European Review for Medical and Pharmacological Sciences 2017; 21: 2392-2396

Prognostic and diagnostic significance of long non-coding RNA AGAP2-AS1 levels in patients with non-small cell lung cancer

K.-J. FAN, Y. LIU, B. YANG, X.-D. TIAN, C.-R. LI, B. WANG

Department of Thoracic Surgery, Chinese PLA General Hospital, Beijing, China

Abstract. – OBJECTIVE: The purpose of this study was to explore whether long non-coding RNA AGAP2-AS1 (AGAP2-AS1) could serve as a novel biomarker for non-small-cell lung cancer (NSCLC).

PATIENTS AND METHODS: Cancer and matched normal lung tissues were collected from 198 patients. AGAP2-AS1 levels were examined by RT-PCR, and the associations of AGAP2-AS1 levels with clinicopathological characteristics evaluated. Overall survival was evaluated using the Kaplan-Meier method. Cox proportional hazard modeling was performed for univariate and multivariate analysis to determine the effects of variables on survival. Receiver-operating characteristic. Besides, the receiver operating characteristic (ROC) curve analysis were applied to analyze its diagnostic value.

RESULTS: Expression of AGAP2-AS1 was up-regulated in the NSCLC tissues compared with the adjacent normal tissues ($p < 0.01$). Furthermore, The level of AGAP2-AS19 in NSCLC was strongly correlated with tumor stage ($p = 0.001$) and lymph nodes metastasis ($p = 0.005$). Kaplan-Meier analysis demonstrated patients with higher AGAP2-AS1 expression had a shorter overall survival time than those with lower AGAP2-AS1 expression ($p < 0.0001$). The multivariate analysis showed that AGAP2-AS1 expression is an independent prognostic factor of overall survival in patients with NSCLC. The results of ROC curve analysis showed that AGAP2-AS1 might be a promising diagnostic marker of NSCLC with an AUC of 0.846.

CONCLUSIONS: Our findings revealed that AGAP2-AS1 might be a potential biomarker for the diagnosis and prognosis of NSCLC. However, to completely elucidate its role as a biomarker, further studies are required.

Key Words:
Long non-coding RNA, AGAP2-AS1, Diagnosis, Prognosis, NSCLC.

Introduction

Non-small cell lung cancer (NSCLC) is the most important type of lung cancer which ranks first in terms of cancer-related mortality in both developing and developed countries[1,2]. Although new methods for NSCLC diagnosis and treatment have continuously emerged, survival rates for patients with NSCLC remain gloomy because the majority (57%) are diagnosed after cancer has metastasized[3,4]. Up to date, susceptive markers that can predict the patients who have a potential higher risk of recurrence, metastasis are very few. Therefore, the discovery of new diagnostic and prognostic biomarkers is of particular importance.

LncRNAs are non-protein coding RNA molecules greater than 200 nucleotides in length[5]. LncRNAs is involved in almost every aspect of cell biology, including remodeling, transcription, and post-transcriptional processing[6,7]. Recently, more and more evidences showed that lncRNAs play an important in the progression of the disease, including cancer[8,9]. Indeed, some lncRNAs have emerged as new members in cancer research and function as tumor suppressor genes or oncogenes[10,11]. A previous study[12] revealed that the abnormal expression patterns of lncRNAs can also be employed for the diagnosis and prognosis of human cancer. Of note, some papers have identified some important lncRNA as important biomarkers, such as CASC2 in lung cancer[13], MVIH in breast cancer[14], and HMlincRNA717 in pancreatic cancer[15]. However, the clinical significance of AGAP2-AS1 in NSCLC is still unclear.

In our study, we aimed to detect the expression of AGAP2-AS1 in 198 NSCLC patients. We analyzed the correlation between the expression of AGAP2-AS1 and the clinicopathological features of this malignancy.

Patients and Methods

Patients and Tissue Samples

NSCLC tissues and adjacent non-tumor tissues from patients who had undergone curative

Corresponding Author: Yang Liu, MD; e-mail: ly147wt@yeah.net

resection were collected from Department of Thoracic Surgery, Chinese PLA General Hospital. All surgical tissues were examined by a pathologist. There were altogether 132 males and 68 females with a mean age of 63.6 years (ranging from 21 to 86 years). All patients recruited to this study did not receive any pre-operative treatments. This study was conducted in accordance with the Declaration of Helsinki and with approval from the Ethics Committee of Chinese PLA General Hospital. Written informed consent was obtained from all of the patients. The clinical feature of NSCLC patients was summarized in Table I.

