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MicroRNA-155-5p suppresses the migration and invasion of lung adenocarcinoma A549 cells by targeting *Smad2*

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Abstract. Lung cancer is one of the major causes of cancer-related deaths worldwide. Notably, miR-155-5p is one of the most amplified miRNAs in non-small cell lung carcinoma (NSCLC). However, the role of miR-155-5p in lung cancer metastasis has not been fully evaluated. In the present study, miR-155-5p mimic and inhibitor were used to investigate the effects of miR-155-5p on the metastasis of human lung carcinoma A549 cells. The study indicated that transfection of miR-155-5p mimic significantly suppressed cell proliferation, migration and invasion of A549 cells, whereas its inhibition significantly promoted cell proliferation, migration and invasion of A549 cells, suggesting a potential therapeutic application of miR-155-5p in controlling lung cancer metastasis. Moreover, transfection of miR-155-5p mimic suppressed the expression of *Smad2/3*, *ZEB1*, *ZEB2* and N-cadherin and induced that of E-cadherin, whereas its inhibition significantly upregulated the expression of Smad2/3, ZEB1, ZEB2 and N-cadherin and downregulated that of E-cadherin. Collectively, the findings suggest that miR-155-5p suppresses the proliferation, migration and invasion of A549 cells. Therefore, loss of miR-155-5p may serve an essential role in tumorigenesis and tumour progression in lung cancers.

Introduction

Lung cancer is a particularly aggressive disease, resulting in over 158,000 deaths a year in the United States (1,2). Non-small cell lung cancer (NSCLC) accounts for ~80% of all lung cancers (3,4). Metastatic progression is the main factor that results in the poor prognosis of patients (5). Although advanced clinical treatments have been developed, the 5-year survival rate of lung cancer is 18%, which is not markedly improved compared with the previous one (13%) (1,6). Hence, there is an urgent need to further explore the biological mechanism underlying the metastasis in patients with NSCLC.

Epithelial-mesenchymal transition (EMT) is a biological process in which epithelial cells lose their polarity and cell-cell adhesion, and is a critical step for the initiation of cancer metastasis (7-10). After acquiring a mesenchymal phenotype due to EMT, carcinoma cells invade adjacent tissues, penetrate the basement membrane and eventually enter the bloodstream, thereby leading to cancer metastasis (7-12). Transforming growth factor-β (TGF-β) plays an essential role in cancer metastasis through the phosphorylation of Smad2/3 (13,14). Furthermore, the activation of Smad2/3 induces the metastasis of lung carcinoma cells by binding to Smad4 and translocating to the nucleus to regulate the expression of target genes, including members of the zinc finger E-box-binding homeobox (*ZEB*) transcription factor family (11,12,15,16). On activation, *ZEB* transcription factors suppress epithelial marker gene expression (E-cadherin) and induce mesenchymal gene expression (N-cadherin), leading to EMT and cancer metastasis (10,17).

MicroRNAs (miRNAs) are small non-coding RNA molecules, typically 19-24 nucleotides long, that regulate hundreds of target genes primarily by translational inhibition or mRNA degradation (18,19). Consequently, miRNAs are involved in various biological processes, including cell proliferation, apoptosis, metastasis, metabolism, differentiation, immune function and oncogenesis (20-24). An increasing number of studies have indicated that miRNA dysregulation in lung cancer contributes to the development and progression of lung cancer, thus acting as oncogenes or tumour suppressor genes (25,26). Recent studies have revealed that miR-155 is upregulated in several tumour tissues, including lung cancer (27-31), and that high expression of miR-155-5p is significantly associated with poor overall survival of patients with lung cancer (32,33). Moreover, functional studies have indicated that upregulation of miR-155-5p significantly promotes proliferation, migration

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Abbreviations: miR-155-5p, microRNA-155-5p; NSCLC, non-small cell lung cancer; EMT, epithelial-mesenchymal transition

Key words: microRNA-155-5p, non-small cell lung cancer, migration, invasion, *Smad2*

and invasion, but inhibits apoptosis of lung cancer cells *in vitro* and *in vivo* (31,34,35). Therefore, miR-155 is predominantly thought to be an onco-miRNA. Interestingly, recently studies have also indicated that increase of miR-155 in primary breast tumor was correlated with better outcome in patients and significantly suppressed the development of metastasis (36,37). In addition, miR-155 inhibits the extravasation and colonisation of cancer cells in distant organs (38) and promotes the apoptosis of ovarian cancer cells (39). These findings highlight the urgent need to further confirm the role of miR-155.

miR-155-5p plays an important role in TGF-β-mediated fibrosis, angiogenesis and immunity by directly suppressing *Smad2* expression in human fibroblasts (40,41), which led us to hypothesise that miR-155 may play an important role in EMT via the TGF-β/Smad2 signalling pathway. Therefore, in the present study, we investigated the role of miR-155-5p in EMT and in controlling the expression of *Smad2*. we also assessed the role of miR-155-5p on proliferation, migration and invasion of A549 cells by transfection of its mimic or inhibitor, and further assessed its regulatory effect on its target gene, i.e. *Smad2* and downstream genes.

