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

Chaowen He (chaowh0331@163.com)
Shenzhen longhua district central hospital

Dongxuan Huang
Shenzhen longhua district central hospital

Fan Yang
Shenzhen longhua district central hospital

Dongsheng Huang
Shenzhen longhua district central hospital

Yahui Cao
Shenzhen longhua district central hospital

Jianfeng Peng
Shenzhen longhua district central hospital

Xiaohua Luo
Shenzhen longhua district central hospital

Research Article

Keywords: Non-small cell lung cancer, Long non-coding RNA HEIH, Prognosis, Carcinoembryonic antigen, Lung squamous cell carcinoma, Lung adenocarcinoma, Peripheral blood

DOI: https://doi.org/10.21203/rs.3.rs-739482/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License
Abstract

Objective: 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. According to the median expression of lncRNA HEIH, LUSC and LUAD patients were divided into lncRNA HEIH high/low expression group. The correlation between lncRNA HEIH and clinical indicators of patients was analyzed. Receiver-operating characteristic (ROC) curve was used to evaluate the diagnostic efficacy of lncRNA HEIH and carcinoembryonic antigen (CEA) in LUSC and 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.

Results: LncRNA HEIH in peripheral blood of LUSC and LUAD patients was higher than that in healthy controls, with no evident difference between LUSC and LUAD groups. LUSC and LUAD patients with high lncRNA HEIH expression had larger 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.

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

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]. 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 [5]. 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 [6]. At present, several IncRNAs [7] 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 [8–10]. High expression in hepatocellular carcinoma (HEIH) is a lncRNA originally found in HBV-induced hepatocellular carcinoma [11]. In patients with hepatocellular carcinoma, high expression of HEIH is associated with increased risk of recurrence and decreased overall survival after surgery. Recent studies have shown that HEIH is also highly expressed in other types of cancers including colorectal cancer, melanoma and NSCLC [12–14]. Kegang Jia et al. found that HEIH was significantly overexpressed in NSCLC tissues and cell lines, which promoted the proliferation and metastasis of NSCLC cells [13]. 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

A total of 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 [15], 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 and other baseline clinical data of enrolled subjects were recorded, as well as tumor size (≤ 3 cm; >3 cm), TNM stage (I; II/III), lymph node metastasis (Absent; Present), distal metastasis and carcinoembryonic antigen (CEA) (µg/mL). 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 10 s, annealing at 60°C for 20 s, and extension at 72°C for 34 s. Glyceraldehyde-3-phosphate dehydrogenase(GAPDH) was used as internal reference, and the data were analyzed by the 2^{-\Delta\Delta CT} method [16]. The primers were synthesized by Sangon Bioengineering Shanghai Co., Ltd (Shanghai, China), and the sequences are shown in Table 1.

| Gene       | Forward 5’-3’            | Reverse 5’-3’             |
|------------|--------------------------|---------------------------|
| LncRNA HEIH| GCGAGGAGAGACTTCCACAG     | GGGTTGAACAAAGTGAGA        |
| GAPDH      | CTCAGACACCATGGGAAGGTGA   | ATGATCTTGAAGCTTGTTCATA    |

**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. Shapiro-Wilk (W test) test showed that the data were normally distributed and the variable data 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 groups, and Tukey's multiple comparisons test was used for post hoc test. 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. 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 found no significant difference in age, gender and smoking among the three groups. The serum CEA levels in LUSC and LUAD patients were notably higher than those in controls (all $P< 0.05$). The comparative analysis of clinical baseline data showed no obvious difference in age, gender, smoking, tumor size, TNM stage, lymph node metastasis and distal metastasis between LUSC and LUAD patients (all $P> 0.05$) (Table 2).

| 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            |       |       |       |
| Tumor size                      |                 |               |               |       |       |       |
| ≤ 3 cm                          | -               | 35            | 44            | -     | -     | 0.510 |
| > 3 cm                          | -               | 35            | 36            | -     | -     |       |
| TNM stage                       |                 |               |               |       |       |       |
| I                               | -               | 47            | 52            | -     | -     | 0.863 |
| II-III                          | -               | 23            | 28            | -     | -     |       |
| Lymph node metastasis          |                 |               |               |       |       |       |
| Absent                          | -               | 32            | 35            | -     | -     | 0.422 |
| Present                         | -               | 38            | 55            | -     | -     |       |
| 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 |

Note: 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). $P$: LUSC group was compared with the control group; $P_b$: LUAD group was compared with the control group; $P_c$: LUSC group was compared with LUAD group.
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 ($P>0.05$) (Fig. 1A).

