RESEARCH ARTICLE

MicroRNA-27b suppresses growth and invasion of NSCLC cells by targeting Sp1

Jun Jiang · Xiaojuan Lv · Liang Fan · Guodong Huang · Yan Zhan · Mengyi Wang · Hongda Lu

Abstract Non-small cell lung cancer (NSCLC) is the major cause of cancer death worldwide. Increasing evidence shows that microRNAs (miRNAs), evolutionally conserved non-coding RNAs, are widely involved in the development and progression of NSCLC. Aberrant alteration of miRNAs expression has been implicated in NSCLC initiation and progression. Herein, we studied the role of miR-27b in NSCLC cells. We found that miR-27b was significantly decreased in several NSCLC cell lines. Forced overexpression of miR-27 inhibited both the growth and invasion of NSCLC cells. Furthermore, we identified Sp1 transcription factor (Sp1) as a target of miR-27b in NSCLC cells. Moreover, we found that miR-27 suppressed growth and invasion of NSCLC cells partially by targeting Sp1. Our data indicate that miR-27b may play a critical role in the development of NSCLC.

Keywords NSCLC · miR-27b · Sp1 · Proliferation · Invasion

Introduction

Lung cancer ranks one of the most frequent causes of cancer-related mortality worldwide, and non-small cell lung cancer (NSCLC) accounts for more than 80 % of all lung cancer cases [1]. The prognosis of NSCLC is very poor, and most patients are diagnosed at an advanced stage, with an overall 5-year survival rate of 11 % [2]. Therefore, it is urgent to further investigate the underlying mechanisms of NSCLC.

J. Jiang · X. Lv · L. Fan · G. Huang · Y. Zhan · M. Wang · H. Lu (✉)
Department of Oncology, The Central Hospital of Wuhan, 430014 Wuhan, China
e-mail: lu_hongda@126.com

MicroRNAs (miRNAs) are a family of small, non-coding RNAs that bind to the 3′-untranslated region (3′-UTR) of target messenger RNA (mRNA), inducing mRNA cleavage or translational repression [3]. miRNAs regulate a variety of biological processes, including development, differentiation, migration, invasion, and apoptosis [4]. They function as oncogenes or tumor suppressors via targeting different targets [5, 6]. Many miRNAs have been aberrantly altered in NSCLC and contribute to the development and progression of NSCLC [7, 8]. miR-27b has been found to be decreased in NSCLC [9, 10]. However, the detailed role of miR-27b in NSCLC carcinogenesis is poorly understood.
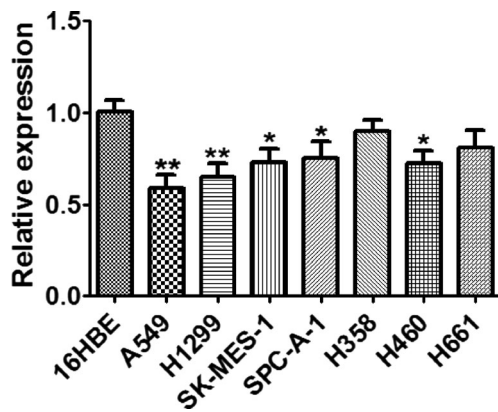
In this study, we found that miR-27b was significantly decreased in several NSCLC cell lines and miR-27b suppressed growth and invasion of NSCLC cells. Moreover, we identified Sp1 transcription factor (Sp1) as a target of miR-27b in NSCLC cells. Finally, we found that miR-27 suppressed growth and invasion of NSCLC cells partially by targeting Sp1.

Materials and methods

NSCLC cell lines

Seven NSCLC cell lines, A549, H1299, SK-MES-1, SPC-A-1, H358, H460, and H661, and a normal bronchial epithelial cell line (16HBE) were purchased from the Institute of Biochemistry and Cell Biology (Shanghai, China). Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) (Invitrogen, Carlsbad, CA, USA) with 10 % fetal bovine serum (FBS) in a humidified atmosphere with 5 % CO_2 at 37 °C. Cell transfection was performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol.

Fig. 1 miR-27b was decreased in NSCLC cell lines. Expression of miR-27b in seven NSCLC cell lines, A549, H1299, SK-MES-1, SPC-A-1, H358, H460, and H661, and normal bronchial epithelial cell line (16HBE) was measured by qRT-PCR. Data were drawn from four independent experiments. *$P<0.05$, **$P<0.01$ compared with control

RNA extraction and quantitative real-time PCR

Total RNAs were extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). miRNAs were extracted using a miRNA extraction kit (Tiangen, Beijing, China). The quantitative real-time PCR (qRT-PCR) was performed on ABI 7900 (ABI, Foster City, CA, USA) using a SYBR Green mix (Takara, Tokyo, Japan). Primers of U6 and miR-27b were obtained from GeneCopoeia (Carlsbad, CA, USA). The primers for Sp1 were 5′-CTGCCGCTCCCAACTTAC-3′ and 5′-TTGC CTCCACTTCCTCGA-3′ and for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) 5′-CCACTCCTCCAC CTTTGAC-3′ and 5′-ACCCTGTTGCTGTAGCCA-3′. The expression of Sp1 was normalized with GAPDH, and the fold change was calculated using the $2^{-\Delta\Delta CT}$ method.

