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LncRNA NBAT-1 is down-regulated in lung cancer and influences cell proliferation, apoptosis and cell cycle

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Abstract. – OBJECTIVE: To explore the expression and role of lncRNA NBAT-1 in lung cancer.

PATIENTS AND METHODS: LncRNA NBAT-1 expression in lung cancer tissues and adjacent areas was detected via reverse transcriptase-polymerase chain reaction (RT-PCR). RAC1 protein was analyzed via Western blotting assay. Cell counting kit-8 (CCK-8) and flow cytometry were used to evaluate cell proliferation and apoptosis as well as cell cycle.

RESULTS: The expression level of lncRNA NBAT-1 in cancer specimen was remarkably lower than that in adjacent areas. Furthermore, the low expression of lncRNA NBAT-1 had a significant correlation with patient's tumor size, differentiation degree of tumor cells and lymph node metastasis. The overexpression of lncRNA NBAT-1 could inhibit the proliferation and cell cycle, promote the apoptosis of A549 cells, and down-regulate the expression level of RAC1.

CONCLUSIONS: The low expression of lncRNA NBAT-1 is involved in the progression of lung cancer.

Key Words:
lncRNA NBAT-1, Lung cancer, Proliferation, Apoptosis.

Introduction

Lung cancer is one of the most highly malignant cancers in the world, with more than 1.8 million new cases annually worldwide[1]. There are many pathological types of lung cancer, of which about 85% of lung cancer patients with non-small cell lung cancer (NSCLC); the 5-year survival rate was only 10%[2]. Despite the remarkable progress made in the clinical diagnosis and treatment of lung cancer in recent years, its prognosis is still not optimistic. Therefore, further research on the related molecules of the occurrence and development of NSCLC is quite necessary[3].

The human genome includes about 20,000 protein-coding genes, yet these encoding genes make up only about 2% of the total. More than 90% of the transcripts are non-coding RNAs. Long non-coding RNAs (lncRNAs) are a class of non-coding RNA molecules with transcripts longer than 200 nucleotides[4]. In recent years, more and more evidence has proved that lncRNA plays an indispensable role in tumor cell proliferation, growth and apoptosis. It participates in the development and progression of tumors by interfering with the major biological processes of tumorigenesis[5]. Studies[6,7] have confirmed that aberrant lncRNA expression is associated with a variety of cancers and can lead to a variety of diseases including lung cancer, although the exact mechanism is unclear. In this investigation, by detecting the expression of lncRNA NBAT-1 in 69 NSCLC pathological specimens, the correlation between the expression of lncRNA NBAT-1 and the clinical features of NSCLC was analyzed, thus providing new biomarkers for the prediction and diagnosis of patients with NSCLC.

Patients and Methods

Samples

A total of 69 samples of NSCLC tissues and cancer-adjacent lung tissues were obtained from patients who were diagnosed with NSCLC and underwent surgery in The Second Affiliated Hospital of Dalian Medical University (Dalian, China). All patients underwent no local or systemic chemoradiation prior to surgery. The lung cancer tissues were definitely diagnosed with NSCLC by pathology. Immediately after all samples had been removed, they were frozen in liquid nitrogen and stored in a refrigerator at -80°C. The study was approved by the Ethics Committee of our Institution. All the patients signed the informed consent. NSCLC adenocarcinoma cell line A549 cells were cultured in Roswell Park Memorial In-

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stitute-1640 (RPMI-1640) medium (10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/L streptomycin) and incubated at 37°C with 5% CO_2.

RNA Extraction and Quantitative Polymerase Chain Reaction (qPCR) Assays

RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Total RNA was prepared into 20 μL cDNA using Prime-Script kit (TaKaRa, Dalian, China). Real-time PCR reaction was conducted according to the instructions of STBR Premix Ex Taq (TaKaRa, Dalian, China). The cDNA was taken for qRT-PCR.

Plasmid Construction and Cell Transfection

The lncRNA NBAT-1 sequence was synthesized and subcloned into pcDNA3.1 vector (Kingsbury, Nanjing, China). A549 cells were transfected with empty pcDNA3.1 vector and lncRNA NBAT1 pcDNA3.1 vector, respectively, according to the instruction manual. Cells transfected with empty pcDNA3.1 vector were identified as the control group.

