European Review for Medical and Pharmacological Sciences 2018; 22: 2328-2333

LncRNA GHET1 predicts a poor prognosis of the patients with non-small cell lung cancer

Q.-M. SHEN[1,2], H.-Y. WANG[1,2], S. XU[1,2]

[1]China Medical University, Shenyang, China
[2]Department of Thoracic Surgery, The First Hospital Of China Medical University, Shenyang, China

Abstract. – OBJECTIVE: Long non-coding RNAs (LcRNAs) is related to lung cancer progression. This study aimed at exploring lncRNA GHET1 in non-small cell lung cancer (NSCLC).

PATIENTS AND METHODS: RT-PCR was used to detect lncRNA GHET1 expression in lung cancer specimen and cancer-adjacent areas. Kaplan-Meier assay was applied to investigate the prognosis of the patients with lung cancer.

RESULTS: The expression of lncRNA GHET1 in lung cancer specimen was significantly higher than that in the cancer-adjacent tissues, which was related to the tumor size, differentiation degree of tumor cells, and lymph node metastasis of clinical specimens. Moreover, lncRNA GHET1 predicted a poor prognosis for the patients with lung cancer.

CONCLUSIONS: LncRNA GHET1 might be a biomarker and molecular target of NSCLC, providing a potential therapeutic target of NSCLC.

Key Words:
LncRNA, Kaplan-Meier, NSCLC, Prognosis.

Introduction

Non-Small Cell Lung Cancer (NSCLC) accounts for 85% of all lung cancers. In recent years, with the development of molecular biology of lung cancer, there have been many new drugs, but the 5-year survival rate of patients with advanced lung cancer is still only 15%[1,2]. Therefore, it is a hot topic to explore new ways for treatment of the patients with NSCLC, especially the genesis, and to find effective targets of therapy.

Long non-coding RNAs (LncRNAs) are a class of RNAs longer than 200 nucleotides, but do not encode proteins and are considered as "dark matter" of gene transcription[3]. In recent years, it has been found that the abnormal expression of lncRNA was closely related to human diseases, and it may be a new therapy target in the processes of tumorigenesis, growth, invasion, metastasis, recurrence and drug resistance[4]. Highly expressed lncRNA GHET1 promotes cancer cell proliferation, inhibits apoptosis and can be used as a molecular marker for pancreatic cancer[5]. In gastric cancer, highly expressed lncRNA GHET1 promotes the multidrug resistance involving in Bax, Bcl-2, MDR1 and MRP1 expression[6]. Down-regulation of GHET1 suppresses cell proliferation, invasion and migration, enhances cell apoptosis and G1 phase, increases E-cadherin, and reduces fibronectin and vimentin[7]. In addition, Yang et al[8] reported that lncRNA GHET1 promotes gastric cancer cell proliferation by increasing c-Myc mRNA stability. In this work, the expression of lncRNA GHET1 in NSCLC patients and its corresponding normal lung tissues were detected, and the relationship between lncRNA GHET1 and clinical factors was analyzed so as to determine the potential role of lncRNA GHET1 in the early diagnosis and prognosis for NSCLC.

Patients and Methods

Clinical Specimen

A total of 105 NSCLC patients with complete clinical data were enrolled in our hospital. The study was approved by the Ethics Committee of the First Hospital of China Medical University, and all patients signed informed consent. Telephone and outpatient follow-ups were conducted for 105 patients including general information and clinical symptoms. The follow-ups started from the date of surgery or pathological biopsy until September 30, 2017.

Real-Time Polymerase Chain Reaction (PCR) Detection

Total RNA of tissue samples was extracted using TRIzol (Invitrogen, Carlsbad, CA, USA), and then reversely transcribed using the reverse transcription kit instructions (20 μL system).

Corresponding Author: Shun Xu, MD; e-mail: drxushun@sina.com

Real-time PCR reaction was conducted using 2 × SYBR Green PCR Master Mix, and the primer concentration was 0.4 μmol/L. The upstream and downstream primers were amplified with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as an internal reference.

