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Original Article

Diminished LINC00173 expression induced miR-182-5p accumulation promotes cell proliferation, migration and apoptosis inhibition via AGER/NF-κB pathway in non-small-cell lung cancer

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Abstract: Non-small cell lung cancer (NSCLC) is one of the most common malignant tumors in the world, and non-coding RNA (ncRNA) has recently been widely reported to participate in the development of NSCLC. Some ncRNAs, especially microRNAs (miRNAs), are widely reported as tumor drug targets due to their short transcript length and easiness for processing into small molecule compounds. Therefore, exploring the potential roles of specific miRNAs in NSCLC may provide a better understanding of the molecular etiology. In this study, we downloaded the large-scale RNA-seq data from the Cancer Genome Atlas (TCGA) database, and identified 211 differentially expressed miRNAs (121 up-regulated and 90 down-regulated) in NSCLC. Similar to the TCGA database, miR-182-5p was significantly up-regulated in the serum and tissue samples of NSCLC patients. Clinicopathological parameters revealed the positive correlation between miR-182-5p expression and advanced TNM stage. Functional tests showed miR-182-5p overexpression promoted cell proliferation, migration and apoptosis inhibition, while miR-182-5p knockdown weakened the above phenotypes. Besides, advanced glycosylation end-product specific receptor (AGER) was identified as a direct downstream target of miR-182-5p. Alteration of AGER expression or NF-κB inhibitor could partially counteract the bioactive roles induced by miR-182-5p overexpression or knockdown. Further study disclosed down-regulated LINC00173 was negatively corrected with miR-182-5p in NSCLC tissues. LINC00173 could regulate miR-182-5p expression and reversed functional behaviors mediated by miR-182-5p/AGER/NF-κB axis. Taken together, miR-182-5p mediated the malignant phenotypes through NF-κB pathway via targeting AGER, and LINC00173 acted as a potential negative regulator of miR-182-5p in NSCLC cells.

Keywords: miR-182-5p, LINC00173, advanced glycosylation end-product specific receptor (AGER), nuclear translocation, non-small-cell lung cancer (NSCLC)

Introduction

Lung cancer is one of the most prevalent cancer and the leading cause of cancer-related death in the world [1]. Among the cases of newly diagnosed lung cancer, the vast majority were classified as non-small cell lung cancer (NSCLC), accounting for about 85% [2]. Due to lack of specific clinical symptoms or novel cancer-specific biomarkers, most diagnosis was confirmed in an advanced tumor stage, accompanied by the five-year survival rate of less than 18% [3]. Therefore it is crucial to elucidate the potential molecular mechanisms of NSCLC and identify new therapeutic targets for NSCLC patients.

MicroRNAs (miRNAs) are short non-coding RNA (ncRNA) sequences with 19~22 nucleotides in length. It was reported to alter gene expression mainly through post-transcriptional regulation. Specifically, miRNAs could target the 3'UTR of different mRNAs via RNA-induced silencing complex, thereby leading to translational repression or mRNA degradation [4]. Increasing studies demonstrated that miRNAs could play important roles not only in several key physiological processes, such as differentiation and proliferation, but also in tumorigenesis, including migration, apoptosis and epidermal to mesenchymal transition (EMT). The long non-coding RNA (lncRNA) is another kind of ncRNAs different from miRNAs. In terms of length, lncRNA

owns a transcripts length >200 nucleotides, much larger than miRNAs, adding diversity to its biological function. For example, if there are certain sequences paired with bases of miRNAs, then the lncRNA can be viewed as decoy. Under this circumstance, lncRNAs act as molecular sponges for miRNAs, which attract miRNAs to leave from or bind to target gene, thereby leading to gene activation or silencing [5]. Therefore, exploring potential miRNAs closely related to lung cancer, applying them as novel therapeutic targets, and further explaining the regulatory network of ncRNAs, can provide a new sight for the treatment of lung cancer.

MiR-182, a novel cancer-related miRNA, was widely reported to be deregulated in various tumor types. Liu et al. [6] found that miR-182 was up-regulated in colorectal cancer tissues and was involved with the resistance to 5-FU in colorectal cancer. Yu et al. [7] demonstrated miR-182 was involved in a negative feedback loop of TGFβ signaling. In short, TGFβ-induced miR-182 suppressed SMAD7 (a negative regulator of TGFβ signalling) translation via directly targeting its 3'-UTR, thereby promoting TGFβ-induced cell invasion and EMT in breast cancer [7]. In fact, the function of miR-182 was far more complicated than imagined. Some scholars found that it also had tumor-suppressive characteristics by targeting insulin-like growth factor 1 receptor in clear cell renal cell carcinoma [8]. Besides, in proliferative vitreoretinopathy development, miR-182 could inhibit the proliferative and migratory activity of retinal pigment epithelial by down-regulating its target gene c-Met [9]. From the above, tumor specificity may account for the paradoxical roles of miR-182 in human cancers. Though several studies reported the oncogenic role of miR-182 in NSCLC [10-12], yet the clinical utility and its molecular mechanisms in NSCLC were not fully clear.

In present study, we identified 221 differentially expressed miRNAs in NSCLC using the Cancer Genome Atlas (TCGA) database. Following initial validation in NSCLC tissues, we further verified the differential expression of miR-182-5p in serum samples. We found that miR-182-5p expression was significantly upregulated in NSCLC tissues and serum samples, and was positively correlated with advanced TNM stage. Gain- and loss- of function tests showed miR-182-5p promoted cell proliferation, migration and apoptosis inhibition in vitro. Notably, the regulatory network containing LINC00173 and advanced glycosylation end-product specific receptor (AGER)/NF-κB signaling axis was identified to be involved in this process.

Materials and methods

Samples collection and cell culture

Serum samples obtained from 67 NSCLC patients, 39 healthy donors and 13 benign pulmonary diseases (BPD) patients were collected in the Affiliated Hospital of Nantong University. Fresh cancerous tissues and adjacent non-cancerous tissues were collected from NSCLC patients who underwent surgery in the Affiliated Hospital of Nantong University and Nantong Tumor Hospital from September 2016 to December 2018. None of the patients received chemotherapy before surgery. Our study was conducted according to authorized guidelines of local hospitals and approved by the Institutional Ethics Committee. All the samples described above were carried out in accordance with the Code of Ethics of the World Medical Association and informed consent was obtained for experimentation with human subjects.

Human NSCLC cell lines, including A549 and NCI-H1299, were kindly provided by the Stem Cell Bank, Chinese Academy of Sciences (Shanghai, China). The above two cell lines were cultured in RPMI 1640 medium (Corning, Manassas, Virginia, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco, Grand Island, NY, USA), 1% penicillin and streptomycin (100 IU/ml) at 37°C in a humidified incubator containing 5% CO_2.

RNA extraction and quantitative real-time PCR (qRT-PCR)

Total RNA from NSCLC tissues and cells were extracted using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. As for serum RNA, the Total RNA Pure and Isolation Kit with Spin Column (BioTeke, Beijing, Chia) was used without separating red blood cells beforehand. After RNA extraction, the RevertAid RT Reverse Transcription Kit (Thermo Fisher Scientific) was used for reverse transcription of RNA into cDNA. All amplified

Table 1. The primer sequences used in this study were listed in detail

Gene	Primer sequence
miR-182-5p	F 5'-ACACTCCAGCTGGGTTTGGCAATGGTAGAACT-3'
	R 5'-TGGTGTCGTGGAGTCG-3'
U6	F 5'-CTCGCTTCGGCAGCACA-3'
	R 5'-AACGCTTCACGAATTTGCGT-3'
AGER	F 5'-CGGCTGGTGTTCCCAATAA-3'
	R 5'-TGTTCCTTCACAGATACTCCCT-3'
LINC00173	F 5'-GCCACCTTGCTCCGCTGTTC-3'
	R 5'-CCGAGGCTTGGAGAGGAGG-3'
18sRNA	F 5'-GTAACCCGTTGAACCCCATT-3'
	R 5'-CCATCCAATCGGTAGTAGCG-3'

Aberration: F, forward primer; R, reverse primer; AGER, advanced glycosylation end-product specific receptor.

procedures were performed on the ABI 7500 PCR Detection System (ABI, USA), and SYBR Green I (Roche, Germany) was used as the fluorescent signal. We used a relatively quantitative ($2^{-\Delta\Delta Ct}$) method to calculate the relative expression level of target gene. In view of their higher stability and consistency, small nuclear RNA U6 and 18S rRNA were used as internal controls to normalize RNA input. All the primer sequences used in this study were shown in **Table 1**.

Western blot analysis

Total proteins from NSCLC cells and tissues were lysed in RIPA lysis buffer supplemented with 1 mM of PMSF (Beyotime, Beijing, China). Then we kept lysates on ice for about 30 minutes, centrifuged the mixture at 14,000 g for 15 minutes, and collected the supernatants. The Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime, Beijing, China) was used to purify nuclear proteins according to the manufacturer's instruction. The proteins were quantified by the enhanced BCA Protein Assay Kit (Beyotime, Beijing, China) to obtain consistent protein loading per well. The bound antibodies were detected on chemiluminescence imaging system (BIO-RAD, USA). The Lamin B1 was used as the marker for nuclear protein and β-actin for cytoplasmic protein.

Plasmid construction and cell transfection

Both miR-182-5p mimic and inhibitor were designed by RiboBio Corporation (Guangzhou, China). The knockdown and overexpression of LINC00173 were achieved by plasmids, designed by GenePharma (Suzhou, China). Lipofectamine 2000 (Thermo Fisher Scientific) was used for cell transfection when 60-80% cell confluence was obtained.

Cell counting kit-8 (CCK-8) and colony formation assay

A549 and NCI-H1299 cells were seeded in a 96-well plate with a density of 3000 cells per well. The inoculation volume of each well was 100 μl, and then 10 μl CCK-8 reagents (Dojindo, Kumamoto, Japan) was added to each well at 0 h, 24 h, 48 h and 72 h, respectively. The absorbance was measured at 450 nm (650 nm as reference) when incubating for additional 2 h at 37°C in the humidified incubator. Three repeated wells were designed for each group and the experiment was performed in triplicate.

