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LncRNA KCNQ1OT1 is overexpressed in non-small cell lung cancer and its expression level is related to clinicopathology

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Abstract. – OBJECTIVE: The aim of this study was to explore the expression of long non-coding RNA (lncRNA) KCNQ1OT1 in non-small cell lung cancer (NSCLC), and to elucidate its clinical significance.

PATIENTS AND METHODS: The quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) was used to detect the expression of lncRNA KCNQ1OT1 in NSCLC tissues and para-cancer tissues (5 cm or above away from the tumor). The relation between lncRNA KCNQ1OT1 expression and the clinical pathological data was analyzed by the multivariate logistic regression analysis. Furthermore, the survival analysis was performed by the Kaplan-Meier method.

RESULTS: The expression of lncRNA KCNQ1OT1 increased significantly in NSCLC tissues than that of in para-cancer tissues. According to the median expression of lncRNA KCNQ1OT1, NSCLC patients were divided into two groups, including high expression group and low expression group. Meanwhile, the lncRNA KCNQ1OT1 expression was correlated with tumor size, tumor node metastasis (TNM) staging, and lymph node metastasis of NSCLC patients. Both univariate analysis and multivariate analysis indicated that the high expression of lncRNA KCNQ1OT1 was closely related to TNM staging and lymph node metastasis. In addition, the Kaplan-Meier analysis showed that the overall survival and progression-free survival time of patients with higher lncRNA KCNQ1OT1 expression were significantly worse than those with lower lncRNA KCNQ1OT1 expression.

CONCLUSIONS: LncRNA KCNQ1OT1 might contribute to the development of NSCLC.

Key Words:
Non-small cell lung cancer (NSCLC), LncRNA KCNQ1OT1, Overall survival, Progression-free survival.

Introduction

Lung cancer is one of the most common malignant tumors with the highest morbidity and mortality rates. It is reported that the fatality rate of lung cancer ranks 1st worldwide, seriously threatening human life and health[1]. In the last decade, the morbidity and mortality rates of lung cancer have been on the rise, especially in more developed countries. Moreover, the morbidity rate of lung cancer will continue to increase year by year in the future, which may become the first killer of human health. Therefore, it is of great significance to explore the mechanism of lung cancer occurrence and development, and to search for molecular markers for early diagnosis and clinical treatment of lung cancer.

Lung cancer can be divided into small cell lung cancer and non-small cell lung cancer (NSCLC) according to its pathological features[2]. The NSCLC is the most common type of lung cancer, accounting for approximately 80-85% of total patients. However, more than half of NSCLC patients are definitely diagnosed late due to the lack of molecular markers for early diagnosis[1]. Currently, the diagnosis of lung cancer still depends on imaging examinations. However, most of the patients have already been in the middle-advanced stage when diagnosed. Meanwhile, invasion and metastasis have occurred in cancer cells. As a result, the best opportunity for operation is missed, leading to the 5-year overall survival of only about 17%[1]. Currently, the traditional treatment of lung cancer is still surgical excision and conventional chemoradiotherapy. The emergence of targeted therapy significantly improves the life

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quality of patients, prolonging that of patients with advanced lung cancer as well[3]. However, targeted immune therapy, such as anti-tumor therapies, is still facing with the problem of drug resistance[4]. It can be found that the pathogenesis of lung cancer and the specific mechanism of tumor invasion and metastasis remain unclear. Therefore, searching for sensitive, efficient and specific early diagnostic markers, as well as drug resistance monitoring and prognostic evaluation indexes is the main direction for lung cancer research[5,6].

In recent years, increasingly more studies have demonstrated that the occurrence and deterioration of lung cancer are closely associated with the gene expression dysregulation and abnormal gene function. Dysregulation of gene expression is the intrinsic factor and molecular basis for the initiation, deterioration, and metastasis of lung cancer. Studies have found that only a small number of human DNA sequences (<3%) encode proteins. The vast majority of DNA sequences (about 87.3%) are transcribed into RNAs which cannot be translated into proteins. Therefore, such RNAs are called as non-coding RNAs[7]. Non-coding RNAs are divided into two major types, namely housekeeping ncRNAs and regulatory ncRNAs. Moreover, regulatory ncRNAs are further divided into two subtypes according to the transcript length, including miRNAs with transcripts less than 200 bp and long non-coding RNAs (lncRNAs) with transcripts between 200 bp and 100 kb. Besides, previous studies[8,9] have confirmed that miRNAs are involved in the mRNA degradation or regulation of mRNA translation. Meanwhile, lncRNAs control biological processes regulating gene transcription and translation. In addition, lncRNAs can directly interact with DNA, mRNA or protein to regulate chromatin modification or structure, transcription, splicing, and translation. Eventually, this may regulate a variety of physiological and pathological processes, such as cell proliferation or differentiation, stem cell rearrangement, tumor occurrence and development or drug resistance[10,11]. These are the potential prognostic markers and therapeutic targets for malignant tumors.

