Original Paper

MicroRNA-196b Inhibits Cell Growth and Metastasis of Lung Cancer Cells by Targeting Runx2

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Key Words

MiR-196b • Lung cancer • Runx2 • PI3K/AKT/GSK3 β pathway • Smad pathway • JNK pathway

Abstract

Background/Aims: Lung cancer is one of the most common causes of cancer related deaths worldwide. The role of several microRNAs (miRNAs) including miR-196b in different cancers has already been established. The study was aimed to explore the role of miR-196b in lung cancer and its possible underlying mechanism. **Methods:** Human lung cancer cell line A549 was transfected with miR-196b mimic, miR-196b inhibitor and corresponding controls. Then cell viability, migration, invasion, and apoptosis of A549 lung cancer cells either with overexpression or with suppression of miR-196b were estimated sequentially. Next, dual luciferase activity assay was performed to clarify whether Runx2 was a direct target of miR-196b. Finally, the expressions of main factors associated with epithelial mesenchymal transition (EMT), PI3K/AKT/GSK3 β , Smad, and JNK pathways were detected by western blot. **Results:** MiR-196b expression was significantly decreased in A549, H1650 and H1299 cell lines compared with in WI-38 and HEL-1 cell lines. Overexpression of miR-196b suppressed cell viability, migration, invasion, and induced apoptosis as well as inhibited TGF- β induced EMT process in A549 cells. In addition, Runx2 was a putative target of miR-196b, and Runx2 silence remarkably increased cell apoptosis and abolished the promotive effects of miR-196b suppression on cell viability, migration and invasion. Finally, miR-196b also mediated its action by inactivation of PI3K/AKT/GSK3 β , Smad, and JNK pathways by down-regulation of Runx2. **Conclusion:** MiR-196b functions as a tumor suppressor that inhibited cell growth and metastasis of lung cancer cells by targeting Runx2. These findings provided further evidences for treatment of lung cancer.

Introduction

Lung cancer is one of the most common malignancies and remains the leading cause of cancer-related deaths worldwide [1]. In the various histological subtypes, non-small cell

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lung cancer (NSCLC) is the most prevalent subtype, which accounts for approximately 80% in all cases of diagnosed with lung cancer [1, 2]. NSCLC is characterized by a high mortality, and the overall five-year survival rate remains less than 15% [2]. Seriously, most of the patients are diagnosed in the latest stage of lung cancer, especially in stage IV [3]. Despite the different therapeutic methods such as chemotherapy, radiotherapy, and surgery were utilized to improve the survival rate of patients with lung cancer, the inevitable metastasis remain a great challenge for the treatment of lung cancer [4, 5]. Hence, there is an urgent need to introduce novel and effective therapies for treating lung cancer.

MicroRNAs (miRNAs or miRs) are a small class of single-stranded and non-coding RNAs, which are participated in post-transcriptional regulation of more than 70% human genes expression [5, 6]. Accumulating evidences indicated that miRNAs play crucial roles in a wide range of cellular biological processes, including cell division, proliferation, differentiation, metastasis and apoptosis of various cancers [7, 8]. In the studies of lung cancer, Yan et al. demonstrated that miR-761 promoted cell proliferation and metastasis of NSCLC by targeting ING4 and TIMP2 [9]. In addition, Jiang et al. revealed that miR-21 suppressed the proliferation and migration of lung cancer A549 cells [10]. Although miRNAs were involved in the development, progression and metastasis of lung cancer, the effect of miR-196b on lung cancer was not straightforward [11].

MiR-196b is a member of miR-196 family, which has been validated as tumor suppressor in various cancers [12, 13]. As Rebutti et al. displayed that miR-196b as a tumor suppressor that suppressed cell proliferation and induced apoptosis in HepG2 cells [13]. Moreover, Bhatia et al. also found that the role of miR-196b as tumor suppressor in blood cancers [14, 15]. In lung cancer, recent studies showed that miR-196b promoted cell invasion and induced epithelial mesenchymal transition (EMT) process in NSCLC cells [1, 16]. Simultaneously, Tellez et al. found that miR-196b as a tumor suppressor whose silencing early in lung carcinogenesis might provide a selective growth advantage to pre-malignant cells [17]. However, the detail roles of miR-196b in lung cancer cells proliferation, metastasis and apoptosis have not been investigated.