Plasmids and dual luciferase activity assay

miR-27b and the control mimics were obtained from RiboBio (Guangzhou, China). Sp1 3′-UTR (CCCTCGAGGTTAGAGA GCTTTTCACTGTGAGGCGGCCGCAA) containing the potential binding sites of miR-27b (position 1663–1669) were synthesized and ligased into psiCHECK-2 vector (Promega, Wisconsin, WI, USA) within XhoI/NotI restriction sites. The coding sequence of Sp1 was amplified using 5′-GGGGTACC CGCCCTCTGACCAAGATC-3′ and 5′-TTGCGGCCGCTG CTCATCAAAGCGTGGC-3′. The PCR product was inserted into pcDNA3 within KpnI/NotI restriction sites.

For dual luciferase activity assay, 1×10^5 HEK293 cells were grown in 24-well plates, co-transfected with miR-27b or control mimics and wild-type (WT) or mutant (Mut) 3′-UTR of Sp1. Cells were collected and lysed 48 h later, and the dual luciferase activity was assayed using a Dual-Luciferase Reporter Assay System (Promega, Wisconsin, WI, USA).

MTT cell proliferation assay

Cell viability was detected using the MTT assay. Briefly, 1,500 transfected A549 cells were plated in 96-well plates for 24 h. Then, A549 cells were incubated with MTT (0.5 mg/ml) for 4 h at 37 °C. Finally, 100 μl DMSO was added to each well, and the absorbance values at 490 nm was measured.

In vitro invasion assays

Twenty-four hours after transfection, 1×10^5 cells in serum-free medium were seeded into the top chamber of an insert precoated with Matrigel (BD, San Jose, CA, USA). DMEM with 10 % FBS was added to the lower chamber. Cells remaining on the upper surface were removed 24 h later, whereas cells that invaded into the lower surface were fixed, stained with 0.05 % crystal violet, and counted under a microscope (Olympus, Tokyo, Japan). Four random fields were counted for each group.

Western blotting

Proteins were lysed with RIPA buffer (Beyotime, Shanghai, China), separated on 10 % SDS-PAGE, and then transferred to PVDF membranes. Membranes were blocked with 3 % non-fat dried milk solution for 0.5 h at room temperature and then
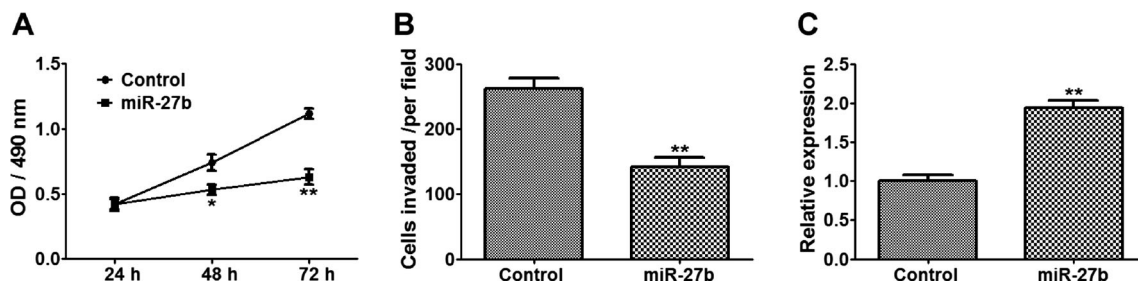
Fig. 2 miR-27b suppressed growth and invasion of NSCLC cells. **a** A549 cells were transfected with 100 μM miR-27b or the control mimics, and MTT assay was performed at different time points. **b** In vitro invasion assay. **c** A549 cells were transfected with 100 μM miR-27b or the control mimics, and the expression of miR-27b was measured by qRT-PCR. Data were drawn from four independent experiments. *$P<0.05$, **$P<0.01$ compared with control

