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ORIGINAL RESEARCH

High Expression of lncRNA HEIH is Helpful in the Diagnosis of Non-Small Cell Lung Cancer and Predicts Poor Prognosis

Chaowen He, Dongxuan Huang, Fan Yang, Dongsheng Huang, Yahui Cao, Jianfeng Peng, Xiaohua Luo

Department of Pulmonary and Critical Care Medicine, Shenzhen Longhua District Central Hospital, Shenzhen, 518110, Guangdong, People's Republic of China

Correspondence: Chaowen He, Department of Pulmonary and Critical Care Medicine, Shenzhen Longhua District Central Hospital, No. 187 Guanlan Avenue, Longhua District, Shenzhen, 518110, Guangdong, People's Republic of China, Tel +86-18123964996, Email chaowh0331@163.com

Background: This study aims to investigate the expression and clinical value of long non-coding RNA (lncRNA) HEIH in peripheral blood of patients with non-small cell lung cancer (NSCLC).

Methods: Healthy subjects (N=70), patients with lung squamous cell carcinoma (LUSC, N=70) and patients with lung adenocarcinoma (LUAD, N=80) were included. LncRNA HEIH expression in peripheral blood of included subjects was detected using RT-qPCR. According to the median expression of lncRNA HEIH, LUSC and LUAD patients were allocated into lncRNA HEIH high/low expression groups. The correlation between lncRNA HEIH and clinical indicators of patients was analyzed; Logistic multifactor regression was used to analyze the independent risk factors influencing lncRNA HEIH level. Receiver-operating characteristic (ROC) curve was used to evaluate the diagnostic efficacy of lncRNA HEIH and carcinoembryonic antigen (CEA) in LUSC/LUAD patients. MedCalc-Comparison of ROC curves was used to compare the area under ROC curve. The cumulative survival rates of lncRNA HEIH high/low expression group were analyzed by Kaplan–Meier curve. COX multivariate analysis was used to assess the independent factors affecting prognosis of NSCLC.

Results: LncRNA HEIH in peripheral blood of LUSC/LUAD patients was higher than that in healthy controls, with no evident difference between LUSC and LUAD groups. In LUSC/LUAD patients, TNM stage, lymph node metastasis, distal metastasis, and CEA were independent risk factors affecting lncRNA HEIH; patients with high lncRNA HEIH expression had larger pack-years and tumor size, higher CEA level and tumor stage, and higher risk of lymph node metastasis and distal metastasis. LncRNA HEIH had higher diagnostic efficiency than CEA in NSCLC patients. High expression of lncRNA HEIH predicted poor prognosis in patients with NSCLC and was an independent risk factor for prognosis of NSCLC.

Conclusion: High expression of lncRNA HEIH is helpful in the diagnosis of NSCLC and predicts poor prognosis.

Keywords: non-small cell lung cancer, long non-coding RNA HEIH, prognosis, carcinoembryonic antigen, lung squamous cell carcinoma, lung adenocarcinoma, peripheral blood

Introduction

According to the data from China Cancer Registry Center, the incidence and mortality of lung cancer rank the first among malignant tumors in the world,[1] and about 2 million patients die of lung cancer every year,[2] among which non-small cell lung cancer (NSCLC) accounted for 80%~85%.[3] NSCLC can be divided into lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUCC) and large cell carcinoma (LCC) according to its pathological features. LUAD and LUSC accounted for approximately 50% and 40% of NSCLC patients, respectively.[4] LUAD is more common in women and smokers and is the main pathological subtype of lung tumors; there are no special symptoms in the early stage, mainly manifesting as respiratory diseases, including cough, low fever, and chest pain.[5,6] LUSC is more common in older men and has historically been strongly associated with smoking, and the main symptoms are fever, cough, hemoptysis, and chest pain.[7] In addition, genes in LUSC and LUAD patients were expressed differently.[8] For example, the diagnostic

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marker CEP55 was significantly differentially expressed in LUSC and LUAD.[9] Due to the atypical early clinical manifestations of the disease and the lack of biological markers for early diagnosis, about 75% of the patients were already in the middle and advanced stage when detected, and the tumor cells had lymphatic metastasis and distant metastasis, thus losing the best opportunity for surgical treatment and resulting in poor prognosis.[10] Therefore, it is of great significance to study the mechanism of the occurrence and development of NSCLC and to obtain novel biomarkers that can be used for early diagnosis, prognosis evaluation and treatment of NSCLC.

