

# miR-202 functions as a tumor suppressor in non-small cell lung cancer by targeting STAT3

ZHONGHAI ZHAO<sup>1</sup>, BIN LV<sup>1</sup>, LI ZHANG<sup>1</sup>, NANA ZHAO<sup>1</sup> and YAN LV<sup>2</sup>

<sup>1</sup>Department of Thoracic Surgery, Yidu Central Hospital of Weifang, Qingzhou, Shandong 262500;

<sup>2</sup>Department of Thoracic Surgery, Anqiu People's Hospital, Anqiu, Shandong 262100, P.R. China

Received May 2, 2016; Accepted March 31, 2017

DOI: 10.3892/mmr.2017.6841

**Abstract.** MicroRNAs (miRNAs) are a group of non-protein-coding, short single-stranded RNAs, which are considered as promising molecular markers and therapeutic targets in several cancers. The present study explored the expression patterns and functional roles of miR-202 in non-small cell lung cancer (NSCLC). The expression levels of miR-202 were determined in NSCLC tissues and cell lines using reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The functional impact of miR-202 overexpression on NSCLC cell viability, migration and invasion were evaluated using Cell Counting Kit-8 reagent and Transwell migration and invasion assays, respectively. The molecular mechanism underlying the tumor suppressive roles of miR-202 on NSCLC was examined using bioinformatics analysis, luciferase reporter assay, RT-qPCR and western blot analysis. In addition, signal transducer and activator of transcription (STAT) 3 was overexpressed to investigate the impact on miR-202-mediated tumor suppression in NSCLC. The results indicated that miR-202 was downregulated in NSCLC tissues and cell lines, and was associated with tumor node metastasis stage and lymph node metastasis. Exogenous miR-202 expression reduced NSCLC cell viability, migration and invasion. Furthermore, STAT3 was identified as a direct target gene of miR-202 in NSCLC. STAT3 overexpression improved miR-202-impaired cell viability, migration and invasion. In conclusion, the present study revealed novel anticancer effects induced by miR-202 upregulation in NSCLC, and indicated that STAT3 may be a molecular target of miR-202.

## Introduction

Lung cancer is globally the most common cancer, and harbors the highest mortality and morbidity rate amongst all kinds of malignant tumors (1,2). An estimated 224,390 new cases and 158,080 deaths due to lung cancer are expected in the United States in 2016 (3). Currently, environmental pollution, tobacco use, radon exposure and occupational carcinogens have been considered as risk factors for lung cancer (4-7). Non-small cell lung cancer (NSCLC), including squamous cell carcinoma, adenocarcinoma and large cell carcinoma, is the predominant form of lung cancer which accounts for ~80-85% of newly diagnosed lung cancer cases (8,9). Despite the combined therapy of surgery, chemotherapy, radiation therapy and targeted biologic agents, as well as significant progress in understanding the pathophysiological mechanisms in NSCLC, the prognosis for NSCLC remains poor, with a 5-year survival rate of ~11% (10,11). Diagnosis at advanced stage, local invasion and/or distant metastases, and a high rate of recurrence following surgery are the most important challenges in the treatment of patients with NSCLC (12,13). Therefore, a greater understanding of the molecular mechanisms underlying NSCLC occurrence and progression are essential for improving the diagnosis, prevention and treatment of this disease.

Recently, microRNAs (miRNAs) have been considered as promising molecular markers and therapeutic targets in several cancers, including NSCLC (14). miRNAs are a group of non-protein-coding, single-stranded, endogenous, short RNA molecules ~19-25 nucleotides in length (15). miRNAs can repress gene expression at the posttranscriptional level by binding to target genes at complementary sites in the 3'-untranslated regions (3'UTRs), which results in mRNA cleavage or inhibition of protein synthesis (16,17). Bioinformatic predictions suggest that miRNAs could regulate ~30-60% of the protein-coding genes in the human genome (18). miRNAs participate in numerous biological processes including cell proliferation, cell cycle, apoptosis, development, metabolism, invasion, migration and metastasis (19). Several studies have reported miRNA dysregulation in a variety of malignant tumors, including bladder cancer (20), thyroid carcinoma (21), colorectal cancer (22), NSCLC (23) and others. In addition, miRNAs have been demonstrated to contribute to the carcinogenesis and progression of cancer (24). Highly expressed

*Correspondence to:* Professor Yan Lv, Department of Thoracic Surgery, Anqiu People's Hospital, 246 Jiankang Road, Anqiu, Shandong 262100, P.R. China  
E-mail: lvyang\_yidu@163.com

**Key words:** miR-202, non-small cell lung cancer, signal transducer and activator of transcription 3, tumor suppressor

miRNAs function as oncogenes by negatively regulating tumor suppressor genes. In contrast, miRNAs expressed at low levels may serve as tumor suppressors, by clocking oncogenes (25,26). Therefore, exploring miRNA expression and function is important to understand their roles in cancer initiation and progression, as they may be useful targets for anticancer therapy.

In the present study, miR-202 was demonstrated to be downregulated in NSCLC tissues and cell lines. Based on this novel finding, the correlation between miR-202 expression and clinicopathological features was explored. Furthermore, the roles of miR-202 on NSCLC carcinogenesis and progression were investigated. Through bioinformatics analysis and functional experiments, including luciferase reporter assay, reverse transcription polymerase chain reaction (RT-qPCR), western blotting and recombinant gene overexpression, signal transducer and activator of transcription (STAT) 3 was demonstrated as a direct target of miR-202 in NSCLC.

## Materials and methods

**Clinical sample collection.** The present study was approved by the Ethical Committee of Yidu Central Hospital of Weifang (Qingzhou, China), and performed in compliance with the Helsinki Declaration. All participants provided written informed consent. A total of 56 fresh NSCLC tissues and paired adjacent non-cancerous tissues were obtained from patients who had undergone surgical NSCLC resection between 2011 and 2013, at Yidu Central Hospital of Weifang. Patients were diagnosed with NSCLC by histopathological analysis and did not receive local nor systemic treatment prior to the surgery. Patient clinical information is listed in Table I. Regarding the pathological evaluation of surgical specimens, the TNM stage and differentiation scores of HCC were determined in accordance with the 2009 American Joint Committee on Cancer (27). All tissues were excised, washed with PBS (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA), snap-frozen in liquid nitrogen, and stored at -80°C until use.