For colony formation assay, cells were seeded in 6-well plates at a concentration of 5×10^2 per well and incubated in complete medium at 37°C for 15 days. The complete medium was replaced every four days. The colonies were then fixed with methanol and stained with 0.1% crystal violet (Beyotime, China). Visible colonies were counted manually.

Cell migration and apoptosis assay

For migration assay, about 100 μl cell suspension (5×10^4 cells) was seeded into the upper chambers (Corning, 8 μm) and 600 μl complete medium (containing 20% FBS) was added into the lower chambers. After incubating at 37°C for 24 h, cells on the upper surface of each membrane were softly removed with a cotton swab, while those adhered to the bottom surface were fixed by 4% formaldehyde and stained with 0.1% crystal violet. The number of migrated cells was determined from five random fields.

For the apoptosis assay, NSCLC cells were mixed with a double staining Annexin V and 7-AAD Apoptosis Detection Kit (BD Pharmingen, San Jose, CA, USA) in the dark and then incubated at room temperature for 15 min. Then the fluorescence was analyzed by flow cytometry according to the manufacturer's instruction.

Luciferase reporter assay

Luciferase reporter was constructed using a psiCHECK-2 vector with both AGER 3'-UTR and its mutant variant being prepared. Then miR-182 mimics were co-transfected with wide-type or mutant 3'-UTR of AGER vector into 293T cells using Lipofectamine 2000 reagent. After 48 h, cells were harvested and analyzed by the Dual-Luciferase Reporter Assay Kit (Promega, Wisconsin, USA) according to the manufacturer's instruction.

Statistical analysis

Data were presented as the mean ± SD and performed at least three independent replicates. SPSS 20.0 software (SPSS, Inc., Chicago, IL, USA) and Graphpad Prism 6.0 (San Diago, CA, USA) were used for data analysis and presentation. Student's t test was performed on data of two groups, and paired t tests was used for comparison of cancerous tissues and corresponding non-cancerous tissues. When there were more than two groups of data to compare, we used one-way ANOVA. Two-way ANOVA was used for cck-8 analysis, and spearman analysis was used to evaluate the correlation between two genes. The NIH Image J software was used to quantify western blot bands. A *P* value <0.05 was considered statistically significant.

Results

miR-182-5p was significantly increased in NSCLC samples

TCGA database was a kind of open-access tumor database that provided detailed and comprehensive genomic datasets for researchers to download and analyze. In this study, to identify differentially expressed miRNAs in NSCLC, we searched the TCGA database (https://cancergenome.nih.gov/). A total of 453 miRNAs was found in the database of NSCLC patients (**Figure 1A**). Of the 211 differentially expressed miRNAs, approximately 121 miRNAs were up-regulated and 90 were down-regulated (**Figure 1B**), and miR-182-5p was one of the most significantly up-regulated miRNAs in NSCLC samples (**Figure 1C**; Tables S1 and S2). To validate the significant difference, we detected miR-182-5p expression using qRT-PCR in 22 newly diagnosed NSCLC tissues. Compared with that in adjacent non-cancerous tissues, miR-182-5p was significantly upregulated in cancerous tissues, similar to that in TCGA database (P<0.001, **Figure 1D**).

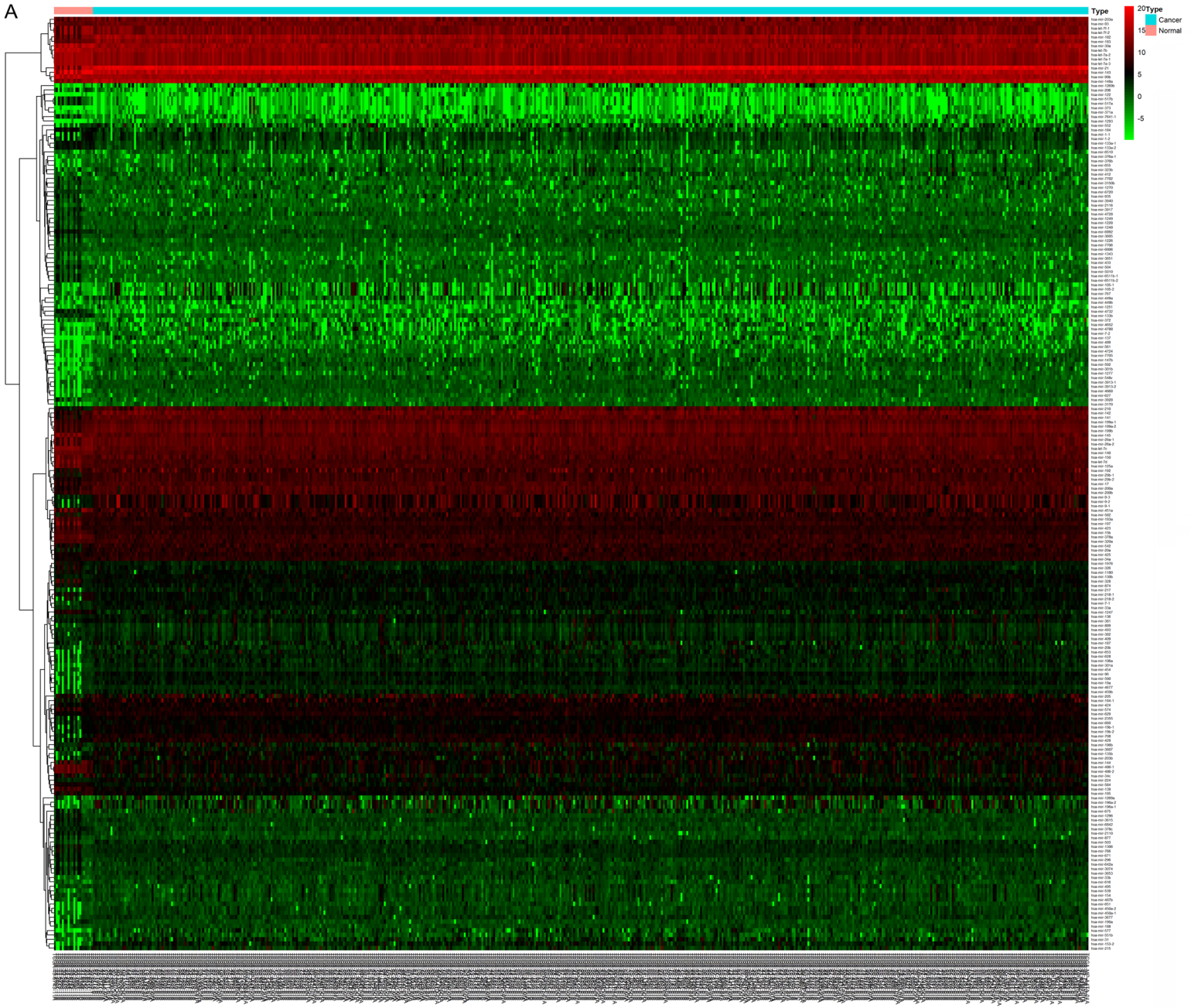
To explore the value of clinical utility, we collected the serum samples from 67 NSCLC patients, 39 healthy donors and 13 BPD patients. Results showed serum miR-182-5p expression in NSCLC patients (1.776±0.807) was significantly higher than that in healthy donors (0.511±2.555, P<0.001) and BPD patients (0.636±1.359, P=0.014, **Figure 1E**). To figure out whether serum miR-182-5p could distinguish NSCLC patients from healthy controls and BPD patients, we adopt the receiver operating characteristic (ROC) curve analysis. When comparing NSCLC and healthy control groups, the related sensitivity and specificity were 80.60% and 76.92% respectively at the cut-off value of 1.003. The area under curve (AUC) was 0.8259 (95% CI: 0.6911-0.8924, **Figure 1F**). In terms of comparison between NSCLC and BPD patients, related sensitivity and specificity were 77.61% and 69.23% respectively at the cutoff value of 1.093 (AUC= 0.7164, 95% CI: 0.6578-0.8689, **Figure 1G**). These results indicated that miR-182 was upregulated in both NSCLC tissues and serum samples, and it could be a potential non-invasive biochemical indicator for differential diagnosis of NSCLC.

High serum miR-182-5p expression was associated with poor prognosis in NSCLC patients

The associations between clinicopathological factors and serum miR-182-5p levels were illustrated in **Table 2**. No significant association was identified between miR-182-5p expression and gender (P=0.302), age (P=0.737), tumor size (P=0.878) and histology (P=0.471). However, serum miR-182-5p expression were positively correlated with tumor stage (P=0.001) and lymph node metastasis (P=0.017), indicating that high serum miR-182-5p expression may account for advanced stage and poor prognosis of NSCLC patients.