Fig. 3 Sp1 was a target of miR-27b in NSCLC cells. **a** SP1 contained potential binding sites of miR-27b. **b** HEK293 cells were co-transfected with miR-27b or control mimics and wild-type (*WT*) or mutant (*Mut*) 3′-UTR of Sp1. Dual luciferase activity was measured 48 h later. **c** A549 cells were transfected with miR-27b or control mimics. The protein level of Sp1 was detected by Western blotting 48 h later. Data were drawn from four independent experiments. **P<0.01 compared with control
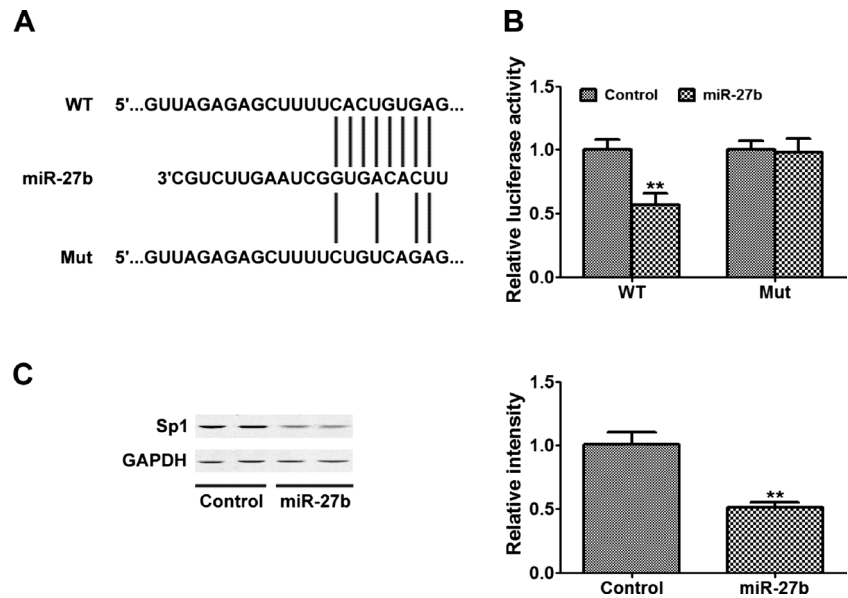
probed with primary antibodies overnight at 4 °C. The membranes were further developed with HRP-conjugated secondary antibodies for 1 h at 37 °C. The blots were visualized with ECL kit (Thermo Scientific, Rockford, IL, USA).

Statistical analysis

All data were presented as mean±SD and analyzed by using SPSS 16.0. Two-tail Student's *t* test and ANOVA were performed to determine the differences. *P*<0.05 was considered statistically significant.

Results

miR-27b was decreased in NSCLC cell lines

To explore the role of miR-27b in NSCLC, we first measured the expression of miR-27b in seven NSCLC cell lines (A549,

H1299, SK-MES-1, SPC-A-1, H358, H460, and H661). We found that miR-27b was significantly decreased in most NSCLC cell lines compared with that in the normal bronchial epithelial cell line (16HBE) (Fig. 1). These data suggest that miR-27b may contribute to the development of NSCLC.

miR-27b suppressed growth and invasion of NSCLC cells

We then investigated the role of miR-27b in the regulation of growth and invasion of NSCLC cells. MTT assay was used to examine the proliferation, and results showed that miR-27b remarkably inhibited the growth of A549 cells (Fig. 2a). Transwell invasion assay also found that miR-27b significantly suppressed the invasion of A549 cells (Fig. 2b). The expression of miR-27b was increased after miR-27b mimics transfection (Fig. 2c). These data indicate that miR-27 may suppress the development and progression of NSCLC.
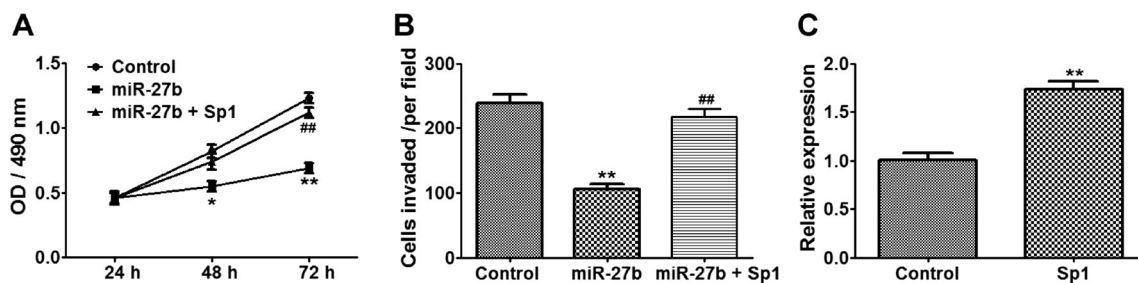
Fig. 4 miR-27b suppressed NSCLC progression by targeting Sp1. A549 cells were co-transfected with miR-27b or control mimics and Sp1 overexpression plasmid, pcDNA-Sp1. MTT assay (**a**) and Transwell invasion assay (**b**) were performed. **c** A549 cells were transfected with Sp1 overexpression plasmid, pcDNA-Sp1, or the control vector, pcDNA3; 48 h later, cells were collected and the expression of Sp1 was measured by qRT-PCR. Data were drawn from four independent experiments. *P<0.05, **P<0.01 compared with control. ##*P*<0.01 compared with the miR-27 group

