European Review for Medical and Pharmacological Sciences 2020; 24: 6139-6149

MicroRNA-448/EPHA7 axis regulates cell proliferation, invasion and migration *via* regulation of PI3K/AKT signaling pathway and epithelial-to-mesenchymal transition in non-small cell lung cancer

H.-Y. LIU[1], J. CHANG[1], G.-D. LI[1], Z.-H. ZHANG[1], J. TIAN[2], Y.-S. MU[1]

[1]Thoracic Surgery, The Second Affiliated Hospital of Shandong First Medical University, Tai'an, China
[2]Shandong Cancer Hospital and Institute, Shandong First Medical University and Shandong Academy of Medical Sciences, Tai'an, China

Haiyan Liu and Jin Chang contributed equally to this work

Abstract. – OBJECTIVE: Non-small cell lung cancer (NSCLC) is a primary subtype of lung cancers which has a high morbidity and poor prognosis. Emerging evidence has demonstrated that aberrantly expressed microRNAs (miRNAs) were implicated in the regulatory functions of multiple processes during tumorigenesis. In the current study, we explored the functional roles and underlying mechanisms of miR-448 in NSCLC.

PATIENTS AND METHODS: Quantitative real-time polymerase chain reaction assays were conducted to measure miR-448 expressions in 51 pairs of NSCLC tissues and corresponding normal tissues. Moreover, the relationship between miR-448 expressions and clinicopathological characteristics of NSCLC patients was also determined. We then performed transwell assays to explore the functions of miR-448 in NSCLC cell invasion and migration. As we had identified EPHA7 as a functional target of miR-448 in NSCLC cells, the clinical significance of EPHA7 in NSCLC patients was further investigated. Finally, we detected the influence of miR-448 on tumor growth rate and tumor size of NSCLC using tumor xenografts.

RESULTS: Underexpressed miR-448 was identified in NSCLC, and low miR-448 expression was confirmed to be associated with the poor prognosis and adverse clinicopathologic features of NSCLC patients. Moreover, functional assays demonstrated that miR-448 overexpression suppressed NSCLC cell proliferation, invasion and migration. EPHA7 was identified as a direct target of miR-448. Additionally, miR-448 restoration suppressed *in vivo* NSCLC cell growth. Finally, our studies also indicated that miR-448 exerted anti-NSCLC functions *via* regulating PI3K/AKT signaling pathway and EMT.

CONCLUSIONS: These results showed that miR-448/EPHA7 axis maybe one of the useful diagnostic and prognostic biomarkers for NSCLC patients.

Key Words:
NSCLC, MiR-448, EPHA7, Proliferation, Invasion, Migration.

Introduction

Non-small cell lung cancer (NSCLC) is one of the most common subtypes of lung cancers. Histologically, about 80% of lung cancer cases are NSCLC[1]. As one common malignant tumor, NSCLC remains a leading factor for tumor mortalities in the world[2]. Nowadays, the standard therapy for NSCLC is surgery. However, certain patients are considered as medically inoperable or high-risk due to their poor general conditions, advanced ages, and other medical comorbidities[3]. In addition, NSCLC cells which have strong metastatic abilities can evade the regulation from apoptosis and division, leading to treatment failure[4]. Gene alterations play crucial functions in cancer genesis and development. In recent years, numbers of oncogenes and cancer suppressive genes, including both coding and non-coding genes, have been found to influence the NSCLC

Corresponding Author: Yushu Mu, MM; e-mail: tyfymys@163.com

progression. Therefore, identification of reliable biomarkers which could unravel the complex heterogeneities of NSCLC is necessary to better manage individualized therapies for NSCLC patients.

MicroRNAs (miRNAs/miRs) are a class of endogenous, non-coding small RNA molecules with important gene regulatory functions, which could base pair with the 3'UTR of target mRNAs, thereby inducing either transcript degradation or translational repression[5]. Guo et al[6] demonstrated that over half of known miRNAs were located in tumor-associated genomic regions, suggesting that miRNA dysregulation possibly played a key role in tumorigenesis. Yang et al[7] found that miR-613 promoted colon cancer progression by regulating ATOH1; Gao et al[8] indicated that miR-142-3p inhibited ovarian cancer cell proliferation and chemoresistance *via* targeting sirtuin 1; Song et al[9] demonstrated that miR-195 inhibited cervical cancer behaviors by directly targeting HDGF. Recent studies have reported that miR-448 can inhibit NSCLC growth[10]; however, the exact mechanism by which miR-448 affects the progression of NSCLC remains largely unclear. Therefore, the current study focused on the functions of miR-448 in NSCLC to better understand the pathogenesis of NSCLC and identify reliable therapeutic biomarkers.

The erythropoietin-producing hepatoma-amplified sequence (Eph) receptors are a large family of receptor tyrosine kinases, which have been classified into EphA and EphB receptors according to their sequence homology and the nature of their corresponding ligands[11]. Accumulating evidence has demonstrated that some Eph genes are overexpressed in diverse tumors and play important roles in tumor cell angiogenesis, survival, proliferation, and migration[12-14]. Ephrin receptor A7 (EPHA7) is a member of Eph/ephrin family, playing key roles in carcinogenesis. For instance, upregulated EPHA7 expressions have been found to correlate with poor outcomes of primary and recurrent glioblastoma multiforme patients[15]; EPHA7 silence repressed human laryngeal cancer cell invasion, proliferation and promoted apoptosis[16]. However, little attentions have been paid to EPHA7 in NSCLC progression, and its functional roles in NSCLC remain to be explored. The present study aimed to untangle EPHA7 expressions in NSCLC to explore the functions and potential mechanism of EPHA7/miR-448 axis in NSCLC.

Patients and Methods

Patients and Tissue Samples

All the 51 pairs of NSCLC tissues and matched para-carcinoma tissue samples were collected from surgical resections on NSCLC patients in the Second Affiliated Hospital of Shandong First Medical University between March 2016 and July 2018. The tissues were frozen in liquid nitrogen and stored at -80°C for further use. Written informed consent was obtained from all the participants. None of the patients had received chemotherapy or radiation therapy treatment prior to the operation. This investigation was approved by the Ethics Committee of The Second Affiliated Hospital of Shandong First Medical University. The selection of patients was based on the guideline proposed by the Union for International Cancer Control (UICC). The entire investigation complied with the principles outlined in the Helsinki Declaration.

