 (anti-Ago2) or anti-IgG for 12 h at 4°C. Next, the RNA was isolated and qRT-PCR was performed to detect the enrichment level of miR-493-3p, circ_0007385 and RAB22A.

### RNA pulldown

MiR-493-3p mimic-biotin (Bio-miR-493-3p) and its negative control (Bio-NC) were synthesized from RiboBio. A Pierce Magnetic RNA-Protein Pull-Down Kit (Sigma) was used to identify the relationship between circ_0007385 and miR-493-3p. Briefly, 1 × 10^7 A549 and H1299 cells were lysed and incubated with the probe-bead complex at 4°C for 3 h. Then, the beads were collected and incubated with RIP wash buffer containing proteinase K at room temperature for 1 h to remove the protein and DNA. Finally, the RNA was eluted using the RNeasy Mini Kit (Sigma) and qRT-PCR was performed to detect the expression level of circ_0007385 and miR-493-3p.

### Xenograft models

The animal experiments were approved by the guidance of the Animal Care and Use Committee of People’s Hospital of Beilun district. A total of 36 female nude mice (5 weeks of age; 18–22 g) were obtained from Beijing Vital River Laboratory Animal Technology. A549 cell lines stable expressing sh-NC, sh-circ_0007385, sh-circ_0007385+anti-miR-NC, sh-circ_0007385+anti-miR-493-3p,
sh-circ_0007385-pcDNA, or sh-circ_0007385+RAB22A were established and purchased from Hanbio (Shanghai, China). Then, 4 × 10^6 A549 stable cell lines were injected into the mice (6 mice for each group), separately. Tumor volume was measured once per week according to the formula: tumor volume = length × width^2 × 0.535 days upon injection. All mice were euthanized, and tumor tissues were removed and weighed. The levels of circ_0007385, miR-493-3p and RAB22A in xenograft tumor tissues were then assessed.

**Statistical analysis**

All data were collected from at least three repeats and presented as mean ± standard deviation (SD). Pearson’s correlation analysis was applied to measure the correlation between the two groups. The difference between two or multiple groups was examined by the Student’s t-test or ANOVA by using SPSS (version 17.0; SPSS Inc). A p-value <0.05 was considered statistically significant.

**RESULTS**

**Circ_0007385 expression was upregulated in NSCLC tissues and cells**

To further explore the role of circ_0007385 in NSCLC, qRT-PCR assay was conducted to measure the expression level of circ_0007385 in NSCLC tissues (n = 60) and adjacent normal tissues (n = 60), and the results indicated that the level of circ_0007385 was significantly increased in NSCLC tissues (Figure 1a). Moreover, our data also suggested that circ_0007385 was upregulated in NSCLC cell lines (A549 and H1299) relative to the control cell line (16HBE) (Figure 1b). In addition, circ_0007385 was mainly distributed in the cytoplasm, rather than the nucleus (Figure 1c and d). These results revealed a high expression of circ_0007385 in NSCLC tissues and cells.

**Silencing of circ_0007385 promoted cell apoptosis, whereas restrained cell proliferation and stemness in NSCLC cells**

A549 and H1299 cells were transfected with si-circ_0007385#1, si-circ_0007385#2 or si-NC, and the transfection efficiency of si-circ_0007385#1 and si-circ_0007385#2 was detected by qRT-PCR. The results indicated that circ_0007385 expression was significantly decreased in A549 and H1299 cells transfected with si-circ_0007385#1 or si-circ_0007385#2 compared to the si-NC group (Figure 2a). Functionally, CCK8 assay uncovered that knockdown of circ_0007385 decreased the cell vitality (Figure 2b and c). The flow cytometry assay demonstrated that circ_0007385 knockdown elevated the percentage of NSCLC cells in the G0/G1 phase, which significantly blocked NSCLC cells in the G0/G1 phase (Figure 2d and e). Next, EdU assays unfolded that knockdown of circ_0007385 significantly lessened the proliferation of A549 and H1299 cells in contrast to controls (Figure 2f). In addition, circ_0007385 knockdown remarkably induced cell apoptosis in A549 and H1299 cells (Figure 2g). Bax and Bcl-2 were proved to be involved in the apoptosis of NSCLC cells, and we verified that si-circ_0007385 transfection conspicuously reduced the

