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# MicroRNA-381 inhibits lung adenocarcinoma cell biological progression by directly targeting LMO3 through regulation of the PI3K/Akt signaling pathway and epithelial-to-mesenchymal transition

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**Abstract. – OBJECTIVE: To investigate the role of miR-381 in the progression of lung adenocarcinoma (LA) and its underlying mechanism.**

**PATIENTS AND METHODS: A total of 54 pairs of LA tissues and para-carcinoma tissues were obtained from May 2015 to April 2017 in our hospital. Four human LA cell lines (A549, SPC-A1, H1299, and PC-9) and one normal human pulmonary epithelial cell line BEAS–2B were obtained and cultured. The protein and mRNA expression levels were detected by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) and Western blot, respectively. Additionally, cell proliferation assays and cell migration and invasion assays were used. Furthermore, tumor xenograft model in nude mice was made in this study.**

**RESULTS: miR-381 was notably downregulated in LA tissues. Moreover, low miR-381 expression was confirmed to be strongly correlated with poor prognosis and aggressive clinicopathological characteristics of LA patients. Exogenous miR-381 overexpression was found to notably restrict LA cell proliferation, migration, and invasion; additionally, miR-381 overexpression could significantly reduce tumor growth *in vivo*. Mechanistically, LMO3 was determined as a novel direct target for miR-381 in LA cells. In clinical LA tissues, the LMO3 expressions were clearly overexpressed. Furthermore, miR-381 overexpression affected the PI3K/Akt pathway and EMT in LA.**

**CONCLUSIONS: MiR-381 played key roles in LA progression, partially *via* directly targeting LMO3 and regulating the PI3K/Akt signaling pathway and EMT. Thus, the miR-381/ LMO3 axis has clinical significance in the therapy of patients with LA.**

*Key Words:*
MicroRNA-381, Lung adenocarcinoma, Epithelial-to-mesenchymal transition, PI3K/Akt, LMO3.

## Introduction

Lung adenocarcinoma (LA) is the main hypotype of non-small cell lung cancers which account for about 85% of lung cancers[1,2]. Due to early metastasis and high invasiveness, patients with LA usually present with locally metastatic or advanced disease[3]. In current clinical management, metastasis and invasiveness of LA cells still remain critical challenges[4]. Although existing therapies, such as chemotherapy and molecular targeted therapy, it have increasingly improved recently and the survival rates of LA patients remain poor[5]. Thus, there is an urgent need to elucidate the molecular mechanism underlying LA progression for identification of molecular therapeutic targets and novel prognostic markers to improve the diagnosis, therapies, and prevention of human LA.

Accumulating studies[6,7] demonstrated that aberrant miRNA expressions were involved in multiple pathological and biological processes, such as tumorigenesis, cell apoptosis, differentiation, and proliferation. MiRNAs often function as tumour suppressors or oncogenes, playing crucial functions in multiple tumors. For instance, Sheng et al[8] found that miR-145 repressed human cell migration and invasion in colorectal cancer *via*

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PAK4-dependent pathway; Li et al[9] proposed that miR-509-5p suppressed pancreatic carcinoma cell proliferation and migration *via* regulation of MDM2; Sun et al[10] found that miR-610 suppressed cell proliferation and invasion in colorectal carcinoma by inhibiting hepatoma-derived growth factor. Therefore, Lin et al[11] have indicated that aberrant expressions of miRNAs have prognostic significance for different kinds of cancers, including LA. However, the expressions and biofunctions of miR-381 in LA need to be further elucidated.

The PI3K/Akt pathway can influence the normal physiological activities of cells. Hou et al[12] have demonstrated that the aberrant activations of the PI3K/Akt pathway play vital functions in the tumorigenesis and progression of various tumors, including bladder carcinoma, non-small cell lung cancer[13], as well as gastric carcinoma[14]. In addition, the PI3K/Akt pathway exerts vital functions in LA progression, and activation of the PI3K/Akt pathway may influence LA cell apoptosis and proliferation[15]. Epithelial-mesenchymal transition (EMT) is a conserved developmental process in which polarized immotile epithelial cells were converted into motile mesenchymal cells[16]. Several signaling pathways and oncogenic events are implicated in EMT and several novel EMT inducing transcription factors have recently been identified, including CXCL5[17], ADAM12[18], and UBE2C[19]. During the progression of EMT, epithelial cells are loss of cell-cell contacts and E-cadherin expressions, changing the apical-basal polarities and differentiating into mesenchymal cells[20]. EMT is generally considered as a crucial progress in the metastasis of various malignancies, including breast carcinoma[21], hepatocellular carcinoma[22], and gastric carcinoma[23]. We also aimed to investigate whether miR-381 inhibited LA invasion and migration by regulating EMT.

LIM-only protein 3 (LMO3) is a member of the LIM-only protein family[24]. LMO3 plays crucial roles in various tumor progresses, such as cell growth, invasion, and metastasis[25,26]. Aberrant expressions of LMO3 have been found in multiple cancers, playing oncogenic functions. For example, Aoyama et al[27] found that LMO3 exerted oncogenic functions in neuroblastoma by interacting with HEN2. In addition, Isogai et al[28] demonstrated that LMO3 promoted neuroblastoma cell growth through transactivation of Mash1. However, to date, little attention has been paid to the oncogenic functions of LMO3 in LA progression.

## Patients and Methods

### Tissue Samples

A total of 54 pairs of LA tissues and para-carcinoma tissues were obtained from the General Hospital of Southern Theatre Command from May 2015 to April 2017. None of the LA patients involved in current research accepted radiotherapy or chemotherapy strategies before the tissue collection. All tissue samples were frozen in liquid nitrogen immediately after resection and preserved at –80°C. This study was approved by the Ethics Committee of the General Hospital of Southern Theatre Command. All participants signed the written informed consent prior to the study.

### Cell Cultures

Four human LA cell lines (A549, SPC-A1, H1299, and PC-9) and one normal human pulmonary epithelial cell line BEAS–2B were obtained from the American Type Culture Collection (Manassas, VA, USA) and cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (Invitrogen, Carlsbad, CA, USA) which contained 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA) in a humidified incubator with 5% $CO_2$ at 37°C.

### Cell Transfections

MiR-381 mimics and inhibitors designed to overexpress and interfere endogenous mature miR-381 were purchased from RiboBio (Guangzhou, China). Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA) was applied to transfect miR-381 mimics and inhibitor into LA cells following the manufacturer's instructions.

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

TRIzol (Invitrogen, Carlsbad, CA, USA) was applied to isolate the total RNAs from LA tissues and cultured cells following the manufacturers' instructions. Reverse transcription for complementary deoxyribose nucleic acid (cDNA) was performed using TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). Then, an SYBR Green mix kit and the ABI 7900 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) were utilized to amplify the cDNA. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as endogenous controls for LMO3. U6 served as the internal control for miR-381. The relative ex-

pressions were determined using the $2^{-\Delta\Delta Ct}$ method. The sequences of the primers were described in Table I.