**Cell lines and culture conditions.** Five NSCLC cell lines (SK-MES-1, H1299, SPC-A1, H520, A549), and a normal human bronchial epithelial cell line (16HBE) were purchased from the American Type Culture Collection (Manassas, VA, USA). The HEK293T cell line was acquired from the Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China). Cells were incubated at 37°C with 5% CO<sub>2</sub> in Dulbecco's modified Eagle's medium or RPMI-1640 medium, containing 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 mg/ml streptomycin (all reagents from Gibco; Thermo Fisher Scientific, Inc.).

**RT-qPCR analysis.** Total RNA was extracted using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. For miR-202 expression, first-strand cDNA was generated using a PrimeScript RT Reagent kit (Takara Biotechnology, Co., Dalian, China), and RT-qPCR was performed using a TaqMan miRNA assay kit (Applied Biosystems; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol, with U6 as an endogenous control. The cycling conditions for qPCR

Table I. Association of miR-202 expression with clinicopathological characteristics in non-small cell lung cancer patients.

Clinicopathological characteristics	Cases	miR-202 expression		P-value
		High	Low	
Sex				0.120
Male	35	11	24	
Female	21	11	10	
Age (years)				0.516
<60	17	5	12	
≥60	39	17	22	
Smoking status				0.813
Non-smoker	24	9	15	
Smoker	32	13	19	
Differentiation				0.719
I-II	22	8	14	
III-IV	34	14	20	
TNM stage				0.004
I-II	25	15	10	
III-IV	31	7	24	
Lymph node metastasis				0.016
Absent	27	15	12	
Present	29	7	22	

miR, microRNA; TNM, tumor node metastasis.

were as follows: 40 cycles of denaturation at 95°C for 15 sec and annealing/extension at 60°C for 60 sec. To quantify the STAT3 mRNA expression, total RNA was reverse transcribed into cDNA using M-MLV Reverse Transcriptase (Promega Corporation, Madison, WI, USA) and qPCR was conducted using a SYBR-Green PCR Master Mix (Takara Biotechnology, Co.) according to the manufacturer's protocol, with GAPDH as an internal control. qPCR was performed as follows: 95°C for 10 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min. qPCR was performed on an Applied Biosystems Real-Time 7500 Sequence Detection System (Applied Biosystems; Thermo Fisher Scientific, Inc.). The primer sequences were as follows: miR-202 forward, 5'-CCTCCCAGGCTCACGAGGCT-3' and reverse, 5'-GGTGCAGGTGCACTGGTGC-3'; U6 forward, 5'-CTCGCTTCGCGAGCACA-3' and reverse, 5'-AACGCTTCACGAATTTGCGT-3'; STAT3 forward, 5'-CCAAGGAGGAGGCATTCG-3' and reverse, 5'-ACATCGGCAGGTCAATGG-3'; GAPDH forward, 5'-CGGAGTCAACGGATTTGGTCGTAT-3' and reverse, 5'-AGCCTTCTCCATGGTGGTGAAGAC-3'. The relative fold expressions of miR-202 and STAT3 mRNA were calculated using the 2<sup>-ΔΔC<sub>q</sub></sup> method (28).

**Cell transfection.** Cells were seeded into 6-well plates at a density of 60-70% confluence. Following overnight incubation, cells were transfected with miRNA mimic (50 pmol/ml), negative control (NC; 50 pmol/ml) or STAT3 overexpression

plasmid vectors (2  $\mu$ g) using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. miR-202 mimic and miRNA NC were purchased from Shanghai GenePharma Co., Ltd. (Shanghai, China). The sequence of miR-202 mimics was 5'-UUC CUA UGC AUA UAC UUC UUUG-3' and for miRNA NC the sequence was 5'-UUC UCC GAA CGU GUC ACG UTT-3'. The STAT3 plasmid vector (pcDNA3.1-STAT3) and empty control vector (pcDNA3.1-Ctl) were synthesized by Guangzhou RiboBio Co., Ltd. (Guangzhou, China).

**Cell Counting Kit-8 (CCK-8) assay.** CCK-8 (Dojindo, Kumamoto, Japan) reagent was used to detect cell viability. Cells were seeded in 96-well plates at a density of  $2 \times 10^3$  cells/well in 100  $\mu$ l culture medium. Cells were incubated at 37°C with 5% CO<sub>2</sub> for 0, 24, 48, 72 or 96 h. Briefly, 10  $\mu$ l CCK-8 solution was added to each well, and the plates were incubated at 37°C for a further 2 h. The absorbance at 450 nm was detected using an ELISA plate reader (Bio-Rad Laboratories, Inc., Hercules, CA, USA). Each experiment was repeated at least three times.

**Cell migration and invasion assays.** Cells were transfected as aforementioned. A total of 24 h post-transfection, cells were harvested and resuspended to create a single cell suspension. For the cell migration assay,  $5 \times 10^4$  cells in 200  $\mu$ l FBS-free culture medium were added to the upper chamber of a transwell insert (8  $\mu$ m pore size; BD Biosciences, San Jose, CA, USA). For the cell invasion assay,  $5 \times 10^4$  cells in 200  $\mu$ l FBS-free culture medium were added to the upper chamber of a Matrigel-coated insert (BD Biosciences). For both assays, the lower chambers were filled with 500  $\mu$ l culture medium containing 20% FBS. Following 48 h incubation, cells remaining on the membranes of the upper chamber were removed with a cotton swab. The migrated and invaded cells were fixed with methanol and stained with 0.1% crystal violet (Beyotime Institute of Biotechnology, Haimen, China). Cells were counted using an inverted microscope (Olympus Corporation, Tokyo, Japan). Cell numbers were calculated across five random fields for each chamber.

**Luciferase reporter assays.** Luciferase reporter assay was performed in HEK293T cells. The wild-type and mutant type STAT3-3'UTR luciferase reporter vectors (pmirGLO-STAT3-3'UTR Wt and pmirGLO-STAT3-3'UTR Mut) were cloned by GenePharma Co., Ltd. HEK293T cells were transfected with miR-202 mimic (20 pmol) or NC (20 pmol), together with pmirGLO-STAT3-3'UTR Wt (1  $\mu$ g) or pmirGLO-STAT3-3'UTR Mut (1  $\mu$ g), using Lipofectamine 2000. At 48 h post-transfection, cells were washed with PBS and luciferase activities were measured using the Dual-Luciferase Reporter assay system (Promega Corporation). Firefly luciferase activities were normalized using co-transfected *Renilla* luciferase vectors. All experiments were performed in triplicate.

