NOB1 Assumes Signifying Capacity in Lung Cancer Diagnosis.

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Abstract

**Background:** *NIN1/RPN12 binding protein 1 homolog (NOB1)* gene was reported to play a key role in the oncogenesis and prognosis of carcinomas. The aim of the present study was to investigate the expression of *NOB1* and its clinical significance in lung cancer, and further explore the diagnostic value of *NOB1* in lung cancer patients.

**Methods:** Quantitative real-time polymerase chain reaction (qRT-PCR) analysis was used to characterize the expression of *NOB1* in 97 lung cancer patients and 55 healthy controls. The associations of *NOB1* mRNA expression with clinicopathological factors of lung cancer patients were analyzed by Chi-square test. The receiver operating characteristics (ROC) curve was used to evaluate the diagnostic value of *NOB1* in lung cancer.

**Results:** The expression levels of *NOB1* mRNA in lung cancer samples were significantly up-regulated compared with healthy controls (*P*<0.001). And the *NOB1* expression was significant correlated with differentiation (*P*=0.005) and invasion depth (*P*=0.008). No associations were found between *NOB1* expression and other factors (All *P*>0.05). ROC curve revealed that the area under ROC curve (AUC) was 0.885, with a sensitivity of 76.3% and a specificity of 89.1%. And the cutoff value of *NOB1* was 2.225.

**Conclusion:** *NOB1* is a sensitive and specific diagnostic marker for lung cancer, and provide a new therapeutic target for lung cancer treatment.

**Background**

Lung cancer is the most common diagnosed cancer and the leading cause of death in economically developed countries and the second leading cause in developing countries [1]. This disease accounts for a higher percentage of deaths than any other cancer in both men and women [2]. According to the pathological and clinical features, lung cancer can be divided into two major histopathological groups: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) [3]. SCLC accounted for about 16.8% of the total number of lung cancer, and the NSCLC accounted for approximately 80.4% [4]. Because lack of effective screening tools for early-stage diagnosis, the 5-years survival rate in patients with lung cancer is less than 10% in the past [5-7]. With the improvement of diagnosis and treatment technology of lung cancer, the 5-year survival rate of lung cancer has increased to 15% currently. However, there is still lack of effective methods for the treatment of lung cancer [8]. Due to the incurable nature and the low survival rate, a huge attention has been focused on lung cancer to find early detection strategies [9].

*NIN1/RPN12 binding protein 1 homolog (NOB1)* is located in chromosome 16q22.1 [10]. This gene was firstly identified in *Saccharomyces cerevisiae*, encodes the essential protein Nin one binding protein (NOB1p) [11]. Previous studies have indicated that NOB1p joins the 20S proteasome with the 19S regulatory particle in the nucleus and facilitates the biogenesis of the 26S proteasome, which plays a role in maintaining cellular homeostasis by controlling protein degradation [12-14]. Besides, *NOB1* may play an essential role in cell cycle progression, drug resistance and tumor genesis [15, 16]. In particular,
increasing studies have shown that \textit{NOB1} participates in the occurrence and development of various cancers such as human ovarian cancer and colorectal cancer [17-19]. However, the role of \textit{NOB1} in diagnosis of human lung cancer has not been fully investigated.

In this study, we explored the potential role of \textit{NOB1} in lung cancer by detecting the expression level of \textit{NOB1} mRNA and the associations with clinical characteristics of lung cancer patients. Besides, we also estimated the diagnostic value of \textit{NOB1} in lung cancer.

\textbf{Materials And Methods}

\textbf{Patients and samples}

Clinical specimens were obtained from The First Affiliated Hospital of Xinxiang Medical University. Blood samples were collected from 97 lung cancer patients and 55 healthy volunteers as the control. All patients had never received any radiotherapy or chemotherapy before sampling and none of the healthy volunteers had formerly been diagnosed with any malignancy. The samples from patients and healthy controls were severally stored at -80°C until use. Besides, the study protocols were approved by the institutional Ethical Committee, and all patients provided written informed consents.

