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Original article

Long non-coding RNA SNHG1 regulates zinc finger E-box binding homeobox 1 expression by interacting with TAp63 and promotes cell metastasis and invasion in Lung squamous cell carcinoma

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ABSTRACT

The long non-coding RNAs (lncRNAs) have been recently shown to participate in the progression of a variety of cancers. However, the biological role of lncRNAs and the underlying mechanisms in Lung squamous cell carcinoma (SCC) or lung adenocarcinoma (AD) remain unclear. Herein, we investigated expression of 5 lncRNAS (SNHG1, NCBP2-AS2, LINC01206, SOX2-OT and LINC01419) in SCC and AD tissues. SNHG1 was one of over-expressed lncRNAs in SCC tissues. Knockdown of SNHG1 significantly inhibited the proliferation, metastasis, invasive ability and induced apoptosis of SCC cells. Moreover, SNHG1 affected the expression of zinc finger E-box binding homeobox 1(ZEB1), which has also been observed to be up-regulated in SCC and to promote cell metastasis and invasion. Rather than direct interaction, SNHG1 regulated ZEB1expression by suppressing the activity of p63 TA isoform (TAp63), which is a repressor of ZEB1 and physically associates with SNHG1. Furthermore, SNHG1 promoted ZEB1 expression and promoted cell proliferation, metastasis, invasive but inhibited apoptosis of SCC cells via the TAp63/ZEB1 pathway. Taken together, our findings suggested that SNHG1 might play an oncogenic role in SCC through ZEB1 signaling pathway by inhibiting TAp63 and might serve as a valuable prognostic biomarker and therapeutic target for SCC patients.

1. Introduction

Lung cancer is the main cause of cancer related death in males and females worldwide. It is estimated to be responsible for nearly 1.6 million deaths worldwide one year or nearly 20% of cancer mortality as a whole [1,2]. Most lung cancers (~90%) are non-small cell lung cancers (NSCLCs), which comprise a number of subtypes driven by various activated oncogenes [3,4]. Lung squamous cell carcinoma (SCC) and lung adenocarcinoma (AD) are the primary histological classification of NSCLC. The differences between SCC and AD are not clear for molecular pathogenesis. Therefore, understanding the molecular pathogenesis and uncovering novel therapeutic targets of SCC or AD would facilitate early detection and improve the survival of patients.

During the past decade, large-scale sequencing efforts have revealed that a large fraction of the human noncoding genome is transcribed. The long non-coding RNAs (lncRNAs) are important new members of the ncRNA family. They greater than 200 nt, with limited or no protein-coding capacity. Altered lncRNAs levels have been observed in gastric cancer [5], colorectal cancer [6], renal cell carcinoma [7], and hepatocellular carcinoma [8], indicating that aberrant expression of certain lncRNAs contributes to carcinogenesis. LncRNAs recently have been known to participate in numerous biological processes, including modulation of apoptosis and migration, reprogramming stem cell pluripotency, and parental imprinting. These findings indicate that lncRNAs play a major role in the regulation of the eukaryotic genome [9–11]. However, although over a decade's research has led to considerable progress in understanding lncRNAs, the precise function of most remains unknown.

Here we firstly investigate expression level of five lncRNAs in SCC or AD samples, including SNHG1, NCBP2-AS2, LINC01206, SOX2-OT and LINC01419. These lncRNAs are reported in microarray data set GSE10245 from the Gene Expression Omnibus (GEO) and some of them are reported direct relate to cancers or cancer-related pathways [12–14]. Long non-coding RNA small nucleolar RNA host gene 1 (lncRNA SNHG1) was found one of over expression lncRNAs in SCC tissues compared to AD tissues in this study. Accumulating evidence shows that SNHG1 is over-expressed in diverse cancer such as hepatocellular carcinoma [15], prostate

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cancer [16], lymphoblastoid cells [17], non small cell lung cancer [18]. However, the biological role of SNHG1 and the underlying mechanisms in cancers has not yet been clarified.

We predicted that SNHG1 may interact with one p63 TA isoform (TAp63) which was known as a repressor of zinc finger E-box binding homeobox 1(ZEB1) [19] from the URL http://pridb.gdcb.iastate.edu/RPISeq/results.php. We next evaluated the effects of SNHG1 and ZEB1 on the metastasis and invasive ability of SCC cells and explored their potential relationship in SCC. The results showed that both SNHG1 and ZEB1 promote metastasis and invasion by SCC cells. SNHG1 enhanced ZEB1 expression by interacting with TAp63 and inhibiting its activity. Furthermore, the TAp63/ZEB1 pathway mediated the regulatory effect of SNHG1 on metastasis and invasion by SCC cells. Our data suggest an important role of SNHG1 in SCC metastasis and invasion.

2. Materials and methods

2.1. Tissue collection

In this study, we obtained 76 AD tissues, 62 SCC tissues and 60 non-tumor lung tissues from patients who underwent surgery at Shengjing hospital of China medical university between 2013 and 2015. All the specimens including cancer tissues were diagnosed with NSCLC (stages I, II, and III) based on histopathological evaluation. The clinicopathological characteristics of the SCC are summarized in Table 1. There were no local or systemic treatment conducted in these patients before surgery. All collected tissues were placed in liquid nitrogen immediately and stored at −80 °C until required. Our study was approved by the Research Ethics Committee of Shengjing Hospital of China Medical University.