Long non-coding RNA (lncRNA) is a class of RNA molecules with a transcript length of more than 200 nt without coding protein function, which can regulate gene expression level, post-transcriptional modification, binding to transcription factors or miRNAs, and play a regulatory role in many biological processes.[11] A study has discovered that lncRNAs regulate cell proliferation, growth, and apoptosis, and the abnormal expression of lncRNA is closely linked to the occurrence of tumor.[12] More and more evidence indicates the involvement of lncRNAs in the pathogenesis and development of NSCLC.[13,14] For instance, lncRNA FeZF1-AS1 is up-regulated in lung cancer and promotes NSCLC through the ITGA11/miR-516b-5p axis;[15] LncRNA LCAT1, as an oncogene, can inhibit the growth of lung cancer cells and inhibit the tumorigenesis and metastasis of xenograft mice after knockout.[16] Additionally, lncRNA PCAT6 is highly expressed in NSCLC as an oncogene, and knockdown of PCAT6 inhibits NSCLC cell growth by inducing cell cycle arrest and apoptosis at the G1 phase.[17] At present, several lncRNAs[18] that can be used as candidate tumor biomarkers have been detected in the body fluids of patients, and their research as NSCLC specific biomarkers has been widely reported.[19–21] High expression in hepatocellular carcinoma (HEIH) is a lncRNA originally found in HBV-induced hepatocellular carcinoma.[22] In patients with hepatocellular carcinoma, high expression of HEIH is associated with an increased risk of recurrence and declined overall survival after surgery. Recent studies have demonstrated that HEIH is also highly expressed in other types of cancers including colorectal cancer, melanoma and NSCLC.[23–25] Jia et al found that HEIH was markedly overexpressed in NSCLC tissues and cell lines, which promoted the proliferation and metastasis of NSCLC cells.[24] However, the expression level of HEIH in peripheral blood of NSCLC and its clinical value in the diagnosis and prognosis of NSCLC have not been reported yet. This study herein investigated the expression level of lncRNA HEIH in peripheral blood of NSCLC patients and explored its clinical value in the diagnosis and prognosis of NSCLC.

Materials and Methods

Ethics Statement

The recruitment procedure was in accordance with the principles of the Declaration of Helsinki of the World Medical Association. All subjects signed a written informed consent. The study protocol was approved by the ethics committee of Shenzhen Longhua district central hospital (AF/SC-08/01.0).

Study Subjects

We used the software Gpower to pre-estimate the samples. Briefly, we took the effect value as the median value of the system recommendation (effect size d = 0.5), set the parameter $\alpha = 0.05$, and set the statistical efficacy $1 - \beta = 0.9$. *P* value was obtained from the bilateral test, and the sample ratio of normal group/disease group = 1/2. The calculated sample size was as follows: normal group ≥ 64, and the NSCLC group ≥ 128. Considering the sample loss (10–15%) and the grouping of NSCLC (LUSC and LUAD groups), we finally obtained a sample size of 70 cases in the normal group and 150 cases in the NSCLC group (LUSC: 70 cases, LUAD: 80 cases) (Supplementary Figure 1). Gpower software was used to calculate the statistical power of the lncRNA HEIH expression difference between the normal group and NSCLC group. According to the effect size d = mean difference/mean standard variance, we calculated that effect size d =1.91. Meanwhile, we set the parameter α=0.05, the sample size was 70 and 150 and calculated the statistical efficacy as $1-\beta >$ 0.95 (Supplementary Figure 2), indicating that the selected sample size was statistically significant.

Based on the Gpower analysis, 150 patients with NSCLC admitted to the Department of Respiratory Medicine of Shenzhen Longhua district central hospital from December 2013 to December 2015 were selected as the study subjects. According to the 2004 World Health Organization (WHO) classification of lung tumors,[26] NSCLC patients were further divided into 80 lung adenocarcinoma (LUAD) patients and 70 lung squamous cell carcinoma (LUSC) patients. Inclusion

criteria for NSCLC were as follows: (a) Having typical clinical manifestations of lung cancer; (b) All cases were diagnosed by histopathology or cytology; and (c) All cases were new and had not received surgery, radiotherapy, chemotherapy or targeted therapy. Exclusion criteria were as follows: (a) A history of tuberculosis; (b) Patients with diabetes; (c) Accompanied by hypertension, hyperlipidemia or hyperglycemia; (d) Suffering from other malignant tumors; (e) Prolonged use of immunosuppressant and steroid hormones; (f) Failure to follow up regularly; and (g) Pregnant or lactating women. Meanwhile, 70 healthy volunteers who came for physical examination at the same period were selected as the control group.

Data and Sample Collection

The age (≤60; >60), gender, smoking, pack-years (< 20; ≥20), comorbidities, and other baseline clinical data of enrolled subjects were recorded, as well as tumor size (≤3 cm; >3 cm), tumor-node-metastasis (TNM) stage (I; II/III), lymph node metastasis (Absent; Present), distal metastasis and carcinoembryonic antigen (CEA) (μg/mL). Among them, comorbidities refer to patients complicated with diseases in the heart, cerebrovascular, respiratory and urinary system or dysfunction. The cumulative illness rating scale for geriatrics was adopted to score the comorbidities of 14 organ systems. According to the severity of disease, the disease score on each scale was rated on a scale of 0–4 points (0: no disease; 1: the current mild disease or past serious disease; 2: moderate dysfunction or disease, requiring first-line treatment; 3: serious/persistent aboriginal diseases; 4: extremely severe damage or failure of the function that needs immediate treatment). The severity index (SI) was used as the evaluation index of comorbidities (SI = total score/number of comorbidities). According to SI, the comorbidities were classified into 3 levels: no comorbidities (SI = 0), mild comorbidities (SI ≤ 2), and severe comorbidities (SI > 2). A total of 2 mL fasting peripheral blood was collected from the vein of all patients without preoperative chemotherapy or radiotherapy, and centrifuged at 4°C and 2000 g for 10 min, and the supernatant was transferred to an EP tube and stored at −80°C until determination.

