

## Original Article

# MEG3 regulates the invasion and migration of lung cancer via targeting miR-219a and through MAPK pathway

Chengyan Jin, Jindong Li, Donglei Shi, Peiyan Hua, Guangxin Zhang, Bin Wang

*Department of Thoracic Surgery, The Second Hospital of Jilin University, Changchun 130041, China*

Received March 26, 2017; Accepted May 24, 2017; Epub June 1, 2017; Published June 15, 2017

**Abstract:** Objective: To investigate the expression of MEG3 in lung cancer and the role of MEG3 in the invasion and migration of lung cancer cells and its mechanism. Methods: The expression of MEG3 in lung cancer and adjacent lung cancer cells was detected by qPCR. Transwell invasion assay was used to detect the invasion ability of lung cancer cells after silencing MEG3. The migration ability of lung cancer cells after silencing MEG3 was detected by scratch test. The expression of miR-219a was detected by flow cytometry. The expression of miR-219a in lung cancer and adjacent tissues and different lung cancer cells was detected by qPCR. Transwell invasion assay was used to detect the effect of miR-219a on the invasion of lung cancer cells after silencing MEG3. The effect of miR-219a on the tumor size and volume of lung cancer was detected by subcutaneous tumorigenesis of MEG3 in nude mice. The effect of miR-219a on the tumor size and volume of lung cancer cells was detected by scintigraphy. Western blotting was used to detect the expression of Notch pathway protein after silencing MEG3. The morphological changes of microcapsule microtubules after silencing MEG3 were detected by phalloidin staining. Results: The expression of MEG3 was significantly increased in lung cancer tissues compared with adjacent tissues; The expression of MEG3 was the highest in lung cancer cell A549; MEG3 could specifically bind to 3'UTR of miR-219a; The expression of miR-219a was significantly decreased in lung cancer tissues compared with adjacent tissues; Inhibition of miR-219a could promote the invasion and migration of lung cancer cells after silencing MEG3; The tumor volume and weight of MEG3-siRNA + miR-219a-inhibitor group were significantly increased compared with MEG3-siRNA group. The expression of Notch pathway protein was down-regulated after silencing MEG3. The number of microfilament microtubules decreased and the number of pseudopods decreased after silencing MEG3. Conclusion: MEG3 plays an important role in the development of lung cancer. MEG3 can regulate the invasion and migration ability of miR-219a through Notch signaling pathway.

**Keywords:** MEG3, lung cancer, MiR-219a, transwell

## Introduction

In recent years, the incidence of lung cancer and mortality rate shows a sharp upward trend, lung cancer ranks first in the respiratory malignancy, the mortality rate accounts for the third rate of malignant tumor mortality [1]. Early diagnosis is difficult due to the lack of specificity in the early stage and diagnosis is more than in the late, 5-year survival rate is low [2]. Therefore, finding effective early diagnosis, targeted therapy and prognostic markers is a hot topic for current research. The new molecular targeting therapy strategy of lung cancer has been gradually promoted to the direction of diagnosis and treatment of lung cancer with the deepening of molecular biology research.

MEG3 maternal expression gene 3 (MEG3) is a functional lncRNA with a tumor suppressor [3]. It has been reported that MEG3 expression in a variety of malignant tumors, such as breast cancer, liver cancer and prostate cancer, its expression level is relatively reduced or even missing [4-6]. It was found that the demethylation of MEG3 promoter and the change of gene region are the main reasons for the abnormal expression of MEG3 in tumors [7]. Overexpression of MEG3 gene can induce cell growth cycle arrest and increase cell apoptosis in human breast cancer cell lines [8]. However, the level of MEG3 expression in lung cancer and lung cancer progression has not yet been reported.

# The role of meG3 in invasion and migration of lung cancer cells and its mechanism

MicroRNA (miRNAs) is an endogenous single-stranded small molecule RNA that regulates protein expression by inhibiting or degrading messenger RNA [9]. MiRNAs plays a unique role in the diagnosis, treatment and prognosis of tumors [10]. MiR-219a is relatively less studied in the field of miRNA, it's reported that the expression of specific in brain tissue, but also in the brain glioma has a certain role in the inhibition of cancer [11]. Recent investigations have shown that miR-219a expression in lung cancer, breast cancer and other malignant tumors, may be involved in the process of tumor invasion and migration [12]. Mitogen activated protein kinase (MAPK) signal transduction pathway is also known as extracellular signal-regulated kinase (ERK) cascade pathway [13]. The structure of MAPK family members is highly conservative, and plays an important role in cell proliferation and differentiation, and plays a different role in the occurrence and development of different tumors. This pathway is a signal transduction pathway will stimulate signal transmitted to the nucleus to the cell surface and internal mediated cell response in most a major pathway, such as growth, differentiation, division and apoptosis are regulated by MAPK signal transduction pathway [14]. This investigation intends to analyze the expression of MEG3 in lung cancer, and further investigate the interaction between MEG3 and miR-219a, and investigate the role and mechanism of MAPK pathway in the invasion and metastasis of lung cancer.

## Materials and methods

### *Samples collection*

50 cases of lung cancer patients and 50 cases of adjacent tissues were collected admitted to our hospital from March 2015 to May 2016. All patients had no chemotherapy or radiotherapy before operation, and they were postoperative pathological staging by two sub-high above the pathologist jointly read the tumor tissue in vitro then put into RNA preservation solution quickly.