### Immunohistochemistry (IHC)

For LMO3 immunohistochemistry, 10% formalin fixed and paraffin embedded tissue sections were dewaxed and rehydrated with xylene and graded alcohols. After pretreatment with citrate buffer in a microwave oven for the endogenous antigen-retrieval, the endogenous peroxidase activities were suppressed by 3% hydrogen peroxide in ethanol for 10 min. Subsequently, slides were incubated with primary LMO3 antibody (1:400, ab230490, Abcam, Cambridge, MA, USA) overnight in a humidified chamber at 4°C, followed by incubation with biotinylated goat anti-rabbit antibodies (1:500, ab7090, Abcam, Cambridge, MA, USA) for 30 min at room temperature. The slides were stained with diaminobenzidine (DAB) as the chromogen and counterstained with hematoxylin. The LMO3 expression was detected based on the ratio of positive cells. Briefly, stained cells/all cells >25% was identified as positive (+), whereas positive ratio < 25% was negative (-). Positive rate of expression = number of positive cells/total number of cells.

### Cell Proliferation Assays

The proliferation of the LA cells treated with miR-381 mimics or inhibitors and was detected by performing MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay (Sigma-Aldrich, St. Louis, MO, USA). The transfected cells were harvested and plated into a 96-well plate. After incubation at 37°C for 0 h, 24 h, 48 h, and 72 h, MTT (10 μL, 5 mg/mL) was added into each well and cultivated for 4h. Afterward, 100 μL dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, MO, USA) was added into each well to dissolve the crystals. The optical density $(OD)_{490}$ was measured with a microplate reader (Thermo Fisher Scientifc, Inc., Waltham, MA, USA).

### Cell Migration and Invasion Assays

Transwell chamber (8.0 μm pore size, Corning Incorporated, Corning, NY, USA) was applied to determine the invasion and migration abilities with or without the Matrigel (Corning Incorporated) being pre-coated. For the invasion assay, LA cells in serum-free medium were added into top chambers of the inserts which had been precoated with Matrigel. In the meantime, culture medium containing 10% FBS, as a chemoattractant, was placed into bottom chambers. After incubated at 37°C for 48h in a 5% $CO_2$ atmosphere, cells maintained on the top side of the inserts were wiped off with cotton swabs while the invaded cells on the bottom side were fixed with 0.1% paraformaldehyde and then stained with 0.1% crystal violet. After that, the stained cells were counted under a microscope (Olympus, Tokyo, Japan) from five randomly selected visual fields. The difference between the migration and invasion assays was that the inserts were not plated with Matrigel for the migration assays.

### Dual-Luciferase Reporter Assay

The LMO3 3'UTR containing the miR-381 binding sequences was amplified by PCR and cloned into the pGL3 plasmids (Promega, Madison, WI, USA), and LMO3-3'UTR-MUT vector was also synthesized with point mutation in the seed sequence. The LA cells were treated with miR-381 mimics and the LMO3-3'UTR-WT or LMO3-3'UTR-MUT vector. The dual-luciferase reporter assay kit (Promega, Madison, WI, USA) was applied to determine the luciferase activity after 48 h of transfections.

**Table I.** Primer sequences for qRT-PCR.

| Primer | Sequence |
|---|---|
| miR-381 forward | 5'-AGTCTATACAAGGGCAAGCTCTC-3' |
| miR-381 reverse | 5'-ATCCATGACAGATCCCTACCG-3' |
| U6 forward | 5'-CTCGCTTCGGCAGCACA-3' |
| U6 reverse | 5'-AACGCTTCACGAATTTGCGT-3' |
| LMO3 forward | 5'-TCTGAGGCTCTTTGGTGTAACG-3' |
| LMO3 reverse | 5'-CCAGGTGGTAAACATTGTCCTTG-3' |
| GAPDH forward | 5'-ACCTGACCTGCCGTCTAGAA-3' |
| GAPDH reverse | 5'-TCCACCACCCTGTTGCTGTA-3' |

U6: small nuclear RNA, snRNA; LMO3: LIM-only protein 3; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

### Western Blotting Analysis

Iced lysis buffer containing protease and phosphatase inhibitors (Roche, Basel, Switzerland) were used to lyse LA cells and extract total proteins. The bicinchoninic acid (BCA) protein assay kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA) was used to detect the concentrations of total proteins. Then, protein samples were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene difluoride (PVDF) membrane (Roche, Basel, Switzerland). The membrane was blocked with 5% non-fat milk at room temperature in Tris-Buffered Saline & Tween (TBST) for 2 hours and then incubated overnight at 4°C with the respective primary antibodies against LMO3 (1:1000, ab230490, Abcam), E-cadherin (1:1000, ab15148, Abcam, Cambridge, MA, USA), Vimentin (1:1000, ab92547, Abcam, Cambridge, MA, USA), PI3K (1:1000, ab86714, Abcam, Cambridge, MA, USA), Akt (1:1000, sc-56878, Santa Cruz Biotechnology, Santa Cruz, CA, USA), p-PI3K (1:1000, ab182651, Abcam, Cambridge, MA, USA), p-Akt (1:1000, sc-81433, Santa Cruz Biotechnology, Santa Cruz, CA, USA), and GAPDH (1:1000, ab9485, Abcam, Cambridge, MA, USA). Membranes were then exposed to the corresponding HRP-conjugated secondary antibodies (1:2000, ab7090, Abcam, Cambridge, MA, USA) at room temperature for 2 h. An enhanced chemiluminescence system (Thermo Fisher Scientific, Waltham, MA, USA) was then utilized to visualize the protein bands. GAPDH served as an internal control.

### Tumor Xenograft Model in Nude Mice

This study was performed strictly following the recommendations in the Guide for the Care and Use of Laboratory Animals of the Southern Medical University. Six-week-old female nude mice were randomly divided into two groups. The A549 cells were stably transfected with lentiviral miR-381 (lenti-miR-381) or the negative lentiviral miR-control (lenti-control) and injected subcutaneously into the flank of the mice in different groups. Tumor size was measured at 3-day intervals using calipers (volume=longest diameter × shortest diameter$^2$/2). 26 days after injection, the mice were killed and their tumors were dissected and trimmed.

### Statistical Analysis

All experiments were repeated at least 3 times. Statistical Product and Service Solutions (SPSS) software version 17.0 (SPSS Inc., Chicago, IL, USA) was applied for the statistical analysis. The Student's *t*-test, ANOVA and Scheffe's post-hoc analysis were applied, where appropriate. The Kaplan-Meier method and log-rank test were applied to estimate the survival rates and compare the survival curves respectively. $p<0.05$ was regarded as a statistically significant difference.

