

miR-1225-5p inhibits non-small cell lung cancer cell proliferation, migration and invasion, and may be a prognostic biomarker

BIN LI¹, FENGMING ZHANG² and HONG LI³

Departments of ¹Respiratory Medicine, ²Obstetrics and ³Public Health, Shouguang People's Hospital, Shouguang, Shandong 262700, P.R. China

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Abstract. Non-small cell lung cancer (NSCLC) is a malignant tumor, which presents with a high 5-year mortality rate owing to the lack of an effective early screening tool and the absence of obvious early symptoms. MicroRNAs (miRs/miRNAs) have attracted increasing attention due to their significant clinical value in the diagnosis and prognosis of various human malignancies. The present study aimed to investigate the expression levels of microRNA (miR)-1225-5p in NSCLC and to analyze its prognostic value and biological role. The expression levels of miR-1225-5p in the tissues of patients with NSCLC and NSCLC cell lines were analyzed using reverse transcription-quantitative PCR. The association between miR-1225-5p expression levels and the clinicopathological features of patients with NSCLC was analyzed using a χ^2 test. The prognostic value of miR-1225-5p in NSCLC was analyzed using both Kaplan Meier survival and Cox regression analyses, and the effects of miR-1225-5p on NSCLC cell proliferation, migration and invasion were examined. The results revealed that the expression levels of miR-1225-5p were significantly downregulated in NSCLC tissues compared with normal control tissues. Furthermore, miR-1225-5p was discovered to be a potential independent prognostic factor in NSCLC. The inhibition of miR-1225-5p in NSCLC cell lines led to increased cell proliferation, migration and invasion, whereas miR-1225-5p overexpression exerted the opposite effects in these cells. In conclusion, the findings of the present study indicated that the downregulated expression levels of miR-1225-5p in NSCLC may predict a poor prognosis in patients and suggested miR-1225-5p as a potential therapeutic target for NSCLC.

Introduction

Non-small cell lung cancer (NSCLC) is a malignant tumor, accounting for >85% of all newly diagnosed lung cancer cases (1). There were ~730,000 new patients with lung cancer and ~610,000 lung cancer-related deaths in China in 2015 (2). There are no obvious symptoms or efficient routine screening methods available for the early diagnosis of NSCLC; thus, at diagnosis, the majority of patients with NSCLC are at advanced stages and miss the best time for effective surgical treatment (3). Currently, the existing regular or low dose CT screening method is insufficient, and the specificity of serum tumor antigen markers to NSCLC is low, which limits their clinical applications (4). Cytotoxic chemotherapy is still an important method used for the treatment of metastatic NSCLC (5,6); however, the prognosis following the treatment remains inadequate. This may be attributed to the lack of effective prognostic biomarkers that can be used to monitor the progression and metastasis of NSCLC and help clinicians develop more appropriate treatment strategies for the patients (7). Therefore, it remains a priority to identify efficient prognostic biomarkers and to develop effective treatment strategies for NSCLC.

MicroRNAs (miRNAs/miRs) are small non-coding RNAs that are widely present in animal and plant cells (8). miRNAs are involved in numerous biological processes, including cell proliferation, migration and apoptosis, through post-transcriptional regulation (7). With the development of targeted and immune therapies, miRNAs have gradually attracted increasing attention (9,10), and emerging evidence has indicated that abnormally expressed miRNAs may be involved in the occurrence and development of different types of cancer, in addition to being closely associated with the prognosis of patients (11-13). At present, it has been confirmed that miR-1225-5p serves a role in several types of cancers (14-17). For example, the downregulated expression levels of miR-1225-5p in thyroid cancer tissues and cell lines was reported to regulate tumor cell proliferation, apoptosis, migration and invasion by targeting sirtuin-3 (15). In addition, a previous study by Zhang *et al* (17) also illustrated the tumor suppressive role of miR-1225-5p in osteosarcoma, in which the overexpression of miR-1225-5p suppressed osteosarcoma cell proliferation, migration and invasion. However, to the best of our knowledge, the expression levels and functional role of miR-1225-5p in NSCLC remains unclear.

Correspondence to: Dr Hong Li, Department of Public Health, Shouguang People's Hospital, 3173 Jiankang Road, Shouguang, Shandong 262700, P.R. China
E-mail: hongman1215@163.com

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The present study aimed to investigate the expression levels of miR-1225-5p in NSCLC, in addition to analyzing its prognostic value and biological role. The expression levels of miR-1225-5p in NSCLC tissues and cell lines were analyzed using reverse transcription-quantitative PCR (RT-qPCR), and Kaplan-Meier survival curves and Cox regression analyses were performed to evaluate the prognostic value of miR-1225-5p in NSCLC. In addition, the effects of miR-1225-5p on cell proliferation, migration and invasion were investigated. The findings of the present study may indicate a novel biomarker and therapeutic target for NSCLC, and the potential dysregulation of miR-1225-5p in NSCLC progression may assist to further uncover the mechanisms underlying NSCLC pathogenesis.

