Oncology Research, Vol. 25, pp. 147–154
Printed in the USA.

0965-0407/17 $90.00 + .00
DOI: https://doi.org/10.3727/096504016X14732772150505
E-ISSN 1555-3906
www.cognizantcommunication.com

Overexpression of MicroRNA-27b Inhibits Proliferation, Migration, and Invasion via Suppression of MET Expression

Hui Zhou,*† Yanglin Liu,† Ling Xiao,‡ Zhengmao Hu,* and Kun Xia*§

*The State Key Laboratory of Medical Genetics, School of Life Sciences, Central South University, Changsha, Hunan, P.R. China
†The Affiliated Cancer Hospital of Xiangya School of Medicine, Central South University, Changsha, Hunan, P.R. China
‡Department of Histology and Embryology, School of Basic Medical Sciences, Central South University, Changsha, Hunan, P.R. China
§Key Laboratory of Medical Information Research, Central South University, Changsha, P.R. China

MicroRNA-27b (miR-27b) was recently found to be significantly downregulated in different human cancers. However, evidence of the function of miR-27b in non-small cell lung cancer (NSCLC) remains limited. In this study, we aimed to investigate novel miR-27b-mediated targets or signaling pathways associated with the tumorigenesis and metastasis of NSCLC. Real-time (RT) PCR was performed to examine miR-27b expression in NSCLC specimens. MTT assay, wound-healing assay, and Transwell assay were used to determine cell proliferation, migration, and invasion. Our data indicated that the miR-27b levels were significantly decreased in NSCLC specimens and cell lines (SK-MES-1, H358, H460, A549, and H1229) when compared to matched normal adjacent tissues and normal human lung epithelial cell lines, respectively. Restoration of miR-27b significantly inhibited the proliferation, migration, and invasion of A549 cells. We then conducted in silico analysis and luciferase reporter gene assay and identified MET, a receptor tyrosine kinase, as a direct target of miR-27b in NSCLC cells. Moreover, overexpression of MET rescued the suppressive effect of miR-27b on the proliferation, migration, and invasion of A549 cells, suggesting that MET acts as a downstream effecter of miR-27b in NSCLC cells. In summary, our study identified a novel miR-27b/MET signaling pathway involved in the cell proliferation, migration, and invasion of NSCLC, and identification of miR-27b-mediated novel signaling pathways may help reveal the molecular mechanism underlying the development and malignant progression of this disease.

Key words: Non-small cell lung cancer (NSCLC); MicroRNA-27b (miR-27b); Tumor suppressor; MET; Oncogene

INTRODUCTION

Lung cancer is the leading cause of cancer death, and its incidence has been increasing worldwide (1). Non-small cell lung cancer (NSCLC) is the most common lung cancer, accounting for approximately 90% of lung cancer cases (2). The most common types of NSCLC are adenocarcinoma, squamous cell carcinoma, and large cell carcinoma (3). Despite recent advances in the combination of treatment strategies, including surgery, chemotherapy, radiotherapy, and molecularly targeted therapy, the prognosis of NSCLC patients remains poor due to the high rate of recurrence and metastasis (4). Exploring the molecular oncogenic processes and metastatic pathways underlying NSCLC may help improve the diagnosis and therapy of NSCLC.

MicroRNAs (miRs) are endogenous small noncoding RNAs that regulate the expression of protein-coding or non-protein-coding genes by degradation of mRNA or inhibition of translation in a sequence-specific manner (5,6). In the recent decade, increasing evidence has shown deregulated miRs in a variety of human cancers (7–9). By affecting the expression levels of oncogenic or tumor suppressive genes, many miRs have been found to play a critical role in cancer initiation, progression, and metastasis (10–12). Therefore, identification of deregulated miRs is crucial for understanding the miR-mediated gene networks in human cancers.

Recently, miR-27b was frequently downregulated in several types of cancer tissue (13,14). Through inhibition of protein expression of its targeting oncogenes, miR-27b generally plays a suppressive role in human cancers (15–17). Hirota et al. compared the levels of miRs in normal lung tissue and lung tumors and found that the miR-27b levels were significantly lower in the tumors than in

Address correspondence to Professor Kun Xia, The State Key Laboratory of Medical Genetics, School of Life Sciences, Central South University, Xiangya Road 110, Changsha, Hunan 410008, P.R. China. Tel: +86-731-84805357; Fax: +86-731-84478152; E-mail: xiakun@sklmg.edu.cn

the normal tissue (18). Hennessey et al. showed that a combination of miR-15b and miR-27b could discriminate NSCLC from healthy controls with a specificity of 84%, sensitivity of 100%, negative predictive value (NPV) of 100%, and positive predictive value (PPV) of 82% (19). Moreover, miR-27b was recently found to inhibit the growth and invasion of NSCLC cells via directly targeting SP1 and LIMK1 (20,21). Therefore, miR-27b acts as a tumor suppressor in NSCLC. However, the regulatory mechanism of miR-27b in the malignant phenotypes of NSCLC cells has yet to be fully uncovered.

In the present study, we aimed to investigate the expression and role of miR-27b in NSCLC tissues and cell lines. Moreover, we explored the miR-27b-mediated target gene and its function in NSCLC cells. The findings in the present study may help facilitate the development of therapeutic strategies for the treatment of NSCLC.

MATERIALS AND METHODS

Clinical Specimens

This study was approved by the Ethics Committee of the Tumor Hospital of Hunan Province, Changsha, P.R. China. A total of 43 cases of NSCLC tissues and matched adjacent normal tissues were collected at the Tumor Hospital of Hunan Province from April 2013 to January 2014. All tissues were confirmed by pathologists in our hospital. Patients involved in this study received neither radiation therapy nor chemotherapy before surgical resection. Written informed consents were obtained from all NSCLC patients. All specimens were immediately snap frozen in liquid nitrogen and stored at −80°C until use.

