Original Article
Effects and mechanism of miR-133a on invasion and migration of lung cancer cells

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Abstract: Objectives: To study the role of miR-133a expression in the invasion, proliferation, migration, and apoptosis of lung cancer cells and its mechanism. Methods: miR-133a expression levels in human normal lung epithelial cells (BEAS-2B), H441 cell lines and NSCLC tissues were detected by qPCR. The influence of miR-133a mimics on the migration, proliferation and invasion of H441 cells was examined by CCK-8 assay, transwell migration assay, and invasion assay, respectively. Expression of MMP-9 and LASP1 in H441 cellstreated by miR-133a mimics was determined by western blot. Pearson's test was conducted to study the association of miR-133a expression with clinical characteristics of NSCLC patients. The targeted regulation of miR-133a on LASP1 gene expression was detected by the luciferase reporter gene assay. Results: miR-133a expression was decreased in H441 cells in contrast to that in BEAS-2B cells (P<0.05). Compared with para-carcinoma tissues, miR-133a levels were markedly down-regulated in NSCLC tissues. miR-133a overexpression inhibited the invasion, proliferation, and migration ability of H441 cells and promoted cell apoptosis (all P<0.05). MMP-9 expression levels were also reduced in the miR-133a mimic group. Moreover, miR-133a expression levels were correlated with tumor size and TNM stage. miR-133a overexpression decreased the expression of LASP1, which is the targeted gene of miR-133a. Conclusions: miR-133a overexpression can reduce the invasion, proliferation, migration, and matrix metalloproteinase expression of NSCLC cells and promote cell apoptosis. This may be correlated to targeted down-regulation of LASP1 expression.

Keywords: miR-133a, proliferation, invasion, migration, NSCLC, matrix metalloproteinase

Introduction

Lung cancer is common worldwide, and the morbidity rate is high [1, 2]. The morbidity and mortality are the top cause of cancer-attributable death [3]. Lung tumor is subdivided into non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC is the main type and accounts for about 80%-85% of all lung cancer [4]. With the development of medical science and technology, the diagnosis and treatment of lung cancer has achieved great progress. However, the prognosis of NSCLC patients is still poor [5, 6]. Some studies have reported that overall 5-year survival rate ranged from 10% to 15% in patients with lung cancer [7]. The mechanisms underlying the development of NSCLC are not yet fully understood. The main reason for poor prognosis of lung cancer is the high rate of recurrence and metastasis. Moreover, many studies revealed that deg-radation of extracellular matrix was necessary for the invasion and metastasis of lung tumor cells and MMP-9 degrading extracellular matrix played a critical role in the invasion and metastasis of tumor cells [8, 9]. Therefore, the identification of effective new targets and clarification of the molecular mechanisms may be helpful to improve the prognosis of lung cancer patients.

miRNAs are single-stranded non-coding small RNAs that are an endogenous and conserved cohort of 19-25 oligonucleotides in length [10]. They are involved in post-transcriptional regulation of different genes by binding the 3'-UTR of the target gene's mRNA [11]. miRNAs regulate various crucial biologic processes including proliferation, invasion, apoptosis, differentiation, and metastasis [12]. Some trials have confirmed that miRNAs play a very critical function in the development of NSCLC [13]. miRNAs may be targets for NSCLC therapy.

miR-133a is a new cancer-associated miRNA, and its expression differs in various tumor tissues. Some studies revealed that miR-133a was lowly expressed in esophageal squamous cell carcinoma [14]. In addition, it was reported that miR-133a has great suppressive roles on the invasion of bladder cancer cells [15]. Furthermore, the level of miR-133a was negatively correlated with the metastasis of breast cancer [16]. Those findings revealed that miR-133a has important functions in the development of cancer. A clear mechanism through which miR-133a affects the development of NSCLC is still unknown. In order to explore the roles of miR-133a expression in invasion, proliferation, migration, and apoptosis of lung tumor cells, in this study, we examined the expression of miR-133a in lung cancer cells and lung cancer tissues, respectively. H441 cells were transfected with miR-133a mimics by the method of lipofection transfection to explore the possible mechanisms and effects of miR-133a on the invasion, proliferation, apoptosis, and metastasis of lung cancer cells. The results of this research would offer an experimental and theoretical basis to consider miR-133a as a new therapeutic target against lung cancer.

Materials and methods

Tissue specimens

Twenty patients who provided written informed consent were included in this study which was approved by the ethic committee of our hospital (No. 2021-258).

The inclusion criteria: (1) Patients with a diagnosis of NSCLC confirmed by pathologic examination. (2) Patients without radiotherapy, chemotherapy or any other therapy prior to operation.

The exclusion criteria: (1) Patients who were unwilling to participate in this research. (2) Patients who were diagnosed with other cancers. (3) Patients with systemic diseases including systemic lupus erythematosus, rheumatoid arthritis, and diabetes.

The specimens from NSCLC patients and their paired adjacent normal tissues after operation were instantly frozen and stored in liquid nitrogen for later detection.

Cell lines

Lung cancer cells line H441 with high metastasis and human normal lung epithelial cells (BEAS-2B) were prepared for the experiments. Those cell lines were cultured in DMEM containing 10% fetal bovine serum (FBS) under 37°C and 5% CO_2 conditions.

