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HBB as a Novel Biomarker for the Diagnosis and Monitoring of Lung Cancer Regulates Cell Proliferation via ERK1/2 Pathway

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Abstract

Objective: Recent studies have revealed that hemoglobin beta (HBB) plays an important role not only in blood disorders but also in malignancies. The aim of this study is to investigate the clinical significance, diagnostic value, and biological function of HBB in lung cancer. **Methods:** HBB expression was examined in lung cancer tissues and plasma samples using quantitative real-time polymerase chain reaction, and its relationship with clinical pathological characteristics was analyzed. Receiver operating characteristic (ROC) curves were constructed to evaluate the diagnostic value of HBB in lung cancer. The proliferation of A549 and SPCA1 cells was analyzed using a cell counting kit-8 assay and protein expressions were detected by western blot. **Results:** The expressions of HBB were found to be down-regulated in both lung cancer tissues and plasma samples. Notably, plasma HBB levels were significantly elevated in postoperative samples when compared to their preoperative counterparts. Across 66 cases of lung cancer tissues, a correlation was observed between HBB levels and both gender and tumor, node, metastasis staging. ROC curve analysis further confirmed the high diagnostic potential of HBB expression in lung cancer. Moreover, the combination of HBB and carcinoembryonic antigen (CEA) had greater significance than HBB or CEA alone in the diagnosis of lung cancer. Knocking out or overexpressing HBB could affect lung cancer cell proliferation through the ERK1/2 signaling pathway. **Conclusion:** HBB can serve as a novel biomarker for the diagnosis and monitoring of lung cancer, regulating cell proliferation via the ERK1/2 pathway and playing a pivotal role in the oncogenesis and progression of the disease.

Keywords

hemoglobin beta, lung cancer, biomarker, diagnosis, proliferation

Abbreviations

AC, diagnostic accuracy; ATC, Anaplastic thyroid cancer; AUC, Area under the receiver operating characteristic curve; CCK8, cell counting kit-8; CEA, Carcinoembryonic antigen; CYFRA21-1, Cytokeratin 19 soluble fragment; HBB, Hemoglobin beta; NPV, negative predictive value; NSE, Neuron-specific enolase; PPV, positive predictive value; qRT-PCR, quantitative real-time polymerase chain reaction; ROC, Receiver operating characteristic; ROS, Reactive oxygen species; SCC, Squamous-cell carcinoma antigen; SEN, sensitivity; SPE, specificity; TNM, Tumor, node, metastasis.

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Introduction

Lung cancer is a prevalent malignant neoplasm affecting the respiratory system. The incidence of lung cancer is increasing year by year, ranking as the second highest among all malignant tumors but with the highest mortality rate of all tumors, which has seriously threatened human health.[1,2] Generally, the tumor markers are abnormally expressed in the early stages of malignant tumors, such as carcinoembryonic antigen (CEA),

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squamous-cell carcinoma antigen (SCC), cytokeratin 19 soluble fragment (CYFRA21-1), and neuron-specific enolase (NSE).[3] It is essential to search for early diagnostic indicators of lung cancer and predictors of tumor metastasis in order to improve the therapeutic effect and prognosis of patients with lung cancer.

The human hemoglobin beta (HBB) gene, located on chromosome 11q15.5,[4] is one of the pathogenic genes encoding β-globin. β-globin, together with α-globin constitutes the dimeric subunits of adult hemoglobin, which is one of the most important oxygen-carrying proteins in red blood cells.[5] Previous studies have found that HBB is mainly involved in various blood diseases,[6] the role of HBB in cancers has been widely concerned in recent years. The expression of HBB in invasive breast carcinoma was significantly higher than that of carcinoma in situ, while it was negative in normal breast epithelium. HBB could promote the proliferation, migration, and metastasis of breast cancer cells, and was related to tumor-mediated angiogenesis. Similar results were observed in 4T1 mice.[7] In contrast, HBB has been found to be involved in tumorigenesis and development as a tumor suppressor gene. HBB was expressed in both normal and thyroid tissues, and was obviously decreased in anaplastic thyroid cancer (ATC), which might be related to promoter methylation. Moreover, the exogenous expression of HBB could significantly suppress the growth of KTA2 cells.[8] These results indicated the possibility of the tumor suppressor activity of HBB. At present, research on HBB in lung cancer is still relatively scarce. It had been found that HBB could suppress the proliferation of nonsmall-cell lung carcinoma through P38/MAPK and JNK pathways.[9] The objective of our research is to verify the potential of HBB as a molecular indicator for diagnosing and prognosticating lung cancer, as well as its role in modulating cellular proliferation.