Cell Lines

Five human NSCLC cell lines (SK-MES-1, H358, H460, A549, and H1229) and normal human lung epithelial BEAS-2B cells were purchased from the Cell Bank of Central South University (Changsha, P.R. China). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Life Technologies) supplemented with 10% fetal bovine serum (FBS; Life Technologies), 100 IU/ml penicillin, and 100 IU/ml streptomycin. Cells were cultured at 37°C in a humidified atmosphere with 5% CO_2.

RNA Isolation and Quantitative RT-PCR

Total RNA of tissues and cell lines was extracted using TRIzol reagent, in accordance with the manufacturer's instruction. The TaqMan quantitative real-time PCR kit was used to examine the miR-27b expression (assay ID: 000409) on the ABI 7500 thermocycler (Applied Biosystems, Thermo Fisher, USA). RNU48 (assay ID: 001006) was used as an internal control. In addition, the TaqMan probes and primers for MET (P/N: Hs01565584_m1) and GAPDH (P/N: Hs02758991_g1) were obtained from Applied Biosystems. The PCR steps were 95°C for 5 min, and 40 cycles of denaturation at 95°C for 15 s and annealing/elongation step at 60°C for 30 s. The relative miR-27b expression was normalized to U6. The relative expression was analyzed by the $2^{-\Delta\Delta Ct}$ method.

Western Blotting

Cells were solubilized in cold RIPA lysis buffer. The BCA protein assay kit was used to examine the protein concentration. Proteins were separated with 12% SDS-PAGE, and transferred onto a polyvinylidene difluoride (PVDF) membrane. Afterward, the PVDF membrane was incubated with TBST containing 5% milk at room temperature for 3 h and then with mouse anti-MET (1:400) and mouse anti-GAPDH (1:200) antibodies at room temperature for 3 h. After being washed with PBS three times, the PVDF membrane was incubated with rabbit anti-mouse secondary antibody (1:5,000) at room temperature for 1 h. Chemiluminescence detection was performed using an ECL kit. The relative protein expression was analyzed by Image-Pro Plus software 6.0, represented as the density ratio versus GAPDH.

MTT Assay

The 3-(4,5-dimethylthiazal-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay was used to examine cell proliferation. A549 cells were plated at a density of 10,000 cells per well in 96-well plates. After being cultured for 0, 24, 48, and 72 h, the cells were incubated with MTT at a final concentration of 0.5 mg/ml for 4 h at 37°C. After the removal of the medium, 150 mM DMSO solutions were added to dissolve the formazan crystals. The absorbance was read at 570 nm using a BioTek™ ELx800™ Absorbance Microplate Reader.

Cell Migration Assay

Wound healing assay was performed to determine the migration of A549 cells. In brief, A549 cells were cultured to full confluence. Wounds of approximately 1-mm width were created with a plastic scriber. Cells were washed and incubated in a serum-free medium. After wounding for 24 h, cells were incubated in a medium including 10% FBS. After being cultured for 48 h, cells were fixed and observed under a microscope (Olympus, Tokyo, Japan).

Cell Invasion Assay

Cell invasion assay was performed using Transwell chambers (BD, USA), which were precoated with Matrigel. Cell suspension containing 10^6 cells/ml was prepared in serum-free media, and 300 µl of cell suspension was added into the upper chamber. Then 400 µl of DMEM with 10% FBS was added into the lower chamber. Cells were incubated for 24 h. A cotton-tipped swab was used to wipe

out the cells that did not migrate or invade through the pores. The filters were fixed in 90% alcohol, stained by crystal violet, and observed under an inverted microscope (Olympus).

Identification of Putative Target Genes of miR-27b by In Silico Analysis

The TargetScan database (http://www.targetscan.org/) was used for in silico identification of putative target genes of miR-27b.

Dual-Luciferase Reporter Gene Assay

Luciferase reporter gene assay was used to determine the relationship between miR-27b and MET. Total cDNA from A549 cells was used to amplify the 3′-UTR of MET, which was then cloned into pMir-Report vector. Mutations were introduced within the potential seed sequences of the 3′-UTR of MET using the QuikChange Site-Directed Mutagenesis Kit. Cells were then cotransfected with the pMir-Report vectors containing the wild type (WT) or mutant type (MT) of MET 3′-UTR, and miR-133b mimics or miR-NC, respectively. After 48 h, the luciferase activity was examined using the Dual-Glo substrate system on LD400 luminometer (Beckman Coulter, Brea, CA, USA). Data are presented as a ratio of Renilla luciferase to Firefly luciferase.

Statistical Analysis

Data were expressed as mean±standard deviation from at least three separate experiments. SPSS19.0 was used to perform statistical analysis. Independent *t*-tests were used to compare the differences between two groups. One-way ANOVA with Bonferroni post hoc tests was performed to compare the differences among three or more groups. A value of $p<0.05$ was considered statistically significant.