### *Cell lines*

Human cells PGCI3, H1299, 95D, A549 were purchased from Shanghai Zhongshan cell bank and they were cultured and passaged in culture medium containing 10% fetal bovine serum in 5% CO<sub>2</sub> incubator at 37°C. Fetal bovine serum, RPMI 1640 medium were purchased from

Hyclone Corporation (Hyclone, Logan, UT). Transwell Chamber was purchased from Millipore (Millipore, Billerica, MA), Matrigel was purchased from Bio-Rad (Bio-Rad, Madrid, Spain). Lipofectamine 2000 and miR-219a-inhibitor were purchased from (Gnenpharma Co., Shanghai, China). Trierol was purchased from Ambion (Ambion Inc., Austin, TX, USA), reverse transcription kit (FSQ-101) was purchased from Japan TOYOBO Corporation (TOYOBO, FSQ-101, Japan), PCR kit was purchased from Sigma (KapaBiosystems Inc., Boston, US). The luciferase activity assay kit was purchased from Promega Corporation (Promega Biotech Co., Beijing, China). The luciferase reporter vector was synthesized by Promega Corporation (Promega Biotech Co., Beijing, China).

### *Quantitative real-time polymerase chain reaction*

Total RNA from tumor tissues and cell lines were extracted using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Total RNA was eluted with RNase-free water and stored at -80°C. qRT-PCR was performed by using SYBR-green PCR Master Mix in a Fast Real-time PCR 7500 System (Applied Biosystems). The RT-PCR primers were purchased from GeneCopoeia (SanDiego, California, USA). GAPDH was used as the internal control of the mRNA or miRNA, respectively. Fold change of MEG3 or miR-219 was calculated by the equation  $2^{-\Delta\Delta Ct}$ .

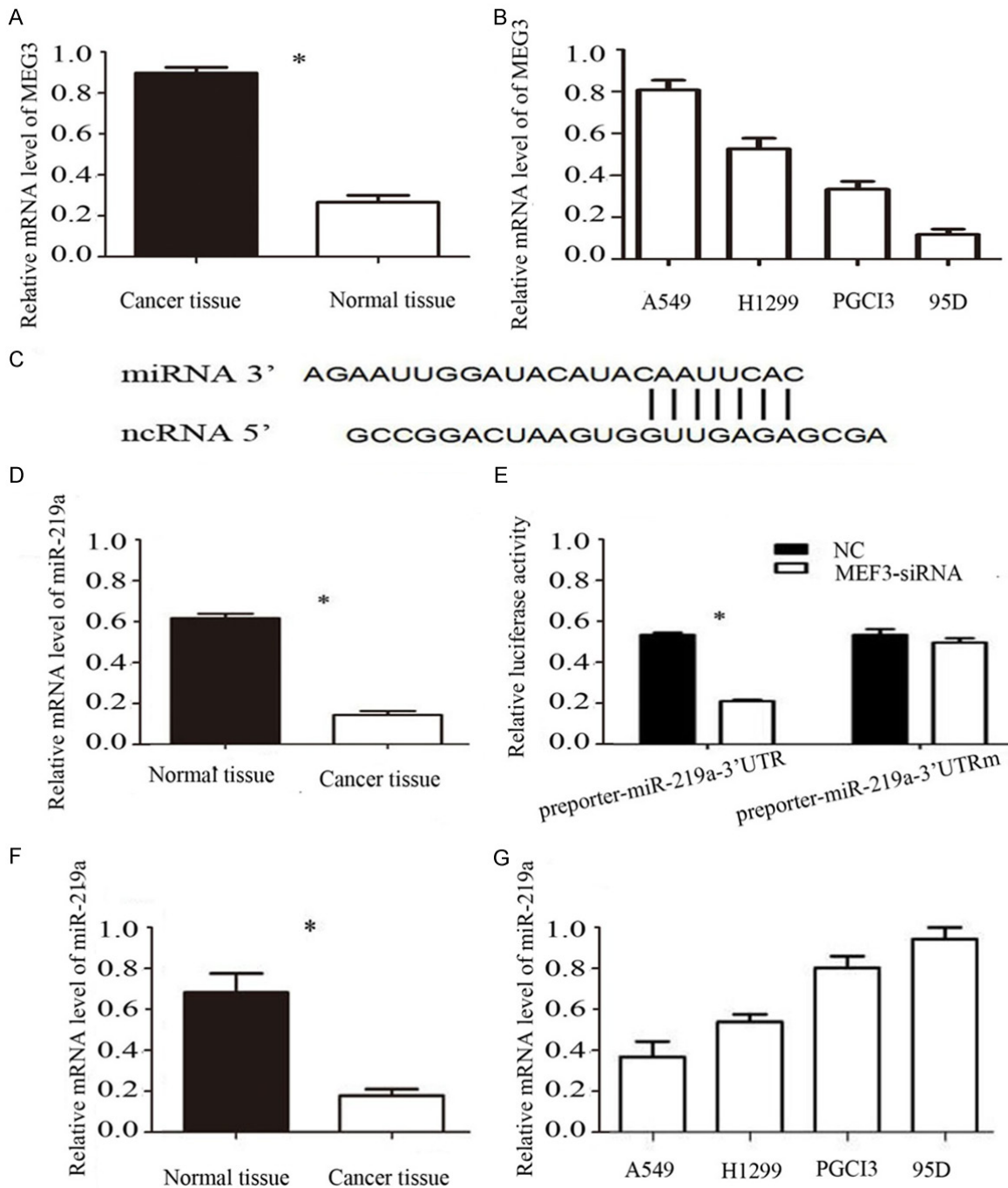
### *Cell transfection*

Mimics/inhibitors specific for miR-219 and short hairpin RNA (shRNA)/scramble fragments targeting MEG3 were designed and purchased from Invitrogen (USA). Cells were seeded in 24-well plates at  $1 \times 10^5$  cells per well. Mimics/inhibitors or shRNA/scramble fragments was transfected into cells with lipofectamine 3000 (Invitrogen) following the manufacturer's instructions for 24 h, respectively. After transfection, the cells were allowed to recover by incubating them for 4 h at 37°C. The experiment was replicated thrice for data calculations.