Materials and methods

Patient studies. NSCLC tissues and adjacent normal tissues (at least 3 cm from the edge of the tumor) were collected from 118 patients at Shouguang People's Hospital (Shouguang, China) from May 2011 to April 2014. The patients included 52 females and 66 males with a mean age of 59.88 ± 11.39 years (range, 38-84 years). All collected tissues were stored at -80°C for subsequent experiments. The inclusion criteria were as follows: i) All patients were pathologically diagnosed with NSCLC during surgery; ii) None of the patients had received any cancer treatment prior to the operation; and iii) The electronic medical records of the patients were complete. The following exclusion criteria were also used: i) Patients with a history of other types of cancer other than NSCLC; ii) Patients who had received any kind of antitumor therapy; iii) Patients with incomplete records of clinicopathological characteristics; and iv) Patients who had died from causes other than NSCLC. The demographic and clinicopathological characteristics of the patients are summarized in Table I. The personal information of each patient was kept confidential and each patient provided written informed consent. The study was approved by the Ethics Committee of Shouguang People's Hospital. Each patient was followed up for a 5-year period following surgery to record survival information. The cases who died from other unrelated events were excluded from the study.

Cell culture and transfection. NSCLC cell lines, A549, HCC827, H1299 and NCI-H460, and a normal human bronchial epithelial cell line, NHBE, were obtained from The Cell Bank of Type Culture Collection of the Chinese Academy of Sciences. All cell lines were cultured in DMEM (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.), and maintained at 37°C in an atmosphere of 5% CO_2 in a cell incubator.

For the cell transfections, 4×10^4 A549 and H1299 cells/well were seeded into six-well plates and cultured in a humidified incubator at 37°C with 5% CO_2 . Following 24 h of incubation, the cells were transfected with 50 nM miR-1225-5p mimic, 100 nM miR-1225-5p inhibitor, 50 nM mimic negative control (NC) and 100 nM inhibitor NC (all purchased from Shanghai GenePharma Co., Ltd.) using Lipofectamine[®] 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol, to regulate the expression levels of miR-1225-5p in NSCLC cells. The sequences used for cell transfection were as follows: miR-1225-5p mimic,

5'-GUGGGUACGGCCCAGUGGGGGG-3', miR-1225-5p inhibitor, 5'-CCCCCAGUGGGCCGUACCCAC-5', mimic NC, 5'-UUCUCCGAACGUGUCACGU-3' and inhibitor NC, 5'-CAGUACUUUGUGUAGUACAA-3'. Cells transfected with transfection reagent alone were set as the mock group. Following 6 h of incubation at 37°C , the culture medium was replaced, and subsequent cell experiments were performed at 48 h post-transfection.

RT-qPCR. Total RNA was extracted from NSCLC tissues (50 mg) and cells (1×10^6 cells) using 1 ml TRIzol[®] reagent (Invitrogen; Thermo Fisher Scientific, Inc.). Total RNA was reverse transcribed into cDNA using a PrimeScript RT reagent kit (Takara Bio, Inc.) at 42°C for 30 min and 85°C for 10 min. qPCR was subsequently performed using the SYBR Green PCR Master Mix (Thermo Fisher Scientific, Inc.) on a 7300 Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The following thermocycling conditions were used for the qPCR: Initial denaturation at 95°C for 10 min; followed by 40 cycles of 95°C for 20 sec, 60°C for 15 sec and 72°C for 20 sec. The following primer sequences were used for the qPCR: miR-1225-5p, forward 5'-GCCGAGGTGGGTACGGCCCA-3', reverse 5'-CTCAAC TGGTGTCTGGA-3'; and U6, forward 5'-CTCGCTTCG GCAGACA-3', reverse 5'-AACGCTTCACGAATTTGC GT-3'. U6 was used as the internal reference gene. The relative expression levels of miR-1225-5p were quantified using the $2^{-\Delta\Delta\text{C}_q}$ method (18) and normalized to U6.

Cell viability assay. H1299 and A549 cells were seeded into 96-well plates at a density of 2×10^5 cells/well and cultured in an incubator at 37°C for 24, 48 or 72 h. Following the incubation, 10 μl MTT was added to each well for 4 h at 37°C . Subsequently, 150 μl DMSO was added to the wells and incubated for 1 h at 37°C to dissolve the purple formazan. The optical density was measured at 570 nm on a microplate reader.