LncRNA KCNQ1OT1 is expressed in a variety of malignant tumors and is involved in regulating tumor occurrence and development[12-15]. However, its exact role in NSCLC remains unclear. Therefore, the aim of the present study was to investigate the expression of lncRNA KCNQ1OT1 in NSCLC and its clinical significance.

Patients and Methods

Tissue Specimens

NSCLC tissues and para-cancer tissues (more than 5 cm away from cancer tissues) were harvested from 200 patients undergoing radical or palliative resection from April 2016 to December 2018. Personal information and detailed clinical data of patients were collected, including gender, age, smoking history, tumor size, lymph node metastasis, and pathological type. All collected tissues were pathologically diagnosed and confirmed with NSCLC. Freshly-resected specimens were immediately cryopreserved in liquid nitrogen for subsequent use. No patients received chemoradiotherapy before the operation. Moreover, all the patients were followed up for general conditions, clinical symptoms and imaging examinations by telephone and review after discharge from April 2016 to December 2018. This investigation was approved by the Ethics Committee of Liangshan People's Hospital. Written and informed consent was obtained from all participants before the study.

Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR) Analysis

The total RNA was extracted from tissues and cells using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA). Subsequently, the extracted total RNA was reverse transcribed into complementary deoxyribose nucleic acid (cDNA) according to the instructions of AMV reverse transcription kit (2 μg total RNA added into 20 μL system). The qRT-PCR was performed by a PCR instrument using 2×SYBR Green PCR Mastermix (Thermo Fisher Scientific, Waltham, MA, USA). An appropriate amount of cDNA was taken as templates. The corresponding forward and reverse primers were designed and synthesized according to target genes. Then, amplification (primer concentration: 0.4 μmol/L, 15 μL system) was conducted, with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an internal reference. Three replicates were set for each sample. Primers used in this study were as follows: KCNQ1OT1: forward primer: 5'-CCCAGAAATCCACACCTCGG-3', reverse primer: 5'-TCCTCAGTGAGCAGATGGAGA-3'. This experiment was repeated for three times. The relative expression level of the gene was calculated by the $RQ=2^{-\Delta\Delta Ct}$ method.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 17.0 software (SPSS Inc., Chicago, IL, USA) was used for all statistical analysis. The association of lncRNA KCNQ1OT1 expression with clinical pathological data was analyzed by the multivariate logistic regression analysis. The Kaplan-Meier curve and Log-rank test were applied for survival analysis. $p<0.05$ was considered statistically significant.

Results

LncRNA KCNQ1OT1 was Highly Expressed in NSCLC Tissues

The expression of lncRNA KCNQ1OT1 in 200 NSCLC tissues and para-cancer tissues was first detected by qRT-PCR. The results showed that the expression of lncRNA KCNQ1OT1 in the NSCLC tissues was 7.413 ±0.570, which was significantly higher than that of para-cancer tissues (1.302 ± 0.128) ($p<0.001$) (Figure 1).

According to the mean expression level of lncRNA KCNQ1OT1 (7.413), NSCLC patients were divided into two groups, including lncRNA KCNQ1OT1 high-expression group (n=107) and low-expression group (n=93). The association between lncRNA KCNQ1OT1 expression and clinicopathological features of patients was analyzed.

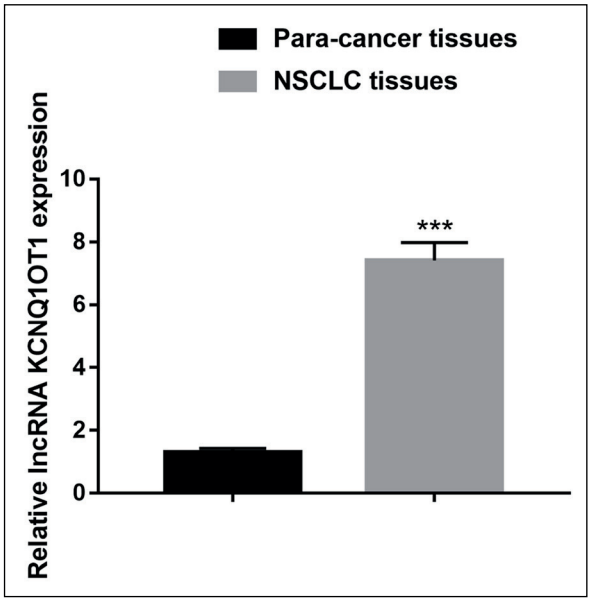

Figure 1. The expression of lncRNA KCNQ1OT1 in NSCLC and para-cancer tissues was measured by qRT-PCR.