### *Transwell invasion assays*

The two transwell invasion chambers with Matrigel (1 mg/ml) (Becton-Dickinson, New Jersey, USA) were used in assay invasion assays of cells in vitro. Firstly, 200  $\mu$ l serum-free medium containing  $1 \times 10^5$  cells/well was added into the

The role of meg3 in invasion and migration of lung cancer cells and its mechanism



**Figure 1.** A. The mRNA expression of MEG3 were detected by qPCR in lung cancer tissue and normal tissue. B. The mRNA expression of MEG3 were detected by qPCR in different lung cancer cells. C, D. MEG3 binds to miR-219a. E. Luciferase report gene detects miR-219a the direct target of MEG3-siRNA. F. The mRNA expression of miR-219a were detected by qPCR in pancreatic lung cancer tissue and normal tissue. G. The mRNA expression of miR-219a were detected by qPCR in different pancreatic cancer cells. Error bars represent standard error. \*P < 0.05.

upper chamber, and the lower chamber contained 0.6 ml medium containing 20% FBS. After incubation at 37°C for 24 h, non-invading cells on the upper membranes were removed with a cotton swab. The migrated or invaded cells were fixed in

95% ethanol, stained with hematoxylin. The cell numbers were counted by ImageJ software and photographed under an inverted microscope on 10 random fields in each well. Each experiment was independently repeated in triplicate.

# The role of meg3 in invasion and migration of lung cancer cells and its mechanism

## Wound-healing assays

Wound-healing assay was performed to evaluate the migration rate of cells transfected with MEG3-siRNA/MEG3 scramble/miR-219 inhibitor. To accomplish this,  $1.5 \times 10^6$  cells/well were seeded in 6-well plates and cultured overnight until the cells reached 90% confluence. A straight scratch was created by a sterile pipette tip. The destroyed cells were rinsed off with PBS 3 times gently and cultured in medium for another 24 h. Cell migration was observed and imaged at 0 h, 24, 48, 72 h with a digital camera (Leica DFC300FX).

## Luciferase activity assays

The luciferase reporter vector was co-transfected with A549 cells with MEG3-siRNA. The transfected pRL-TK was used as standard internal control. The cells were harvested after transfection for 36 h. The luciferase activity of A549 cells was detected by Promega's luciferase activity assay kit. Calculate relative luciferase activity = firefly luciferase activity value/bloody luciferase activity value.

## Lung cancer xenografts

Nude mice are selected from 4 to 6 weeks old. The lung cancer MEG3-siRNA cells and MEG3-siRNA + miR-219a-inhibitor cells were cultured in logarithmic phase. Adjusted to cell concentration of  $2 \times 10^8$ /ml. Take 0.1 ml cell suspension injection in each nude mice left forelimb axillary subcutaneous, a total of 10. Within 4 weeks after injection, the survival, weight, and survival status of the mice were monitored, and the size and weight of the tumor in the immediately-dead mice were measured.

## Statistical analysis

The results are presented as the mean  $\pm$  the standard error of the mean of 3 replicates. Differences between means were analyzed using Student's t test. The difference was considered statistically significant at  $P < 0.05$ .

## Results

### Expression of MEG3 mRNA in lung cancer tissues, adjacent tissues and lung cancer cells

The results (**Figure 1A**) of qPCR showed that the expression of MEG3 mRNA in lung cancer tissues was significantly higher than that in

adjacent tissues [(0.86 $\pm$ 0.05) vs (0.22 $\pm$ 0.03),  $P < 0.05$ ], the difference was statistically significant; The expression level of MEG3 in A549 cells was the highest in different lung cancer cells (**Figure 1B**). We selected A549 for further experimental cell lines combined with the above results and consider MEG3 in lung cancer play a role in cancer.

### Luciferase reporter gene detection of MEG3 and miR-219a relationship

We use the bioinformatics predictor to make clear that MEG3 may interact directly with miR-219a to clarify the case of miRNAs associated with MEG3. MEG3-siRNA and miR-219a were co-transfected into lung cancer cell A549 to confirm whether MEG3 binds to miR-219a 3'UTR. The luciferase reporter gene results showed that MEG3-siRNA significantly inhibited luciferase activity in miR-219a (**Figure 1C-E**). The results show that MEG3-siRNA can specifically bind to the 3'UTR of miR-219a.