Quantitative Real-time RT-PCR

Total RNA was extracted from tissue samples using the miRVANA Kit (Ambion, Carlsbad, MA, USA) according to the manufacturer instruction. 1 μg of total RNA was reversed transcribed using QuantiTect Reverse Transcription Kit (QIAGEN, Hilden, Germany). The expressions of AGAP2-AS1 were measured by quantitative PCR (qPCR) using SYBR Green assays (TaKaRa, Dalian, Niaoning, China). AGAP2-AS1 expression was measured using the following primers: forward, 5'- TACCTTGACCTTGCTGCTCTC-3′ and reverse, 5′-TGTCCCTTAATGACCCCATCC-3′. The expression levels of the genes were normalized to that of the housekeeping gene β-actin, as a control. The relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical Analysis

Statistical analyses were performed using SPSS software (version 16.0, SPSS Inc., Chicago, IL, USA). Chi-square test and Fisher's exact test were applied to assess the relationship between AGAP2-AS1 expression and clinicopathological characteristics. Overall survival curves were generated according to the Kaplan-Meier method. The Cox regression analysis in a forward stepwise method was used to evaluate the effect of multiple independent prognostic factors on survival outcome. ROC curve and the area under the ROC curve (AUC) were applied to assess the diagnosis value of GAP2-AS1. $p < 0.05$ was considered statistically significant.

Results

AGAP2-AS1 is Upregulated in NSCLC Tissues

To determine whether AGAP2-AS1 is involved in NSCLC tumorigenesis, We determined the expression levels of AGAP2-AS1 in NSCLC tissues and matched normal lung tissues by RT-qPCR. As shown in Figure 1, the results showed that the median expression level of AGAP2-AS1 was higher in the NSCLC tissues compared with the median level in the paired normal lung tissues ($p < 0.01$).

Table I. Correlation between AGAP2-AS1 expression and patient characteristics.

Variable	Number	AGAP2-AS1 expression		*p*-value
		High	Low	
Age (years)				0.640
< 60	78	41	37	
≥ 60	120	59	61	
Gender				0.482
Male	132	69	63	
Female	68	31	35	
Tumor size (cm)				0.322
< 3	96	45	51	
≥ 3	102	55	47	
Histologic type				0.890
Squamous	102	52	50	
Adenoma	96	48	48	
Tumor stage				0.001
I-II	91	33	58	
III	108	67	40	
Lymph nodes metastasis				0.005
No	93	37	56	
Yes	105	63	42	

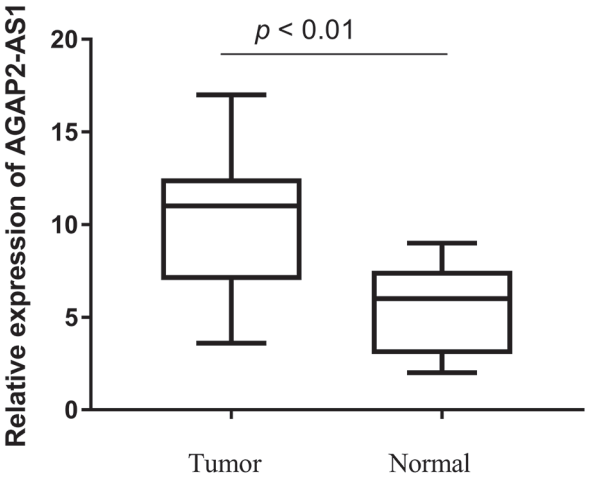

Figure 1. AGAP2-AS1 expression in 198 pairs of NSCLC tissue and matched adjacent normal tissue specimens by qRT-PCR.

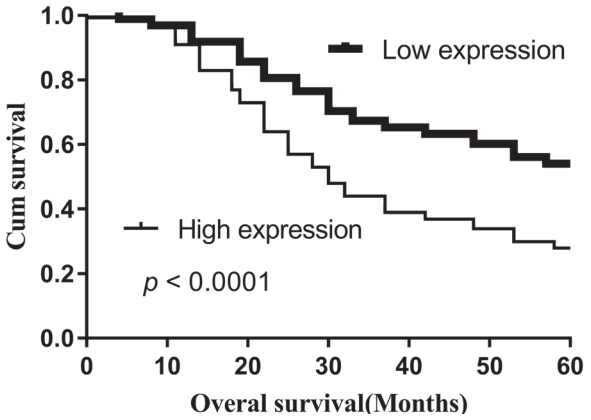

Figure 2. The survival curves comparing the overall survival of NSCLC with high or low AGAP2-AS1 expression (Kaplan-Meier curves with log-rank tests).