Statistical Analysis

Statistical Product and Service Solutions (SPSS Inc., Chicago, IL, USA) 17.0 was used for data analysis. One-way ANOVA followed by Post Hoc Test (Least Significant Difference) was used to analyze the expression of lncRNA GHET1 in each group. The correlation of lncRNA GHET1 with clinicopathological parameters was analyzed by x^2-test. The correlation between lncRNA GHET1 expression and prognosis was analyzed by Kaplan-Meier method. The log-rank test was used for the prognosis of patients.

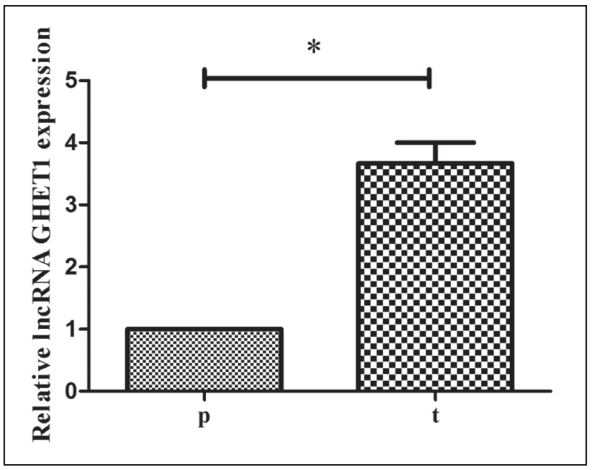

Figure 1. RT-PCR analysis showed that the relative lncRNA GHET1 expression is increased in tumor tissues compared to that in cancer-adjacent tissues. *$p < 0.05$.

Results

LncRNA GHET1 Was Over-Expressed in Lung Cancer

To explore the underlying functions of lncRNA GHET1 on the cancer progression, firstly, the expression level of lncRNA GHET1 was detected by using RT-PCR method. Figure 1 shows that the expression level of lncRNA GHET1 in NSCLC is higher than that in cancer-adjacent tissues, and the difference was statistically significant ($p < 0.05$). This finding indicated that dysregulation of lncRNA GHET1 expression may be an oncogene which plays a potential role in the development of lung cancer.

Over-Expression of LncRNA GHET1 Was Implicated in the Clinical Characteristics of NSCLC Patients

According to the expression level of lncRNA GHET1, 105 NSCLC patients were divided into lncRNA GHET1 high expression group (n=53) and low expression group (n=52). Analysis of the clinical characteristics of NSCLC patients showed that lncRNA GHET1 expression was correlated with disease stage, lymph node metastasis and tumor size, and the difference was statistically significant. However, there were no differences in the patient's age, gender and smoking history (Table I) ($p > 0.05$).

Aberrant of LncRNA GHET1 Expression Might be Related to the Survival Analysis of the Patients with NSCLC

Kaplan-Meier method was used to evaluate whether aberrant lncRNA GHET1 expression might be related to the survival analysis of the patients with NSCLC. Figure 2 shows that the overall survival (OS) of lncRNA GHET1 in low expression group was higher than that in high expression group, and the difference was statistically significant ($p < 0.001$). The progression-free survival (PFS) of patients with lncRNA GHET1 overexpression was shorter than that of the patients with low expression, and the difference was statistically significant ($p < 0.001$, Figure 3).

Table I. Correlation between lncRNA GHET1 expression and clinicopathological features in NSCLC patients.

Influencing factor	Regression coefficient	SE	Wald value	*p*-value	RR	95% CI
GHET1 expression	-0.618	0.782	0.017	< 0.05	0.838	0.389-2.729
Lymph node metastasis	-1.187	0.547	2.081	< 0.05	0.625	0.208-1.822
Disease stage	-1.212	0.621	1.572	< 0.05	0.448	0.127-1.489

SE: Standard error, RR: relative risk, CI: confidence interval.