Enzyme-Linked Immunosorbent Assay (ELISA)

Human CEA levels in peripheral blood of NSCLC patients and healthy subjects were detected using human CEA ELISA Kit (Amyjet Scientific., Wuhan, China).

Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR)

Trizol reagent (Invitrogen, Carlsbad, CA, USA) was used to extract total RNA. The total RNA was transcribed into cDNA by Primescript RT reagent kit (Takara, Dalian, China) and the qPCR assay was performed on the ABI7900HT Fast PCR Real-Time System (Applied Biosystems, Foster city, CA, USA) using SYBR® Prepremix Ex Taq™ II (Takara, Dalian, China). The reaction conditions included pre-denaturation at 95°C for 10 min, and 40 cycles of denaturation at 95°C for 10s, annealing at 60°C for 20s, and extension at 72°C for 34s. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as internal reference, and the data were analyzed by the $2^{-\Delta\Delta CT}$ method.[27] The primers were synthesized by Sangon Bioengineering Shanghai Co., Ltd (Shanghai, China), and the sequences are shown in Table 1.

Statistical Analysis

Statistical software SPSS 21.0 (IBM Corp. Armonk, NY, USA), GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA) and Medcalc® version 15.0 (Medcalc Software Ltd, Ostend, Belgium) were used for data analysis and map plotting. Gpower was used for sample size pre-estimation. Shapiro–Wilk (*W*-test) test showed that data of numerical variables were normally distributed and were expressed as mean ± standard deviation. Unpaired *t*-test was used for comparison between two groups, one-way analysis of variance (ANOVA) was used for comparison among multiple

Table 1 Primer Sequence

Gene	Forward 5'-3'	Reverse 5'-3'
LncRNA HEIH	GCGAGGAGAGACTTCCACAG	GGGGTTGAACAAAGTGGAGA
GAPDH	CTCAGACACCATGGGGAAGGTGA	ATGATCTTGAGGCTGTTGTCATA

groups, and Tukey's multiple comparisons test was used for post hoc test. Fisher's exact test was used for comparative analysis of classification variables. Receiver operating characteristic curve (ROC) was used to analyze the diagnostic value of lncRNA HEIH for NSCLC. Kaplan-Meier method was used to analyze the effect of lncRNA HEIH on the prognosis of NSCLC patients. Logistic multifactor regression analysis was used to evaluate the influencing factors of lncRNA HEIH expression. COX regression test was used to analyze independent prognostic factors of NSCLC. The area difference under the ROC curve was analyzed by MedCalc-comparison of ROC curves. A value of $P < 0.05$ was indicative of statistically significant.

Results

Comparison of Clinicopathological Features Between Patients with NSCLC and Healthy Subjects

A total of 220 subjects were included in this study, including 70 healthy subjects, 70 LUSC patients, and 80 LUAD patients. The comparative analysis of the clinical data of LUSC and LUAD patients and healthy subjects manifested that there were no significant differences in age, gender and smoking among the three groups ($P > 0.05$), while the pack-years, comorbidities, and serum CEA were significantly different in LUSC and LUAD patients compared with the control group (all $P < 0.05$). The comparative analysis of clinical baseline data showed no obvious difference in age, gender, smoking, pack-years, tumor size, TNM stage, lymph node metastasis, distal metastasis, CEA level and comorbidities between LUSC and LUAD patients (all $P > 0.05$) (Table 2).

High Expression of lncRNA HEIH in Peripheral Blood of NSCLC Patients

We detected the expression of lncRNA HEIH in peripheral blood of LUSC, LUAD patients and healthy subjects by qRT-PCR. LncRNA HEIH in peripheral blood of LUSC and LUAD patients was obviously higher than that of healthy controls (all $P < 0.01$), with no significant difference between LUSC and LUAD groups ($P > 0.05$) (Figure 1).

Correlation Analysis Between lncRNA HEIH Levels and Clinical Indexes of NSCLC Patients

To further study the relationship between lncRNA HEIH expression and clinical indicators of NSCLC patients, we assigned LUSC and LUAD patients into lncRNA HEIH low expression group and lncRNA HEIH high expression group according to lncRNA HEIH median level in LUSC and LUAD. In LUSC and LUAD patients, there was no evident difference in age, gender, smoking, and comorbidities between the lncRNA HEIH low and high expression groups, while the lncRNA HEIH high expression group had larger pack-years, tumor size, higher tumor stage and higher CEA level, and higher risk of lymph node metastasis and distal metastasis (all $P < 0.05$) (Table 3).

In addition, Logistic regression was conducted to analyze the factors influencing the expression of lncRNA HEIH in peripheral blood of NSCLC patients. With lncRNA HEIH expression as a dependent variable, the pack-years, tumor size, TNM stage, lymph node metastasis, distant metastasis, and CEA ($P < 0.1$) in Table 3 were incorporated into the binary Logistic regression equation as independent variables. The results showed that TNM stage, lymph node metastasis, distal metastasis, and CEA were independent risk factors affecting the expression of lncRNA HEIH in LUSC and LUAD (all $P < 0.05$) (Table 4).