Cell migration and invasion assays. Transwell chambers (Corning, Inc.) were used to analyze the migratory and invasive abilities of NSCLC cells. The chambers used for the invasion assay were precoated with Matrigel (Corning, Inc.) at 37°C for 1 h, whereas the chambers for the migration assay were not precoated. A549 and H1299 cells (cell density, 5×10^5 cells/well) in serum-free DMEM were seeded into the upper chambers of the Transwell plates. The lower chambers were filled with DMEM supplemented with 10% FBS. Following 48 h of incubation at 37°C , the cells in the lower chambers were fixed with 4% paraformaldehyde for 10 min at room temperature and stained using 0.1% crystal violet at room temperature for 20 min. The number of migratory or invasive cells in five randomly selected fields were counted under an inverted light microscope at $\times 200$ magnification (Olympus Corporation).

Western blotting. Total protein was extracted from A549 and H1299 cells (1×10^7 cells) using 100 μl RIPA buffer (Thermo Fisher Scientific, Inc.) and quantified using a BCA method. A total of 20 μg protein/lane was separated by 10% SDS-PAGE and subsequently transferred onto polyvinylidene fluoride membranes (EMD Millipore). The membranes were blocked with 5% skimmed milk at room temperature

Table I. Association between miR-1225-5p expression levels and the clinicopathological features of patients with non-small cell lung cancer.

Clinicopathological characteristic	Total (n=118)	miR-1225-5p expression level		P-value
		Low (n=64)	High (n=54)	
Sex				0.940
Female	52	28	24	
Male	66	36	30	
Age, years				0.959
≤60	44	24	20	
>60	74	40	34	
Tumor size, cm				0.185
≤3	71	35	36	
>3	47	29	18	
Smoking history				0.691
Negative	46	26	20	
Positive	72	38	34	
Lymph node metastasis				0.012
Negative	53	22	31	
Positive	65	42	23	
TNM stage				0.002
I-II	54	21	33	
III-IV	64	43	21	

miR, microRNA.

for 2 h then incubated at 4°C overnight with the following primary antibodies: Anti-proliferating cell nuclear antigen (PCNA; rabbit; 1:1,000; cat. no. ab92552; Abcam), anti-Ki67 (rabbit; 1:1,000; cat. no. ab16667; Abcam) and anti-GAPDH (rabbit; 1:2,500; cat. no. ab9485; Abcam). Following the primary antibody incubation, the membranes were washed with PBS +0.05% Tween-20 and incubated with a horse-radish peroxidase-conjugated anti-rabbit secondary antibody (1:10,000; cat. no. 111-035-045; Jackson ImmunoResearch Europe, Ltd.) at 37°C for 2 h. Protein bands were visualized using an ECL system (EMD Millipore) and analyzed using ImageJ v1.46 software (National Institutes of Health).

Target gene prediction and dual-luciferase reporter assay. The target genes of miR-1225-5p were predicted using miRanda (<http://www.microrna.org>) (19). To confirm the interaction between miR-1225-5p and Sox9 in NSCLC cells, 4x10⁴ A549 and H1299 cells/well were plated into six-well plates and cultured at 37°C for 24 h. Subsequently, the wild-type (WT) or mutant (MUT) 3'-UTR of Sox9 was cloned into the luciferase reporter vector PsiCheck2 (Promega Corporation). The MUT sequence was generated using a site-directed mutagenesis kit (Promega Corporation). The WT or MUT Sox9 luciferase plasmid (100 ng) was co-transfected into A549 and H1299 cells with the miR-1225-5p mimic (50 nM), miR-1225-5p inhibitor (100 nM) or the respective NCs (50 nM mimic NC; 100 nM inhibitor NC) using Lipofectamine 2000, according to the manufacturer's protocol. Following

incubation for 48 h at 37°C, the relative luciferase activity was measured using a Dual-Luciferase Reporter Assay System (Promega Corporation). Firefly luciferase activity was normalized to *Renilla* luciferase activity.

Statistical analysis. Statistical analysis was performed using SPSS 21.0 software (IBM Corp.) and data are presented as the mean ± SD. All experiments were performed at least three times. Statistical differences between groups were analyzed using a paired Student's t-test or a one-way ANOVA followed by Tukey's post hoc test. The association between miR-1225-5p expression levels and the clinicopathological features of patients with NSCLC was determined using a χ^2 test. Kaplan-Meier survival, followed by log-rank, and Cox regression analyses were adopted to determine the prognostic value of miR-1225-5p. P<0.05 was considered to indicate a statistically significant difference.