Patients and Methods

66 paired lung cancer tissues and para-carcinoma tissues (the distance to the lesion was > 5 cm) were collected from patients who underwent surgical excision without any other therapy from Nantong Tumor Hospital and Affliated Hospital 2 of Nantong University. Inclusive criteria: Lung cancer was diagnosed by histopathology. All patients received no radiotherapy or chemotherapy treatment before surgery and signed the informed consent. Exclusion criteria: Any patient who had received surgical treatment; lung cancer combined with other tumors; any patient suffering from severe liver and kidney disease and autoimmune disease. The surgical specimens were quickly frozen with liquid nitrogen or stored in a −80 °C refrigerator after leaving the body. 80 whole blood samples of lung cancer patients before any anticancer treatment, such as surgery, radiotherapy, and chemotherapy, and 80 whole blood samples from healthy controls were collected from Affliated Hospital 2 of Nantong University. The healthy controls excluded those with other cancers and benign lung diseases including tuberculosis, pulmonary nodules, benign lung tumors, and pneumonia. There was no statistically significant difference in the comparison of general information between the 2 groups. Plasma was isolated from whole blood by centrifugation at 845 g for 6 min at 4 °C. In addition, 25 paired preoperative and postoperative plasma samples were collected and stored in a −20 °C refrigerator. The study was approved by the Ethics Committee of Nantong Tumor Hospital and Affliated Hospital 2 of Nantong University, and our the Ethics Committee study protocol number is 2020KT048, with an approval date of July 21, 2020. Furthermore, we obtained informed consent from 80 lung cancer patients. The expression level of CEA was detected using the Abbott I2000 immunoanalyzer (Abbott, IL, USA) and matching reagents. All operations were performed in strict accordance with the laboratory's standard operating procedures.

Cell Culture

The human pulmonary epithelial normal cell line (BEAS-2B) and lung cancer lines (H1299, A549, SPCA1, H1650, and H1975) were obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). All cell lines were grown in Roswell Park Memorial Institute-1640 (RPMI-1640) media from Gibco (Rockville, MD, USA) with 10% fetal bovine serum (FBS) in a 37 °C incubator in a 5% CO_2 atmosphere.

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

Total RNAs of lung cancer tissues were isolated by TRIzol (Invitrogen, Carlsbad, CA, USA). The concentration and purity of RNA were detected by Nano Drop 1000 micronucleic acid protein analyzer (Thermo Scientific, USA). RNA with a concentration of 100 to 500 ng/μL and a purity (A260/A280) of 1.9 to 2.1 was selected to synthesize cDNA according to the instructions of the Takara reverse transcription kit (TaKaRa, Kusatsu, Japan). Then, the qRT-PCR reactions were performed using the SYBR Green reagent (TaKaRa, Kusatsu, Japan) by the ABI 7500 Real Time-PCR System (ABI, Foster City, CA, USA). Each experiment was repeated 3 times. The reaction mixture consisted of 2 μL CDNA, 0.8 μL of 10 μmol/L forward and reverse primers, respectively, 10 μL of the Mix system and 6.4 μL of DEPC water. The fluorescence signal was collected at 60 °C and the melting curve was analyzed with the software for the ABI step one plus real-time PCR system (ABI, Foster City, CA, USA). The relative expression levels of HBB relative to β-actin were calculated by the $2^{-\Delta\Delta CT}$ method. The following primer sequences were used for qRT-PCR reactions: HBB F: 5′-TCCTTTGTTCCC TAAGTCCAACTAC-3′, R: 5′-TTAGGCAGAATCCAGA TGCTCAA-3′; β-actin, F: 5′-TCAAGATCATTGCTCCTCC TGAG-3′, and R: 5′-ACATCTGCTGGAAGGTGGACA-3′.

Cell Transfection

The negative controls (siRNA-NC), interference sequence (siRNA-HBB), and pCDNA3.1-HBB expression plasmid were synthesized and constructed by Ruibo Biotechnology Company (Guangzhou, China). The cells were seeded in 6-well plates and transfections were performed according to the manufacturer's instructions for Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA). The transfection efficiency was detected by qRT-PCR and western blot after 24 h and 48 h.

CCK-8 Assay

Cell proliferation was assessed using the Cell Counting Kit-8 (CCK-8) assay (Thermo Fisher Scientific, Waltham, MA, USA). Treated cells were seeded at a density of 5×10^3 cells/well in 96-well plates and cultured for 0, 24, 48, and 72 h. Then, 10 μL CCK-8 reagent was added to each well and incubated for 2 h. The absorbance at OD450 was measured with an enzyme-labeled instrument (Thermo Fisher Scientific, Waltham, MA, USA). The experiment was performed in triplicate.

Western Blot

The treated A549 and SPCA1 cells were lysed, and total protein was extracted with radio-immunoprecipitation assay (RIPA) buffer and quantified using a bicinchoninic acid (BCA) kit (Pierce, Rockford IL, USA), according to the manufacturer's instructions. The cell lysates were separated by 10% sodium dodecyl sulfate-poly-acrylamide gel electrophoresis (SDS-PAGE) (New Cell and Molecular Biotech, Suzhou, China) and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After blocking with 5% nonfat milk in TBST at room temperature for 2 h, the membranes were incubated with primary antibodies β-actin 1:50 000 (Abcam, Cambridge, MA, USA), catalog number: ab8227, HBB 1:2000 (Santa Cruz, CA, USA), catalog number: sc-21757, p-ERK1/2 1:2000 catalog number: #4370 and ERK1/2 1:2000 (Cell Signaling Technology, Danvers, MA, USA) catalog number: #9107, at 4 °C overnight. Then the membranes were washed 3 times and incubated with secondary antibody (HRP goat anti-rabbit IgG (AS014), Abclonal, Wuhan, China 1:10 000 and HRP goat anti-mouse IgG (7076P2), Cell Signaling Technology, Danvers, MA, USA, 1: 2000) at room temperature for 2 h. Finally, the target bands were captured by enhanced chemiluminescence (ECL) (New Cell and Molecular Biotech, Suzhou, China). The experiment was performed in triplicate.