LncRNA HEIH Has High Diagnostic Value in NSCLC Patients

Tumor marker CEA has been reported as a biomarker for the auxiliary diagnosis and treatment effect of NSCLC.[28–31] We evaluated the diagnostic efficacy of lncRNA HEIH and CEA in LUSC and LUAD patients through ROC curve analysis. The area under ROC curve of CEA in the diagnosis of LUSC patients was 0.706, the sensitivity was 31.43%, and the specificity was 100.00%. The area under ROC curve for lncRNA HEIH in the diagnosis of LUSC patients was 0.860, the sensitivity was 72.86%, and the specificity was 95.71% (Figure 2A). MedCalc-comparison of ROC curves showed that the area under ROC curve of lncRNA HEIH was significantly higher than that of CEA ($P = 0.0031$; 95% CI = 0.052~0.255), indicating that lncRNA HEIH had a higher diagnostic efficiency for LUSC than CEA. In addition, the

Table 2 Comparative Analysis of Clinical Data Between NSCLC Patients and Healthy Subjects

Parameters		Control (N=70)	LUSC (N=70)	LUAD (N=80)	P_a	P_b	P_c
Age (years)	≤ 60	33	28	30	0.495	0.250	0.867
	> 60	37	42	50			
Gender	Male	38	41	49	0.733	0.411	0.742
	Female	32	29	31			
Smoke	Yes	32	36	43	0.612	0.413	0.870
	No	38	34	37			
Pack-years	< 20	59	47	51	0.029	0.005	0.732
	≥ 20	11	23	29			
Tumor size	≤ 3 cm	-	35	44	-	-	
	> 3 cm	-	35	36	-	-	0.510
TNM stage	I	-	47	52	-	-	0.863
	II–III	-	23	28	-	-	
Lymph node metastasis	Absent	-	32	35	-	-	0.870
	Present	-	38	45	-	-	
Distant metastasis	Absent	-	39	46	-	-	0.870
	Present	-	31	34	-	-	
CEA (μg/L)		5.24 ± 1.32	6.33 ± 1.36	6.69 ± 1.41	< 0.001	< 0.001	0.127
Comorbidities	No	32	18	19	0.047	0.018	0.962
	Mild case	21	29	34			
	Severe case	17	23	27			

Notes: Fisher's Exact test was used for comparative analysis of classification variables. Unpaired *t* test was used for comparison of continuous variables between two groups. A value of $P < 0.05$ was indicative of statistically significant. Pa: LUSC group was compared with the control group; Pb: LUAD group was compared with the control group; Pc: LUSC group was compared with LUAD group.

Abbreviations: LUSC, lung squamous cell carcinoma; LUAD, lung adenocarcinoma; TNM, T: extent of the primary tumor; N: lymph node involvement; M: metastatic disease; CEA, carcinoma embryonic antigen.

area under ROC curve of lncRNA HEIH in the diagnosis of LUAD patients was 0.905, the sensitivity was 77.50%, and the specificity was 95.71%. The area under ROC curve of LUAD patients diagnosed by CEA was 0.763, the sensitivity was 50.00%, and the specificity was 90.00% (Figure 2B). MedCalc-comparison of ROC curves showed that the area under ROC curve of lncRNA HEIH was significantly higher than that of CEA ($P = 0.0011$; 95% CI = 0.057–0.228), indicating that lncRNA HEIH had a higher diagnostic efficacy than CEA for LUAD. The above data indicate that lncRNA HEIH has a high diagnostic efficacy in NSCLC patients.

High Expression of lncRNA HEIH Predicts Poor Prognosis of NSCLC

Furthermore, we analyzed the prognostic value of lncRNA HEIH in peripheral blood on NSCLC. According to the median level of lncRNA HEIH in LUSC and LUAD, patients with LUSC and LUAD were divided into lncRNA HEIH low expression group and lncRNA HEIH high expression group. Patients with NSCLC after the operation were followed up every 3 months for 60 months and the survival of the patients was recorded. The follow-up results showed that during the follow-up period, a total of 27 LUSC patients died at the end of the follow-up, including 18 cases in the high expression group and 9 cases in the low expression group. The cumulative survival rate in the lncRNA HEIH high expression group was evidently lower than that in the low expression group ($P = 0.0252$) (Figure 3A). A total of 34 LUAD patients died at the end of follow-up, including 24 cases in the high expression group and 10 cases in the low expression group. The cumulative survival rate in the lncRNA HEIH high expression group was notably lower than that of the low expression group ($P = 0.0027$) (Figure 3B). These results suggest that lncRNA HEIH overexpression predicts poor prognosis in patients with NSCLC.