Results

Expression levels of miR-1225-5p are downregulated in NSCLC tissues and cell lines. The expression levels of miR-1225-5p in NSCLC tissues and cell lines were quantified using RT-qPCR, which demonstrated that the expression levels of miR-1225-5p were significantly lower in NSCLC tissues compared with the levels of expression in adjacent normal tissues (P<0.001; Fig. 1A). Consistent with these findings, the expression levels of miR-1225-5p in NSCLC cell lines were

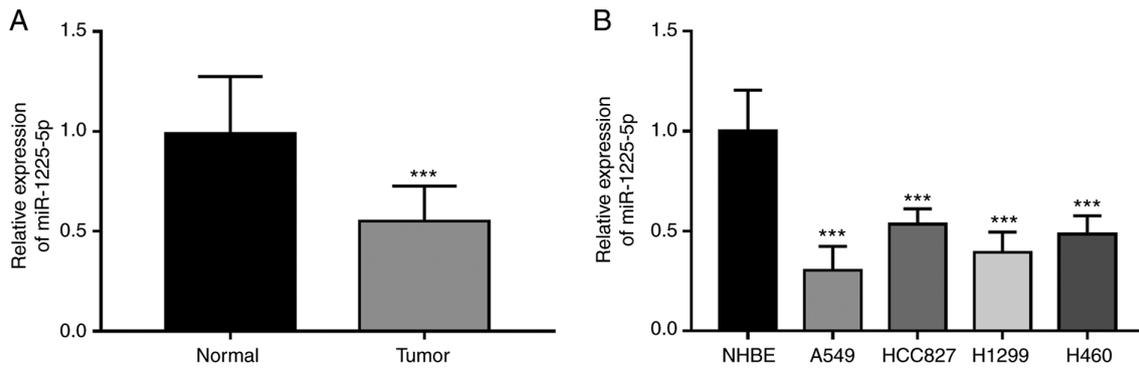


Figure 1. Expression levels of miR-1225-5p in NSCLC tissues and cell lines. (A) RT-qPCR demonstrated that the expression levels of miR-1225-5p were downregulated in the tumoral tissues compared with the normal adjacent tissues in patients with NSCLC. *** $P < 0.001$ vs. Normal. (B) RT-qPCR demonstrated that the expression levels of miR-1225-5p in four NSCLC cell lines were downregulated compared with the normal human bronchial epithelial cell line, NHBE. *** $P < 0.001$ vs. NHBE. miR, microRNA; NSCLC, non-small cell lung cancer; RT-qPCR, reverse transcription-quantitative PCR.

also significantly lower compared with the control NHBE cells (all $P < 0.001$; Fig. 1B). Since A549 and H1299 cells showed the lowest miR-1225-5p expression, these cells were used for subsequent cell experiments.

Association between miR-1225-5p expression levels and the clinicopathological characteristics of patients with NSCLC. Through determining the association between miR-1225-5p expression levels and the clinicopathological data of patients, it was identified that miR-1225-5p may be involved in NSCLC development. The mean expression value (0.550) of miR-1225-5p was used as the cut-off value to classify the patients into low ($n = 64$) and high ($n = 54$) miR-1225-5p expression groups. miR-1225-5p expression levels were discovered to be significantly associated with lymph node metastasis ($P = 0.012$) and TNM stage ($P = 0.002$) (Table I). By contrast, no significant associations were identified between miR-1225-5p expression levels and the other parameters, including sex, age, tumor size and smoking history (all $P > 0.05$).

Clinical significance of miR-1225-5p expression levels for the prognosis of NSCLC. Kaplan-Meier survival curves and Cox regression analyses were performed to determine the prognostic value of miR-1225-5p. Patients with high expression levels of miR-1225-5p exhibited a significantly increased overall survival compared with those with low miR-1225-5p expression levels ($P = 0.002$; Fig. 2). In addition, the clinicopathological indicators and miR-1225-5p that might be related with survival of patients were included in univariate and multivariate Cox regression analysis. The results indicated that miR-1225-5p expression levels and TNM stage were associated with the prognosis of NSCLC as two independent prognostic factors (miR-1225-5p: Hazard ratio = 2.240, 95% CI = 1.184-4.239; $P = 0.013$; TNM stage: Hazard ratio = 2.059, 95% CI = 1.109-4.187; $P = 0.032$; Table II). However, other indicators, including sex, age, tumor size, smoking history and lymph node metastasis, showed no independent association with the overall survival of patients with NSCLC (all $P > 0.05$).

miR-1225-5p inhibits NSCLC cell proliferation. Cell proliferation assays were performed to determine the function of

