MicroRNA-148b regulates tumor growth of non-small cell lung cancer through targeting MAPK/JNK pathway

Lin Lu\textsuperscript{1,2}, Qiyao Liu\textsuperscript{1,2}\textsuperscript{†}, Peipei Wang\textsuperscript{1,2}\textsuperscript{†}, Yong Wu\textsuperscript{1,2}, Xia Liu\textsuperscript{1,2}, Chengyin Weng\textsuperscript{1,2}, Xisheng Fang\textsuperscript{1,2}, Baoxiu Li\textsuperscript{1,2}, Xiaofei Cao\textsuperscript{1,2}, Haibo Mao\textsuperscript{1,2}, Lina Wang\textsuperscript{1,2}, Mingmei Guan\textsuperscript{1,2*}, Wei Wang\textsuperscript{3*} and Guolong Liu\textsuperscript{1,2*}

Abstract

Background: MicroRNA-148b (miR-148b) has been detected in various types of tumors, and is generally viewed as a tumor suppressor. Our previous study found the decreased expression of miR-148b in human non small cell lung cancer (NSCLC) specimens and cell lines. However, the underlying mechanisms of miR-148b in regulating tumor progression remain unclear.

Methods: Firstly animal experiments were performed to verify whether miR-148b could inhibit the tumor growth. Then, the underlying mechanisms were studied by transfecting recombinant plasmids containing a miR-148b mimic or a negative control (NC) mimic (shRNA control) into NSCLC cell lines PC14/B and A549 cells. Tumor cells transfected with unpackaged lentiviral vectors was used as blank control. Cell proliferation capabilities were measured by using CCK-8 kit and colony formation assay. Cell cycle arrest was compared to clarify the mechanism underlying the tumor cell proliferation. Annexin V-FITC Apoptosis Detection kit was applied to investigate the effect of miR-148b on cell apoptosis. Furthermore, western blot analysis were performed to study the targeting pathway.

Results: We found that over-expression of miR148b could significantly inhibit tumor growth, while knocking down miR148b could obviously promote tumor growth. Further experiment showed that miR-148b inhibited tumor cell proliferation. Besides, over-expression of miR148b decreased the G2/M phase population of the cell cycle by preventing NSCLC cells from entering the mitotic phase and enhanced tumor cell apoptosis. Further western blot analysis indicated that miR148b could inhibit mitogen-activated protein kinase/Jun N-terminal kinase (MAPK/JNK) signaling by decreasing the expression of phosphorylated (p) JNK.

Conclusions: These results demonstrate that miR-148b could inhibit the tumor growth and act as tumor suppressor by inhibiting the proliferation and inducing apoptosis of NSCLC cells by blocking the MAPK/JNK pathway.

Keywords: microRNA-148b, Non small cell lung cancer (NSCLC);tumor suppressor, Proliferation, Apoptosis, MAPK/JNK pathway

* Correspondence: 13808815499@163.com; wangwei@sysucc.org.cn; eyglliu@scut.edu.cn; liugl108@163.com
\textsuperscript{†}Lin Lu, Qiyao Liu and Peipei Wang contributed equally to this work.
\textsuperscript{1}Department of Medical Oncology, Guangzhou First People’s Hospital, Guangzhou Medical University, Guangzhou 510180, Guangdong, China
\textsuperscript{2}Department of Experimental Research and State Key Laboratory of Oncology in Southern China, Sun Yat-Sen University Cancer Center, Guangzhou 510080, Guangdong, China

Full list of author information is available at the end of the article

© The Author(s). 2019 Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
Background
Lung cancer is the most frequent cause of cancer-related deaths worldwide. Each year, 1.8 million people are diagnosed with lung cancer, and 1.6 million people die from this disease. Five-year survival rates of lung cancer patients vary from 4 to 17%, depending on their tumor stages and regional differences [1]. Lung cancer are usually classified into non-small cell lung cancer (NSCLC) and small-cell lung cancer (SCLC) depending on their pathological and histological characteristics [2]. Most NSCLC patients were present with metastatic disease at the time of diagnosis [3]. The brain or central nervous system is a common metastatic site for NSCLC, with 40–50% of patients developing brain metastasis [4–6]. Patients with NSCLC and brain metastases have a poor prognosis, with a median overall survival between 4 and 9 months with chemotherapy, and only 7 months for patients receiving whole-brain radiation therapy [7]. Therefore, innovative therapeutic approaches hold great promise in the management of NSCLC.