Materials and methods

Materials and reagents. Roswell Park Memorial Institute (RPMI)-1640 medium, fetal bovine serum (FBS), penicillin-streptomycin, trypsin-EDTA, trypan blue, Opti-MEM medium, Lipofectamine RNAiMAX transfection reagent, BCA Protein Assay Kit and Chemiluminescence (ECL) Detection Kit were obtained from Thermo Fisher Scientific, Inc. (Waltham, MA, USA). RNAiso for small RNA, Mir-X miRNA First-Strand Synthesis Kit and SYBR PrimeScript miRNA RT-PCR Kit were provided by Dalian Takara Biotechnology Co., Ltd. (Dalian, China). Crystal violet reagent was purchased from Amresco (Solon, OH, USA). Transwell chambers were obtained from Corning Life Sciences (Tewksbury, MA, USA). BD BioCoat Matrigel invasion chambers were purchased from BD Biosciences (San Jose, CA, USA). N-cadherin and E-cadherin antibodies were purchased from Abcam (HK) Ltd. (Hong Kong, China). Smad2/3, ZEB1, ZEB2 and β-actin antibodies and horseradish peroxidase (HRP)-conjugated secondary antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA). All other chemicals, unless otherwise stated, were obtained from Sigma-Aldrich; Merck KGaA (Darmstadt, Germany).

Cell culture. Human lung carcinoma A549 cells were obtained from the cell bank of the Chinese Academy of Science (Shanghai, China). The cells were grown in RPMI-1640 containing 10% (v/v) FBS, 100 U/ml penicillin and 100 μg/ml streptomycin. Cells were cultured at 37˚C in a humidified incubator with 5% CO_2.

The transfection of miR-155-5p mimic or inhibitor. To elucidate the effect of miR-155-5p on the metastasis of A549 cells, the cells were transfected with miR-155-5p mimic or inhibitor (25, 50 or 100 nM) or respective negative control (NC) according to the manufacturer's protocol. Briefly, the cells were seeded onto 6-well plates at a density of $2x10^5$ cells/well in 2 ml of medium and allowed to adhere overnight until they reached 30-50% confluence. Then, the cells were transfected with various concentrations of miR-155-5p mimic or inhibitor using Lipofectamine RNAiMAX transfection reagent according to the manufacturer's instructions for 6 h, after which a complete medium without miR-155-5p mimic or inhibitor was added. After 48 h of transfection, the cells were used for further analysis. Human miR-155-5p mimic and inhibitor as well as corresponding NCs of the miRNA mimic (NC) and inhibitor (iNC) were synthesised by Shanghai GenePharma Co., Ltd. (Shanghai, China) with the following sequences: human miR-155-5p mimics (sense, 5'-UUAAUGCUAAUCGUGAUAGGGGU-3' and antisense, 5'-CCCUAUCACGAUUAGCAUUAAUU-3'); NC (sense, 5'-UUCCUCCGAACGUGUCACGUTT-3' and antisense, 5'-ACGUGACACGUUCGGAGAATT-3'); human miR-155-5p inhibitor (5'-ACCCCUAUCACGAUUAGCAUUAA-3') and iNC (5'-CAGUACUUUUGUGUAGUACAA-3').

Observation of cell morphology and calculation of cell number. A549 cells were transfected with different concentrations of miR-155-5p mimic or inhibitor for 48 h. The cell morphology was observed using a phase-contrast microscope (Leica Microsystems Ltd., Wetzlar, Germany). Photographs were recorded at a magnification of 200x. Trypan blue exclusion test was then used to calculate the number of live cells using a Countstar automatic cell counter (Shanghai Ruiyu Biotech Co., Ltd., Shanghai, China) according to the manufacturer's protocol.

Colony formation assay. A549 cells were seeded onto 6-well plates at a density of $2x10^5$ cells/well in 2 ml of medium. After transfection with miR-155-5p mimic or inhibitor (25, 50 and 100 nM) for 48 h, the cells were collected and diluted in a medium without the miR-155-5p mimic or inhibitor and then reseeded onto 12-well plates at a density of $5x10^2$ cells/well. Following incubation for 8-10 days at 37˚C in a humidified incubator with 5% CO_2, the colonies were fixed with 4% paraformaldehyde, stained with 0.01% crystal violet and counted. Cell survival was calculated by normalising the colonies of the control cells as 100%.

Wound-healing assay. The migration of A549 cells was examined using the wound-healing assay. After transfection with the indicated concentrations of miR-155-5p mimic or inhibitor for 48 h, the cells were collected and diluted in fresh medium and then reseeded onto 12-well plates at a density of $5x10^5$ cells/well. After 24 h of incubation, the cells were vertically scraped from each well using a P100 pipette tip. Three randomly selected views along the scraped line were photographed in each well using a phase-contrast microscope at a magnification of x100. Another set of images was obtained using the same method. A reduction in the scraped area was calculated and indicated cell migration.