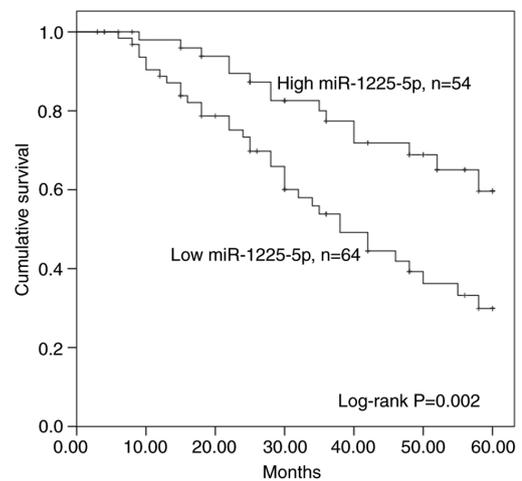


Figure 2. Kaplan-Meier survival curves for patients with non-small cell lung cancer with either high or low miR-1225-5p expression levels. Patients with low miR-1225-5p expression levels had a shorter overall survival time compared with those with high miR-1225-5p expression levels. $P = 0.002$. miR, microRNA.

miR-1225-5p in the progression of NSCLC. Following the transfection with the miR-1225-5p mimic or miR-1225-5p inhibitor, the expression levels of miR-1225-5p were significantly upregulated or downregulated compared with the corresponding NCs, respectively, in both A549 and H1299 cells (all $P < 0.001$; Fig. 3A). Furthermore, the inhibition of miR-1225-5p significantly increased NSCLC cell proliferation compared with cells transfected with inhibitor NC (all $P < 0.05$), whereas the overexpression of miR-1225-5p significantly suppressed cell proliferation compared with the cells transfected with mimic NC, in both A549 and H1299 cells (all $P < 0.05$; Fig. 3B). Furthermore, compared to the cells with mimic NC transfection, both A549 and H1299 cells with overexpression of miR-1225-5p showed significantly downregulated protein expression levels of PCNA and Ki67, which are two proliferation-related proteins (19), whereas the inhibition of miR-1225-5p expression levels significantly upregulated the expression levels of PCNA and Ki67 in both cell lines compared with cells transfected with inhibitor NC (all $P < 0.05$; Fig. 3C).