MiR-182-5p mediated the malignant phenotype of NSCLC cells in vitro

To further investigate the biological function of miR-182-5p in NSCLC, we artificially overexpressed and knocked down the miR-182-5p in NSCLC cells by transfecting miR-182-5p mimics (M) and inhibitor (I). Following verification of transfection efficiency, miR-182-5p was found to be effectively upregulated or downregulated in A549 and H1299 cells (**Figure 2A**). The cck-8 assay showed miR-182-5p overexpression could promote cell proliferation, while miR-182-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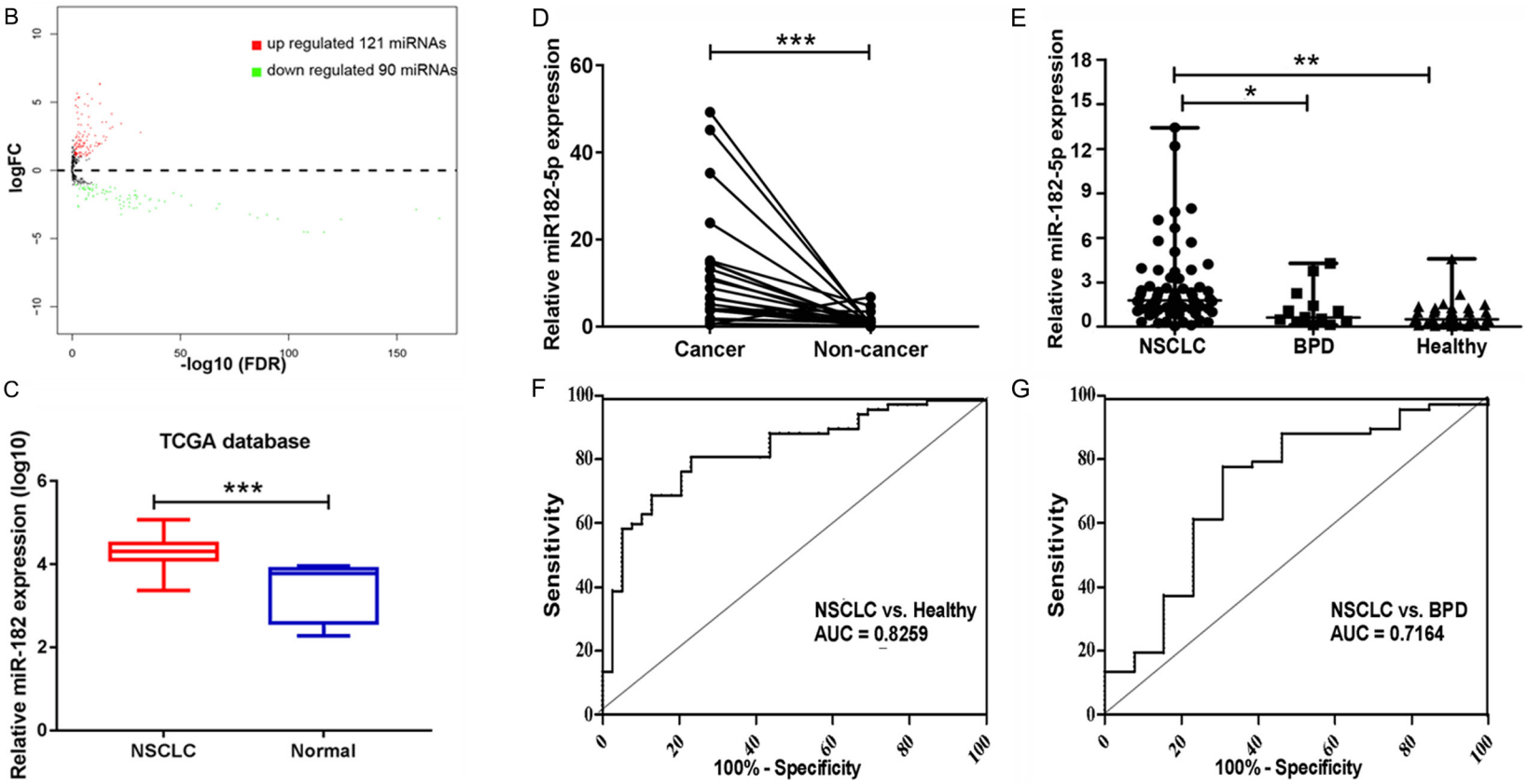


Figure 1. MiR-182-5p was significantly up-regulated in NSCLC samples. A. Heatmap of 453 miRNAs in 510 cases of NSCLC patients in TCGA database. B. Volcano plot of differentially expressed miRNAs in NSCLC patients. C. Relative miR-182-5p expression in 510 NSCLC patients in TCGA database. D. Validation of miR-182 expression in 22 pairs of NSCLC tissues. E. MiR-182-5p level was compared between 67 cases of newly diagnosed NSCLC patients, 13 cases of BPD patients and 39 cases of healthy donors. Small nuclear RNA U6 was used as an internal control. F, G. ROC curve anylysis was conducted to evaluate the diagnosic performance of miR-182-5p to distinguish NSCLC patients from healthy donors and BPD patients. **P*<0.05, ***P*<0.01, ****P*<0.001.

Table 2. Associations between relative expression of serum miR-182-5p and clinicopathological characteristics of NSCLC patients

Clinicopathological characteristics	No.	miR-182-5p Median (IQR 25-75)	U-value	*P*-value
Gender			498.5	0.550
Male	39	2.167 (1.676-2.813)		
Female	28	2.435 (1.829-3.217)		
Mean age (years)			444.5	0.155
≤60	31	2.357 (1.785-3.333)		
>60	36	2.283 (1.782-2.990)		
Tumor size (cm)			493.5	0.557
≤2	40	2.092 (1.376-3.130)		
>2	27	2.281 (1.856-2.870)		
Histology			433.0	0.754
Adenocarcinoma	50	1.843 (1.335-3.308)		
Squamous cell carcinoma	17	2.435 (1.924-3.117)		
Tumor stage (TNM)			249.0	0.001**
I-II	43	2.090 (1.489-2.941)		
III-IV	24	2.329 (1.788-3.230)		
Lymph node metastasis			333.0	0.017*
Negative	40	2.013 (1.642-2.566)		
Positive	27	2.731 (1.928-3.363)		

Aberration: IQR, interquartile range; *P<0.05, **P<0.01.

5p inhibitor exerted the opposite effect (**Figure 2B**). Colony formation assay revealed that overexpressed miR-182-5p in A549 and H1299 cells formed significantly more colonies than normal controls (**Figure 2C**), suggesting the positive role of miR-182-5p in cell proliferation. To assess cell migration ability, transwell assays were performed. As presented in **Figure 2D**, enhanced miR-182-5p expression significantly strengthen the ability of cell migration in A549 and H1299 cells. Next, western blot assay showed miR-182-5p overexpression in A549 cells markedly increased anti-apoptotic protein Bcl-2 and decreased pro-apoptotic protein Bax (**Figure 2E**). Similar results were also observed in H1299 cell lines (date not shown). In contrast, miR-182-5p knockdown led to decreased proliferation, migration and increased apoptosis in NSCLC cells (**Figure 2B-E**). These results demonstrated that miR-182-5p was involved with the malignant phenotype of NSCLC cells.

AGER was a direct target of miR-182-5p in NSCLC

As mentioned above, miRNAs was able to silence gene expression via directly binding to the 3'UTR. To identify target genes involved in miR-182-mediated malignant phenotype, we searched bioinformatics website TargerScan (http://www.targetscan.org/vert_72/) to predict potential target genes. Results showed the 3'-UTR of AGER matched the 'seed sequence' of miR-182-5p (**Figure 3A**). To demonstrate the possibility of binding, we simultaneously transferred the mutated 3'UTR (AGER-mut) and wild-type sequences of AGER into the 293T cells and then co-translated the miR-182 mimic and negative control (NC) for comparison. We found increased miR-182-5p obviously weakened the luciferase activity of the wild-type, while no influence was observed on AGER-mut (P<0.05, **Figure 3B**). Besides, qRT-PCR and western blot demonstrated miR-182-5p overexpression markedly suppressed the expression of AGER mRNA and protein in A549 and H1299 cells (**Figure 3C**, **3D**). Meanwhile, miR-182-5p inhibitor significantly increased AGER expression in A549 cells (**Figure 3C**, **3D**). In addition, we also verified AGER expression in NSCLC tissues at both mRNA and protein levels. Results showed both the expression of AGER protein and mRNA were lower in cancerous tissues than that in adjacent non-cancerous tissues (**Figure 3E** and **3F**). Meanwhile, the expression level of AGER mRNA in NSCLC tissues was negatively correlated with miR-182-5p expression (R^2=0.4461, P<0.001, **Figure 3G**). In conclusion, these data suggested AGER was a direct downstream target of miR-182-5p in NSCLC.

Alteration of AGER expression reversed miR-182-mediated malignant phenotype in NSCLC cells

Although miR-182 was demonstrated to bind to the 3'UTR of AGER, it was unclear whether