RESULTS

miR-27b Was Downregulated in NSCLC

First, RT-PCR was used to examine the miR-27b levels in a total of 43 NSCLC clinical specimens and their matched adjacent normal tissues. Our data indicated that miR-27b was significantly downregulated in NSCLC tissues when compared with that in their matched adjacent normal tissues (Fig. 1A). We further examined its expression in five human NSCLC cell lines (SK-MES-1, H358, H460, A549, and H1229) and normal human lung epithelial BEAS-2B cells. As shown in Figure 1B, the miR-27b levels were also reduced in NSCLC cell lines compared to BEAS-2B cells. Accordingly, our findings demonstrated that miR-27b was downregulated in NSCLC.

miR-27b Inhibited the Proliferation, Migration, and Invasion of NSCLC Cells

To restore the expression of miR-27b, A549 cells were transfected with miR-27b mimic or miR-NC, respectively. RT-PCR indicated that the miR-27b levels were significantly increased after transfection with the miR-27b mimic (Fig. 2A). We further examined the role of miR-27b in regulating the malignant phenotypes of NSCLC cells. MTT assay demonstrated that the proliferation of A549 cells was significantly reduced after transfection with the miR-27b mimic when compared with the control group (Fig. 2B). Moreover, wound-healing assay and Transwell assay further demonstrated that the migration and invasion activities of A549 cells were significantly decreased after restoration of miR-27b when compared to the control group (Fig. 2C and D). Taken together, we demonstrated that miR-27b inhibited the proliferation, migration, and invasion of NSCLC A549 cells.

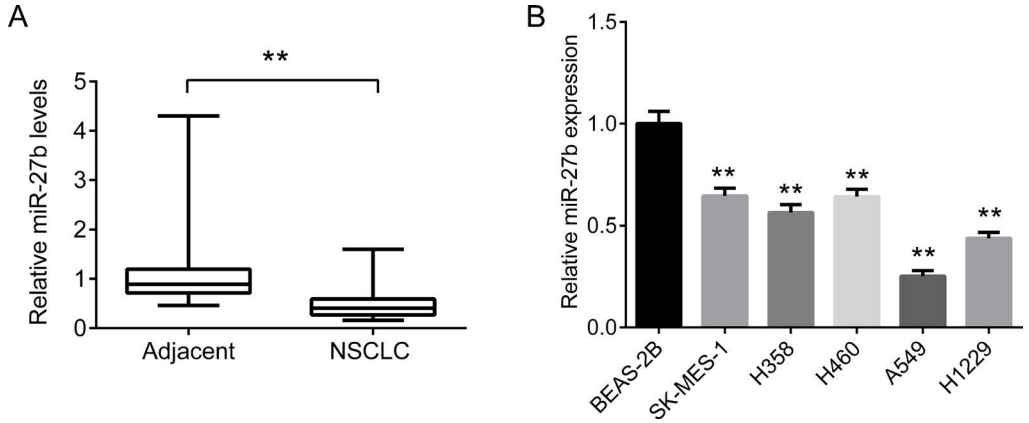

Figure 1. (A) RT-PCR was used to examine the miR-27b levels in a total of 43 NSCLC clinical specimens and their matched adjacent normal tissues. $^{**}p<0.01$ versus Adjacent. (B) RT-PCR was used to examine the miR-27b levels in five human NSCLC cell lines (SK-MES-1, H358, H460, A549, and H1229) and normal human lung epithelial BEAS-2B cells. $^{**}p<0.01$ versus BEAS-2B.