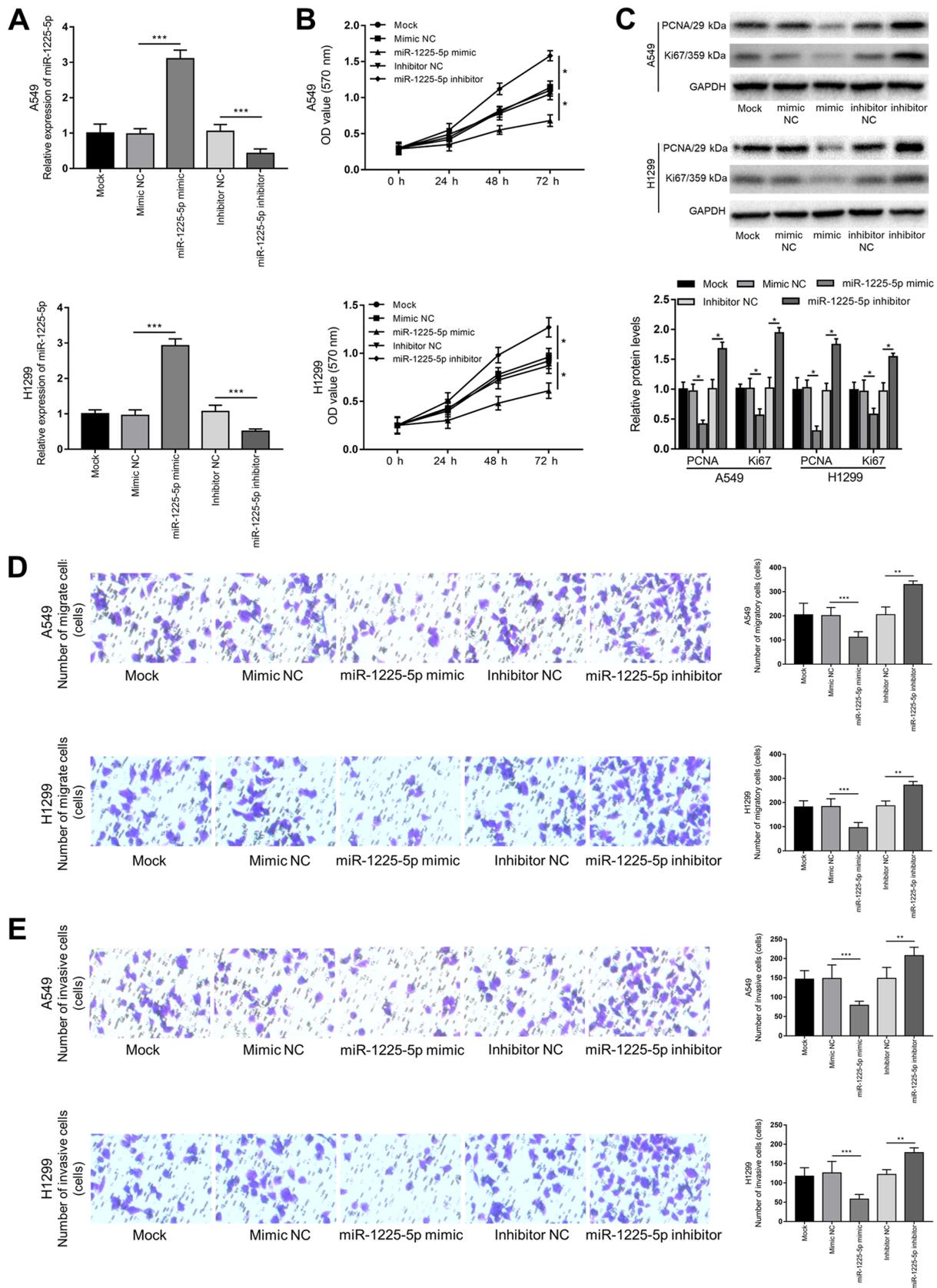


Figure 3. Effects of miR-1225-5p on A549 and H1299 cell proliferation, migration and invasion. (A) Reverse transcription-quantitative PCR demonstrated that the expression levels of miR-1225-5p were upregulated by the miR-1225-5p mimic but downregulated by the miR-1225-5p inhibitor. ***P<0.001. (B) MTT assay demonstrated that miR-1225-5p overexpression inhibited NSCLC cell proliferation, whereas the inhibition of miR-1225-5p promoted tumor cell proliferation. *P<0.05. (C) The expressional changes of proliferation-related proteins PCNA and Ki67 in NSCLC cells with deregulated miR-1225-5p. *P<0.05. (D) Transwell assay results demonstrated that NSCLC cell migration was promoted by the inhibition of miR-1225-5p but inhibited following the overexpression of miR-1225-5p. Magnification x200. (E) Transwell Matrigel assay demonstrated that the overexpression of miR-1225-5p in NSCLC cells inhibited cell invasion, whereas the inhibition of miR-1225-5p expression promoted the opposite results. Magnification, x200. **P<0.01, ***P<0.001. miR, microRNA; NC, negative control; NSCLC, non-small cell lung cancer; OD, optical density; PCNA, proliferating cell nuclear antigen.

Table II. Multivariate Cox regression analysis for miR-1225-5p expression levels in patients with non-small cell lung cancer.

Variable	Univariate Cox analysis			Multivariate Cox analysis		
	Hazard ratio	95% CI	P-value	Hazard ratio	95% CI	P-value
miR-1225-5p	2.474	1.348-4.539	0.003	2.240	1.184-4.239	0.013
Sex	1.513	0.867-2.641	0.145	1.418	0.807-2.492	0.225
Age	1.090	0.612-1.942	0.771	1.244	0.676-2.290	0.483
Tumor size	1.556	0.891-2.715	0.120	1.520	0.851-2.715	0.157
Smoking history	1.408	0.783-2.531	0.253	1.325	0.727-2.415	0.357
Lymph node metastasis	1.896	0.921-2.954	0.053	1.788	1.012-3.859	0.068
TNM stage	2.159	1.238-4.215	0.021	2.059	1.109-4.187	0.032

miR, microRNA.