Correlation analysis between lncRNA HEIH and clinical indexes of NSCLC patients

To further study the relationship between lncRNA HEIH expression and clinical indicators of NSCLC patients, we divided LUSC and LUAD patients into lncRNA HEIH low expression group and lncRNA HEIH high expression group according to lncRNA HEIH median expression level in LUSC and LUAD. In LUSC and LUAD patients, there was no evident difference in age, gender and smoking indexes between the lncRNA HEIH low and high expression groups, while the lncRNA HEIH high expression group had larger 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).
### Table 3
Correlation analysis of lncRNA HEIH expression in peripheral blood of NSCLC patients and clinical indicators

| Parameters               | LUSC                      |              |              |              | LUAD                      |              |              |              |
|--------------------------|---------------------------|--------------|--------------|--------------|---------------------------|--------------|--------------|--------------|
|                          | Total (N=70)              | Low (N=35)   | High (N=35)  | P            | Total (N=80)              | Low (N=40)   | High (N=40)  | P            |
| 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           | > 0.999      | 49                        | 27           | 22           | 0.359        |
| Female                   | 29                        | 15           | 14           |              | 31                        | 13           | 18           |              |
| Smoke                    |                           |              |              |              |                           |              |              |              |
| Yes                      | 36                        | 17           | 19           | 0.811        | 43                        | 23           | 20           | 0.846        |
| No                       | 34                        | 18           | 16           |              | 37                        | 17           | 20           |              |
| 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           | <0.001       | 52                        | 34           | 18           | 0.034        |
| II-III                   | 23                        | 4            | 19           |              | 28                        | 16           | 22           |              |
| Lymph node metastasis    |                           |              |              |              |                           |              |              |              |
| Absent                   | 32                        | 22           | 10           | 0.008        | 35                        | 23           | 12           | 0.024        |
| Present                  | 38                        | 13           | 25           |              | 55                        | 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       |

Note: 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)

**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 [17–20]. 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 LSC patients was 0.860, the sensitivity was 72.86%, and the specificity was 95.71% (Fig. 2A). MedCalc-comparison of ROC curves showed that the area under ROC curve of
IncRNA HEIH was significantly higher than that of CEA ($P = 0.0031; 95\%CI = 0.052 \sim 0.255$), indicating that IncRNA HEIH had a higher diagnostic efficiency for LUSC than CEA. In addition, the area under ROC curve of IncRNA 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% (Fig. 2B). MedCalc-comparison of ROC curves showed that the area under ROC curve of IncRNA HEIH was significantly higher than that of CEA ($P = 0.0011; 95\%CI = 0.057 \sim 0.228$), indicating that IncRNA HEIH had a higher diagnostic efficacy than CEA for LUAD. The above data indicate that IncRNA HEIH has a high diagnostic efficacy in NSCLC patients.

**High expression of IncRNA HEIH predicts poor prognosis of NSCLC**

Furthermore, we analyzed the prognostic value of IncRNA HEIH in peripheral blood on NSCLC. According to the median expression level of IncRNA HEIH in LUSC and LUAD, patients with LUSC and LUAD were divided into IncRNA HEIH low expression group and IncRNA 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 IncRNA HEIH high expression group was evidently lower than that in the low expression group ($P = 0.0252$) (Fig. 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 IncRNA HEIH high expression group was notably lower than that of the low expression group ($P = 0.0027$) (Fig. 3B). These results suggest that IncRNA HEIH overexpression predicts poor prognosis in patients with NSCLC.

**Discussion**

LncRNAs are important in gene regulation [21]. Many lncRNAs have been shown to be associated with the diagnosis and prognosis of NSCLC [22]. HEIH is a lncRNA [11] 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 [13]. In this paper highlighted that the high expression of IncRNA 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 [23]. 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 [19, 20]. These results suggest that high serum CEA level can be used for the preliminary diagnosis of NSCLC.
Next, the expression of lncRNA HEIH in peripheral blood of LUSC and LUAD patients and healthy subjects was detected by qRT-PCR. 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 [13]. 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 based on the median expression of lncRNA HEIH. In LUSC and LUAD patients, 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 [14]. LncRNA HEIH high expression in gastric cancer patients is closely related to medium-high differentiation, distant metastasis, lymph node metastasis, and deeper tumor invasion [24]. Altogether, high expression of lncRNA HEIH is associated with poorer clinical indicators and higher cancer staging.