Kaplan-Meier Plotter Survival Analysis

The instructions for logging on to the Kaplan-Meier plotter database (http://kmplot.com/analysis/) to analyze the relationship between HBB and survival of lung cancer patients are as follows: Set the conditions to include gene: HBB, cancer: lung cancer, split patients: median, survival: OS and follow-up threshold: 60 months.

Statistical Analysis

All data were analyzed by SPSS 25.0 (IBM SPSS Statistics, Chicago, USA) and the figures were drawn using GraphPad 5.0 software. Quantitative data were detected by the Normality test, normally distributed data were presented as $x \pm s$ and an independent-sample t-test was used for comparison between 2 groups, while one-way ANOVA was employed for the comparison of multiple groups, and the Tukey test was used for pairwise comparison between groups. Data that were nonnormal distribution were expressed as median M (X25%-X75%) and the Mann-Whitney *U*-test was used for comparison between groups. The relationship between HBB levels and clinicopathological characteristics was analyzed using the chi-square test, and the *P*-values were adjusted using the Benjamini-Hochberg method. $P < .05$ was considered statistically significant.

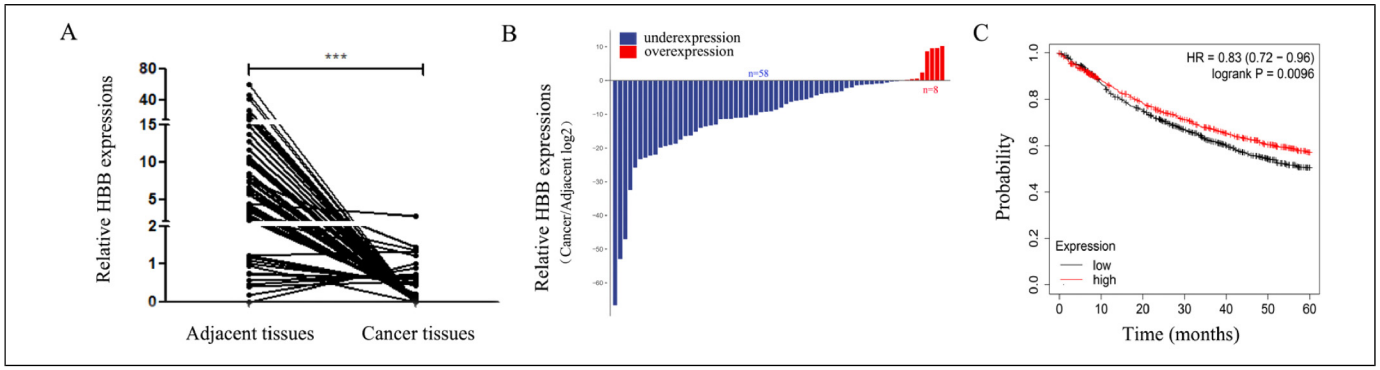

Figure 1. (A) The expressions of HBB in lung cancer tissues and adjacent tissues, ***$P < .001$. (B) HBB expression was significantly down-regulated in 87.9% (58 of 66 paired). The results were normalized to adjacent tissues and shown as log2 ($2^{-\triangle\triangle Ct}$). (C) Kaplan-Meier survival curve of patients with lung cancer downloaded from Kaplan-Meier plotter database.
Abbreviations: HBB, hemoglobin beta.

Results

HBB Was Down-Regulated in Lung Cancer Tissue and Indicated Better Survival

The expressions of HBB in 66 pairs of lung cancer tissues and their corresponding para-cancerous tissues were detected by qRT-PCR. Our findings showed that the HBB levels in lung cancer tissues were significantly lower than those in adjacent tissues (Figure 1A). The waterfall plot indicated that HBB expression was down-regulated in 87.9% (58 of 66 paired) of lung cancer tissues (Figure 1B). Besides, the cohort of lung cancer patients was divided into high and low-expression groups based on the mean value. We analyzed the relationship between HBB expression and clinicopathological parameters of lung cancer patients. The results displayed that HBB levels were correlated with gender and tumor, node, metastasis staging but were not correlated with age, smoking history, pathological type, tumor size, or lymphatic metastasis (Table 1). The Kaplan-Meier survival curve for lung cancer patients was downloaded from the Kaplan-Meier plotter database, which demonstrated that high expression of HBB was associated with better overall survival (Figure 1C).

Table 1. Correlation Between HBB Expressions and Clinicopathologic Features of Lung Cancer Patients.

