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Long non-coding RNA HNF1A-AS1 up-regulation in non-small cell lung cancer correlates to poor survival

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Abstract. – OBJECTIVE: Accumulating studies have focused on the role of the newly identified lncRNAs in the tumor. We aimed to investigate the clinical correlation between long non-coding RNA HNF1A-AS (HNF1A-AS1) expression and non-small cell lung cancer (NSCLC).

PATIENTS AND METHODS: Real-time polymerase chain reaction (PCR) was employed to detect HNF1A-AS expression in NSCLC tissues and corresponding adjacent tissues. The χ^2-test was used to compare the clinicopathological characteristics between different groups. Survival analysis was performed using the Kaplan-Meier method. The univariate and multivariate analyses were performed to investigate the prognostic significance of HNF1A-AS for NSCLC.

RESULTS: The level of HNF1A-AS was higher in tumor tissues than that in normal tissues ($p < 0.01$). In addition, HNF1A-AS level was significantly associated with TNM stage ($p = 0.002$) and Lymph node metastasis ($p = 0.005$). Kaplan-Meier analysis indicated that a high level of HNF1A-AS expression predicted unfavorable overall survival ($p < 0.001$). The univariate and multivariate analysis identified HNF1A-AS as an independent poor prognostic factor for overall survival in NSCLC patients ($p < 0.05$).

CONCLUSIONS: These results demonstrated that overexpression of HNF1A-AS might act a poor prognosis indicator in patients with NSCLC.

Key Words:
lncRNAs, HNF1A-AS1, Non-small cell lung cancer, Quantitative real-time PCR, Prognosis.

Introduction

Lung cancer is the leading cause of cancer-related death worldwide, with nearly 1.4 million deaths recorded annually[1]. Non-small cell lung cancer (NSCLC) represents 85% of patients diagnosed with lung cancer[2]. Despite the improvements in diagnosis and therapy made during the past few years, the 5-year survival rate of NSCLC is still approximately 15% because of the high rate of recurrence and metastasis after surgical resection[3,4]. Therefore, it is considered important to clarify the mechanism of tumor biology and search for new therapeutic targets and markers, to improve the clinical outcome of patients with NSCLC.

LncRNA is a transcribed RNA molecule longer than 200 nt and is not participate in expression of protein[5,6]. In the past decade, miRNAs has been identified to play an important role in the tumor progression[7]. Similar to miRNAs, previous studies[8,9] have indicated that lncRNAs play critical roles in malignant tumors including NSCLC. For instance, lncRNA HOTAIR acted as an oncogene in different tumor entities, such as NSCLC[10], liver cancer[11] and cervical cancer[12] and its expression may be used for diagnostic and therapeutic purposes. lncRNA UCA1 contributed to the progression of advanced clinical stages in tumors, such as breast cancer[13], osteosarcoma[14] and esophageal squamous cell carcinoma[15]. These findings suggested that functional lncRNAs might be a new class of cancer biomarkers and therapeutic targets.

The long non-coding RNA HNF1A-AS1 (HNF1A-AS1) was a newly found lncRNA[16]. A few studies were reported about the role of HNF1A-AS1 in the tumor. The aim of this study was to examine the role of HNF1A-AS1 in NSCLC and to confirm whether the expression of HNF1A-AS1 was aberrant in NSCLC tissues and whether it was associated with poor prognosis in NSCLC.

Patients and Methods

Patients and Tissue Samples

The study included 177 NSCLC patients who were recruited from Chinese PLA General Hos-

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pital from January 2009 to May 2011. None of the patients received chemotherapy or radiotherapy before surgery. All NSCLC cases were diagnosed through pathology or cytology examination and further confirmed with CT, MRI, and other imaging modalities. The histological grade and clinical stage were determined according to the TNM classification of the International Union Against Cancer[17]. There were no serious perioperative complications. The clinicopathological characteristics of the 177 patients are listed in Table I. The present study was approved by the Ethical Committee of Chinese PLA General Hospital.