MicroRNAs (miRNAs) are single stranded small non-coding RNAs [8]. By completely or incompletely binding with the 3′-untranslated region of the miRNA (3′-UTR), miRNAs regulate protein expression [9–11]. Abnormal expression of miRNAs may lead to alterations in processes that are important in the tumor development, including cellular differentiation, proliferation, apoptosis and metastasis [12–14]. Numerous studies have proved that the structures of artificial miRNAs and endogenous miRNAs are similar and will not cause an endogenous reaction [15]. Thus, miRNA therapy is considered a potentially safe and promising treatment method for cancer patients [16].

MiR-148b has been detected downregulated in several types of human tumors, including hepatocellular carcinoma [17, 18], chronic myeloid leukemia [19], breast cancer [20, 21], lung cancer [22], pancreatic cancer [23], gastric cancer [24] and colorectal cancer [25]. Our previous study also detected decreased expression of miR-148b in human non small cell lung cancer (NSCLC) tissues and cell lines [22]. However, the contribution of miR-148b to tumor progression and its potential mechanisms have not been fully explored. To better understand the biological significance of miR-148b in NSCLC, we firstly performed animal experiments to verify the tumor suppressor function of miR-148. Then, we studied the apoptosis, and target signal pathway was investigated.

Methods
Cell culture and transfection
Human non small cell lung cancer cell lines PC14/B and A549 were purchased from the Committee of the Type Culture Collection of the Chinese Academy of Sciences, Shanghai, China in the year of 2016. Cell lines cultured in RPMI-1640 medium (Gibco, Thermo Fisher Scientific, USA) supplemented with 10% fetal bovine serum (Gibco, Thermo Fisher Scientific, USA). The identity of the cell lines have been validated by short-tandem repeat analyses. They were free of mycoplasma contamination. The 293 T normal renal cell line was maintained in Dulbecco’s modified Eagle’s medium (Gibco, Thermo Fisher Scientific) supplemented with 10% FBS (18). MiR-148b mimic (UCAGUGCAUCACAGAACUUUGU) and negative control (NC) mimic (shRNA) were synthesized by RiboBio Co. (China). miR-148b mimic was used to construct the lentiviral rLv-miR-148b vector as a miR-148b overexpression group, and NC mimic was used to construct lentiviral rLv-shRNAvector to interfere with the expression of miR-148b as a negative control group. The lentiviral vector was packaged by co-transfection of 293 T cells with a recombinant plasmid containing a miR-148b mimic or a negative control mock. After concentration and purification of the lentiviral vector, the titer of the miR-148b lentiviral vector is determined to be 1.25X10⁸ transducing units(TU)/ml, and the titer of the NC lentiviral is 1.5X10⁸ transducing units(TU)/ml. The multiplicity of infection(MOI) was determined to be 50 based on the effect of cell infection. The viral vector rLv-miR-148b or the viral vector rLv-NC was then stably transfected into PC14/B and A549 cells by Lipofectamine TM2000 according to the manufacture’s instructions. The blank group was transfected with the unrecombined lentiviral vector rLv-Blank at the same MOI. The puromycin resistance gene carried together in the lentiviral vector is used for cell screening by puromycin drugs after stable cell transfection. After the stable transfected was successfully constructed, it was continuously cultured using a low concentration of puromycin-containing medium to ensure stable cell transfection.

Mice
Female BALB/c nude mice were supplied by the Laboratory Animals Center of Sun Yat-Sen University. The mice were housed in specific pathogen-free condition at the animal facilities of South China University of Technology. Guangzhou First People’s Hospital approved all animal protocols.

Tumorigenicity
The mice at the age of 6 to 8 weeks were randomly divided into three groups and each group contained at least 8 mice. PC14/B (1 x 10⁶/mouse in 0.2 ml of PBS) and A549 cells (2 x 10⁶/mouse in 0.2 ml of PBS) were subcutaneously inoculated into the right flank of the nude mice. The tumor size was measured every 3 days by measuring the length (L) and width (W). And the
tumor volume was calculated using formula: (L x W^2) / 2. After 6 weeks implantation and the last measurement of tumor volume, animals were euthanized by carbon dioxide inhalation followed by cervical dislocation and the subcutaneous tumors were collected and weighted.

**Quantitative real-time PCR (qRT-PCR)**

Total RNA was extracted using TRIzol (Invitrogen, USA) according to the manufacturer’s protocol. cDNA was synthesized with the MLV transcriptase Kit (Invitrogen, USA). The quantitative analysis of miR-148b expression was assayed using a Bulge-Loop TM miRNA qRT-PCR primer (forward, 5′-ACACTCCAGCTGGTGCATC-3′ and reverse, 5′-CTCAACTGTCTGGTGGAA-3′; RiboBio, China) and Platinum SYBR Green qPCR SuperMix-UDG with ROX (Invitrogen, USA) on an ABI PRISM 7500 Sequence Detection System (Applied Biosystems, Foster, CA). U6 small nuclear RNA (forward, 5′-CTCGCTTCG GCAGCAACA-3′ and reverse, 5′-AAGGCTTCACGGAAT TTGCAGT-3′) was used as an internal control (RiboBio, China). The fold changes were calculated through relative quantification using the 2−ΔΔCt method (18).