Reagent and instruments

BEAS-2B and H441 were purchased from American Type Culture Collection (ATCC, USA). FBS and high glucose medium DMEM were purchased from Gibco (USA), Trizol reagent, and Lipofectamine TM 2000 transfection reagents were purchased from Invirogen (USA); Crystal violet was purchased from Sigma (USA); Anti-human matrix metalloproteinase-3 (MMP-3) and MMP-9 antibody, GAPDH antibody were obtained from Abcam (England); Transwell chambers were purchased from Corning (USA); pcDNA3.1-LASP1 and pcDNA3.1-Vector plasmids, miR-133a mimic and negative mimics control were purchased from GenePharma (China).

qPCR analysis

Total miRNA of lung tumor cells and tissues was extracted by the one-step method for miRNA. The above miRNAs were reversely transcribed into cDNA by TaqMan MicroRNA reverse transcription test kit. The analysis of qPCR was conducted with the SYBR Premix Ex Taq for the detection of miRNA. The primers were designed as follows, miR-133a forward primer: CTGCGATATGACCTCTCAC-3', backward primer: 5'-AGATCTTGAGT GCTTTTCC-3'. U6 forward primer: 5'-CTCGCTTCGGCAG CACACA-3', U6 backward primer: 5'-AACGCTTCACGAATTTGC-GT-3'. LASP1 forward primer: 5'-GAGAGGAAC-AAGCTGGCTGC-3', LASP1 backward primer: 5'-GCTTCTC CTTCTCCTTCTGC-3'. MMP-9 forward primer: 5'-CGGAGCACGGAGACG GGTAT-3', MMP-9 backward primer: 5'-TGAAGGGGAAGA-CGCACAGC-3'. GAPDH forward primer: 5'-CGA-GATCCCTCCAAAATCAA-3', GAPDH backward primer: 5'-AGGTCCACCACTGACACGTT-3'. The miR-133a expression level was normalized to U6. GAPDH was used as an internal reference for detecting the levels of LASP1 and MMP-9 mRNA. The relative level of miRNA expression was obtained by the $2^{-\Delta\Delta Ct}$ method.

CCK-8 assay

The proliferation rate of cells was detected using CCK-8 assay. The lung tumor cells were seeded in a 96-well plate with a density of 2×10^3 cells/well. miR-133a mimics or pcDNA3.1-LASP1 were transfected after static culture. 10 μL CCK-8 solution was treated after culturing for 24 h, 48 h, or 72 h. Optical density (OD) at 450 nm was examined using a microplate reader.

Cell migration experiment

The lung cancer cell line H441 was inoculated in a 96-well plate. After static culture, miR-133a mimics or pcDNA3.1-LASP1 were transfected by Lipo2000 transfection reagent with miR-133a NC as control. After being further incubated for 48 h, the cells were seeded into transwell chambers with 3 complex wells in each group. Serum-free DMEM culture medium was added in the upper chamber, and DMEM culture medium with FBS was placed in the lower chamber. After further culture for 24 h, non-migrated cells were cleaned and PBS was used to wash off invaded cells. Then the migrated cells were dyed using 0.1% crystal violet. Under the light microscope, the migrated cells were calculated from six independent fields. Next, the crystal violet was totally eluted using 33% acetic acid dehydrating, and optical density at 450 nm in a microplate reader was detected. The value of optical density indicated the migration ability of lung cancer cells.

Cell invasion experiment

Matrigel solution was spread evenly on the membranella (8 μm) of the Transwell chamber. The subsequent experiments were prepared according to the instructions of cell migration assay. Finally, the cells were dyed using crystal violet and counted. Then, the crystal violet was eluted completely with 33% acetic acid and detected the OD value, suggesting the invasion ability of the cells.

Apoptosis assay

The analysis of apoptosis was determined by Caspase-3 activity assay. These kits were obtained from R&D Science, USA. The cells were further cultured for 48 h after transfection for 48 h. The Caspase-3 Assay Kit was applied for examining the activity of caspase-3. The

procedures were strictly followed the instructions on the kits.

Western blot analysis

After transfection for 48 h, the total protein was obtained with lysis buffer RIPA solutions using proteinase inhibitor. The concentration of protein was examined through a BCA Reagent Kit following the instructions. The proteins were purified using SDS-PAGE gel electrophoresis. They were transferred to the PVDF membrane. Then the membrane was kept in the TBST solutions with 5% non-fat milk powder, and blocked at room temperature for 2 h. The primary antibody was incubated overnight in the shaker at 4°C. After being washed, PVDF membrane was kept with the secondary antibody. Finally, ECL reagents were used to detect the target proteins, and GAPDH served as a loading control.

Construction and activity detection of luciferase reporter gene

According to LASP1 sequencing and Target Scan prediction, 3'UTR sequence in the LASP1 DNA amplified and constructed by PCR was cloned into pGL-Basic reporter gene plasmid (pGL-UTR), sequenced, and inserted into luciferase reporter vector pGL3-MUT-LASP1-3'UTR-mutant type and pGL3-WT-LASP1-3'UTR-wild type. Sequencing was completed by commissioned Takara Bio (Dalian). Lipo2000 liposomes mediated the two plasmids and chemically-synthesized miR-133a analogs, respectively, and 70% of pooled HepG cells were transfected by nonsense control sequence. At 48 h, the activity of luciferase was detected using dual luciferase assay kits (Promega, US).
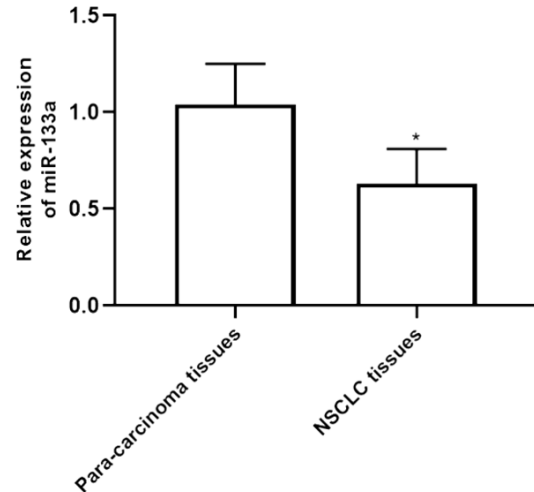
Statistical methods

