Biochemical and Biophysical Research Communications 471 (2016) 82–88

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

MicroRNA-187-3p mitigates non-small cell lung cancer (NSCLC) development through down-regulation of BCL6

Chengcao Sun [a], Shujun Li [a, b], Cuili Yang [a], Yongyong Xi [a], Liang Wang [a], Feng Zhang [a], Dejia Li [a, *]

[a] Department of Occupational and Environmental Health, School of Public Health, Wuhan University, Wuhan, PR China
[b] Wuhan Hospital for the Prevention and Treatment of Occupational Diseases, 430071 Wuhan, PR China

ARTICLE INFO

Article history:
Received 8 January 2016
Received in revised form
18 January 2016
Accepted 28 January 2016
Available online 1 February 2016

Keywords:
Hsa-miRNA-187-3p (miR-187-3p)
BCL6
Non-small cell lung cancer (NSCLC)
Proliferation
Apoptosis

ABSTRACT

Hsa-microRNA-187-3p (miR-187-3p) has recently been discovered having anticancer efficacy in different organs. However, the role of miR-187-3p on non-small cell lung cancer (NSCLC) is still ambiguous. In this study, we investigated the role of miR-187-3p on the development of NSCLC. The results indicated that miR-187-3p was significantly down-regulated in primary tumor tissues and very low levels were found in NSCLC cell lines. Ectopic expression of miR-187-3p in NSCLC cell lines significantly suppressed cell growth as evidenced by cell viability assay and colony formation assay, through inhibition of BCL6. In addition, miR-187-3p induced apoptosis, as indicated by concomitantly with up-regulation of the activities of caspase-3 and caspase-7, and inhibited cellular migration and invasiveness through inhibition of BCL6. Further, oncogene *BCL6* was revealed to be a putative target of miR-187-3p, which was inversely correlated with miR-187-3p expression in NSCLC. Taken together, our results demonstrated that miR-187-3p played a pivotal role on NSCLC through inhibiting cell proliferation, migration, invasion, and promoting apoptosis by targeting oncogenic *BCL6*.

1. Introduction

MicroRNAs (miRNAs) are a class of small, highly conserved, and non-coding RNAs that directly bind to some sequence-specific sites of target genes' 3′-UTRs (3′ untranslated regions), which lead to inhibition of these genes expression [1,2]. Increasing evidences have confirmed that ectopic miRNAs are key regulatory factors in various types of cancers [3–7]. Selective miRNA expression contributes to tumor proliferation, apoptosis, senescence, cell identity, stem cell maintenance and metastasis [8–13]. Though recent researches of miRNAs have brought mind-blowing insight into our knowledge of human cancers, there are still large amount of unknown details that need to be explored further.

Lung cancer is one of the most frequently diagnosed cancers and is the leading cause of cancer-associated death both in men and women around the world. There are estimated to be 1.80 million new cases in 2012, killing about 1.59 million people per year globally, extrapolating from a 2012 International Agency for Research on Cancer (IARC) risk assessment [14], and this trend is expected to continue until 2030. Generally, approximately 85% of lung cancers are classified histopathologically as non-small cell lung carcinomas (NSCLC). Treatment advances have been made with the use of platinum-based chemotherapy [15–20], but the 5-year overall survival (OS) rate of just 16% for all stages [21]. These changes are attributed to silencing of tumor suppressor genes, dysregulation of proto-oncogenes, and an up-regulation of genes that promote cell growth and transformation and ultimately tumor development [22,23].

MiR-187-3p, a recognized tumor-suppressing miRNA, has been shown to be down-regulated in a variety of diseases, including type 2 diabetes [24] and several cancers, such as retinal ganglion [25], prostate cancer [26,27], clear cell renal cell carcinoma [28] and breast cancer [29]. Recently, Mirzadeh and his colleagues reported miR-187 was significantly down-regulated in lung tumors [30], which suggest tumor-suppressive functions of miR-187-3p in lung cancer but up to now this suggestion has not been rigorously tested.

The goal for our current study is to investigate the biological functions of miR-187-3p in non-small cell lung cancer and to explore the underlying mechanisms of action. We show for the first time that miR-187-3p directly targets and regulates the full-length

* Corresponding author. No.115 Donghu Road, Wuchang District, Wuhan, China.
E-mail address: lodjlwhu@sina.com (D. Li).

http://dx.doi.org/10.1016/j.bbrc.2016.01.175
0006-291X/

3′-UTR of the human B-cell CLL/lymphoma 6 (BCL6) mRNA, which is up-regulated in many cancers, including lung cancer. Bcl6 is encoded by *BCL6* gene, and plays a key role in the control of invasive growth during tumorigenesis [31]. Here, we reported that miR-187-3p is indeed suppressed in primary lung cancers compared with the matching adjacent normal tissues, and found 3′-UTR of the human BCL6 mRNA is really a target of miR-187-3p. Collectively, we discovered that miR-187-3p inhibits NSCLC cell growth, migration, invasion and colony formation, and promoted cell apoptosis by targeting 3′-UTR of *BCL6*.