### Expression of miR-219a mRNA in lung cancer tissue, adjacent tissues and lung cancer cells

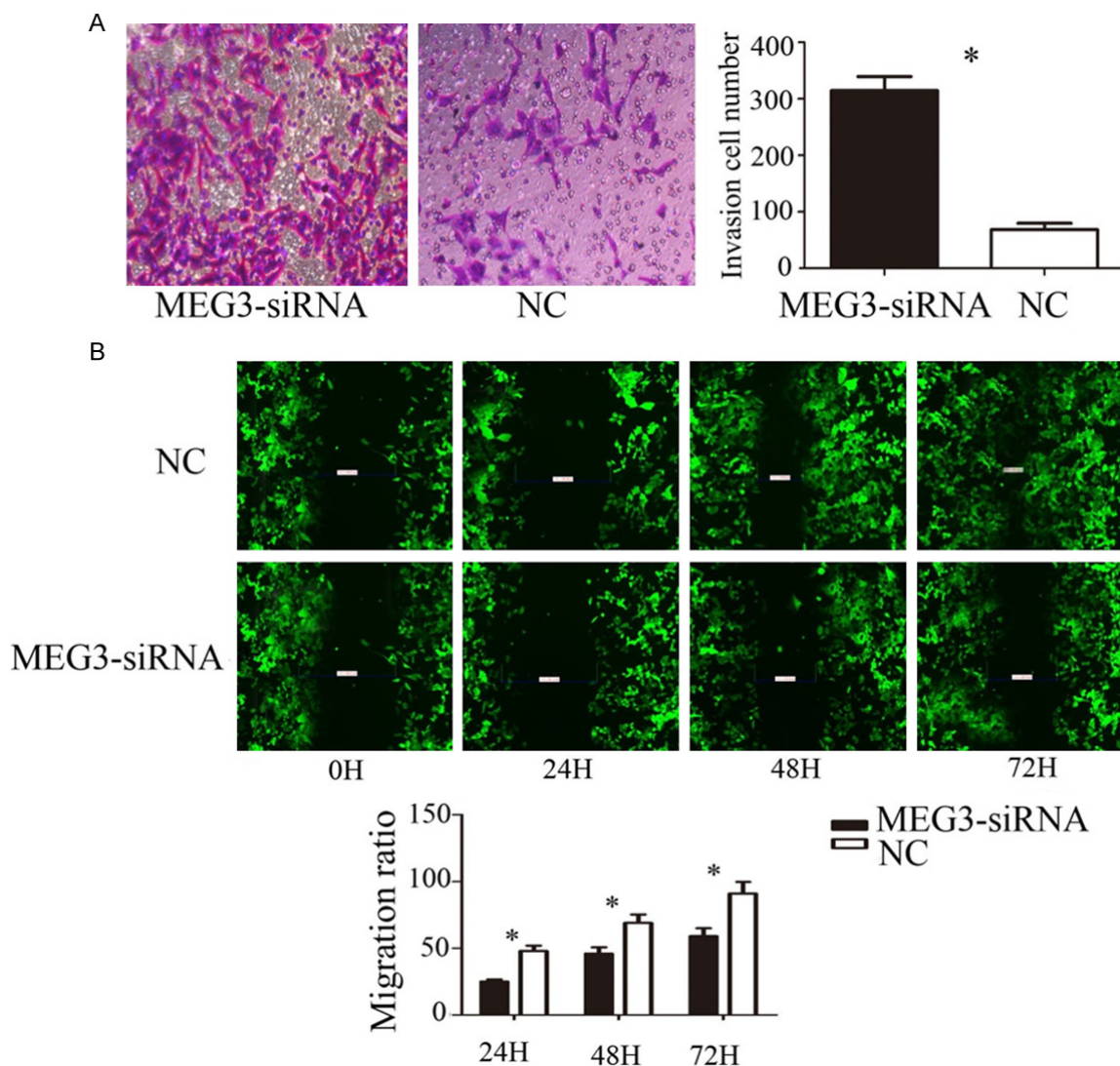
The results of qPCR showed that the expression of miR-219a mRNA in lung cancer tissues was significantly lower than that in adjacent tissues [(0.78 $\pm$ 0.03) vs (0.29 $\pm$ 0.01), ( $P < 0.05$ )] (**Figure 1F**), the difference was statistically significant; The expression level of MEG3 was the lowest in A549 cells in different lung cancer cells (**Figure 1G**).

### Effect of MEG3-siRNA on invasion and migration of human lung cancer cell line A549

The ability of cells to penetrate through Matrigel can reflect the ability of cells to invade. The results of Transwell showed that the number of cells in Matrigel matrix was (179.2 $\pm$ 7.4) (**Figure 2A**), which was significantly higher than that of MEG3-siRNA group (44.8 $\pm$ 2.9), the difference was statistically significant ( $P < 0.01$ ). It indicated that MEG3-siRNA can inhibit the invasion of human lung cancer cell A549. The results of scratches showed that the mobility of MEG3-siRNA group was significantly decreased at 24 h, 48 h and 72 h compared with NC group [24 h (34.2 $\pm$ 2.3)% vs (18.6 $\pm$ 1.3)%,  $P < 0.05$  and 48 h (62.8 $\pm$ 4.8)% vs (39.9 $\pm$ 3.1)%,  $P < 0.05$ ; 72 h (86.4 $\pm$ 7.8)% vs (52.1 $\pm$ 5.0)%,  $P < 0.01$ ] (**Figure 2B**), the difference was statistically significant. It indicated that MEG3-siRNA can inhibit the



## The role of meg3 in invasion and migration of lung cancer cells and its mechanism



**Figure 2.** A. Effect of MEG3-siRNA on the invasion ability of A549 cells were detected by Transwell matrigel invasion assays. B. Effect of MEG3-siRNA on the A549 cells migration ability were detected by wound healing assays. Error bars represent standard error. \* $P < 0.05$ .

migration ability of human lung cancer cell A549.

