

Original Article

Increased expression of lncRNA ZEB1-AS1 in non-small cell lung cancer is associated with poor prognosis

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Abstract: Background: Dysregulated long non-coding RNAs (lncRNAs) play critical roles in tumorigenesis and tumor progression. The purpose of this study was to investigate the relationship between lncRNA ZEB1-AS1 expression and non-small cell lung cancer (NSCLC) clinicopathological characteristics and prognosis. Methods: Expression levels of lncRNA ZEB1-AS1 in 183 NSCLC specimens were determined by quantitative real-time PCR (qRT-PCR). To clarify the clinical significance of lncRNA ZEB1-AS1 in NSCLC, we further explored the relationship between lncRNA ZEB1-AS1 expression and overall survival (OS). Results: In the present study, we found that lncRNA ZEB1-AS1 was upregulated in NSCLC tissues compared to adjacent non-tumor tissues. In addition, upregulated lncRNA ZEB1-AS1 expression was significantly associated with lymph node metastasis and TNM stage ($P < 0.05$). Furthermore, patients with increased expression of lncRNA ZEB1-AS1 had poor OS (HR=3.202, 95% CI=2.018-5.078, $P < 0.001$). Multivariate Cox proportional hazards model analysis demonstrated that high lncRNA ZEB1-AS1 expression was an independent poor prognostic factor for NSCLC patients. Conclusion: Our study suggests that increased expression of lncRNA ZEB1-AS1 is related to adverse prognosis of NSCLC and may be a new prognostic biomarker and potential therapeutic target for NSCLC intervention.

Keywords: NSCLC, lncRNA ZEB1-AS1, prognosis

Introduction

Lung cancer is the leading cause of cancer-related deaths in men and the second leading cause in women, worldwide [1]. The mortality rate of lung cancer is almost 90%. It is estimated that 1.8 million people are diagnosed with lung cancer, yearly, and 1.6 million die of the disease [2]. Lung cancer is divided into two groups by pathologists: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) [3]. NSCLC accounts for 85% of lung cancers [3]. Considerable progress has been made in research, screening, and therapy in NSCLC [1]. The prognosis is still unfavorable, however, and overall 5-year survival rates vary from 4-17% depending on stage and regional differences [4].

Long non-coding RNAs (lncRNAs) are clusters of RNA that have not been translated to protein and with lengths of more than 200 nucleo-

tides. Increasing evidence has been found that lncRNAs widely take part in regulation of gene expression by binding to and targeting chromatin regulators, directly interacting with chromatin, and acting as enhancers [5-7]. Also, lncRNAs have been found to function as decoys, scaffolds, and guides in gene expression regulation [6, 7]. Furthermore, lncRNAs not only are downstream targets of chemokine and hormonal pathways but also regulate cell signal pathways such as AKT, Notch, p53, and NF- κ B [5, 8]. For example, lncRNA AKO23948 functionally interacts with DHX9 and p85 and positively regulates AKT activity in breast cancer [9]. Therefore, lncRNAs take part in multiple processes of cancers such as angiogenesis, viability, proliferation, motility, and immortality [5]. lncRNA LUNAR1 is a downstream target of Notch1 which can upregulate insulin-like growth factor 1 receptor expression and signaling and promote T-cell acute lymphoblastic leukemia cell proliferation [10]. lncRNA lncTCF7, which is

Table 1. Correlation between lncRNA ZEB1-AS1 expression and clinicopathological factors of NSCLC patients

Features	ZEB1-AS1		χ ²	P
	Low	High		
Gender			1.759	0.185
Male	60	76		
Female	26	21		
Age			1.017	0.313
≥60	61	62		
<60	25	35		
Histological classification			0.000	0.999
Squamous	39	44		
Adenocarcinoma	47	53		
Differentiation			6.160	0.013
High/Middle	43	31		
Low	43	66		
Tumor size			1.854	0.173
<5 cm	33	28		
≥5 cm	53	69		
T Stage			2.747	0.097
I/II	46	40		
III	40	57		
N stage			1.625	0.202
No	69	70		
Yes	17	27		
TNM Stage			14.971	<0.001
I	60	40		
II/III	26	57		

highly expressed in hepatocellular carcinoma (HCC) and liver cancer stem cells, can recruit the SWI/SNF complex to the promoter of TCF7 and regulate its expression, resulting in activation of Wnt signaling and promoting liver CSC self-renewal and tumor propagation [11]. lncRNA-ATB can be induced by transforming growth factor β in HCC cells, promoting epithelial to mesenchymal transition (EMT), cellular invasion, and organ colonization [12]. These demonstrate that lncRNAs may be a new class of biomarkers and therapeutic targets of cancers.

lncRNA ZEB1-AS1 is the antisense RNA of Zinc finger E-box-binding homeobox1 (ZEB1). Its expression and biological role in NSCLC development and progression remains unknown. In the present study, we investigated expression levels of lncRNA ZEB1-AS1 in NSCLC tissues and explored association between its expres-

sion and clinicopathologic characteristics of patients with NSCLC.