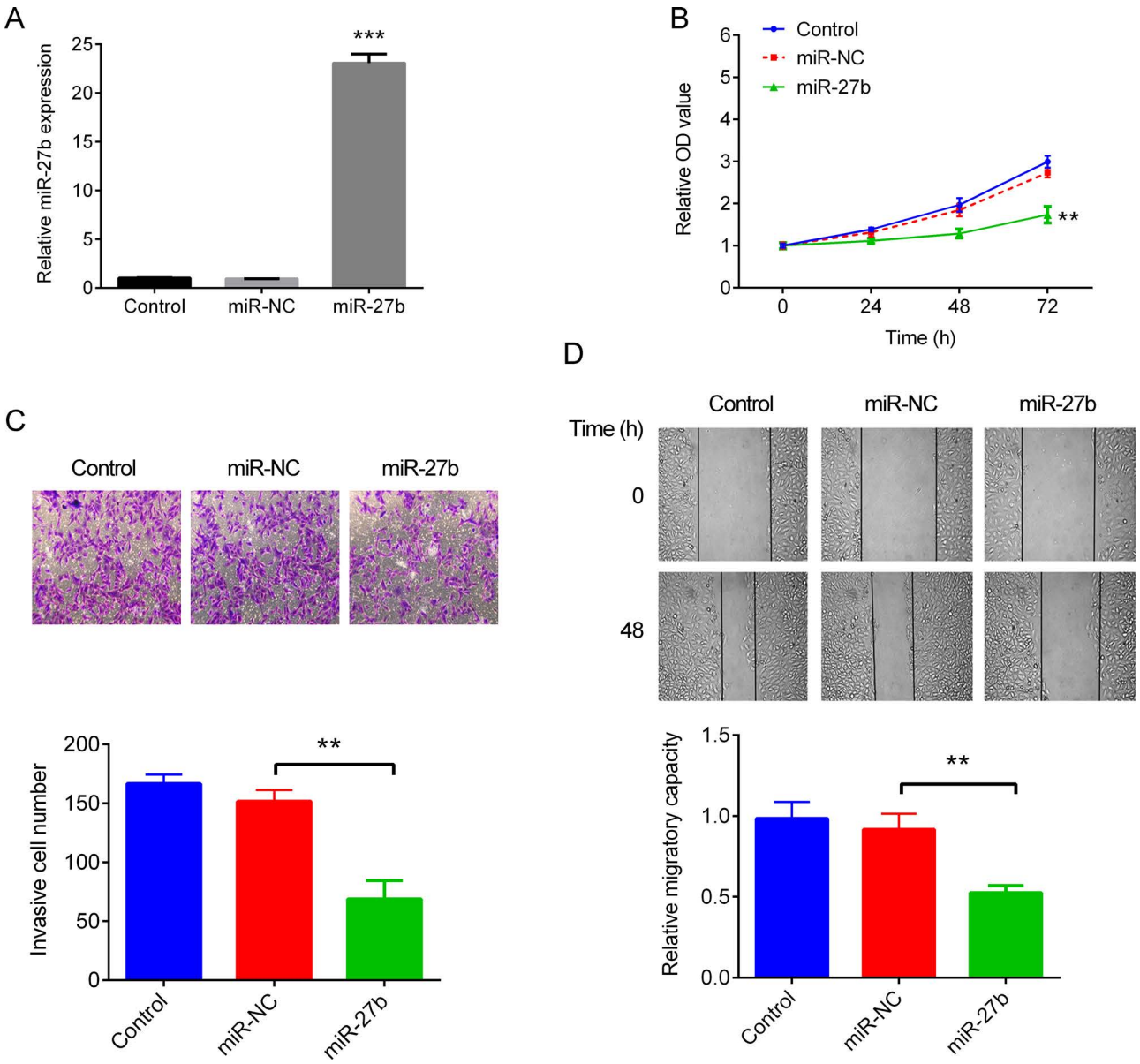

Figure 2. (A) RT-PCR was used to examine the miR-27b levels in A549 cells transfected with miR-27b mimic or scramble miR (miR-NC), respectively. Nontransfected A549 cells were used as Control. (B) MTT assay, (C) Transwell assay, and (D) wound healing assay were used to examine the cell proliferation, invasion, and migration. $^{**}p<0.01$ versus Control.

miR-27b Directly Targets MET in NSCLC Cells

We performed in silico analyses to identify the putative target genes of miR-27b. The TargetScan database was used to screen the putative targets of miR-27b. MET, a known oncogene in some cancers including NSCLC, attracted our attention (http://www.targetscan.org/cgi-bin/targetscan/vert_70/view_gene.cgi?rs=ENST00000397752.3&taxid=9606&members=&showcnc=0&shownc=0&showncf=&subset=1#miR-27b-3p). To study the relationship between miR-27b and MET, we examined the mRNA and protein levels in NSCLC A549 cells after overexpression of miR-27b. As indicated in Figure 3A and B, both the mRNA and protein levels of MET were significantly reduced in A549 cells after miR-27b upregulation. Therefore, miR-27b could reduce the expression of MET in A549 cells, suggesting that MET might be a direct target gene of miR-27b in NSCLC cells.

Afterward, we conducted luciferase reporter gene assay in A549 cells to determine whether miR-27b could directly bind to the seed sequences within the 3′-UTR of MET mRNA. We found that the luciferase activity was significantly decreased in A549 cells transfected with