Sp1 was a target of miR-27b in NSCLC cells

We further explored the downstream molecular target of miR-27b in NSCLC. TargetScan 6.2 (www.targetscan.org) was used, and we found that Sp1 contained potential binding sites of miR-27b (Fig. 3a). The dual luciferase activity assay showed that miR-27b significantly inhibited the luciferase activity of the wild-type (WT) 3′-UTR of Sp1, without effect on its mutant (Mut) (Fig. 3b). Moreover, Western blotting confirmed that miR-27b suppressed the protein level of Sp1 in A549 cells (Fig. 3c). These data suggest that Sp1 was a target of miR-27b in NSCLC cells.

miR-27b suppressed NSCLC progression by targeting Sp1

We further studied whether miR-27b suppressed NSCLC progression by targeting SP1. A549 cells were co-transfected with miR-27b or control mimics and Sp1 overexpression plasmid, pcDNA-Sp1. MTT and invasion assays found that overexpression of Sp1 dramatically attenuated the tumor suppressive effects of miR-27b on A549 cells (Fig. 4a, b). The effect of Sp1 overexpression was confirmed by qRT-PCR (Fig. 4c). These results suggest that miR-27b may suppress NSCLC progression by targeting Sp1.

Discussion

In this study, we identified a tumor suppressive role of miR-27b in NSCLC cells. We found that miR-27b was decreased in several NSCLC cell lines. Overexpression of miR-27b suppressed proliferation and invasion of NSCLC cells. Sp1 was identified as a target of miR-27b in NSCLC cells. Moreover, supplement of Sp1 could reverse the tumor suppressive effects of miR-27b in NSCLC cells.

Accumulating evidence reveals the important function of miRNAs in NSCLC development and progression [11]. Cui et al. found that miR-186 suppressed the growth and metastasis of NSCLC cells through targeting rho-associated protein kinase 1 (ROCK1) [12]. miR-195 acted as a tumor suppressor in NSCLC by targeting different targets, such as insulin-like growth factor 1 receptor (IGF1R) and hepatoma-derived growth factor (HDGF) [5, 13]. On the other hand, several miRNAs function as oncogenes in NSCLC. Chen et al. showed that miR-95 induced proliferation and chemo- or radioresistance of NSCLC cells by targeting sorting nexin 1 (SNX1) [14]. Lang et al. found that miR-429 induced tumorigenesis of NSCLC via targeting multiple tumor suppressor genes [15]. miR-27b acts as a tumor suppressor in some cancers. Lee et al. found that miR-27b suppressed growth, tumor progression, and inflammatory response of neuroblastoma cells via inhibiting peroxisome proliferator-activated

receptor γ (PPARγ) [16]. Ye et al. reported that miR-27b inhibited tumor progression and angiogenesis of colorectal cancer cells via targeting vascular endothelial growth factor C (VEGFC) [17]. Ishteiwy et al. found that in castration-resistant prostate cancer cells, miR-23b/miR-27b cluster inhibited the metastatic phenotype [18]. In NSCLC, Wan et al. reported that miR-27b was decreased in NSCLC tissues and miR-27b functioned as a tumor suppressor by targeting LIM kinase 1 (LIMK1) in NSCLC [10]. Our work further expanded the tumor suppressive role of miR-27b in NSCLC.

Sp1 is a well-known transcription factor which modulates transcription of TATA-less genes via interacting directly with and mediating the recruitment to basal transcription machinery [19]. Sp1 is constitutively increased in several cancers, including, pancreatic, lung, and gastric cancers [20–22]. Sp1 contributes to cancer progression and was regulated by many miRNAs. Wang et al. reported that miR-335 induced apoptosis and suppressed invasion of NSCLC cells by targeting Bcl-w and Sp1 [23]. Mao et al. found that miR-330 suppressed prostate cancer cells motility by targeting Sp1 [24]. Wang et al. showed that miR-375 inhibited squamous cervical cancer cell migration and invasion by targeting Sp1 [25]. In our study, we found that Sp1 could also be regulated by miR-27b in NSCLC, supporting its oncogenic role in NSCLC.

In summary, we reveal the tumor suppressive role of miR-27b in NSCLC. miR-27b suppressed the proliferation and invasion of NSCLC cells by targeting Sp1. Our data suggest that miR-27b may provide a potential therapeutic target for NSCLC treatment.

Conflicts of interest None

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