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Original article

LncRNA FEZF1-AS1 enhances epithelial-mesenchymal transition (EMT) through suppressing E-cadherin and regulating WNT pathway in non-small cell lung cancer (NSCLC)

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ABSTRACT

Objective: Recent discoveries verify that long non-coding RNAs (lncRNAs) are important functional regulators involved in non-small cell lung cancer (NSCLC) progression. However, long non-coding RNA FEZF1-AS1 was not been investigated in NSCLC so for.

Methods: We applied the quantitative real time polymerase chain reaction (qRT-PCR) assays to detect the expression of lncRNA FEZF1-AS1 in NSCLC tissues and adjacent normal tissues. Cell proliferation and invasion capacities were evaluated by MTT, colony formation, and cell invasion assays. Chromatin immunoprecipitation (ChIP) and RNA immunoprecipitation (RIP) methods demonstrated the association between lncRNA FEZF1-AS1 expression and E-cadherin. The relative protein expression levels were analyzed by western blot analysis.

Results: LncRNA FEZF1-AS1 was significantly up-regulated in NSCLC tissues compared with adjacent normal tissues. Higher lncRNA FEZF1-AS1 expression levels associated with lymph node metastasis, poor differentiation grade and advanced TNM stage. In vitro, we revealed that down-regulation of lncRNA FEZF1-AS1 inhibited cell proliferation and cell invasion capacities in NSCLC. Moreover, down-regulation of lncRNA FEZF1-AS1 suppressed cell epithelial-mesenchymal transition (EMT) process by increasing the expression of E-cadherin and ZO-1, whereas, decreasing the expression of Slug, Twist and Vimentin in NSCLC cells. Furthermore, we demonstrated lncRNA FEZF1-AS1 could epigenetically repress the expression of E-cadherin via binding with LSD1 and EZH2 in NSCLC cells. We also revealed that knockdown of lncRNA FEZF1-AS1 suppressed Wnt/β-catenin signaling in NSCLC.

Conclusion: These results demonstrated that lncRNA FEZF1-AS1 could function as a tumor promoting regulator in NSCLC, which may provide a target of treatment in NSCLC.

1. Introduction

Lung cancer is one of the most common causes of cancer related death of the worldwide [1]. The non-small cell lung cancer (NSCLC) is the main subtype of lung cancer and accounts for approximately 80–85% of new cases in lung cancer [2]. In spite of larger advances including surgery, and radio- or chemotherapy and molecular targeted therapies for NSCLC, the overall survival rate of patients with advanced disease still remains low [3,4]. Thus, to explore new target for NSCLC treatment is important.

Recent evidence shows that long noncoding RNAs (lncRNAs) are involved in the regulation of gene expression and cancer progression including NSCLC [5,6]. For example, over-expression of lncRNA SNHG1 contributes to progression of non-small cell lung cancer through inhibition of miR-101-3p and activation of WNT/β-catenin signaling pathway [7]. Long noncoding RNA LINC01186, regulated by TGF-β/SMAD3, inhibits cell migration and invasion through epithelial-mesenchymal-transition in lung cancer [8]. Higher lncRNA-HIT expression promotes migration and invasion of non-small cell lung cancer cells by association with ZEB1[9]. Long non-coding RNA UCA1 induces non-T790M acquired resistance to EGFR-TKIs by activating the AKT/mTOR pathway in EGFR-mutant non-small cell lung cancer [10].

Long non-coding RNA FEZF1-AS1 was reported to be up-regulated in some tumors, such as, long non-coding RNA FEZF1-AS1 facilitates cell proliferation and migration in colorectal carcinoma [11]. LncRNA FEZF1-AS1 represses p21 expression to promote gastric cancer

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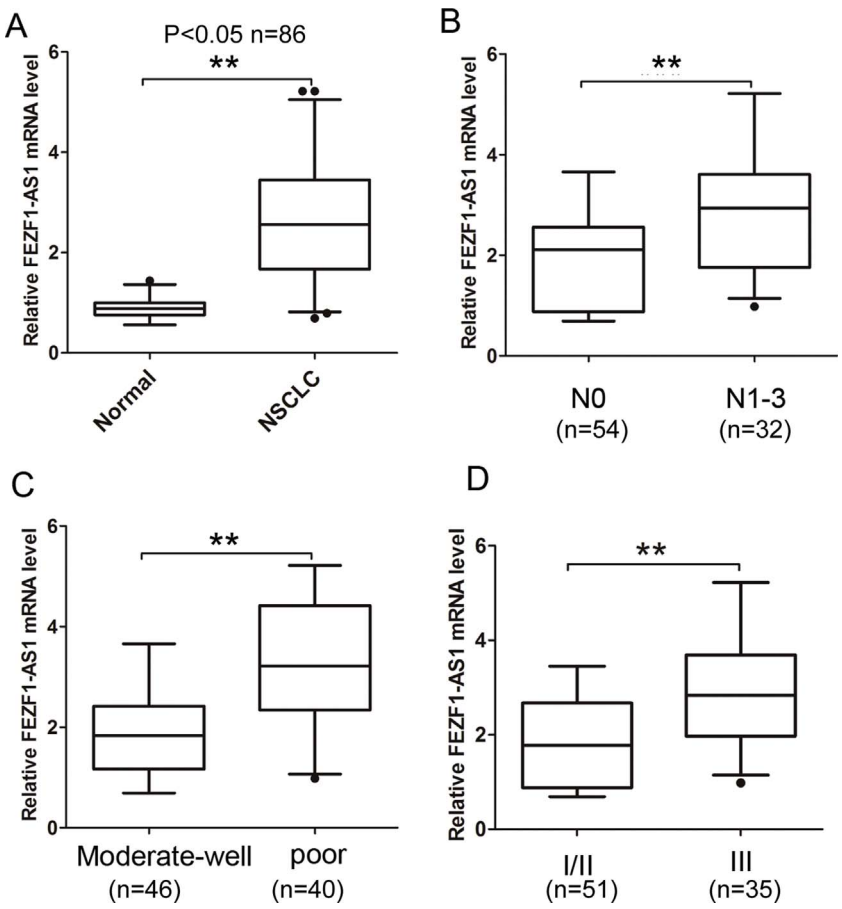