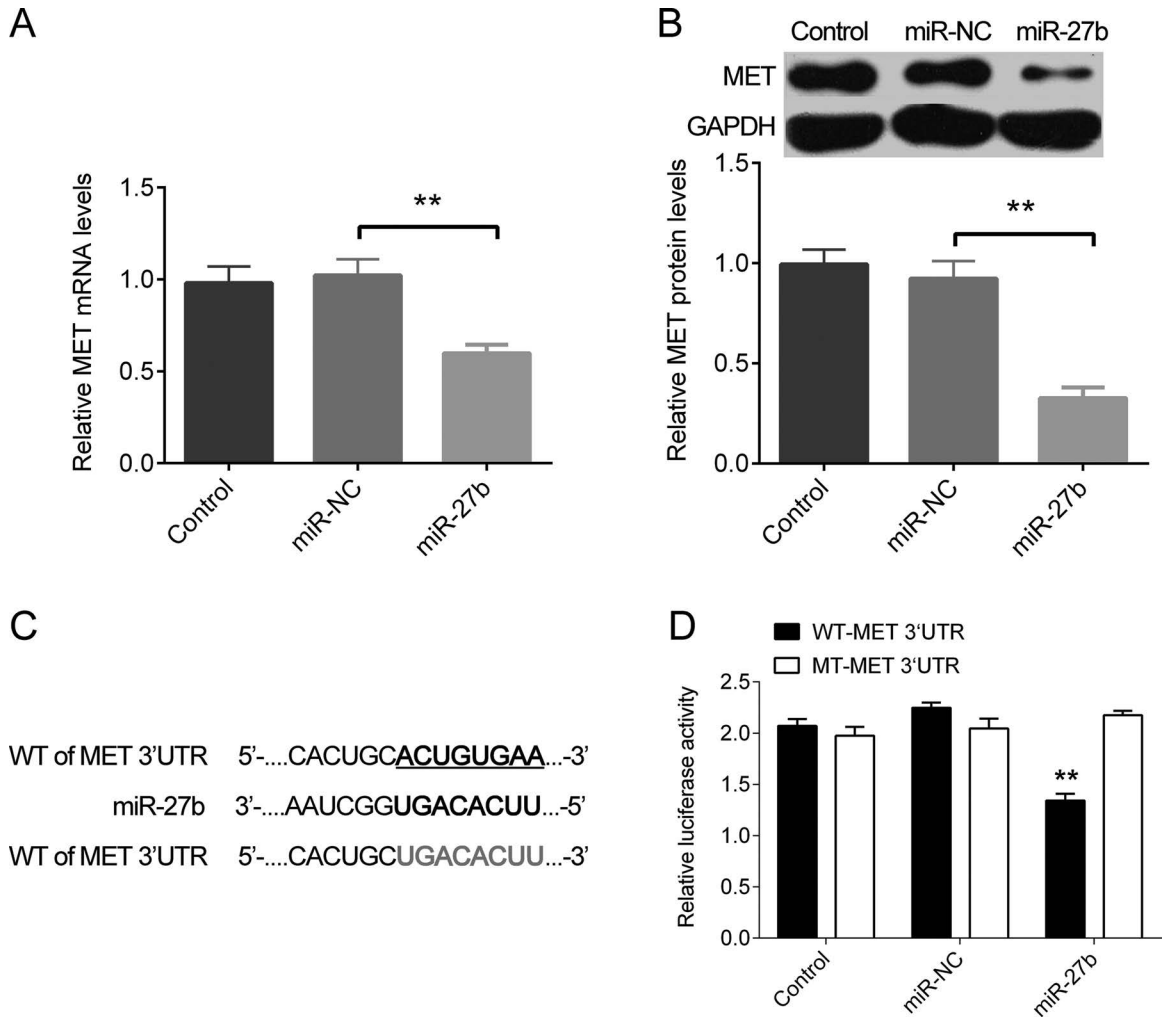

Figure 3. (A) RT-PCR and (B) Western blot assay were used to examine the mRNA and protein expression of MET in A549 cells transfected with miR-27b mimic or scramble miR (miR-NC), respectively. Nontransfected A549 cells were used as Control. $^{**}p<0.01$ versus Control. (C) The wild type (WT) and mutant type (MT) of MET 3′-UTR are shown. (D) A549 cells were then cotransfected with the pMir-Report vectors containing the WT or MT of MET 3′-UTR, and miR-133b mimics or miR-NC, respectively. The luciferase activity was significantly decreased in A549 cells transfected with miR-27b mimic and WT-MET-3′-UTR reporter plasmid, which was abolished by MT-MET-3′-UTR reporter plasmid. $^{**}p<0.01$ versus Control.

miR-27b mimic and WT-MET-3′-UTR reporter plasmid, which was abolished by MT-MET-3′-UTR reporter plasmid (Fig. 3C and D). These data confirmed that MET was a direct target gene of miR-27b.

Overexpression of MET Rescued the Effect of miR-27b Upregulation on the Malignant Phenotypes of A549 Cells

We speculated that MET might be involved in miR-27b-mediated inhibition of the malignant phenotypes of NSCLC cells. To clarify this speculation, the miR-27b-overexpressing A549 cells were transfected with pcDNA3.1-MET plasmid to upregulate its expression. After transfection, Western blot data showed that the protein level of MET was significantly increased (Fig. 4A). The cell proliferation, migration, and invasion were then examined, respectively. As demonstrated in Figure 4B–D, the proliferation, migration, and invasion capacities of A549 cells were significantly increased in the miR-27b + MET group when compared with those in the miR-27b group, respectively. Accordingly, these findings indicated that overexpression of MET rescued the effect of miR-27b upregulation on the malignant phenotypes of NSCLC cells.

MET Was Upregulated in NSCLC

Finally, we examined the MET mRNA levels in 43 NSCLC tissues and cell lines. RT-PCR data revealed that MET was significantly upregulated in NSCLC tissues compared to adjacent normal tissues (Fig. 5).

