European Review for Medical and Pharmacological Sciences 2016; 20: 3031-3034

Over-expression of lncRNA SBF2-AS1 is associated with advanced tumor progression and poor prognosis in patients with non-small cell lung cancer

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Abstract. – OBJECTIVE: Emerging evidence suggest that long non-coding RNAs (lncRNAs) may play important roles in human cancers. The aim of this study was to investigate the expression of SBF2-AS1 in non small cell lung cancer (NSCLC) and its correlation with clinicopathological features and prognosis in NSCLC.

PATIENTS AND METHODS: The expression of lncRNA SBF2-AS1 was measured in 174 NSCLC samples and their matched non-tumor tissues by using RT-PCR. Association of SBF2-AS1 expression with clinicopathological features was analyzed in NSCLC. Kaplan–Meier analysis was performed to evaluate the overall survival of NSCLC patients.

RESULTS: The expression of SBF2-AS1 was higher in NSCLC tissues compared with adjacent non-tumor tissues ($p < 0.01$). Additionally, high expression level of SBF2-AS1 was significantly associated with NSCLC histological grade, and lymph node metastasis. Further¬more, a higher SBF2-AS1 expression was demonstrated to be associated with poor overall survival times in NSCLC patients ($p < 0.001$). Multivariate analysis suggested that SBF2-AS1 expression was an independent prognostic factor for overall survival of patients with NSCLC ($p = 0.013$).

CONCLUSIONS: Our data suggest that SBF2-AS1 could represent a novel prognostic marker and potential therapeutic target in patients with NSCLC.

Key Words
Long non-coding RNA, SBF2-AS1, Non small cell lung cancer, Prognosis.

Introduction

Lung cancer is the most common cause of cancer-associated deaths worldwide, especially for male[1]. Approximately 85% of all lung cancer cases are categorized as non-small cell lung cancer (NSCLC)[2]. Characteristically, NSCLC cells can invade local tissue and spread to distant sites even in the early stage, even though there has been a great improvement on traditional treatments, such as surgery, supplemented with radiotherapy and chemotherapy. The overall 5-year survival rate of lung cancer patients remains poor[3]. Therefore, it is of great significance to explore prognostic markers for early detection and targeted treatment of NSCLC.

The long non-coding RNAs (lncRNAs) are a class of non-coding RNA over 200 nucleotides with no protein-coding potential[4,5]. lncRNAs was disregarded as transcriptional noise. But more and more evidence shows that lncRNAs function as new regulators in the cancer paradigm[6,7]. Recent reports indicate that lncRNAs contribute to the development and progression of multiple cancers. Abnormal expression of lncRNAs has been observed in different tumors, including lung cancer, breast cancer, esophageal squamous carcinoma, gallbladder carcinoma[8-11]. Indeed, lncRNAs could act as oncogenes or tumor suppressors.

SBF2-AS1 is a 2708 nt antisense RNA to SBF2, which is located at the 11p15.1 locus. As a newly found lncRNA, the prognosis value of SBF2-AS1

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in NSCLC have not been reported. In the present report, we examine the expression level of SBF2-AS1 in NSCLC and adjacent non-tumor tissues, as well as explore its association with overall survival of patients.

Patients and Methods

Patients and tissue samples

A total of 174 primary NSCLC patients were obtained from Department of Surgery, Linyi People's Hospital during 2008-2014. None of the patients received preoperative chemotherapy or radiotherapy. Clinical and pathological variables analyzed are shown in Table I. After surgical resection, tumor specimens and adjacent normal renal tissues were collected and stored in liquid nitrogen until use. All patients were recruited in accordance with Institutional Ethics Guidelines. Written informed consent was obtained from all subjects.

Quantitative real-time RT-PCR.

Total RNA was extracted from cancerous/non-cancerous specimens using TRIzol® reagent (Invitrogen, Carlsbad, CA, USA). RNA was reverse transcribed using SuperScript First Strand cDNA System (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The reverse transcription was performed at 37°C for 15 min, then 85°C for 5 s. Each sample was run in triplicate for analysis. The relative expression fold change of mRNAs was calculated by the $2^{-\Delta\Delta CT}$ method.