**Western blot analysis.** Cells were transfected as aforementioned. A total of 72 h post-transfection, cells were harvested and total cellular protein was isolated using radioimmunoprecipitation assay cell lysis buffer (Beyotime Institute of Biotechnology) supplemented with Protease Inhibitor Cocktail (Roche Diagnostics GmbH, Mannheim, Germany).

Equal amounts of protein (30  $\mu$ g) were separated by 10% SDS-PAGE, transferred to polyvinylidene fluoride membranes (Bio-Rad Laboratories, Inc.) and blocked with 5% skimmed milk powder at room temperature for 1 h. Membranes were subsequently probed with primary antibodies overnight at 4°C. The primary antibodies were a mouse anti-human STAT3 monoclonal antibody (1:1,000; sc-8019) and a mouse anti-human GADPH monoclonal antibody (1:1,000; sc-59540) (both from Santa Cruz Biotechnology, Inc., Dallas, TX, USA). Membranes were then washed three times with TBS solution containing 0.1% Tween-20 and incubated with the goat anti-mouse horseradish peroxidase-conjugated secondary antibody (1:5,000; sc-2005; Santa Cruz Biotechnology, Inc.) at room temperature for 2 h. An enhanced chemiluminescence detection system (Amersham; GE Healthcare Life Sciences, Chalfont, UK) was used to visualize the bands and the intensity of the bands was quantified by densitometry (Image J 1.47; National Institutes of Health, Bethesda, MD, USA).

**Statistical analysis.** Results were presented as the mean  $\pm$  standard deviation and compared using Student's t-test or one-way analysis of variance, followed by the Student-Newman-Keuls multiple comparison test. A Chi-square test was used to investigate the association between miR-202 and the clinicopathological features of patients with HCC. SPSS 16.0 (SPSS Inc., Chicago, IL, USA) was used to perform all statistical analyses.  $P < 0.05$  was considered to indicate a statistically significant difference.

## Results

**miR-202 is downregulated in NSCLC and is associated with TNM stage and lymph node metastasis.** To explore whether miR-202 expression is altered in NSCLC, miR-202 expression levels were measured in 56 pairs of NSCLC tissues and adjacent non-cancerous tissues by RT-qPCR. miR-202 expression levels were significantly reduced in NSCLC tissues compared with adjacent non-cancerous tissues ( $P < 0.05$ ; Fig. 1A). Next, miR-202 expression was explored in five NSCLC cell lines (SK-MES-1, H1299, SPC-A1, H520, A549) and a normal human bronchial epithelial cell line (16HBE). miR-202 was downregulated in all five NSCLC cell lines compared with 16HBE ( $P < 0.05$ ; Fig. 1B).

To investigate the clinical significance of miR-202 downregulation in NSCLC, the association between miR-202 expression and clinicopathological features was evaluated (Table I). All NSCLC patients were divided into two groups according to the median miR-202 value: Those below the median became the low-miR-202 group and those above the median were the high-miR-202 group. Low miR-202 expression levels were significantly correlated with advanced TNM stage ( $P = 0.004$ ) and lymph node metastasis ( $P = 0.016$ ). However, miR-202 expression was not associated with other clinicopathological features in NSCLC, including gender ( $P = 0.120$ ), age ( $P = 0.516$ ), smoking status ( $P = 0.813$ ) and differentiation grade ( $P = 0.719$ ). Taken together, these findings suggested that miR-202 expression may be associated with the progression and development of NSCLC.

**miR-202 reduces viability, migration and invasion of NSCLC cells in vitro.** To investigate the biological functions of



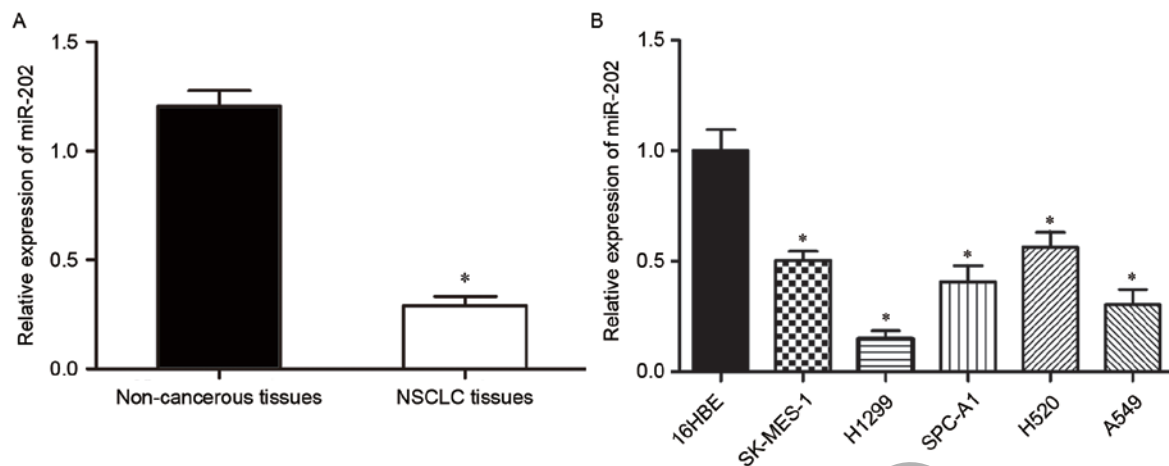


Figure 1. miR-202 expression levels in NSCLC. (A) Expression of miR-202 in 56 pairs of NSCLC tissues and paired adjacent non-cancerous tissues. \* $P < 0.05$  vs. non-cancerous tissues. (B) Expression of miR-202 in five NSCLC cell lines and a normal human bronchial epithelial cell line (16HBE). \* $P < 0.05$  vs. 16HBE. NSCLC, non-small cell lung cancer.

miR-202, a miR-202 mimic was used to increase the expression of miR-202 in NSCLC cells. miR-202 expression was lowest in H1299 and A549 cells; these cells were therefore selected to perform functional experiments. Following transfection with the miR-202 mimic for 48 h, RT-qPCR analysis indicated that miR-202 was markedly upregulated in miR-202 mimic-transfected H1299 and A549 cells compared with NC-transfected cells ( $P < 0.05$ ; Fig. 2A).