		HBB expression				
Characteristics	n	High	Low	χ^2	*P*-value	*P*-adjust
Gender				7.542	.006**	.032*
Male	42	15	27			
Female	24	17	7			
Age (years)				0.910	.340	.389
< 65	41	18	23			
≥ 65	25	14	11			
Smoking history (years)				1.331	.249	.332
≤ 20	45	24	21			
> 20	21	8	13			
Pathological type						
Adenocarcinoma	37	19	18	0.387	.534	.534
Squamous cell carcinoma	21	9	12			
Others	8					
Tumor size (cm)				2.236	.135	.216
< 5	35	20	15			
≥ 5	31	12	19			
TNM staging				6.307	.012*	.032*
I + II	37	23	14			
III+IV	29	9	20			
Lymphatic metastasis				2.920	.088	.176
Yes	30	18	12			
No	36	14	22			
Survival time (years)				6.567	.010*	.032*
< 5	38	14	24			
≥ 5	16	12	4			
Unclear	12					

Abbreviations: HBB, hemoglobin beta; TNM, tumor, node, metastasis.

HBB Was Down-Regulated in Lung Cancer Plasma Samples

To explore the value of clinical utility, 80 serum samples were collected from patients with lung cancer and healthy controls. Results displayed serum HBB expressions were obviously lower in patients with lung cancer than in healthy controls (Figure 2A). To determine whether serum HBB expression could distinguish lung cancer patients from healthy controls, the receiver operating characteristic (ROC) curve was analyzed. The area under the receiver operating characteristic curve (AUC) for HBB was 0.862, when the critical value was 1.456, the sensitivity was 78.8% and the specificity was 80.0% (Figure 2B, Table 2). Moreover, serum carcinoembryonic antigen (CEA) levels were measured in patients with lung cancer and healthy controls. Our findings found the expressions of CEA were greatly higher in lung cancer patients than in healthy controls (Figure 2C). The AUC for CEA was 0.825 (Figure 2D), which was lower than that for HBB. Next, we combined HBB with CEA for diagnosing lung cancer. The combination of HBB and CEA could markedly increase the diagnostic efficiency for lung cancer, with related sensitivity and specificity of 90.0% and 85.0% respectively (AUC = 0.930, 95% CI: 0.892-0.969, Figure 2E). Besides, HBB levels were analyzed in 25 paired preoperative and postoperative plasma samples from lung cancer patients. Our results found that plasma HBB levels were remarkably higher postoperatively than preoperatively (Figure 2F). The results demonstrated that HBB could be used to monitor patients with lung cancer after surgery.

HBB Knockdown Promotes A549 and SPCA1 Cell Proliferation

To explore the function of HBB in lung cancer, we first detected the expression of HBB in lung cancer cell lines. Our findings revealed that the HBB level was significantly lower in lung cancer cells than in the normal bronchial epithelial cell line BEAS-2B (Figure 3A). We chose A549 and SPCA1 cells, whose expressions were in the middle, for subsequent experiments. Next, the proliferation of A549 and SPCA1 cells was detected after transfection with siRNA-NC or siRNA-HBB, the results showed that the HBB level was markedly reduced after transfection with siRNA-HBB (Figure 3C), while the proliferation of A549 and SPCA1 was promoted (Figure 3D). Moreover, the protein level of p-ERK1/2 was increased in HBB-knockdown A549 and SPCA1 cells (Figure 3C).

HBB Overexpression Inhibits A549 and SPCA1 Cell Proliferation

We next overexpressed HBB in A549 and SPCA1 cells (Figure 4A and B). The overexpression of HBB significantly