Cell Lines

Human NSCLC cell lines (A549, NCI-H1299, NCI-H460, and SPC-A1) and normal bronchial epithelium cell line BEAS-2B were obtained from the cell bank of the Chinese Academy of Sciences (Shanghai, China). All the above cell lines were maintained in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, Rockville, MD, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA) at 37°C in a humidified atmosphere containing 5% CO_2.

Cell Transfections

MiR-448 mimics/inhibitor or the negative miRNA controls (NC) were purchased from RiboBio Co., Ltd. (Guangzhou, China). For cell transfections, Lipofectamine® 2000 (Invitrogen, Carlsbad, CA, USA) was utilized to transfect the above miRNAs into NSCLC cells following the manufacturer's protocols.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

qRT-PCR analysis was performed to determine the expressions of miR-448 and EPHA7 mRNA in NSCLC tissues and cells. Total RNA was isolated from cells and tissues by TRIzol (Invitrogen, Carlsbad, CA, USA). Reverse transcription

was performed using PrimeScript reverse transcription reagent kit (Thermo Fisher Scientific, Waltham, MA, USA). qRT-PCR was carried out using SYBR® Premix Ex Taq™ II (TaKaRa, Dalian, China) with the 7500 real-time RT-PCR system (Applied Biosystems, Foster City, CA, USA) in accordance with the manufacturer's instructions. U6 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were used as the internal controls for miR-448 and EPHA7 mRNA respectively. The $2^{-\Delta\Delta Ct}$ method was used to determine relative expressions. The primer sequences were list in Table I.

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyl Tetrazolium Bromide) Assay

The MTT assays (Sigma-Aldrich, St. Louis, MO, USA) were carried out to detect cell proliferation ability in strict line with the manufacturer's instructions. NSCLC cells transfected with miR-448 mimics or inhibitor were plated into 96-well plates. Then, 24, 48 and 72 h post-transfections, MTT (5 mg/mL) was added into the plates and incubated for another 4 h at 37°C. The resulting MTT-formazan crystals were solubilized by 150 μL dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, MO, USA). Finally, the results were quantified with a microplate reader (BioTek, Winooski, VT, USA) at 490 nm.

Transwell Assays

8-μm pore sized transwell chamber (BD Biosciences, San Jose, CA, USA) coated with or without Matrigel was used to detect cell invasion and migration ability, respectively. The transfected cells were plated into the upper chambers which were filled with serum-free medium. On the other hand, medium containing 10% FBS was added into the lower champers as a chemoattractant. After incubation for 48 h at 37°C, cells that invaded or migrated to the bottom chamber were fixed and stained with methanol and crystal violet. In the meantime, the non-invading or -migrating cells remained on the top chamber were removed with a cotton swab. Finally, the results were photographed and quantified in five randomly selected fields under the microscope (Olympus, Tokyo, Japan).

Western Blot

Total proteins were isolated from the cells with different treatment by radioimmunoprecipitation assay (RIPA) buffer (Pierce, Rockford, IL, USA) and quantified using a bicinchoninic acid (BCA) Protein Assay Kit (Pierce, Rockford, IL, USA). Then, equal amounts of lysates were subjected to separation by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). The membrane was blocked with 5% skimmed milk in tris buffered saline and tween-20 (TBST) for 2 h at room temperature. Thereafter, the membrane was then incubated with specific primary antibodies against EPHA7 (1:1000, Abcam, Cambridge, MA, USA), AKT (1:1000, Abcam, Cambridge, MA, USA), p-AKT (1:1000, Abcam, Cambridge, MA, USA), PI3K (1:1000, Abcam, Cambridge, MA, USA), p-PI3K (1:2000, Abcam, Cambridge, MA, USA), E-cadherin (1:2000, Abcam, Cambridge, MA, USA), N-cadherin(1:1000, Abcam, Cambridge, MA, USA), Vimentin (1:1000, Abcam, Cambridge, MA, USA) and GAPDH (1:1000, Abcam, Cambridge, MA, USA). After that, the membrane was incubated for 2 h at room temperature with horseradish peroxidase (HRP)-labeled secondary antibody (1:3,000, Abcam, Cambridge, MA, USA). Finally, the protein band was detected with en-

Table I. Primer sequences for qRT-PCR.

Primer	Sequence
miR-448 forward	5'- TTGCATATGTAGGATGTCCCAT -3'
miR-448 reverse	5'-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGATGGGACA-3'
U6 forward	5'-CTCGCTTCGGCAGCACA-3'
U6 reverse	5'- AACGCTTCACGAATTTGCGT-3'
EPHA7 forward	5'- GTGAAGATGGGTATTACAGGGC-3'
EPHA7 reverse	5'- CAACTGCACCGCTTACACAAT-3'
GAPDH forward	5'-ACCTGACCTGCCGTCTAGAA-3'
GAPDH reverse	5'-TCCACCACCCTGTTGCTGTA-3'

U6: small nuclear RNA, snRNA; EPHA7: Ephrin receptor A7; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

hanced chemiluminescence (ECL) reagent (Beyotime, Shanghai, China). GAPDH was used as an internal control.

Immunohistochemistry

Tissue samples were fixed with 10% formalin, dehydrated in graded ethanol, embedded in paraffin and then cut into 4 μm sections. Subsequently, the sections were deparaffinized and rehydrated. After that, the antigen retrieval was performed by microwaving the sections in citrate buffer. Then, endogenous peroxidase activities were blocked in 3% H_2O_2. The sections were then subjected to an incubation of primary EPHA7 antibody (1:100, Abcam, Cambridge, MA, USA) at 4°C overnight. After incubation with secondary goat anti-rabbit IgG (1:1,000, Abcam, Cambridge, MA, USA) labeled by HRP, the slides were stained with diaminobenzidine (DAB) as the chromogen and counterstained with hematoxylin. The results were detected under a brightfield microscope (Olympus BX50; Olympus Corporation, Tokyo, Japan). The scores were calculated according to positive cell ratio: stained cells/all cells<25% was considered as negative (-) while >25% was positive (+)[17,18].

Luciferase Reporter Assay

Wildtype (WT) or mutant (MUT) EPHA7 3'UTRs containing the potential miR448 binding sequences were inserted into pGL3 reporter vectors (Promega Corporation, Madison, WI, USA). NSCLC cells were cotransfected with WT or MUT EPHA7-3'UTR and miR-448 mimics and incubated for 48 h. After that, the cells were harvested and the Luciferase activities were examined by Luciferase reporter assay system (Promega Corporation, Madison, WI, USA).