Runx-related transcription factor 2 (Runx2) belongs to the Runx family, which is known to be an important regulator of organogenesis and cell differentiation [18]. Abnormal expressions of Runx genes are associated with variety of cancers, especially breast and osteosarcoma [18, 19]. Moreover, the effect of Runx2 on lung cancer has also been explored in several studies [20-22]. However, whether Runx2 acted as a key regulator in lung cancer cells proliferation, metastasis and apoptosis remain unclear.

In this study, we aimed to explore the effect of miR-196b in lung cancer. Firstly, we examined the expressions of miR-196b in lung cancer cell lines and normal lung cell lines. Then we used CCK-8, Transwell, flow cytometry assays to analyze A549 cells viability, migration, invasion and apoptosis. Lastly, the key factors expressions of apoptosis, EMT, phosphatidylinositol 3 kinase (PI3K)/protein kinase B (AKT)/ glycogen synthase kinase 3 beta (GSK3 β), Smad, and Jun N-terminal kinases (JNK) pathways were detected by western blot. Our study will provide a new idea and theoretical basis for treatment of lung cancer.

Materials and Methods

Cell culture and treatment

Human lung cancer cell lines A549, H1650 and H1299 cells and human lung cell lines WI-38 and HEL-1 cells were purchased from American Type Culture Collection (ATCC, Manassas, VA). All these cells were maintained in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 1 $\times$ antibiotic-antimycotic mixture (Gibco, Grand Island, NY) and 10% fetal bovine serum (FBS, Hyclone Laboratories, Logan, UT) at 37°C in an atmosphere of 5% CO₂-95% air. Transforming growth factor-beta 1 (TGF- β 1, 10 ng/ml) was used to induce EMT.

Cell transfection

MiR-196b mimic, miR-196b inhibitor and corresponding controls were synthesized by GenePharma Co. (Shanghai, China). In addition, Runx2 targeted small interfering RNA (si-Runx2) was also constructed by GenePharma Co. (Shanghai) to inhibit Runx2 expression. In brief, 1×10^5 cells were cultured in 6-well plate and all transfection cells were accomplished using Lipofectamine 3000 reagent (Invitrogen) following the manufacturer's protocol.

Cell viability assay

Cells were seeded in 96-well plate with 5000 cells/well, and cell viability was assessed by a Cell Counting Kit-8 (CCK-8, Dojindo Molecular Technologies, Gaithersburg, MD). Briefly, after transfection for 48 h, the 10 μ l CCK-8 solution was added to the culture medium, and the cultures were incubated for 1 h at 37°C in humidified 95% air and 5% CO₂. The absorbance was measured at 450 nm using a Microplate Reader (Bio-Rad, Hercules, CA).

Migration and invasion assay

Cell migration and invasion assays were determined by using a modified two-chamber chambers with a pore size of 8 mm. For migration assay, cells suspended in 200 μ l of serum-free medium were seeded on the upper compartment of 24-well Transwell culture chamber, and 600 μ l of complete medium was added to the lower compartment. After incubation for 48 h at 37°C, cells were fixed with methanol. Non-traversed cells were removed from the upper surface of the filter carefully with a cotton swab. Traversed cells on the lower side of the filter were stained with 0.1% crystal violet (Merck, Darmstadt, Germany) and counted. For invasion assay, the invasion capacity detection was conducted the same as migration assay, except utilizing Matrigel coated inserts with 8 mm pore size (catalog no. 354480, BD Biosciences) [23].

Apoptosis assay

Cell apoptosis was quantified with a flow cytometry using propidium iodide (PI) and fluorescein isothiocyanate (FITC)-conjugated Annexin V staining (Beyotime Biotechnology). Briefly, cells were washed three times in phosphate buffered saline (PBS) and then were re-suspended in the staining buffer. Subsequently, 10 μ l FITC-Annexin V (20 μ g/ml) and 5 μ l PI (50 μ g/ml) were mixed with these cells. After incubation for 10 min in a dark at room temperature, the mixtures were examined using the FACS can flow cytometry system (Beckman Coulter, Fullerton, CA, USA). The data were analyzed by using FlowJo software (Tree Star, Inc., Ashland, OR, USA).