Correlations Between AGAP2-AS1 Expression and NSCLC Clinicopathologic Characteristics

To investigate the correlation between AGAP2-AS1 expression and clinicopathological variables of the patients, we manually divided the melanoma patients into two groups (high and low group) based on the median expression level. The relationships between AGAP2-AS1 expression levels and clinicopathological characteristics in patients with NSCLC are summarized in Table I. We found that the level of AGAP2-AS19 in NSCLC was strongly correlated with tumor stage ($p = 0.001$) and lymph nodes metastasis ($p = 0.005$). However, AGAP2-AS1 expression in NSCLC was not associated with other parameters such as age, gender, tumor size, histologic type (all $p > 0.05$).

The Association between AGAP2-AS1 Expression and Overall Survival of Patients with NSCLC

Next, we used Kaplan-Meier analysis and log-rank test to explore the correlation of AGAP2-AS1 expression with prognosis in NSCLC patients. As shown in Figure 2, the results showed that higher AGAP2-AS1 expression had a shorter overall survival time than those with lower AGAP2-AS1 expression ($p < 0.0001$). Furthermore, the univariate analysis demonstrated that tumor stage ($p = 0.001$), lymph node metastasis ($p = 0.004$) and AGAP2-AS1 expression ($p = 0.001$) were significantly associated with overall survival of NSCLC patients (Table II). Finally, the multivariate analysis further revealed that AGAP2-AS1 expression ($p = 0.003$) maintained independent prognostic influence on overall survival.

Diagnostic Accuracy of AGAP2-AS1 in NSCLC

To determine whether AGAP2-AS1 can be identified as a biomarker to distinguish NSCLC from normal tissue, the ROC curve analysis was performed. As shown in Figure 3, the results showed that AGAP2-AS1 was a useful marker for discriminating NSCLC tissues from normal lung tissues, with the areas under the ROC

Table II. Univariate and multivariate analyses for overall survival by Cox regression model.

Variables	Univariate analysis		Multivariate analysis	
	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Age	1.326 (0.873-1.882)	0.422	–	–
Gender	0.925 (0.631-1.738)	0.398	–	–
Tumor size	1.631 (0.799-1.933)	0.235	–	–
Histologic type	1.447 (0.823-1.793)	0.319	–	–
Tumor stage	2.349 (1.448-5.698)	0.001	1.983 (1.249-4.885)	0.001
Lymph nodes metastasis	2.238 (1.366-4.459)	0.004	2.033 (1.138-3.763)	0.007
AGAP2-AS1 expression	2.659 (1.546-5.221)	0.001	2.139 (1.147-4.223)	0.003

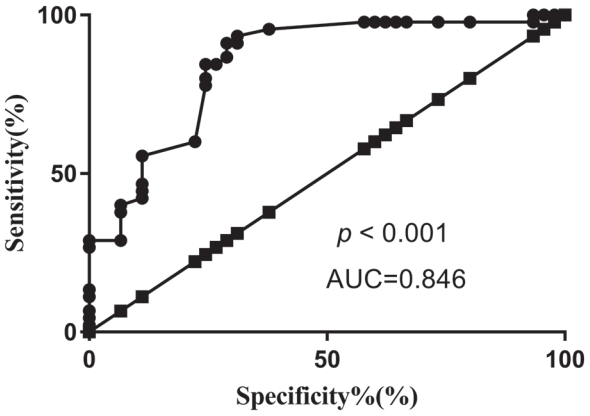

Figure 3. Area under curve (AUC) of receiver operating characteristic (ROC) for AGAP2-AS1.

curve (AUC) of 0.845 (95% confidence interval (CI) 0.764 to 0.927, $p < 0.001$), suggesting that AGAP2-AS1 has potential diagnostic value in NSCLC. $p < 0.05$ was considered statistically significant.