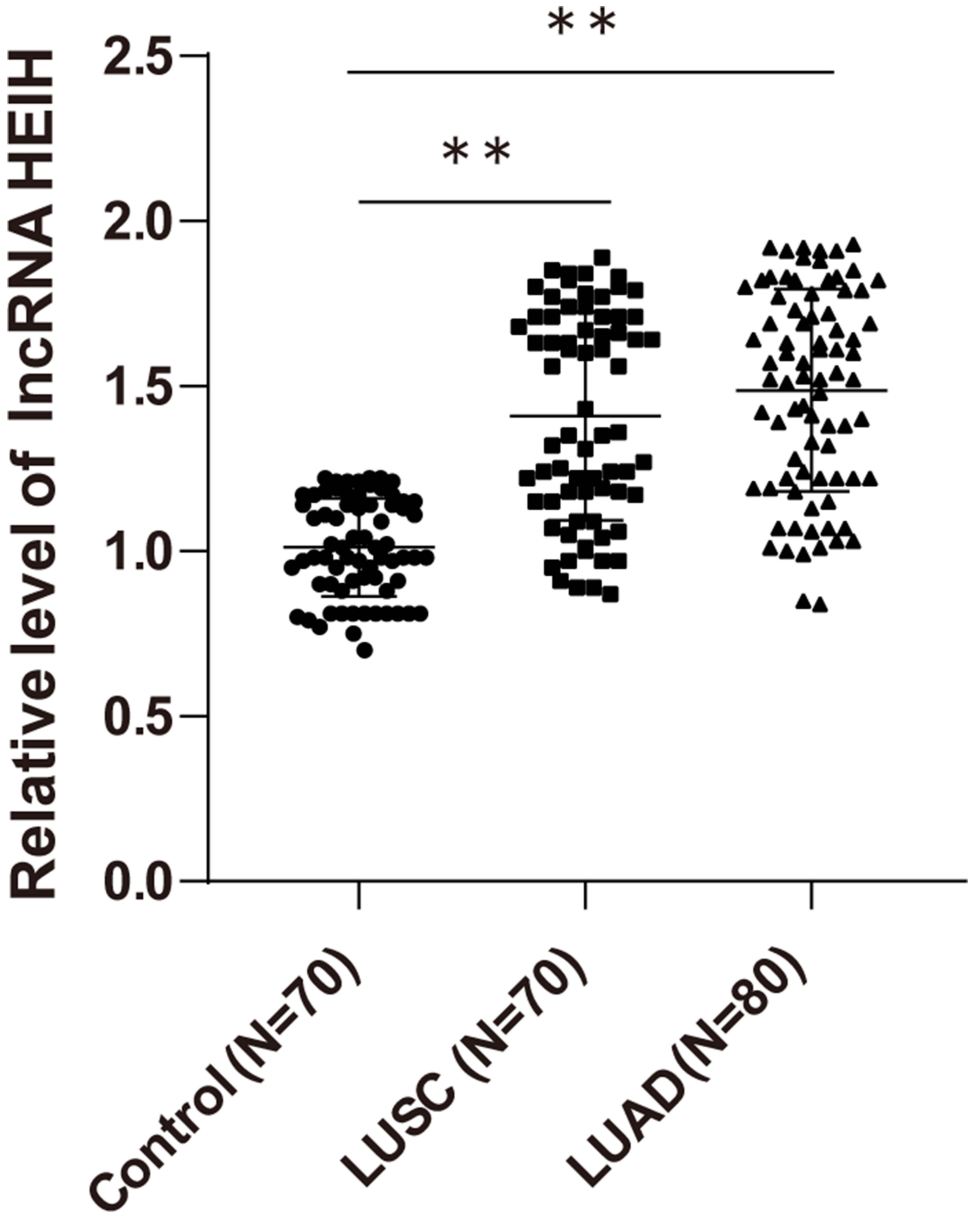

Figure 1 High expression of lncRNA HEIH in peripheral blood of NSCLC patients. The expression of lncRNA HEIH in peripheral blood of NSCLC patients was detected by qRT-PCR. The values were expressed as mean ± standard deviation. One-way ANOVA was used for data comparison among multiple groups. Tukey's multiple comparisons test was used for post hoc test, $^{**}p < 0.01$.

LncRNA HEIH Expression Was an Independent Prognostic Risk Factor for NSCLC

To further evaluate the effect of lncRNA HEIH on the prognosis of NSCLC, we took the survival of patients as the dependent variable, and included age, smoking, pack-years, tumor diameter, TNM stage, lymph node metastasis, distal metastasis, CEA, comorbidities and LncRNA HEIH as independent variables into Cox multivariate regression analysis model according to the analysis in Table 3 and the possible risk factors affecting the prognosis of NSCLC as previously reported.[32] The results showed that lncRNA HEIH was an independent prognostic risk factor for LUSC patients after adjustment for pack-years and CEA level (P=0.035, HR=9.752, 95% CI: 1.175–80.898); After adjusting for pack-years and CEA level, lncRNA HEIH was an independent prognostic risk factor for LUAD patients (P=0.034, HR=6.623, 95% CI: 1.156–37.950) (Tables 5 and 6).

Table 3 Correlation Analysis of lncRNA HEIH Expression in Peripheral Blood of NSCLC Patients and Clinical Indicators

Parameters		LUSC				LUAD			
		Total	Low Expression	High Expression	*P*	Total	Low Expression	High Expression	*P*
		(N=70)	(N = 35)	(N = 35)		(N=80)	(N = 40)	(N = 40)	
Age (years)	≤ 60	28	12	16	0.465	30	14	16	0.818
	> 60	42	23	19		50	26	24	
Gender	Male	41	20	21	>	49	27	22	0.359
	Female	29	15	14	0.999	31	13	18	
Smoke	Yes	36	17	19	0.811	43	23	20	0.846
	No	34	18	16		37	17	20	
Pack-years	< 20	47	29	18	0.01	51	32	19	0.005
	≥ 20	23	6	17		29	8	21	
Tumor size	≤ 3 cm	35	23	12	0.016	44	28	16	0.013
	> 3 cm	35	12	23		36	12	24	
TNM stage	I	47	31	16	<	52	34	18	<
	II–III	23	4	19	0.001	28	6	22	0.001
Lymph node	Absent	32	22	10	0.008	35	23	12	0.024
metastasis	Present	38	13	25		45	17	28	
Distant metastasis	Absent	39	25	14	0.016	46	29	17	0.012
	Present	31	10	21		34	11	23	
CEA (μg/L)		6.33 ± 1.36	5.64 ± 1.22	7.02 ± 1.14	< 0.001	6.69 ± 1.41	6.08 ± 1.45	7.26 ± 1.17	<0.001
Comorbidities	No	18	8	10	0.861	19	10	9	0.361
	Mild case	29	15	14		34	14	20	
	Severe case	23	12	11		27	16	11	