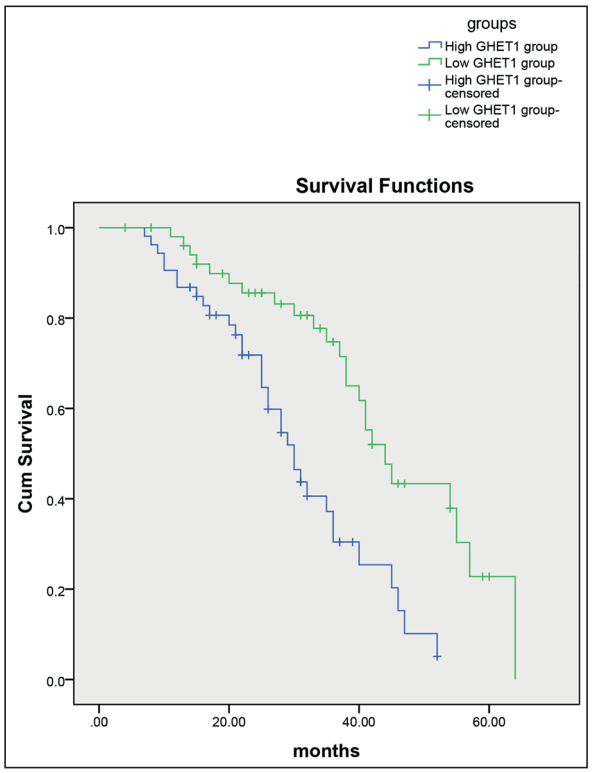

Figure 2. Kaplan-Meier method showed that the overall survival (OS) of lncRNA GHET1 low expression group is higher than the high expression group months.

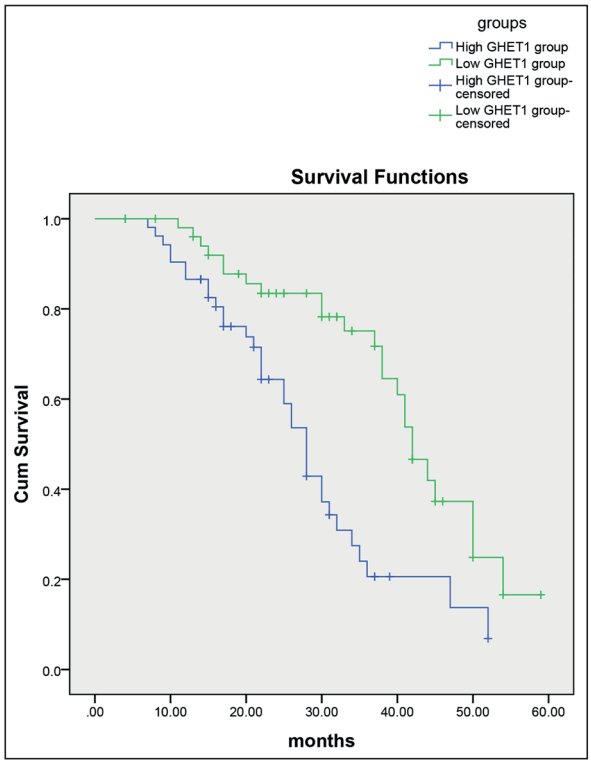

Figure 3. The progression-free survival (PFS) of patients with lncRNA GHET1 overexpression is shorter than that of the patients with low expression.

Furthermore, the multivariate cox analysis results showed lncRNA GHET1 expression, lymph node metastasis and disease stage were independent prognostic factors of NSCLC (Table II).

Table II. The multivariate cox analysis about independent prognostic factors of NSCLC.

		lncRNA GHET1		
Features	**Patients**	**High**	**Low**	***p***
Total	105	53	52	
Age (years)				0.285
≤ 50	49	22	27	
> 50	56	31	25	
Gender				0.382
Male	44	20	24	
Female	61	33	28	
Smoking history				0.759
Yes	59	29	30	
No	46	24	22	
Stage				0.003*
I-II	64	25	39	
III-IV	41	28	13	
Tumor size				0.000*
< 3 cm	60	20	40	
> 3 cm	45	33	12	
Lymph node metastasis				0.000*
No	66	21	45	
Yes	39	32	7	

*$p < 0.05$ was considered as statistically significant.