Statistical Analysis

All statistical analyses were performed using SPSS 20.0 statistical software (SPSS, Inc., Chicago, IL, USA). The chi-square test was used to assess SBF2-AS1 expression with respect to clinicopathological parameters. Survival curves were obtained by using the Kaplan-Meier method and compared by using the log-rank test. A multivariate Cox regression was performed to adjust for other covariates. A value of $p < 0.05$ was considered statistically significant.

Results

Expression of SBF2-AS1 was prominently upregulated in NSCLC samples

We examined SBF2-AS1 expression level in 174 paired NSCLC tissues and adjacent non-tumor tissues by qRT-PCR, and normalized to GAPDH. Our results showed that the SBF2-AS1level was significantly increased in NSCLC tissues compared with adjacent non-tumor tissues ($p < 0.01$, Figure 1).

Table I. Correlation between SBF2-AS1 expression and clinicopathologic features in 174 patients with NSCLC-

	lncRNA SBF2-AS1			
Variable	Number	High	Low	*p*-value
Age (years)				
<55	107	48	59	0.709
≥55	67	32	35	
Gender				
Male	97	44	53	0.855
Female	77	36	41	
Tumor size (cm)				
<3	77	39	38	0.271
≥3	97	41	56	
Histology				
Adeno	64	33	31	0.259
Squamous	110	47	63	
Histological grade				
I	91	31	60	0.001
II-III	83	49	34	
Lymph nodes metastasis				
No	87	29	58	0.001
Yes	87	51	36	

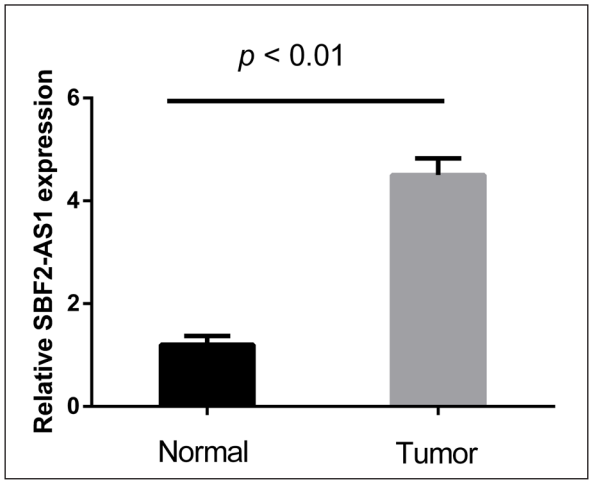

Figure 1. Over-expression of SBF2-AS1 in NSCLC. Higher SBF2-AS1 expression level was observed in NSCLC compared to that in the adjacent non-cancerous lung ($p < 0.01$).

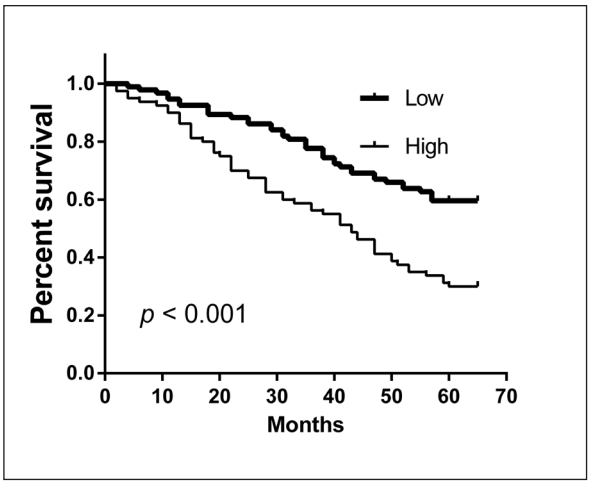

Figure 2. The Kaplan-Meier survival curve for the implication of SBF2-AS1 expression in NSCLC patients.

