Biochemical and Biophysical Research Communications xxx (2017) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Long non-coding RNA PVT1-5 promotes cell proliferation by regulating miR-126/SLC7A5 axis in lung cancer

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ARTICLE INFO

Article history:
Received 7 December 2017
Accepted 20 December 2017
Available online xxx

Keywords:
Lung cancer
lncRNA-PVT1-5
miR-126
SLC7A5
Competing endogenous RNA

ABSTRACT

Dysregulated long non-coding RNAs (lncRNAs) and microRNAs (miRNAs) play key roles in the development of human cancers. The lncRNA plasmacytoma variant translocation 1 (PVT1) is reported to be an oncogene in a variety of cancers. However, the roles of PVT1-5 and its related miRNAs in lung cancer are poorly understood. In this study, we found that PVT1-5 expression was significantly increased in lung cancer tissues and cell lines. By using biotin-labeled lncRNA-PVT1-5 probe for miRNA in vivo precipitation (miRIP) in lung cancer cells and dual-luciferase reporterassays, we identified that miR-126 was associated with lncRNA-PVT1-5. Furthermore, knockdown of lncRNA-PVT1-5 in cells could down-regulate the expression of SLC7A5, the target of oncogenic miR-126, resulting in the cell proliferation. Conversely, inhibiting the expression of miR-126 markedly increased the expression of SLC7A5 and alleviated cell proliferation inhibition. Thus, our results indicated that lncRNA-PVT1-5 may function as a competing endogenous RNA (ceRNA) for miR-126 to promote cell proliferation by regulating the miR-126/SLC7A5 pathway, suggesting that lncRNA-PVT1-5 plays a crucial role in lung cancer progression and lncRNA-PVT1-5/miR-126/SLC7A5 regulatory network may shed light on tumorigenesis in lung cancer.

1. Introduction

Lung cancer has been the most common cancer diagnosed and the leading cause of death from cancer in the world, and it is estimated that 700,000 new cases are diagnosed and causes about 60,000 deaths in China annually [1]. Due to its early aggressive nature of lung cancer and lacking of early diagnostic and prognostic markers, the overall prognosis remains unsatisfied, with 5-year survival rate to be ~17%. Hence, it is urgent to explore the underlying molecular mechanisms of carcinogenesis and progression of lung cancer and thetargeted signaling pathways for cancer treatment.

Long non-coding RNAs (lncRNAs) with more than 200 bases in length, are consist of exons and introns in structure, without ORFs, are not highly conserved. Recent reports suggest that lncRNAs play important roles in regulation of diverse cellular processes such as cell growth, apoptosis and cancer metastasis [2]. Hu et al. reported that lncRNA taurine-upregulated gene 1 (TUG1) was significantly overexpressed in cervical cancer and it was associated with larger tumor size and advanced international federation of gynecology

Abbreviations: LncRNAs, long non-coding RNAs; miRNAs, microRNAs; PVT1, plasmacytoma variant translocation 1; miRIP, miRNA in vivo precipitation; ceRNA, competing endogenous RNA; TUG1, taurine-upregulated gene 1; FIGO, federation of gynecology and obstetrics; CASC9, cancer susceptibility candidate 9; EGFL7, epidermal growth factor-like protein 7; NSCLC, non-small cell lung cancer.

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https://doi.org/10.1016/j.bbrc.2017.12.114
0006-291X/

and obstetrics (FIGO) stage [3]. Pan et al. found that lncRNA cancer susceptibility candidate 9 (CASC9) was markedly up-regulated in esophageal squamous cell carcinoma tissues and knockdown of lncRNA CASC9 significantly suppressed cell migration and invasion [4]. The lncRNA plasmacytoma variant translocation 1(lncRNA-PVT1) is encoded by a gene located in the well-known cancer risk region8q24 [5]. Recently, increased expression of lncRNA-PVT1 has been found in several kinds of cancers, such as pancreatic cancer [6], gastric cancer [7], ovarian cancer and thyroid cancer [8]. Moreover, upregulation of lncRNA-PVT1 is significantly correlated with histological grade and lymphnode metastasis [9]. Increased lncRNA-PVT1 expression also correlates with advanced stage and hormone resistance of prostate cancer [10]. However, the biological role and underlying molecular mechanism of lncRNA-PVT1-5 in lung cancer remain unknown.

Currently, a new regulatory mechanism has been identified in which crosstalk between lncRNAs and mRNAs occurs by competing for shared microRNAs (miRNAs) response elements. In this case, lncRNAs may function as competing endogenous RNAs (ceRNAs) to sponge miRNAs, thereby modulating the depression of miRNA targets and imposing an additional level of post-transcriptional regulation. Up to date, it was estimated that miRNAs may potentially regulate up to 30% of all human protein-coding genes [11]. As an important member of the miRNA family, miR-126, located within the seventh intron of epidermal growth factor-like protein 7 (EGFL7) gene [12], was participated in a wide range of biological functions. For instance, miR-126 could inhibit the proliferation of non-small cell lung cancer (NSCLC) through EGFL7 [13]. The up-regulation of miR-126 in A549 cells could reduce the expression of the target gene PIK3R2 and influence the PTEN/PI3K/AKT signaling pathway, suppressing the proliferation, migration, and invasive abilities of A549 cells [14]. In addition, decreased miR-126 expression could enhance the adhesion, migration and invasion of NSCLC cells through increased Crk protein [15]. These results suggested that miR-126 may be function as an important regulatory gene in the development of NSCLC. However, limited knowledge is available concerning whether lncRNA-PVT1-5 could act as a sponge for miR-126 to affect the biological processes of lung cancer and the potential primary mechanism among lncRNA-PVT1-5, miR-126, and the target gene of miR-126 in lung cancer progression remains unknown.