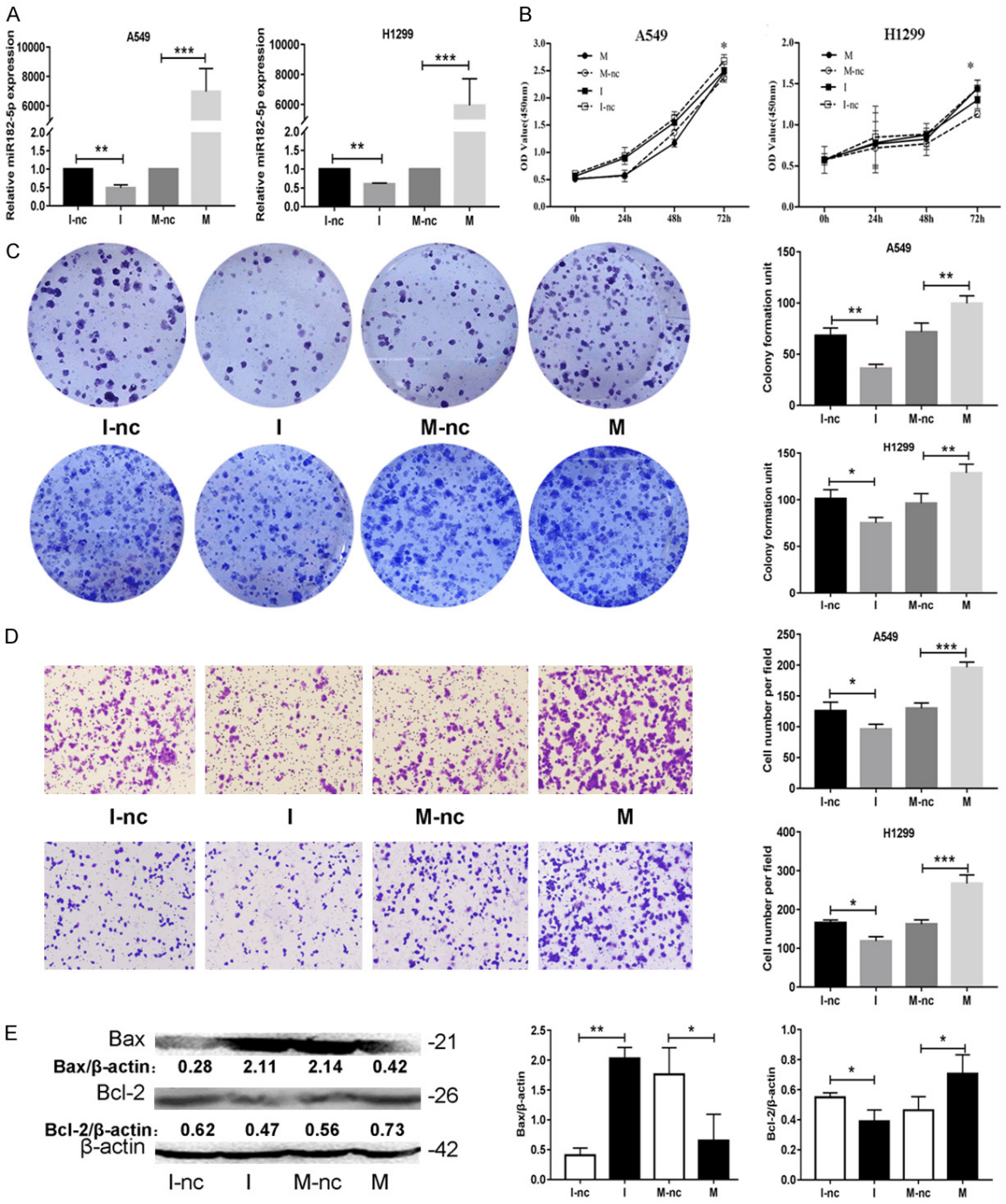


Figure 2. MiR-182-5p mediated the malignant phenotype of NSCLC cells in vitro. A. A549 and H1299 cells stably transfected with miR-182-5p mimics and inhibitor were subjected to qRT-PCR for miR-182-5p. B. Cell proliferation curve was depicted with the absorbance at 450 nm after the treatment of miR-182-5p mimics or inhibitors at different time points. C. Overexpressed miR-182-5p accelerated cell division, as characterized by an increase of clony units. D. miR-185-5p mimics treatment increased cell migration on the bottom surface of transwell, which was reversed by miR-182-5p inhibitors. E. Bax and Bcl-2 protein expression were examined via transfecting miR-182-5p mimic and inhibitor into A549 cells. **P*<0.05, ***P*<0.01, ****P*<0.001. Abbreviations: I, inhibitor; I-nc, negative control of miR-182-5p inhibitor; M, mimic; M-nc, negative control of miR-182-5p mimic.

AGER was involved in the miR-182-mediated malignant phenotype. Therefore, we constructed both the overexpressing (OV) plasmid and small interfering (Si) plasmids of AGER. Western

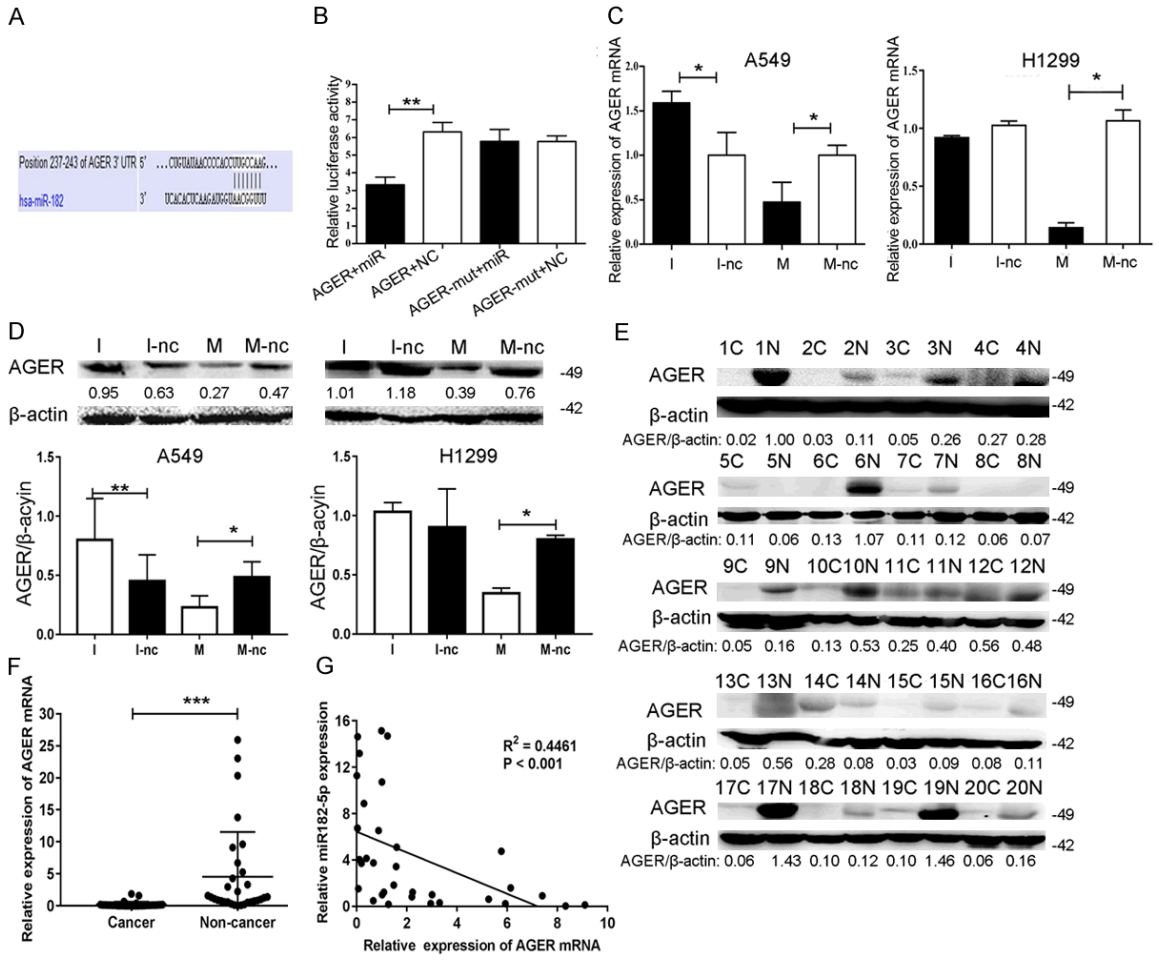


Figure 3. AGER was a direct target of miR-182-5p in NSCLC cells. A. Targetscan showed the complementary sequences in AGER-3'-UTR and miR-182-5p. B. The plasmids inserting wild-type or mutant 3'-UTR (AGER-mut) were co-transfected into 293T cells with either miR-182-5p or miR-NC and luciferase activity was quantified. C, D. AGER mRNA and protein expression levels were detected following miR-182-5p mimics and inhibitors transfection into A549 and H1299 cells. E. AGER protein expression was detected by western blot in 20 pairs of NSCLC tissues. F. AGER mRNA expression was further verified by qRT-PCR in 22 pairs of fresh NSCLC tissues. G. A negative correction between AGER mRNA and miR-182-5p expression was observed in NSCLC tissues. **P*<0.05, ***P*<0.01, ****P*<0.001.

blot confirmed the efficiency of AGER overexpression and knockdown at the protein level (**Figure 4A**). Then we restored AGER expression by transfecting its OV plasmid in A549 cells which were co-transfected miR-182-5p inhibitors. Resulted showed AGER restoration partially strengthened the anti-proliferative effect of miR-182-5p inhibitor, manifesting as significantly decreased colony formation units and cell migration rate in A549 cells (**Figure 4B** and **4C**).

NF-κB pathway was involved in miR-182-5p/AGER regulatory axis

To explore the underlying mechanisms responsible for malignant phenotype, we used pathDIP to look for the significantly enriched pathways, and finally chose NF-κB pathway for further analysis (**Figure 5A**). Western blot showed that overexpressed miR-182-5p decreased the IκBα protein and increased the translocation of p65, rather than p52, from the cytoplasm to the nucleus, while miR-182-5p inhibitor exerted opposite effect (**Figure 5B**). As for functional test, we then treated A549 and H1299 cells with both miR-182-5p mimics and the NF-κB pathway inhibitor BAY-11-7082. Results showed BAY-11-7082 partially weakened the promotion of cell proliferation, migration and apoptosis inhibition induced by oncogeic miR-182 in NSCLC cells (**Figure 5C-E**). In conclusion, our results indicated that NF-κB pathway was