The tumor marker CEA has been well established as a biomarker for the diagnosis and treatment of NSCLC [17, 18]. We evaluated the diagnostic efficacy of lncRNA HEIH and CEA in patients with LSC and LUAD through ROC curve analysis, and the results showed 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 divided LUSC and LUAD patients into a low-expression group and a high-expression group, based on the median expression of lncRNA HEIH in LUSC and LUAD, and then followed up the patients. The results showed that in LUSC and LUAD patients, the cumulative survival rate in the group with high expression of lncRNA HEIH was lower than that in the low expression group. These results suggest that 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 [25]. Oesophageal squamous cell carcinoma patients with high lncRNA HEIH expression have poorer prognosis than those with low expression [26]. These results are consistent with the trend of our results.

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. However, 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. 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. More studies are required to explore the molecular regulatory mechanism of lncRNA HEIH in the occurrence and development of NSCLC.
Declarations

Funding
Not applicable.

Author Contributions
CWH is the guarantor of integrity of the entire study; CWH contributed to the study concepts, study design, definition of intellectual content, literature research, manuscript preparation and manuscript editing and review; DXH contributed to the experimental studies; FY contributed to the data acquisition; DSH contributed to the data analysis; YHC contributed to the statistical analysis; JFP contributed to the manuscript preparation; XHL contributed to the data acquisition and data analysis; All authors read and approved the final manuscript.

Acknowledgements
Not applicable.

References
1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68(6):394–424. https://doi.org/10.3322/caac.21492
2. Hirsch FR, Scagliotti GV, Mulshine JL, Kwon R, Curran WJ Jr, Wu YL et al (2017) Lung cancer: current therapies and new targeted treatments. Lancet 389(10066):299–311. https://doi.org/10.1016/S0140-6736(16)30958-8
3. Ettinger DS, Wood DE, Aggarwal C, Aisner DL, Akerley W, Bauman JR et al (2019) NCCN Guidelines Insights: Non-Small Cell Lung Cancer, Version 1.2020. J Natl Compr Canc Netw 17(12):1464–1472. https://doi.org/10.6004/jnccn.2019.0059
4. Zhang J, Zhu N, Chen X (2015) A novel long noncoding RNA LINC01133 is upregulated in lung squamous cell cancer and predicts survival. Tumour Biol 36(10):7465–7471. https://doi.org/10.1007/s13277-015-3460-9
5. Souza CP, Cinegaglia NC, Felix TF, Evangelista AF, Oliveira RA, Hasimoto EN et al (2020) Deregulated microRNAs Are Associated with Patient Survival and Predicted to Target Genes That Modulate Lung Cancer Signaling Pathways. Cancers (Basel) 12(9): https://doi.org/10.3390/cancers12092711
6. Beylerli OA, Azizova ST, Konovalov NA, Akhmedov AD, Gareev IF, Belogurov AA (2020) [Non-coding RNAs as therapeutic targets in spinal cord injury]. Zh Vopr Neirokhir Im N N Burdenko 84(4):104–110. https://doi.org/10.17116/neiro202084031104
7. Reis EM, Verjovski-Almeida S (2012) Perspectives of Long Non-Coding RNAs in Cancer Diagnostics. Front Genet 3:32. https://doi.org/10.3389/fgene.2012.00032

8. Luo J, Li Q, Pan J, Li L, Fang L, Zhang Y (2018) Expression level of long noncoding RNA H19 in plasma of patients with nonsmall cell lung cancer and its clinical significance. J Cancer Res Ther 14(4):860–863. https://doi.org/10.4103/jcrt.JCRT_733_17

9. Abbastabar M, Sarfi M, Golestani A, Khalili E (2018) IncRNA involvement in hepatocellular carcinoma metastasis and prognosis. EXCLI J 17:900–913. https://doi.org/10.17179/excli2018-1541

10. Xie Y, Zhang Y, Du L, Jiang X, Yan S, Duan W et al (2018) Circulating long noncoding RNA act as potential novel biomarkers for diagnosis and prognosis of non-small cell lung cancer. Mol Oncol 12(5):648–658. https://doi.org/10.1002/1878-0261.12188

11. Yang F, Zhang L, Huo XS, Yuan JH, Xu D, Yuan SX et al (2011) Long noncoding RNA high expression in hepatocellular carcinoma facilitates tumor growth through enhancer of zeste homolog 2 in humans. Hepatology 54(5):1679–1689. https://doi.org/10.1002/hep.24563