**Western blot analysis**

Proteins were extracted from PC14/B and A549 cells using RIPA buffer (Beyotime Biotechnology Company, China). Equal amounts (20 μg) of protein lysate were separated on parallel lanes of a 12% SDS PAGE gel and then electrotransferred onto a PVDF membrane (Millipore, USA). Following blocking with 5% non-fat milk in PBST (phosphate-buffered saline containing Tween 20) for 1 h, the membranes were incubated overnight at 4 °C with the following antibodies: phosphor (p)-JNK (Thr183/Tyr185) rabbit polyclonal antibody (1:500 dilution; Immuno Way, USA), (t) JNK mouse monoclonal antibody (1:500 dilution; Proteintech, USA), mitogen activated protein kinase kinase 4 (MKK4) rabbit polyclonal antibody (1:200 dilution; Proteintech, USA), or mitogen activated protein kinase kinase 7 (MKK7) rabbit polyclonal antibody (1:200 dilution; Proteintech, USA), glyceraldehyde 3-phosphate dehydrogenase (GAPDH) rabbit monoclonal antibody (1:10,000 dilution; Abcam, USA) was used as a protein loading control. After being washed thrice with PBST, the membranes were incubated with the corresponding secondary antibody HRP-conjugated goat anti-mouse immunoglobulin (Ig)G (1:2000 dilution; Abcam, USA). After being incubated for 1 h at room temperature, the protein bands were developed using an Immobilon Western Chemiluminescent HRP Substrate kit (Millipore, USA) and visualized using a ChemiDoc XRS+ Imaging system (Bio-Rad Laboratories, Inc., USA). Gray value analysis of protein bands was conducted for quantification of the protein levels by Image Lab software (Bio-Rad Laboratories, Inc., USA).

**Cell counting kit-8 (CCK-8) assay**

Tumor cells (1 × 10^3 cells per well) were seeded into 96-well plates in a final volume of 100 μl and incubated overnight at 37 °C. The proliferation of the treated cells was determined by adding 10 μl of CCK-8 per well (KeyGen Biotech, China). Following incubation at 37 °C for 4 h, the absorbance values of each well were measured at 450 nm using a microplate reader (Olympus, Japan).

**Colony formation assay**

Tumor cells were seeded in 6-well plates at a concentration of 500 cells per well, and medium was replaced with fresh medium every 3 days as the general colony formation assay method [26, 27]. After cultivation for 14 days, the cells were washed and fixed using 75% ethanol for 15 min. Following staining with 0.5% crystal violet for 15 min, the cells were washed and air dried at room temperature. The cells were then visualized under a microscope, and colonies comprising 50 or more cells were counted.

**Cell cycle assay**

PC14/B and A549 cells were rinsed thrice with cold PBS. Then the cells were collected and cell concentration were adjusted to 1 × 10^6 /ml. 1 ml of single cell suspension was taken to perform the cell cycle assay. After removing the supernatant, the cells were fixed in cold 75% ethanol overnight at − 20 °C. After three rinses with cold PBS, the cells were treated with RNase A (KeyGen Biotech, China) in a 37 °C thermostat water bath for 30 min and stained with PI (KeyGen Biotech, China) in the dark at 4 °C for 30 min. Subsequently, the cells were analyzed by flow cytometry (BD Biosciences, USA).

**Cell apoptosis assay**

The apoptotic cell rates were determined using the Annexin V-FITC Apoptosis Detection kit (KeyGen Biotech, China) according to the manufacturer’s instructions. Cells were rinsed twice with cold PBS and re-suspended in binding buffer. Following incubation with Annexin V-FITC reagent and PI in the dark at room temperature for 15 min, the cell suspension was analyzed by flow cytometry (BD Biosciences, USA).