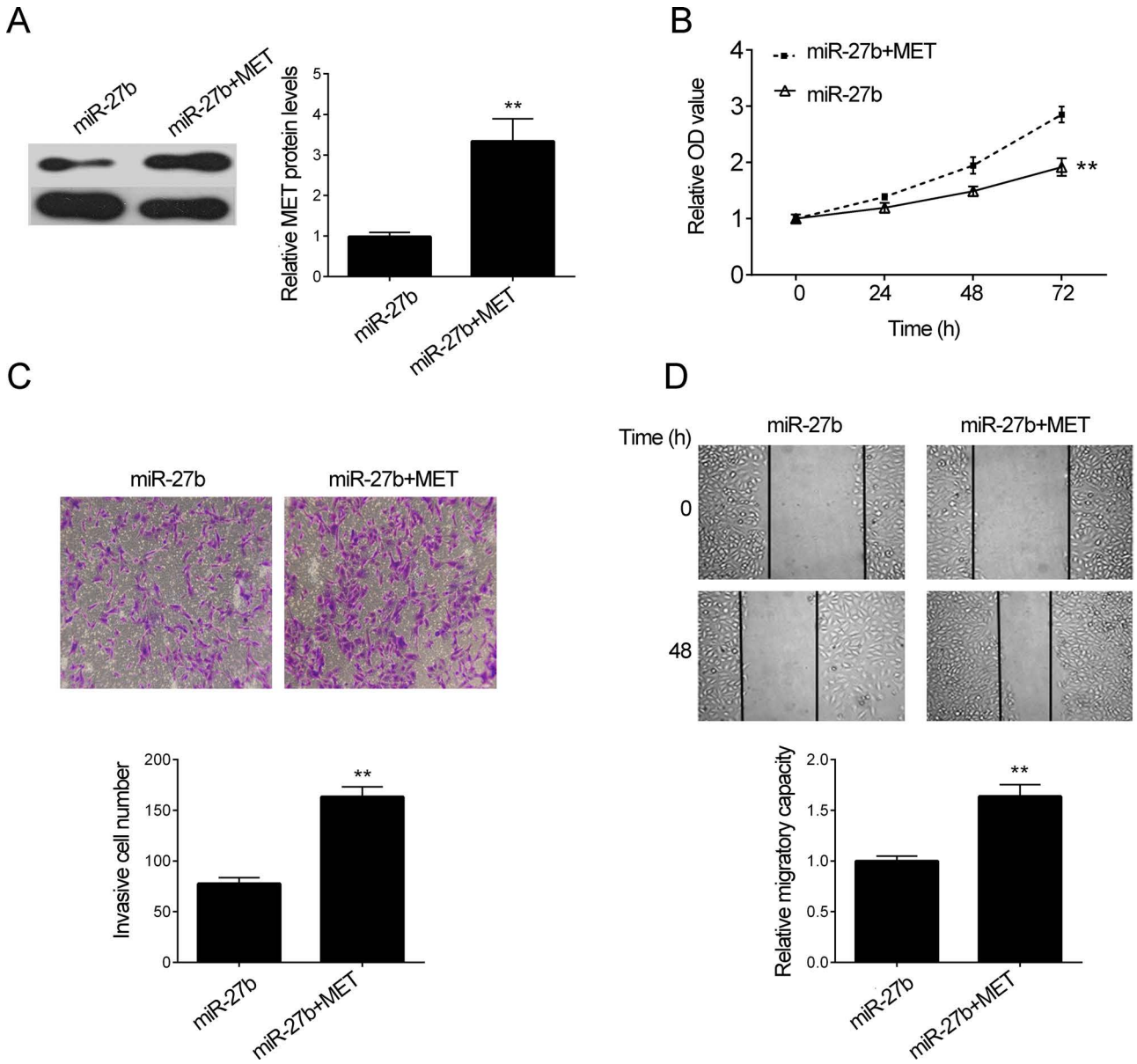

Figure 4. (A) Western blot assay was used to examine the protein expression of MET in A549 cells transfected with miR-27b mimic or cotransfected with miR-27b mimic and MET plasmid. (B) MTT assay, (C) Transwell assay, and (D) wound-healing assay were used to examine the cell proliferation, invasion, and migration. $^{**}p<0.01$ versus miR-27b.

DISCUSSION

The aberrantly expressed miRs have been implicated in the tumorigensis and malignant progression of NSCLC (22,23). Therefore, exploration of the miR-mediated targets genes and signaling pathways can help in the understanding of the molecular mechanism underlying NSCLC and improve the development of novel therapeutic strategies. In this study, we investigated the expression and regulatory role of miR-27b in NSCLC. We found that miR-27b was markedly downregulated in cancer specimens and that restoration of miR-27b significantly inhibited the proliferation, migration, and invasion of NSCLC A549 cells. These findings strongly suggest that miR-27b functions as a tumor suppressor in NSCLC cells.

In fact, other studies showed that miR-27b generally plays a suppressive role in human cancers, including prostate cancer, renal cell carcinoma, bladder cancer, gastric cancer, and so forth (24). For instance, miR-27b was downregulated in prostate cancer tissue, and low expression of miR-27b predicted a short duration of progression to castration-resistant prostate cancer (25). Moreover, a gain-of-function study indicated that miR-27b could

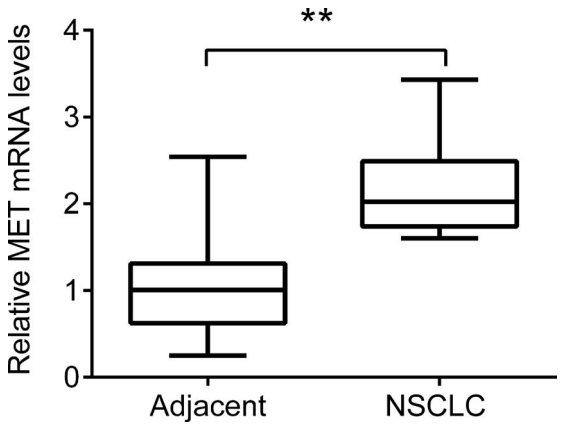

Figure 5. RT-PCR was used to examine the mRNA levels of MET in a total of 43 NSCLC clinical specimens and their matched adjacent normal tissues. $^{**}p<0.01$ versus Adjacent.

inhibit the proliferation, migration, and invasion of prostate cancer cells (25). In clear cell renal cell carcinoma, miR-27b was also significantly decreased in cancer tissues and associated with pathological grade and stage. In addition, lower expression of miR-27b predicated poorer prognosis of patients with clear cell renal cell carcinoma, and restoration of miR-27b significantly inhibited cancer cell proliferation, migration, and invasion (26). Contrary to the suppressive role of miR-27b in some human cancers, the expression of miR-27b was also found to be increased in cervical carcinoma, breast cancer, and glioma (27–30). Yao et al. showed that miR-27b was upregulated in cervical carcinoma and promoted cell growth and invasion by regulating CDH11 and epithelial–mesenchymal transition (EMT) (27). Zhang et al. reported that elevation of miR-27b by HPV16 E7 inhibited PPARγ expression and promoted proliferation and invasion of cervical carcinoma cells (28). In addition, the expression of miR-27b was elevated in breast cancer tissues, correlating with poor prognosis of patients, and knockdown of miR-27b significantly repressed breast cancer cell growth (31). These results suggest that the detailed role of miR-27b is tumor specific due to different target genes in different tumor microenvironments, and thus elucidation of the molecular mechanisms controlling the miR-27b expression is critical.