Relationship between SBF2-AS1 expression and clinic pathological factors in patients with NSCLC

The median value of SBF2-AS1 expression levels in NSCLC tissues was chosen as a cutoff value and used to assign the 174 patients with EOC to the high HOTAIR group or the low HOTAIR group. As shown in Table I, the SBF2-AS1 level was associated with histological grade ($p < 0.01$), and lymph node metastasis ($p < 0.01$). However, lncRNA SBF2-AS1 expression was not associated significantly with age, gender, tumor size, histology (all $p > 0.05$).

Low expression levels of SBF2-AS1 was correlated with unfavorable survival in NSCLC

To investigate further the correlations of SBF2-AS1 expression with survival of NSCLC patients, we measured the association between the levels of lncRNA SBF2-AS1 expression and survival rate of patients using Kaplan-Meier analysis with the log-rank test. Our data showed that lncRNA SBF2-AS1 expression was significantly associated with NSCLC patients' OS ($p < 0.001$, Figure 2). Moreover, The multivariate Cox regression analysis showed that SBF2-AS1 expression was an independent prognostic factor for overall survival ($p = 0.013$, Table II).

Discussion

Identification of early disease indicators for prognosis and efficacious treatment is urgently required. To date, most studies are focusing on dysregulated protein coding genes to identify potential oncogenes and tumor suppressors[12,13]. Accumulate evidence informed that lncRNAs can be considered as a new diagnostic and therapeutic gold mine in cancer[14]. Furthermore, researches show that lncRNAs may function as a potential prognostic factor in patients with cancer. In the present study, our attention focuses on SBF2-AS1

Many authors informed that the expression levels of lncRNAs are dysregulated in different kinds of tumors, including NSCLC. For example, Zeng

Table II. Prognostic factors in Cox proportional hazards model.

	Univariate analysis			Multivariate analysis		
Variable	**Risk ratio**	**95% CI**	***p***	**Risk Ratio**	**95% CI**	***p***
Age (years)						
≥55 vs. <55	0.823	0.564-1.772	0.496			
Gender						
Male vs. Female	1.478	0.729-2.519	2.239			
Tumor size						
≥3 cm vs. <3 cm	1.336	0.515-2.893	0.391			
Histology						
Adeno vs. Squamous	0.771	0.467-1.571	0.214			
Histological grade						
II, III vs. I	2.991	1.237-7.655	0.016	2.337	1.159-6.331	0.019
Lymph nodes metastasis						
Yes vs. No	3.445	2.113-8.744	0.005	3.037	1.672-8.339	0.008
SBF2-AS1						
low vs. high	2.464	1.358-6.672	0.003	2.341	1.236-5.881	0.013

et al[15] showed that AFAP1-AS1 is significantly upregulated in lung cancer. AFAP1-AS1 knockdown significantly inhibited the cell invasive and migration capability in the lung cancer cells. Zhang et al[16] found that TUG1 was downregulated and associated with the cell proliferation of NSCLC through being regulated by P53. Yang et al[17] showed that the increased expression of the lncRNA PVT1 promoted tumorigenesis in non-small cell lung cancer. Recently, Lv et al[18] firstly reported that lncRNA SBF2-AS1 is upregulated in NSCLC, and could promote proliferation of NSCLC cells *in vitro* and *in vivo*. So, those results revealed that SBF2-AS1 may function as a tumor promoter.

In the present work, our data showed that SBF2-AS1 expression was significantly higher in NSCLC tissues compared with that in adjacent normal tissues. We also found that the relative expression level of SBF2-AS1 was associated with histological grade and lymph nodes metastasis. Furthermore, Kaplan-Meier analysis with the log-rank test indicated that patients with NSCLC had significantly shorter overall survival.

Multivariate analysis confirmed that SBF2-AS1 expression was a poor independent prognostic predictor for NSCLC patients.

Conclusions

SBF2-AS1 might play an oncogenic role in NSCLC and is a poor prognostic factor. Large well-designed studies with diverse populations and functional evaluations are warranted to confirm and extend our findings.

Conflict of Interests

The authors declare no conflicts of interest.

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