Notes: Fisher's Exact test was used for comparative analysis of classification variables. Unpaired *t* test was used for comparison of continuous variables between two groups. A value of $P < 0.05$ was indicative of statistically significant.

Abbreviations: LUSC, lung squamous cell carcinoma; LUAD, lung adenocarcinoma; TNM, T: extent of the primary tumor; N: lymph node involvement; M: metastatic disease; CEA, carcinoma embryonic antigen.

Table 4 Logistic Multifactor Regression Analysis of Influencing lncRNA HEIH Expression

Clinical Features	LUSC			LUAD		
	P value	OR	95% CI	*P* value	OR	95% CI
Pack-years	0.094	5.779	0.743~44.943	0.128	3.1	0.723~13.288
Tumor size	0.137	4.665	0.612~35.581	0.255	2.08	0.589~7.339
TNM stage	0.003	21.482	2.769~166.635	0.005	6.942	1.794~26.857
Lymph node metastasis	0.018	9.273	1.477~58.232	0.023	4.667	1.243~17.531
Distant metastasis	0.008	15.199	2.010~114.915	0.021	4.707	1.264~17.525
CEA	0.011	2.913	1.280~6.629	0.018	1.945	1.121~3.374

Discussion

LncRNAs are important in gene regulation.[33] LncRNA interacts with many transcription factors and affects lung cancer growth and spread.[13,34] To date, many lncRNAs have been shown to be associated with the diagnosis and prognosis of NSCLC.[35] It was reported that lncRNA DNS-AS1 up-regulated the protein level of anti-apoptotic factor Bcl-2 and promoted the growth, migration, and invasion of LUAD cells.[36,37] Meanwhile, lncRNA XIST showed carcinogenic properties in