2.2. RNA extraction and real-time PCR analysis

Total RNA was extracted from tissue samples or cells using the Trizol reagent (Invitrogen) following manufacturer's instructions.

Table 1
Correlation between SNHG1 expression and clinicopathological characteristics of SCC patients ($n = 62$).

Characteristics	SNHG1		*P*
	High expression cases	Low expression cases	Chi-squared test *P*-value
Age (years)			0.384
≤65	14	13	
>65	22	13	
Gender			0.973
Male	22	16	
Female	14	10	
Smoking history			0.934
Smokers	19	14	
Non-smokers	17	12	
Tumor size			0.003*
≤5 cm	14	20	
>5 cm	22	6	
TNM stage			0.032*
I + II	15	18	
III	21	8	
Lymph node metastasis			0.039*
Negative	14	17	
Positive	22	9	

* Overall $P < 0.05$.

The cDNA was generated using the Reverse Transcription Kit (Takara, China). The real-time PCR was performed using Takara SYBR Premix Ex TaqII (RR820) according to the manufacturer's protocol on the Applied Biosystems 7500 Fast Real-time PCR System (Applied Biosystems, Foster City, USA). The primers were as follows: for SNHG1, 5′-TTCTACTGCTCGTGGATT-3′ and 5′-CATAAAGATGGGTCTTGTC-3′; for NCBP2-AS2, 5′-CCCGCTCCGTTGAAGTCT-3′ and 5′-TCTCCGTAGGTGCCGTGT-3′; for LINC01206, 5′-ATCCAAACGACCATCAGC-3′ and 5′-GAGCCTTTCCCTTCTTCA-3′; for SOX2-OT, 5′-GCCAGGCAACAGCCATAT-3′ and 5′-TGCAAGCCAGGTCACAAT-3′; for LINC01419, 5′-GAGGGTCCACGGCTTCAT-3′ and 5′-CGGTTCTCCTGCTGGTTG-3′; for TAp63 5′-TGCAGCATTGTCAGGATC-3′ and 5′-TGTAGAGGCACTGCTTGG-3′; for ZEB1 5′-TGCTGATGTGGCTTTATG-3′ and 5′-CTCTTTCACTGCTCCTCC-3′. Dissociation curves were generated to ensure that a single and specific product was amplified. Cycle threshold values (Ct) were analyzed by the SDS2.4 software and GAPDH was as an internal control.

2.3. Western blot analysis

Total proteins were extracted from SCC tissues, non-tumor tissues and SCC cells. Then we determined the concentrations of proteins. Proteins were transferred to PVDF membranes (Bio-Rad, USA) at 4 °C. The 5% (wt/vol) nonfat milk was used for membranes blocked. The membranes were incubated with primary antibody specific for TAp63 (legend, USA), ZEB1 (Cell Signaling Technology, USA), GAPDH (Shanghai Kangcheng, China) in 1% (wt/vol) BSA overnight at 4 °C and incubated with secondary antibodies (Santa Cruz Biotechnology, USA) at room temperature for 2 h. Luminescent assay was carried out on an ECL instrument (Gene Co, China).

2.4. Cell culture

H-266 and SK-MES-1 SCC cell lines were purchased from Shanghai cell bank of Chinese academy of sciences and cultured in RPMI 1640 medium containing 10% (v/v) heat-inactivated fetal bovine serum (FBS, Gibco, Grand Island, NY, USA), 50 units/mL penicillin, and 100 μg/mL streptomycin (Invitrogen, Carlsbad, CA, USA) at 37 °C in an incubator containing humidified air with 5% (v/v) CO_2.

2.5. RNA interference

Two kinds of knockdown siRNAs were used: for SNHG1, 5′-GGACAACCUAGCUGUUGAATT-3′ and 5′-UUCAACAGCUAGGUUGUCCTT-3′ (GenePharma, Shanghai, China), for TAp63, 5′-CCAGAGCACACAGACAAAUTT-3′ and 5′-AUUUGUCUGUGUGCUCUGGTT-3′ (GenePharma, Shanghai, China). Negative control siRNAs were used as a control for unspecific side effects. H-266 and SK-MES-1 Cells were transfected using the Lipofectamine 2000 reagent (Life technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. Cytoplasmic RNA was extracted from H-266 and SK-MES-1 Cells 48 h after transfection using PARIS™ Kit (ThermoFisher, USA) and was prepared for real-time PCR to determine the efficiency of gene expression ablation.

2.6. Cell proliferation assay

Cell proliferation was measured by the Cell Counting Kit-8 system (CCK-8, Dojindo, Japan) according to the manufacturer's instructions. Briefly, H-266 or SK-MES-1 cells were transfected with SNHG1 siRNA respectively. 24 h after transfection, cells were transferred into 96-well plates (BD Bioscience, USA) with density of 2 × 103 per well. The proliferation of transfected cells was