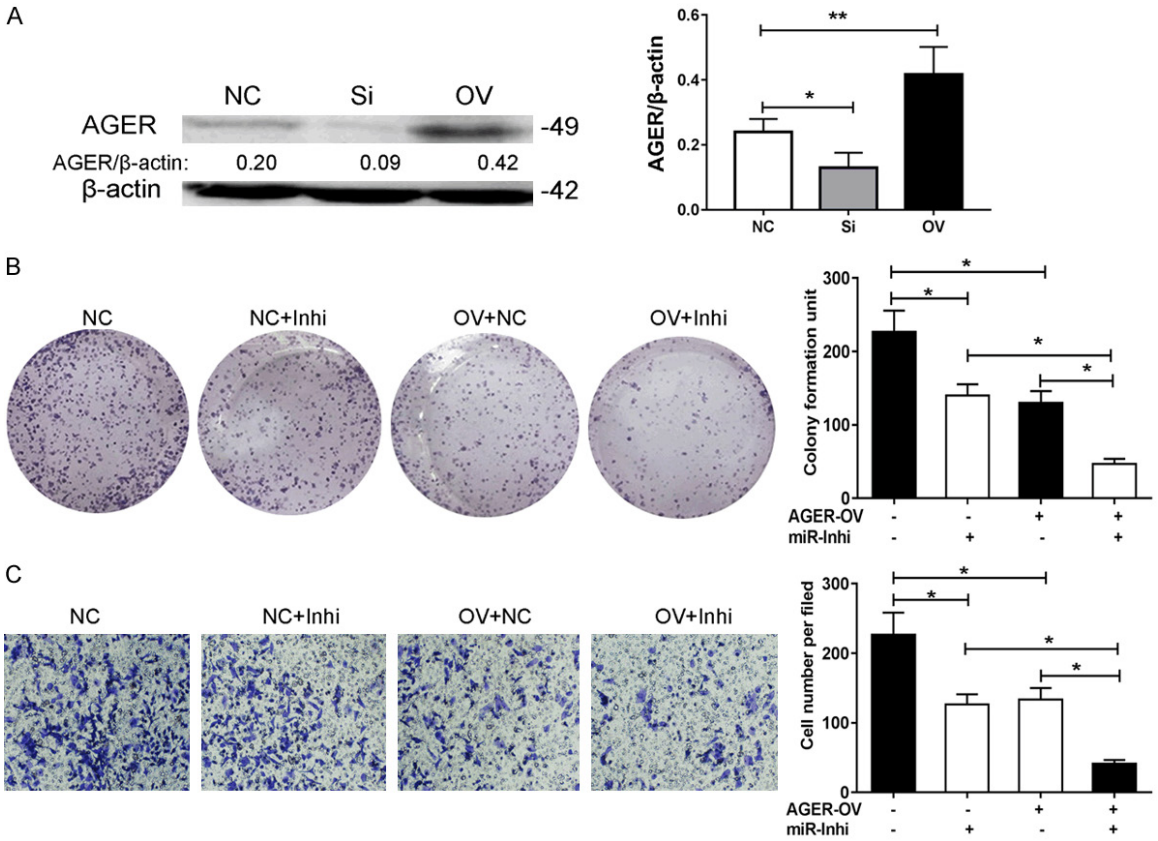
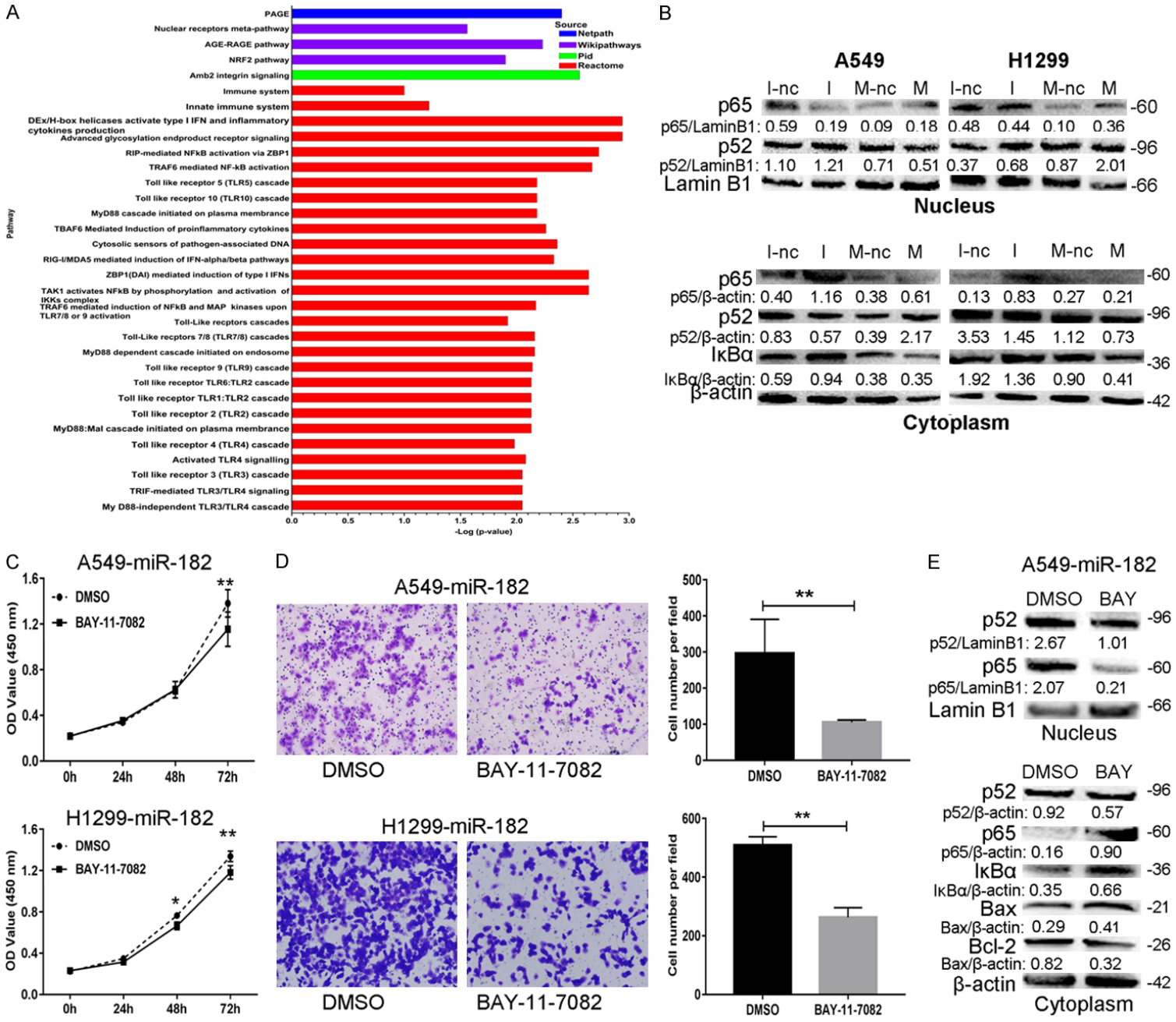


Figure 4. Alteration of AGER expression reversed miR-182-mediated malignant phenotypes in NSCLC cells. A. AGER protein expression level detected by western blot following siRNAs and overexpression plasmid transfection in A549 cells. B, C. Crystal violet staining at day 15 showed AGER restoration partially strengthened the anti-proliferative effect of miR-182-5p inhibitor, manifesting as significantly decreased colony formation units and cell migration rate in A549 cells. **P*<0.05, ***P*<0.01, ****P*<0.001.

involved in miR-182-5p-mediated biological function on NSCLC cells.

MiR-182-5p was negatively regulated by LINC00173 in NSCLC cells

To trace the mechanism of overexpressed miR-182-5p in NSCLC, we used starBase v2.0 (http://starbase.sysu.edu.cn/starbase2/) to predict the upstream gene. We discovered LINC00173 owned partial sequences complementary to miR-182-5p, implying LINC00173 might act as a molecular sponge. Next, our qRT-PCR data revealed LINC00173 expression in NSCLC tissues were notably lower than that in adjacent non-cancerous tissues (**Figure 6A**). Spearman correlation analysis revealed LINC00173 expression was negatively correlated with miR-182-5p in NSCLC tissues (R^2=0.2129, P<0.01, **Figure 6B**). Then, we used shRNA and pcDNA to artificially down- and up-regulate LINC00173 expression in A549 and H1299 cells (**Figure 6C**). QRT-PCR results showed LINC00173 overexpression led to a reduction of miR-182-5p in NSCLC cells, while LINC00173 inhibition induced an increase of miR-182-5p expression, suggesting the negative regulatory role of LINC00173 (**Figure 6D**). Furthermore, LINC00173 knockdown promoted cell proliferation, migration and inhibited apoptosis in NSCLC cells, while LINC00173 overexpression exerted the opposite effect (**Figures 6E-G**). Since LINC0173 was negatively correlated with miR-182-5p at the mRNA level, whether LINC00173 was a regulator of miR-182-5p/AGER/NF-κB axis raised our curiosity. We then artificially up-regulated LINC00173 in A549 and H1299 cells that were previously transfected with miR-182-5p mimics. Interestingly, LINC00173 overexpression partly counteracted the promotion of cell proliferation and migration mediated by miR-182 overexpression (**Figure 6H** and **6I**). Western blot also showed LINC00173 overexpression increased the expression level of AGER and


A
PAGE
Nuclear receptors meta-pathway
AGE-RAGE pathway
NRF2 pathway
Amb2 integrin signaling
Immune system
Innate immune system
DEx/H-box helicases activate type I IFN and inflammatory cytokines production
Advanced glycosylation endproduct receptor signaling
RIP-mediated NFkB activation via ZBP1
TRAF6 mediated NF-kB activation
Toll like receptor 5 (TLR5) cascade
Toll like receptor 10 (TLR10) cascade
MyD88 cascade initiated on plasma membrance
TBAF6 Mediated Induction of proinflammatory cytokines
Cytosolic sensors of pathogen-associated DNA
RIG-I/MDA5 mediated induction of IFN-alpha/beta pathways
ZBP1(DAI) mediated induction of type I IFNs
TAK1 activates NFkB by phosphorylation and activation of IKKs complex
TRAF6 mediated induction of NFkB and MAP kinases upon TLR7/8 or 9 activation
Toll-Like recptors cascades
Toll-Like recptors 7/8 (TLR7/8) cascades
MyD88 dependent cascade initiated on endosome
Toll like receptor 9 (TLR9) cascade
Toll like receptor TLR6:TLR2 cascade
Toll like receptor TLR1:TLR2 cascade
Toll like receptor 2 (TLR2) cascade
MyD88:Mal cascade initiated on plasma membrance
Toll like receptor 4 (TLR4) cascade
Activated TLR4 signalling
Toll like receptor 3 (TLR3) cascade
TRIF-mediated TLR3/TLR4 signaling
My D88-independent TLR3/TLR4 cascade
Pathway
Source
Netpath
Wikipathways
Pid
Reactome
0.0 0.5 1.0 1.5 2.0 2.5 3.0
-Log (p-value)
B
A549
H1299
I-nc I M-nc M I-nc I M-nc M
p65
p65/LaminB1: 0.59 0.19 0.09 0.18 0.48 0.44 0.10 0.36
p52
p52/LaminB1: 1.10 1.21 0.71 0.51 0.37 0.68 0.87 2.01
Lamin B1
-60
-96
-66
Nucleus
I-nc I M-nc M I-nc I M-nc M
p65
p65/β-actin: 0.40 1.16 0.38 0.61 0.13 0.83 0.27 0.21
p52
p52/β-actin: 0.83 0.57 0.39 2.17 3.53 1.45 1.12 0.73
IκBα
IκBα/β-actin: 0.59 0.94 0.38 0.35 1.92 1.36 0.90 0.41
β-actin
-60
-96
-36
-42
Cytoplasm
C
A549-miR-182
DMSO
BAY-11-7082
OD Value (450 nm)
0.0 0.4 0.8 1.2 1.6
0h 24h 48h 72h
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H1299-miR-182
DMSO
BAY-11-7082
OD Value (450 nm)
0.0 0.4 0.8 1.2 1.6
0h 24h 48h 72h
*
**
D
A549-miR-182
DMSO
BAY-11-7082
Cell number per field
0 100 200 300 400 500
DMSO BAY-11-7082
**
H1299-miR-182
DMSO
BAY-11-7082
Cell number per field
0 200 400 600
DMSO BAY-11-7082
**
E
A549-miR-182
DMSO BAY
p52
p52/LaminB1: 2.67 1.01
p65
p65/LaminB1: 2.07 0.21
Lamin B1
-96
-60
-66
Nucleus
DMSO BAY
p52
p52/β-actin: 0.92 0.57
p65
p65/β-actin: 0.16 0.90
IκBα
IκBα/β-actin: 0.35 0.66
Bax
Bax/β-actin: 0.29 0.41
Bcl-2
Bax/β-actin: 0.82 0.32
β-actin
-96
-60
-36
-21
-26
-42
Cytoplasm

Figure 5. NF-κB pathway was involved in miR-182-5p/AGER regulatory axis. A. Bioinformatics software pathDIP identified significantly enriched pathways of AGER. B. The p65, p52 and IκBα protein expression both in the nucleus and cytoplasm were detected by western blot. The Lamin B1 protein was used as the marker for nuclear protein and β-actin for cytoplasmic protein. C. Proliferation curve was depicted with the absorbance at 450 nm after the co-treatment with miR-182-5p mimics and 10 nM BAY-11-7082 at different time points. D. Cells migrating to the upper bottom of transwell were counted following treatment with miR-182-5p mimics and BAY-11-7082 for 24 h. E. Western blot analysis showed the changes in the distribution of p65 protein in nucleus and cytoplasm of A549 cells that stably expressed miR-182-5p after treating with BAY-11-7082 for 24 h. **P*<0.05, ***P*<0.01, ****P*<0.001.