Discussion

Due to the lack of early diagnosis, many patients with NSCLC are already late to be diagnosed, thus missing the treatment[9, 10]. Targeted molecular therapy of patients with lung cancer can significantly improve the survival rate, but there are still a large number of patients with ineffective treatment with the 5-year survival rate of ≤ 20%[11-13]. Therefore, investigation of the molecular markers for the early diagnosis and treatment of NSCLC is particularly important.

As lung cancer-associated miRNAs are confirmed, lots of researchers are beginning to focus on another non-coding RNA, lncRNAs[14]. LncRNA is longer than 200 nucleotides with no or limited protein-coding ability. Depending on the intracellular location, lncRNAs regulate chromatin and gene regulation[15]. To date, more than 10,000 lncRNAs have been discovered but only < 1% have been annotated[16]. It is found that lncRNA regulates cell proliferation, growth and apoptosis. The abnormal expression of lncRNAs is closely related to tumorigenesis. More and more evidence shows that lncRNAs are involved in the pathogenesis and development of NSCLC[17,18]. LncRNA H19 regulates lung cancer cells resistance to cisplatin, and can be used as a prognostic biomarker[19]. LncRNA RMRP can promote the expression of KRAS, FMNL2 and SOX9 in lung cancer cells by inhibiting the expression of miR-206[20]. LncRNA-LET inhibits the growth of lung adenocarcinoma cells[21] and plays a crucial role in the epigenetic process[22]. The up-regulation of lncRNA-HIT promotes the migration and invasion of NSCLC by acting on ZEB1[23]. LncRNA-SNHG7 promotes the proliferation and migration of lung cancer by up-regulating FAIM2 expression[24]. MALAT1 can be used as an independent prognostic marker for the prognosis of patients with early stage lung adenocarcinoma[25].

LncRNA GHET1 predicts the poor prognosis in hepatocellular carcinoma and promotes cell proliferation by silencing KLF2[26]. LncRNA GHET1 activated by H3K27 acetylation promotes cell tumorigenesis through regulating ATF1 in hepatocellular carcinoma[27]. LncRNA GHET1 expression is significantly increased in ESCC tissues and cell lines, lncRNAGHET1 inhibition significantly decreases the expression of vimentin and N-cadherin, while it increases the expression of E-cadherin[28]. Knockdown of lncRNA GHET1 inhibits cell proliferation and invasion of colorectal cancer[29]. LncRNA GHET1 knockdown suppresses the proliferation and invasion of bladder cancer cells, and the inhibition of lncRNA GHET1 reversed the epithelial-mesenchymal-transition[30]. To date, lncRNA GHET1 expression in NSCLC and its significance are rarely reported. Here, we compared the expression of lncRNA GHET1 in 105 cases of NSCLC tissues and adjacent non-cancerous tissues, and found that the expression of lncRNA GHET1 in lung cancer tissues was significantly higher than that in paracancerous tissues. The expression of lncRNA GHET1 had no correlation with the age, sex and smoking history of patients, and was significantly correlated with the stage, tumor size, and lymph node metastasis. In addition, both OS and PFS of patients with high expression of lncRNA GHET1 were shorter as compared to low expression of lncRNA GHET1. Furthermore, the expression of lncRNA GHET1, lymphatic metastasis and disease staging were independent prognostic factors of NSCLC in multivariate Cox analysis.

Conclusions

The above results suggested that lncRNA GHET1 was involved in the regulation of the development of NSCLC and might be used as a potential molecular marker for the diagnosis and prognosis of NSCLC. However, the specific mechanism of lncRNA GHET1 in the regulation of the development of NSCLC and the signaling pathways need to be further studied.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Corallo S, D'Argento E, Strippoli A, Basso M, Monterisi S, Rossi S, Cassano A, Barone CM. Treatment options for EGFR T790M-negative EGFR tyrosine kinase inhibitor-resistant non-small cell lung cancer. Target Oncol 2017; 12: 153-161.