## Results

### MiR-381 Expressions Were Declined in LA Tissues

To determine the functional effects of miR-381 in LA, we firstly measured the miR-381 expressions in LA tissues using qRT-PCR. The results demonstrated that, compared to the normal tissues, the LA tissues presented significant lower expression level of miR-381 (Figure 1A). Furthermore, we divided the LA patients into low and high miR-381 expressing groups using the mean miR-381 expression level as the cut-off to elucidate the prognostic functions of miR-381. As shown in Table II, the low miR-381 expressions were notably related to worse clinicopathologic features of LA patients. Additionally, the Kaplan-Meier analysis indicated that LA patients with low miR-381 expressions had poorer overall survivals (Figure 1B).

### MiR-381 Inhibited LA Cell Proliferation

To investigate the biofunctions about miR-381 in LA development, we further measured the expressions of miR-381 in LA cells. qRT-PCR analysis demonstrated that the miR-381 expressions in LA cells were notably reduced in contrast with that in BEAS–2B (Figure 2A). Subsequently, miR-381 mimic or inhibitor was transfected into the SPC-A1 and A549 cells, which expressed relatively lower and higher endogenous miR-381 among the four LA cell lines. Results revealed that miR-381 was prominently upregulated in SPC-A1 cells with transfections of miR-381 mimic and markedly downregulated in A549 cells transfected with miR-381 inhibitor (Figure 2B and 2C). MTT assay was conducted to determine the proliferation capacity of LA cells with different transfections and results revealed that miR-381 overexpression remarkably repressed the SPC-A1 proliferation while miR-381 inhibition markedly enhanced the proliferation capacity of A549 cells (Figure 2D).

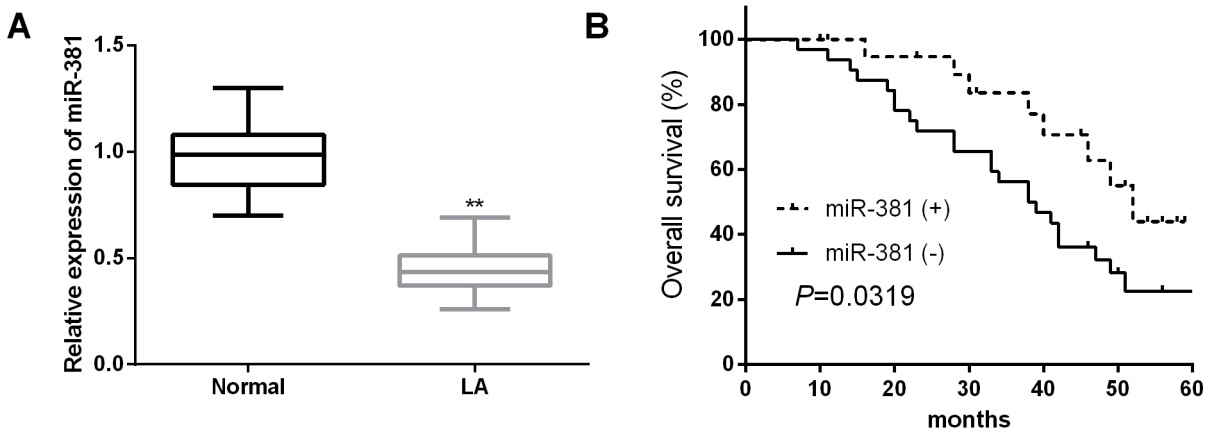


**Figure 1.** miR-381 was down-regulated in LA. **A,** miR-381 expressions in LA tissues and normal tissue samples were detected using qRT-PCR. **B,** Kaplan-Meier analysis of LA patients with different miR-381 expressions. $^{**}p<0.01$

### *MiR-381 Suppressed the Migration and Invasion of LA Cells*

Subsequently, the functions of miR-381 on LA cell migration and invasion abilities were determined by performing transwell assay. Results revealed that miR-381 overexpression significantly repressed SPC-A1 migration and invasion abilities (Figure 3A and 3B). On the other hand, miR-381 suppression in A549 cells markedly enhanced the invasion and migration capacities (Figure 3C and 3D). These findings suggested that miR-381 exerted anti-tumor functions in LA cells.

### *LMO3 Was a Direct Target of MiR-381 in LA Cells*

Bioinformatics analysis was performed to explore candidate targets for elucidating the mechanism of miR-381-mediated inhibition of LA pro-

**Table II.** Correlation of miR-381 expression with the clinicopathological characteristics of the LA patients.

| Clinicopathological features | Cases (n=54) | miR-381# expression | | *p*-value |
|---|---|---|---|---|
| | | High (n=22) | Low (n=32) | |
| Age (years) | | | | 0.4636 |
| >60 | 30 | 13 | 17 | |
| ≤60 | 24 | 9 | 15 | |
| Gender | | | | 0.3734 |
| Male | 28 | 10 | 18 | |
| Female | 26 | 12 | 14 | |
| Tumor size (cm) | | | | 0.1034 |
| ≥ 5.0 | 26 | 7 | 19 | |
| < 5.0 | 28 | 15 | 13 | |
| Lymph node metastasis | | | | 0.0021* |
| Yes | 24 | 18 | 6 | |
| No | 30 | 4 | 26 | |
| TNM stage | | | | 0.0015* |
| I +II | 25 | 16 | 9 | |
| III+IV | 29 | 6 | 23 | |
| Smoker | | | | 0.3781 |
| Yes | 32 | 15 | 17 | |
| No | 22 | 7 | 15 | |

LA: Lung adenocarcinoma; TNM: tumor-node-metastasis. #The mean expression level of miR-381 was used as the cutoff. *Statistically significant.