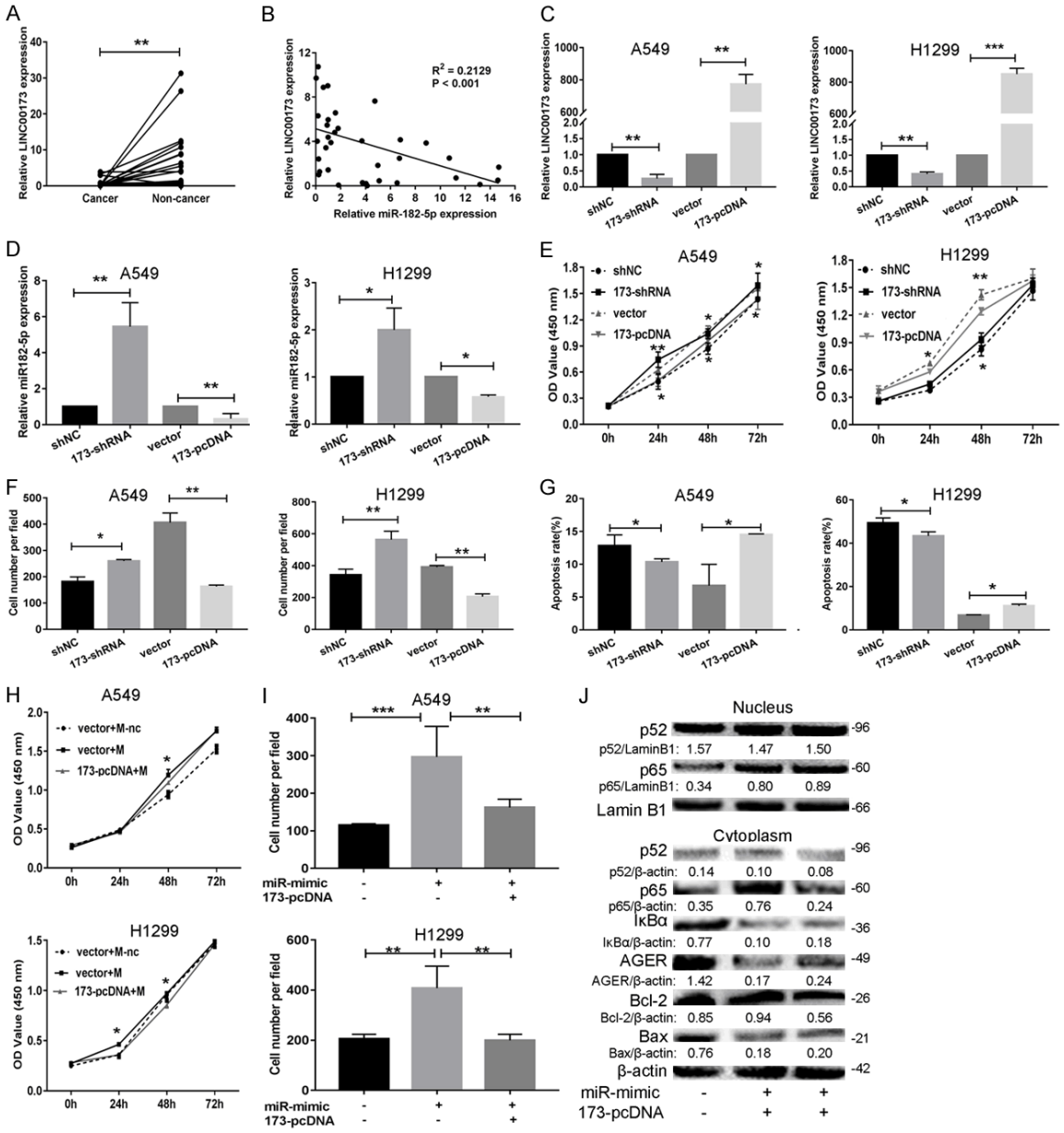

Figure 6. MiR-182-5p was negatively regulated by LINC00173 in NSCLC cells. A. Detection of LINC00173 expression in 22 pairs of NSCLC tissues by qRT-PCR. 18S rRNA was used as an internal control. B. A negative correction between miR-182-5p and LINC00173 expression was observed in NSCLC tissues. C, D. LINC00173 and miR-182-5p expression detected by qRT-PCR following shRNA and overexpression plasmid transfection in A549 cells and H1299 cells. E-G. LINC00173 knockdown increased proliferation, migration and apoptosis rate of NSCLC cells, which was reversed by LINC00173 overexpression. H, I. Overexpressed LINC00173 in A549 cells ransfected with miR-182-5p mimic partly counteracted miR-182-5p mediated promotion of cell proliferation and migration. J. Western blot analysis of downstream AGER/p65/IκBα protein in A549 cells that were co-transfected with miR-182-5p mimic and LINC00173 overexpressed vector. **P*<0.05, ***P*<0.01, ****P*<0.001.

IκBα protein, and inhibited the nuclear translocation of p65 in miR-182-overexpressed A549 cells (**Figure 6J**). Thus, we might conclude that LINC00173 participated in AGER/NF-κB axis possibly by negatively regulating miR-182-5p in NSCLC cells.

Discussion

Increasing studies reported ncRNAs played important roles in tumor initiation and progression. Therefore, figuring out differentially expressed ncRNAs and explaining the specific mechanism were of great value in cancer diagnosis and treatment. Due to their short transcript length and easy access to the cell, miRNAs received widely attention among clinicians and drug explorers. The treatment of MRX34 (a liposomal miR-34a mimic) plus dexamethasone premedication showed satisfactory curative effects in patients with refractory advanced solid tumors [13]. Though miR-182-5p was known to be differentially expressed in various types of cancers, the mechanism remained largely unexplored. In this study, we identified 211 differentially expressed miRNAs in NSCLC patients of TCGA database. Among 121 up-regulated miRNAs, miR-182 was of significant difference. Further validation in tissue and serum samples showed miR-182-5p was significantly up-regulated in NSCLC samples. Besides, exploring circulating tumor DNA or RNA as diagnosic marker, which was also called "liquid biopsies", obtained widespread attention owing to its non-invasiveness and low cost [14]. In the present study, ROC curve demonstrated serum miR-182-5p could distinguish NSCLC patients from healthy controls and BPD patients. Analysis on clinicopathological parameters showed high miR-182-5p expression was positively correlated with advanced tumor stage and lymphatic metastasis. The data above disclosed the potential role of serum miR-182-5p in differential diagnosis and prognosis evaluation of NSCLC.

Previous studies showed that miR-182 was differentially expressed in different tumor types. Kulkarni et al. [15] reported that miR-182-5p was downregulated in clear cell renal carcinoma and exerted an inhibitory effect on cell proliferation, colony formation, apoptosis, and cell-cycle arrest by directly targeting lncRNA MALAT-1. However, in medullary thyroid carcinoma, miR-182 was significantly upregulated and responsible for the invasive and migratory properties of cancer [16]. Besides, even in tumors of the same organ origin, the role of miR-182 was contradictory. On the one hand, miR-182 was frequently downregulated in metastatic NSCLC cells and was able to inhibit cell migration, invasion and EMT [16]. On the other hand, miR-182 was reported to function as an oncogene resulting in increased cell proliferation [10], radioresistance [12] and glucose metabolism [11] in NSCLC cells. Therefore, the exact mechanism of miR-182 in NSCLC was complex and needed to be further clarified. To some extent, the dual role of miR-182 in NSCLC might be attributed to different target genes, and it was difficult to say which specific target gene or some genes were really active during tumor progression. In our study, we discovered that miR-182-5p mediated malignant phenotype of NSCLC cells via targeting AGER, manifesting as accelerated cell proliferation, increased migration ability and reduced apoptosis rate.

AGER was a multi-ligand cell surface protein that belonged to the immunoglobulin superfamily. Except for AGEs, AGER could also identify amyloid protein, S100 calgranulin and high mobility group protein B1 [17]. Studies showed abnormal AGER expression was closely associated with immunoinflammatory response and tumorigenesis [18, 19]. Interestingly, RAGE was reported to be upregulated in most cancerous tissues except for the lung cancer [20]. AGER was thought to be an adhesion molecule rather than a truly receptor [21], and decreased AGER expression in NSCLC increased migration and invasion in cancer cells [22]. Here, low AGER expression was found in NSCLC tissues. Bioinformatics software disclosed a complementary binding sequence existed in miR-182-5p and 3'UTR of AGER. Luciferase reporter assay further confirmed miR-182-5p could negatively regulate the AGER expression by directly targeting its 3'UTR. Besides, we found miR-182-5p was negatively correlated with AGER in NSCLC tissues and AGER overexpression partly abrogated the simulative effects of miR-182-5p on cell proliferation and migration.