Patients and methods

Patients and tissue samples

In this study, a total of 183 patients, histopathologically diagnosed with NSCLC, were enrolled from the Affiliated Hospital of Jiangnan University. None of the patients had received chemotherapy, radiotherapy, or any other treatments before surgery. Additionally, 85 adjacent non-cancerous lung tissues were collected as controls. This study was approved by the Ethical Committee of the hospital and written informed consent of tissue use in research was provided in advance by all participants. Detailed clinicopathological characteristics of patients are shown in **Table 1**. 5-year follow up was performed using the telephone or outpatient services. Patients that died from other diseases or unexpected events were excluded from this study.

RT-PCR

Total RNA samples were isolated using RNAiso Plus (TaKaRa, Japan) and reverse transcription was conducted by PrimeScript™ II 1st Strand cDNA Synthesis (TaKaRa, Japan), according to manufacturer instructions. Realtime PCR (RT-PCR) was conducted using SYBR® Premix Ex Taq™ II (TaKaRa, Japan). Using GAPDH as internal control, sequences of primers for lncRNA ZEB1-AS1 and GAPDH were as follows: lncRNA ZEB1-AS1, forward-5'-GGAGTTGAAAGGGACGCC-3', and reverse-5'-GCAAGCGGAACCTCTAGCC-3'; GAPDH, forward-5'-CTCCTCCGGGTGATGCTTTT-3', and reverse-5'-ATGAAGGGGTCATTGATGGCA-3'. All samples were detected in triplicate and relative mRNA quantification of lncRNA ZEB1-AS1 expression was calculated using 2^{-ΔΔCt} method.

Statistics

All statistical analysis were performed with IBM SPSS statistics package 22.0 software and expression data of lncRNA ZEB1-AS1 are expressed as mean ± standard deviation (SD). Student's t test was carried out to evaluate differences between the two groups. Chi-square test was used to analyze the relationship between lncRNA ZEB1-AS1 expression and clinical characteristics. Kaplan-Meier analysis with

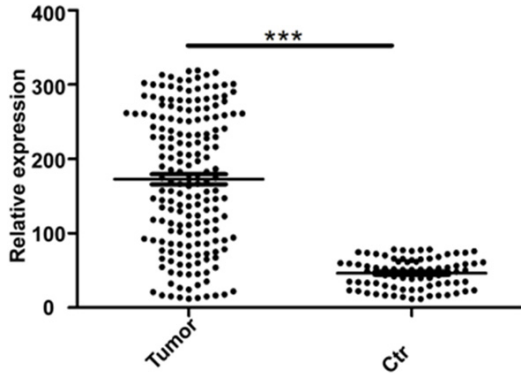


Figure 1. Relative expression of lncRNA ZEB1-AS1 in NSCLCs quantified by real time PCR.

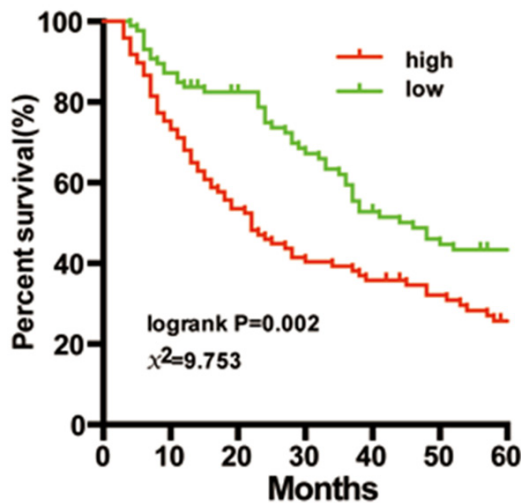


Figure 2. Kaplan-Meier's plot of lncRNA ZEB1-AS1 in NSCLCs patients.

log rank test was used to analyze overall survival of patients with different lncRNA ZEB1-AS1 expression. Univariate and multivariate analysis with Cox regression was used to assess the prognostic value of lncRNA ZEB1-AS1 in NSCLC. $P < 0.05$ was considered statistically significant.

Results

Expression of lncRNA ZEB1-AS1 was upregulated in NSCLCs

Relative expression levels of lncRNA ZEB1-AS1 of NSCLC cancer and control tissues were identified by qRT-PCR. Our results indicated that lncRNA ZEB1-AS1 expression was significantly higher in cancer tissues than control tissues of

NSCLC patients ($P < 0.001$, **Figure 1**). We then determined the mean of relative lncRNA ZEB1-AS1 expression of cancers as a cutoff value and divided NSCLCs into two groups: low expression group (expression value $<$ the mean value) and high expression group (expression value \geq the mean value). We assessed association between lncRNA ZEB1-AS1 expression and clinicopathological characteristics of NSCLCs patients. As shown in **Table 1**, high lncRNA ZEB1-AS1 expression was closely correlated with advanced TNM stage ($P < 0.001$). There was no obvious association found, however, between lncRNA ZEB1-AS1 expression and age, gender, tumor size, T stage, N stage, histological grade, or histological classification (all, $P > 0.05$, **Table 1**).