In Vivo Tumor Xenograft Mouse Model

This investigation was approved by the Animal Ethics Committee of Shandong First Medical University Animal Center. Mice were randomly divided into two groups and inoculated subcutaneously on the flank with the stable expressing A549 cells which were transfected either with lentiviral miR-448 (lenti-miR-448) or the negative lentiviral miR-control (lenti-control). The length and width of the tumor diameters were measured every three days. Tumor volumes (mm^3) = length×width ^{2}/2. Following treatments for 28 days, all of the mice were sacrificed for tumor isolation.

Statistical Analysis

All above experiments were repeated at least thrice. Statistical analysis was performed with Statistical Product and Service Solutions (SPSS) software version 17.0 (SPSS Inc., Chicago, IL, USA). Differences between two groups were analyzed using the Student's *t*-test. Comparison between multiple groups was done using One-way ANOVA test followed by Post-Hoc Test (Least Significant Difference). The Kaplan-Meier curve together with log-rank test was used to analyze the overall survival of NSCLC patients. $p<0.05$ indicated statistically significant differences.

Results

Downregulation of MiR-448 Indicated Poor Prognosis and Adverse Clinicopathological Features

First, qRT-PCR assays were conducted to determine the miR-448 expressions in 51 NSCLC tissues and matched normal controls. As shown in Figure 1A, we found that miR-448 expressions were significantly decreased in NSCLC tissues when compared to the matched non-tumor tissue samples. Moreover, we also analyzed the relationship between miR-448 expressions and clinicopathological features of NSCLC patients. All NSCLCL patients enrolled in our study were subdivided into high and low miR-448 expression groups according to the mean expression of miR-448. As shown in Table II, patients with low miR-448 expressions exhibited poor clinicopathological characteristics in comparison to those with high miR-448 expressions. Moreover, Kaplan-Meier analysis and log-rank test showed that downregulation of miR-448 was associated with poor prognosis in NSCLC patients (Figure 1B).

MiR-448 Upregulation Repressed NSCLC Cell Proliferation

The miR-448 expressions in NSCLC cells were also examined. As expected, miR-448 was prominently downregulated in all selected NSCLC cell lines in comparison to BEAS-2B (Figure 2A). Next, we transfected miR-448 mimics or inhibitor into A549 cells and qRT-PCR analysis demonstrated that miR-448 was evidently overexpressed by miR-448 mimics while significantly inhibited by miR-448 inhibitor in A549 cells

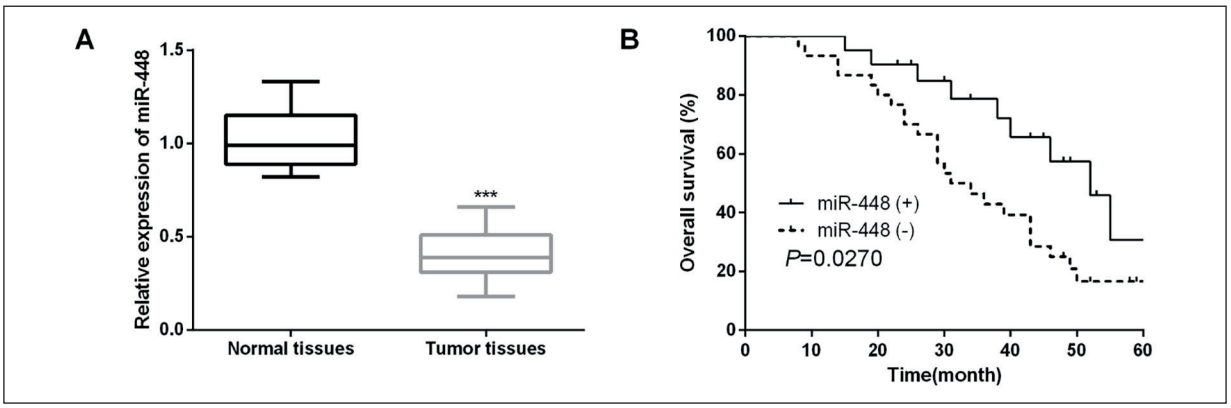

Figure 1. Downregulated miR-448 expression in NSCLC patients indicated poor prognosis. **A,** qRT-PCR analysis was performed to detect miR-448 expressions in NSCLC tissue samples. **B,** As indicated by Kaplan-Meier analysis, NSCLC patients with low miR-448 expressions presented obviously shorter overall survival (OS) than patients with high miR-448 expressions.

(Figure 2B and 2C). After that, MTT assay was further performed to investigate the influence of miR-448 on NSCLC cell proliferation capacity. As shown in Figure 2D and 2E, miR-448 overexpression significantly suppressed NSCLC proliferation, while on the other hand, miR-448 knockdown had the opposite functions. Taken together, these findings demonstrated that miR-448 inhibited NSCLC cell proliferation.

MiR-448 Overexpression Repressed NSCLC Cell Invasion and Migration

We, then, performed transwell assays to explore the functional roles of miR-448 in NSCLC cell invasion and migration. Results showed that miR-448 overexpression significantly inhibited A549 cell invasion and migration (Figure 3A and 3B). On the contrary, miR-448 downregulation markedly promoted A549 cell invasion and

Table II. Correlation of miR-448 expression with the clinicopathological characteristics of the NSCLC patients.

Clinicopathological features	Cases (n = 51)	miR-448# expression		*p*-value
		High (n = 20)	Low (n = 31)	
Age (years)				0.2783
> 60	28	12	16	
≤ 60	23	8	15	
Gender				0.3152
Male	27	9	18	
Female	24	11	13	
Tumor size (cm)				0.1974
≥ 5.0	23	6	17	
< 5.0	28	14	14	
Lymph node metastasis				0.0018*
Yes	21	17	4	
No	30	3	27	
Histology				0.3267
Squamous cell carcinoma	25	11	14	
Adenocarcinoma	26	9	17	
TNM stage				0.0024*
I +II	22	15	7	
III+IV	29	5	24	
Smoker				0.3205
Yes	28	12	16	
No	23	8	15	

NSCLC: non-small cell lung cancer; TNM: tumor-node-metastasis. #The mean expression level of miR-448 was used as the cutoff; *Statistically significant.