The ability of miR-202 to modulate the biological functions of NSCLC cells was explored. Analysis using a CCK-8 assay indicated that the number of viable cells was suppressed in H1299 and A549 cells transfected with miR-202 mimics, compared with NC groups ( $P < 0.05$ ; Fig. 2B). To investigate whether miR-202 served a functional role in facilitating cell migration and invasion in NSCLC, cell migration and invasion assays were performed. Restoration of miR-202 expression by mimics transfection significantly impaired the migration and invasion abilities of H1299 and A549 cells compared with NC-transfected cells (Fig. 2C). Collectively, the present results indicated that miR-202 may reduce total cell numbers, migration and invasion of NSCLC H1299 and A549 cells.

*STAT3 is a direct target of miR-202.* The molecular mechanisms underlying the inhibitory effect of miR-202 on NSCLC cell viability, migration and invasion were further investigated. STAT3 was identified as a potential target of miR-202 using publicly available databases, including TargetScan (<http://www.targetscan.org/>) and microRNA.org (<http://www.microRNA.org/microRNA/>). Bioinformatics analysis identified two potential target regions at the 3' UTR of the *STAT3* gene. To evaluate whether STAT3 was a direct target gene of miR-202, luciferase reporter plasmids were constructed including the sequences of the two predicted target regions and mutated sequences as controls (Fig. 3A). The results indicated that miR-202 was able to significantly suppress the luciferase activities of the pmirGLO-STAT3-3'UTR Wt-transfected cells, however, luciferase activity was not suppressed in the pmirGLO-STAT3-3'UTR Mut-transfected HEK293T cells ( $P < 0.05$ ; Fig. 3B), which indicates that miR-202 directly

interacted with the two target regions in the 3-UTR of STAT3. RT-qPCR and western blot analysis were performed to further investigate STAT3 as a direct target gene of miR-202. The results revealed that treatment with exogenous miR-202 mimic suppressed STAT3 mRNA and protein expression levels in H1299 and A549 cells ( $P < 0.05$ ; Fig. 3C and D). These results suggested that STAT3 is a direct target gene of miR-202 in H1299 and A549 NSCLC cells.

*STAT3 overexpression reverses the effect of miR-202 overexpression in NSCLC cells.* The potential role of STAT3 in mediating the miR-202-induced tumor suppressive roles in NSCLC was investigated. pcDNA3.1-STAT3 was transfected into H1299 and A549 cells to induce STAT3 overexpression. Following transfection for 48 h, STAT3 overexpression was confirmed by RT-qPCR ( $P < 0.05$ ; Fig. 4A) and western blot analysis ( $P < 0.05$ ; Fig. 4B). A dual-transfection experiment was subsequently performed in H1299 and A549 cells. Cells were transfected with miR-202 mimics for 24 h, followed by transfection with either pcDNA3.1-STAT3 or pcDNA3.1-Ctl. Analysis by CCK-8 assay demonstrated that STAT3 overexpression significantly improved the cell growth inhibition in H1299 and A549 cells induced by miR-202 overexpression ( $P < 0.05$ ; Fig. 4C). Cell migration and invasion assays also revealed that impaired migration and invasion abilities induced by miR-202 overexpression were improved by STAT3 overexpression in H1299 and A549 cells ( $P < 0.05$ ; Fig. 4D). These results indicated that the tumor suppressive roles of miR-202 occur via STAT3 in H1299 and A549 NSCLC cells.

## Discussion

NSCLC has a high global morbidity and mortality rate, and therefore it commands significant research interest (29). miRNAs are a large family of small RNA molecules that negatively modulate the expression of their target genes in a sequence-specific manner (16). The abnormal expression of miRNAs has frequently been observed in human malignancies. Several of these deregulated miRNAs are involved in tumorigenesis and tumor development, and