qRT-PCR

Total RNA was extracted from cells using Trizol reagent (Life Technologies Corporation, Carlsbad, CA, USA) according to the manufacturer's instructions. Then the first-strand complementary DNAs (cDNAs) were synthesized using 1 μ g RNAs by PrimeScript 1st Strand cDNA Synthesis Kit (TaKaRa Bio, Siga, Japan). The One Step SYBR® PrimeScript®PLUS RT-RNA PCR Kit (TaKaRa) was used for the Real-Time PCR analysis to test the expression levels of miR-196b in cells. U6 RNA was used to normalize the miR-196b levels and GAPDH was used to normalize other mRNAs levels. Data were analyzed with the $2^{-\Delta\Delta Ct}$ method.

Dual luciferase activity assay

The 3'UTR target site was generated by PCR and the luciferase reporter constructs with the Runx2 3'UTR carrying a putative miR-196b-binding site into pMiR-report vector (Promega, Madison, WI, USA) were amplified by PCR. Cells were co-transfected with the reporter construct, control vector and miR-196b mimic or mimic control using Lipofectamine 3000 (Life Technologies). Reporter assays were done using the dual-luciferase assay system (Promega) following to the manufacturer's information.

Western blot

The protein used for western blot was extracted using radio immunoprecipitation assay (RIPA) lysis buffer (Beyotime Biotechnology, Shanghai, China) supplemented with protease inhibitors (Roche, Basle, Switzerland). The concentrations of protein extracts were quantified using the BCA™ Protein Assay Kit (Pierce, Appleton, WI, USA). The western blot system was established using a Bio-Rad Bis-Tris Gel system according to the manufacturer's instructions. Primary antibodies were prepared in 5% blocking buffer

at a dilution of 1:1,000 and were incubated with the membranes at 4°C overnight. Thereafter, the membranes were washed and incubated with secondary antibody (dilution of 1:5000) marked by horseradish peroxidase for 1 h at room temperature. After rinsing, the polyvinylidene difluoride (PVDF) membranes (Millipore, Bedford, MA, USA) carried blots and antibodies were transferred into the Bio-Rad ChemiDoc™ XRS system. The immunoreactive protein bands were visualized using chemiluminescence (PerkinElmer, Wellesley, MA, USA).

Statistical analysis

All experiments were repeated three times. The results of multiple experiments are presented as mean ± standard derivations (SD). Statistical analyses were performed using SPSS 19.0 statistical software (SPSS Inc., Chicago, IL). The *P*-values were calculated using one-way analysis of variance (ANOVA). A *P*-value of < 0.05 was considered to indicate a statistically significant result.

Results

MiR-196b was low expressed in different lung cancer cells

The expression levels of miR-196b were assessed in different cell lines of lung cancer cell lines A549, H1650 and H1299 and human normal lung cell lines WI-38 and HEL-1 by qRT-PCR. All the lung cancer cell lines revealed reduced expression levels of miR-196b compared to human lung cell lines ($P < 0.01$ or $P < 0.001$, Fig. 1). The expression level was maximally reduced in the A549 lung cancer cell line, hence A549 cell line was chosen in the following experiments.

MiR-196b suppressed cell viability, migration, invasion, and induced apoptosis

To assess the role of miR-196b in cell viability, migration, invasion, and apoptosis, A549 cells were transfected with miR-196b mimic, miR-196b inhibitor and corresponding controls. Results showed that the expression level of miR-196b was significantly up-regulated by miR-196b mimic and down-regulated by miR-196b inhibitor ($P < 0.01$ or $P < 0.001$, Fig. 2A). In terms of cell viability, migration and invasion, miR-196b overexpression remarkably decreased cells viability, migration and invasion in A549 cells compared to its control group ($P < 0.05$). At same time, these effects were reversed by miR-196b suppression ($P < 0.05$, Fig. 2B-2D).

In terms of cell apoptosis, the percentage of apoptotic cells was significantly enhanced by miR-196b overexpression compared to control ($P < 0.001$, Fig. 2E). There was no obviously effect of miR-196b suppression on cell apoptosis. Meanwhile, the western blot results revealed that miR-196b overexpression down-regulated Bcl-2 expression, up-regulated Bax expression as well as activated cleaved-caspase-3 and cleaved-caspase-9 expressions. MiR-196b suppression showed an opposite results of Bcl-2 and Bax expressions. There were no obviously changes of pro-caspase-3 and pro-caspase-9 (Fig. 2F). Taken together, these data indicated that miR-196b inhibited cell growth and metastasis of A549 cells.