### *Effects of miR-219a on the invasion and migration ability of human lung cancer cell line A549 after silencing MEG3*

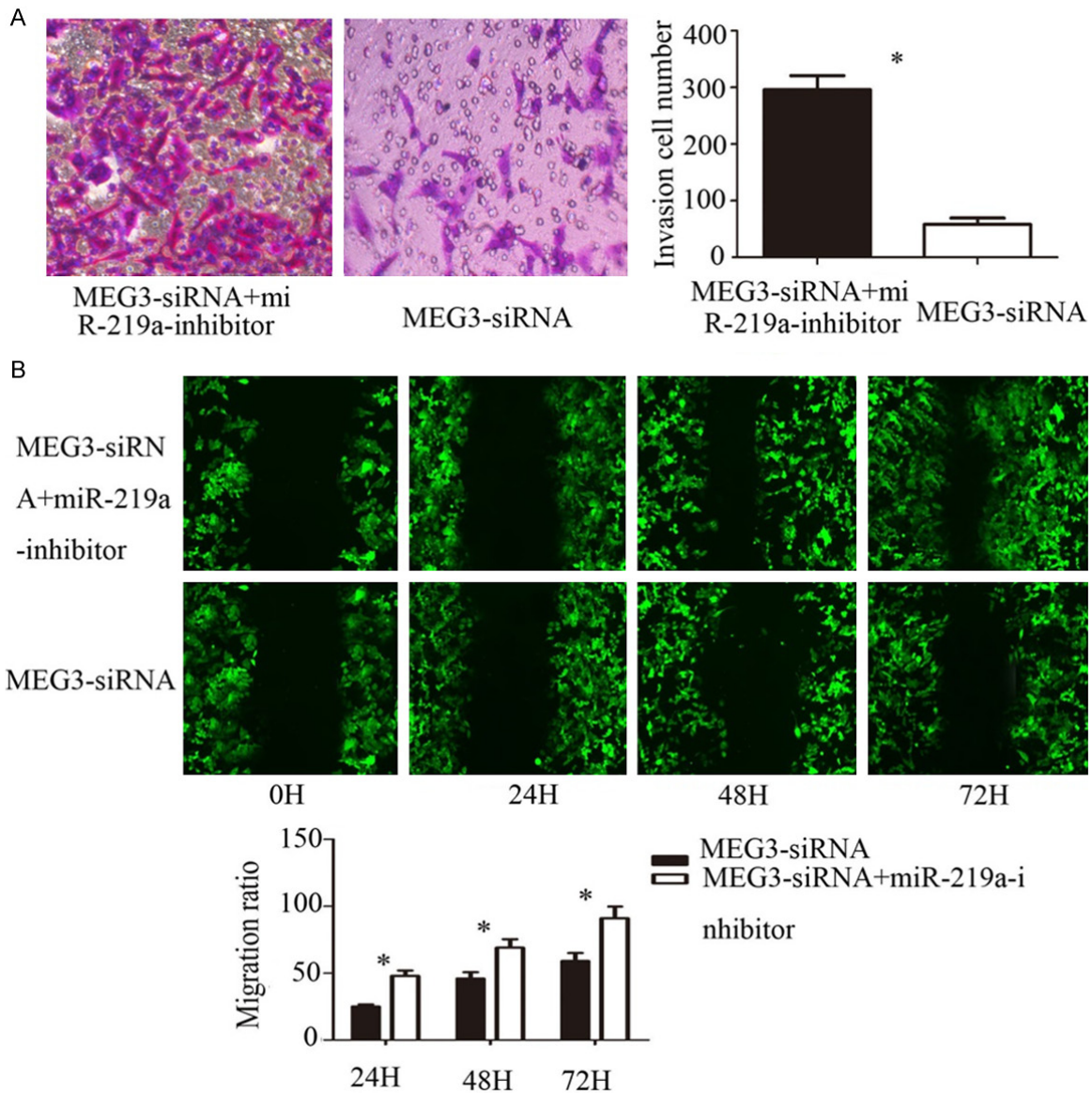
The results of Transwell showed that the number of cells in Matrigel matrix was  $(63.4 \pm 5.3)$ , which was significantly less than that of MEG3-siRNA + miR-219a-inhibitor group  $(187.2 \pm 6.9)$  (Figure 3A), the difference was statistically significant ( $P < 0.01$ ). The results indicated that inhibition of miR-219a could promote the invasion of human lung cancer cell line A549 after silencing MEG3. The results of scratches showed that the mobility of MEG3-siRNA + miR-

219a-inhibitor group was significantly higher than that of MEG3-siRNA group at 24 h, 48 h and 72 h [24 h  $(29.2 \pm 2.4)\%$  vs  $(12.1 \pm 1.3)\%$ ,  $P < 0.05$ ; 48 h  $(62.4 \pm 4.9)\%$  vs  $(38.2 \pm 2.8)\%$ ,  $P < 0.05$ ; 72 h  $(89.5 \pm 9.1)\%$  vs  $(53.4 \pm 3.8)\%$ ,  $P < 0.01$ ] (Figure 3B), the difference was statistically significant. It indicated that inhibition of miR-219a could promote the migration of human lung cancer cell line A549 after silencing MEG3.

### *Effect of miR-219a on tumor growth after silencing MEG3 showed by the experiment of subcutaneous tumor formation in nude mice*

The survival time of tumor-bearing mice was 4-9 weeks, the median survival time was 6.5

The role of meg3 in invasion and migration of lung cancer cells and its mechanism



**Figure 3.** A. Effect of miR-219a on the invasion ability of A549 cells were detected after silencing MEG3 by Transwell matrigel invasion assays. B. Effect of miR-219a on the A549 cells migration ability were detected after silencing MEG3 by wound healing assays. Error bars represent standard error. \*P < 0.05.