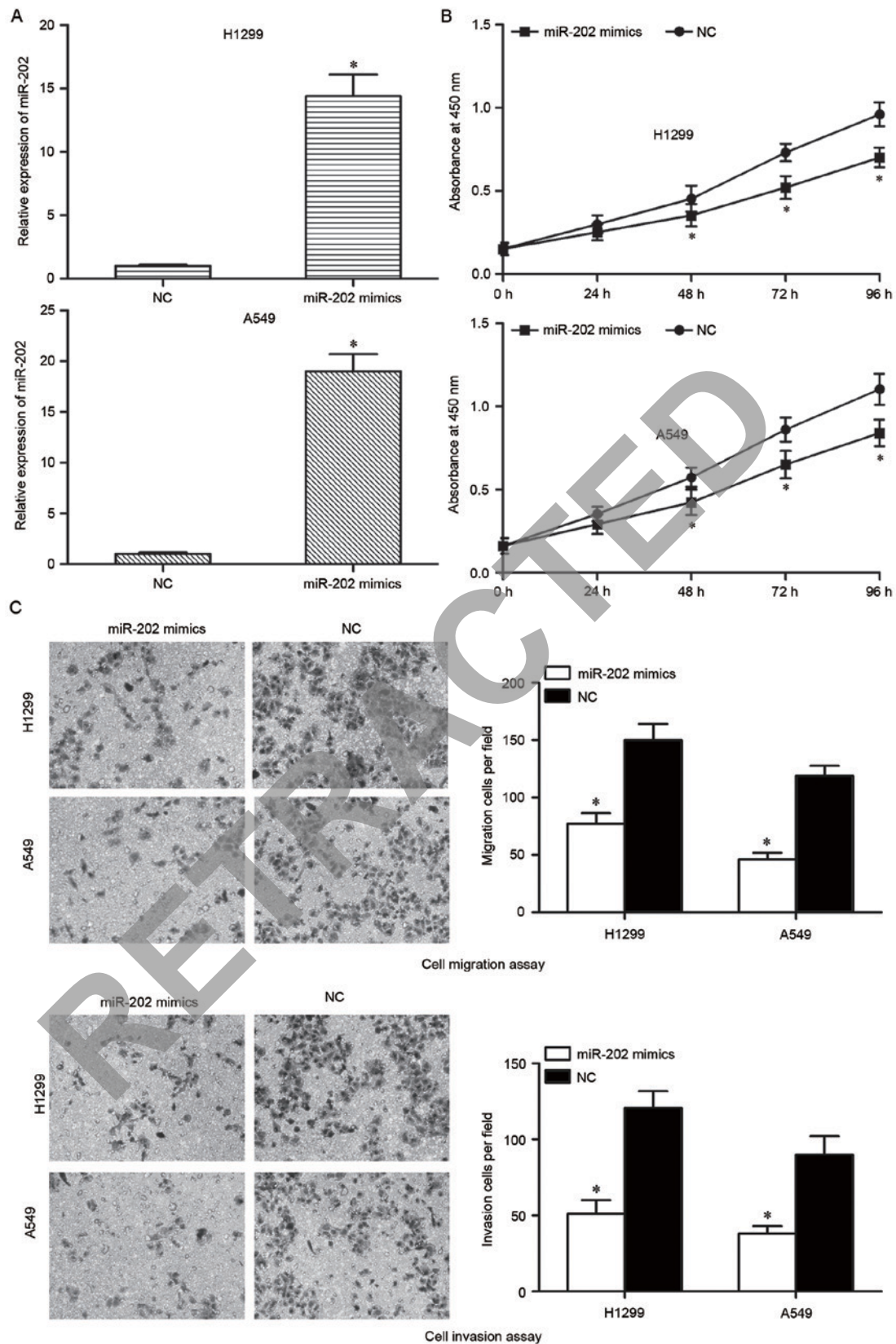


Figure 2. Effects of miR-202 on viability, migration and invasion of NSCLC cells. (A) Reverse transcription-quantitative polymerase chain reaction analysis of miR-202 expression in H1299 and A549 cells, following treatment with miR-202 mimics or NC. (B) Cell Counting Kit-8 assay indicated that miR-202 overexpression significantly inhibited the number of viable H1299 and A549 cells. (C) Transwell migration and invasion assay results for miR-202 mimic- and NC-transfected H1299 and A549 cells. \* $P < 0.05$  vs. NC. NSCLC, non-small cell lung cancer; NC, negative scramble control.

are closely correlated with clinicopathological factors and patient prognosis (30,31). Furthermore, previous studies have

demonstrated an important role for miRNAs in the prognosis of NSCLC (32,33). Therefore, it is very important to

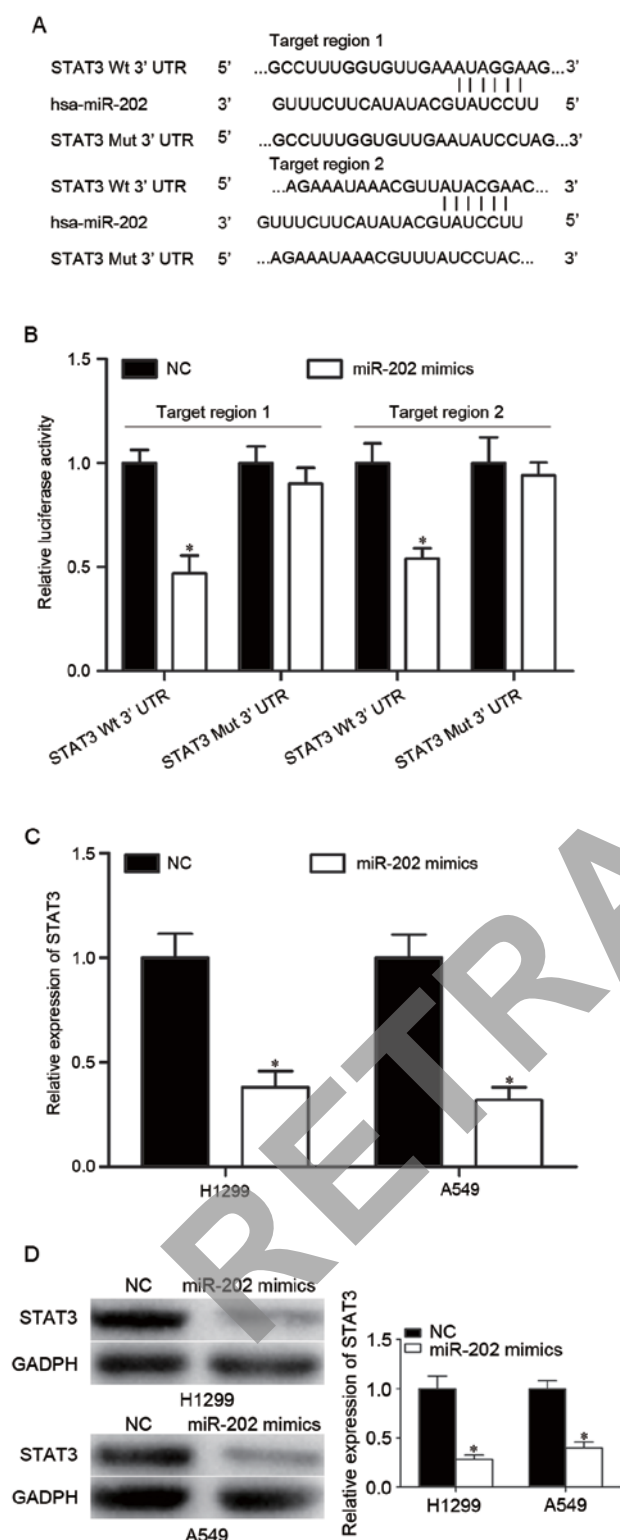


Figure 3. miR-202 decreases STAT3 expression via binding its 3'UTR. (A) The sequence of the two predicted target regions in the STAT3 3'UTR for miR-202 binding are shown. As a control, mutations were introduced at both target regions. (B) Relative luciferase activities in HEK293T cells following transfection with miR-202 mimics or NC, along with pmirGLO-STAT3-3'UTR Wt or pmirGLO-STAT3-3'UTR Mut for each target region. (C) miR-202 overexpression by mimics transfection decreased the mRNA expression levels of STAT3 in H1299 and A549 cells. (D) miR-202 overexpression by mimics transfection suppressed STAT3 protein expression in H1299 and A549 cells. \* $P < 0.05$  vs. NC. STAT3, signal transducer and activator of transcription 3; UTR, untranslated region; NC, negative scramble control; Wt, wild-type; Mut, mutant.

investigate the expression and functional roles of miRNAs in NSCLC.

To our knowledge, this is the first study to evaluate the expression, the biological function and the clinical significance of miR-202 in NSCLC. miR-202 expression levels were reduced in NSCLC tissues and cell lines compared with adjacent noncancerous tissues and a normal human bronchial epithelial cell line, respectively. Statistical analysis indicated that low miR-202 expression levels were significantly correlated with advanced TNM stage and lymph node metastasis. *In vitro*, exogenous miR-202 expression reduced NSCLC cell viability, migration and invasion. Furthermore, STAT3 was identified as a direct target gene of miR-202, and overexpression of STAT3 reversed cell miR-202-impaired viability, migration and invasion. These findings indicated that miR-202 may be involved in NSCLC carcinogenesis and progression, and may serve as a novel therapeutic target in the therapy of this disease.