The analysis of data included in this research was performed by SPSS 18.0 software. The measured data were expressed as mean ± standard deviation (SD), and T test was conducted for the comparison between two groups. Counted data were presented by rate and χ^2 test was conducted to compare between two groups. Pearson's test was conducted to determine the association of miR-133a expression levels with the clinical characteristics in patients with NSCLC and the relationship between LASP1 and MMP-9 expression. $P < 0.05$ indicated a significant difference.
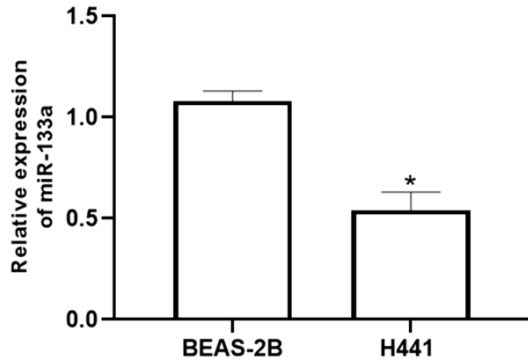
Figure 1. Expression level of miR-133a in lung cancer cell lines. Compared to BEAS-2B, *P<0.05.

Results

Comparison of miR-133a expression levels in lung cancer cells

As shown in **Figure 1**, qPCR test revealed that the expression level of miR-133a in high metastatic lung cancer H441 cell lines (0.54±0.09) was much lower than that in BEAS-2B (1.08±0.05) cells, and the difference was significant (P<0.05).

The comparison of miR-133a expression levels in lung cancer tissues

qPCR experiment was applied to examine the miR-133a expression in the samples of lung cancer tissues and para-carcinoma tissues. miR-133a expression in lung tumor tissues was significantly downregulated in contrast to para-carcinoma tissues (0.63±0.18 vs 1.04±0.21, P<0.05), as show in **Figure 2**, indicating that miR-133a might be dysregulated in lung cancer tissues.

LASP1 expression levels in lung cancer cells and NSCLC tissues

As shown in **Figure 3**, compared to that in BEAS-2B cells, the mRNA and protein expression levels of LASP1 in H441 cells were increased (P<0.05). Moreover, mRNA and protein expression of LASP1 in NSCLC tissues was increased as compared with that of para-carcinoma tissues (P<0.05).

miR-133a mimics reduced proliferation of lung cancer cells

To further investigate the biologic functions of miR-133a in lung tumors, the expression of

Figure 2. The expression level of miR-133a in lung cancer tissues. *P<0.05.

miR-133a was increased with miR-133a mimics. CCK-8 assay was applied to explore the roles of miR-133a in the proliferation of lung tumor cells, and the results found that overexpression of miR-133a could inhibit the proliferation of H441 and BEAS-2B cells. Statistical differences were observed at transfection for 48 h and 72 h compared to the miR-133a NC group, as seen in **Figure 4A**. However, LASP1 overexpression in miR-133a transfected H441 cells increased the proliferation of cells, as shown in **Figure 4B** (P<0.05).

miR-133a mimics inhibited migration of lung cancer cells

As shown in **Figure 5A**, after miR-133a mimic transfection for 48 h, in contrast to control group (OD value: 1.75±0.23), miR-133a mimic significantly decreased the migration ability of H441 cells (OD value: 0.57±0.11) (P<0.05).

However, LASP1 overexpression in miR-133a transfected H441 cells increased the migration of cells (P<0.05), as seen in **Figure 5B**.