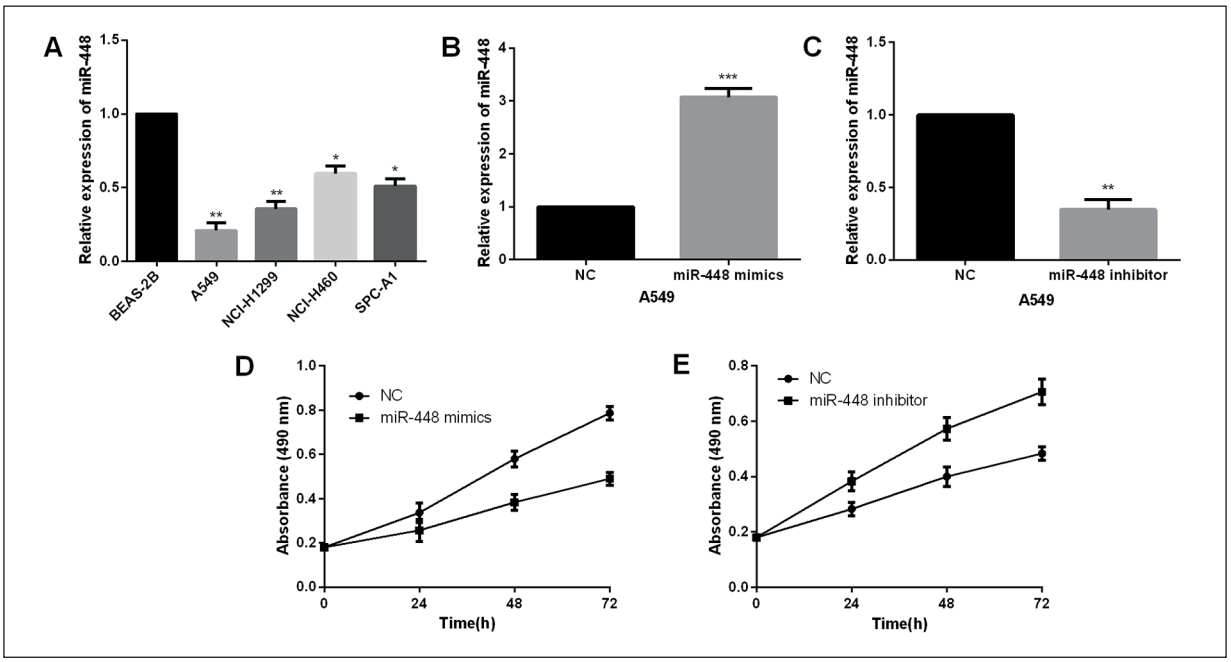

Figure 2. MiR-448 overexpression inhibited NSCLC cell proliferation. **A,** MiR-448 expressions in NSCLC cells were detected using qRT-PCR. **B, C,** Relative miR-448 expressions in A549 cells transfected with miR-448 mimics or inhibitor were examined by qRT-PCR. **D, E,** MTT assays showed that miR-448 upregulation inhibited whereas proliferation ability miR-448 silence promoted A459 cell proliferation ability.

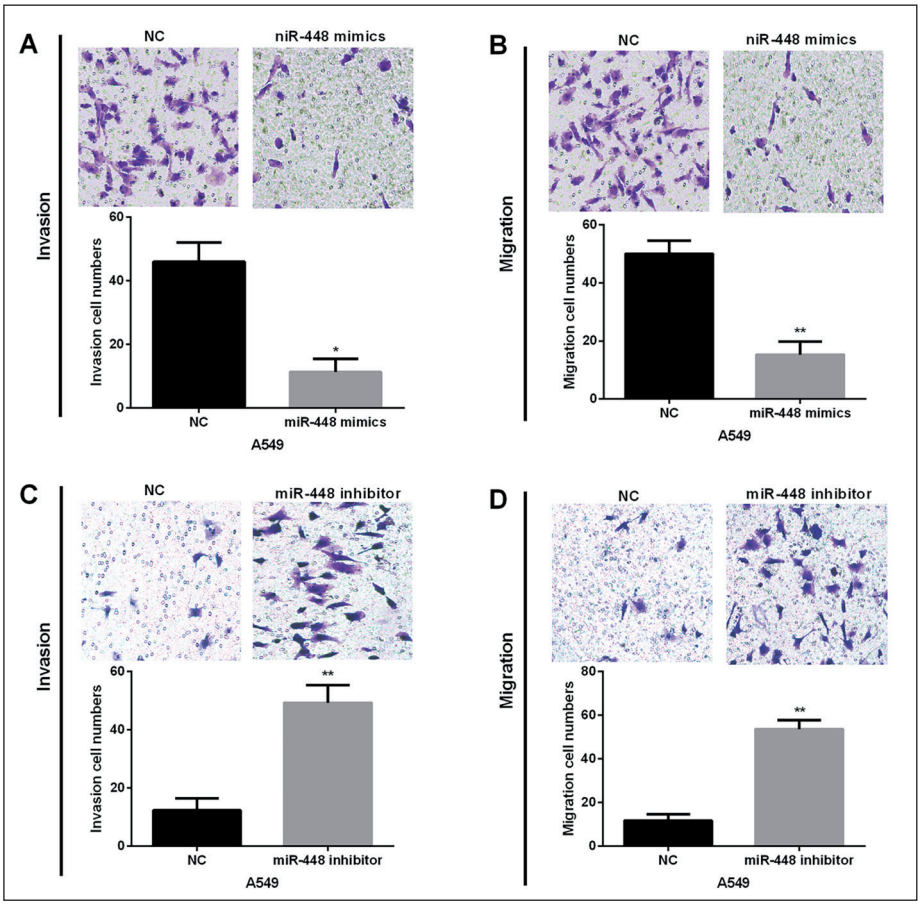

Figure 3. MiR-448 overexpression suppressed NSCLC cell invasion and migration. **A, B,** Transwell assays showed that miR-448 overexpression inhibited NSCLC cell invasion and migration capacities. (magnification: 200×). **C, D,** MiR-448 inhibition led to significant enhancement of A549 invasion and migration capacities. (magnification: 200×).

migration (Figure 3C and 3D), indicating that miR-448 exerted anti-tumor functions in NSCLC progression.