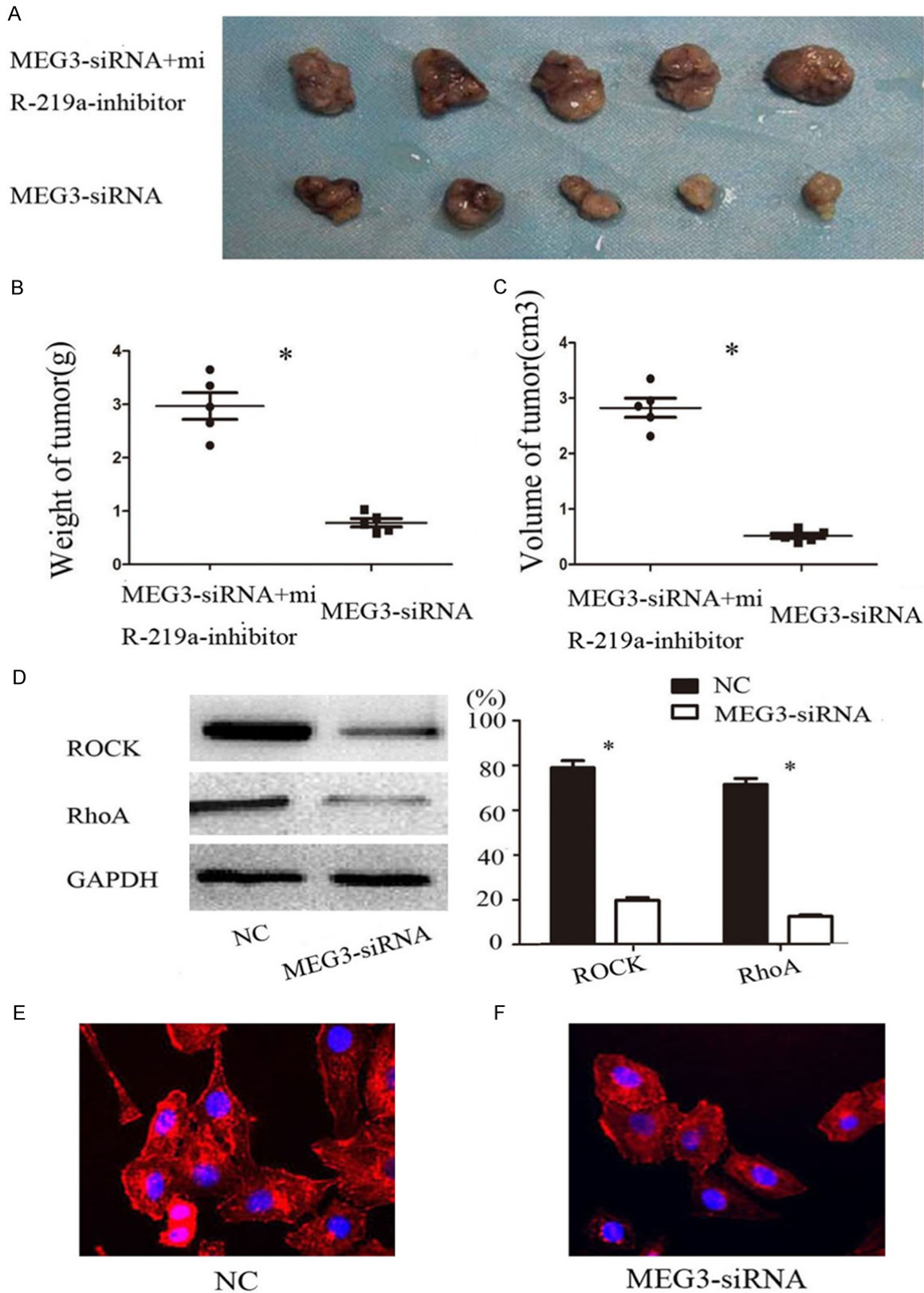
weeks. The autopsy showed that all the tumors in the left armpit were growing, and the tumors were gray white, solid, round or oval, with nodular protrusions on the surface and fish in the profile. The tumor formation rate was 100%. The tumor growth in nude mice (**Figure 4A**): the tumor size of MEG3-siRNA group was significantly higher than that of MEG3-siRNA group compared with MEG3-siRNA group. The tumor weight and volume of contrast (**Figure 4B, 4C**): MEG3-siRNA miR-219a-inhibitor group the tumor volume and weight were significantly higher compared with MEG3-siRNA group [vol-

ume (2.99 + 0.35) cm<sup>3</sup> vs (0.29 + 0.11) cm<sup>3</sup>, P < 0.05; weight (3.12 + 0.34) g vs (0.36 + 0.07) g, P < 0.05].

*Changes in cytoskeleton morphology and ROCK family protein levels*

The changes of ROCK family protein and the changes of cytoskeleton morphology after silencing MEG3 were detected by phalloidin staining. The results (**Figure 4D**) showed that the expression levels of ROCK and RhoA were decreased after the MEG3 silencing

The role of meg3 in invasion and migration of lung cancer cells and its mechanism



**Figure 4.** Effect of miR-219a on tumorigenesis after silencing MEG3 in vivo. A. Comparison of the tumor size of nude mice. B, C. Compare the volume and weight of the tumor in nude mice. D. Western blotting was used to detect the changes of ROCK and RhoA protein levels after silencing MEG3. E, F. phalloidin staining to detect changes in cytoskeleton morphology. Error bars represent standard error. \*P < 0.05.



## The role of meg3 in invasion and migration of lung cancer cells and its mechanism

[(81.26%±5.23%) vs (19.62±1.62),  $P < 0.05$ ; (78.28±8.15) vs (11.31±2.61) g,  $P < 0.05$ ]. It was detected by Phalloidin staining (**Figure 4E, 4F**) that microfilament microtubule cytoskeleton in MEG3 silencing pseudopodia increased intracellular reduction, burr like structure, which can influence the movement and migration of cells.