miR-133a mimics inhibited invasion of lung cancer cells

It was showed that the invasion ability of lung cancer H441 cells was down-regulated (OD: 0.71±0.16) when miR-133a was overexpressed, as shown in **Figure 6A** (P<0.05), indicating that miR-133a mimics could decrease
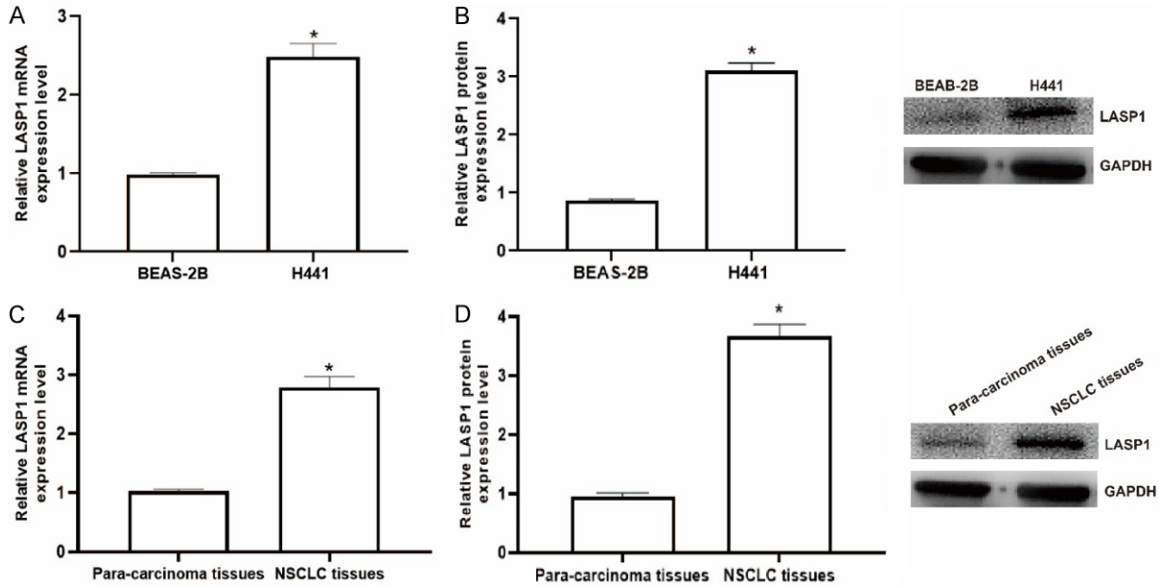
Figure 3. Expression level of LASP1 in lung cancer cells and NSCLC tissues. A: LASP1 levels were detected in BEAS-2B and H441 by qRT-PCR. B: LASP1 levels were detected in BEAS-2B and H441 by western blot. C: LASP1 levels were detected in para-carcinoma tissues and NSCLC tissues by qRT-PCR. D: LASP1 levels were detected in para-carcinoma tissues and NSCLC tissues by western blot. *$P<0.05$.
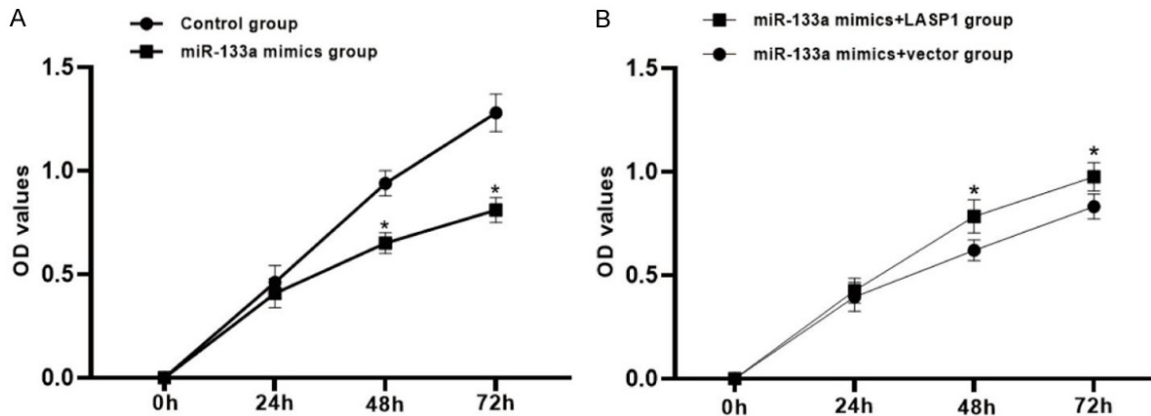
Figure 4. miR-133a regulates the proliferation of lung cancer H441 cells through targeting LASP1. A: The effect of miR-133a mimics on the proliferation of H441. B: The proliferation of H441 cells co-transfected with miR-133a mimics and pcDNA3.1-LASP1. *$P<0.05$.

the cell invasion. However, LASP1 overexpression in miR-133a transfected H441 cells increased the invasion of cells, as seen in **Figure 6B** ($P<0.05$).