Cell Counting Kit-8 (CCK-8) Analysis

A549 cells transfected with pcDNA3.1-lncRNA NBAT-1 and pcDNA3.1 empty plasmid were cultured in 96-well plates (3,000 to 5,000 cells per well) with 5 replicate wells in each group. After the incubation for 24 h, 36 h, 48 h and 72 h, CCK-8 reagent (10 μL) was added to each well. Cell proliferation activity (OD value) was measured according to the instruction manual.

Flow Cytometry

Cell apoptosis was evaluated with an Annexin V assay kit (Miltenyi Biotec., Bergisch Gladbach, Germany). Annexin V-fluorescein isothiocyanate (FITC) and propidium iodide (PI) were added into cells according to the instructions. Cell cycle was also detected by flow cytometry assay. Cells were stained using the BD Cycletest Plus DNA Reagent Kit (BD Biosciences, Shanghai, China). The percentage of cells in subG0/G1, S or G2/M phase was compared. The results were measured by the flow cytometry with FACS Calibur (BD Bioscience, San Jose, CA, USA).

Western Blotting

The protein was extracted from A549 cells. The bicinchoninic acid (BCA) protein concentration kit was used to determine the protein concentration. The protein was subjected to 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to polyvinylidene difluoride (PVDF) membrane and then incubated with 1:500 diluted RAC1 antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA). GAPDH was taken as an internal reference. The experiment was repeated 3 times independently.

Statistical Analysis

LncRNA NBAT-1 expression in lung cancer and cancer-adjacent tissues was calculated with Statistical Product and Service Solutions (SPSS) 17.0 software (SPSS Inc., Chicago, IL, USA). The correlation of the expression of lncRNA NBAT-1 with the clinicopathological parameters was analyzed by x^2-test. p-values < 0.05 were considered statistically significant.

Results

LncRNA NBAT-1 Was Reduced in Lung Cancer Tissues

In 69 NSCLC patients, qRT-PCR was used to detect the expression level of lncRNA NBAT-1 in lung cancer tissues and cancer-adjacent tissues, respectively. The expression level of lncRNA NBAT-1 in lung cancer tissues was significantly lower than that in cancer-adjacent tissues ($p<0.05$, Figure 1). Furthermore, the plasmid pcDNA3.1-lncRNA NBAT-1 or empty plasmid pcDNA3.1 was transfected into A549 cells. The detection of lncRNA NBAT-1 expression was performed via qRT-PCR, which revealed that the expression of lncRNA NBAT-1 in cells transfected with the recombinant plasmid pcDNA3.1-lncRNA NBAT-1 was significantly higher than that in cells transfected with the empty plasmid (Figure 2).

The Expression of lncRNA NBAT-1 was Related to the Clinicopathological Features

According to the expression of lncRNA NBAT-1 in NSCLC tissues, the correlation of lncRNA NBAT-1 expression with clinicopathological features was analyzed. The data suggested that the expression level of lncRNA NBAT-1 in NSCLC has significant correlation with patient's tumor size, differentiation degree of tumor cells and lymph node metastasis ($p<0.05$), but not with patient's sex, age and smoking status ($p>0.05$, Table I).

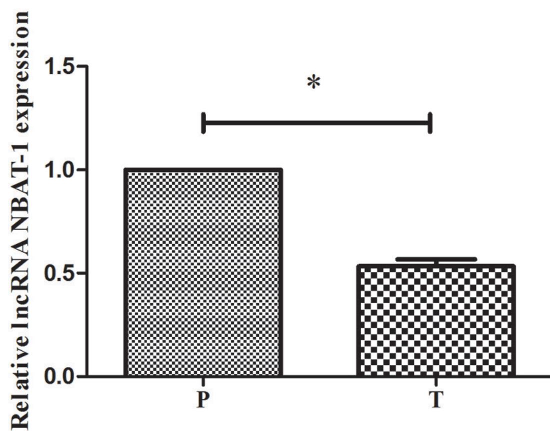

Figure 1. The relative lncRNA NBAT-1 expression is detected between lung cancer tissues and the adjacent tissues by RT-PCR method. *$p<0.05$.