2) Daks A, Petukhov A, Fedorova O, Shuvalov O, Merkulov V, Vasileva E, Antonov A, Barlev NA. E3 ubiquitin ligase Pirh2 enhances tumorigenic properties of human non-small cell lung carcinoma cells. Genes Cancer 2016; 7: 383-393.

3) Tani H. Short-lived non-coding transcripts (SLiTs): clues to regulatory long non-coding RNA. Drug Discov Ther 2017; 11: 20-24.
4) Zhang D, Sun G, Zhang H, Tian J, Li Y. Long non-coding RNA ANRIL indicates a poor prognosis of cervical cancer and promotes carcinogenesis via PI3K/Akt pathways. Biomed Pharmacother 2017; 85: 511-516.
5) Zhou HY, Zhu H, Wu XY, Chen XD, Qiao ZG, Ling X, Yao XM, Tang JH. Expression and clinical significance of long-non-coding RNA GHET1 in pancreatic cancer. Eur Rev Med Pharmacol Sci 2017; 21: 5081-5088.
6) Zhang X, Bo P, Liu L, Zhang X, Li J. Overexpression of long non-coding RNA GHET1 promotes the development of multidrug resistance in gastric cancer cells. Biomed Pharmacother 2017; 92: 580-585.
7) Huang H, Liao W, Zhu X, Liu H, Cai L. Knockdown of long noncoding RNA GHET1 inhibits cell activation of gastric cancer. Biomed Pharmacother 2017; 92: 562-568.
8) Yang F, Xue X, Zheng L, Bi J, Zhou Y, Zhi K, Gu Y, Fang G. Long non-coding RNA GHET1 promotes gastric carcinoma cell proliferation by increasing c-Myc mRNA stability. FEBS J 2014; 281: 802-813.
9) Heist RS. First-line systemic therapy for non-small cell lung cancer. Hematol Oncol Clin North Am 2017; 31: 59-70.
10) Dinglin XX, Ma SX, Wang F, Li DL, Liang JZ, Chen XR, Liu Q, Zeng YD, Chen LK. Establishment of an adjusted prognosis analysis model for initially diagnosed non-small-cell lung cancer with brain metastases from sun Yat-Sen university cancer center. Clin Lung Cancer 2017; 18: e179-e186.
11) Han Y, Tian H, Chen P, Lin Q. TRIM47 overexpression is a poor prognostic factor and contributes to carcinogenesis in non-small cell lung carcinoma. Oncotarget 2017; 8: 22730-22740.
12) Park CK, Oh IJ, Kim KS, Choi YD, Jang TW, Kim YS, Lee KH, Shin KC, Jung CY, Yang SH, Ryu JS, Jang SH, Yoo SS, Yong SJ, Lee KY, In KH, Lee MK, Kim YC. Randomized phase III study of docetaxel plus cisplatin versus pemetrexed plus cisplatin as first-line treatment of nonsquamous non-small-cell lung cancer: A TRAIL trial. Clin Lung Cancer 2017; 18: e289-e296.
13) Raphael J, Chan K, Karim S, Kerbel R, Lam H, Santos KD, Saluja R, Verma S. Antiangiogenic therapy in advanced non-small-cell lung cancer: a meta-analysis of phase III randomized trials. Clin Lung Cancer 2017; 18: 345-353.
14) Liu Y, Xiao N, Xu SF. Decreased expression of long non-coding RNA LINC00261 is a prognostic marker for patients with non-small cell lung cancer: a preliminary study. Eur Rev Med Pharmacol Sci 2017; 21: 5691-5695.
15) Guo J, Cai H, Zheng J, Liu X, Liu Y, Ma J, Que Z, Gong W, Gao Y, Tao W, Xue Y. Long non-coding RNA NEAT1 regulates permeability of the blood-tumor barrier via miR-181d-5p-mediated expression changes in ZO-1, occludin, and claudin-5. Biochim Biophys Acta 2017; 1863: 2240-2254.