RNA Extraction and Real-Time Quantitative PCR (qPCR)

Total RNA was extracted from tissues using TRIzol reagent (Invitrogen; Carlsbad, CA, USA). The reaction mixture (20 μl) containing 2 μg of total RNA was reversely transcribed to cDNA by using PrimeScript RT-polymerase (Takara, Dalian, China). Quantitative real time-PCR (qRT-PCR) was carried out to determine expression levels of HNF1A-AS1 using a Brilliant SYBR Green II qRT-PCR kit (Strategene, Santa Clara, CA, USA) according to manufacturer's protocol. The relative expression of HNF1A-AS1 was calculated and normalized using the $2^{-\Delta\Delta Ct}$ method about GAPDH. Each experiment was performed in triplicate and repeated at least three times. The primer sequences were shown in Table I.

Statistical Analysis

SPSS software, version 16.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The X^2 and Mann-Whitney tests were used to assess the correlation between the HNF1A-AS1 and clinicopathological variables. Survival parameters were compared using Kaplan-Meier curves and log-rank test. The Cox proportional hazards regression model was used to perform univariate and multivariate analyses. All p-values in the study considered significant at values of $p < 0.05$.

Table I. Sequences of primers for qRT-PCR and HNF1-AS1 related sequence.

Name	Sequences (5′ to 3′)
HNF1-AS1(Forward)	5′-TCAAGAAATGGTGGCTAT-3′
HNF1-AS1 (Reverse)	5′-GCTCTGAGACTGGCTGAA-3′
GAPDH (Forward)	5′-GGGAGCCAAAAGGGTCAT-3′
GAPDH (Reverse)	5′-GAGTCCTTCCACGATACCAA-3′

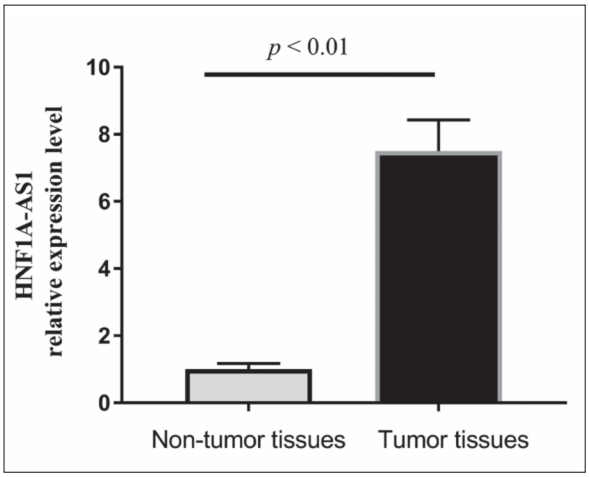

Figure 1. Relative expression of HNF1A-AS1 was evaluated by TaqMan (RT-qPCR). HNF1A-AS1 expression was significantly higher in NSCLC tissues than in the corresponding non-tumorous samples ($p < 0.01$).

Results

HNF1A-AS1 Expression is Significantly Up-Regulated in NSCLC

To determine expression levels of HNF1A-AS1, we performed qRT-PCR using RNA isolated from NSCLC samples. Significantly, we found that higher HNF1A-AS1 level was expressed in NSCLC tissues than that in adjacent tissues ($p < 0.01$, Figure 1).

Expression of HNF1A-AS1 in NSCLC and Correlation with Clinicopathological Parameters

Next, we investigated the associations between NSCLC expression and clinicopathologic features of NSCLC patients. The median expression level of HNF1A-AS1 was used as a cutoff point to divide all 177 patients into two groups (high groups and low groups). As shown in Table II, HNF1A-AS1 expression level was higher in NSCLC patients with advanced TMN stage ($p = 0.002$). Moreover, higher expression of HNF1A-AS1 was more frequently detected in patients with lymph nodes metastasis ($p = 0.005$). HNF1A-AS1 expression exhibited no significant association with other clinicopathological char-

Table II. Relationship between HNF1A-AS1 expression and clinicopathological features of 177 patients.