**FIGURE 1** Circ_0007385 expression was enhanced in NSCLC tissues and cells. (a) The expression of circ_0007385 in NSCLC tumor tissues (n = 60) and normal tissues (n = 60) was detected by qRT-PCR. (b) Circ_0007385 expression in 16HBE, A549 and H1299 cells was determined by qRT-PCR. (c and d) The nuclear–cytoplasm separation experiment was used to confirm the subcellular localization of circ_0007385 in NSCLC cells. *p < 0.05
protein level of Bcl-2, but increased the protein level of Bax in A549 and H1299 cells compared to the si-NC group (Figure 2h). Moreover, tumor sphere-formation assay showed that knockdown of circ_0007385 inhibited the cell stemness of A549 and H1299 cells (Figure 2i). OCT4, NANOG and CD133 were proven to be involved in stemness of NSCLC cells, and transfection of si-circ_0007385#1 or si-circ_0007385#2 conspicuously reduced the protein levels of OCT4, NANOG and CD133 in A549 and H1299 cells compared to the si-NC group (Figure 2j). Our results indicated that circ_0007385 knockdown inhibited cell proliferation, and stemness, but promoted apoptosis in NSCLC cells. si-circ_0007385#2 also exhibited more significant effects in NSCLC cells, and was chosen for subsequent research.

**MiR-493-3p acted as the target of circ_0007385 in NSCLC cells**

StarBase predicted that miR-493-3p is a target of circ_0007385 (Figure 3a). Then, the relative levels of miR-493-3p in A549 and H1299 cells transfected with miR-493-3p mimic and miR-NC were determined by qRT-PCR. After the addition of miR-493-3p mimic, the expression of miR-493-3p significantly increased (Figure 3b). The luciferase activity was significantly decreased in the circ_0007385-WT and miR-493-3p cotransfected group in A549 and H1299 cells compared to miR-NC groups, while no difference was found in the luciferase activity of the circ_0007385-MUT and miR-493-3p cotransfected group (Figure 3c and d). The RIP assays and RNA pulldown assay further validated a direct correlation between miR-493-3p and circ_0007385 in A549 and H1299 cells (Figure 3e-g). Moreover, we discovered that miR-493-3p expression increased in NSCLC cells with si-circ_0007385#2 transfection (Figure 3h). Meanwhile, the data demonstrated that miR-493-3p expression was markedly decreased in NSCLC tumor tissues (n = 60) compared with that in normal tissues (n = 60) (Figure 3i). In addition, Pearson’s correlation analysis unfolded that miR-493-3p expression was negatively correlated with circ_0007385 in NSCLC tissues (Figure 3j). miR-493-3p expression was also markedly decreased in A549 and H1299 cells compared with that in 16HBE cells (Figure 3k). In summary, circ_0007385 acted as a sponge for miR-493-3p in NSCLC, and it may therefore play an important role in NSCLC development.

**Circ_0007385 facilitated NSCLC progression by sponging miR-493-3p**

In order to further research the correlation and function of circ_0007385 and miR-493-3p in NSCLC progression, we...
first validated the interfering efficiency of miR-493-3p inhibitors. First, qRT-PCR revealed that miR-493-3p expression was significantly decreased by miR-493-3p inhibitors in A549 and H1299 cells (Figure 4a). CCK8 assay showed that circ_0007385 silencing diminished cell vitality; however, this effect was dramatically impaired by miR-493-3p knockdown (Figure 4b and c). The flow cytometry assay demonstrated that circ_0007385 silencing blocked NSCLC cells in the G0/G1 phase, whereas this effect was notably lessened by miR-493-3p knockdown (Figure 4d and e). The luciferase activity of RAB22A 3'UTR-WT was notably downregulated after miR-493-3p mimic transfection. However, the luciferase activity of RAB22A 3'UTR-MUT was not significantly changed by miR-493-3p (Figure 5b and c). RIP assay demonstrated an interaction between miR-493-3p and RAB22A in A549 and H1299 cells (Figure 5d and e). Figure 5f and g showed that compared with the control group, the expression level of RAB22A was significantly increased by miR-493-3p inhibitors and significantly decreased by miR-493-3p mimics. Moreover, the expression level of RAB22A was examined by qRT-PCR and Western blot in NSCLC tumor tissues. The results showed that RAB22A expression at mRNA levels and protein levels were remarkably upregulated in NSCLC tumor tissues when compared with the control (Figure 5h and i). In addition, Pearson’s correlation analysis validated that miR-493-3p expression was negatively correlated with circ_0007385 silencing hindered NSCLC development by sponging miR-493-3p.