In this study, we found that the expression of lncRNA-PVT1-5 was increased in lung cancer and that lncRNA-PVT1-5 may be a promising prognostic or progression marker for lung cancer. Additionally, down-regulation of lncRNA-PVT1-5 suppressed cell proliferation. Moreover, mechanistic analysis revealed that lncRNA-PVT1-5 may function as a ceRNA for miR-126 to regulate the expression of SLC7A5 to promote cell proliferation, thus playing an important role in lung cancer pathogenesis. Here, we provide the first evidence for the lncRNA-PVT1-5/miR-126/SLC7A5 axis, shedding new light on the mechanism of lung cancer.

2. Materials and methods

2.1. Specimens

In this study, 80 paired lung cancer and adjacent non-tumor specimens were collected from the Department of Thoracic Surgery, the Third Affiliated Hospital, Kunming Medical University (Kunming, Yunnan, China). The protocol used in this study was approved by the Human Investigation Ethics Committee of the School of Medicine, Yunnan University, with written informed consent obtained from all patients. All tissue samples were flash-frozen in liquid nitrogen immediately after collection and stored at −80 °C until use. Both tumor and non-tumor samples were confirmed by pathological examination. No patients received chemotherapy or radiotherapy prior to surgery.

2.2. Cell culture

The human lung cell line BESA-2B and four lung cancer cell lines (A549, H226, H1299 and H446) were purchased from American Type Culture Collection (ATCC) and were cultured in the supplemented media as ATCC recommendation. HEK293 cells were purchased from ATCC and were cultured in Dulbecco's Modified Eagle Medium (DMEM; Sigma-Aldrich, St. Louis, Mo., USA) media containing 10% (vol/vol) fetal bovine serum (FBS). Cells were maintained at 37 °C in a humidified atmosphere with 5% CO2.

2.3. RNA isolation and qRT-PCR

Total RNA was isolated from lung cancer tissues, adjacent non-tumor tissues and lung cancer cell lines using Trizol according to the manufacturer's instructions. Purified mRNA and lncRNA were detected by qRT-PCR assay. All primers were listed in Table 1. GAPDH was used as an internal control for normalization and quantification of lncRNA-PVT1-5 expression.

2.4. Luciferase assays

Luciferase reporter plasmid was constructed by cloning human lncRNA-PVT1-5 mRNA sequence into pMIR-Report construct (Ambion, Austin, USA). All the primers were listed in Table 1. Luciferase reporter assays were performed as followed protocol. HEK 293T cells plated in a 96-well plate were co-transfected with 50 nM miR-126 mimics or negative control oligonucleotides, 20 ng of firefly luciferase reporter and 10 ng of pRL-TK (Promega, USA) using the INTERFER in reagent (Polyplus-transfection, France). Cells were collected 36 h after transfection and analyzed using Dual-Luciferase Reporter Assay System (Promega).

2.5. Oligonucleotides and plasmids transfection

RNA oligos were chemically synthesized and purified by Genepharma Co. Ltd., (Shanghai, China). The sequences of lncRNA-PVT1-5 siRNA was: 5′- CUAUUAAGGGGAAACAAAATT -3' (sense) and 5′-UUUUGUUUCCCCUUAAUAGTT-3' (antisense), Control siRNA was: 5′-UUC UCC GAA CGU GUC ACG UTT-3' (sense), 5′-ACG UGA CAC GUU CGU AGA ATT-3' (antisense). Sequence of human miR-126 mimics was 5′-UCGUACCGUGAGUAAUAAUGCG-3'. Negative control oligonucleotides for miRNA was 5′-CAG UAC UUU UGU GUA GUA CAA-3'. Sequence of human miR-126 inhibitors was 5′-CGCAUUAUUACUCACGGUACGA-3'. Negative control oligonucleotides for miRNA was 5′-CAG UAC UUU UGU GUA GUA CAA-3'. The transfections were performed with INTERFER in reagent (Polyplus-transfection). The final concentration of miRNA mimics was 50 nM, the final concentration of miRNA inhibitors was 100 nM and the final concentration of siRNAs was 20 nM.

2.6. MTT assay

The *in vitro* growth of lung cancer cells was measured using the MTT assay. 5000 cells were seeded into each well of 96-well plates and transfected with lncRNA-PVT1-5 siRNA or negative control oligonucleotides at a final concentration of 50 nM respectively. On the day of harvest, 100 μL of spent medium was replaced with an equal volume of fresh medium containing MTT 0.5 mg/ml. Plates were incubated at 37 °C for 4 h, then the medium was replaced by 100 μLof DMSO (Sigma) and plates shaken at room temperature for 10 min. The absorbance was measured at 570 nm.

Please cite this article in press as: H. Li, et al., Long non-coding RNA PVT1-5 promotes cell proliferation by regulating miR-126/SLC7A5 axis in lung cancer, Biochemical and Biophysical Research Communications (2017), https://doi.org/10.1016/j.bbrc.2017.12.114