**Statistical analysis**

All of the data were analyzed using GraphPad Prism 6 software (GraphPad Inc., USA). Numerical data were expressed as the mean ± standard deviation (SD) of at least three independent experiments. Differences between two groups were determined by the Student’s t-test, and correlation analysis was evaluated by Pearson’s correlation. Statistical significance was considered when P < 0.05.
Results

Over-expression of miR-148b inhibited tumor growth

Our previous study detected decreased expression of miR-148b in human NSCLC tissues and cell lines [22], which indicating that miR-148b might work as a tumor suppressor in NSCLC. Thus, in this study, we further investigated the effect and underlying mechanisms of miR-148b in tumor progression. Firstly, miR-148b mimics or negative control mimics (shRNA control) were stably transfected into PC14/B and A549 cells. The blank groups were transfected with unpackaged lentiviral vectors at the same MOI. Changes in the expression of miR-148b in PC14/B and A549 cells were assessed by qRT-PCR (Fig. 1).

We performed mouse models to verify our previous results that miR-148b might work as a tumor suppressor. As shown in Fig. 2, PC14/B and A549 cells transfected with miR-148b mimics developed much smaller local tumors than blank groups (P = 0.001 and P < 0.001, respectively, Fig. 2). Besides, knocking down miR-148b by transfecting tumor cells with a negative control (NC) mimic could significantly promote the tumor growth compared to blank groups (P = 0.009 and P = 0.003, respectively, Fig. 2). Same results were generated both in PC14/B (Fig. 2a) and A549 (Fig. 2b) models. These results provided evidence that miR-148b could be viewed as a tumor suppressor in NSCLC.

The effect of miR-148b on the proliferation of NSCLC cells

It's generally accepted that tumor cell proliferation and apoptosis play important roles in tumor development. Thus we further investigate the functional contribution of miR-148b in cell proliferation and apoptosis in NSCLC. As shown in Fig. 3a and b, over-expression of miR-148b significantly inhibited PC14/B (P < 0.001, Fig. 3a) and A549 cell (P = 0.002, Fig. 3b) proliferation compared to the blank group. And knocking down miR-148b expression through transfecting negative control mimics (NC) could reverse miR-148b-mediated proliferation (P < 0.001 for PC14/B and P = 0.001 for A549, respectively, Fig. 3a and b).

Representative graphs of colony formation were presented in Fig. 3c. Over-expression of miR-148b could significantly inhibit PC14/B and A549 cell colony formation compared to the blank group (P < 0.001, Fig. 3d). Besides, compared to the blank group, knocking down miR-148b expression by transfecting negative control mimics (NC) could significantly promoted the colony formation capabilities of PC14/B and A549 cells (P < 0.001, Fig. 3d). These results indicate that miR-148b could regulate the proliferation of NSCLC cells.

The effect of miR-148b on the cell cycle of NSCLC

To explore the effect of miR-148b on the cell cycle of NSCLC cells, we examined the cell cycle of PC14/B and A549 cells after being transfected with different mimics. The cell population in G2/M phase was significantly decreased in PC14/B (Fig. 4a, b and d) and A549 (Fig. 4a, c and e) cells after being transfected with miR-148b mimics compared to the blank group, while the G2/M phase was significantly increased after being transfected with negative control mimics. These results indicated that miR-148b suppressed NSCLC cell entry to the mitotic phase, although it had no effect on DNA replication activity (S phase). These data suggest that miR-148b could work on the cell cycle.

The effect of miR-148b on the apoptosis of NSCLC cells

To examine whether miR-148b influences the apoptotic rate of PC14/B and A549 cells, Annexin V-FITC/propidium iodide (PI) double staining and flow cytometric analysis were conducted. As demonstrated in Fig. 5a,
over-expression of miR-148b could induce apoptosis of tumor cell. The apoptotic rate of the miR-148b-overexpression cells was higher than the blank groups ($P < 0.001$, Fig. 5b). While the apoptotic rate of the miR-148b low-expression cells (NC group) was lower than the blank group ($P < 0.001$, Fig. 5b). These data indicate that miR-148b could work on cell apoptosis.

miR-148b inhibited the MAPK/JNK signaling pathway by decreasing the expression of phosphorylated (p) JNK in NSCLC cells

It is well known that JNK belongs to the MAPK family. JNK activity regulates several important cellular functions, including cell proliferation, differentiation, survival and apoptosis. It is activated by dual phosphorylation of MKK4 and MKK7. Therefore, we further investigated the role of MAPK/JNK signaling pathway in regulating the proliferation and apoptosis of miR-148b in NSCLC. Representative western blot results were demonstrated in Fig. 6a. Over-expression of miR-148b obviously decreased the protein levels of phosphorylated JNK in PC14/B and A549 cells compared to the blank group ($P = 0.002$ and $P < 0.001$, respectively, Fig. 6b), while the protein levels of MKK4, MKK7 and unphosphorylated (t) JNK was not affected. Phosphorylation of JNK was re-activated by knocking down miR-148b expression ($P = 0.002$ and $P = 0.007$, respectively, Fig. 6b). Our results showed that miR-148b could inhibit the MAPK/JNK signaling pathway by decreasing the expression of phosphorylated (p) JNK in NSCLC cells.