**MiR-493-3p targeted RAB22A in NSCLC cells**

StarBase was used to predict the binding sites of miR-493-3p in RAB22A 3'UTR (Figure 5a). Dual-luciferase reporter assay validated that the luciferase activity of RAB22A 3'UTR-WT was notably downregulated after miR-493-3p mimic transfection. However, the luciferase activity of RAB22A 3'UTR-MUT was not significantly changed by miR-493-3p (Figure 5b and c). RIP assay demonstrated an interaction between miR-493-3p and RAB22A in A549 and H1299 cells (Figure 5d and e). Figure 5f and g showed that compared with the control group, the expression level of RAB22A was significantly increased by miR-493-3p inhibitors and significantly decreased by miR-493-3p mimics. Moreover, the expression level of RAB22A was examined by qRT-PCR and Western blot in NSCLC tumor tissues. The results showed that RAB22A expression at mRNA levels and protein levels were remarkably upregulated in NSCLC tumor tissues when compared with the control (Figure 5h and i). In addition, Pearson’s correlation analysis validated that miR-493-3p expression was negatively correlated with
RAB22A mRNA expression (Figure 5j). Moreover, we discovered that RAB22A expression was remarkably higher in A549 and H1299 cells compared with 16HBE (Figure 5k and l). Collectively, these results suggested that miR-493-3p could interact with RAB22A to inhibit its expression.

**MiR-493-3p suppressed NSCLC progression by targeting RAB22A**

First, qRT-PCR and western blot assay assessed that RAB22A expression was significantly increased by transfected pcDNA-RAB22A compared with the pcDNA group in A549 and H1299 cells (Figure 6a and b). Subsequently, CCK8 assay revealed that miR-493-3p mimic restrained the cell viability, but this impact was significantly attenuated by RAB22A overexpression (Figure 6c and d). Flow cytometry assay demonstrated that miR-493-3p mimic blocked NSCLC cells in the G0/G1 phase, whereas this effect was notably lessened by RAB22A overexpression (Figure 4e and f). EdU assay confirmed that miR-493-3p mimic inhibited cell stemness, however, this effect was dramatically impaired by RAB22A overexpression (Figure 6j). Moreover, the results showed that RAB22A overexpression lessened the suppressive impacts of miR-493-3p on the protein levels of OCT4, NANOG and CD133 in A549 and H1299 cells (Figure 6k). In addition, Pearson’s correlation analysis validated that circ_0007385 expression was positively correlated with RAB22A expression in NSCLC tissues (Figure 7a). Meanwhile, RAB22A expression was diminished by si-circ_0007385#2, whereas anti-miR-493-3p could partially lessen these influences (Figure 7b and c). In summary, all data illustrated that miR-493-3p regulated NSCLC cell progression by targeting RAB22A.

**Circ_0007385 knockdown restricted tumor growth in vivo**

A xenograft tumor model was established to further research the clinical application of circ_0007385/miR-493-3p/RAB22A axis in NSCLC in vivo. As shown in Figures 8a and b, injection of sh-circ_0007385 dramatically inhibited tumor volume and weight compared with sh-NC group, whereas miR-493-3p downregulation or RAB22A overexpression overturned these effects. Additionally, expression levels of circ_0007385, miR-493-3p and RAB22A in xenograft tumor tissues were measured. The results revealed that circ_0007385 and RAB22A expression levels were conspicuously reduced, whereas miR-493-3p expression was notably increased in tumor tissues from the sh-circ_0007385 group.
compared with the sh-NC group (Figure 8c-f). In addition, miR-493-3p downregulation or RAB22A overexpression significantly overturned the effects of circ_0007385 silence on circ_0007385, miR-493-3p and RAB22A expression levels in xenograft tumor tissues (Figure 8c-f). These results confirmed that circ_0007385 knockdown inhibited xenograft tumor growth by regulating the miR-493-3p/RAB22A axis in vivo.