MiR-448 Directly Targeted EPHA7 in NSCLC Cells

To verify the molecular basis for the regulatory roles of miR-448 in NSCLC progression, TargetScan was utilized to identify the potential target genes of miR-448. Among the predicted targets, EPHA7 was selected for further investigations due to its key roles in facilitating tumor development. EPHA7 harbored the putative binding sites of miR-448 in its 3'-UTRs (Figure 4A) and Luciferase reporter assays were further conducted to confirm this association. Results showed that miR-448 mimics could prominently decrease the Luciferase activities of EPHA7-3'UTR-WT, while had no notable influence on the Luciferase activities of EPHA7-3'UTR-MUT (Figure 4B). Thereafter, the regulatory functions of miR-448 in EPHA7 expressions were also investigated. As shown in Figure 4C and 4D, it was found that when miR-448 was overexpressed, EPHA7 expression was decreased; conversely, with low expression levels of miR-448, EPHA7 expression was increased. These results demonstrated that miR-448 directly suppressed EPHA7 expressions *via* targeting its 3'-UTR.

MiR-448 Restoration Inhibited Epithelial-Mesenchymal Transition (EMT) and Phosphatidylinositol 3-Kinase/Protein Kinase B (PI3K/Akt) Pathway in NSCLC Cells

As EPHA7 had been identified as a functional target of miR-448 in NSCLC cells, its clinical significance in NSCLC patients was further investigated. The EPHA7 expression in NSCLC tissues was determined by performing IHC assays. Results showed that EPHA7 was mainly located on the membrane (Figure 5A). Additionally, significantly upregulated EPHA7 expressions in NSCLC tissue samples were identified when compared to the adjacent noncancerous tissue samples (Figure 5B). Moreover, results of Kaplan-Meier analysis and log-rank test also indicated that patients with high EPHA7 expressions exhibited poorer prognosis than patients with low EPHA7 expressions (Figure 5C). Next, Western blot was

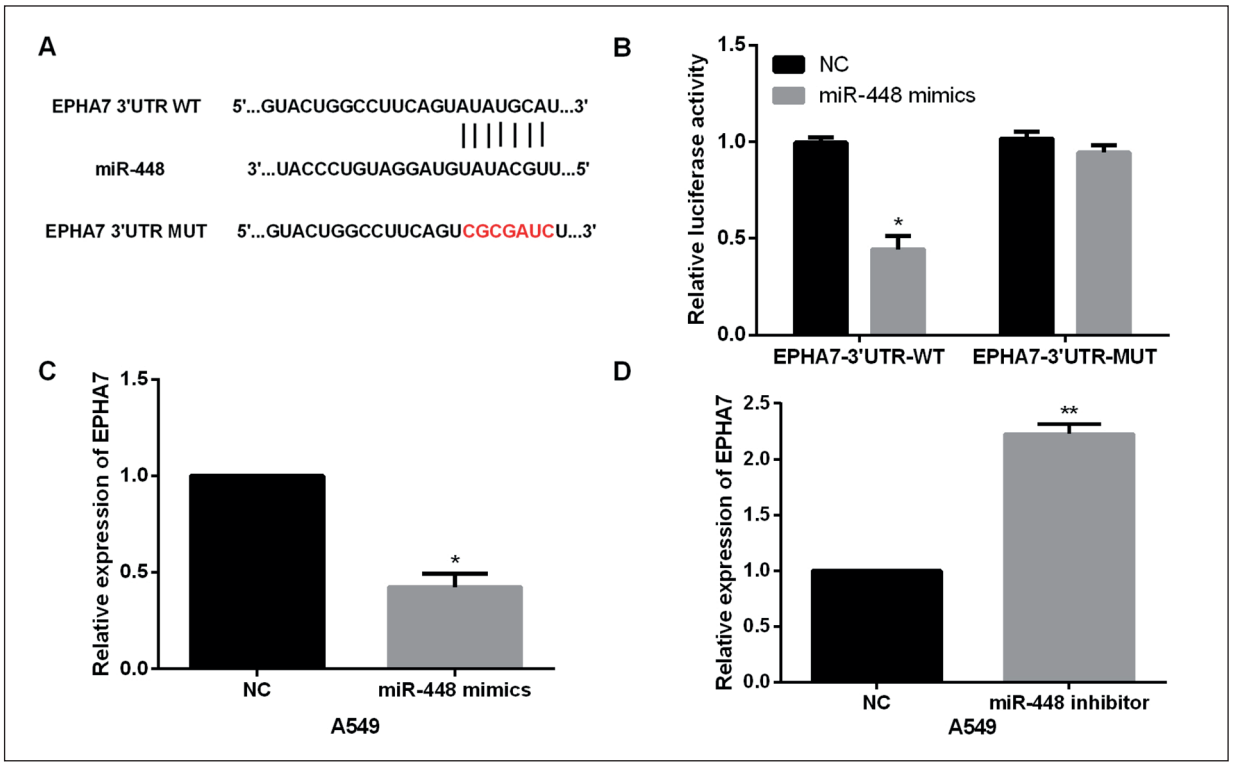

Figure 4. EPHA7 was directly targeted by miR-448 in NSCLC cells. **A,** The potential binding sites of miR-448 in EPHA7-3'-UTR were predicted. **B,** Luciferase activities of EPHA7-3'-UTR-WT or EPHA7-3'-UTR-MUT luciferase activity vector were detected. **C, D,** The regulatory functions of miR-448 on EPHA7 expression levels were determined by qRT-PCR.