miR-133a mimics promoted apoptosis of lung cancer cells

Compared with the control group, miR-133a mimic could significantly promote the apoptosis of lung cancer H441 cells ($P<0.05$). How-
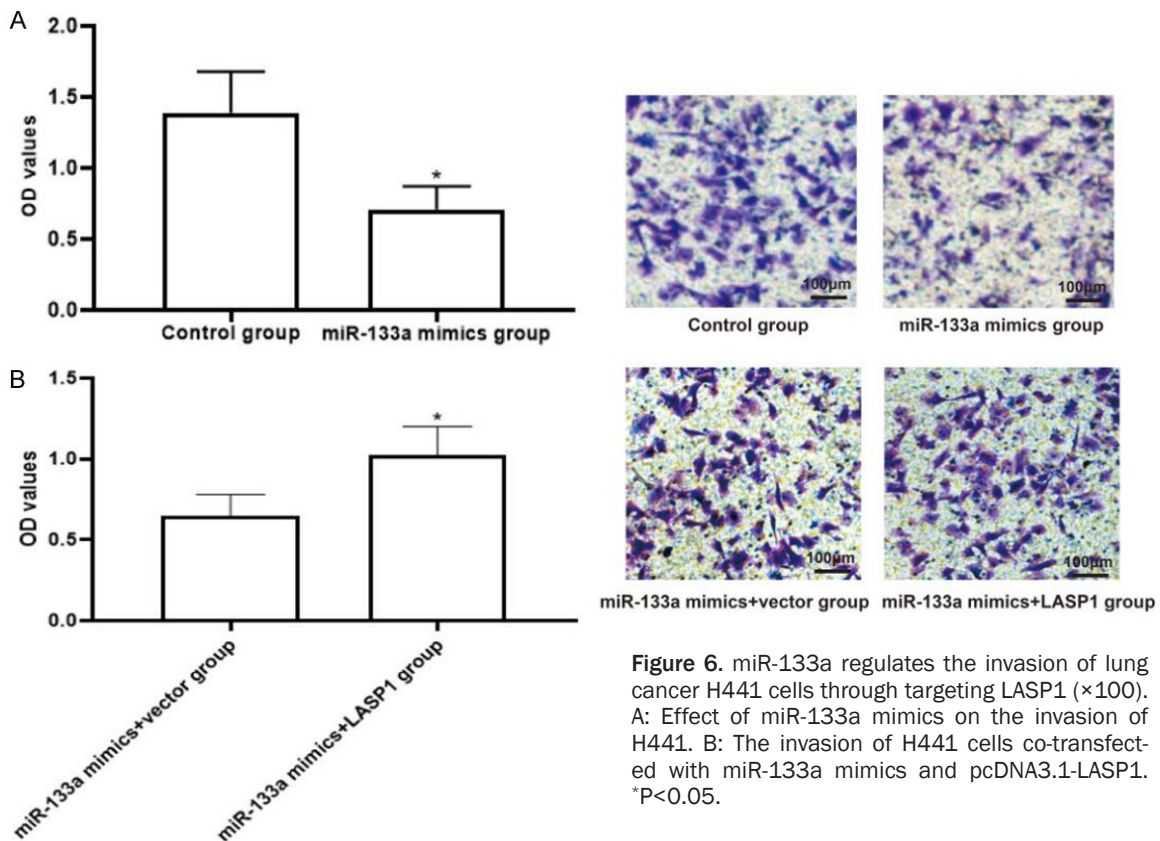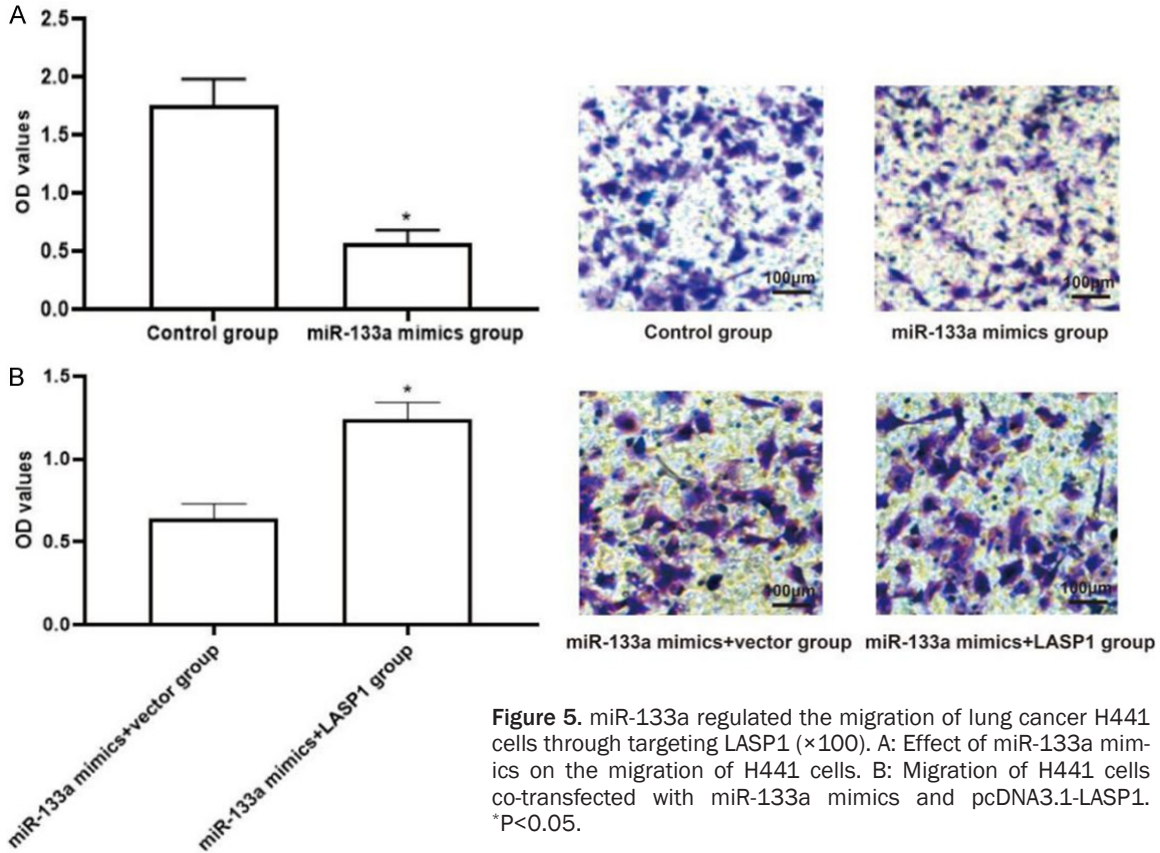
ever, LASP1 overexpression in miR-133a transfected H441 cells could decrease the cell apoptosis, as shown in **Figure 7** ($P<0.05$).
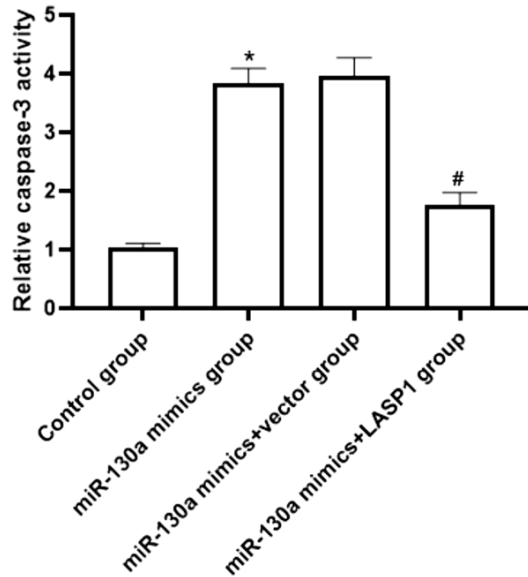
The relationship between LASP1 and MMP-9 expression

As shown in **Figure 8**, the Pearson correlation analysis of the levels of LASP1 and MMP-9 expression in the NSCLC tissues revealed that they were positively associated (r=0.613; $P<0.001$).