The poor prognosis of patients with NSCLC is mainly attributed to the metastasis of cancer cells (32). Thus, identification of tumor suppressive miRs that regulate metastasis-promoting genes may improve our understanding of NSCLC progression and metastasis. In this study, we performed in silico analyses using TargetScan database to identify the putative target genes of miR-27b and focused on the MET oncogene, as MET has been widely demonstrated to activate signaling that contributes to cancer cell proliferation and metastasis (33). The MET signaling pathway can be abnormally activated by many mechanisms such as mutation, amplification, and overexpression, and hypomethylation and acetylation of the MET gene have been associated with its high expression in some cancers (33,34). Moreover, MET plays a promoting role in the development and malignant progression of NSCLC, as well as in the tolerance of epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) (34). Therefore, the control of MET oncogenic signaling seems to be a promising therapeutic target for the treatment of NSCLC. In this study, we confirmed that MET was a direct target gene of miR-27b, and its expression was negatively mediated by miR-27b in A549 cells. Moreover, we found that overexpression of MET rescued the suppressive effect of miR-27b upregulation on the proliferation, migration, and invasion of NSCLC cells, suggesting that MET acts as a downstream effector in the miR-27b-mediated malignant phenotypes of NSCLC cells. Besides, our data indicated that MET was significantly upregulated in NSCLC tissues and cell lines. Therefore, we suggest that the upregulation of MET in NSCLC may be due to the decreased expression of miR-27b.

In summary, our study demonstrated that miR-27b was frequently downregulated in NSCLC tissues and cell lines and could inhibit the malignant phenotypes of NSCLC cells by directly targeting the MET oncogene. The identification of miR-27b-mediated novel signaling pathways may help reveal the molecular mechanism underlying the development and malignant progression of this disease.

ACKNOWLEDGMENTS: This work was supported by the Natural Science Foundation of Hunan Province of China (2015JJ6058) and the Research Project of Health and Family Planning Commission of Hunan Province (B2015-111). The authors declare no conflicts of interest.

REFERENCES

1. DeSantis, C.; Ma, J.; Bryan, L.; Jemal, A. Breast cancer statistics, 2013. CA Cancer J. Clin. 64:52–62; 2014.
2. Jemal, A.; Bray, F.; Center, M. M.; Ferlay, J.; Ward, E.; Forman, D. Global cancer statistics. CA Cancer J. Clin. 61:69–90; 2011.
3. Dimou, A.; Papadimitrakopoulou, V. Non-small cell lung cancer beyond biomarkers: The evolving landscape of clinical trial design. J. Pers. Med. 4:386–401; 2014.
4. Chouaid, C.; Crequit, P.; Borget, I.; Vergnenegre, A. Economic evaluation of first-line and maintenance treatments for advanced non-small cell lung cancer: A systematic review. Clinicoecon. Outcomes Res. 7:9–15; 2015.
5. Ambros, V. The functions of animal microRNAs. Nature 431:350–355; 2004.
6. John, B.; Enright, A. J.; Aravin, A.; Tuschl, T.; Sander, C.; Marks, D. S. Human microRNA targets. PLoS Biol. 2:e363; 2004.
7. Lu, J.; Getz, G.; Miska, E. A.; Alvarez-Saavedra, E.; Lamb, J.; Peck, D.; Sweet-Cordero, A.; Ebert, B. L.; Mak,