**FIGURE 5** MiR-493-3p targeted RAB22A in NSCLC cells. (a) The binding site between miR-493-3p and RAB22A was analyzed by StarBase. (b and c) Dual-luciferase reporter assay was used to confirm the relationship between miR-493-3p and RAB22A. RAB22A 3'UTR-WT: RAB22A 3'UTR wild-type, RAB22A 3'UTR-MUT: RAB22A 3'UTR mutant type. (d and e) RIP analysis was utilized to confirm the relationship of RAB22A and miR-493-3p. (f and g) The mRNA and protein levels of RAB22A in NSCLC cells with miR-NC, miR-493-3p, anti-miR-NC or anti-miR-493-3p transfection was detected by qRT-PCR and western blot. (h and i) RAB22A expression in NSCLC tumor tissues and normal tissues was detected by qRT-PCR and western blot. *p < 0.05
DISCUSSION

Over the past 20 years, with the emergence and maturity of molecular technologies, treatments for NSCLC have greatly improved. OS rates for patients with lung cancer have been increasing slowly in recent years. The main treatment methods for NSCLC include surgery, chemotherapy and targeted therapy. Previous sequencing results have shown that there are many differentially expressed circRNAs in NSCLC tissues. However, the role of circRNA in NSCLC is still unclear. Therefore, our study further investigated the role of circ_0007385.

Previous studies have discovered that some emerging circRNAs are crucial in NSCLC. Circ_103762 has been found to promote multidrug resistance in NSCLC, and circ_001010 to promote proliferation and metastasis of NSCLC. Circ_102179 has been reported to promote the proliferation and invasion in NSCLC. In our study, we found that circ_0007385 could regulate NSCLC progression. Our results indicated that circ_0007385 deficiency promoted cell apoptosis, whereas it restrained cell proliferation and stemness in NSCLC cells. In addition, in vivo studies further discovered that knockdown of circ_0007385 impaired tumor growth. An increasing number of reports have disclosed that circRNAs can impact specific gene expression by competitively binding to miRNAs. For example, circ_102179 could target miR-330-5p and circCDR1as could regulate miR-219a-5p in NSCLC. In this study, circ_0007385 could accelerate NSCLC progression by sponging miR-493-3p, which is similar to previous findings.

According to previous reports, miR-493-3p could regulate the progression of laryngeal squamous cell carcinoma, ovarian cancer and breast cancer. These results showed that miR-493-3p participates in the development of human cancers. In this study, we found that miR-493-3p could regulate NSCLC progression. We also demonstrated...
the prohibitive role of miR-493-3p in cell development by targeting RAB22A. The results validated that miR-493-3p may participate in NSCLC progression.

It has previously been reported that RAB22A plays an important role in breast cancer invasion and metastasis. Moreover, that RAB22A could regulate osteosarcoma progression. In this study, RAB22A expression was prominently upregulated in NSCLC tissues and cells. More importantly, we demonstrated that miR-493-3p upregulation inhibited cell development and this impact was lessened by RAB22A. We also discovered that miR-493-3p inhibitor repressed the inhibitory effect of circ_0007385/miR-493-3p/RAB22A in NSCLC cells. This study has some significant findings; however, it still has some limitations. For example, the results obtained from commercial cell lines and the conclusions did not fully represent the actual clinical
situation. In the following study, we will increase statistical support for clinical trials to further indicate the role of circ_0007385 in clinical application.

In conclusion, the study confirmed that circ_0007385 and RAB22A were highly expressed, while miR-493-3p was lowly expressed in NSCLC tissues and cells. Furthermore, our study demonstrated that circ_0007385 knockdown suppressed NSCLC cell proliferation and stemness in vitro and curbed tumor growth in vivo by regulating miR-493-3p/RAB22A axis. We believe that this knowledge could provide a new mechanism for the development of NSCLC treatments.

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None.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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