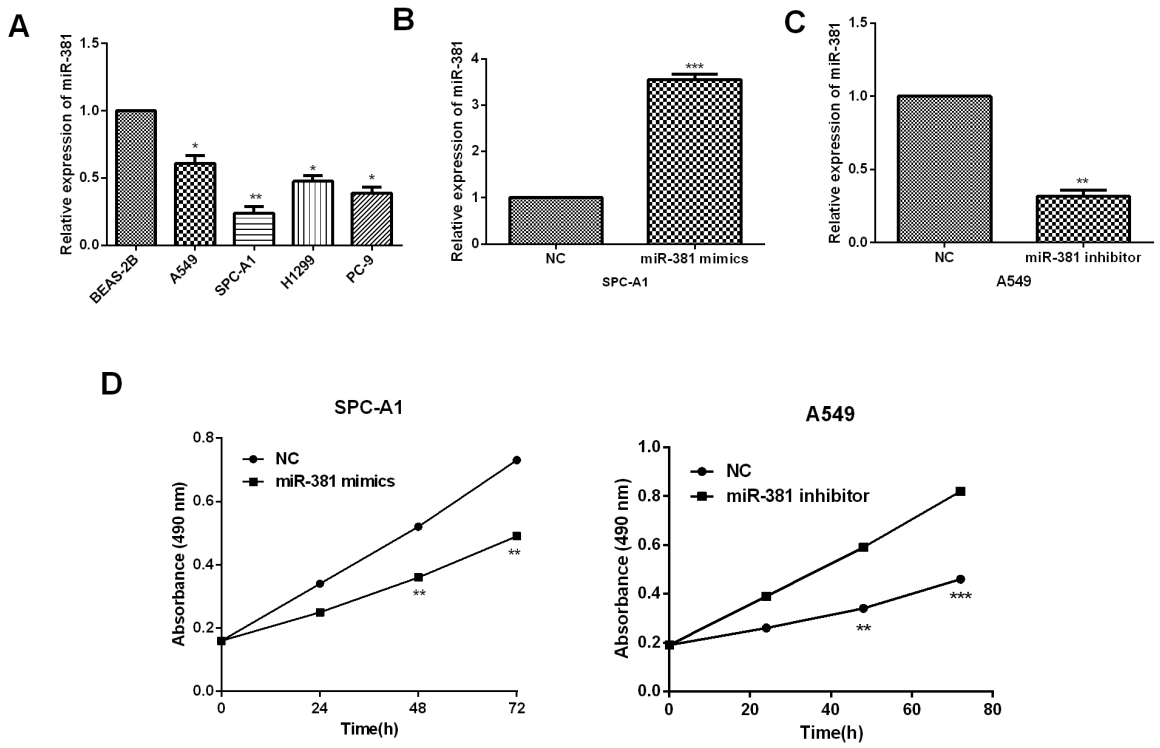


**Figure 2.** MiR-381 overexpression suppressed LA cell proliferation. **A,** qRT-PCR analysis was utilized to detect miR-381 expression in the normal pulmonary epithelial cell BEAS–2B and LA cells. **B,** MiR-381 expressions in SPC-A1 with transfections of miR-381 mimics. **C,** MiR-381 expressions in A549 with transfection of miR-381 inhibitor. **D,** Cell proliferation was observed by MTT assays in SPC-A1 or A549 cells treated with miR-381 mimics or inhibitor. ***$p$<0.001, **$p$<0.01, *$p$<0.05.

gression. The analysis demonstrated a potentially complementary region between LMO3 3'-UTR and miR-381 (Figure 4A). Then, the luciferase reporter assay was further conducted to confirm the interaction between miR-381 and LMO3 3'-UTR. As shown in Figure 4B, the overexpression of miR-381 led to a significant decline of luciferase activities of LMO3-3'UTR-WT, with no notable change in luciferase activities of LMO3-3'UTR-MUT. Next, the regulatory effects of miR-381 on the endogenous LMO3 expressions were confirmed by qRT-PCR in response to miR-381 overexpression or inhibition in SPC-A1 and A549 cells. As expected, findings revealed that the miR-381 upregulation significantly suppressed the LMO3 expression while the miR-381 inhibition markedly enhanced the LMO3 expression (Figure 4C and 4D). Taken together, these results indicated that miR-381 directly targeted LMO3.

### *MiR-381 Regulated the PI3K/AKT Signaling Pathway and EMT in LA Cells*

The potential mechanisms of the repressive functions mediated by miR-381 in LA progression were next investigated. IHC assays were carried out to determine the LMO3 expressions in LA tissues. Results revealed that LMO3 mainly localized at the nucleus and upregulated in LA tissues compared to the normal tissues (Figure 5A and 5B). Moreover, the Kaplan-Meier analysis demonstrated that LA patients with high LMO3 expression levels had poorer OS than those with low LMO3 expressions (Figure 5C). To determine whether miR-381 affected PI3K/Akt signaling pathway and EMT in LA, Western blot was performed to examine the expressions of PI3K, Akt, p-PI3K, and p-Akt, as well as the EMT related markers in SPC-A1 or A549 cells with transfection of miR-381 mimic or inhibitor. Data demonstrated that miR-381 overexpression prominently decreased the p-PI3K and p-Akt protein expressions in SPC-A1 cells while had no notable effects on the PI3K and Akt expressions. In contrast, the miR-381 inhibition in A549 cells remarkably enhanced the p-PI3K and p-Akt protein expressions (Figure 5D). The functions of miR-381 in regulating LA EMT phenotypes were also investigated. We found that E-cadherin expressions were

significantly increased while N-cadherin and vimentin expressions were remarkably declined in SPC-A1 cells by miR-381 overexpression. On the other hand, the miR-381 inhibition in A549 cells significantly decreased E-cadherin expressions and enhanced N-cadherin and vimentin expressions (Figure 5D). Above results suggested that miR-381 suppressed LA progression by regulating EMT and PI3K/Akt pathway.

### *MiR-381 Repressed the Tumor Growth of LA In Vivo*

We further investigated whether ectopic miR-381expression inhibited tumor growth *in vivo*. A549 cells stably treated with lenti-miR-381 or lenti-control were subcutaneously injected into nude mice and the tumor size was measured every 3 days. The results showed that mice in the lenti-miR-381 group had a significantly decreased tumor growth rate and tumor volume in comparison to the control group (Figure 6A and 6B).

## Discussion

Recently, due to the high incidence and mortality rate, lung adenocarcinoma still remains a major health challenge for human globally. Tumor-associated molecular abnormality plays a key role in the progression and therapies of lung