Discussion

Increasing evidence suggests that lncRNAs play key roles in the regulation of cell function in the mammalian genome[16]. Recently, some studies are just beginning to explore the biology of lncRNAs in NSCLC. For instance, Wu et al[17] showed that overexpression of lncRNA HNF1A-AS1 promoted lung adenocarcinoma cells proliferation and metastasis by regulating cyclin D1, E-cadherin, N-cadherin and β-catenin expression. Han et al[18] found that PANDAR overexpression inhibited tumor growth of NSCLC cell *in vivo*. They also indicated that ectopic PANDAR expression could induce caspase-3-dependent apoptosis in NSCLC cells. These results suggested that ectopic expression of lncRNA may be associated with prognosis in patients with NSCLC. Indeed, previous studies showed that the expression levels of lncRNAs were strangely associated with the overall survival of patients with NSCLC, such as ZFAS1[19] and AFAP1-AS1[20]. Unfortunately, the emerging functional role of lncRNAs in NSCLC remains largely unknown.

AGAP2-AS1 which transcribed from a gene located at 12q14.1 is 1567 nt in length. Previous studies demonstrated that AGAP2-AS1 played important roles in the tumor development. For example, Qi et al[21] showed that AGAP2-AS1 was highly expressed in the gastric cancer tissues and cell lines, and high s AGAP2-AS1 expression levels predicted poor survival in NSCLC. Besides, they found that over-expression of AGAP2-AS1 promoted cell migration and invasion in GC. Wang et al[22] revealed that knockdown of AGAP2-AS1 can inhibit the glioma cell proliferation, migration, and invasion, while increase the apoptosis cell rates *in vitro*. They also showed glioma patients with high AGAP2-AS1 expression tended to have shorter overall survival. More important, finding by Li et al[23] indicated the AGAP2-AS1 expression level was significantly upregulated in NSCLC tissues. Furthermore, they found that AGAP2-AS1 could suppress NSCLC cell proliferation partly through the down-regulation of LATS2 expression. The above results indicated the potential effect of AGAP2-AS1 served as a useful marker for diagnosis and prognosis in NSCLC.

In the present study, we confirmed that AGAP2-AS1 was up-regulated in NSCLC patients by RT-PCR. Next, we analyzed the relationship of AGAP2-AS1 with various clinical features of NSCLC patients. Our results showed that the level of AGAP2-AS19 in NSCLC was strongly correlated with tumor stage and lymph nodes metastasis, indicating that AGAP2-AS19 may function as a tumor promoter in NSCLC. Moreover, Kaplan-Meier analysis of clinical survival showed that AGAP2-AS1expression was associated with poor clinical survival of patients with NSCLC. These results were consistent with a previous study[23]. Importantly, our results were more evident because we collected 198 patients. Then, multivariate analysis indicated that overexpression of AGAP2-AS19 was an independent predictor of overall survival of patients with NSCLC. Finally, we firstly performed ROC analysis to explore whether AGAP2-AS1 could distinguish NSCLC tissues from normal tissue. Our results confirmed our hypothesis. AGAP2-AS1 has potential diagnostic value in NSCLC.