Characteristic	N (177)	Low-HNF1A-AS1 (n = 90)	High-HNF1A-AS1 (n = 87)	*p*-value
Gender				0.206
Male	112	61	51	
Female	65	29	36	
Age (years)				0.426
< 60	66	31	35	
≥ 60	111	59	52	
Smoking index[a]				0.374
< 400	71	39	32	
≥ 400	106	51	55	
History of COPD				0.509
Yes	55	30	25	
No	122	60	62	
Histological type				0.354
Squamous	95	51	44	
Adenocarcinoma	58	28	30	
Other	24	11	13	
Differentiation				0.422
Well	81	43	38	
Moderately	49	27	22	
Poorly	47	20	27	
TNM stage				0.002
I-II	94	58	36	
III-IV	83	32	51	
Lymph node metastasis				0.005
Yes	87	35	52	
No	90	55	35	

[a]Sort by 400 cigarettes/years.

acteristics, such as the sex and age.

Upregulation of HNF1A-AS1 Associated with Poor Prognosis in Patients with NSCLC

Survival analysis was conducted using Kaplan-Meier curves for overall survival. All patients were followed up for at least 5 years. As shown in Figure 2, NSCLC tissues with high expressions of HNF1A-AS1 was correlated with a shorter overall survival ($p < 0.01$).

We further examined overall survival using Cox regression hazard analyses to determine whether HNF1A-AS1 levels could serve as a useful prognostic factor in NSCLC. On univariate survival analysis, TNM stage ($p = 0.015$), lymph nodes metastasis ($p = 0.008$) and HNF1A-AS1 levels ($p = 0.001$) were associated with overall survival (Table III). Furthermore, multivariate analysis using Cox's regression model was performed as shown in Table IV. HNF1A-AS expression ($p = 0.002$), TNM stage ($p = 0.009$), and lymph node status ($p = 0.006$) were independent prognostic factors.

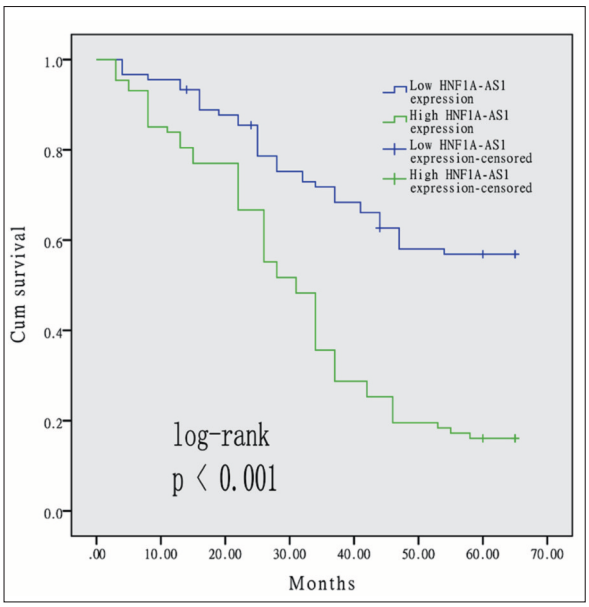

Figure 2. Kaplan-Meier plot of the overall survival in 177 patients with NSCLC. High HNF1A-AS1 was associated with poorer overall survival ($p < 0.001$, log-rank test).

Table III. Univariate Cox proportional hazard regression analysis for clinical outcome in 177 NSCLC patients.