Cell migration and invasion assays. Migration assays were performed using Transwell cell culture chambers with 8-μm pore filters (Corning Life Sciences). Following the transfection of miR-155-5p mimic or inhibitor (25, 50 or 100 nM) for 48 h, A549 cells were trypsinised and resuspended in a serum-free medium. A total of $5x10^4$ cells in 200 μl of serum-free RPMI-1640 were plated in the upper chambers. RPMI-1640

medium containing 10% (v/v) FBS was used in the lower chambers as a chemoattractant. The cells were allowed to migrate for 12 h in a 37˚C humidified incubator, following which the non-migrated cells were removed from the upper surface of the Transwell membrane using a cotton swab. Membranes were then stained with 0.01% crystal violet. For quantification, the average number of migrating cells per field was assessed by counting three random fields under a Leica phase-contrast microscope at a magnification of x100. For the cell invasion assay, after transfection with 50 nM of miR-155-5p mimic or inhibitor for 48 h, similar procedure as in the migration assay was used; however, the upper chambers were coated with Matrigel matrix (BD Biosciences), and cell invasion was allowed to proceed for 24 h.

RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA from A549 cells transfected with miR-155-5p mimic or inhibitor (25, 50 or 100 nM) for 48 h was isolated using RNAiso for small RNA and reverse-transcribed using the Mir-X miRNA First-Strand Synthesis Kit according to the manufacturer's instructions. The resulting cDNA was used to determine the expression of miR-155-5p; U6 was used as an internal control.

Western blot analysis. After transfection with 50 nM of miR-155-5p mimic or inhibitor for 48 h, the cells were washed with PBS and lysed in RIPA buffer plus protease inhibitors (Roche Applied Science, Mannheim, Germany). The supernatants were collected by centrifugation at 14,000 rpm at 4˚C for 20 min and stored at -80˚C. The protein concentrations were measured using the BCA Protein Assay Kit. Total protein (50 μg) was separated by electrophoresis on a 12% sodium dodecyl sulphate (SDS)-polyacrylamide gel and transferred to NC membranes (EMD Millipore, Darmstadt, Germany). Non-specific protein interactions were blocked by incubation with 5% non-fat milk in a Tris-buffered saline with Tween-20 (TBST) buffer at room temperature for 2 h. The membranes were incubated with primary antibodies (1:1,000 dilution) overnight, followed by incubation with HRP-conjugated secondary antibodies (1:5,000 dilution). Proteins were detected using a chemiluminescence (ECL) detection system and visualised using an enhanced chemiluminescence method (Bio-Rad, Hercules, CA, USA). The protein expression was normalised to an endogenous reference (β-actin) relative to the control.

Statistical analysis. Data were expressed as the mean ± SD. Statistical analysis of the data was performed using the SPSS package for Windows (version 16.0). The differences between the groups were analysed using the Student's t-test or one-way analysis of variance (ANOVA). A P-value of <0.05 was considered to be statistically significant.

Results

Effects of miR-155-5p mimic or inhibitor on the expression of miR-155-5p in A549 cells. To explore the physiological function of miR-155-5p in human lung cancer cells, A549 cells were transfected with miR-155-5p mimic or inhibitor, and RT-qPCR was performed to determine the expression of miR-155-5p. As shown in Fig. 1, the expression of miR-155-5p was significantly upregulated following mimic treatment and downregulated following inhibitor treatment compared with corresponding NCs (P<0.05).

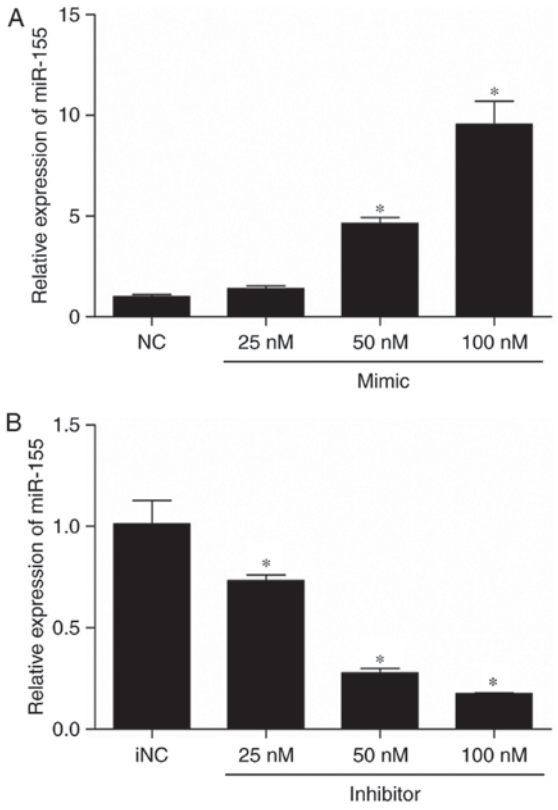

Figure 1. Effect of miR-155-5p expression following inhibitor or mimic treatment in A549 cells. The expression of miR-155-5p in A549 cells was analysed by using RT-qPCR assay after transfection with miR-155-5p mimic (A) or inhibitor (B) for 48 h. U6 was used as an internal control. *P<0.05 vs. NC or iNC. NC, negative control; iNC, inhibitor negative control.