Previous studies showed the p65 could regulate AGER expression by binding to its promoter in high glucose conditions [23]. Besides, AGER could be down-regulated by oxidant-dependent activiation of p38 MAPK and NF-kB in A549

cells [24]. Here we found miR-182-5p exerted its oncogenic effect through promoting translocation of p65 from cytoplasm to nucleus, and the inhibitor of NF-κB signal (BAY-11-7082) could counteract the effect mediated by overexpressed miR-182-5p. Because of the same miRNA response elements, lncRNA was able to attracted miRNAs to leave from their target genes, thus down-regulating miRNAs and up-regulating the target genes [25, 26]. Once the upstream regulator diminished, the downstream miRNAs accumulated to bring about bad results. Therefore, we tried to explain whether high miR-182-5p expression in NSCLC tissues was regulated by a certain lncRNA. We then found diminished LINC00173 expression was negatively correlated with miR-182-5p in NSCLC tissues. Decreased LINC00173 expression promoted cell growth, migration and apoptosis inhibition, while increased LINC00173 exerted the opposite trend. Besides, miR-182-5p was negatively regulated by LINC00173, and LINC00173 overexpression could partly abrogate the malignant phenotypes mediated by miR-182-5p in NSCLC cells.

In summary, we identified the significantly up-regulated miR-182-5p in NSCLC samples. The potential of miR-182-5p as a serum marker for NSCLC was also demonstrated. Functional tests disclosed the oncogenic characteristics of miR-182-5p in NSCLC cells. Furthermore, diminished LINC00173-induced miR-182 accumulation was responsible for the accelerated cell proliferation, migration and the inhibited apoptosis via AGER /NF-κB axis in NSCLC cells.

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Disclosure of conflict of interest

None.

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Table S1. A total of 121 miRNAs were up-regulated in NSCLC samples from the TCGA database

ID	logFC	logCPM	*P* Value	FDR
hsa-mir-21	2.781362801	18.49555939	9.42E-34	1.86E-32
hsa-mir-708	3.428150073	6.920691792	1.39E-24	1.62E-23
hsa-mir-96	3.184847811	4.900558523	1.80E-20	1.73E-19
hsa-mir-210	4.144083751	10.16821956	4.17E-20	3.94E-19
hsa-mir-142	3.079497681	11.50688563	2.11E-19	1.87E-18
hsa-mir-182	2.444822934	14.60513617	1.22E-17	1.02E-16
hsa-mir-301a	3.549684891	4.220750165	1.56E-17	1.29E-16
hsa-mir-141	2.227112453	10.74341135	3.53E-17	2.80E-16
hsa-mir-183	2.499485311	13.58662134	2.52E-16	1.94E-15
hsa-mir-135b	3.535232704	6.251031129	1.27E-15	9.14E-15
hsa-mir-7-1	1.957160932	4.599827713	3.13E-15	2.18E-14
hsa-mir-153-2	4.907773185	4.470539547	1.31E-14	9.01E-14
hsa-mir-9-2	6.371594842	10.13841369	1.50E-14	1.01E-13
hsa-mir-9-1	6.360219033	10.138135	1.61E-14	1.07E-13
hsa-mir-9-3	6.312169382	10.14031044	1.68E-14	1.10E-13
hsa-mir-590	1.98025975	4.347835591	3.11E-14	2.01E-13
hsa-mir-148a	1.898786742	15.60567971	4.79E-14	3.05E-13
hsa-mir-29b-1	1.975050928	8.97655957	7.01E-14	4.41E-13
hsa-mir-29b-2	1.781770654	9.065301754	2.43E-13	1.51E-12
hsa-mir-200a	2.056400044	9.912877934	9.83E-13	5.78E-12
hsa-mir-19a	2.556216421	4.714266473	2.47E-12	1.43E-11
hsa-mir-20a	1.803636875	7.93440915	3.43E-12	1.97E-11
hsa-mir-93	1.440997311	11.98174547	4.56E-11	2.40E-10
hsa-mir-577	5.245530922	3.350556955	1.04E-10	5.32E-10
hsa-mir-429	1.836016214	7.061500236	3.86E-10	1.90E-09
hsa-mir-4668	3.113871732	1.960370637	3.96E-10	1.93E-09
hsa-mir-454	1.709818076	3.35779456	5.20E-10	2.51E-09
hsa-mir-199a-1	1.287140658	10.14705483	5.68E-10	2.71E-09
hsa-mir-199a-2	1.290587129	10.87019694	8.12E-10	3.83E-09
hsa-mir-2355	1.482823172	6.105340885	2.88E-09	1.29E-08
hsa-mir-147b	3.9278976	2.369203084	3.32E-09	1.47E-08
hsa-mir-450a-2	2.640930184	2.758408309	3.43E-09	1.51E-08
hsa-mir-199b	1.273530159	11.20549613	4.28E-09	1.85E-08
hsa-mir-4677	1.599206356	3.458221344	8.15E-09	3.36E-08
hsa-mir-301b	3.339905193	2.148392147	8.65E-09	3.52E-08
hsa-mir-31	4.738961864	4.610826733	8.70E-09	3.52E-08
hsa-mir-425	1.159224519	7.629493484	1.33E-08	5.33E-08
hsa-mir-196a-2	5.606536935	6.043901695	1.50E-08	5.90E-08
hsa-mir-629	1.127376915	7.02768265	2.02E-08	7.84E-08
hsa-mir-224	2.4782645	5.974133936	2.37E-08	9.11E-08
hsa-mir-34a	1.149325777	7.87737221	2.51E-08	9.54E-08
hsa-mir-33a	1.720585658	4.747700188	2.83E-08	1.07E-07
hsa-mir-450a-1	2.216529556	2.779386846	3.39E-08	1.27E-07
hsa-mir-130b	1.364993213	5.282599556	9.07E-08	3.29E-07
hsa-mir-17	1.066299958	9.498338329	1.11E-07	3.98E-07
hsa-mir-19b-1	1.239771088	6.004210099	1.65E-07	5.81E-07
hsa-mir-196a-1	4.865224903	5.925219717	2.89E-07	9.93E-07
hsa-mir-548v	2.837431802	2.070425789	3.18E-07	1.08E-06

hsa-mir-628	1.972092202	4.59484044	4.63E-07	1.55E-06
hsa-mir-33b	2.044724206	2.821370204	5.13E-07	1.70E-06
hsa-mir-450b	1.822726515	4.35961933	9.55E-07	3.11E-06
hsa-mir-217	2.600910427	5.504196336	1.79E-06	5.62E-06
hsa-mir-651	1.98266926	2.33162609	1.85E-06	5.78E-06
hsa-mir-19b-2	1.182449032	5.838538052	1.88E-06	5.83E-06
hsa-mir-3677	1.790363147	3.03817412	2.28E-06	7.02E-06
hsa-mir-592	2.466522246	2.214408117	3.70E-06	1.13E-05
hsa-mir-106a	1.749141836	4.229246162	6.35E-06	1.88E-05
hsa-mir-137	4.118133257	2.075825647	8.15E-06	2.37E-05
hsa-mir-424	1.286346224	6.671613445	1.07E-05	3.07E-05
hsa-mir-7705	2.171254462	1.719737785	1.61E-05	4.57E-05
hsa-mir-660	1.075824124	5.650391804	1.70E-05	4.79E-05
hsa-mir-4652	3.338672567	2.020445598	2.04E-05	5.63E-05
hsa-mir-203b	1.823559653	5.946754505	2.62E-05	7.12E-05
hsa-mir-136	1.694779839	5.149604529	2.83E-05	7.59E-05
hsa-mir-653	2.259555114	5.039938441	4.19E-05	0.000110896
hsa-mir-616	1.320060938	2.522077784	6.14E-05	0.000161646
hsa-mir-196b	2.692291996	7.022123329	6.46E-05	0.000169216
hsa-mir-542	1.118999633	8.178772282	6.85E-05	0.000177822
hsa-mir-503	1.301634051	3.600327476	6.87E-05	0.000177822
hsa-mir-1277	1.994239339	1.670064434	6.98E-05	0.000179562
hsa-mir-200b	1.031628659	9.593745113	8.43E-05	0.000215641
hsa-mir-188	1.264153346	2.311833584	9.95E-05	0.000251689
hsa-mir-3170	1.325956871	1.677061116	0.00011079	0.000277281
hsa-mir-539	2.164411655	3.180750307	0.00011725	0.000291837
hsa-mir-20b	1.882950903	4.628039995	0.000123927	0.000305102
hsa-mir-3607	1.709176338	5.859225158	0.000140158	0.000341353
hsa-mir-105-1	5.344217145	4.966311763	0.000145116	0.000351537
hsa-mir-192	2.44065515	11.28223123	0.00017307	0.000417025
hsa-mir-105-2	5.386596447	4.972606264	0.000174656	0.00041862
hsa-mir-4724	1.919917644	1.676629142	0.000267457	0.000631032
hsa-mir-194-1	2.240933307	8.928315417	0.000372765	0.000861545
hsa-mir-627	1.734542627	1.78337548	0.000375242	0.000862866
hsa-mir-215	2.81133749	5.715814094	0.000399608	0.000909742
hsa-mir-1293	3.355134579	1.66792254	0.000399644	0.000909742
hsa-mir-1269a	3.802751883	7.263179592	0.000417755	0.000946215
hsa-mir-889	1.766262777	4.452413865	0.00055999	0.001249633
hsa-mir-1269b	5.320023423	4.011690738	0.000690876	0.001526667
hsa-mir-767	4.624962529	4.793515766	0.000760728	0.001672864
hsa-mir-551b	1.738626272	2.675168808	0.000862715	0.001887971
hsa-mir-203a	1.228258294	12.6083526	0.000976456	0.002106354
hsa-mir-4788	4.248023123	3.858676947	0.001381055	0.002951028
hsa-mir-372	5.646609916	6.096806785	0.001562538	0.003292232
hsa-mir-582	1.269954513	8.780188929	0.001970088	0.004093807
hsa-mir-489	2.591945511	1.698795427	0.002072481	0.004286913
hsa-mir-449a	2.461166284	3.197196306	0.002163263	0.004454355
hsa-mir-187	1.581428151	5.217663506	0.002506174	0.00511395
hsa-mir-205	2.08887292	9.071663147	0.002669828	0.00539925
hsa-mir-323b	1.979694195	3.30139748	0.003009018	0.006058156