### Discussion

Lung cancer is the most common malignant tumor of the respiratory system, its rapid development of the disease, recurrence rate and mortality is high, the prognosis is very poor, which has become a serious threat to human health and life in China and even malignant disease [2]. So the prognosis of lung cancer patients and early intervention is very important. DNA abnormal methylation can lead to microRNA and other tumor suppressor gene inactivation and oncogene activation in the occurrence and development of tumor [15]. Therefore, miR-219a may be associated with lung cancer progression and prognosis.

It was reported that only about 2% of the genome sequence was translated into protein, most of which were transcribed as non-coding RNA with the deepening of research and the understanding of genomics deepened [16]. Non-coding RNA is divided into lncRNA and small non-coding RNA according to the sequence of the transcribed gene. lncRNA is a class of RNA molecules of about 200 nucleotides in length, and there is no relatively complete open reading frame that was once considered to have no biological function. However, recent researches have shown that lncRNA is closely related to a variety of diseases, such as some malignant tumors, cardiovascular disease, neurodegenerative diseases, autoimmune diseases [17].

MEG3 is located on chromosome 14q32.3, about 1.6 kb, and is initially found to have a class of lncRNA with anti-cancer effect. Researches have shown that MEG3 expression in the liver, brain, lung and ovary and other normal tissues is relatively high, but in a variety of malignant tumor cells show the phenomenon of down-regulation or deletion [18, 19]. The current investigation confirmed that MEG3 its anti-tumor effect and p53 pathway are partially related. Kobayashi et al. [20] found that MEG3

expression decreased in liver cancer and cell lines. By increasing the expression of MEG3 and found that p53 gene activity increased, suggesting that MEG3 P53 pathway may inhibit the proliferation and invasion of liver cancer cells, and promote its apoptosis. It has been reported that MEG3 can play a role in tumor suppressing by a variety of ways, and its mechanism is related to DNA methylation, P53 pathway, Rb pathway and angiogenesis [21]. In this investigation, the expression of lncRNA MEG3 in lung cancer tissue was significantly down-regulated compared with normal lung tissue. Follow-up of MEG3 inhibition in lung cancer cell lines was performed in order to verify the role of MEG3 expression in the development of lung cancer. These findings suggest that lncRNA MEG3 lung cancer plays a role in the development of tumor suppressor genes, and its deletion or downregulation is involved in the development and progression of lung cancer. Therefore, MEG3 plays an important role in lung cancer and can serve as a target for future lung cancer treatment. Mature miR-219a is generally expressed in tissues such as gastric cancer, lung cancer and breast cancer. Torres et al. [22] demonstrated that miR-219a expression in gastric cancer was down-regulated and was closely related to the up-regulation of telomerase reverse transcriptase by detecting the expression of miR-219a in gastric cancer. In this investigation, the expression of miR-219a in lung cancer tissues and normal lung tissues was confirmed by qPCR. The expression of miR-219a in lung cancer was significantly down-regulated, indicating that miR-219a had an anti-cancer effect in lung cancer. The effect of miR-219a on the invasion and migration of lung cancer cells was further studied by silencing MEG3 and inhibiting the expression of miR-219a in combination with the previous experimental results. The results showed that inhibition of miR-219a expression after silencing MEG3 could promote the invasion and migration of lung cancer cells. In vivo experiments using subcutaneous tumor formation in nude mice also have similar results. Similar results were observed in vivo experiments using nude mice subcutaneously.

In this investigation, we used qPCR to detect the expression of MEG3 and miR-138 in lung cancer and adjacent tissues, and to further investigate the interaction between MEG3 and



# The role of meg3 in invasion and migration of lung cancer cells and its mechanism

miR-138. The role of MEG3 and miR-138 in the invasion and migration of lung cancer cells was further investigated. The results showed that MEG3 was up-regulated in lung cancer, miR-138 was down regulated in lung cancer, HOTAIR and miR-138 had direct interaction. MEG3 can target the migration and invasion of lung cancer by miR-138, which indicated that MEG3 and miR-138 may be involved in lung cancer cell invasion and migration process, there may be a prognostic marker for predicting lung cancer progression, and monitoring the effect of treatment.

## Acknowledgements

The Natural Science Foundation of China (#81272472).

## Disclosure of conflict of interest

None.

**Address correspondence to:** Bin Wang, Department of Thoracic Surgery, The Second Hospital of Jilin University, 218 Ziqiang Street, Changchun 130041, China. Tel: 13353111009; E-mail: wangbin@163.com