16) Wang A, Meng M, Zhao X, Kong L. Long non-coding RNA ENST00462717 suppresses the proliferation, survival, and migration by inhibiting MDM2/MAPK pathway in glioma. Biochem Biophys Res Commun 2017; 485: 513-521.
17) Xia S, Ji R, Zhan W. Long noncoding RNA papillary thyroid carcinoma susceptibility candidate 3 (PTCSC3) inhibits proliferation and invasion of glioma cells by suppressing the Wnt/beta-catenin signaling pathway. BMC Neurol 2017; 17: 30.
18) Xing B, Bai X, Guo H, Chen J, Hua L, Zhang J, Ma Q, Ren Q, Wang H, Wang J. Long non-coding RNA analysis of muscular responses to testosterone deficiency in Huainan male pigs. Anim Sci J 2017; 88: 1451-1456.
19) Wang Q, Cheng N, Li X, Pan H, Li C, Ren S, Su C, Cai W, Zhao C, Zhang L, Zhou C. Correlation of long non-coding RNA H19 expression with cisplatin-resistance and clinical outcome in lung adenocarcinoma. Oncotarget 2017; 8: 2558-2567.
20) Meng Q, Ren M, Li Y, Song X. LncRNA-RMRP acts as an oncogene in lung cancer. PLoS One 2016; 11: e164845.
21) Liu B, Pan CF, He ZC, Wang J, Wang PL, Ma T, Xia Y, Chen YJ. Long noncoding RNA-LET suppresses tumor growth and EMT in lung adenocarcinoma. Biomed Res Int 2016; 2016: 4693471.
22) Terashima M, Tange S, Ishimura A, Suzuki T. MEG3 long noncoding RNA contributes to the epigenetic regulation of epithelial-mesenchymal transition in lung cancer cell lines. J Biol Chem 2017; 292: 82-99.
23) Jia X, Wang Z, Qiu L, Yang Y, Wang Y, Chen Z, Liu Z, Yu L. Upregulation of LncRNA-HIT promotes migration and invasion of non-small cell lung cancer cells by association with ZEB1. Cancer Med 2016; 5: 3555-3563.
24) She K, Huang J, Zhou H, Huang T, Chen G, He J. LncRNA-SNHG7 promotes the proliferation, migration and invasion and inhibits apoptosis of lung cancer cells by enhancing the FAIM2 expression. Oncol Rep 2016; 36: 2673-2680.
25) Huang NS, Chi YY, Xue JY, Liu MY, Huang S, Mo M, Zhou SL, Wu J. Long non-coding RNA metastasis associated in lung adenocarcinoma transcript 1 (MALAT1) interacts with estrogen receptor and predicted poor survival in breast cancer. Oncotarget 2016; 7: 37957-37965.
26) Jin L, He Y, Tang S, Huang S. LncRNA GHET1 predicts poor prognosis in hepatocellular carcinoma and promotes cell proliferation by silencing KLF2. J Cell Physiol 2018; 233: 4726-4734.
27) Ding G, Li W, Liu J, Zeng Y, Mao C, Kang Y, Shang J. LncRNA GHET1 activated by H3K27 acetyl-

ation promotes cell tumorigenesis through regulating ATF1 in hepatocellular carcinoma. Biomed Pharmacother 2017; 94: 326-331.

28) Liu H, Zhen Q, Fan Y. LncRNA GHET1 promotes esophageal squamous cell carcinoma cells proliferation and invasion via induction of EMT. Int J Biol Markers 2017; 32: e403-e408.

29) Zhou J, Li X, Wu M, Lin C, Guo Y, Tian B. Knockdown of long noncoding RNA GHET1 inhibits cell proliferation and invasion of colorectal cancer. Oncol Res 2016; 23: 303-309.

30) Li LJ, Zhu JL, Bao WS, Chen DK, Huang WW, Weng ZL. Long noncoding RNA GHET1 promotes the development of bladder cancer. Int J Clin Exp Pathol 2014; 7: 7196-7205.