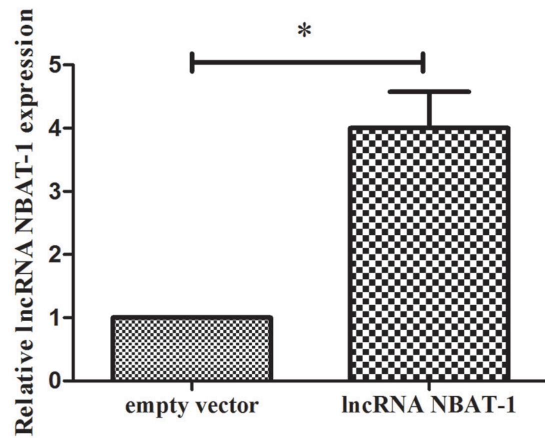

Figure 2. The relative lncRNA NBAT-1 expression is detected between cells transfected with empty vector and lncRNA NBAT1. *$p<0.05$.

Up-regulated lncRNA NBAT-1 Inhibited Cell Proliferation and Cell Cycle, but Promoted Cell Apoptosis

In addition, CCK-8 assay was used to investigate the influence of lncRNA NBAT-1 on cell proliferation. The experimental results showed that the proliferation of A549 cells was inhibited in cells overexpressing lncRNA NBAT-1 related to the control group (Figure 3A). Furthermore, the flow cytometry analysis showed that the up-regulation of lncRNA NBAT-1 inhibited cell cycle, but promoted cells apoptosis (Figure 3B and C).

Overexpressed lncRNA NBAT-1 Could Down-regulate RAC1 Protein Expression in A549 Cells

Finally, Western blotting was conducted to analyze the expression of RAC1 protein based on the overexpression of lncRNA NBAT-1. The data presented that the expression level of RAC1 in A549 cells overexpressing lncRNA NBAT-1 was significantly lower than that in the control group (Figure 4).

Table I. The relationship between aberrant lncRNA NBAT-1 expression and clinical features of NSCLC patients.

Features	Patients	lncRNA NBAT-1		p
		Low	High	
Total	69	35	34	
Age (years)				0.900
≤ 50	33	17	16	
> 50	36	18	18	
Gender				0.729
Male	29	14	15	
Female	40	21	19	
Smoking status				0.552
Yes	32	15	17	
No	37	20	17	
Differentiation				0.019
Poor/median	41	16	25	
High	28	19	9	
Tumor size				0.002
< 3 cm	44	16	28	
> 3 cm	25	19	6	
Lymph node metastasis				0.009
No	42	16	26	
Yes	27	19	8	

*$p<0.05$.

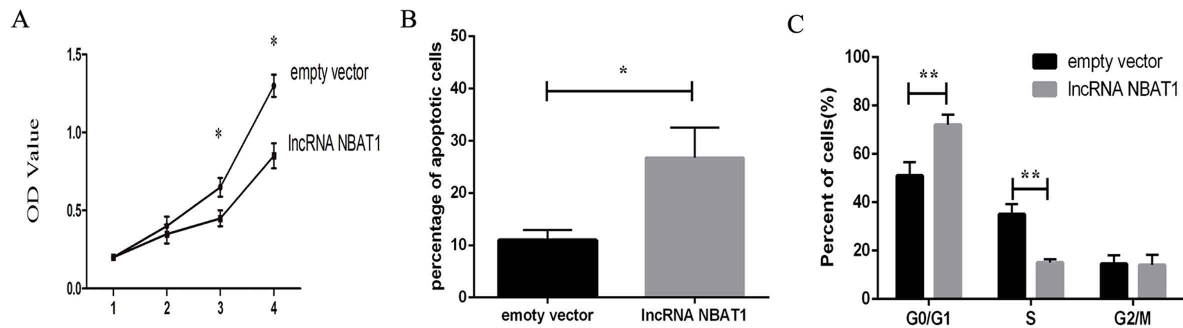

Figure 3. ***A,*** Cells proliferation was detected by CCK-8 assay. OD value is examined between cells transfected with empty vector and lncRNA NBAT-1. *$p<0.05$. 1: 24 h; 2: 36 h; 3: 48 h; 4: 72 h. ***B*** and ***C,*** Flow cytometry methods were used to evaluate cells apoptosis and cell cycle.