Discussion

It is generally accepted that during cancer progression, multiple oncogene proteins are up-regulated. In contrast, numerous tumor-suppressing proteins are down-regulated. The mechanisms of regulating changes in protein expression are very complex. Among these tumor promoting and suppressive proteins, miRNAs have been recognized to play an important role in epigenetic changes [28, 29]. Dysregulated miRNAs expression takes part in multiple
biological processes, including regulation of oncogenes and suppressor genes expression, tumor progression and metastasis [30, 31]. Down-regulation of miR-148b has been identified in various types of human cancer, and miR-148b was regarded as a tumor-suppressive miRNA [21, 32, 33]. The lncRNAs (long non-coding RNAs) controlled by miR-148b are thought to play an important role in immune system and are associated with the development and progression of gastric cancer [34]. MiR-148b was reported to increase the radio-sensitivity of non-Hodgkin’s cells in non-Hodgkin’s Lymphoma [35]. Additionally, miR-148b exerted an effect on cell proliferation by regulating the expression of the cholecystokinin-2 receptor gene in colorectal cancer [18]. These findings indicated that dysregulation of miR-148b closely related to malignancies. Besides, our previous studies found that miR-148b was down-regulated in NSCLC tissues, suggesting that miR-148b might participate in the malignant degeneration of NSCLC.

In this study, we further investigate the tumor suppressive functions and underlying mechanisms of miR-148b in NSCLC by both over-expressing and knocking down the expression of miR-148b in NSCLC cell lines. Firstly, we carried out mouse model to verify the tumor suppressive function of miR-148b. Our research found that over-expression of miR-148b could significantly inhibited tumor growth, while knocking down miR-148b significantly promoted tumor growth. There were positive correlations between the xenograft model, the CCK8 assay, and the colony formation assay. When the CCK8 assay of the miR48b group demonstrated a small OD value, the corresponding colony formation was smaller, and the volume of the subcutaneous tumor of the mouse was smaller. In contrast, when the CCK8 assay demonstrated a large OD value in the NC group, the colony formation of the NC group was larger, and the volume of the subcutaneous tumor was larger. These results combined with our previous study revealed that miR-148b could be viewed as a tumor suppressor in NSCLC [22]. Consistently, Ge et al. detected decreased expression of miR-148b in NSCLC tumor tissues, and decreased expression of miR-148b was correlated with poor survival [36]. Li et al. detected the expression of miR-148b in serum of NSCLC patients, and they found that downregulation of miR-148b was correlated with more aggressive tumors [37].

We further studied the mechanisms underlying the miR-148b-mediated anti-cancer process, and found that
Fig. 4 miR-148b blocks the cell cycle at the G2/M phase. 

(a) Cell cycle distribution of PC14/B and A549 cells after being transfected with different mimics. Red color represents G0/G1 phase, pink color represents G2/M phase, blue color represents S phase. 

(b) and (d) The percentage of cell cycle distribution in PC14/B cells. 

(c) and (e) The percentage of cell cycle distribution in A549 cells. The results are expressed as the mean ± standard deviation. Blank, cells transfected with unpackaged lentiviral vectors; NC, negative control; miR, microRNA.
over-expression of miR-148b could significantly inhibit NSCLC cell proliferation, colony formation, and induce cell apoptosis. Knocking down the miR-148b expression could reverse these effects. Over-expression of miR-148b significantly decreased the G2/M phase population in NSCLC cells by preventing NSCLC cells from entering the mitotic phase to inhibit the cell proliferation. Taken together, these results suggested that miR-148b might function as a tumor-suppressor in NSCLC by regulating cell proliferation and apoptosis.

As we all know that the miRNAs regulated multiple malignant incidences via regulation of specific pathways [38–40]. To further understand the target network involved in the function of miR-148b, we studied the MAPK/JNK signaling pathway. JNK belongs to the MAPK family, and regulates several important cellular functions including cell proliferation, differentiation, survival and apoptosis [41–43]. JNK can be activated by dual phosphorylation of MKK4 and MKK7 [44]. Therefore, we investigated whether miR-148b regulates the tumor growth of non-small cell lung cancer through targeting MAPK/JNK pathway. The results demonstrated that over-expression of miR-148b significantly decreased the protein expression of phosphorylated (p) JNK in NSCLC cells, while the protein expression of MKK4, MKK7 and unphosphorylated (t) JNK was not affected. Our results confirm that miR-148b could inhibit the MAPK/JNK signaling pathway by decreasing the expression of phosphorylated (p) JNK in NSCLC cells. Knocking down miR-148b expression could enhance the