MiR-196b suppressed EMT process in A549 cells

To explore the effect of miR-196b on EMT process, A549 cells were transfected with miR-196b mimic, miR-196b inhibitor and corresponding controls. After inducing by 10 ng/ml of

Fig. 1. MiR-196b was low expressed in lung cancer cell lines. MiR-196b was significantly down-regulated in lung cancer cell lines (A549, H-1650 and H-1299) compared to normal lung cell lines (WI-38, HEL-1). MiR-196b: microRNA-196b; **, $P < 0.01$; ***, $P < 0.001$ when compared with WI-38 cell line; ##, $P < 0.01$; ###, $P < 0.001$ when compared with HEL-1 cell line.

Fig. 2. MiR-196b inhibited cell viability, migration, invasion, and promoted apoptosis. A549 cells were transfected with miR-196b mimic, mimic control, miR-196b inhibitor and inhibitor control. A: MiR-196b expression was up-regulated in miR-196b mimic transfected cells but down-regulated in miR-196b inhibitor transfected cells; B-D: Overexpression of miR-196b inhibited cell viability, migration and invasion, but suppression of miR-196b promoted cell viability, migration and invasion in A549 cells; E: Overexpression of miR-196b promoted cell apoptosis in A549 cells; F: Overexpression of miR-196b promoted apoptosis-related proteins expressions, and suppression of miR-196b inhibited apoptosis-related proteins expressions in A549 cells. MiR-196b: microRNA-196b; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

TGF- β 1, the protein levels of EMT-related factors were detected by western blot. As results revealed in Fig. 3, miR-196b overexpression promoted E-cadherin expression but inhibited N-cadherin, Vimentin, ZEB1, and Snail expressions. Of contrast, miR-196b suppression led to opposite results for these five factors expressions. These results demonstrated that miR-196b could suppress EMT process.

Fig. 3. MiR-196b inhibited EMT process. A549 cells were transfected with miR-196b mimic, miR-196b inhibitor and corresponding controls and then induced by 10 ng/ml of TGF- β 1. Overexpression of miR-196b promoted E-cadherin expression but inhibited N-cadherin, Vimentin, ZEB1 and Snail expressions. MiR-196b: microRNA-196b; EMT: epithelial-mesenchymal transition; TGF- β 1: transforming growth factor-beta 1; ZEB1: zinc-finger E-box binding homeobox 1.

Fig. 4. Runx2 was a target of miR-196b. A: miR-196b negatively regulated the mRNA and protein expressions of Runx2 in A549 cells; B: Runx2 was a target of miR-196b in A549 cells. Runx2: Runt-related transcription factor 2; MiR-196b: microRNA-196b; *, $P < 0.05$; **, $P < 0.01$.

Runx2 was a target of miR-196b

To explore the relationship between miR-196b and Runx2, A549 cells were transfected with miR-196b mimic, miR-196b inhibitor and corresponding controls. qRT-PCR and western blot assays were used to examine the mRNA and protein levels of Runx2 in different experiment groups. The results displayed that the mRNA expression of Runx2 was significantly down-regulated by miR-196b overexpression but up-regulated by miR-196b suppression compared to corresponding controls ($P < 0.05$ or $P < 0.01$). Western blot analysis with a similar trend to mRNA level of Runx2 (Fig. 4A).

Further, dual luciferase activity assay was performed to verify whether miR-196b was able to directly bind to its site sequences in the Runx2 3'-UTR in A549 cells; Runx2-wt and Runx2-mt containing the wild-type and mutant binding sequences of miR-196b within the 3'-UTR of Runx2 mRNA were generated. Results showed that miR-196b overexpression reduced the activity of luciferase reporter fused to the Runx2-wt ($P < 0.01$). However, there was no significant difference in A549 cells co-transfected with Runx2-mt when compared with the control group (Fig. 4B). Overall, these data indicated that miR-196b inhibited Runx2 expression by directly targeting its 3'-UTR.