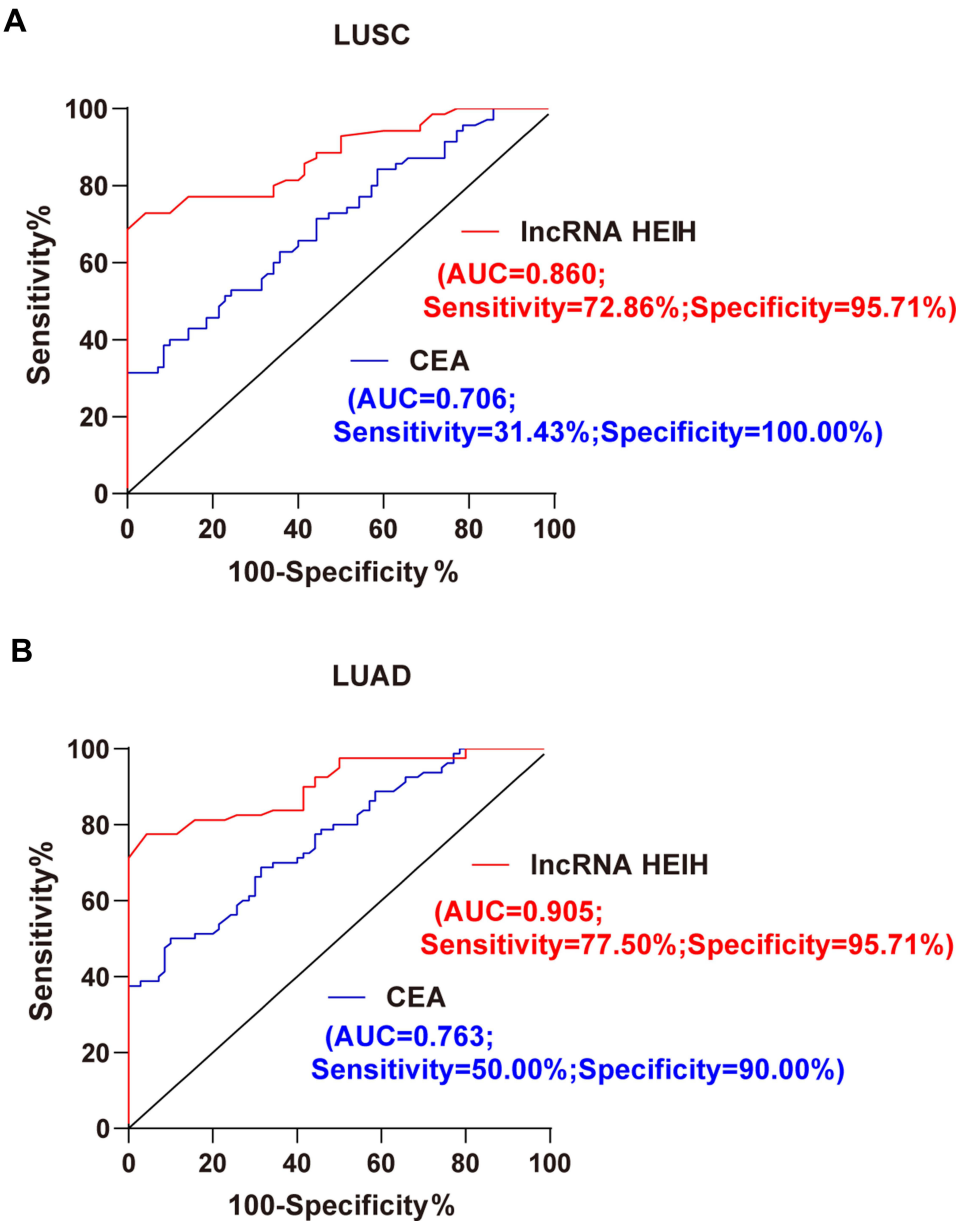

Figure 2 LncRNA HEIH has a high diagnostic efficacy in NSCLC patients. (**A**) The diagnostic efficacy of lncRNA HEIH and CEA in LUSC patients was evaluated by ROC curve analysis; (**B**) the diagnostic efficacy of lncRNA HEIH and CEA in LUAD patients was evaluated by ROC curve analysis. MedCalc-comparison of ROC curves was used to compare and analyze the area difference under the ROC curve.

NSCLC by regulating miR-449a and Bcl-2.[38] Moreover, knockdown of lncRNA NEAT 1 attenuated the expression of hypoxia-inducible factor 1, thus affecting the tumor-like phenotype of pulmonary bronchial epithelial cells.[39] HEIH is a lncRNA[22] originally found in HBV-induced hepatocellular carcinoma, and is highly expressed in NSCLC tissues and cell lines, which can promote the proliferation and metastasis of NSCLC.[24] This paper highlighted that the high expression of lncRNA HEIH in peripheral blood of NSCLC patients can assist in the diagnosis of NSCLC and predict poor prognosis.

A total of 220 subjects were included in this study, including 70 healthy subjects, 70 patients with LUSC, and 80 patients with LUAD. As a biomarker, tumor marker CEA can play a role as a predictor and prognostic factor in cancer patients.[40,41] Intraoperative CEA monitoring can provide more valuable prognostic information for patients with LUAD, and patients with normal or elevated postoperative CEA level have worse overall survival than those with normal preoperative CEA level.[42] Stage-specific embryonic antigen-4 was reported to be expressed in basal-like lung cancer and

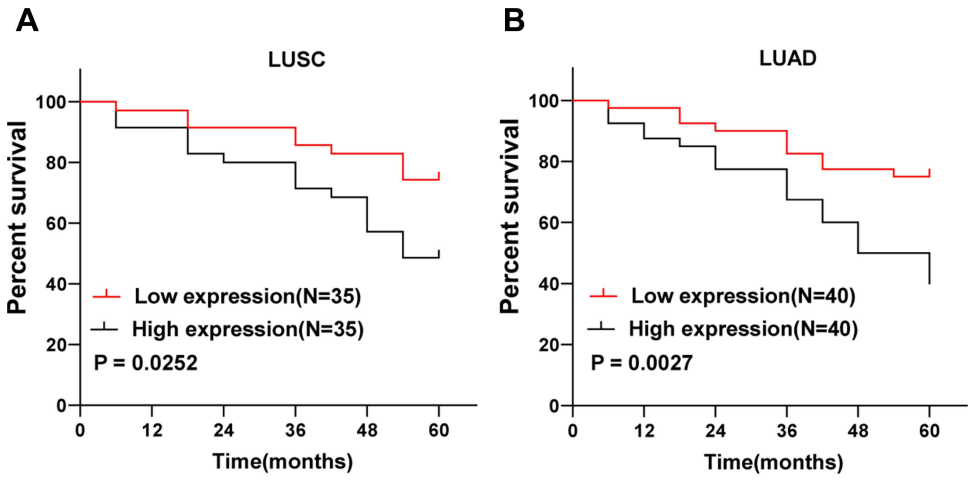

Figure 3 LncRNA HEIH high expression predicts poor prognosis of NSCLC. In (**A**) LUSC and (**B**) LUAD patients, the cumulative survival rates of lncRNA HEIH high expression group and low expression group were analyzed by Kaplan–Meier curve, and the difference in cumulative survival rates between groups was determined by Log rank test.

is associated with poor prognosis.[32] Smoking cessation therapy can obviously improve lung function parameters and reduce serum CEA levels.[43] However, CEA as a traditional tumor marker has limited sensitivity and specificity, and miRNAs have higher diagnostic and prognostic value.[44] In the present study, serum CEA levels in LUSC and LUAD patients were higher than those in controls. Consistently, CEA has been identified as a biomarker to assist in the diagnosis of NSCLC.[30,31] Taken together, high serum CEA level can be used for the preliminary diagnosis of NSCLC.