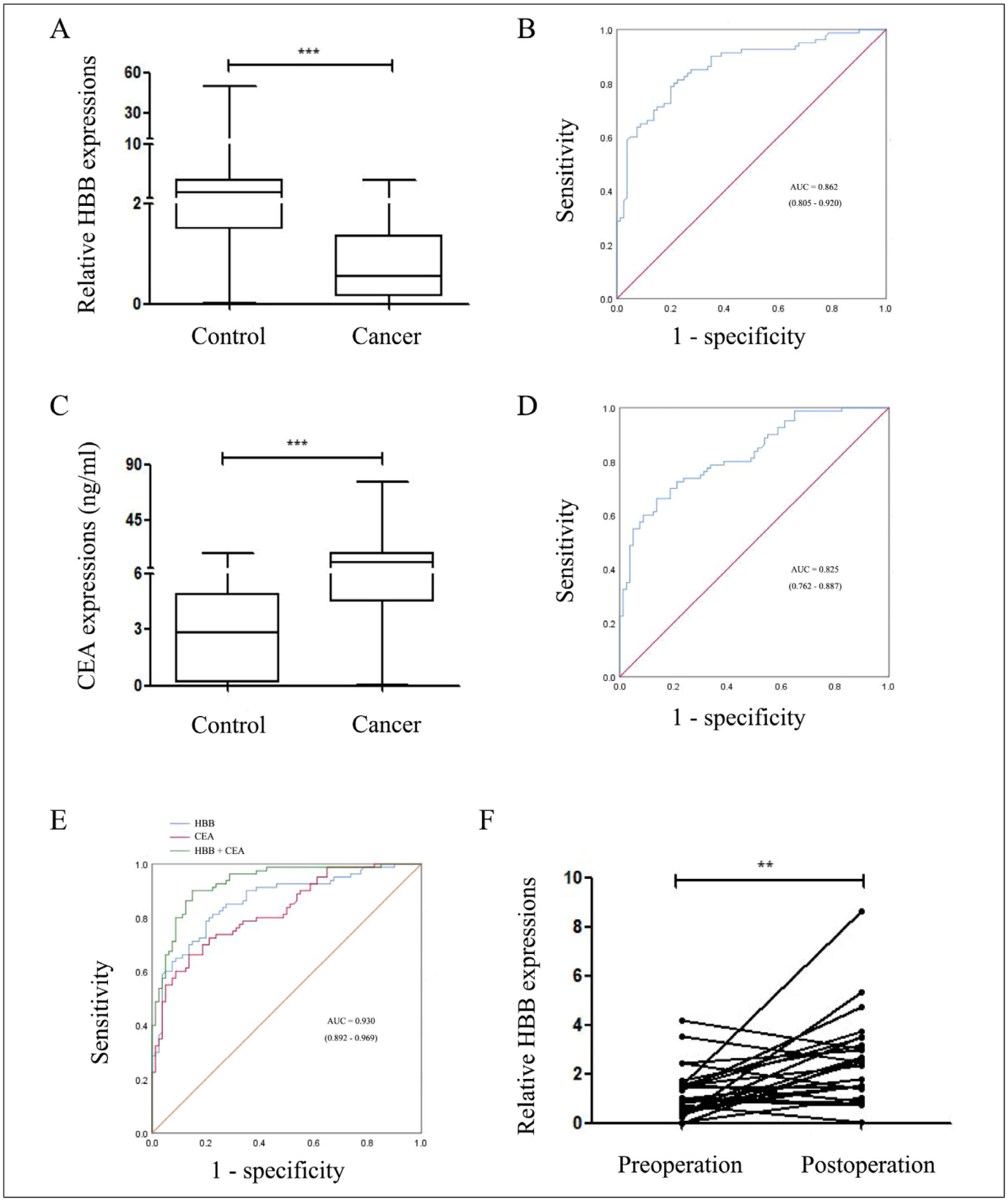

Figure 2. (A) The expressions of plasma HBB in lung cancer patients (n = 80) and healthy controls (n = 80), ***$P < .001$. (B) ROC curve for prediction of lung cancer based on HBB expression level, the AUC was 0.862 (95% CI: 0.805-0.920). (C) The expressions of plasma CEA in lung cancer patients (n = 80) and healthy controls (n = 80), ***$P < 0.001$. (D) ROC curve for prediction of lung cancer based on CEA expression level, the AUC was 0.825 (95% CI: 0.762-0.887). (E) ROC curve for prediction of lung cancer based on a combination of HBB and CEA expression levels, the AUC was 0.930 (95% CI: 0.892-0.969). (F) Comparison of plasma HBB levels between pre- and postoperative samples, **$P < .01$. Abbreviations: HBB, hemoglobin beta; ROC, receiver operating characteristic; AUC, Area under the receiver operating characteristic curve; CEA, carcinoembryonic antigen.

Table 2. Use of HBB and CEA Levels to Distinguish Lung Cancer Patients From Healthy Controls.

	SEN	SPE	AC	PPV	NPV
HBB	78.8%	80.0%	79.4%	79.0%	79.8%
CEA	73.8%	76.2%	75.0%	75.6%	74.4%
HBB + CEA	90.0%	85.0%	87.5%	85.7%	89.5%

Abbreviations: SEN, sensitivity; SPE, specificity; AC, diagnostic accuracy; PPV, positive predictive value; NPV, negative predictive value; HBB, hemoglobin beta; CEA, carcinoembryonic antigen.

reduced the ability of cell proliferation (Figure 4C). Besides, the protein level of p-ERK1/2 was decreased in HBB-overexpression A549 and SPCA1 cells (Figure 4B).

Discussion

Currently, the treatment of lung cancer has greatly improved, but due to the lack of effective early diagnostic and prognostic monitoring indicators, the treatment effect is still poor. Some oncogenes and tumor suppressor genes related to lung cancer have been reported, including ALK, EGFR, PI3K, BRAF, p53, and so on.[10–14] However, many potential genes remain to be discovered, and the molecular mechanisms are still unclear. Therefore, it is urgent to search for new biomarkers for the diagnosis and monitoring to explore the mechanism of the occurrence and development of lung cancer.

Hemoglobin is a tetramer consisting of 2 alpha and 2 beta chains, which are involved in oxygen transport. At first, it was thought that hemoglobin was expressed exclusively in erythroid cell lines,[15,16] but later studies found that hemoglobin chains were expressed in a variety of nonerythroid cells, such as nerve cells, mesangium cells, macrophages, and hepatocytes.[17–19] HBB, a member of the globin family, is widely expressed in human thyroid tissues and in both epithelial and endothelial cells of rat alveoli.[5] Many previous studies have suggested that HBB was involved in blood diseases such as sickle cell anemia and thalassemia.[20] In recent years, the relationship between HBB and tumors has been of interest. It is located on chromosome 11p15.5, and loss of heterozygosity at this locus has been found in tumors and is associated with a poor prognosis for nonsmall-cell lung carcinoma.[21,22] The expression of HBB was significantly down-regulated, and