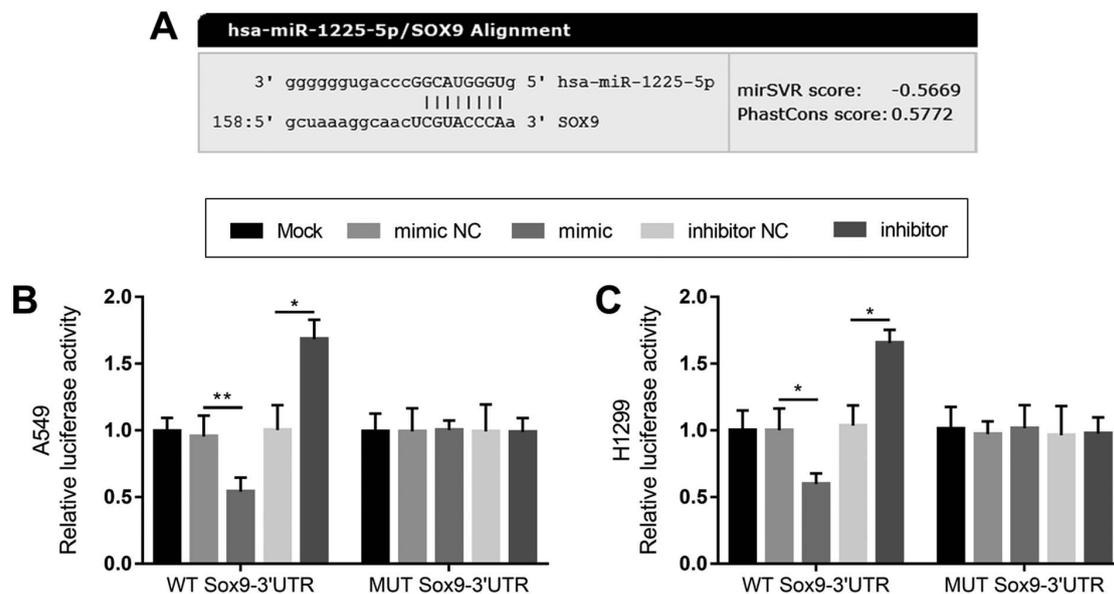


Figure 4. miR-1225-5p directly binds to the 3'-UTR of Sox9 in non-small cell lung cancer cells. (A) Putative binding site of miR-1225-5p was identified in the 3'-UTR of Sox9. (B) Relative luciferase activity of the WT Sox9-3'-UTR was inhibited following miR-1225-5p overexpression but was enhanced by miR-1225-5p inhibition in A549 cells. (C) Relative luciferase activity of the WT Sox9-3'-UTR group was inhibited following miR-1225-5p overexpression but increased by miR-1225-5p inhibition in H1299 cells. * $P < 0.05$, ** $P < 0.01$. miR, microRNA; NC, negative control; UTR, untranslated region; 158, the initial position of the sequence.

Effect of miR-1225-5p on the migration and invasion of NSCLC cells. Transwell assays were performed to determine the influence of miR-1225-5p on NSCLC cell migration and invasion. Compared with the cells transfected with mimic NC or inhibitor NC, miR-1225-5p overexpression significantly decreased NSCLC cell migration and invasion (all $P < 0.001$), whereas the inhibition of miR-1225-5p significantly induced NSCLC cell migration and invasion ($P < 0.01$ and $P < 0.001$; Fig. 3D and E).

Sox9 is a direct target of miR-1225-5p in NSCLC cells.

Through target gene prediction, a putative binding site of miR-1225-5p was identified in the 3'-UTR of Sox9 (Fig. 4A). Subsequently, a dual-luciferase reporter assay was performed to confirm the interaction between miR-1225-5p and Sox9. The relative luciferase activity of the WT Sox9-3'-UTR was significantly inhibited following the overexpression of miR-1225-5p

(all $P < 0.05$), whereas it was significantly increased following the inhibition of miR-1225-5p when compared to the respective NCs, in both A549 and H1299 cells ($P < 0.05$ and $P < 0.01$; Fig. 4B and C). Conversely, no significant differences were observed in the relative luciferase activity of the MUT Sox9-3'-UTR across all groups in both cell lines (all $P > 0.05$). These findings indicated that miR-1225-5p may directly bind to the 3'-UTR of Sox9 in NSCLC cells.

Discussion

The aberrant expression of miRNAs have been identified to be closely associated to the occurrence of numerous types of human malignant tumor, in which they were reportedly involved in cell apoptosis, proliferation, the cell cycle and metastasis by altering post-transcriptional gene expression (20-22). miRNAs have been identified to serve as both oncogenes or tumor

suppressor genes in tumorigenesis; for example, the abnormal expression of miRNAs, such as miR-146a (23), miR-375 (24) and miR-449c (25), was determined to be closely associated with gastric tumorigenesis. In addition, a significant association was reported between tumor budding and the downregulated expression levels of miRNAs, such as miR-148a (26) and miR-625-3p (27). miR-21, miR-144-3p and miR-148a were also reported to be abnormally expressed in NSCLC tissues (28-30). The results of the present study also confirmed the dysregulation of miR-1225-5p in NSCLC cells. Clinically effective prognostic biomarkers can not only indicate the progression and metastatic status of potential cancers, but they can also help clinicians develop more appropriate treatment strategies for patients with cancer (31,32). Therefore, it is important to identify further potential functional miRNAs for the treatment of NSCLC.