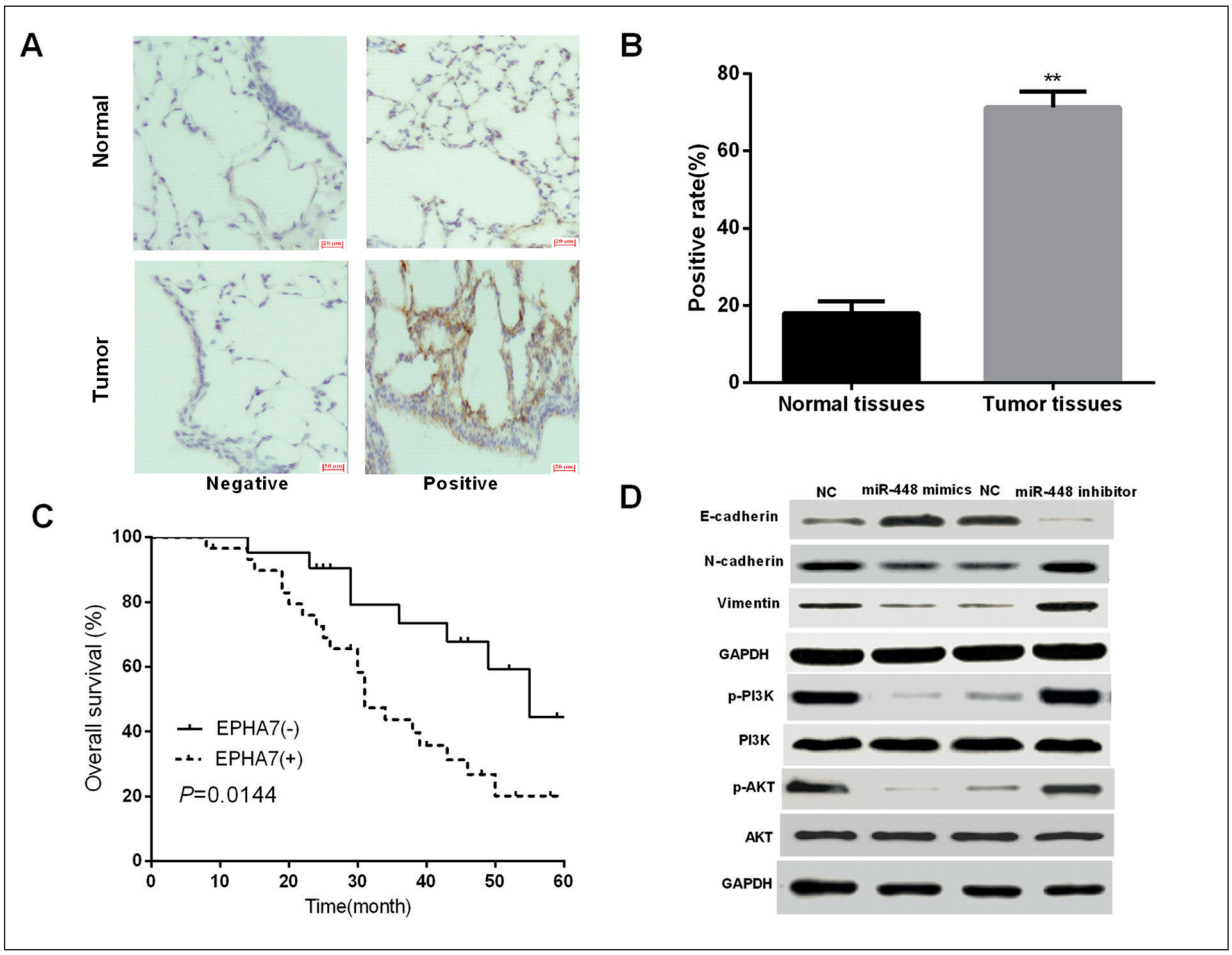

Figure 5. MiR-448 inhibited PI3K/Akt pathway and EMT in NSCLC cells. **A, B,** IHC assays were performed to determine the EPHA7 expressions in NSCLC tissue samples, (magnification: 200×). **C,** Kaplan-Meier analysis of NSCLC patients with different EPHA7 expressions showed that high EPHA7 expression demonstrated shorter OS. **D,** MiR-448 inhibited NSCLC cell PI3K/Akt pathway and EMT.

conducted to determine the influence of miR-448 on PI3K/AKT pathway in NSCLC cells. As shown in Figure 5D, in miR-448-overexpressed cells, expressions of p-PI3K and p-AKT were prominently downregulated while there were no evident changes in PI3K and AKT expressions. On the contrary, p-PI3K and p-AKT expressions were significantly promoted by miR-448 knockdown. Furthermore, Western blot analysis was also conducted to detect the expressions of EMT markers after miR-448 upregulation or silencing. Results demonstrated that rescuing miR-448 expressions significantly upregulated E-cadherin expressions whereas downregulated N-cadherin and Vimentin expressions. Moreover, miR-448 silencing led to upregulated N-cadherin and Vimentin, and downregulated E-cadherin (Figure 5D). Collectively, the above results demonstrated that miR-448 suppressed NSCLC progression *via* the inhibition of PI3K/AKT signaling pathway and EMT.

MiR448 Overexpression Inhibited In Vivo Tumor Growth

We detected the influence of miR-448 on tumor growth rate and tumor size of NSCLC using tumor xenografts. Results showed that subcutaneous tumors in the miR-448 groups were notably smaller than those in the control groups, and tumor growth rate in miR-448 groups was also prominently lower than the control groups (Figure 6A and 6B). These results indicated that miR-448 overexpression could inhibit the *in vivo* tumor growth of NSCLC.

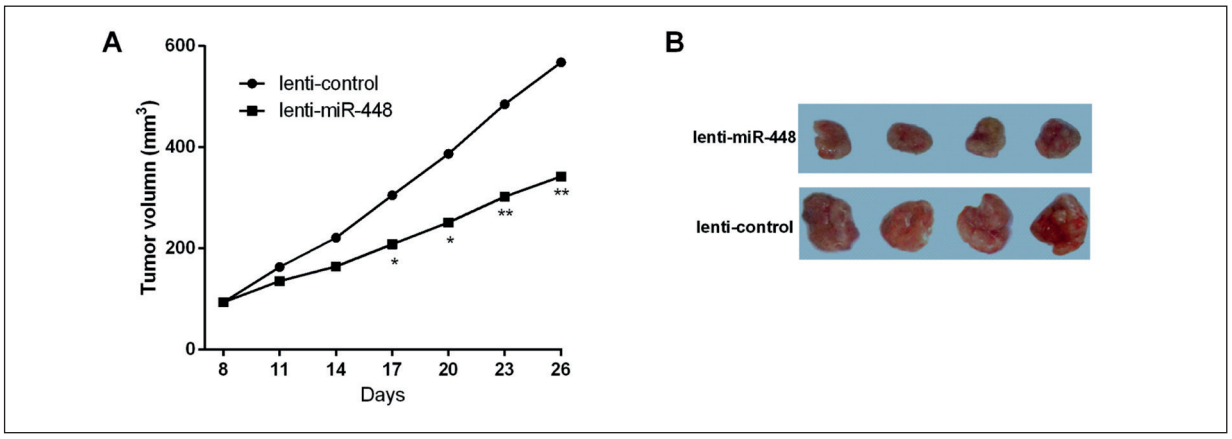

Figure 6. MiR-448 suppressed NSCLC growth *in vivo.* **A,** Growth curves of tumor volumes in xenografts. **B,** Representative images of tumors in different groups.