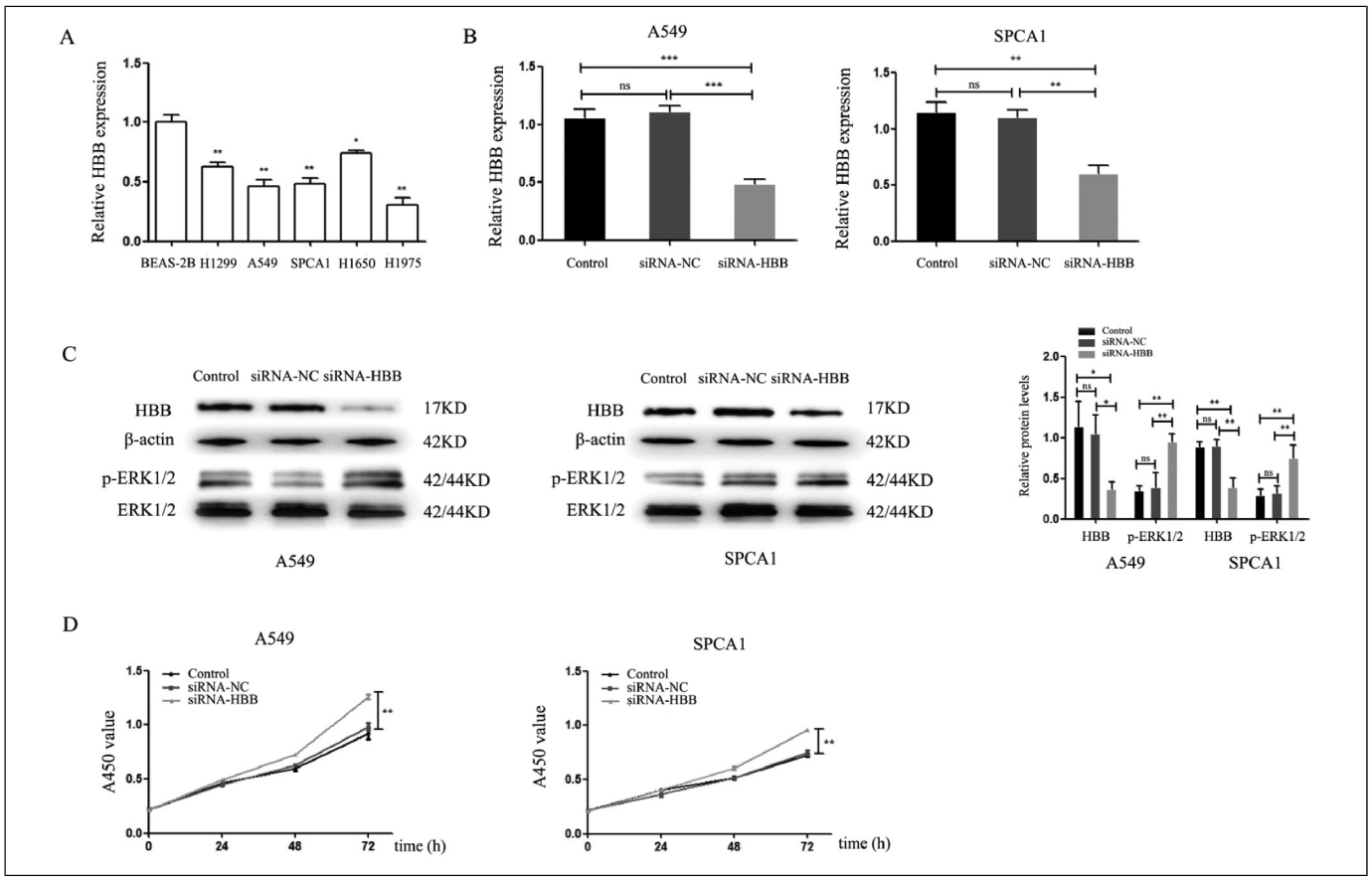

Figure 3. (A) The level of HBB in normal bronchial epithelial cell line (BEAS-2B) and lung cancer cell lines (H1299, A549, SPCA1, H1650, and H1975). (B and C) qRT-PCR and western blot demonstrated that the HBB level was significantly decreased, while the expression of p-ERK1/2 markedly increased after transfection with siRNA-HBB in A549 and SPCA1 cells. (D) CCK8 assay revealed that the proliferation of A549 and SPCA1 cells was promoted after silencing HBB for 72 h. All experiments were repeated 3 times, $*P < .05$, $**P < .01$, $***P < .001$.
Abbreviations: HBB, hemoglobin beta; qRT-PCR, quantitative real-time polymerase chain reaction; CCK8, cell counting kit-8.