Aberrant miR-202 expression has been observed in several human cancers. miR-202 is downregulated in esophageal squamous cell carcinoma, and weak miR-202 expression is correlated with the degree of cell differentiation and lymph node metastasis (34). Downregulation of miR-202 has also been reported in multiple myeloma (35) and hepatocellular carcinoma (36). However, studies have produced conflicting results in osteosarcoma. Sun *et al* (37) demonstrated a decrease in miR-202 expression in human osteosarcoma cell lines and tissue samples, however, Lin *et al* (38) reported that miR-202 is highly expressed in osteosarcoma tissues. Collectively, the present results and the previous studies indicate that miR-202 may be involved in tumor occurrence and development.

miR-202 has demonstrated a tumor suppressor role in numerous human cancers. In esophageal squamous cell carcinoma, miR-202 significantly represses cell proliferation, migration and invasion, and induces cell apoptosis (34,39). Shen *et al* (35) revealed that miR-202 overexpression inhibits multiple myeloma cell growth and adhesion, and renders cells more sensitive to bortezomib. Recently, Zhang *et al* (36) reported that exogenous miR-202 expression suppresses cell proliferation and tumorigenicity, whereas downregulation of miR-202 enhances the cells proliferative capacity in hepatocellular carcinoma. Sun *et al* (37) revealed that overexpression of miR-202 suppresses osteosarcoma cell proliferation, induces cell apoptosis and decreases tumor growth in nude mice models. In the present study, miR-202 was identified as a potential tumor suppressor in NSCLC, through inhibition of tumor cell viability and metastasis. These results indicated that downregulation of miR-202 may represent a novel therapeutic mechanism for NSCLC treatment.

The underlying mechanistic action of miRNAs involves target regulation via mRNA degradation or translation suppression. Numerous miR-202 targets, including laminin  $\alpha 1$  (39), sex determining region Y-box 6 (40), programmed cell death 4 (38), B cell-activating factor (35) and low-density lipoprotein receptor-related protein 6 (36) have been identified, mediating the miR-202-induced effects in different biological functions. To explore the regulatory mechanism of miR-202 in NSCLC, bioinformatics analysis was performed using TargetScan and microRNA.org to predict potential direct targets of miR-202.



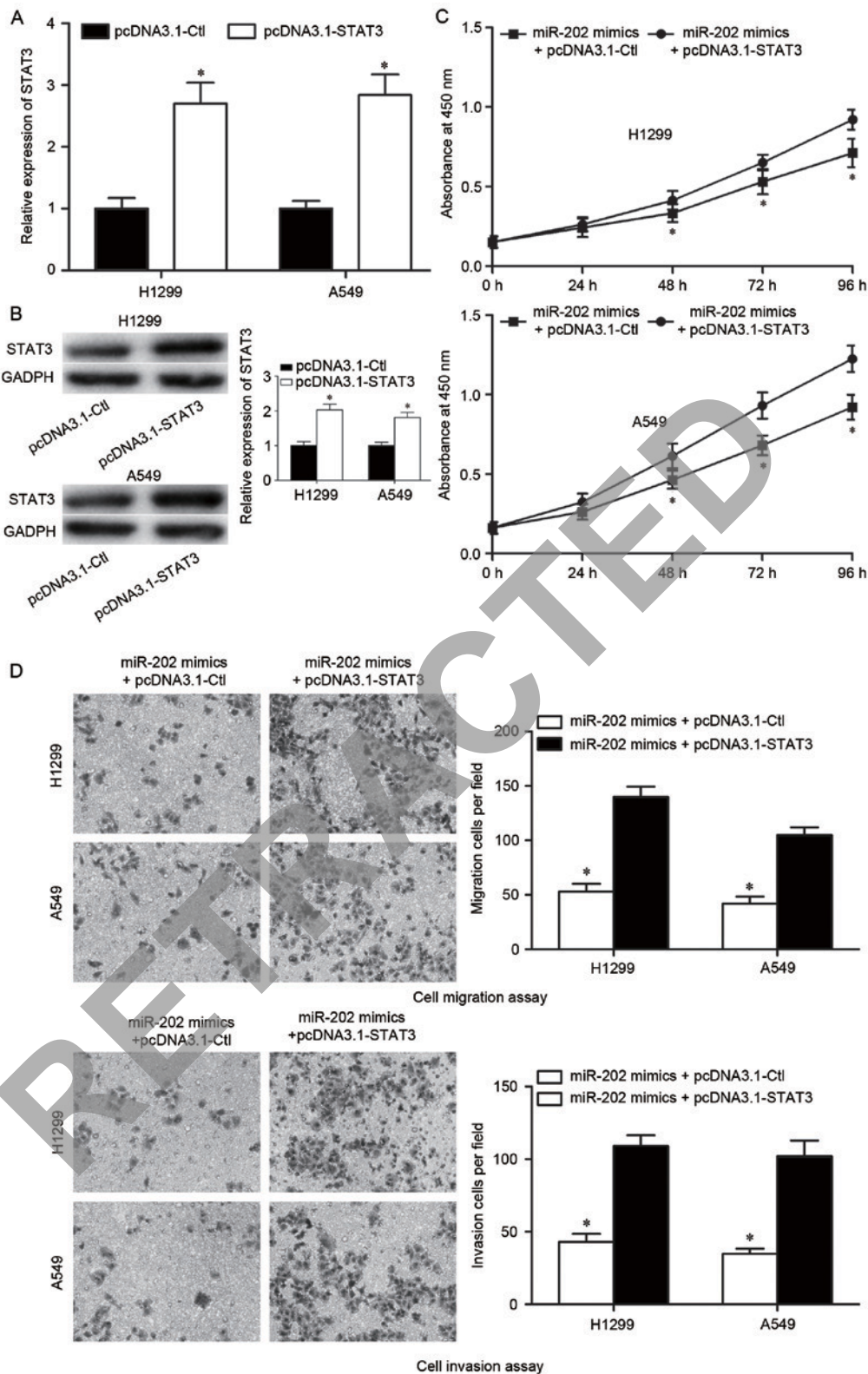


Figure 4. STAT3 overexpression increases miR-202-impaired cell viability, migration and invasion. (A) Reverse transcription quantitative-polymerase chain reaction was performed to detect STAT3 mRNA expression levels in H1299 and A549 cells transfected with pcDNA3.1-STAT3 or pcDNA3.1-Ctl. \* $P < 0.05$  vs. pcDNA3.1-Ctl. (B) Western blot analysis of STAT3 protein expression levels in H1299 and A549 cells transfected with pcDNA3.1-STAT3 or pcDNA3.1-Ctl. \* $P < 0.05$  vs. pcDNA3.1-Ctl. (C) Cell Counting Kit-8 assay indicated that STAT3 overexpression significantly improved the cell viability inhibition in H1299 and A549 cells induced by miR-202 overexpression. \* $P < 0.05$  vs. miR-202 mimic + pcDNA3.1-Ctl. (D) Transwell migration and invasion assays revealed that impaired migration and invasion capacities induced by miR-202 overexpression were reversed by STAT3 overexpression in H1299 and A549 cells. \* $P < 0.05$  vs. miR-202 mimic + pcDNA3.1-Ctl. STAT3, signal transducer and activator of transcription 3; Ctl, control.