Figure 5. miR-133a regulated the migration of lung cancer H441 cells through targeting LASP1 (×100). A: Effect of miR-133a mimics on the migration of H441 cells. B: Migration of H441 cells co-transfected with miR-133a mimics and pcDNA3.1-LASP1. *P<0.05.

Figure 6. miR-133a regulates the invasion of lung cancer H441 cells through targeting LASP1 (×100). A: Effect of miR-133a mimics on the invasion of H441. B: The invasion of H441 cells co-transfected with miR-133a mimics and pcDNA3.1-LASP1. *P<0.05.

Figure 7. miR-133a regulates the apoptosis of lung cancer H441 cells through targeting LASP1. Compared to Control group, *P<0.05; Compared to miR-133a mimics+vector group, #P<0.05.

miR-133a mimics inhibited the MMP-9 expression in lung cancer cells

As seen in **Figure 9**, in contrast to the control group, miR-133a mimics could decrease the MMP-9 expression in H441 cells (P<0.05). However, LASP1 overexpression in miR-133a transfected H441 cells increased the expression levels of MMP-9 (P<0.05).

The relationship of miR-133a expression with clinicopathologic characteristics in lung tumor

The relationship of miR-133a expression with the clinicopathologic characteristics of NSCLC was investigated further. As shown in **Figures 10** and **11**, low expression of miR-133a was correlated to TNM stage and tumor size in NSCLC patients, but not related to other clinical parameters, including gender, age, differentiation and type of tumor, as shown in **Table 1**. The above findings suggest that miR-133a expression was related to NSCLC progression.

miR-133a regulated the LASP1 expression in lung tumor cells

As seen in **Figure 12**, miR-133a mimics significantly decreased the LASP1 expression levels in H441 cells, compared with that in control group (P<0.05).
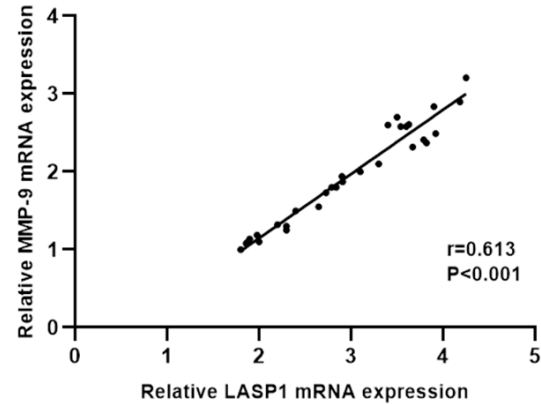
Figure 8. The relationship between LASP1 and MMP-9 expression levels was examined in NISCLC samples (N=30).

*LASP1 targeted regulation by miR-133a as detected by the luciferase reporter gene*

For the LASP1-mutant type, there were merely small differences in the activity of luciferase between the miR-338-3p mimic group and control group (P>0.05). However, for the LASP1 wild type, significantly lower activity of luciferase was found in the miR-133a mimic group (P<0.05), as seen in **Figure 13**.

Discussion

In recent years, the morbidity of NSCLC has been the highest among cancers worldwide. Chemotherapy and operation are the main treatment methods for NSCLC. But the prognosis of patients is unsatisfactory, as result of the postoperative recurrence is common in NSCLC patients [17]. The main causes of recurrence in patients with NSCLC are metastasis and invasive growth, which have been considered as a complex biologic process. It was suggested that in this process, multiple factors influenced one another and interacted with each other and it may involve invasion and migration of tumor cells, extracellular matrix and decomposition of basement membrane, the decreasing of adhesion ability among cells, and other factors [18].

With the developments of molecular biology, targeted treatment is available for cancers. At present, miRNAs have become diagnostic or prognostic markers in different kinds of cancers. The emergence of miRNA has provided new insight into the therapy of tumors and
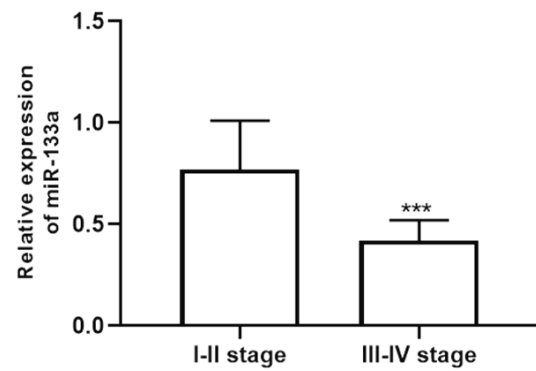
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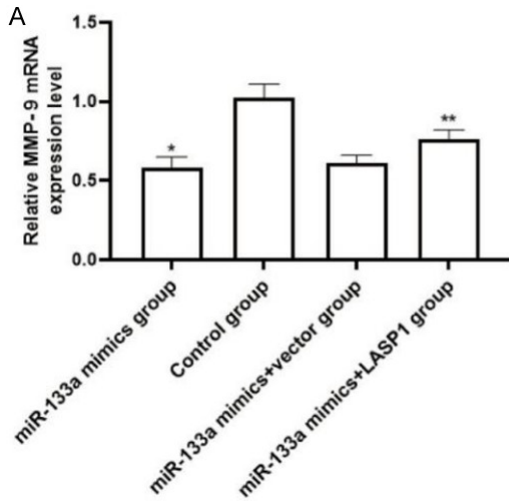
Figure 9. miR-133a regulates MMP-9 expression levels in lung cancer H441 cells through targeting LASP1. A: Relative levels of MMP-9 mRNA expression. B: Relative levels of MMP-9 protein expression. Compared to the Control group, *P<0.05; Compared to the miR-133a mimics+vector group, **P<0.01.