Conclusions

We observed that AGAP2-AS1 was potential biomarkers for detection and prognosis of NSCLC. However, the clinical value and wide application of AGAP2-AS1 in diagnosing NSCLC still require further investigation and optimization.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.
2) Xue C, Hu Z, Jiang W, Zhao Y, Xu F, Huang Y, Zhao H, Wu J, Zhang Y, Zhao L, Zhang J, Chen L, Zhang L. National survey of the medical treatment status for non-small cell lung cancer (NSCLC) in China. Lung Cancer 2012; 77: 371-375.
3) Margaritora S1, Cesario A, Cusumano G, Dall'armi V, Porziella V, Meacci E, Lococo F, D'Angelillo R, Congedo MT, Granone P. Pneumonectomy with and without induction chemo-radiotherapy for non-small cell lung cancer: short and long-term results from a single centre. Eur Rev Med Pharmacol Sci 2013; 17: 29-40.
4) Winton T, Livingston R, Johnson D, Rigas J, Johnston M, Butts C, Cormier Y, Goss G, Inculet R, Vallieres E. Vinorelbine plus cisplatin vs. observation in resected non-small-cell lung cancer. N Engl J Med 2005; 352: 2589-2597.
5) Gutschner T, Diederichs S. The hallmarks of cancer: a long non-coding RNA point of view. RNA Biol 2012; 9: 703-719.
6) Fatica A, Bozzoni I. Long non-coding RNAs: new players in cell differentiation and development. Nat Rev Genet 2014; 15: 7-21.
7) Prensner JR, Chinnaiyan AM. The emergence of lncRNAs in cancer biology. Cancer Discov 2011; 1: 391-407.
8) Li CH, Chen Y. Targeting long non-coding RNAs in cancers: progress and prospects. Int J Biochem Cell Biol 2013; 45: 1895-1910.
9) Hart RP, Goff LA. Long noncoding RNAs: Central to nervous system development. Int J Dev Neurosci 2016; 55: 109-116
10) Li DS, Ainiwaer JL, Sheyhiding I, Zhang Z, Zhang LW. Identification of key long non-coding RNAs as competing endogenous RNAs for miRNA-mRNA in lung adenocarcinoma. Eur Rev Med Pharmacol Sci 2016; 20: 2285-2295.
11) Venkatesh T, Suresh PS, Tsutsumi R. Non-coding RNAs: Functions and applications in endocrine-related cancer. Mol Cell Endocrinol 2015; 416: 88-96.
12) Ricciuti B, Mencaroni C, Paglialunga L, Paciullo F, Crinò L, Chiari R, Metro G. Long noncoding RNAs: new insights into non-small cell lung cancer biology, diagnosis and therapy. Med Oncol 2016; 33: 18.
13) He X, Liu Z, Su J, Yang J, Yin D, Han L, De W, Guo R. Low expression of long noncoding RNA CASC2 indicates a poor prognosis and regulates cell proliferation in non-small cell lung cancer. Tumour Biol 2016; 37: 9503-9510.
14) Lei B, Xu SP, Liang XS, Li YW, Zhang JF, Zhang GQ, Pang D. Long non-coding RNA MVIH is associated with poor prognosis and malignant biological behavior in breast cancer. Tumour Biol 2016 ;37: 5257-5264.
15) Sun XL, Cao GH, Cao Y, Jiang X, Li XK, Ye XH, Wang DH, Yan SX. Association of LncRNA HMlincRNA717 with prognosis in pancreatic cancer. Eur Rev Med Pharmacol Sci 2016; 20: 2230-2234.
16) Tsai MC, Spitale RC, Chang HY. Long intergenic noncoding RNAs: new links in cancer progression. Cancer Res 2011; 71: 3-7.
17) Wu Y, Liu H, Shi X, Yao Y, Yang W, Song Y. The long non-coding RNA HNF1A-AS1 regulates proliferation and metastasis in lung adenocarcinoma. Oncotarget 2015; 6: 9160-9172.
18) Han L, Zhang EB, Yin DD, Kong R, Xu TP, Chen WM, Xia R, Shu YQ, De W. Low expression of long noncoding RNA PANDAR predicts a poor prognosis of non-small cell lung cancer and affects cell apoptosis by regulating Bcl-2. Cell Death Dis 2015; 6: e1665.
19) Tian FM, Meng FQ, Wang XB. Overexpression of long-noncoding RNA ZFAS1 decreases survival in human NSCLC patients. Eur Rev Med Pharmacol Sci 2016; 20: 5126-5131.
20) Deng J, Liang Y, Liu C, He S, Wang S. The up-regulation of long non-coding RNA AFAP1-AS1 is associated with the poor prognosis of NSCLC patients. Biomed Pharmacother 2015; 75: 8-11.
21) Qi F, Liu X, Wu H, Yu X, Wei C, Huang X, Ji G, Nie F, Wang K. Long noncoding AGAP2-AS1 is activated by SP1 and promotes cell proliferation and invasion in gastric cancer. J Hematol Oncol 2017; 10: 48.
22) Wang W, Yang F, Zhang L, Chen J, Zhao Z, Wang H, Wu F, Liang T, Yan X, Li J, Lan Q, Wang J, Zhao J. LncRNA profile study reveals four-lncRNA signature associated with the prognosis of patients with anaplastic gliomas. Oncotarget 2016; 7: 77225-77236.
23) Li W, Sun M, Zang C, Ma P, He J, Zhang M, Huang Z, Ding Y, Shu Y. Upregulated long non-coding RNA AGAP2-AS1 represses LATS2 and KLF2 expression through interacting with EZH2 and LSD1 in non-small-cell lung cancer cells. Cell Death Dis 2016; 7: e2225.