It was found that the lncRNA KCNQ1OT1 expression was not associated with patients' gender, age, and histological classification, displaying no statistically significant differences ($p>0.05$). However, its expression was significantly correlated with smoking history, tumor size, lymph nodes metastasis, and TNM stage of NSCLC patients ($p<0.05$) (Table I).

Univariate and Multivariate Analysis of lncRNA KCNQ1OT1 Expression and NSCLC Clinicopathological Data

The univariate analysis showed that the overexpression of lncRNA KCNQ1OT1 was closely associated with smoking, tumor size, lymph nodes metastasis and TNM stage in NSCLC ($p<0.05$), whereas was not correlated with gender and age ($p>0.05$, Table II).

As shown in Table III, the multivariate analysis showed that the high expression of lncRNA KCNQ1OT1 was related to smoking, tumor size, lymph nodes metastasis and TNM stage of NSCLC patients ($p<0.05$). However, no significant correlation was found between lncRNA KCNQ1OT1 expression with gender and age ($p>0.05$). Therefore, smoking, tumor size, lymph nodes metastasis and TNM stage and lncRNA KCNQ1OT1 expression were independent risk factors for NSCLC.

Effect of lncRNA KCNQ1OT1 on the Prognosis of Patients with NSCLC

The correlation between the lncRNA KCNQ1OT1 expression and the survival time of patients was estimated using the Kaplan-Meier method. The results revealed that the progression-free survival time of patients in lncRNA KCNQ1OT1 high-expression group and low-expression group was (33.00±1.72) and (21.00±2.82) months, respectively, showing a statistically significant difference ($p<0.001$) (Figure 2A). Meanwhile, the overall survival time of patients in low-expression group ((41.00±3.37) months) was significantly longer than that of patients in high-expression group ((28.00±3.75) months) ($p<0.001$) (Figure 2B).

Discussion

Lung cancer is one of the most common malignant tumors with the highest morbidity and mortality rates in the world. The invasion and metastasis of tumor cells are the major reasons for poor

Table I. lncRNA KCNQ1OT1 expression and clinical features of patients with NSCLC cancer.

Features	No.	lncRNA KCNQ1OT1		p
		High	Low	
No.	200	107	93	
Gender				0.463
Male	116	63	53	
Female	84	44	40	
Age (years)				0.727
< 60	93	49	44	
≥ 60	107	48	49	
Smoking				0.022
Yes	105	81	24	
No	95	26	69	
Tumor size (cm)				0.001
< 5	81	24	57	
≥ 5	119	83	36	
Lymph nodes metastasis				0.014
Negative	127	44	83	
Positive	73	63	10	
TNM stage				0.000
I-II	103	19	84	
III-IV	97	88	9	
Histological classifcation				0.240
Squamous cell carcinoma	101	60	51	
Adenocarcinoma carcinoma	84	39	46	
Magnocellular carcinoma	15	8	7	

prognosis. However, the mechanism of inducing and promoting the invasion and metastasis of lung cancer cells remains unclear. Furthermore, the lack of early diagnosis and metastasis of tumor markers is one of the great challenges in the treatment of lung cancer[16].

In the 1990s, with the development of whole-genome sequencing, especially the emergence of gene chips and second-generation sequencing, people began to believe that the genome is actually "generally transcribed"[17,18]. For example, studies have found that up to 70-90% sequences in the

Table II. Univariate analyses of lncRNA KCNQ1OT1 expression in 200 NSCLC patients.

Variables	Hazard ratio/CI (95%)	p
Gender	1.643/(0.583-2.215)	0.540
Age (years)	1.035/(0.672-2.033)	0.208
Smoking (years)	3.721/(1.814-5.943)	0.016
Tumor size (cm)	3.408/(1.232-7.167)	0.001
Lymph nodes metastasis	4.117/(3.204-6.553)	0.001
TNM stage	2.339/(1.530-4.365)	0.029

Table III. Multivariate analyses of lncRNA KCNQ1OT1 expression in 200 NSCLC patients.