miR-1225-5p, which was investigated in the current study, has been considered as an important regulator in various types of tumor. Previous studies have demonstrated that compared with its expression levels in normal tissues, the expression levels of miR-1225-5p were downregulated in osteosarcoma (17), thyroid cancer (15) and pancreatic cancer (33), in which it was discovered to be associated with tumor grade and poor prognosis. In the present study, the expression levels of miR-1225-5p in tumor tissues of patients with NSCLC and NSCLC cell lines were significantly downregulated compared with expression in adjacent normal lung tissues and human bronchial epithelial cells. The low expression levels were subsequently illustrated to be associated with lymph node metastasis, TNM stage and poor prognosis, thus it was suggested that miR-1225-5p may be a potential tumor suppressor gene. These findings indicated that miR-1225-5p may serve an important role in the development and progression of NSCLC tumors and may be used clinically to predict the metastasis of NSCLC.

Given the abnormal expression of miR-1225-5p in NSCLC tissues, the present study analyzed the clinical significance of its diagnostic and prognostic values in NSCLC. Biomarkers can provide clinicians with an effective guide during the early stages of treatment of patients with NSCLC (34-36). The prognostic value of miR-1225-5p was evaluated based on the 5-year survival information of patients with NSCLC. The Kaplan-Meier survival curve illustrated that patients with low miR-1225-5p expression levels had a worse overall survival compared with patients with high expression levels. In addition, miR-1225-5p was independently related to overall survival, suggesting that miR-1225-5p may be a potential prognostic biomarker for NSCLC.

A previous study reported that the aberrant expression levels of miR-145 in NSCLC mediated chemoresistance and brain metastasis, and its downregulation was associated with the poor prognosis of NSCLC (37). The overexpression of miR-142-5p was discovered to downregulate the expression levels of phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit α isoform at both the mRNA and protein levels, thereby inhibiting the proliferation of NSCLC cells (38,39). The results of the present study revealed that miR-1225-5p inhibition promoted the proliferation, migration and invasion of NSCLC cells, while miR-1225-5p overexpression inhibited tumor cell proliferation, migration and invasion, which suggested that miR-1225-5p may serve a tumor suppressive role in NSCLC development. However, to the best of our knowledge, the mechanism of action of miR-1225-5p in NSCLC remains unknown. Zhang *et al* (17) discovered that miR-1225-5p served a tumor

suppressive role in osteosarcoma by targeting Sox9. In addition, Sun *et al* (14) demonstrated that miR-1225 inhibited laryngeal cancer cell proliferation and survival by targeting the 3'-UTR of dual-specificity protein phosphatase CDC14B, leading to G₁/S phase cell cycle arrest. Based on the existing research and conclusions, the present study hypothesized that miR-1225-5p may regulate proliferation and/or metastasis of NSCLC cells by binding to the 3'-UTR region of target genes, thereby exerting the anticancer effect of miR-1225-5p. Subsequently, a dual-luciferase reporter assay was used to confirm the interaction between miR-1225-5p and Sox9; the results revealed that miR-1225-5p directly bound to the 3'-UTR of Sox9 in NSCLC cells. Sox9 was found to be upregulated in NSCLC tissues, which was correlated with histological stage and shorter survival time of patients with NSCLC (40), and serves as an oncogene in NSCLC progression (40). Thus, these findings suggested that the tumor suppressive role of miR-1225-5p in NSCLC progression may be achieved by targeting Sox9. To further confirm the functional role and underlying mechanisms of miR-1225-5p in NSCLC pathogenesis, further investigations with *in vivo* experiments are required.

In conclusion, the findings of the present study demonstrated that miR-1225-5p expression levels were downregulated in NSCLC, which may serve as an independent prognostic biomarker. Therefore, the current study provided a novel, potential non-invasive biomarker to predict the survival outcomes of patients with NSCLC. In addition, the aberrant expression levels of miR-1225-5p in NSCLC progression may help to further elucidate the pathogenesis of this malignancy. The overexpression of miR-1225-5p was revealed to inhibit NSCLC cell proliferation, migration and invasion, indicating that miR-1225-5p may be a potential therapeutic target, and that methods to upregulate miR-1225-5p expression levels may be novel approaches for NSCLC therapy.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

BL and HL designed and conceived the study, conducted the clinical studies, analyzed the clinical data and wrote the manuscript. FZ conducted the *in vitro* experiments and analyzed the cell experimental data. All authors read and approved the manuscript.

Ethics approval and consent to participate

The personal information of each patient involved remains confidential and each patient provided written informed

consent. The study was approved by the Ethics Committee of Shouguang People's Hospital (Shouguang, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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