Characteristics	For death	
	Hazard Ratio (95% confidence interval)	*p*-value
Gender (Male vs. Female)	1.432 (0.391-2.113)	0.651
Age (years) (< 60 *vs.* ≥ 60)	1.179 (0.633-1.642)	0.236
Smoking index (< 400 *vs.* ≥ 400)	0.831 (0.513-1.822)	0.451
History of COPD (No *vs.* Yes)	0.361 (0.148-0.933)	0.177
Histological type		
Squamous	2.218 (0.925-3.783)	0.317
Adenocarcinoma	1.782 (0.663-2.931)	0.441
Other	1	
Differentiation		
Well	0.793 (0.237-1.515)	0.314
Moderately	0.731 (0.442-1.348)	0.279
Poorly	1	
TNM stage (I-II *vs.* III- IV)	2.532 (0.883-3.137)	0.015
Lymph nodes metastasis (Yes *vs.* No)	2.144 (0.981-4.239)	0.008
HNF1A-AS1 (Low *vs.* High)	3.166 (1.451-5.332	0.001

Discussion

In the past decade, plenty of energy focused on the findings of molecular markers that can predict the presence, diagnosis and prognosis of tumor[18,19]. Lots of relevant articles[20,21] have reported the potential of targeting specific miRs in cancer therapy. However, to date, a few effective molecular markers has been found for the recurrence of malignant NSCLC. Zeng et al[22] pointed to lncRNAs as new candidate molecules to be incorporated into the arsenal of therapeutic targets in lung cancer. However, the role of HNF1A-AS1 in NSCLC cells remains unclear. Therefore, in our present study, we focused on HNF1A-AS1.

The long non-coding RNA HNF1A antisense RNA 1 (lncRNA HNF1A-AS1) located at chromosome 12 with 2455 nucleotides in length was a newly discovered lncRNA[16]. Previous studies reported the role of HNF1A-AS1 in several tumors. For instance, Yang et al[16] showed that HNF1A-AS1 was markedly upregulated in oesophageal adenocarcinoma and HNF1A-AS1 knockdown significantly inhibited cell migration and invasion. Zhao et al[23] found that HNF1A-AS1 was markedly up-regulated in osteosarcoma tissues and promotes cell proliferation and metastasis through activation of the Wnt/β-catenin signaling pathway. Liu et al[24] reported that over-expression of HNF1A-AS1 was closely related to larger tumor size, multiple tumor lesions, poor differentiation and advanced TNM stage. Furthermore, they found that HNF1A-AS1 promoted cell proliferation, invasion and migration in hepatocellular carcinoma through sponging hsa-miR-30b-5p24. Dang et al[25] also found that abnormal expression levels of HNF1A-AS1 in gastric cancer, whether HNF1A-AS1 played a similar role in NSCLC has not reported.

In the current study, we investigated the clinical role of HNF1A-AS1 in NSCLC. Not surprisingly, we found elevated expressions of HNF1A-AS1 in NSCLC tissues compared with the adjacent noncancerous tissues. Next, we analyzed the association of HNF1A-AS1 with clinicopatho-

Table IV. Multivariate Cox proportional hazard regression analysis of prognostic factor in 177 NSCLC patients.

Characteristics	For death	
	Hazard Ratio (95% confidence interval)	*p*-value
TNM stage (I-II *vs.* III- IV)	2.146 (0.634-2.885)	0.009
Lymph nodes metastasis (Yes *vs.* No)	1.532 (0.733-3.816)	0.006
HNF1A-AS1 (Low *vs.* High)	3.014 (1.137-4.418)	0.002

logical features. The results showed that increased HNF1A-AS1 expression was significantly associated with advanced TNM stage and Lymph node metastasis. Furthermore, we found that increased HNF1A-AS1 expression predicts poor overall survival of patients with NSCLC. At last, univariate and multivariate analysis indicated that high HNF1A-AS1, advanced TMN and lymph nodes metastasis, were independent prognostic factors for a poor overall survival rate of NSCLC patients. These results indicated the prognostic potential of HNF1A-AS1 for NSCLC.

Conclusions

HNF1A-AS1 showed a potential as a favorable prognostic marker in NSCLC. In the future, additional studies are necessary to explore the molecular mechanism of HNF1A-AS1 regulating the NSCLC cells.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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