12. Cui C, Zhai D, Cai L, Duan Q, Xie L, Yu J (2018) Long Noncoding RNA HEIH Promotes Colorectal Cancer Tumorigenesis via Counteracting miR-939Mediated Transcriptional Repression of Bcl-xL. Cancer Res Treat 50(3):992–1008. https://doi.org/10.4143/crt.2017.226

13. Jia K, Chen F, Xu L (2019) Long noncoding RNA HEIH promotes the proliferation and metastasis of non-small cell lung cancer. J Cell Biochem 120(3):3529–3538. https://doi.org/10.1002/jcb.27629

14. Zhao H, Xing G, Wang Y, Luo Z, Liu G, Meng H (2017) Long noncoding RNA HEIH promotes melanoma cell proliferation, migration and invasion via inhibition of miR-200b/a/429. Biosci Rep 37(3): https://doi.org/10.1042/BSR20170682

15. Beasley MB, Brambilla E, Travis WD (2005) The 2004 World Health Organization classification of lung tumors. Semin Roentgenol 40(2):90–97. https://doi.org/10.1053/j.ro.2005.01.001

16. Schmittgen TD, Livak KJ (2008) Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc 3(6):1101–1108. https://doi.org/10.1038/nprot.2008.73

17. Clevers MR, Kastelijn EA, Peters BJM, Kelder H, Schramel F (2021) Evaluation of Serum Biomarker CEA and Ca-125 as Immunotherapy Response Predictors in Metastatic Non-small Cell Lung Cancer. Anticancer Res 41(2):869–876. https://doi.org/10.21873/anticanres.14839

18. Dal Bello MG, Filiberti RA, Alama A, Orenge AM, Mussap M, Coco S et al (2019) The role of CEA, CYFRA21-1 and NSE in monitoring tumor response to Nivolumab in advanced non-small cell lung cancer (NSCLC) patients. J Transl Med 17(1):74. https://doi.org/10.1186/s12967-019-1828-0

19. Li C, Lv Y, Shao C, Chen C, Zhang T, Wei Y et al (2019) Tumor-derived exosomal IncRNA GAS5 as a biomarker for early-stage non-small-cell lung cancer diagnosis. J Cell Physiol 234(11):20721–20727. https://doi.org/10.1002/jcp.28678

20. Bai N, Ma Y, Zhao J, Li B (2020) Knockdown of IncRNA HCP5 Suppresses the Progression of Colorectal Cancer by miR-299-3p/PFN1/AKT Axis. Cancer Manag Res 12:4747–4758. https://doi.org/10.2147/CMAR.S255866
21. Wang M, Dai M, Wang D, Tang T, Xiong F, Xiang B et al (2021) The long noncoding RNA AATBC promotes breast cancer migration and invasion by interacting with YBX1 and activating the YAP1/Hippo signaling pathway. Cancer Lett. https://doi.org/10.1016/j.canlet.2021.04.025

22. Zhang C, Gong C, Li J, Tang J (2021) Downregulation of long non-coding RNA LINC-PINT serves as a diagnostic and prognostic biomarker in patients with non-small cell lung cancer. Oncol Lett 21(3):210. https://doi.org/10.3892/ol.2021.12471

23. Iacuzzo C, Germani P, Troian M, Cipolat Mis T, Giudici F, Osenda E et al (2021) Serum carcinoembryonic antigen pre-operative level in colorectal cancer: revisiting risk stratification. ANZ J Surg. https://doi.org/10.1111/ans.16861

24. Chen X, Sun X, Li X, Xu L, Yu W (2021) LncRNA-HEIH is a Novel Diagnostic and Predictive Biomarker in Gastric Cancer. Genet Test Mol Biomarkers 25(4):284–292. https://doi.org/10.1089/gtmb.2020.0270

25. Si L, Chen J, Yang S, Liu Z, Chen Y, Peng M et al (2020) IncRNA HEIH accelerates cell proliferation and inhibits cell senescence by targeting miR-3619-5p/CTTNBP2 axis in ovarian cancer. Menopause 27(11):1302–1314. https://doi.org/10.1097/GME.0000000000001655

26. Ding X, Qi C, Min J, Xu Z, Huang K, Tang H (2020) Long non-coding RNA HEIH suppresses the expression of TP53 through enhancer of zeste homolog 2 in oesophageal squamous cell carcinoma. J Cell Mol Med 24(18):10551–10559. https://doi.org/10.1111/jcmm.15673

**Figures**
High expression of IncRNA HEIH in peripheral blood of NSCLC patients. The expression of IncRNA 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 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.

Figure 2
Figure 3

LncRNA OSER1-AS1 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.