![Fig. 5 miR-148b induces apoptosis in NSCLC cells. a Annexin V-FITC/PI double staining and flow cytometric analysis was applied to detect the apoptotic rate of PC14/B and A549 cells after being transfected with different mimics. b Early apoptotic rates obtained by quantification of results generated from graph A, the apoptotic rate of the miR-148b-overexpression cells was higher than the blank group, while the apoptotic rate of the miR-148b-lowexpression cells was below the blank group. The results are expressed as the mean ± standard deviation. PI, propidium iodide; Blank, cells transfected with unpackaged lentiviral vectors; NC, negative control; miR, microRNA]
phosphorylation of JNK. These results support our previous hypothesis that miR-148b exert anti-cancer activity in NSCLC by targeting MAPK/JNK pathway. In accordance with our study, Fang et al. found that decreased miR-148b could enhance tumor cell proliferation and inhibit apoptosis in human renal cancer cells by targeting MAP3K9, which is a upstream activator of MAPK/JNK pathway [45]. At the same time, several studies have confirmed that miR148b works through the regulation of MAPK/JNK [46, 47].

Conclusions
In summary, our study demonstrated that miR-148b could significantly inhibit tumor growth. MiR-148b inhibits NSCLC cell proliferation, colony formation, and induces apoptosis through targeting the MAKP/JNK pathway. All of these results suggest that miR-148b could be viewed as a tumor suppressor and might serve as a potential therapeutic candidate in the developing novel strategy for NSCLC patients.

Abbreviations
GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; miRNAs: MicroRNAs; MKK4: Mitogen activated protein kinase kinase 4; MKK7: Mitogen activated protein kinase kinase 7; MOI: Multiplicity of Infection; NC: Negative control; NSCLC: Non-small cell lung cancer; qRT-PCR: Real time quantitative polymerase chain reaction; SCLC: Small-cell lung cancer

Acknowledgements
Not applicable

Funding
This research about cell function assay in vitro was funded by the National Natural Science Foundation of China (No. 81672900 and No. 81502563). The over-expression and knockdown of cell models was funded by the National Science Foundation of Guangdong Province, China (No. 2016A030310109). Animal experiment was funded by the Fundamental Research Funds for the Central Universities (No. X2ykD2175050). The funders had no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

Availability of data and materials
All date generated or analyzed during this study are included within the article. The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions
LL, GLL, MMG and WW designed the project; QYL and PPW performed the research; YW and XL collected and analyzed the data; CYW, XSF and BXL analyzed the data; LL and QYL wrote the manuscript; XFC, HBM and LNW did the statistical analysis. All the authors have read and approved the final manuscript.

Ethics approval
The Ethics Committee of Guangzhou First People’s Hospital approved all the animal experiments. This study did not involve in any human subjects or human material. The cell lines used in this study did not require ethics approval for the use.

Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interests.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details
1Department of Medical Oncology, Guangzhou First People’s Hospital, Guangzhou Medical University, Guangzhou 510180, Guangdong, China.
2Department of Medical Oncology, Guangzhou First People’s Hospital, School of Medicine, South China University of Technology, Guangzhou 510180.
Guangdong, China. \*Department of Experimental Research and State Key Laboratory of Oncology in Southern China, Sun Yat-Sen University Cancer Center, Guangzhou 510080, Guangdong, China.

Received: 25 September 2018 Accepted: 21 February 2019

Published online: 08 March 2019

References

1. Hirsch FR, Scagliotti GV, Mulshine JL, Kwon R, Curran WJ Jr, Wu YL, Paz-Ares L. Lung cancer: current therapies and new targeted treatments. Lancet. 2017;389(10066):299–311.

2. Calbo J, Miyawaki S, van Montfort E, van Tellingen O, Berns A. Genotype-phenotype relationships in a mouse model for human small-cell lung cancer. Cold Spring Harb Symp Quant Biol. 2005;70:225–32.

3. Peters S, Bexelius M, Vunk V, Leight N. The impact of brain metastasis on quality of life, resource utilization and survival in patients with non-small-cell lung cancer. Cancer Treat Rev. 2016;45:139–62.

4. Sorensen JB, Hansen HH, Hansen M, Dombernowsky P. Brain metastases in adenocarcinoma of the lung: frequency, risk groups, and prognosis. J Clin Oncol. 1988;6(9):1474–80.

5. Patchell RA. The management of brain metastases. Cancer Treat Rev. 2003;29(6):533–40.

6. Yawn BP, Wollan PC, Schroeder C, Gazzuola L, Mehta M. Temporal and gender-related trends in brain metastases from lung and breast cancer. Minn Med. 2003;86(12):22–7.