## References

- [1] Zatloukal P, Petruzelka L, Zemanova M, Havel L, Janku F, Judas L, Kubik A, Krepela E, Fiala P, Pecan L. Concurrent versus sequential chemoradiotherapy with cisplatin and vinorelbine in locally advanced non-small cell lung cancer: a randomized study. *Lung Cancer* 2004; 46: 87-98.
- [2] Yasufuku K, Chiyo M, Koh E, Moriya Y, Lyoda A, Sekine Y, Shibuya K, Lizasa T, Fujisawa T. Endobronchial ultrasound guided transbronchial needle aspiration for staging of lung cancer. *Lung Cancer* 2005; 50: 347-354.
- [3] Zhou Y, Zhang X, Klibanski A. MEG3 noncoding RNA: a tumor suppressor. *J Mol Endocrinol* 2012; 48: R45-R53.
- [4] Xiu YL, Sun KX, Chen X, Chen S, Zhao Y, Guo QG, Zong ZH. Upregulation of the lncRNA Meg3 induces autophagy to inhibit tumorigenesis and progression of epithelial ovarian carcinoma by regulating activity of ATG3. *Oncotarget* 2017; 8: 31714-31725.
- [5] Miyoshi N, Wagatsuma H, Wakana S, Shiroishi T, Nomura M, Aisaka K, Kohda T, Surani MA, Kaneko-Ishino T, Ishino F. Identification of an imprinted gene, Meg3/Gtl2 and its human homologue MEG3, first mapped on mouse distal chromosome 12 and human chromosome 14q. *Genes Cells* 2000; 5: 211-220.
- [6] Wang P, Ren Z, Sun P. Overexpression of the long non-coding RNA MEG3 impairs in vitro glioma cell proliferation. *J Cell Biochem* 2012; 113: 1868-1874.
- [7] Zhang X, Rice K, Wang Y, Chen W, Zhong Y, Nakayama Y, Zhou Y, Klibanski A. Maternally expressed gene 3 (MEG3) noncoding ribonucleic acid: isoform structure, expression, and functions. *Endocrinology* 2010; 151: 939-947.
- [8] Benetatos L, Vartholomatos G, Hatzimichael E. MEG3 imprinted gene contribution in tumorigenesis. *Int J Cancer* 2011; 129: 773-779.
- [9] Junker A, Hohlfeld R, Meinel E. The emerging role of microRNAs in multiple sclerosis. *Nat Rev Neurol* 2011; 7: 56-59.
- [10] Liu K, Wang R. MicroRNA-mediated regulation in the mammalian circadian rhythm. *J Theor Biol* 2012; 304: 103-110.
- [11] Tang G, Shen X, Lv K, Wu Y, Bi J, Shen Q. Different normalization strategies might cause inconsistent variation in circulating microRNAs in patients with hepatocellular carcinoma. *Med Sci Monit* 2015; 21: 617.
- [12] Dugas JC, Cuellar TL, Scholze A, Ason B, Ibrahim A, Emery B, Zamanian JL, Foo LC, McManus MT, Barres BA. Dicer1 and miR-219 Are required for normal oligodendrocyte differentiation and myelination. *Neuron* 2010; 65: 597-611.
- [13] Bonni A, Brunet A, West AE, Datta SR, Takasu MA, Greenberg ME. Cell survival promoted by the Ras-MAPK signaling pathway by transcription-dependent and-independent mechanisms. *Science* 1999; 286: 1358-1362.
- [14] Roux PP, Blenis J. ERK and p38 MAPK-activated protein kinases: a family of protein kinases with diverse biological functions. *Microbiol Mol Biol Rev* 2004; 68: 320-344.
- [15] Goya T, Asamura H, Yoshimura H, Kato H, Shimokata K, Tsuchiya R, Sohara Y, Miya T, Miyaoaka E; Japanese Joint Committee of Lung Cancer Registry. Prognosis of 6644 resected non-small cell lung cancers in Japan: a Japanese lung cancer registry study. *Lung Cancer* 2005; 50: 227-234.
- [16] Wang Y, Yao J, Meng H, Yu Z, Wang Z, Yuan X, Chen H, Wang A. A novel long non-coding RNA, hypoxia-inducible factor-2 $\alpha$  promoter upstream transcript, functions as an inhibitor of osteosarcoma stem cells in vitro. *Mol Med Rep* 2015; 11: 2534-2540.
- [17] Wang P, Xue Y, Han Y, Lin L, Wu C, Xu S, Jiang Z, Xu J, Liu Q, Cao X. The STAT3-binding long noncoding RNA lnc-DC controls human dendritic cell differentiation. *Science* 2014; 344: 310-313.

## The role of meG3 in invasion and migration of lung cancer cells and its mechanism

- [18] Gejman R, Batista DL, Zhong Y, Zhou Y, Zhang X, Swearingen B, Stratakis CA, Hedley-Whyte ET, Klibanski A. Selective loss of MEG3 expression and intergenic differentially methylated region hypermethylation in the MEG3/DLK1 locus in human clinically nonfunctioning pituitary adenomas. *J Clin Endocrinol Metab* 2008; 93: 4119-4125.
- [19] Benetatos L, Hatzimichael E, Dasoula A, Dranitsaris G, Tsiara S, Syrrou M, Georgiou I, Bourantas KL. CpG methylation analysis of the MEG3 and SNRPN imprinted genes in acute myeloid leukemia and myelodysplastic syndromes. *Leuk Res* 2010; 34: 148-153.
- [20] Kobayashi S, Wagatsuma H, Ono R, Ichikawa H, Yamazaki M, Tashiro H, Aisaka K, Miyoshi N, Kohda T, Ogura A, Ohki M, Kaneko-Ishino T, Ishino F. Mouse Peg9/Dlk1 and human PEG9/DLK1 are paternally expressed imprinted genes closely located to the maternally expressed imprinted genes: mouse Meg3/Gtl2 and human MEG3. *Genes Cells* 2000; 5: 1029-1037.
- [21] Zhao J, Dahle D, Zhou Y, Zhang X, Klibanski A. Hypermethylation of the promoter region is associated with the loss of MEG3 gene expression in human pituitary tumors. *J Clin Endocrinol Metab* 2005; 90: 2179-2186.
- [22] Torres L, Juárez U, García L, Miranda-Ríos J, Frias S. External ear microRNA expression profiles during mouse development. *Int J Dev Biol* 2016; 59: 497-503.