Amongst the predicted target genes, STAT3 was selected for further investigation in the present study.

STAT3 is a signal mediator that is activated by various cytokines, growth factors and interferons (41), and is highly

expressed in a several human tumor tissues and cell lines, including NSCLC (42,43). STAT3 expression is associated with tumor differentiation, clinical stage and lymph node metastasis in NSCLC (44). NSCLC patients with high STAT3 expression displayed a shorter 5-year overall survival rate compared with patients with low STAT3 expression, and multivariate analysis indicated that high STAT3 expression was an independent prognostic factor for NSCLC (44). Furthermore, previous functional studies have demonstrated that STAT3 suppression significantly inhibits cell proliferation and induces apoptosis in NSCLC cells (45). STAT3 is also involved in promoting metastasis in several types of cancer, including pancreatic cancer (46), breast cancer (47), prostate cancer (48) and lung cancer (49). These findings suggest that STAT3 targeting in NSCLC may provide a novel strategy for the treatment of patients with NSCLC.

In conclusion, miR-202 may serve a critical role in the occurrence and development of NSCLC, by reducing tumor cell viability, migration and invasion. Therefore, miR-202 detection and targeting may provide novel diagnostic or therapeutic strategies, respectively, for patients with NSCLC.

## References

- Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A: Global cancer statistics, 2012. *CA Cancer J Clin* 65: 87-108, 2015.
- Billello KS, Murin S and Matthey RA: Epidemiology, etiology, and prevention of lung cancer. *Clin Chest Med* 23: 1-25, 2002.
- Siegel RL, Miller KD and Jemal A: Cancer statistics, 2016. *CA Cancer J Clin* 66: 7-30, 2016.
- Boffetta P and Nyberg F: Contribution of environmental factors to cancer risk. *Br Med Bull* 68: 71-94, 2003.
- Didkowska J, Manczuk M, McNeill A, Powles J and Zatonski W: Lung cancer mortality at ages 35-54 in the European Union: Ecological study of evolving tobacco epidemics. *BMJ* 331: 189-191, 2005.
- Ridge CA, McErlean AM and Ginsberg MS: Epidemiology of lung cancer. *Semin Intervent Radiol* 30: 93-98, 2013.
- Paliogiannis P, Attene F, Cossu A, Budroni M, Cesaraccio R, Tanda F, Trignano M and Palmieri G: Lung cancer epidemiology in North Sardinia, Italy. *Multidiscip Respir Med* 8: 45, 2013.
- Zhang T, Zhang DM, Zhao D, Hou XM, Liu XJ, Ling XL and Ma SC: The prognostic value of osteopontin expression in non-small cell lung cancer: A meta-analysis. *J Mol Histol* 45: 533-540, 2014.
- Dempke WC, Suto T and Reck M: Targeted therapies for non-small cell lung cancer. *Lung Cancer* 67: 257-274, 2010.
- Janku F, Stewart DJ and Kurzrock R: Targeted therapy in non-small-cell lung cancer-is it becoming a reality? *Nat Rev Clin Oncol* 7: 401-414, 2010.
- Reck M, Heigener DF, Mok T, Soria JC and Rabe KF: Management of non-small-cell lung cancer: Recent developments. *Lancet* 382: 709-719, 2013.
- Goldstraw P, Ball D, Jett JR, Le Chevalier T, Lim E, Nicholson AG and Shepherd FA: Non-small-cell lung cancer. *Lancet* 378: 1727-1740, 2011.
- Rosell R, Bivona TG and Karachaliou N: Genetics and biomarkers in personalisation of lung cancer treatment. *Lancet* 382: 720-731, 2013.
- Campayo M, Navarro A, Viñolas N, Diaz T, Tejero R, Gimferrer JM, Molins L, Cabanas ML, Ramirez J, Monzo M and Marrades R: Low miR-145 and high miR-367 are associated with unfavourable prognosis in resected nonsmall cell lung cancer. *Eur Respir J* 41: 1172-1178, 2013.
- Bartel DP: MicroRNAs: Genomics, biogenesis, mechanism, and function. *Cell* 116: 281-297, 2004.
- Ambros V: The functions of animal microRNAs. *Nature* 431: 350-355, 2004.
- Gregory RI and Shiekhattar R: MicroRNA biogenesis and cancer. *Cancer Res* 65: 3509-3512, 2005.
- Filipowicz W, Bhattacharyya SN and Sonenberg N: Mechanisms of post-transcriptional regulation by microRNAs: Are the answers in sight? *Nat Rev Genet* 9: 102-114, 2008.
- Krol J, Loedige I and Filipowicz W: The widespread regulation of microRNA biogenesis, function and decay. *Nat Rev Genet* 11: 597-610, 2010.
- Wu D, Niu X, Pan H, Zhou Y, Qu P and Zhou J: MicroRNA-335 is downregulated in bladder cancer and inhibits cell growth, migration and invasion via targeting ROCK1. *Mol Med Rep* 13: 4379-4385, 2016.
- Rahman MA, Salajegheh A, Smith RA and Lam AK: MicroRNA-126 suppresses proliferation of undifferentiated (BRAF (V600E) and BRAF (WT)) thyroid carcinoma through targeting PIK3R2 gene and repressing PI3K-AKT proliferation-survival signalling pathway. *Exp Cell Res* 339: 342-350, 2015.
- Zheng K, Liu W, Liu Y, Jiang C and Qian Q: MicroRNA-133a suppresses colorectal cancer cell invasion by targeting Fascin1. *Oncol Lett* 9: 869-874, 2015.
- Li Y, Li Y, Liu J, Fan Y, Li X, Dong M, Liu H and Chen J: Expression levels of microRNA-145 and microRNA-10b are associated with metastasis in non-small cell lung cancer. *Cancer Biol Ther* 17: 272-279, 2016.
- Kitade Y and Akao Y: MicroRNAs and their therapeutic potential for human diseases: microRNAs, miR-143 and -145, function as anti-oncomirs and the application of chemically modified miR-143 as an anti-cancer drug. *J Pharmacol Sci* 114: 276-280, 2010.
- Ventura A and Jacks T: MicroRNAs and cancer: Short RNAs go a long way. *Cell* 136: 586-591, 2009.
- Yang T, Thakur A, Chen T, Yang L, Lei G, Liang Y, Zhang S, Ren H and Chen M: MicroRNA-15a induces cell apoptosis and inhibits metastasis by targeting BCL2L2 in non-small cell lung cancer. *Tumour Biol* 36: 4357-4365, 2015.
- Edge SB and Compton CC: The American Joint Committee on Cancer: The 7th edition of the AJCC cancer staging manual and the future of TNM. *Ann Surg Oncol* 17: 1471-1474, 2010.
- Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25: 402-408, 2001.
- Li W and He F: Monocyte to macrophage differentiation-associated (MMD) targeted by miR-140-5p regulates tumor growth in non-small cell lung cancer. *Biochem Biophys Res Commun* 450: 844-850, 2014.
- Landi MT, Zhao Y, Rotunno M, Koshiol J, Liu H, Bergen AW, Rubagotti M, Goldstein AM, Linnoila I, Marincola FM, *et al*: MicroRNA expression differentiates histology and predicts survival of lung cancer. *Clin Cancer Res* 16: 430-441, 2010.
- Izzotti A, Calin GA, Arrigo P, Steele VE, Croce CM and De Flora S: Downregulation of microRNA expression in the lungs of rats exposed to cigarette smoke. *FASEB J* 23: 806-812, 2009.
- Wiggins JF, Ruffino L, Kelnar K, Omotola M, Patrawala L, Brown D and Bader AG: Development of a lung cancer therapeutic based on the tumor suppressor microRNA-34. *Cancer Res* 70: 5923-5930, 2010.
- Hu Z, Chen J, Tian T, Zhou X, Gu H, Xu L, Zeng Y, Miao R, Jin G, Ma H, *et al*: Genetic variants of miRNA sequences and non-small cell lung cancer survival. *J Clin Invest* 118: 2600-2608, 2008.
- Ma G, Zhang F, Dong X, Wang X and Ren Y: Low expression of microRNA-202 is associated with the metastasis of esophageal squamous cell carcinoma. *Exp Ther Med* 11: 951-956, 2016.
- Shen X, Guo Y, Yu J, Qi J, Shi W, Wu X, Ni H and Ju S: miRNA-202 in bone marrow stromal cells affects the growth and adhesion of multiple myeloma cells by regulating B cell-activating factor. *Clin Exp Med* 16: 307-316, 2016.
- Zhang Y, Zheng D, Xiong Y, Xue C, Chen G, Yan B and Ye Q: miR-202 suppresses cell proliferation in human hepatocellular carcinoma by downregulating LRP6 post-transcriptionally. *FEBS Lett* 588: 1913-1920, 2014.
- Sun Z, Zhang T, Hong H, Liu Q and Zhang H: miR-202 suppresses proliferation and induces apoptosis of osteosarcoma cells by downregulating Gli2. *Mol Cell Biochem* 397: 277-283, 2014.
- Lin Z, Song D, Wei H, Yang X, Liu T, Yan W and Xiao J: TGF- $\beta$ 1-induced miR-202 mediates drug resistance by inhibiting apoptosis in human osteosarcoma. *J Cancer Res Clin Oncol* 142: 239-246, 2016.