Discussion

Lung cancer remains the leading factor for tumor-associated deaths globally due to insufficient understanding about the mechanisms underlying lung cancer development. MiRNAs have potentials to function as pivotal diagnostic or prognostic biomarkers for malignant tumors[19,20]. Meanwhile, the crucial implication of miRNAs in NSCLC in modulating gene expressions and cell behaviors has also been widely investigated. Shen et al[21] indicated that miR-451a suppressed NSCLC cell metastasis through regulating ATF2; miR-367 was confirmed to promote NSCLC progression through targeting FBXW7[22]; miR-185 inhibited NSCLC cell viability and invasion *via* regulation of KLF7[23]. These studies elucidated that alterations of miR expressions could influence NSCLC tumorigenesis. Our investigation provides an insight into the roles of miR-448 in controlling NSCLC progression.

The PI3K/Akt pathway, which has been commonly identified in diverse human malignancies, is considered to be a key pathway in carcinogenesis[24]. PI3K/Akt pathway was reported to be involved in the development of various malignancies, including nasopharyngeal carcinoma[25], breast cancer[26], and glioblastoma[27]. Moreover, it was found that PI3K/Akt pathway played crucial roles in NSCLC[28]. EMT has been indicated to play an important role in tumor metastases[29,30]. During EMT progress, cells are characterized by upregulated mesenchymal markers (such as Vimentin and N-cadherin) and downregulated epithelial marker (such as E-cadherin)[31]. Tumor cells that acquire stromal cell-like properties could be isolated from primary tumors and spread to distant locations, which is the vital steps in tumor metastases[32].

Aberrantly expressed miR-448 was implicated in tumorigenesis of various tumors, demonstrating its critical functions in tumor development. MiR-448 negatively regulated ovarian carcinoma cell metastases and growth through regulating CXCL12[33]. MiR-448 promoted oral squamous cell carcinoma proliferation and inhibited apoptosis *via* inhibition of MPPED2[34], However, the specific biofunctions of miR-448 in NSCLC development remain unclear. In the current study, miR-448 was confirmed to be significantly downregulated in NSCLC tissues and correlated with the poor prognosis and adverse phenotypes of NSCLC patients. Moreover, results of functional assays further demonstrated that miR-448 overexpression could suppress the malignancies of NSCLC cells, including proliferation, invasion and migration abilities. Meanwhile, miR-448 upregulation in NSCLC cells significantly inhibited PI3K/Akt pathway and EMT. Several target genes of miR-448 have been identified, such as ADAM10[35] and MEF2C[36]. To gain more insights into the underlying mechanisms about the influence of miR-448 in NSCLC, we next explored its potential target genes and results identified EPHA7 as a functional target of miR-448 in NSCLC cell lines. We also found that miR-448 prominently inhibited NSCLC cell growth *in vivo.* Therefore, above findings together demonstrated that miR-448 exerted anti-NSCLC functions both *in vitro* and *in vivo.*

Conclusions

In brief, the functional roles and potential mechanisms of miR-448 in NSCLC progression were explored. It was found that miR-448 was abnormally underexpressed both in NSCLC tissues and cell lines. The decreased miR-448 in NSCLC patients indicated poor prognosis and malignant clinicopathologic features. Moreover, miR-448 restoration suppressed NSCLC cell proliferation, invasion and migration via downregulation of EPHA7 expressions. Findings also indicated that miR-448 exerted anti-NSCLC functions *via* the regulation of PI3K/Akt pathway and EMT. These data may be beneficial to the development of miR448 as a potential therapeutic biomarker in NSCLC treatment. In conclusion, the novelty of this work was that regulation of EPHA7 as a target for miR-448 can serve as a novel direction in the targeted cancer diagnosis or therapy.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.
2) Torre LA, Siegel RL, Jemal A. Lung cancer statistics. Adv Exp Med Biol 2016; 893: 1-19.
3) Shintani T, Matsuo Y, Iizuka Y, Mitsuyoshi T, Mizowaki T. A Retrospective long-term follow-up study of stereotactic body radiation therapy for non-small cell lung cancer from a single institution: incidence of late local recurrence. Int J Radiat Oncol Biol Phys 2018; 100: 1228-1236.
4) Shi H, Bi H, Sun X, Dong H, Jiang Y, Mu H, Li W, Liu G, Gao R, Su J. Tubeimoside-1 inhibits the proliferation and metastasis by promoting miR-126-5p expression in non-small cell lung cancer cells. Oncol Lett 2018; 16: 3126-3134.
5) Zhou M, Chen X, Wu J, He X, Ren R. MicroRNA-143 regulates cell migration and invasion by targeting GOLM1 in cervical cancer. Oncol Lett 2018; 16: 6393-6400.
6) Guo H, Ingolia NT, Weissman JS, Bartel DP. Mammalian microRNAs predominantly act to decrease target mRNA levels. Nature 2010; 466: 835-840.
7) Yang X, Zhang L, Song X, He W, Zhang D, Lu Q, Wu J, Wu C, Jiang J. MicroRNA-613 promotes colon cancer cell proliferation, invasion and migration by targeting ATOH1. Biochem Biophys Res Commun 2018; 504: 827-833.
8) Gao J, Wu N, Liu X, Xia Y, Chen Y, Li S, Deng Z. MicroRNA-142-3p inhibits cell proliferation and chemoresistance in ovarian cancer via targeting sirtuin 1. Exp Ther Med 2018; 15: 5205-5214.
9) Song R, Cong L, Ni G, Chen M, Sun H, Sun Y, Chen M. MicroRNA-195 inhibits the behavior of cervical cancer tumors by directly targeting HDGF. Oncol Lett 2017; 14: 767-775.
10) Cheng D, Bao C, Zhang X, Lin X, Huang H, Zhao L. LncRNA PRNCR1 interacts with HEY2 to abolish miR-448-mediated growth inhibition in non-small cell lung cancer. Biomed Pharmacother 2018; 107: 1540-1547.
11) Janes PW, Griesshaber B, Atapattu L, Nievergall E, Hii LL, Mensinga A, Chheang C, Day BW, Boyd AW, Bastiaens PI, Jorgensen C, Pawson T, Lackmann M. Eph receptor function is modulated by heterooligomerization of A and B type Eph receptors. J Cell Biol 2011; 195: 1033-1045.
12) Rudno-Rudzinska J, Kielan W, Frejlich E, Kotulski K, Hap W, Kurnol K, Dzierzek P, Zawadzki M, Halon A. A review on Eph/ephrin, angiogenesis and lymphangiogenesis in gastric, colorectal and pancreatic cancers. Chin J Cancer Res 2017; 29: 303-312.
13) Walshe J, Richardson NA, Al AN, Stephenson SA, Harkin DG. A potential role for Eph receptor signalling during migration of corneal endothelial cells. Exp Eye Res 2018; 170: 92-100.
14) Noberini R, Pasquale EB. Proliferation and tumor suppression: not mutually exclusive for Eph receptors. Cancer Cell 2009; 16: 452-454.
15) Wang LF, Fokas E, Juricko J, You A, Rose F, Pagenstecher A, Engenhart-Cabillic R, An HX. Increased expression of EphA7 correlates with adverse outcome in primary and recurrent glioblastoma multiforme patients. BMC Cancer 2008; 8: 79.
16) Xiang C, Lv Y, Wei Y, Wei J, Miao S, Mao X, Gu X, Song K, Jia S. Effect of EphA7 silencing on proliferation, invasion and apoptosis in human laryngeal cancer cell lines Hep-2 and AMC-HN-8. Cell Physiol Biochem 2015; 36: 435-445.
17) Hu XT, Chen W, Zhang FB, Shi QL, Hu JB, Geng SM, He C. Depletion of the proteasome subunit PSMA7 inhibits colorectal cancer cell tumorigenicity and migration. Oncol Rep 2009; 22: 1247-1252.
18) Hu XT, Chen W, Wang D, Shi QL, Zhang FB, Liao YQ, Jin M, He C. The proteasome subunit PSMA7 located on the 20q13 amplicon is overexpressed and associated with liver metastasis in colorectal cancer. Oncol Rep 2008; 19: 441-446.
19) Minguet J, Smith KH, Bramlage P. Targeted therapies for treatment of non-small cell lung cancer--Recent advances and future perspectives. Int J Cancer 2016; 138: 2549-2561.
20) Levy B, Hu ZI, Cordova KN, Close S, Lee K, Becker D. Clinical utility of liquid diagnostic platforms in non-small cell lung cancer. Oncologist 2016; 21: 1121-1130.
21) Shen YY, Cui JY, Yuan J, Wang X. MiR-451a suppressed cell migration and invasion in non-small