Effects of miR-155-5p on A549 cell growth. The effect of miR-155-5p on the growth of A549 cells was assessed by observing the cell confluence using microscopy. As shown in Fig. 2A and B, miR-155-5p upregulation following mimic treatment significantly decreased cell confluence compared with NC, and cells became rounded and shrunken and even detached from each other or floated in the medium. In contrast, miR-155-5p inhibition increased A549 cell confluence. To further verify the results, we counted the cell number and confirmed that miR-155-5p upregulation significantly decreased the cell number in a dose-dependent manner compared with NC (Fig. 2C, P<0.05). Furthermore, the inhibition of miR-155-5p significantly increased A549 cell number compared with iNC (Fig. 2D, P<0.05). Taken together, these data demonstrated that the upregulation of miR-155-5p suppressed cell growth, whereas its downregulation significantly promoted cell growth in A549 cells.

Effects of miR-155-5p on the survival of A549 cells. To evaluate the survival of lung cancer cells after miR-155-5p mimic or inhibitor treatment, we detected cell survival using the colony formation assay. As shown in Fig. 3A and C, the upregulation of miR-155-5p significantly reduced the cell

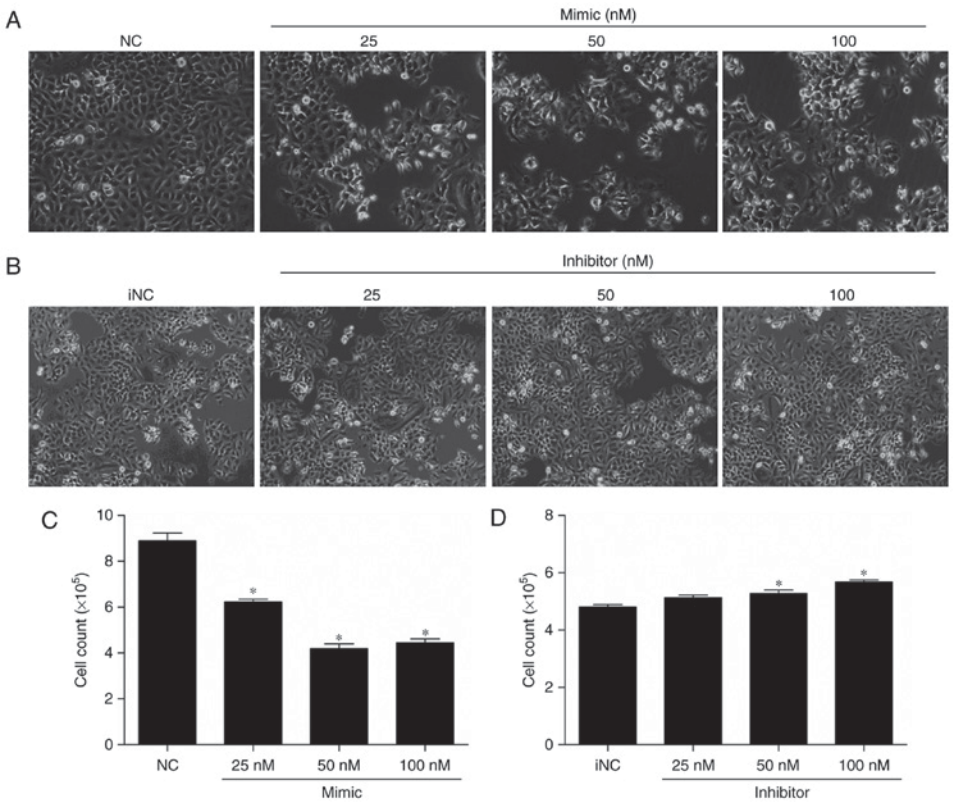

Figure 2. Effect of miR-155-5p on A549 cell growth. A549 cells were transfected with miR-155-5p mimic or inhibitor for 48 h. (A and B) Morphological changes observed using phase-contrast microscopy. The photographs were obtained at a magnification of x200. Images are representative of three independent experiments. (C and D) Cell number was counted using Countstar automatic cell counter and statistically analysed. Data were normalised to the number of surviving control cells and are shown as averages with SD (error bars) from three independent experiments. *P<0.05 vs. NC or iNC. NC, negative control; iNC, inhibitor negative control.