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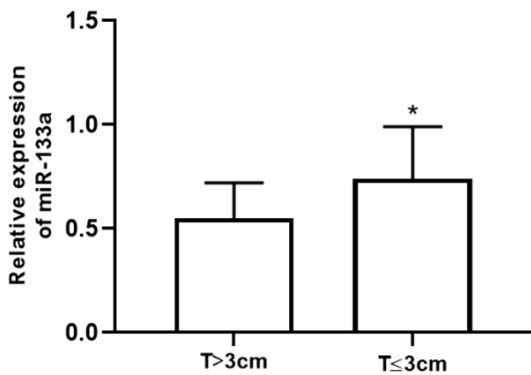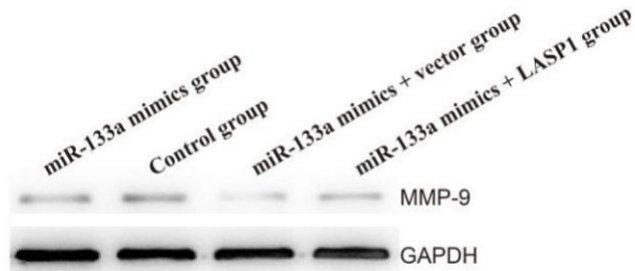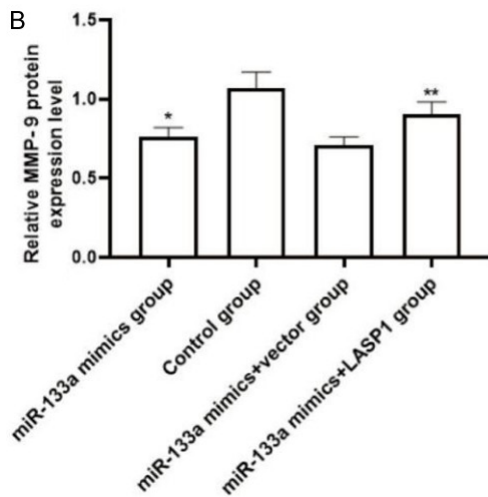
Figure 10. miR-133a expression is reduced in NSCLC tissues with large tumors.

Figure 11. miR-133a expression is reduced in NSCLC tissues with III-IV stages. Compared to the group of stages I-II, ***P<0.001.

molecular mechanisms [10, 19]. miRNAs belong to a class of highly conserved endogenous non-coding small molecule RNA [20]. Increasing evidence has indicated that miRNA dysregulation is closely related to tumor angio-

genesis and extracellular matrix degradation which influence tumor invasion and metastasis. In addition, more miRNAs have been proven to be involved in tumorigenesis and develop-

Table 1. Relationship of miR-133a expression to clinical factors in patients with NSCLC

Factor	Cases	miR-133a expression	T value	P value
Normal lung tissues	40	1.04±0.24	8.644	<0.001
NSCLC	40	0.63±0.18		
Age (years old)				
>60	24	0.65±0.21	0.657	0.515
≤60	16	0.61±0.15		
Gender				
Male	26	0.64±0.23	0.278	0.783
Female	14	0.62±0.19		
Size of Tumor				
>3 cm	13	0.55±0.17	2.471	0.018
≤3 cm	27	0.74±0.25		
Type				
Squamous cell	19	0.66±0.27	0.938	0.354
Adenocarcinoma	21	0.59±0.20		
Differentiation				
Low	15	0.54±0.16	1.860	0.071
Middle and High	25	0.67±0.24		
TNM stage				
I-II stage	27	0.77±0.24	5.025	<0.001
III-IV stage	13	0.42±0.10		

ment of cancer [21, 22]. miR-21 dysregulation was found in breast cancer, and it promoted the migration and invasion of cells through regulating the TIMP-3 expression. Many studies revealed that miR-148b, miR-758, and other miRNAs might influence tumor suppressors or oncogenes which participate in the progression of NSCLC [23, 24]. Another study reported that miR-1258 as a tumor suppressor could suppress the development of NSCLC [25]. Therefore, the miRNA dysregulation is correlated with NSCLC, and studies into the association of NSCLC with miRNAs are promising.

Further studies have revealed that miR-133a expression in a variety of cancers was significantly reduced [26], but the role of miR-133a in NSCLC still remained unclear. To study the effects of miR-133a on the progression of NSCLC, the miR-133a expression was determined in lung cancer H441 cell line with high metastatic ability as well as lung tumor tissues by qPCR in this study. The roles of miR-133a mimics in proliferation, migration, invasion and apoptosis of lung cancer cell lines were examined by CCK-8 test, Transwell migration assay, invasion assay, and caspase-3 activity assay. This study revealed that the miR-133a expres-

sion in H441 cells was lower than that of human normal lung epithelial BEAS-2B cells (P<0.05), and miR-133a overexpression could reduce the proliferation, migration, and invasion of lung cancer cells, and promote cell apoptosis (P< 0.05), indicating that miR-133a negatively regulated the proliferation, invasion and migration of lung cancer cell lines. Moreover, the relationship of miR-133a expression to the clinicopathological characteristics of NSCLC patients was also explored further. The results showed that in NSCLC patients a low level of miR-133a expression was related to TNM stage and tumor size, but the down-regulation of miR-133a expression was not related to other clinical factors, including gender, age, differentiation, and type of tumor. Therefore, it was considered that miR-133a may serve as a suppressor of tumor in controlling the development of NSCLC, which is similar to results reported in other studies [27, 28].