Next, it was found that LncRNA HEIH in peripheral blood of LUSC and LUAD patients was higher than that of healthy controls, while lncRNA HEIH expression between LUSC and LUAD groups had no significant difference. This result is consistent with previous reports that lncRNA HEIH is highly expressed in NSCLC tissues and cell lines.[24] Briefly, lncRNA HEIH level can be used as a potential diagnostic indicator for NSCLC, but it is not yet clear to distinguish LUSC from LUAD. To further study the relationship between lncRNA HEIH expression and clinical indicators in NSCLC patients, LUSC and LUAD patients were divided into a low-expression group and a high-expression group, the lncRNA HEIH overexpression group had larger tumor size, higher tumor stage and higher CEA levels, and higher risk of lymph node metastasis and distal metastasis. Similarly, lncRNA-HEIH is highly expressed in melanoma tissues and cell lines, which is associated with late clinical stage and predicts poor prognosis in melanoma patients.[25] LncRNA HEIH high expression in gastric cancer patients is closely related to medium-high differentiation, distant metastasis, lymph node metastasis, and deeper tumor invasion.[45] Altogether, high expression of lncRNA HEIH is associated with poorer clinical indicators and higher cancer staging.

Table 5 Meaning of Variable and Assignment Method

Factors	Meaning	Assignment
y	Prognosis	y=1 death, y=0 survival
x_1	Age	≤ 60=0, > 60=1
x_2	Smoke	No smoking=0, smoking=1
x_3	Pack-years	< 20=0; ≥ 20=1
x_4	Tumor size	≤ 3cm=0, > 3cm=1
x_5	TNM stage	Grade II–III=1, Grade I=0
x_6	Lymph node metastasis	Present=1, Absent=0
x_7	Distant metastasis	Present=1, Absent=0
x_8	CEA	Continuous variables (μg/L)
x_9	Comorbidities	No=0; Mild=1; Severe=2
x_{10}	LncRNA	Continuous variables (μg/L)

Table 6 Cox Multivariate Analysis of Independent Factors Influencing NSCLC Prognosis

Clinical Features	LUSC			LUAD		
	P value	HR	95% CI	*P* value	HR	95% CI
Age	0.796	0.885	0.352~2.228	0.867	1.077	0.451~2.570
Smoke	0.831	0.904	0.360~2.275	0.918	0.958	0.419~2.189
Pack-years	0.037	2.457	1.057~5.710	0.027	2.897	1.129~7.433
Tumor size	0.281	1.679	0.655~4.306	0.565	1.273	0.559~2.902
TNM stage	0.295	0.588	0.217~1.588	0.493	0.761	0.348~1.662
Lymph node metastasis	0.185	0.559	0.236~1.322	0.08	0.518	0.248~1.082
Distant metastasis	0.302	0.629	0.261~1.516	0.592	0.816	0.387~1.719
CEA	0.007	1.864	1.184~2.935	0.049	1.451	1.001~2.102
Comorbidities (mild case)	0.639	1.32	0.414~4.209	0.686	1.287	0.379~4.367
Comorbidities (severe case)	0.741	0.822	0.258~2.624	0.555	1.433	0.433~4.741
LncRNA	0.035	9.752	1.175~80.898	0.034	6.623	1.156~37.950

The tumor marker CEA has been well established as a biomarker for the diagnosis and treatment of NSCLC.[28,29] We evaluated the diagnostic efficacy of lncRNA HEIH and CEA in patients with LSC and LUAD through ROC curve analysis, which manifested that lncRNA HEIH had a high diagnostic efficacy in patients with NSCLC. Our paper may identify a more effective biomarker for NSCLC diagnosis.

Further, we analyzed the prognostic value of lncRNA HEIH in NSCLC. We assigned LUSC and LUAD patients into a low-expression group and a high-expression group and then followed up the patients. As expected, the cumulative survival rate in patients with high expression of lncRNA HEIH was lower than that in the patients with low expression of lncRNA HEIH in LUSC and LUAD. In short, high expression of lncRNA HEIH predicts poor prognosis in patients with NSCLC. The expression of HEIH is up-regulated in ovarian cancer tissues and cell lines, and high expression of HEIH indicates a poor prognosis.[46] Oesophageal squamous cell carcinoma patients with high lncRNA HEIH expression have poorer prognosis than those with low expression.[47] These results are consistent with the trend of our results.

Conclusion

In conclusion, the high expression of lncRNA HEIH in peripheral blood is helpful to the diagnosis and prognosis prediction of NSCLC, and may provide a new reference for evaluation of NSCLC clinically. Nevertheless, due to the small number of cases and events included in this study, it is necessary to further expand the sample size to further clarify the diagnostic and prognostic ability of lncRNA HEIH. Moreover, the efficacy of predicting lung cancer only by detecting the expression of lncRNA HEIH in peripheral blood is limited, and more studies are still necessary to find suitable combined diagnostic markers. In addition, the role of lncRNA HEIH in the occurrence and development of NSCLC is still poorly understood, and further studies are needed. In future studies, we should carry out a larger multi-center study, expand the sample size and match the control to increase the credibility of the results. Meanwhile, we should further study the combined diagnostic and prognostic value of lncRNA HEIH and CEA as well as other biomarkers in NSCLC patients. More studies are required to explore the molecular regulatory mechanism of lncRNA HEIH in the occurrence and development of NSCLC.

Data Sharing Statement

All the data generated or analyzed during this study are included in this published article.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically

reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no conflicts of interest.

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