R. H.; Ferrando, A. A.; Downing, J. R.; Jacks, T.; Horvitz, H. R.; Golub, T. R. MicroRNA expression profiles classify human cancers. Nature 435:834–838; 2005.
8. Xi, Y.; Formentini, A.; Chien, M.; Weir, D. B.; Russo, J. J.; Ju, J.; Kornmann, M.; Ju, J. Prognostic values of microRNAs in colorectal cancer. Biomark. Insights 2:113–121; 2006.
9. Iorio, M. V.; Visone, R.; Di Leva, G.; Donati, V.; Petrocca, F.; Casalini, P.; Taccioli, C.; Volinia, S.; Liu, C. G.; Alder, H.; Calin, G. A.; Menard, S.; Croce, C. M. MicroRNA signatures in human ovarian cancer. Cancer Res. 67:8699–8707; 2007.
10. Zheng, K.; Liu, W.; Liu, Y.; Jiang, C.; Qian, Q. MicroRNA-133a suppresses colorectal cancer cell invasion by targeting Fascin1. Oncol. Lett. 9:869–874; 2015.
11. Zhu, J.; Zeng, Y.; Xu, C.; Qin, H.; Lei, Z.; Shen, D.; Liu, Z.; Huang, J. A. Expression profile analysis of microRNAs and downregulated miR-486-5p and miR-30a-5p in non-small cell lung cancer. Oncol. Rep. 34:1779–1786; 2015.
12. Liu, G.; Xu, Z.; Hao, D. MicroRNA451 inhibits neuroblastoma proliferation, invasion and migration by targeting macrophage migration inhibitory factor. Mol. Med. Rep. 13(3):2253–2260; 2016.
13. Tao, J.; Zhi, X.; Zhang, X.; Fu, M.; Huang, H.; Fan, Y.; Guan, W.; Zou, C. miR-27b-3p suppresses cell proliferation through targeting receptor tyrosine kinase like orphan receptor 1 in gastric cancer. J. Exp. Clin. Cancer Res. 34:139; 2015.
14. Lee, J. J.; Drakaki, A.; Iliopoulos, D.; Struhl, K. MiR-27b targets PPARgamma to inhibit growth, tumor progression and the inflammatory response in neuroblastoma cells. Oncogene 31:3818–3825; 2012.
15. Geng, Y.; Lu, X.; Wu, X.; Xue, L.; Wang, X.; Xu, J. MicroRNA-27b suppresses Helicobacter pylori-induced gastric tumorigenesis through negatively regulating Frizzled7. Oncol. Rep. 35(4):2441–2450; 2016.
16. Matsuyama, R.; Okuzaki, D.; Okada, M.; Oneyama, C. MicroRNA-27b suppresses tumor progression by regulating ARFGEF1 and focal adhesion signaling. Cancer Sci. 107:28–35; 2016.
17. Liu, H. T.; Xing, A. Y.; Chen, X.; Ma, R. R.; Wang, Y. W.; Shi, D. B.; Zhang, H.; Li, P.; Chen, H. F.; Li, Y. H.; Gao, P. MicroRNA-27b, microRNA-101 and microRNA-128 inhibit angiogenesis by down-regulating vascular endothelial growth factor C expression in gastric cancers. Oncotarget 6:37458–37470; 2015.
18. Hirota, T.; Date, Y.; Nishibatake, Y.; Takane, H.; Fukuoka, Y.; Taniguchi, Y.; Burioka, N.; Shimizu, E.; Nakamura, H.; Otsubo, K.; Ieiri, I. Dihydropyrimidine dehydrogenase (DPD) expression is negatively regulated by certain microRNAs in human lung tissues. Lung Cancer 77:16–23; 2012.
19. Hennessey, P. T.; Sanford, T.; Choudhary, A.; Mydlarz, W. W.; Brown, D.; Adai, A. T.; Ochs, M. F.; Ahrendt, S. A.; Mambo, E.; Califano, J. A. Serum microRNA biomarkers for detection of non-small cell lung cancer. PLoS One 7: e32307; 2012.
20. Wan, L.; Zhang, L.; Fan, K.; Wang, J. MiR-27b targets LIMK1 to inhibit growth and invasion of NSCLC cells. Mol. Cell. Biochem. 390:85–91; 2014.
21. Jiang, J.; Lv, X.; Fan, L.; Huang, G.; Zhan, Y.; Wang, M.; Lu, H. MicroRNA-27b suppresses growth and invasion of NSCLC cells by targeting Sp1. Tumour Biol. 35:10019–10023; 2014.
22. Ceppi, P.; Mudduluru, G.; Kumarswamy, R.; Rapa, I.; Scagliotti, G. V.; Papotti, M.; Allgayer, H. Loss of miR-200c expression induces an aggressive, invasive, and chemoresistant phenotype in non-small cell lung cancer. Mol. Cancer Res. 8:1207–1216; 2010.
23. Chen, Z.; Zeng, H.; Guo, Y.; Liu, P.; Pan, H.; Deng, A.; Hu, J. miRNA-145 inhibits non-small cell lung cancer cell proliferation by targeting c-Myc. J. Exp. Clin. Cancer Res. 29:151; 2010.
24. Chiyomaru, T.; Seki, N.; Inoguchi, S.; Ishihara, T.; Mataki, H.; Matsushita, R.; Goto, Y.; Nishikawa, R.; Tatarano, S.; Itesako, T.; Nakagawa, M.; Enokida, H. Dual regulation of receptor tyrosine kinase genes EGFR and c-Met by the tumor-suppressive microRNA-23b/27b cluster in bladder cancer. Int. J. Oncol. 46:487–496; 2015.
25. Goto, Y.; Kojima, S.; Nishikawa, R.; Enokida, H.; Chiyomaru, T.; Kinoshita, T.; Nakagawa, M.; Naya, Y.; Ichikawa, T.; Seki, N. The microRNA-23b/27b/24-1 cluster is a disease progression marker and tumor suppressor in prostate cancer. Oncotarget 5:7748–7759; 2014.
26. Ishihara, T.; Seki, N.; Inoguchi, S.; Yoshino, H.; Tatarano, S.; Yamada, Y.; Itesako, T.; Goto, Y.; Nishikawa, R.; Nakagawa, M.; Enokida, H. Expression of the tumor suppressive miRNA-23b/27b cluster is a good prognostic marker in clear cell renal cell carcinoma. J. Urol. 192:1822–1830; 2014.
27. Yao, J.; Deng, B.; Zheng, L.; Dou, L.; Guo, Y.; Guo, K. miR-27b is upregulated in cervical carcinogenesis and promotes cell growth and invasion by regulating CDH11 and epithelial-mesenchymal transition. Oncol. Rep. 35:1645–1651; 2016.
28. Zhang, S.; Liu, F.; Mao, X.; Huang, J.; Yang, J.; Yin, X.; Wu, L.; Zheng, L.; Wang, Q. Elevation of miR-27b by HPV16 E7 inhibits PPARgamma expression and promotes proliferation and invasion in cervical carcinoma cells. Int. J. Oncol. 47:1759–1766; 2015.
29. Liu, C.; Liang, S.; Xiao, S.; Lin, Q.; Chen, X.; Wu, Y.; Fu, J. MicroRNA-27b inhibits Spry2 expression and promotes cell invasion in glioma U251 cells. Oncol. Lett. 9: 1393–1397; 2015.
30. Chen, L.; Li, H.; Han, L.; Zhang, K.; Wang, G.; Wang, Y.; Liu, Y.; Zheng, Y.; Jiang, T.; Pu, P.; Jiang, C.; Kang, C. Expression and function of miR-27b in human glioma. Oncol. Rep. 26:1617–1621; 2011.
31. Jin, L.; Wessely, O.; Marcusson, E. G.; Ivan, C.; Calin, G. A.; Alahari, S. K. Prooncogenic factors miR-23b and miR-27b are regulated by Her2/Neu, EGF, and TNF-alpha in breast cancer. Cancer Res. 73:2884–2896; 2013.
32. Hall, R. D.; Le TM; Haggstrom, D. E.; Gentzler, R. D. Angiogenesis inhibition as a therapeutic strategy in non-small cell lung cancer (NSCLC). Transl. Lung Cancer Res. 4:515–523; 2015.
33. Zhang, J.; Babic, A. Regulation of the MET oncogene: Molecular mechanisms. Carcinogenesis 37(4):345–355; 2016.
34. Liang, H.; Wang, M. [Mechanism of c-MET in non-small cell lung cancer and its treatment and testing]. Zhongguo Fei Ai Za Zhi 18:745–751; 2015.