cell lung cancer through targeting ATF2. Eur Rev Med Pharmacol Sci 2018; 22: 5554-5561.

22) Xu J, Wu W, Wang J, Huang C, Wen W, Zhao F, Xu X, Pan X, Wang W, Zhu Q, Chen L. miR-367 promotes the proliferation and invasion of non-small cell lung cancer via targeting FBXW7. Oncol Rep 2017; 37: 1052-1058.

23) Zhao L, Zhang Y, Liu J, Yin W, Jin D, Wang D, Zhang W. MiR-185 inhibits the proliferation and invasion of non-small cell lung cancer by targeting KLF7. Oncol Res 2019; 27: 1015-1023.

24) Fruman DA, Rommel C. PI3K and cancer: lessons, challenges and opportunities. Nat Rev Drug Discov 2014; 13: 140-156.

25) Zhu JF, Huang W, Yi HM, Xiao T, Li JY, Feng J, Yi H, Lu SS, Li XH, Lu RH, He QY, Xiao ZQ. Annexin A1-suppressed autophagy promotes nasopharyngeal carcinoma cell invasion and metastasis by PI3K/AKT signaling activation. Cell Death Dis 2018; 9: 1154.

26) Zhu M, Wang X, Gu Y, Wang F, Li L, Qiu X. MEG3 overexpression inhibits the tumorigenesis of breast cancer by downregulating miR-21 through the PI3K/Akt pathway. Arch Biochem Biophys 2019; 661: 22-30.

27) Zheng Q, Diao S, Wang Q, Zhu C, Sun X, Yin B, Zhang X, Meng X, Wang B. IL-17A promotes cell migration and invasion of glioblastoma cells via activation of PI3K/AKT signalling pathway. J Cell Mol Med 2019; 23: 357-369.

28) Zhao W, Sun Q, Yu Z, Mao S, Jin Y, Li J, Jiang Z, Zhang Y, Chen M, Chen P, Chen D, Xu H, Ding S, Yu Z. MiR-320a-3p/ELF3 axis regulates cell metastasis and invasion in non-small cell lung cancer via PI3K/Akt pathway. Gene 2018; 670: 31-37.

29) Wang H, An X, Yu H, Zhang S, Tang B, Zhang X, Li Z. MiR-29b/TET1/ZEB2 signaling axis regulates metastatic properties and epithelial-mesenchymal transition in breast cancer cells. Oncotarget 2017; 8: 102119-102133.

30) Wu J, Yang Y, Gao S, Jiang H, Wang XQ, Xiao Y, Chen XH, Li P, Xu CD. ERp29 inhibits tumorigenicity by suppressing epithelial mesenchymal transition in gastric cancer. Oncotarget 2017; 8: 78757-78766.

31) Yu K, Li Q, Shi G, Li N. Involvement of epithelial-mesenchymal transition in liver fibrosis. Saudi J Gastroenterol 2018; 24: 5-11.

32) Thiery JP, Sleeman JP. Complex networks orchestrate epithelial-mesenchymal transitions. Nat Rev Mol Cell Biol 2006; 7: 131-142.

33) Lv Y, Lei Y, Hu Y, Ding W, Zhang C, Fang C. MiR-448 negatively regulates ovarian cancer cell growth and metastasis by targeting CXCL12. Clin Transl Oncol 2015; 17: 903-909.

34) Shen L, Liu L, Ge L, Xie L, Liu S, Sang L, Zhan T, Li H. MiR-448 downregulates MPPED2 to promote cancer proliferation and inhibit apoptosis in oral squamous cell carcinoma. Exp Ther Med 2016; 12: 2747-2752.

35) Wu X, Tang H, Liu G, Wang H, Shu J, Sun F. MiR-448 suppressed gastric cancer proliferation and invasion by regulating ADAM10. Tumour Biol 2016; 37: 10545-10551.

36) Zhang R, Sui L, Hong X, Yang M, Li W. MiR-448 promotes vascular smooth muscle cell proliferation and migration in through directly targeting MEF2C. Environ Sci Pollut Res Int 2017; 24: 22294-22300.