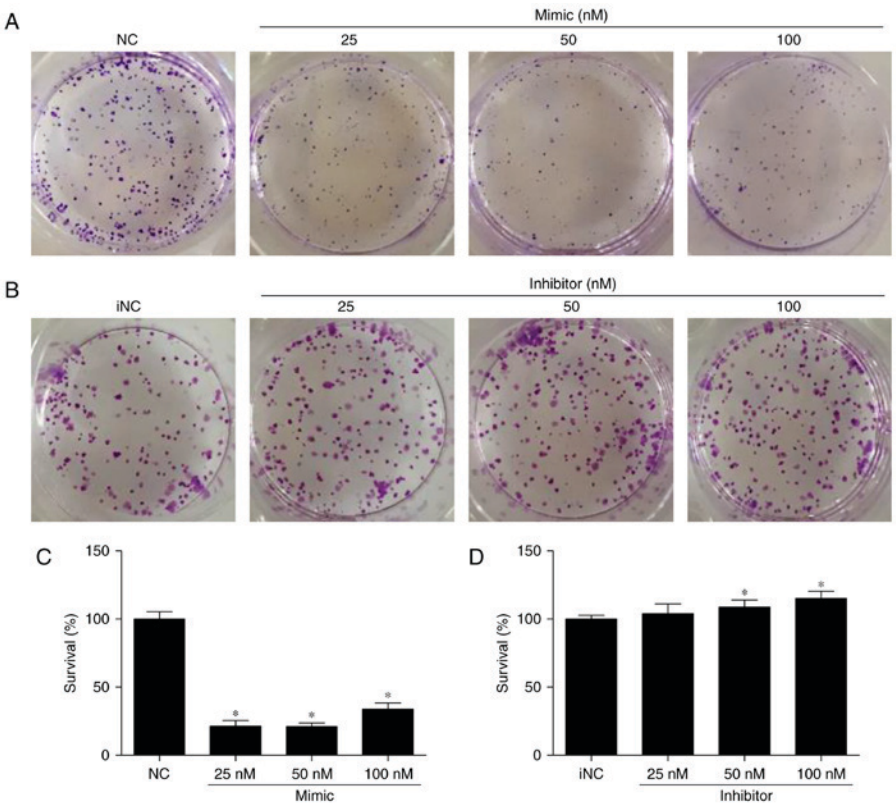

Figure 3. Effects of miR-155-5p on colony formation in A549 cells. Cell survival was determined by colony formation analysis after transfection of A549 cells with miR-155-5p mimic (A and C) or inhibitor (B and D) for 48 h. Images are representative of three independent experiments. Data were normalised to the surviving control cells and are shown as averages with SD (error bars) from three independent experiments. *P<0.05 vs. NC or iNC. NC, negative control; iNC, inhibitor negative control.