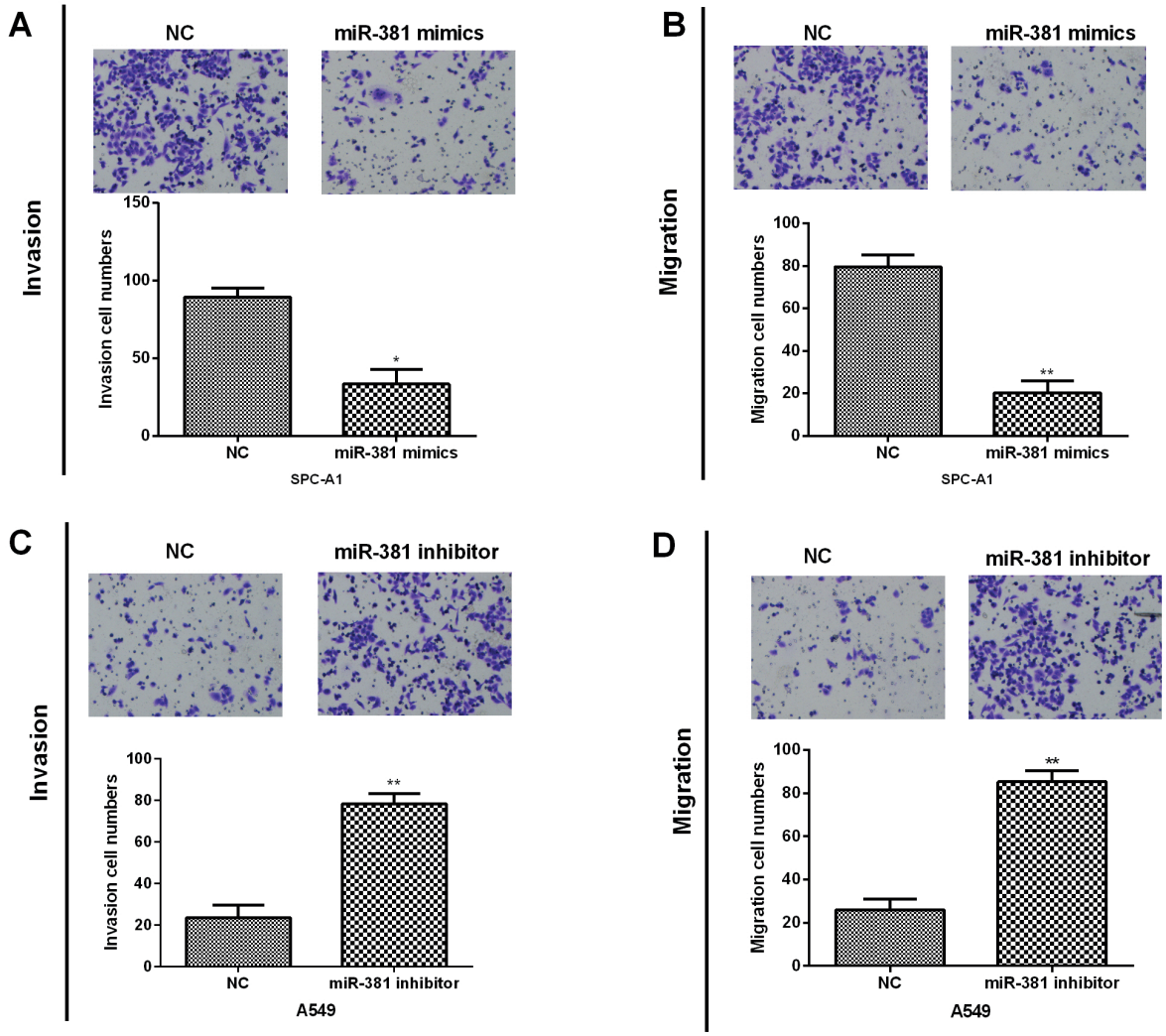


**Figure 3.** MiR-381 overexpression suppressed LA cell invasion and migration. Cell invasion **(A)** and migration abilities **(B)** were assessed with transwell assay in SPC-A1 with transfections of miR-381 mimics. Cell invasion **(C)** and migration abilities **(D)** were observed using transwell assay in A549 with transfection of miR-381 inhibitor (Magnification: 40×). $^{**}p<0.01$, $^{*}p<0.05$.

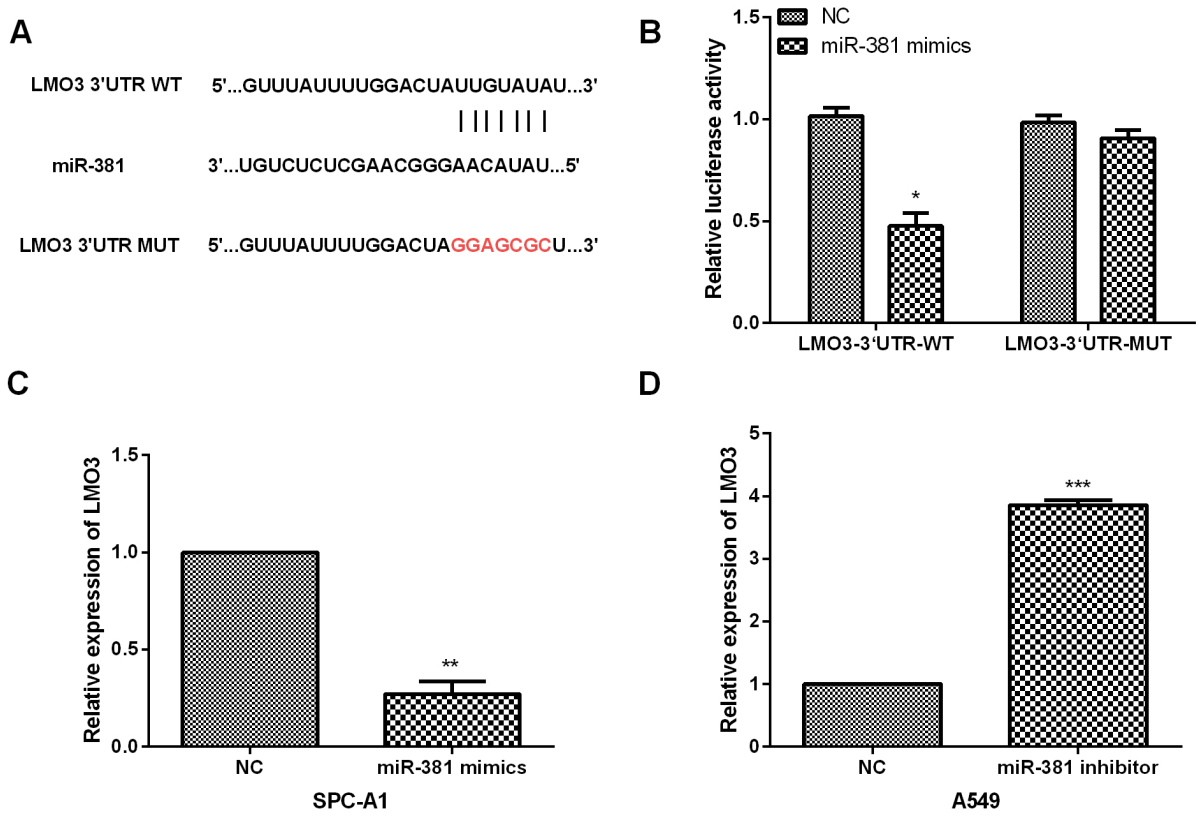


**Figure 4.** LMO3 was a direct target of miR-381 in LA cells. **A,** Putative wild-type (WT) and mutant (MUT) miR-381 binding sites in the 3'-UTR of LMO3. **B,** Relative luciferase activities were analyzed in LA cells cotransfected with WT or MUT reporter plasmids and miR-381 mimics. **C-D,** LMO3 expressions in SPC-A1 or A549 cells with transfections of miR-381 mimics or inhibitor respectively. ***$p$<0.001, **$p$<0.01, *$p$<0.05.