Fig. 1. The expression of lncRNA FEZF1-AS1 is higher in NSCLC tissues. (A) The relative expression of lncRNA FEZF1-AS1 was tested by qRT-PCR assays in NSCLC tissues (n = 86) and adjacent normal tissues (n = 86). (B)–(D) The association between lncRNA FEZF1-AS1 expression and lymph node metastasis, differentiation grade and TNM stage was showed in patients, **P < 0.05.

proliferation through LSD1-Mediated H3K4me2 demethylation [12]. However, in NSCLC development and progression, the understanding role of lncRNA FEZF1-AS1 is still limited. In the study, we demonstrated that lncRNA FEZF1-AS1 was up-regulated in NSCLC tissues compared with adjacent normal tissue. In vitro, inhibition of lncRNA FEZF1-AS1 inhibited cell proliferation, cell invasion and EMT process in NSCLC. Furthermore, we demonstrated lncRNA FEZF1-AS1 could epigenetically repress the expression of E-cadherin via binding with LSD1 and EZH2. We also revealed that lncRNA FEZF1-AS1 modulated Wnt/β-catenin signaling in NSCLC cells. Thus, lncRNA FEZF1-AS1 may be a potential target of NSCLC treatment.

2. Material and methods

2.1. Patients and clinical tissue samples

86 cases of matched tumor tissues and adjacent normal tissues were obtained from NSCLC patients who underwent radical surgery at the Affiliated Hospital of Shan dong University of Traditional Chinese Medicine from April 2010 to July 2013. Patients do not receive local or systemic treatments before surgery. The tissues were immediately frozen in liquid nitrogen and stored at −80 °C until for further analysis. Informed consent was obtained from all patients. The study was approved by Human Research Ethics Committee of Affiliated Hospital of Shang dong University of Traditional Chinese Medicine.

2.2. Cell culture and cell transfection

The NSCLC cell lines including A549, SPC-A1, H1299, H1975, PC9 and a human airway epithelial cells (16HBE) were purchased from the Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences (Shanghai, China). Cells were maintained in RPMI Medium 1640 basic media (GIBICO, Invitrogen, Carlsbad, CA, USA). 16HBE cells were curtured in DMEM (GIBICO, Invitrogen, Carlsbad, CA, USA). Cells were cultured using 10% foetal bovine plasma (FBS, HyClone, Camarillo, CA, USA) and were supplemented with a humidified incubator at 37 °C with 5% CO2. Cells were transfected using Lipofectamine 2000 (Life Technologies). The two si-RNA lncRNA FEZF1-AS1 oligos (si-FEZF1-AS1-1:5′-GGGTTTCTGCAGGAACTTTGA-3′andsi-FEZF1-AS1-2:5′-GCCTGATGTCTA ACAGAAAGG-3′) were purchased from Genechem Company, Shanghai, China.

2.3. Quantitative reverse transcriptase polymerase chain reaction (QRT-PCR) assay

The total RNA was derived from tissue samples and cells using Trizol Reagent (Invitrogen, Carlsbad, CA, USA). The cDNA was reversed using the Primer-Script™ One Step RT-PCR Kit (TAKARA, Dalian, China). Quantitative real-time PCR was performed using a SYBR Premix Ex Taq II kit (TAKARA) according to the manufacturer's instructions at ABI7500 system. The results were normalized to the GAPDH. The mRNA expression levels were determined using the comparative delta–delta CT method ($2^{-\Delta\Delta Ct}$). Primers of lncRNA FEZF1-AS1-F:5′-TTAGGAGGCTTGTTCTGTGT-3′andlncRNAFEZF1-AS1-R:5′-GCGCAGGTACTTAAGAAAGA-3′.GAPDH-F:5′-ACAGTCAGCCGCATCTTCT-3′andGAPDH-R:5′-GACAAGC-TTCCCGTTCTCAG-3′.

2.4. Cell proliferation and colony-formation assays

Cell proliferation was determined using a Cell Proliferation Reagent Kit I (MTT) (Roche Applied Science). Transfected cells were seeded into a 96-well plate and was detected at indicated time at 0, 24, 48, and 72 h. Cells at per well were added 20 μL MTT solution. Plates were