Matrix metalloproteases (MMPs) are a class of proteolytic enzymes that degrade extracellular matrix and play an important role in the migration and invasion of tumors. Among them, MMP-9 not only degrades extracellular matrix, but also breaks down the basement membrane, thereby increasing cell migration ability to promote the metastasis and proliferation of tumor [29]. Many studies reported that the expression of MMP-9 in lung cancer tissues was higher than in normal tissues [30, 31]. MMP-9 overexpression could increase the invasion of the tumor, affecting the prognosis [32]. Previous studies revealed that miRNAs could regulate the expression of MMP-9 in the invasion and migration process of cancer cells [33, 34]. Our study found that miR-133a mimics could inhibit MMP-9 expression in lung tumor cell lines by western blot. This suggested that miR-133a overexpression might affect the migration and invasion of lung tumor cell lines by regulating the MMP-9 expression, thus improving the prognosis of NSCLC patients.

The target genes controlled by miR-133a in the development of NSCL tumor growth are still
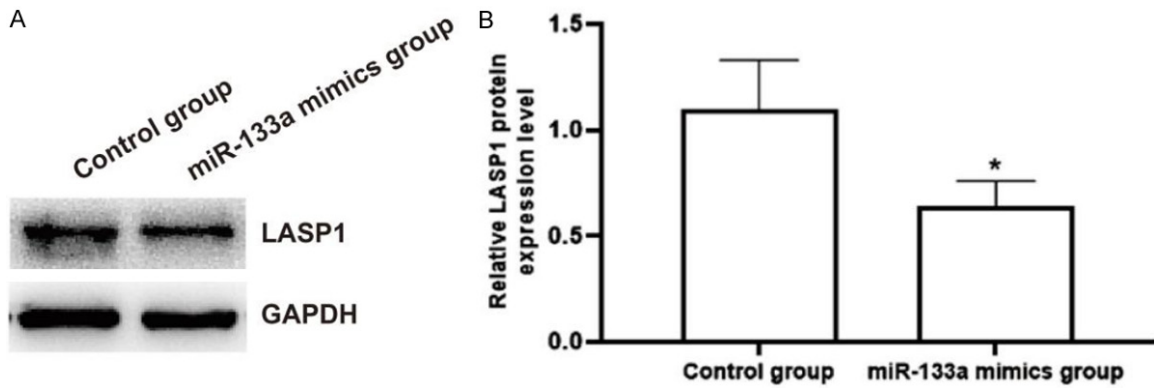
Figure 12. Effects of miR-133a mimics on the expression of LASP1 in H441 cells. A: Western blot of LASP1; B: Relative expression of LASP1 protein. *P<0.05.
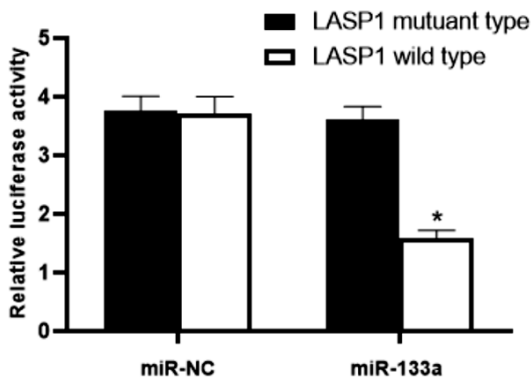
Figure 13. Targeting relationship between LASP1 and miR-133a as detected by luciferase reporter gene assay. *P<0.05.

unclear. In order to investigate the detailed regulating mechanism of miR-133a in the growth of NSCLC, we performed the luciferase reporter gene analysis. The results of this study revealed that LIM and SH3 domain protein 1 (LASP1) were the targeted genes of miR-133a, and western blot assays also showed that miR-133a mimics could significantly reduce the expression levels of LASP1 in H441 cells. LASP1 plays a vital role in some pathological and biological processes. Previous studies reported that LASP1 could promote the growth, invasion and metastasis of cancer cells as an oncogene [35]. It was reported that LASP1 was up-regulated in promote the progression of colorectal tumor cells [36]. In terms of NSCL, it was found that LASP1 overexpression could promote the invasion and proliferation of tumor cells [27, 37]. In this study, the results showed that LASP1 could promote the expression of MMP-9 in H441, which was confirmed to be

associated with development of tumor. The underlying mechanism may be correlated with regulation of transcription factor activator protein-1 expression [38]. However, the specific mechanism is still not clear, and this will need future research. Another study showed that LASP1 expression was related to poor prognosis in lung cancer patients [39]. Taken together, LASP1 is a targeted gene of miR-133a, participates in the regulation of miR-133a in the proliferation, migration, apoptosis, and invasion of NSCLC cells.

This study has some limitations. It was revealed that the level of miR-133a expression was decreased in lung cancer cell lines and NSCLC tissues. However, the number of clinical samples was small. Further confirmation with a larger number of clinical tissues is needed. Moreover, the specific signal transduction pathways for miR-133a regulating the progression of lung cancer are still unclear. The signal transduction pathways following LASP1 expression should be further investigated. In this study, a low level of miR-133a expression was related to TNM stage and size of tumor. However, clinical information is still needed to verify whether miR-133a expression affects the prognosis of NSCLC patients.

In conclusion, miR-133a overexpression could decrease the invasion, proliferation and migration, but promote the apoptosis of lung cancer cells. Moreover, it downregulated the expression of matrix metalloproteases. We also confirmed that miR-133a expression played important roles in NSCLC progression by targeted regulation of LASP1 expression, indicating that

miR-133a may be a potent antitumor target for NSCLC therapy. In future, it is necessary to make further exploration of related signaling pathways that are regulated by miR-133a, so as to provide more evidence for the treatment of NSCLC.

Disclosure of conflict of interest

None.

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