7. Oshinowo TO, Adhikary J, Zelnick A, Shu HK, Shim H, Robin AM, Kalkanis SN, Whitsett TG, Saliba H, Tran NL, et al. Current approaches to the treatment of metastatic brain tumours. Nat Rev Clin Oncol. 2014;11(4):203–22.

8. Lau NC, Lim LP, Weinstein EG, Bartel DP. An abundant class of tiny RNAs with probable regulatory roles in Caenorhabditis elegans. Science. 2001;294(5543):858–62.

9. Lagou Quintana M, Rauhut R, Lendebek W, Tuschl T. Identification of novel genes coding for small expressed RNAs. Science. 2001;294(5543):853–8.

10. Ke XS, Liu CM, Liu DP, Liang CC. MicroRNAs: key participants in gene regulatory networks. Curr Opin Chem Biol. 2003;7(4):16–23.

11. Mohr AM, Mott CL. Overview of microRNA biology. Semin Liver Dis. 2015;35(1):3–11.

12. Zhang Y, Yang PY, Wang XF. Microenvironmental regulation of cancer metastasis by miRNAs. Trends Cell Biol. 2011;21(4):146–53.

13. Wang WX, Corrigan-Cummins M, Hudson J, Maric I, Simakov O, Neelapu SS, Kvak LW, Janik JE, Gause B, Jaffe ES, et al. MicroRNA profiling of follicular lymphoma identifies microRNAs related to cell proliferation and tumor response. Haematol Hematol J. 2012;9(4):586–94.

14. Chittwood DH, Timmermans MC. Small RNAs are on the move. Nature. 2010;467(7314):415–20.

15. Calloni R, Bonatto D, et al. MicroRNA-148b and miR-148b targeting inhibits dissemination of melanoma and breast cancer. Cancer Res. 2016;76(17):5151–62.

16. Liu GL, Liu X, Lv XB, Wang XP, Fang XS, Yang Z, miR-148b functions as a tumor suppressor in non-small cell lung cancer by targeting carcinoembryonic antigen (CEA). Int J Clin Exp Med. 2014;7(8):1990–9.

17. Bloomston M, Frankel WL, Petracca F, Volinia S, Alder H, Hagan JP, Lu CG, Bhatt D, Taccioli C, Croce CM. MicroRNA expression patterns to differentiate pancreatic adenocarcinoma from normal pancreas and chronic pancreatitis. J Am Med Assoc. 2007;297(17):1901–8.

18. Song YX, Yue ZY, Wang ZN, Xu YY, Luo Y, Xu HM, Zhang X, Jiang L, Xing C2, Zhang Y. MicroRNA-148b is frequently down-regulated in gastric cancer and acts as a tumor suppressor by inhibiting cell proliferation. Mol Cancer. 2011;10:1.

19. Schetter AJ, Leung SY, Sohn JJ, Zanetti KA, Bowman ED, Yanaihara N, Yuen ST, Chan TL, Kwong DL, Au GK, et al. MicroRNA expression profiles associated with prognosis and therapeutic outcome in colon adenocarcinoma. JAMA. 2008;299(4):425–36.

20. Rajehi H, Oltvitski C, Georgiadi TS, Gerner K, El-Osta A, Karagiannis TC. Oncogenic assay: adherent cells. J Vis Exp. 2011;49.

21. Crowley LC, Christensen ME, Waterhouse NJ. Measuring Survival of Adherent Cells with the Colony-Forming Assay. Cold Spring Harb Protoc. 2016;2016(8).

22. Okugawa Y, Grady WM, Goel A. Epigenetic Alterations in Colorectal Cancer: Emerging Biomarkers. Gastroenterology. 2015;149(5):1204.

23. Sivastava D, DeWitt N. In vivo cellular reprogramming: the next generation. Cell. 2016;166(6):1386–96.

24. Bhayadia R, Krowocz K, Haetscher N, Jammal R, Emmrich M, Bohlakum S, Fedler J, Schwarzer A, Rouhi A, Heuser M, et al. Endogenous Tumor Suppressor microRNA-193b: Therapeutic and Prognostic Value in Acute Myeloid Leukemia. J Clin Oncol. 2018;36(10):1007.

25. Sachdeva M, Mitro JK, Lee CL, Zhang MS, Li ZZ, Dood D, Cason D, Luo LX, Sargent DJ, van Metser D, Wright MA, et al. MicroRNA-182 drives metastasis of primary breast cancer by targeting multiple genes. J Clin Invest. 2014;124(10):4005–19.