overexpression of HBB remarkably inhibited the growth of anaplastic thyroid cancer KTA2 cells.[8] Meanwhile, HBB could inhibit the growth and metastasis of neuroblastoma.[23] These results indicated that HBB had an inhibitory effect on the occurrence and development of tumors. In our study, we found HBB expressions were down-regulated in both lung cancer tissues and plasma samples, this was consistent with the report by Kang et al.[9] The expression HBB had a high diagnostic value in lung cancer by ROC curve analysis. Moreover, the combination of HBB and CEA was more significant than HBB or CEA alone in the diagnosis of lung cancer. The results demonstrated that HBB could serve as a biomarker for the diagnosis and monitoring of lung cancer. Besides, we discovered that plasma HBB levels were obviously higher in postoperative samples than in preoperative samples, which suggested that HBB levels could reflect tumor burden. After surgical resection of the tumor, significantly increased HBB expression indicated more complete resection and a better prognosis. Unchanged or continuously decreased HBB expression implied potential microscopic residual lesions or a tendency toward metastasis. In addition, HBB levels could be used to evaluate tumor prognosis and predict recurrence. Persistently elevated HBB expression indicated a better prognosis in lung cancer patients. We could determine tumor sensitivity to therapies like surgery and chemoradiotherapy based on changes in HBB expression, and adjust treatment plans accordingly when necessary. Next, we will further investigate the mechanistic role of HBB in tumor occurrence and progression. By elucidating the relationship between changes in HBB expression and tumor cell proliferation, apoptosis, and metastasis, we aim to provide a theoretical basis for developing HBB-targeted therapies.

Gene expression is affected by many factors, such as loss of Heterozygosity, DNA methylation, noncoding RNA regulation, and environmental changes.[24,25] It has been suggested that the promoter of HBB was semimethylated in undifferentiated thyroid cancer, which led to the decrease in HBB expression. Reactive oxygen species (ROS) could promote HBB expression in a KLF4-dependent manner under oxidative stress.[26] However, whether there is a similar mechanism of HBB in lung cancer needs further study. HBB could inhibit Neuroblastoma micrometastasis in the lung, and the neuroblastoma micrometastases might stimulate HBB expression by secreting soluble factors in Alveolar endothelial cells and endothelium. It might be that HBB is paracrine to the extracellular