High expression of lncRNA ZEB1-AS1 favored poor prognosis

To explore the prognostic value of lncRNA ZEB1-AS1 in NSCLCs, Kaplan-Meier analysis with log-rank test was performed. With the follow up data, Kaplan-Meier analysis with log rank test revealed that NSCLC patients with high lncRNA ZEB1-AS1 expression had shorter overall survival than those with low expression of lncRNA ZEB1-AS1 (logrank $P = 0.002$, **Figure 2**). Besides, the results of Cox regression analysis showed that lncRNA ZEB1-AS1 expression ($HR = 1.577$, 95% $CI = 1.071-2.323$, $P = 0.021$) as well as TNM stage ($HR = 2.535$, 95% $CI = 1.604-4.006$, $P < 0.001$) and N stage ($HR = 5.322$, 95% $CI = 2.289-12.370$, $P < 0.001$) were correlated with prognosis of NSCLC patients, indicating that they may be independent prognostic factors for NSCLC (**Table 2**).

Discussion

Recently, many lncRNAs have been found to be dysregulated in NSCLC [13]. It has been reported that lncRNA Sox2ot positively reinforces expression levels of SOX2 and OCT4, maintains stemness, and enhances cell anchorage-independent growth in lung cancer [14]. Hou et al. has also shown that lncRNA Sox2ot is upregulated in NSCLC and is a poor prognostic indicator of survival [15]. Also, some lncRNAs have been demonstrated to be tumor suppressor genes. lncRNA SPRY4-IT1 was first found upregulated in melanoma and located into an intron region within the SPRY4 gene (chromosome 5q31.3) [16]. Sun et al. found that lncRNA

ZEB1-AS1 in NSCLC

Table 2. Univariate and multivariate analyses for overall survival (Cox proportional hazards regression model)

Features	Multivariate		Univariate	
	HR (95% CI)	<i>p</i>	HR (95% CI)	<i>p</i>
Gender	0.450 (0.137, 1.477)	0.651	0.873 (0.564, 1.352)	0.543
Age	1.081 (0.791, 1.477)	0.242	0.979 (0.659, 1.453)	0.915
Classification	1.046 (0.356, 3.074)	0.900	1.068 (0.738, 1.546)	0.728
Differentiation	1.230 (0.819, 1.848)	0.319	1.220 (0.842, 1.766)	0.293
Tumor size	0.426 (0.141, 1.282)	0.129	1.278 (0.852, 1.916)	0.235
T stage	0.000 (0.000, 2.471E35)	0.846	2.123 (1.440, 3.131)	<0.001
N stage	5.322 (2.289, 12.370)	<0.001	1.990 (1.333, 2.970)	0.001
TNM Stage	2.535 (1.604, 4.006)	<0.001	3.620 (2.438, 5.373)	<0.001
ZEB1-AS1	1.577 (1.071, 2.323)	0.021	1.704 (1.169, 2.485)	0.006

SPRY4-IT1 was reduced in NSCLC, in which the promoter was epigenetically silenced by enhancer of zeste homolog 2 (EZH2) [17]. Additionally, downregulation of SPRY4-IT1 promoted epithelial-mesenchymal transition (EMT) and predicted poor prognosis of NSCLC [17]. There are still many dysregulated lncRNAs, however, which have not been well characterized in NSCLC.

lncRNA ZEB1-AS1 has been shown to act as an oncogene in many kinds of cancers including HCC, prostate cancer, colorectal cancer, bladder cancer, osteosarcoma, glioma, and esophageal squamous cell carcinoma [18-24]. As an antisense RNA of ZEB1, lncRNA ZEB1-AS1 is physically located close to ZEB1, directly facilitating transcription of ZEB1 and positively promoting EMT of HCC cells [18]. lncRNA ZEB1-AS1 could directly bind and recruit p300 to promoter of ZEB1 and facilitate transcription of ZEB1 in osteosarcoma [25]. Both in prostate cancer and osteosarcoma, lncRNA could act as a sponge of miR-200 and indirectly promote ZEB1 expression [21, 23]. Additionally, lncRNA ZEB1-AS1 inhibits cell cycle inhibitory protein p15, enhances cell proliferation, and inhibits cell apoptosis [22]. Finally, in these cancers, upregulated lncRNA ZEB1-AS1 has predicted poor prognosis [18-26].

In this study, we quantified expression of lncRNA ZEB1-AS1 in NSCLCs by real time PCR. Our results show that lncRNA ZEB1-AS1 increased in cancer compared to adjacent lung tissues. Increased lncRNA ZEB1-AS1 was also related to advanced TNM stage and predicted poor prognosis. Accordingly, it acts as an oncogene in NSCLCs. The mechanism of lncRNA ZEB1-AS1 increase and action as an oncogene

in NSCLCs remains unknown, however. This should be explored further.

In summary, our results demonstrate that lncRNA ZEB1-AS1 is frequently overexpressed in NSCLCs. It is associated with advanced stage and predicts poor prognosis. These results suggest that lncRNA ZEB1-AS1 contributes to development and progression of NSCLCs and may be a therapeutic target in NSCLCs.

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Disclosure of conflict of interest

None.

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