39. Meng X, Chen X, Lu P, Ma W, Yue D, Song L and Fan Q: MicroRNA-202 inhibits tumor progression by targeting LAMA1 in esophageal squamous cell carcinoma. *Biochem Biophys Res Commun* 473: 821-827, 2016.
40. Zhang D, Li Y, Tian J, Zhang H and Wang S: miR-202 promotes endometriosis by regulating SOX6 expression. *Int J Clin Exp Med* 8: 17757-17764, 2015.
41. Wagner KU and Schmidt JW: The two faces of Janus kinases and their respective STATs in mammary gland development and cancer. *J Carcinog* 10: 32, 2011.
42. Zhang Z, Ma J and Zhang L: STAT3 and ras-MAPK signal transduction pathway in non-small cell lung cancer. *Zhongguo Fei Ai Za Zhi* 8: 23-27, 2005 (In Chinese).
43. Hu FY, Wang LP, Hou LL, Yin G and Wang HC: The expressions of STAT3, WWOX and c-myc in human non small cell lung cancer tissue and correlativity analysis. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi* 26: 1203-1205, 1209, 2010 (In Chinese).
44. Yin Z, Zhang Y, Li Y, Lv T, Liu J and Wang X: Prognostic significance of STAT3 expression and its correlation with chemoresistance of non-small cell lung cancer cells. *Acta Histochem* 114: 151-158, 2012.
45. Zhu BR, Cai JM, Tang GS, Li BL, Gao F, Cui JG and Liu HC: Effects of STAT3 antisense oligonucleotide on proliferation and apoptosis of non-small cell lung cancer cell line A549. *Ai Zheng* 26: 820-827, 2007 (In Chinese).
46. Fofaria NM and Srivastava SK: STAT3 induces anoikis resistance, promotes cell invasion and metastatic potential in pancreatic cancer cells. *Carcinogenesis* 36: 142-150, 2015.
47. Dai L, Cheng L, Zhang X, Jiang Q, Zhang S, Wang S, Li Y, Chen X, DU T, Yang Y, *et al*: Plasmid-based STAT3-siRNA efficiently inhibits breast tumor growth and metastasis in mice. *Neoplasia* 58: 538-547, 2011.
48. Abdulghani J, Gu L, Dagvadorj A, Lutz J, Leiby B, Bonuccelli G, Lisanti MP, Zellweger T, Alanen K, Mirtti T, *et al*: Stat3 promotes metastatic progression of prostate cancer. *Am J Pathol* 172: 1717-1728, 2008.
49. Lin HY, Chiang CH and Hung WC: STAT3 upregulates miR-92a to inhibit RECK expression and to promote invasiveness of lung cancer cells. *Br J Cancer* 109: 731-738, 2013.

RETRACTED