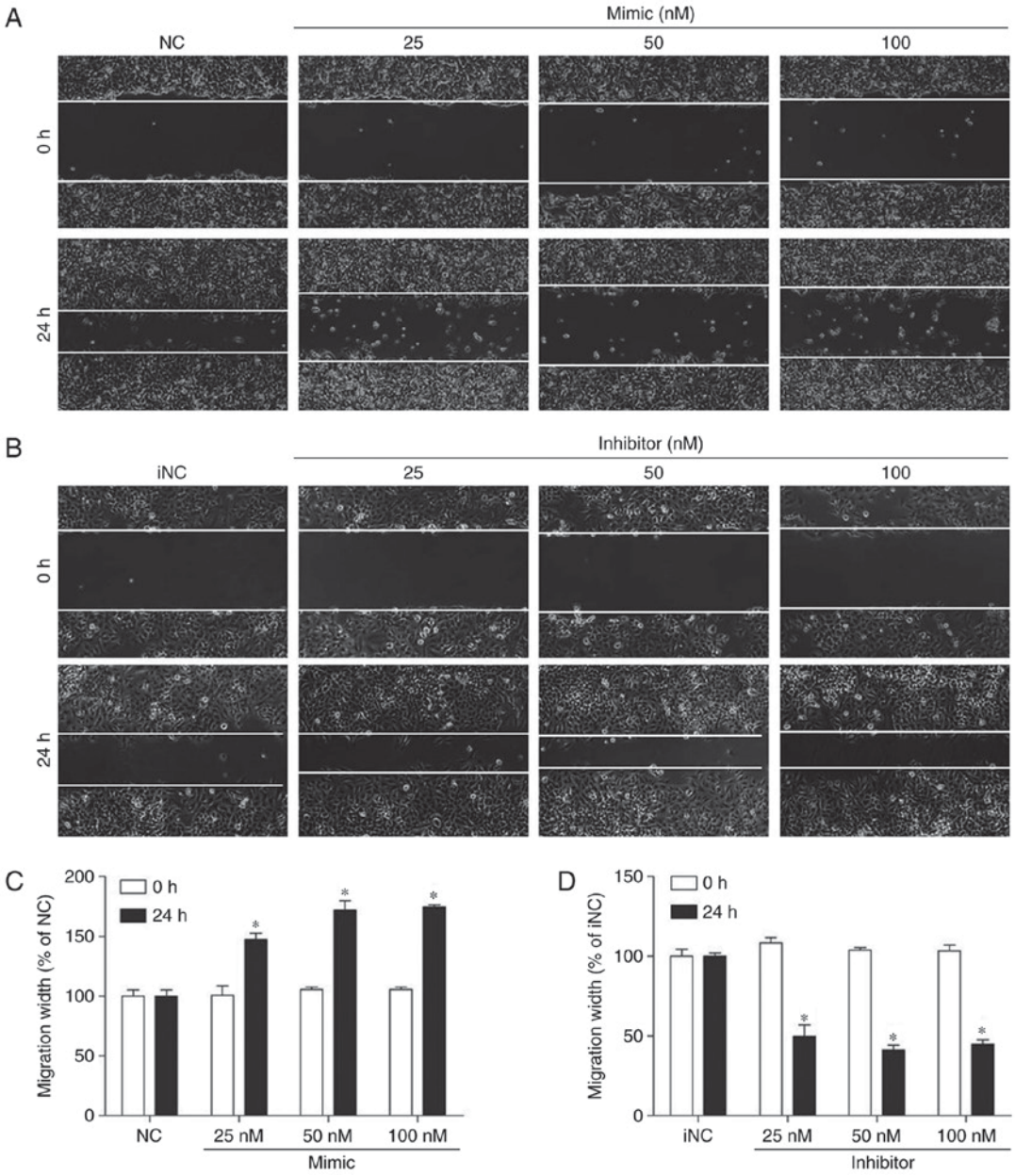

Figure 4. Effect of miR-155-5p on wound healing in A549 cells. After transfection with miR-155-5p mimic (A) or inhibitor (B) for 48 h, the migration of A549 cells was observed using phase-contrast microscopy. Images were captured at a magnification of x100. Images are representative of three independent experiments. (C and D) The distance of A549 cells transfected with miR-155-5p mimic (C) or inhibitor (D) was calculated and were normalised to the distance of control cells and are shown as averages with SD (error bars) from three independent experiments. *P<0.05 vs. NC or iNC. NC, negative control; iNC, inhibitor negative control.

survival rate (P<0.05), whereas its knockdown significantly increased the cell survival rate (Fig. 3B and D, P<0.05). These data suggested that miR-155-5p plays an important role in the survival of lung cancer cells.

Effects of miR-155-5p on A549 cell migration. To explore the role of miR-155-5p in human lung cancer metastasis, we first performed a wound-healing assay to evaluate the effect of miR-155-5p on the migration of A549 cells. As shown in Fig. 4A and B, after wounding for 24 h, NC cells migrated into the clear area. However, transfection with miR-155-5p mimic inhibited A549 cell migration in a dose-dependent manner (Fig. 4A and C), whereas transfection with miR-155-5p inhibitor significantly increased A549 cell migration (Fig. 4B and D). We further verified these results using the Transwell assay. As shown in Fig. 5, transfection with miR-155-5p mimic for 24 h markedly decreased cell migration rate, whereas that with miR-155-5p inhibitor significantly increased the cell migration rate of A549 cells compared with NC or iNC (P<0.05).

Effects of miR-155-5p on A549 cell invasion. We determined the effect of miR-155-5p on the invasion capacity of A549 cells using the Transwell assay. As shown in Fig. 6A and B, the invasion rate of A549 cells following transfection with 50 nM of miR-155-5p mimic was 38.52±1.7% and that with 50 nM of miR-155-5p inhibitor was 132.04±9.70% compared with NC and iNC cells (100%), respectively (P<0.05), suggesting that miR-155-5p upregulation significantly suppressed the invasion capacity, whereas miR-155-5p