26. Huang MX. Down-expression of circulating micro ribonucleic acid (miRNA)-148/152 family in plasma samples of non-small cell lung cancer patients. J Cancer Res Ther. 2016;12(2):671–5.

27. Zhang JG, Shi Y, Hong DF, Song M, Huang D, Wang CY, Zhao G. MicroRNA-148b suppresses cell proliferation and invasion in hepatocellular carcinoma by targeting WNT1/beta-catenin pathway. Sci Rep. 2015;5:8087.

28. Song Y, Sun J, Wu L, Liu J, Liu J, Liu H, Tan C, Ding CM. MicroRNA-148b is down-regulated in non-small cell lung cancer and associated with poor survival. J Int J Exp Pathol. 2015;8(1):800–5.

29. Li J, Chen YY, Li SQ, Huang C, Qin YZ. Expression of microRNA-148-152 family as potential biomarkers in non-small-cell lung cancer. Med Sci Monit. 2015;21(3):1155–61.

30. Thié B, Alter C, Lupinski S, Eckstein A, Tan S, Fuhrer D, Pastille E, Ritz C, Buer J, Hansen W. MicroRNA-183 and microRNA-96 are associated with autoimmune responses by regulating T cell activation. J Autoimmun. 2018.

31. Sachdeva M, Mitro JK, Lee CL, Zhang MS, Li ZZ, Dood D, Cason D, Luo LX, Sargent DJ, van Metser D, Wright MA, et al. MicroRNA-182 drives metastasis of primary breast cancer by targeting multiple genes. J Clin Invest. 2014;124(10):4005–19.

32. Ge H, Li B, Hu WX, Liu J, Jin H, Gao MN, Ding CM. MicroRNA-148b is downregulated in non-small cell lung cancer and associated with poor survival. J Int J Exp Pathol. 2015;8(1):800–5.

33. Li J, Chen YY, Li SQ, Huang C, Qin YZ. Expression of microRNA-148-152 family as potential biomarkers in non-small-cell lung cancer. Med Sci Monit. 2015;21(3):1155–61.

34. Thié B, Alter C, Lupinski S, Eckstein A, Tan S, Fuhrer D, Pastille E, Westendorf AM, Buer J, Hansen W. MicroRNA-183 and microRNA-96 are associated with autoimmune responses by regulating T cell activation. J Autoimmun. 2018.

35. Zhang X, Liu L, Deng X, Li D, Cai H, Ma Y, Jia C, Wu B, Fan Y, Lv Z. MicroRNA-483-3p targets Pard3 to potentiate TGF-beta-induced cell migration, invasion, and epithelial-mesenchymal transition in anaplastic thyroid cancer cells. Oncogene. 2018.

36. Iqbal MA, Arora S, Prakasham C, Carlin GA, Syed MA. MicroRNA in lung cancer: role, mechanisms, pathways and therapeutic relevance. Mol Asp Med. 2018.

37. Daneshkaran DN, Reddy EP. JNK signaling in apoptosis. Oncogene. 2008;27(48):6245–51.

38. Hsu YN, Chen W, Zheng XX. ROS, MAPK/ERK and PKC play distinct roles in EGF-stimulated human corneal cell proliferation and migration. Cell Mol Biol. 2015;61(7):16–11.

39. Ip YT, Davis RJ. Signal transduction by the c-Jun N-terminal kinase (JNK) - from inflammation to development. Curr Opin Cell Biol. 1998;10(2):205–19.

40. Jaeschke A, Davis RJ. Metabolic stress signaling mediated by mixed-lineage kinases. Mol Cell. 2007;27(3):498–508.

41. Nie F, Liu T, Zheng L, Yang X, Liu Y, Xia H, Liu X, Wang X, Liu Z, Zhou L, et al. MicroRNA-148b enhances proliferation and apoptosis in human
renal cancer cells via directly targeting MAP3K9. Mol Med Rep. 2016;13(1):83–90.

46. Kuwagata S, Kume S, Chin-Kanasaki M, Araki H, Araki S, Nakazawa J, Sugaya T, Koya D, Haneda M, Maegawa H, et al. MicroRNA148b-3p inhibits mTORC1-dependent apoptosis in diabetes by repressing TNFR2 in proximal tubular cells. Kidney Int. 2016;90(6):1211–25.

47. Zhao G, Zhang JG, Liu Y, Qin Q, Wang B, Tian K, Liu L, Li X, Niu Y, Deng SC, et al. miR-148b functions as a tumor suppressor in pancreatic Cancer by targeting AMPK alpha 1. Mol Cancer Ther. 2013;12(1):83–93.