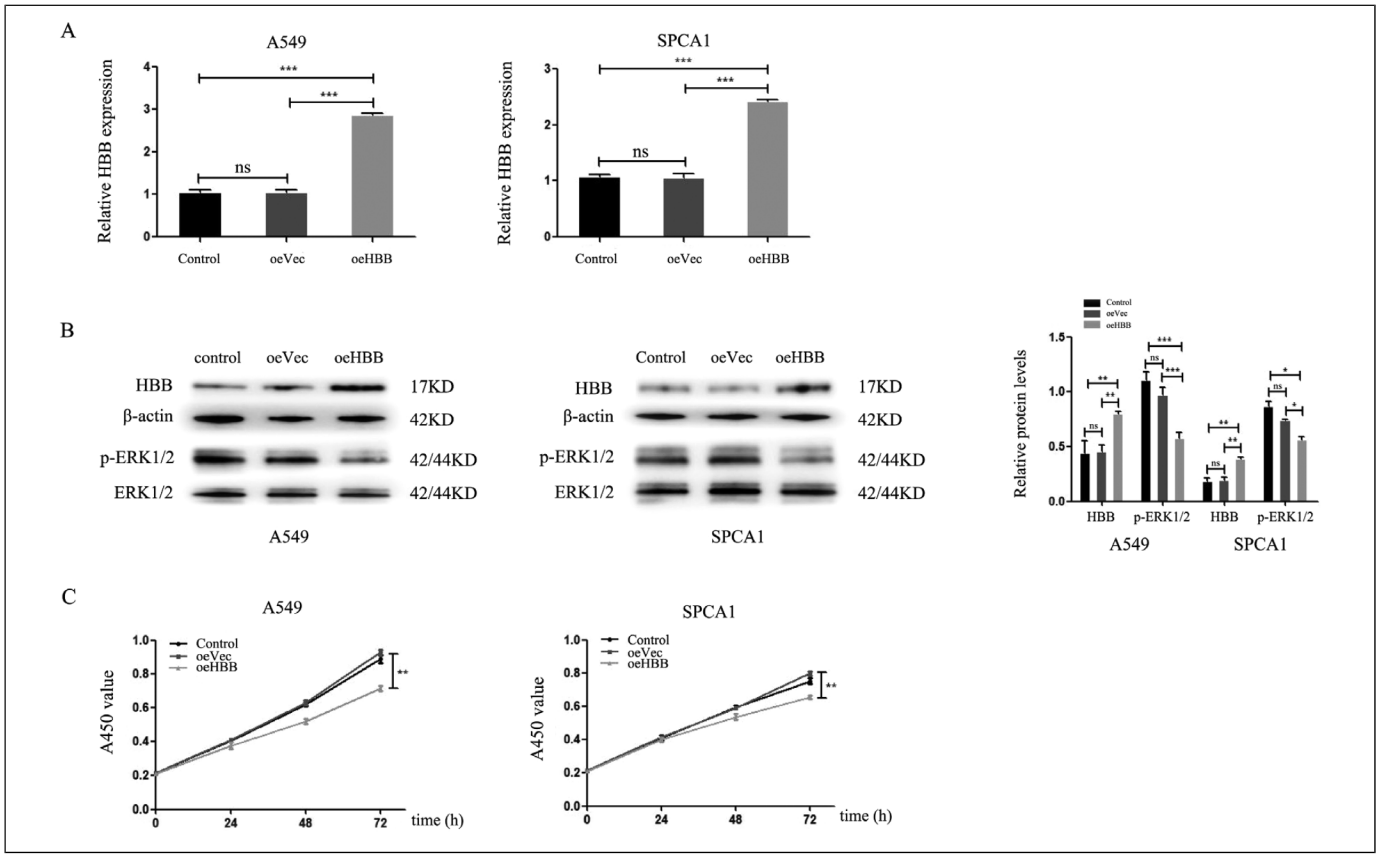

Figure 4. (A and B) qRT-PCR and western blot demonstrated that the HBB level was significantly increased, while the expression of p-ERK1/2 was markedly decreased after transfection with oeHBB in A549 and SPCA1 cells. (C) Overexpression of HBB inhibited the proliferation of A549 and SPCA1 cells for 72 h as shown by CCK8 assay. All experiments were repeated 3 times, $*P < .05$, $**P < .01$, $***P < .001$.
Abbreviations: HBB, hemoglobin beta; qRT-PCR, quantitative real-time polymerase chain reaction; CCK8, cell counting kit-8.

environment and binds to HBB receptors on the surface of tumor cells. Then HBB entered the tumor cells by endocytosis to activate TAK1 and P38 signaling pathways and down-regulated Erk phosphorylation levels, ultimately leading to the growth inhibition and apoptosis of the metastatic tumor cells.[23] In addition, HBB might play a role in the immune escape mechanism of tumors. HBB could be recognized by T cells as an associated antigen in the tumor microenvironment and lead to T cell differentiation into $CD8^+$. BALB/C mice were immunized with $HBB_{33\text{-}42}$ peptide vaccine, which could protect the mice from attack by tumor cells. The protective effect of the vaccine was not directly on tumor cells, but rather through an immune response to HBB in perivascular cells, limiting or disrupting the structure of tumor-associated vessels to suppress cancer.[27] Therefore, HBB expression was decreased in normal lung tissue under the influence of some external factors. On the one hand, the decreased HBB level might directly lead to the malignant transformation of normal alveolar epithelial cells. On the other hand, it might lead to the loss of immune targets in vivo, which makes the tumor microenvironment beneficial to tumor growth and ultimately promotes the occurrence and development of lung cancer. On the contrary, some studies found that HBB expression was significantly higher in the circulating tumor cells from breast cancer, prostate cancer, and nonsmall cell lung cancer.[26] Overexpression of HBB promoted the migration and invasion of breast cancer cells, and the overall survival rate of patients with high expression of HBB was low. In our study, 54 of 66 patients with lung cancer were followed up regularly. Among them, 38 cases survived for < 5 years, of which 24 cases (63.2%) had low HBB expressions and 16 cases survived for > 5 years, of which 4 cases (25%) had low HBB expressions. It showed that lung cancer patients with low expression of HBB had a poor prognosis, and the results were consistent with the Kaplan-Meier survival analysis. However, due to the lack of specific death dates for some specimens and the inability to fully track patients, we were unable to construct survival curves, which reduced the persuasiveness of the results. This will be improved in future experiments by obtaining complete follow-up data. In addition, we collected 66 tissue samples and 80 serum samples, which was a relatively small sample size. In later experiments, we will increase the sample size to make the results more reliable. The role of the ERK signaling pathway in regulating tumor cell proliferation, migration, apoptosis, and EMT has been extensively elucidated. In this study, we observed that HBB knockdown activated the ERK pathway and promoted the proliferation of A549 and SPCA1 cells, while HBB overexpression exerted the opposite effect. We considered that HBB might regulate the proliferation of lung cancer through the ERK signaling pathway. Because of this, we speculated whether the regulation of lung cancer proliferation by HBB is related to cell cycle, apoptosis, migration, and invasion, and whether it can regulate downstream related molecules. Next, we will continue to study the mechanism of HBB in tumor occurrence and development, providing a theoretical basis for the development of HBB-targeted therapy.

Conclusions

The expressions of HBB were significantly reduced in lung cancer tissues and plasma samples. Our study and analysis of the Kaplan-Meier plotter database revealed that the prognosis of lung cancer patients with low expression of HBB was poor. HBB could inhibit lung cancer cell proliferation via the ERK1/2 pathway. The present study indicated that HBB might serve as a novel biomarker for the diagnosis and postoperative monitoring of lung cancer.

Acknowledgments

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Author's Contributions

Fuying Chu conceived and designed this research; Xinxin Xu performed the experiments; Hua Cai and Hongli Liu collected important background information; Jingjing Peng performed the statistical analysis. Fuying Chu and Xinxin Xu edited the manuscript. All authors read and approved the final manuscript.

Availability of Data and Materials

The datasets used and analyzed in the current study are available from the corresponding author upon reasonable request.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval

This study has been approved by the Ethical Committee of Affliated Hospital 2 of Nantong University (Ethical approval number: 2020KT048), and we obtained informed consent from 80 lung cancer patients.

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Informed Consent

All authors and participants consent to the publication of the manuscript.

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