cancers[29]. Accumulating studies have revealed that the idea of targeted molecular therapies has become one attractive topic in human tumor treatments. Thus, identifications of the molecular pathogenesis for LA are crucial to explore novel effective therapies. Emerging evidence indicates that the dysregulations of miRNAs play pivotal roles in LA development *via* targeting varieties of important molecules. For instance, Cho et al[30] reported that miR-145 restoration prominently inhibited LA cell growth with the mutation of epidermal growth factor receptor; Bai et al[31] found that miR-519d overexpression in LA inhibited cell proliferation and invasion *via* regulating eIF4H; Bian et al[32] reported that miR-1236-3p suppressed LA cell invasion and migration *via* regulating KLF8. However, the functional roles of miR-381 in LA still need to be further elucidated.

Aberrant expressions of miR-381 have been found in multiple tumors, playing important roles in tumor progression. For example, Zhang et al[33] found that miR-381 repressed gastric carcinoma cell migration and invasion through down-regulating SOX4; Xue et al[34] reported that miR-381 suppressed cell proliferation, metastasis and EMT in breast cancer by targeting CXCR4; He et al[35] referred that miR-381 exerted tumor suppressive functions in colorectal carcinoma *via* targeting Twist1. In the current study, results demonstrated that miR-381 was notably down-regulated in LA tissues and decreased miR-381 expressions were associated with worse clinicopathological features and poorer prognosis of LA patients. Moreover, miR-381 overexpression could significantly repress the LA cell proliferation, invasion, and migration abilities by modulating the PI3K/Akt pathway and EMT. In addition, miR-381 overexpression significantly inhibited the LA tumor growth rate and tumor size *in vivo*. All the results demonstrated that miR-381 exerted anti-tumor effects in LA.

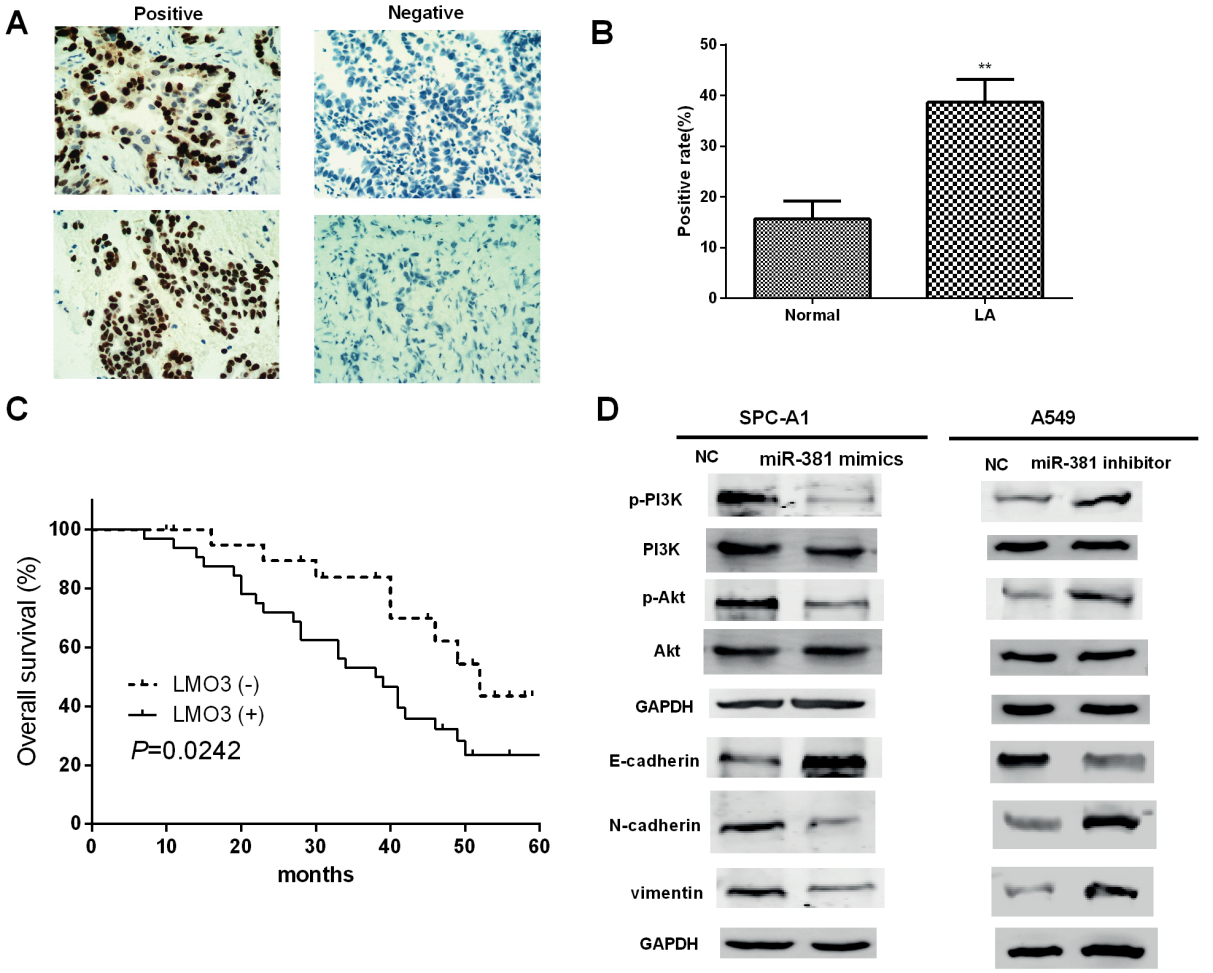


**Figure 5.** MiR-381 regulated PI3K/AKT pathway and EMT in LA cells. **A-B,** LMO3 expressions in LA tissues were measured by IHC (Magnification: 40×). **C,** Survival analysis of LA patients with different LMO3 expressions was performed by Kaplan-Meier survival analysis. **D,** The functions of miR-381 in EMT and PI3K/AKT signaling pathway were determined. $^{**}p<0.01$.