2. Materials and methods

2.1. Tissue collection

Lung cancer tissues and adjacent normal lung tissues were obtained from patients who had undergone surgery at the Zhongnan Hospital of Wuhan University, between 2011 and 2015 and who were diagnosed with lung cancer based on histopathological evaluation. No local or systemic treatment had been conducted in these patients before the operation. All the tissue samples were collected, immediately snap frozen in liquid nitrogen, and stored at −80 °C until RNA extraction. The study was approved by the Research Ethics Committee of Wuhan University (Wuhan, Hubei, PR China). Informed consent was obtained from all patients.

2.2. Cell culture and transfection

The human NSCLC cell lines, namely, A549, SPC-A-1, SPC-A-1, SK-MES-1, NCI−H520, 95D and normal 16HBE cells were grown in RPMI 1640 (Gibco, USA) medium containing 10% heat-inactivated (56 °C, 30 min) fetal calf serum, 2 mmol/L glutamine, penicillin (100 U/mL) and streptomycin (100 U/mL), which was maintained in an incubator at 37 °C with 5% CO_2 in a humidified atmosphere. Hsa-miR-187-3p mimic and mimic negative control, hsa-miR-187-3p inhibitor and inhibitor negative control were purchased from GenePharma Co.,Ltd. (Shanghai, China). The cells were washed with 1 × PBS (pH7.4) and then transiently transfected with 50 nM miR-187-3p mimic or miR mimic NC, 100 nM miR-187-3p inhibitor or miR inhibitor NC, using Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

2.3. Western blot analysis

Western blot was carried out using the protocol described previously [11,12,32−34]. The following primary antibodies were used: rabbit anti-Bcl6 (Santa Cruz, USA), rabbit anti-GAPDH (Santa Cruz, USA).

RNA isolation and quantitative reverse transcription polymerase chain reaction (qRT-PCR).

RNA isolation and qRT-PCR was carried out using the protocol described previously [11,12]. The primer sequences used in this study are in Table S1.

2.3.1. Colony formation assay

Colony formation assay was carried out using the protocol described previously [11,12].

2.3.2. Luciferase reporter assays

Luciferase reporter assays was carried out using the protocol described previously [11,12].

2.3.3. Transwell migration/invasion assay

Transwell migration/invasion assay were carried out using the protocol described previously [11,12].

2.3.4. CCK8 assay

Cell growth was measured using the cell proliferation reagent WST-8 (Roche Biochemicals, Mannheim, Germany). After plating cells in 96-well microtiter plates (Corning Costar, Corning, NY) at 1.0×10^3/well, 10 μL of CCK8 was added to each well at the time of harvest, according to the manufacturer's instructions. One hour after adding CCK8, cellular viability was determined by measuring the absorbance of the converted dye at 450 nm.

2.3.5. Caspase-3/7 activity assay

Caspase-3/7 activity assay was carried out using the protocol described previously [11,12].

2.4. Statistical analysis

All experiments were repeated 3 times independently. The results are presented as the means ± standard error mean (SEM). Two independent sample t-test or One-Way Analysis of Variance (ANOVA) was performed using SPSS 19.0 software in order to detect significant differences in measured variables among groups. A value of $P < 0.05$ was considered to indicate a statistically significant difference.

3. Results

3.1. MiR-187-3p is down-regulated in primary human lung cancer and NSCLC cell lines, and benefits for prognosis

To determine whether miR-187-3p is down-regulated in lung cancer, we measured the mature miR-187-3p level in human primary lung tumors (NSCLC) and pair-matched adjacent lung normal tissues by qRT-PCR. We used U6 that is not deregulated in lung cancer for normalization. The results showed that miR-187-3p expression in the tumors was significantly ($P < 0.05$) reduced (mean = 29% of decrease) in 39 lung cancers relative to their matched controls among 39 samples analyzed (Fig. 1A). Next, we examined miR-187-3p expression in NSCLC cell lines, and results demonstrated a lower expression of miR-187-3p in A549, SPC-A-1, 95D, SK-MES-1, NCI−H520 and SPC-A-1 cell lines, compared with that of in normal lung cells 16HBE (Fig. 1B). In addition, to evaluate the clinical significance of miR-187-3p, we assessed the correlation of its expression with clinic−pathological parameters (i.e., stage, maximum diameter and lymph node metastasis). Results demonstrated miR-187-3p expression levels in NSCLC were significantly associated with tumor size ($P = 0.0019$), smoking history ($P = 0.0172$), TNM stage ($P = 0.0.0191$), and lymph node metastasis ($P = 0.0.0057$). However, miR-187-3p expression was not correlated with other clinical characteristics such as differentiation ($P = 0.1281$), gender ($P = 0.6138$), age ($P = 0.7573$) or histological tumor type ($P = 0.0190$) in NSCLC (Table 1). Additionally, Kaplan−Meier survival analysis revealed that patients with low expression levels (≤29% of decrease, n = 25) of miR-187-3p had shorter overall survival, when compared with patients with high expression levels (>29% of decrease, n = 14) of miR-187-3p (Fig. 1C). These results demonstrated that down-regulation of miR-187-3p was associated with poor prognosis. Thus, it was concluded that the decreased expression of miR-187-3p might play an important role in lung cancer progression and development.

3.2. Expression of Bcl6 is up-regulated in primary human lung cancer and negatively expressed related to miR-187-3p

Bcl6 is important oncogene that shown strong power of oncogenicity, by promotion of cell growth, migration, invasion and epithelial mesenchymal transition (EMT), as well as inhibition of