MiR-196b inhibited cell viability, migration and invasion and promoted apoptosis by regulation of Runx2

To explore the effects of miR-196b and Runx2 on cell viability, migration, invasion and apoptosis, miR-196b inhibitor, miR-196b inhibitor + si-Runx2 and corresponding control were transfected into A549 cells. Results revealed in Fig. 5A, miR-196b inhibitor + si-Runx2 prominently reduced Runx2 expression compared to miR-196b inhibitor group. Then miR-196b suppression significantly elevated cell viability, migration and invasion compared to inhibitor control group ($P < 0.05$). While the promoting effects of miR-196b suppression on cell viability, migration and invasion were abolished by miR-196b inhibitor + si-Runx2 ($P <$

Fig. 5. MiR-196b inhibited cell viability, migration, invasion and promoted apoptosis in A549 cells by regulation of Runx2. MiR-196b inhibitor or/and Runx2 siRNA were transfected into A549 cells. A: Suppression of miR-196b increased Runx2 expression, and co-transfected with miR-196b inhibitor and Runx2 siRNA decreased Runx2 expression in A549 cells; B-D: Suppression of miR-196b promoted cell viability, migration and invasion, while co-transfected with miR-196b inhibitor and Runx2 siRNA inhibited cell viability, migration and invasion in A549 cells; E: Co-transfected with miR-196b inhibitor and Runx2 siRNA promoted cell apoptosis in A549 cells; F: Suppression of miR-196b promoted cell apoptosis-related proteins expressions, and co-transfected with miR-196b inhibitor and Runx2 siRNA inhibited the cell apoptosis-related proteins expressions in A549 cells. MiR-196b: microRNA-196b; Runx2: Runt-related transcription factor 2; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

0.01, Fig. 5B-5D). Furthermore, miR-196b inhibitor + si-Runx2 remarkably promoted cell apoptosis compared to miR-196 inhibitor group ($P < 0.001$, Fig. 5E). Moreover, western blot results showed that miR-196b inhibitor + si-Runx2 inhibited Bcl-2 expression, promoted Bax

Fig. 6. MiR-196b inactivated PI3K/AKT/GSK3 β , Smad, and JNK pathways by regulation of Runx2. A: Overexpression of miR-196b inactivated PI3K/AKT/GSK3 β pathway in A549 cells; B: Overexpression of miR-196b inactivated Smad pathway in A549 cells; C: Overexpression of miR-196b inactivated JNK pathway in A549 cells. MiR-196b: microRNA-196b; Runx2: Runt-related transcription factor 2; PI3K: phosphoinositide-3-Kinase; AKT: protein kinase B; GSK3 β : glycogen synthase kinase 3 beta; JNK: Jun N-terminal kinases.

expression and activated cleaved-caspase-3 and cleaved-caspase-9 expressions compared to miR-196b suppression group. There were no obviously changes of pro-caspase-3 and pro-caspase-9 (Fig. 5F). In a word, these data indicated that Runx2 silence induced apoptosis and abolished the promotive effects of miR-196b suppression on cell viability, migration and invasion.

MiR-196b blocked PI3K/AKT/GSK3 β , Smad, and JNK pathways by regulation of Runx2

To explore the role of miR-196b and Runx2 in PI3K/AKT/GSK3 β , Smad, and JNK signal pathways, A549 cells were transfected with miR-196b mimic, miR-196b inhibitor, miR-196b inhibitor + si-Runx2 and corresponding controls. Results showed in Fig. 6A-6C, the three signal pathways key factors of p-PI3K, p-AKT, p-GSK3 β , p-Smad2, p-Smad3, Smad7, p-JNK and p-c-Jun were significantly down-regulated by miR-196b overexpression, as well as up-regulated by miR-196b suppression. While miR-196b inhibitor + si-Runx2 abolished the activation effects of miR-196b suppression on these eight factors. There were no obviously changes of PI3K, AKT, GSK3 β , Smad2, Smad3, JNK and c-Jun. These data indicated that miR-196b could block PI3K/AKT/GSK3 β , Smad, and JNK pathways by regulation of Runx2.

Discussion

In the present study, we explored the role of miR-196b in lung cancer cells. It demonstrated that miR-196b was low expressed in lung cancer cells. MiR-196 overexpression significantly inhibited cell viability, migration and invasion and promoted apoptosis of A549 cells. In addition, we also found that Runx2 was a direct target of miR-196b, and Runx2 silence significantly induced apoptosis and abolished the promotive effects of miR-196b suppression on cell viability, migration and invasion. Furthermore, miR-196b blocked PI3K/AKT/GSK3 β , Smad, and JNK pathways by regulation of Runx2.