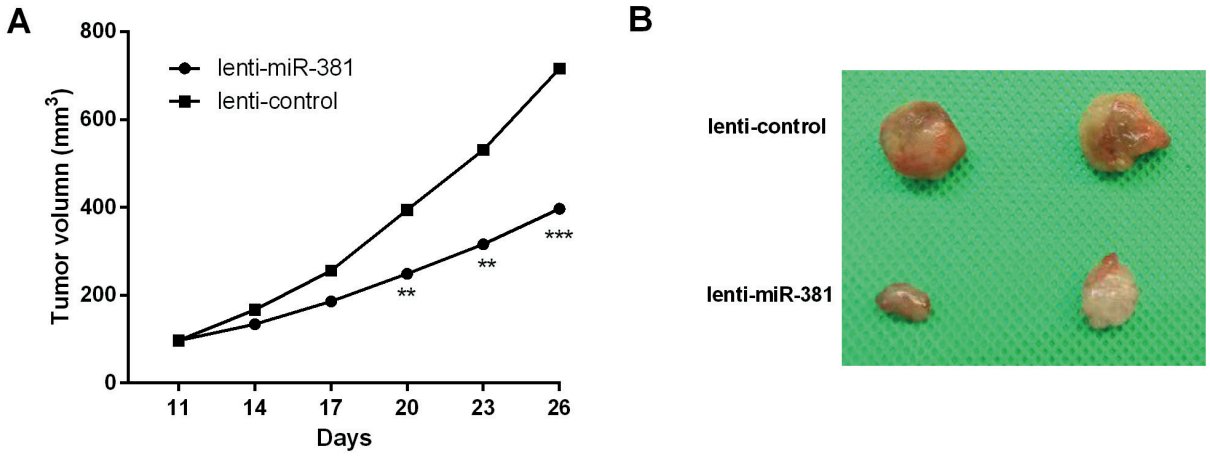


**Figure 6.** MiR-381 inhibited LA tumor growth *in vivo.* **A,** Tumor volume was calculated every 3 days after inoculation from day 11 to 26. **B,** The tumor size in the lenti-miR-381 group was significantly declined. $^{***}p<0.001$, $^{**}p<0.01$.

LMO3 has been found to be abnormally expressed in multiple tumors, such as B-cell lymphoma[36] and glioma[37]. However, the detailed biological functions of LMO3 in LA and the underlying mechanism remained to be fully elucidated. Findings of this research indicated that LMO3 was a direct target for miR-381 and the expressions of LMO3 were significantly enhanced in LA tissues, which were related to poor prognosis of LA patients.

## Conclusions

In summary that miR-381 was downregulated in LA, which was related to the worse clinicopathological characteristics and poorer prognosis of LA patients. Moreover, miR-381 overexpression could repress LA cell proliferation, invasion, and migration capacities *via* the regulation of the PI3K/Akt pathway and EMT. Furthermore, we showed that miR-381 overexpression significantly inhibited the LA tumor growth rate and tumor size *in vivo*. All data revealed that miR-381 served as a tumor suppressor in LA. Additionally, LMO3 confirmed to be a direct target of miR-381 and partially implicated in the repressive functions of miR-381 in LA progression. Accordingly, our novel findings may aid in understanding the LA progression and identifying potential therapeutic strategies for LA patients in the clinic.

### Conflicts of interest

The authors declare no conflicts of interest.

## References


1) Denisenko TV, Budkevich IN, Zhivotovsky B. Cell death-based treatment of lung adenocarcinoma. Cell Death Dis 2018; 9: 117.
2) Li D, Yang W, Zhang J, Yang JY, Guan R, Yang MQ. Transcription factor and lncRNA regulatory networks identify key elements in lung adenocarcinoma. Genes (Basel) 2018; 9: 12.
3) Imielinski M, Berger AH, Hammerman PS, Hernandez B, Pugh TJ, Hodis E, Cho J, Suh J, Capelletti M, Sivachenko A, Sougnez C, Auclair D, Lawrence MS, Stojanov P, Cibulskis K, Choi K, de Waal L, Sharifnia T, Brooks A, Greulich H, Banerji S, Zander T, Seidel D, Leenders F, Ansen S, Ludwig C, Engel-Riedel W, Stoelben E, Wolf J, Goparju C, Thompson K, Winckler W, Kwiatkowski D, Johnson BE, Janne PA, Miller VA, Pao W, Travis WD, Pass HI, Gabriel SB, Lander ES, Thomas RK, Garraway LA, Getz G, Meyerson M. Mapping the hallmarks of lung adenocarcinoma with massively parallel sequencing. Cell 2012; 150: 1107-1120.
4) He P, Yao G, Guan Y, Lin Y, He J. Diagnosis of lung adenocarcinoma in situ and minimally invasive adenocarcinoma from intraoperative frozen sections: an analysis of 136 cases. J Clin Pathol 2016; 69: 1076-1080.
5) Mao H, Han F, Xiang W, Xing Y, Sun X. [The diagnosis and management of focal ground glass opacity of lung adenocarcinoma]. Zhonghua Jie He He Hu Xi Za Zhi 2015; 38: 535-537.
6) Zhang L, Zhou L, Shi M, Kuang Y, Fang L. Downregulation of miRNA-15a and miRNA-16 promote tumor proliferation in multiple myeloma by increasing CABIN1 expression. Oncol Lett 2018; 15: 1287-1296.
7) Liu M, Zeng X, Lu YX, Mo YJ, Liao TH, Gan C, Lu XQ. Study on molecular mechanism of MiRNA-29a in promoting proliferation and invasion of non-small-cell lung cancer by inhibiting MTSS1. Eur Rev Med Pharmacol Sci 2018; 22: 5531-5538.
8) Sheng N, Tan G, You W, Chen H, Gong J, Chen D, Zhang H, Wang Z. MiR-145 inhibits human colorectal cancer cell migration and invasion via PAK4-dependent pathway. Cancer Med 2017; 6: 1331-1340.
9) Li X, Li Y, Wan L, Chen R, Chen F. miR-509-5p inhibits cellular proliferation and migration via targeting MDM2 in pancreatic cancer cells. Onco Targets Ther 2017; 10: 4455-4464.
10) Sun B, Gu X, Chen Z, Xiang J. MiR-610 inhibits cell proliferation and invasion in colorectal cancer by repressing hepatoma-derived growth factor. Am J Cancer Res 2015; 5: 3635-3644.
11) Lin Y, Lv Y, Liang R, Yuan C, Zhang J, He D, Zheng X, Zhang J. Four-miRNA signature as a prognostic tool for lung adenocarcinoma. Onco Targets Ther 2018; 11: 29-36.
12) Hou T, Zhou L, Wang L, Kazobinka G, Zhang X, Chen Z. CLCA4 inhibits bladder cancer cell proliferation, migration, and invasion by suppressing the PI3K/AKT pathway. Oncotarget 2017; 8: 93001-93013.
13) Zhang X, He X, Liu Y, Zhang H, Chen H, Guo S, Liang Y. MiR-101-3p inhibits the growth and metastasis of non-small cell lung cancer through blocking PI3K/AKT signal pathway by targeting MALAT-1. Biomed Pharmacother 2017; 93: 1065-1073.
14) Xu X, Liu X, Zhang Y. Osthole inhibits gastric cancer cell proliferation through regulation of PI3K/AKT. PLoS One 2018; 13: e193449.
15) Liu Y, Yang H, Chen T, Luo Y, Xu Z, Li Y, Yang J. Silencing of receptor tyrosine kinase ROR1 inhibits tumor-cell proliferation via PI3K/AKT/mTOR signaling pathway in lung adenocarcinoma. PLoS One 2015; 10: e127092.
16) Vu T, Datta PK. Regulation of EMT in colorectal cancer: a culprit in metastasis. Cancers (Basel) 2017; 9: 171.
17) Wang C, Li A, Yang S, Qiao R, Zhu X, Zhang J. CXCL5 promotes mitomycin C resistance in non-muscle invasive bladder cancer by activating

EMT and NF-kappaB pathway. Biochem Biophys Res Commun 2018; 498: 862-868.