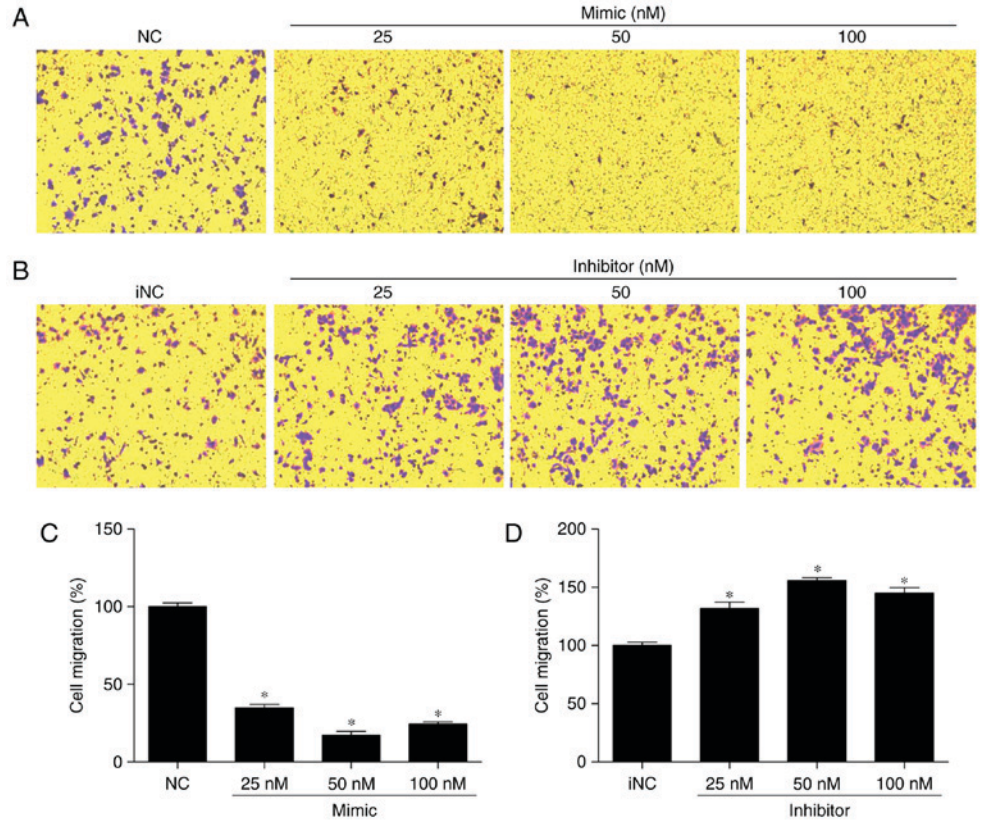

Figure 5. Effect of miR-155-5p on A549 cell migration. A549 cells were transfected with miR-155-5p mimic or inhibitor for 48 h. (A and B) The migration of A549 cells was determined using trans-well cell culture chambers. Cells were stained with 0.01% crystal violet. Photographs were obtained at a magnification of x100. (C and D) The average number of migrated cells was counted in three randomly selected fields. The data were normalised to the migration of A549 cells (100%). Data are shown as averages with SD (error bars) from three independent experiments. *P<0.05 vs. NC or iNC. NC, negative control; iNC, inhibitor negative control.

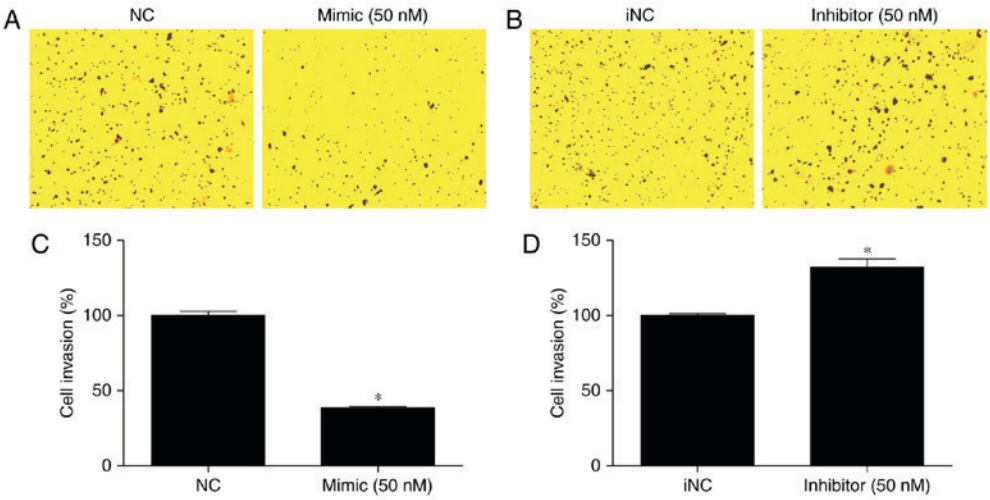

Figure 6. Effect of miR-155-5p on A549 cell invasion. A549 cells were transfected with miR-155-5p mimic or inhibitor for 48 h. (A and B) The invasion capacity of A549 cells was determined using trans-well cell culture chambers with membranes (8 μM) coated with Matrigel matrix. Cells were stained with 0.01% crystal violet. Photographs were obtained at a magnification of x100. (C and D) The average number of invasive cells was counted in three randomly selective fields. The data were normalised to the invasion capacity of A549 cells (100%). Data are shown as averages with SD (error bars) from three independent experiments. *P<0.05 vs. NC or iNC. NC, negative control; iNC, inhibitor negative control.

inhibition significantly promoted the invasion capacity of lung cancer cells.

Modulation of metastasis-related gene expression in A549 cells due to miR-155-5p. To explore the mechanism underlying the suppression of migration and invasion in lung cancer cells by miR-155-5p, we determined the protein expression of *Smad2/3*, which was one of the target genes of miR-155-5p, and its downstream targets, including *ZEB1*, *ZEB2*, N-cadherin and E-cadherin using western blot analysis. As shown in Fig. 7, the protein expression levels of *Smad2/3*, *ZEB1*, *ZEB2* and N-cadherin were downregulated, whereas that of E-cadherin

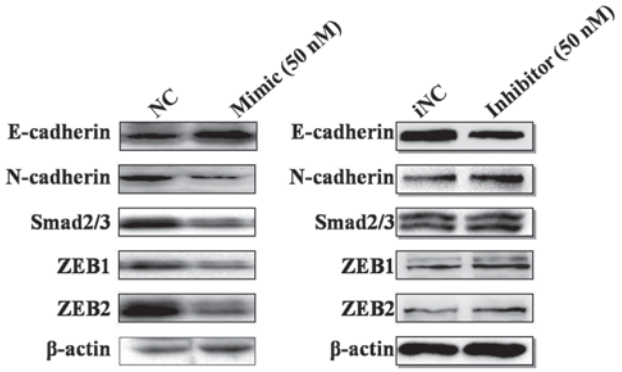

Figure 7. Effect of miR-155-5p on the expression of metastasis-related factors in A549 cells. A549 cells were transfected with miR-155-5p mimic or inhibitor for 48 h. The protein expression levels of E-cadherin, N-cadherin, ZEB1, ZEB2 and Smad2/3 in A549 cells were determined by Western blot analyses. β-actin was used as the internal control. Images are representatives of three independent experiments. ZEB, zinc finger E-box-binding homeobox.

was upregulated after transfection with miR-155-5p mimic. Conversely, the protein expression levels of *Smad2/3*, *ZEB1*, *ZEB2* and N-cadherin were upregulated, whereas that of E-cadherin was downregulated after transfection with miR-155-5p inhibitor. Taken together, the upregulation of *Smad2/3*, *ZEB1*, *ZEB2* and N-cadherin expression and downregulation of E-cadherin expression may be involved in the promotion of migration and invasion by miR-155-5p in lung cancer cells.