Several studies have demonstrated that abnormal expressions of miRNAs have been found in lung cancer, and some of these miRNAs are involved in modulating lung cancer cells proliferation, metastasis and apoptosis [24, 25]. As Chen et al. demonstrated that the expression level of miR-144 was increased in lung cancer cells and up-regulation of miR-144 could suppress lung cancer cells proliferation, induce apoptosis and autophagy [26]. In addition, Bhatia et al. uncovered the tumor suppressor role of miR-196b in B-cell lineage acute lymphoblastic leukemia via promoting cell apoptosis in EB-3 cells [27]. Similar to

previous studies, our study demonstrated that miR-196b was significantly decreased in lung cancer cells and miR-196b acted as a tumor suppressor that inhibited lung cancer cells growth and metastasis.

It is generally known that EMT is a biological phenomenon in which the epithelial cells lose their characteristic cellular properties and acquire those of mesenchymal cells [28]. It is associated with a number of physiological processes like wound healing, invasion and metastasis [29]. Recent studies have proved that miRNAs play a key role in induction EMT [30, 31]. Here, we have demonstrated the inhibitory effects of miR-196b on EMT in lung cancer cells. Similar to our finding, Ma et al. revealed that miR-124 as a tumor suppressor that suppressed EMT process in NSCLC [25]. Whereas, in contrast to our finding, Liao et al. demonstrated that miR-196b promoted EMT process, through decreasing E-cadherin expression and increasing N-cadherin, Vimentin, ZEB1, and Snail expressions in gastric cancer cells [32]. The opposite reason may be due to the difference functions of miR-196b in diverse tumor cells. However, further study need to clarify this hypothesis.

Runx2 is a transcription factor, which is recognized that its regulation activities are associated with increased cell growth, invasion and metastasis in various tumor types [33]. Previous studies have confirmed that the role of Runx2 as proto-oncogene in lung cancer [21, 22]. Moreover, Xie et al. demonstrated that Runx2 was a direct and functional target of miR-218, and Runx2 could execute the former on lung cancer chemo-resistance [34]. Therefore, we supposed whether Runx2 was a target of miR-196b and involved in regulation of lung cancer cells cellular functions. Just as we expected, our study confirmed that Runx2 was a direct target gene of miR-196b and was negatively regulated by miR-196b. Moreover, we also found that Runx2 silence could induce apoptosis and abolish the promotive effects of miR-196b suppression on cell viability, migration and invasion. Thus, the data suggested that miR-196b inhibited cell growth and metastasis by targeting Runx2. Similar to our findings, Zheng et al. have demonstrated that WW domain-containing oxidoreductase (WWOX), which is a tumor suppressor, suppressed the invasiveness of lung cancer cells via regulation of Runx2 expression [20].

Mounting evidences confirmed the beneficial role of suppression of PI3K/AKT/GSK3 β , Smad, and JNK pathways in various cancers [35-37]. As Li et al. reported that miR-196b improved cell proliferation and invasion ability by regulating of PI3K/AKT/mTOR signal pathway in gastric cancer cells [38]. Furthermore, Manish et al. found that Runx2 could regulate PI3K/AKT pathway and play a pivotal role in modulating breast cancer-associated cell survival and invasive properties [39]. However, the cross-regulation between miR-196b and Runx2 in PI3K/AKT/GSK3 β , Smad, and JNK signal pathways remains unclear. In this study, we found that miR-196b suppression activated PI3K/AKT/GSK3 β , Smad, and JNK pathways. However, the activation effects were abolished by Runx2 silence. These data indicated that miR-196b could block PI3K/AKT/GSK3 β , Smad, and JNK pathways through regulation of Runx2 expression in A549 cells.

Collectively, our study demonstrated that miR-196b as a tumor suppressor that inhibited lung cancer cells growth, metastasis and blocked PI3K/AKT/GSK3 β , Smad, and JNK pathways by targeting Runx2. These findings suggested that miR-196b might be considered as a novel drug target in the treatment of lung cancer.

Disclosure Statement

None declared.

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