hsa-mir-382	1.181001576	4.255320739	0.00335078	0.006716386
hsa-mir-561	1.94432037	1.647287746	0.003541917	0.007037231
hsa-mir-493	1.116276354	3.962869045	0.004612042	0.00900541
hsa-mir-3913-1	1.286178235	2.072567082	0.004885521	0.009498459
hsa-mir-376b	1.714391323	1.733096785	0.005130445	0.009889752
hsa-mir-6510	1.548386964	2.257477318	0.006718913	0.012681948
hsa-mir-381	1.111285203	5.620637665	0.007208385	0.013498992
hsa-mir-154	1.144849936	3.012950691	0.007255176	0.013525081
hsa-mir-373	4.754826802	2.454701521	0.007312389	0.013575869
hsa-mir-487b	1.33388816	3.041527175	0.007548459	0.013956947
hsa-mir-655	1.580594326	1.82076093	0.008234193	0.014920358
hsa-mir-552	2.413991449	4.189155134	0.009671546	0.017140294
hsa-mir-3913-2	1.262765012	2.044928185	0.009686347	0.017140294
hsa-mir-371a	5.183837099	3.465838372	0.010227234	0.017957121
hsa-mir-122	4.752924764	2.44297594	0.010380967	0.018156672
hsa-mir-409	1.049936636	4.794851113	0.010443724	0.018196181
hsa-mir-495	1.192388885	3.2164334	0.011566531	0.020075243
hsa-mir-7-2	1.569058918	1.566476272	0.016189983	0.027571662
hsa-mir-376a-1	1.366253598	1.688445006	0.017772303	0.030153009
hsa-mir-1251	1.845010449	1.754740834	0.022996597	0.038583179
hsa-mir-675	1.813554941	6.053727206	0.023947094	0.039882476
hsa-mir-412	1.272315487	2.615616471	0.025742018	0.042714776
hsa-mir-449b	1.952601989	1.940474774	0.026636768	0.044038161

Abbreviations: FC, fold change; CPM, counts per million; FDR, false discovery rate.

Table S2. A total of 90 miRNAs were down-regulated in NSCLC samples from the TCGA database

ID	logFC	logCPM	*P* Value	FDR
hsa-mir-197	-3.515212738	8.613788334	3.45E-173	1.56E-170
hsa-let-7d	-2.883058705	9.456694042	3.73E-162	8.45E-160
hsa-mir-328	-3.584733392	5.676471455	2.70E-127	4.07E-125
hsa-mir-4732	-4.53244544	1.543422011	2.53E-119	2.87E-117
hsa-mir-486-1	-4.526396964	7.219644201	1.16E-111	1.05E-109
hsa-mir-486-2	-4.511949841	7.207729706	7.49E-110	5.65E-108
hsa-mir-6511b-1	-3.563701359	1.783336293	1.15E-97	7.46E-96
hsa-mir-6892	-3.256051402	2.416058635	1.25E-92	7.06E-91
hsa-mir-6511b-2	-3.478864668	1.796157979	2.90E-88	1.46E-86
hsa-mir-139	-3.221424274	6.19259236	1.14E-84	5.17E-83
hsa-mir-1976	-2.458771163	4.218202687	1.62E-70	6.65E-69
hsa-mir-1296	-2.797505358	2.737928257	3.79E-69	1.43E-67
hsa-mir-2110	-2.561786677	2.44106754	2.27E-57	7.91E-56
hsa-mir-125a	-1.868133681	9.524012931	8.14E-53	2.63E-51
hsa-mir-423	-1.663123966	7.57394995	3.45E-49	1.04E-47
hsa-mir-195	-2.281967024	5.649979896	4.43E-45	1.25E-43
hsa-mir-378a	-2.199487207	8.649824964	2.08E-42	5.53E-41
hsa-mir-1343	-2.768129644	1.491592029	5.51E-41	1.39E-39
hsa-mir-3615	-2.087786244	2.934131643	1.53E-38	3.65E-37
hsa-mir-504	-2.990926931	2.029997661	5.77E-38	1.31E-36
hsa-mir-1306	-2.158443272	3.636426535	6.42E-38	1.38E-36
hsa-mir-574	-1.858866463	6.69396571	7.44E-36	1.53E-34

hsa-mir-143	-2.446766614	16.69561978	1.80E-33	3.39E-32
hsa-mir-145	-1.922347739	10.46256415	3.54E-32	6.41E-31
hsa-mir-133a-1	-2.743746723	2.72909142	6.93E-32	1.21E-30
hsa-let-7c	-2.073661458	10.55696295	7.68E-32	1.29E-30
hsa-let-7b	-1.817396938	14.12883895	1.54E-31	2.49E-30
hsa-mir-99b	-1.506020609	14.76802721	3.58E-31	5.59E-30
hsa-mir-193a	-1.822690732	7.79653934	6.20E-31	9.36E-30
hsa-mir-144	-2.777510078	7.336909554	1.55E-30	2.27E-29
hsa-mir-1249	-2.054145612	1.840766892	9.15E-29	1.30E-27
hsa-mir-766	-1.833488396	3.662299964	1.61E-28	2.22E-27
hsa-mir-3917	-2.359270498	1.817666898	1.98E-28	2.63E-27
hsa-mir-133a-2	-2.670817078	2.620869086	2.19E-28	2.83E-27
hsa-mir-1228	-2.095430781	1.737240488	1.18E-27	1.48E-26
hsa-mir-326	-2.314225891	4.790879446	7.57E-26	9.11E-25
hsa-mir-451a	-2.621181941	9.56060829	7.64E-26	9.11E-25
hsa-mir-7641-1	-3.228107759	1.510463884	1.46E-24	1.66E-23
hsa-mir-1247	-2.793367425	4.842324608	1.56E-24	1.73E-23
hsa-mir-3605	-1.883907116	2.308700497	3.85E-24	4.15E-23
hsa-mir-133b	-2.495580902	1.689169671	4.78E-24	5.04E-23
hsa-mir-1226	-2.172917802	1.907737416	9.78E-24	1.01E-22
hsa-mir-5010	-2.046776598	1.626933264	3.27E-22	3.29E-21
hsa-mir-15b	-1.319319066	7.946008245	1.12E-21	1.10E-20
hsa-mir-218-1	-1.567634713	4.948750546	1.92E-19	1.78E-18
hsa-mir-140	-1.231874199	9.954904532	2.09E-19	1.87E-18
hsa-mir-6842	-1.57848259	2.5578861	3.11E-18	2.71E-17
hsa-mir-3940	-2.117927297	1.762579972	1.16E-17	9.89E-17
hsa-mir-218-2	-1.528139287	4.883921749	1.94E-17	1.57E-16
hsa-mir-877	-1.716202354	2.465313187	8.55E-17	6.68E-16
hsa-let-7a-1	-1.441606946	13.55345181	4.22E-16	3.19E-15
hsa-let-7a-3	-1.437279686	13.55586696	4.54E-16	3.37E-15
hsa-let-7a-2	-1.438133523	13.55238784	5.25E-16	3.83E-15
hsa-mir-30a	-1.630163069	14.41572633	1.53E-15	1.08E-14
hsa-mir-3928	-1.722182768	1.681253486	2.78E-13	1.70E-12
hsa-mir-320a	-1.060734063	8.580337183	7.27E-13	4.39E-12
hsa-mir-874	-1.256765689	4.829901919	7.88E-13	4.70E-12
hsa-mir-26a-1	-1.045620227	10.64708216	2.03E-11	1.15E-10
hsa-mir-1-1	-1.89605952	3.139574876	2.28E-11	1.28E-10
hsa-mir-1-2	-1.869384761	3.208698592	2.37E-11	1.31E-10
hsa-mir-26a-2	-1.04002736	10.65150946	2.43E-11	1.32E-10
hsa-mir-1180	-1.347189601	4.931971659	3.13E-11	1.68E-10
hsa-mir-671	-1.036390479	3.286309195	3.15E-11	1.68E-10
hsa-let-7f-1	-1.501137702	12.59218392	5.51E-11	2.87E-10
hsa-let-7f-2	-1.49452975	12.60515584	6.88E-11	3.54E-10
hsa-mir-190a	-1.365892526	2.355560528	2.71E-10	1.35E-09
hsa-mir-7706	-1.340710718	1.878998702	1.43E-09	6.61E-09
hsa-mir-6806	-1.394085457	1.684817714	1.55E-09	7.09E-09
hsa-mir-6720	-1.668201752	1.78371628	3.71E-09	1.61E-08
hsa-mir-3150b	-1.8338225	1.905721418	4.87E-09	2.08E-08
hsa-mir-3074	-1.282005996	2.868046445	5.70E-09	2.41E-08

hsa-mir-150	-1.29683383	10.15499627	5.87E-09	2.46E-08
hsa-mir-378c	-1.153320795	2.819534555	1.52E-08	5.95E-08
hsa-mir-935	-2.059356112	2.30372287	3.65E-08	1.36E-07
hsa-mir-296	-1.370871783	3.151037557	1.21E-07	4.31E-07
hsa-mir-184	-2.11872094	3.427266994	1.42E-07	5.04E-07
hsa-mir-2116	-1.421328613	1.550610019	1.85E-07	6.46E-07
hsa-mir-3653	-1.208231719	3.083614936	4.31E-07	1.46E-06
hsa-mir-642a	-1.201935411	2.888639136	5.06E-07	1.69E-06
hsa-mir-3651	-1.402575588	1.555774092	1.10E-06	3.55E-06
hsa-mir-4728	-1.319867623	2.218411164	1.61E-06	5.10E-06
hsa-mir-1248	-1.294801541	2.110896349	4.57E-06	1.37E-05
hsa-mir-584	-1.143858515	5.406549057	2.91E-05	7.75E-05
hsa-mir-7702	-1.341264408	2.097334297	0.000109499	0.000275572
hsa-mir-517b	-2.670460122	2.324923152	0.000332631	0.000776709
hsa-mir-517a	-2.582208139	2.373368492	0.000598071	0.00132807
hsa-mir-206	-1.875716602	1.611206055	0.00090876	0.001979175
hsa-mir-1270	-1.006231928	2.064099213	0.001704505	0.003574725
hsa-mir-433	-1.063303668	1.761535846	0.005451846	0.010464772
hsa-mir-34c	-1.074491262	6.74755296	0.006554006	0.012422447

Abbreviations: FC, fold change; CPM, counts per million; FDR, false discovery rate.