Discussion

Lung cancer is one of the leading causes of cancer death in the world[8,9]. In recent years, radiotherapy, chemotherapy and molecular targeted therapy for NSCLC have made remarkable progress, but the 5-year survival rate of patients with NSCLC is still poor due to the limitations of early diagnosis[10]. At present, many NSCLC-related lncRNAs have been found[11-13], but the potential molecular mechanism of lncRNAs in NSCLC still needs further study. Therefore, further understanding of the mechanism of lung cancer can provide a better basis for the early diagnosis of NSCLC and provide novel treatment methods. lncRNAs are a class of RNAs that are more than 200 bases in length and lack a complete open reading frame, with little or no protein-coding ability. Researches have shown that lncRNA plays an essential role in cell physiology and pathology, involved in the development and progression of a variety of diseases including tumors[14], and associated with tumor lymphatic metastasis, distant metastasis and prognosis[15]. In this work, the expression level of lncRNA NBAT-1 in NSCLC tissues and adjacent tissues was detected so as to explore the effect of its abnormal expression on NSCLC cell proliferation. The experimental results revealed that the expression level of lncRNA NBAT-1 in NSCLC tissues was lower than that in cancer-adjacent tissues. Overexpression of lncRNA NBAT-1 could inhibit NSCLC cell proliferation and cell cycle, but promoted cell apoptosis. Moreover, as a small molecule G protein, RAC1 plays an essential role in angiogenesis and proliferation of tumors. Studies[16] have shown that RAC1 can inhibit apoptosis and promote cell proliferation. This investigation demonstrated that in A549 cells overexpressing lncRNA NBAT-1, the expression of RAC1 was significantly decreased, and cancer cell proliferative ability was inhibited. We demonstrated that the low expression of lncRNA NBAT-1 in NSCLC was significantly correlated with the tumor size and lymph node metastasis. LncRNA NBAT-1 played a crucial role in the development of NSCLC. The overexpression of lncRNA NBAT-1 could not only significantly inhibit the cell proliferation and cell cycle, but also promote the apoptosis of NSCLC cells via downregulating RAC1. These results suggested that lncRNA NBAT-1 plays an import-

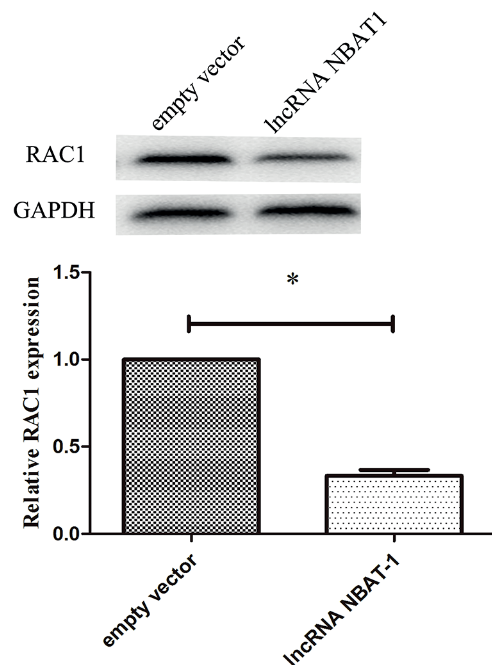

Figure 4. The relative RAC1 expression is detected by Western blotting method in cells transfected with empty vector and lncRNA NBAT-1. *$p<0.05$.

ant role in the progression of NSCLC. However, its exact molecular mechanism remains to be further explored.

Conclusions

We showed that lncRNA NBAT-1 plays an essential role in the progression of lung cancer and provides a good supplement for further exploring the pathogenesis of NSCLC. It can be used as a new biomarker for the detection and treatment of NSCLC in the future.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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