Variables	Hazard ratio/CI (95%)	p
Gender	1.353/(0.643-1.871)	0.559
Age (years)	1.341/(0.523-1.987)	0.538
Smoking (years)	3.335/(1.652-4.433)	0.030
Tumor size (cm)	3.661/(1.802-5.540)	0.001
Lymph nodes metastasis	3.978/(3.074-5.507)	0.001
TNM stage	1.981/(1.141-3.361)	0.031

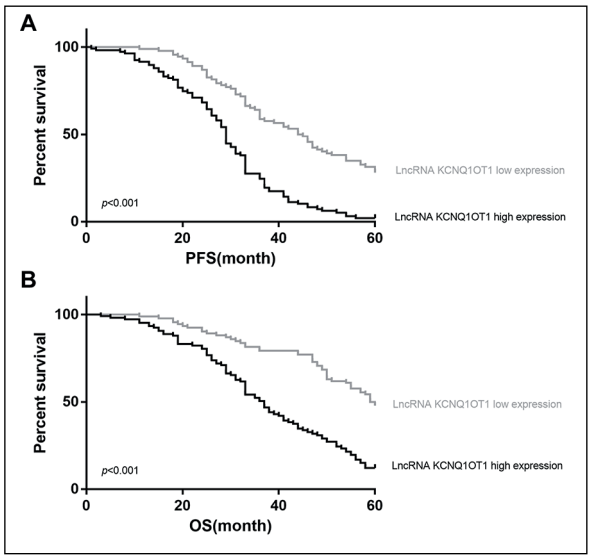

Figure 2. The relation between lncRNA KCNQ1OT1 expression with progression-free survival and overall survival of the NSCLC patients.

human genome are transcribed. Meanwhile, there are a large number of non-coding transcripts in the functional gene spacer. However, it was argued in the past that most of these transcriptions had no function and belonged to "transcriptional noise". In other words, ncRNAs did not exert any function actually. Currently, more and more ncRNAs have been proved to exert special and important functions. Moreover, they are associated with various biological processes, such as tumor formation, invasion, and metastasis. Therefore, ncRNAs are expected to become new tumor markers and therapeutic targets with good clinical application prospects in the diagnosis and treatment of malignancies[19-21].

LncRNAs are a kind of RNAs with about 200 bases in length. They lack a complete open reading frame and have little or no protein-encoding ability. Some studies have demonstrated that lncRNAs play important roles in cellular physiological and pathological activities. They also participate in the occurrence and development of various diseases including tumors. The dysregulation of HOTAIR, which is the most widely studied lncRNA, affects the prognosis of cancer patients[22-24]. Besides, the association between lncRNA expression and clinicopathological features and prognosis of lung cancer has also been studied. For example, MALAT-1, the first lncRNA studied in lung cancer, can serve as an independent prognostic marker for the prognosis of patients with early lung adenocarcinoma[25]. Moreover, it has been found that CCAT2 is highly expressed in the NSCLC tissues. The high expression of CCAT2 is related to lung adenocarcinoma, indicating that CCAT2 may be a specific lncRNA for lung adenocarcinoma[26]. In addition, GAS5 is lowly expressed in the NSCLC tissues, whose expression level is related to tumor size and clinical grade of NSCLC as well[27]. Furthermore, GAS6-AS1 is also lowly expressed in the NSCLC tissues. Its expression level is associated with lymph node metastasis and clinical grade of NSCLC, which can also be used as an independent prognostic marker for NSCLC patients[28]. However, the number of lncRNAs studied in lung cancer is still small.

KCNQ1OT1 is a lncRNA located in the KCNQ1 site. It is an imprinted gene, namely the expression of the homologous gene derived from only one parent, but not from the other parent. KCNQ1OT1 only expresses paternal allele, and its transcript regulates the centromere position located at chromosome 11p15.5[29]. Abnormal expression of the imprinted gene can induce a variety of human diseases, accompanied by complex mutations and phenotypic defects[30,31]. Moreover, the study of KCNQ1OT1 in tumors is still in the early stage. In addition, the exact role of KCNQ1OT1 in NSCLC remains unclear.

In the present work, the expression of lncRNA KCNQ1OT1 in NSCLC tissues and para-cancer tissues was detected *via* qRT-PCR. Meanwhile, its clinical significance was analyzed with clinical data. The results showed that the expression of lncRNA KCNQ1OT1 in NSCLC tissues was significantly higher than that of para-cancer tissues. The expression of lncRNA KCNQ1OT1 was significantly associated with tumor size, lymph node metastasis, TNM stage and smoking history, whereas was not correlated with age, gender, and pathological type. The survival analysis indicated that both overall survival and progression-free survival of patients in lncRNA KCNQ1OT1 high-expression group were remarkably lower than those of patients in the low-expression group. In addition, it was suggested that the lncRNA KCNQ1OT1 could be used as an independent marker for poor prognosis of NSCLC.

Conclusions

We showed that lncRNA KCNQ1OT1 is highly expressed in NSCLC tissues. Meanwhile, its high expression is associated with poor prognosis

of patients, which may be a potential molecular marker for the prognosis of NSCLC. However, due to the small sample size and the lack of relevant molecular biological and laboratory data, the specific role and molecular mechanism of KCNQ1OT1 in NSCLC remains to be further investigated.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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