Discussion

Present study suggested that differential miRNA expression levels in lung cancer may be involved in the development and progression of lung cancer, and may serve as biomarkers for lung cancer diagnosis and prognosis (42-44). Furthermore, miR-155-5p acts as a multi-functional miRNA in many pathophysiological processes, including immunology, inflammation, angiocardiopathy and carcinogenesis (32,45,46). In addition, miR-155-5p is one of the most important miRNAs involved in tumour development and progression. Various studies have shown that miR-155-5p is overexpressed in various tumour types, and is significantly upregulated in lung cancer cells (32,33). Moreover, the downregulation of miR-155 expression significantly suppressed the metastasis of lung cancer cells *in vitro* and *in vivo* (34,35). However, recent studies indicated that a high expression of miR-155 was correlated with better outcomes in patients with triple-negative breast cancer (36,37), and that increase expression of miR-155 significantly suppressed the migration and invasion of cancer cells *in vitro* and *in vivo* (38). The above findings indicated that miR-155 exhibited contrasting roles in the metastasis of different cancer cells. To further confirm the findings of a previous study on lung cancer, we determined the effect of miR-155-5p on the migration and invasion of A549 cells using miR-155-5p mimic and inhibitor.

Using wound-healing and Transwell assays, we found that the upregulation of miR-155-5p significantly suppressed the migration and invasion of A549 cells, whereas its inhibition significantly increased the migration and invasion of A549 cells, which is contradictory to previous studies on lung cancer cells and consistent with studies on breast and other cancer cells (36,37). The contrasting role of miR-155-5p in the same cell line in different studies further highlighted the urgent need to elucidate the various mechanisms through which miR-155-5p is involved in cancer cell metastasis. Indeed, in future studies, we will continue to establish the reasons for the differences in findings between various studies and explore the underlying mechanisms.

EMT is a biological process and critical step for the initiation of cancer metastasis. The TGF-β/Smad2/3 signalling pathway plays an essential role in this process by increasing the transcriptional activity of downstream targets, including *ZEB1/2*, which suppresses epithelial marker gene expression (E-cadherin) and induces mesenchymal biomarker gene expression (N-cadherin), leading to EMT and cancer metastasis (10,17). To further explore the mechanism underlying miR-155-5p involvement in EMT and metastasis, using western blotting, we found that the transfection of miR-155-5p mimic reduced the protein expression of the mesenchymal marker N-cadherin, but increased that of the epithelial marker E-cadherin. In contrast, the inhibition of miR-155-5p increased the protein expression of N-cadherin and decreased that of E-cadherin, indicating that miR-155-5p significantly inhibits EMT and metastasis of lung cancer cells. Because *Smad2/3* has been shown to be the direct target gene for miR-155-5p (40,41), to further explore the mechanism underlying the role of miR-155-5p in EMT, we performed western blotting and found that the expression of *Smad2/3* was significantly upregulated in A549 cells after miR-155-5p knockdown but downregulated after miR-155-5p upregulation. Moreover, it has been reported that the activation of Smad2/3 induces the migration of lung carcinoma cells (15,16) by translocating to the nucleus and regulating the expression of target genes, including *ZEB* transcription factors (11,12), thereby suppressing the epithelial marker gene expression and inducing the mesenchymal gene expression, leading to EMT and cancer metastasis (10,17). Thus, we determined the expressions of *ZEB1* and *ZEB2* and found that miR-155-5p knockdown increased these gene expressions, whereas miR-155-5p upregulation significantly decreased them. These findings indicated that miR-155-5p significantly suppressed the Smad2/ZEB signalling pathway. However, the effect of miR-155-5p on the expression of *Smad2* in lung cancer cells should be further confirmed. Therefore, we will continue to identify and evaluate the effect of miR-155-5p on its target genes involved in metastasis of lung cancer cells.

In conclusion, the present study indicated that miR-155-5p inhibited the migration and invasion of lung cancer cells, and that the downregulation of its direct target gene expression (*Smad2/3*) may be involved as the underlying mechanism. Several limitations need to be addressed in our study. First, we validated the expressions of these target genes only using western blotting. However, additional detailed functional assays, such as qPCR and luciferase reporter assay, should be performed. Second, we only detected the effect of miR-155-5p in migration and invasion of A549 cells *in vitro*; this effect should also be studied *in vivo*. Most importantly, we assessed the effect of miR-155-5p only in A549 cells. However, other lung cancer cell lines and cancer cell lines should be evaluated in further studies.

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Availability of data and materials

The authors declare that all the data supporting the findings of this study are available within the article.

Authors' contributions

WZ conceived of and directed the project. JL and YC designed the experiments. JL, YC, LL and AS performed the experiments. JL, YC and LL conducted the data analysis. YC and AS drafted the manuscript. WZ revised and edited the manuscript. All authors have reviewed the manuscript and approved it for publication.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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