18) Wang J, Zhang Z, Li R, Mao F, Sun W, Chen J, Zhang H, Bartsch JW, Shu K, Lei T. ADAM12 induces EMT and promotes cell migration, invasion and proliferation in pituitary adenomas via EGFR/ERK signaling pathway. Biomed Pharmacother 2018; 97: 1066-1077.

19) Wang R, Song Y, Liu X, Wang Q, Wang Y, Li L, Kang C, Zhang Q. UBE2C induces EMT through Wnt/betacatenin and PI3K/Akt signaling pathways by regulating phosphorylation levels of Aurora-A. Int J Oncol 2017; 50: 1116-1126.

20) Gurzu S, Turdean S, Kovecsi A, Contac AO, Jung I. Epithelial-mesenchymal, mesenchymal-epithelial, and endothelial-mesenchymal transitions in malignant tumors: an update. World J Clin Cases 2015; 3: 393-404.

21) Ogunbolude Y, Dai C, Bagu ET, Goel RK, Miah S, MacAusland-Berg J, Ng CY, Chibbar R, Napper S, Raptis L, Vizeacoumar F, Vizeacoumar F, Bonham K, Lukong KE. FRK inhibits breast cancer cell migration and invasion by suppressing epithelial-mesenchymal transition. Oncotarget 2017; 8: 113034-113065.

22) Dai W, Wang F, He L, Lin C, Wu S, Chen P, Zhang Y, Shen M, Wu D, Wang C, Lu J, Zhou Y, Xu X, Xu L, Guo C. Genistein inhibits hepatocellular carcinoma cell migration by reversing the epithelial-mesenchymal transition: partial mediation by the transcription factor NFAT1. Mol Carcinog 2015; 54: 301-311.

23) Zhang W, Gu J, Chen J, Zhang P, Ji R, Qian H, Xu W, Zhang X. Interaction with neutrophils promotes gastric cancer cell migration and invasion by inducing epithelial-mesenchymal transition. Oncol Rep 2017; 38: 2959-2966.

24) Isogai E, Okumura K, Saito M, Yoshizawa Y, Itoh K, Tando S, Ohira M, Haraguchi S, Nakagawara A, Fushiki S, Nagase H, Wakabayashi Y. Oncogenic Lmo3 cooperates with Hen2 to induce hydrocephalus in mice. Exp Anim 2015; 64: 407-414.

25) Song YF, Hong JF, Liu DL, Lin QA, Lan XP, Lai GX. miR-630 targets LMO3 to regulate cell growth and metastasis in lung cancer. Am J Transl Res 2015; 7: 1271-1279.

26) Qiu YS, Jiang NN, Zhou Y, Yu KY, Gong HY, Liao GJ. LMO3 promotes gastric cancer cell invasion and proliferation through Akt-mTOR and Akt-GSK3beta signaling. Int J Mol Med 2018; 41: 2755-2763.

27) Aoyama M, Ozaki T, Inuzuka H, Tomotsune D, Hirato J, Okamoto Y, Tokita H, Ohira M, Nakagawara A. LMO3 interacts with neuronal transcription factor, HEN2, and acts as an oncogene in neuroblastoma. Cancer Res 2005; 65: 4587-4597.

28) Isogai E, Ohira M, Ozaki T, Oba S, Nakamura Y, Nakagawara A. Oncogenic LMO3 collaborates with HEN2 to enhance neuroblastoma cell growth through transactivation of Mash1. PLoS One 2011; 6: e19297.

29) Rivera MP. Lung cancer in women: differences in epidemiology, biology, histology, and treatment outcomes. Semin Respir Crit Care Med 2013; 34: 792-801.

30) Cho WC, Chow AS, Au JS. Restoration of tumour suppressor hsa-miR-145 inhibits cancer cell growth in lung adenocarcinoma patients with epidermal growth factor receptor mutation. Eur J Cancer 2009; 45: 2197-2206.

31) Bai Y, Lu C, Zhang G, Hou Y, Guo Y, Zhou H, Ma X, Zhao G. Overexpression of miR-519d in lung adenocarcinoma inhibits cell proliferation and invasion via the association of eIF4H. Tumour Biol 2017; 39: 1393395098.

32) Bian T, Jiang D, Liu J, Yuan X, Feng J, Li Q, Zhang Q, Li X, Liu Y, Zhang J. miR-1236-3p suppresses the migration and invasion by targeting KLF8 in lung adenocarcinoma A549 cells. Biochem Biophys Res Commun 2017; 492: 461-467.

33) Zhang M, Huang S, Long D. MiR-381 inhibits migration and invasion in human gastric carcinoma through downregulatedting SOX4. Oncol Lett 2017; 14: 3760-3766.

34) Xue Y, Xu W, Zhao W, Wang W, Zhang D, Wu P. miR-381 inhibited breast cancer cells proliferation, epithelial-to-mesenchymal transition and metastasis by targeting CXCR4. Biomed Pharmacother 2017; 86: 426-433.

35) He X, Wei Y, Wang Y, Liu L, Wang W, Li N. MiR-381 functions as a tumor suppressor in colorectal cancer by targeting Twist1. Onco Targets Ther 2016; 9: 1231-1239.

36) Kobayashi K, Yamaguchi M, Miyazaki K, Imai H, Yokoe K, Ono R, Nosaka T, Katayama N. Expressions of SH3BP5, LMO3, and SNAP25 in diffuse large B-cell lymphoma cells and their association with clinical features. Cancer Med 2016; 5: 1802-1809.

37) Liu X, Lei Q, Yu Z, Xu G, Tang H, Wang W, Wang Z, Li G, Wu M. MiR-101 reverses the hypomethylation of the LMO3 promoter in glioma cells. Oncotarget 2015; 6: 7930-7943.