\textbf{RNA extraction and qRT-PCR analysis}

The total RNA of all blood samples was isolated using TRizol® (Life Technologies, Carlsbad, CA, USA). Quantitative RT-PCR was carried out according to the manufacturer's protocols and was performed in duplicate. The reverse transcription reactions were incubated for 30 min at 50°C followed by heat inactivation at 85°C for 5-10 min. cDNA templates were diluted and added to the PCR master mix. RT-PCR was performed on a 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, United States) with a 96-well block module. Relative amounts of \textit{NOB1} were calculated from the threshold cycle (CT) number using the expression of U6 for normalization.

\textbf{Statistical analysis}

Statistical analyses and graphics were performed using the SPSS 21.0 statistical package (SPSS, Inc., Chicago, IL). The t-test was used to compare the \textit{NOB1} mRNA levels in tumor samples and healthy controls. The Chi-square test was used to analyze the relationship between \textit{NOB1} expression and various clinicopathological characteristics. Receiver operating characteristic (ROC) curves was constructed to determine the diagnostic performance of \textit{NOB1} expression levels in distinguishing patients with lung cancer from the healthy control subjects.

\textbf{Results}

\textbf{Up-regulated expression of \textit{NOB1} in lung cancer}
The expression levels of \textit{NOB1} mRNA were detected in 97 lung cancer samples and 55 healthy controls. Compared with the controls, the expression levels of \textit{NOB1} in lung cancer were significantly up-regulated ($P<0.001$, \textbf{Figure 1}).

**Correlation between \textit{NOB1} expression and clinicopathological characteristics**

We analyzed the associations of \textit{NOB1} expression with various clinicopathological parameters of lung cancer patients. As shown in \textbf{Table 1}, Significant relationships were found between \textit{NOB1} high expression and differentiation ($P=0.005$), invasion depth ($P=0.008$). However, there was no relationship between \textit{NOB1} expression and other factors, including age, gender, tumor size, lymph node metastasis and TNM stage (all, $P>0.05$).

**The diagnostic value of \textit{NOB1} in lung cancer**

Receiver operating characteristic (ROC) curve was built to investigate the diagnostic value of \textit{NOB1} in lung cancer. The results revealed that the area under ROC curve (AUC) was 0.885 (95\% CI = 0.829 to 0.941), with a sensitivity of 76.3\% and a specificity of 89.1\% and cutoff value of 2.225 (\textbf{Figure 2}). All the data might demonstrate that \textit{NOB1} could play a key role in the distinction of the healthy people and the lung cancer patients.

**Discussion**

Lung cancer is the most common cause of cancer deaths worldwide with more than 1.2 million people dying of the disease annually [20, 21]. The study for early detection and treatment of lung cancer is a promising strategy to reduce the mortality of lung cancer. Currently, diagnostic techniques such as spiral computed tomography (CT) and autofluorescence bronchoscopy can detect lung cancers down to the normal range [22, 23]. However, these methods did not improve the 5-year survival rate of lung cancer. An explanation for this poor outcome is that the prognosis for patients with advanced lung cancer remains poor because the cancer is detected at an advanced and typically untreatable stage [24]. Hence, it is very important to investigate some early and more-accurate diagnostic biomarkers for lung cancer to improve the survival rates of patients.

The human \textit{NOB1} gene is a newly discovered gene, which is associated with the regulation of cell cycle and cell transcription. And it may participate in occurrence and development of the various tumors by regulating the gene transcription. \textit{NOB1} is mainly expressed in the liver, lung and spleen, and downregulated in kidney, prostate, colon and ovarian tissues [10]. Increasing studies indicated that \textit{NOB1} has extensive biological functions, for example, it participated in the synthesis and assembling of 26s proteasome and ribosome, regulating cell cycle through the UPP pathway. Besides, \textit{NOB1} may be involved in the regulation of cell cycle by ZNRD1 structure domain [25-29].

With the deepening of more and more research, \textit{NOB1} is found abnormally expressed in a variety of diseases. Yin et al. found that the \textit{NOB1} expression increased with pathological grades and it is
important in oral squamous cell carcinoma development and serves as a candidate indicator of aggressiveness and a therapeutic target of OSCC [30]. Besides, *NOB1* might be involved in tumorigenesis of prostate cancer, and could be a potential molecular target for prostate cancer gene therapy [31]. However, there are few researches about the *NOB1* expression in lung cancer. The study of Li et al. [32] has provided a basis for our research on the diagnostic value of *NOB1* in lung cancer. The results of this study revealed that down-regulation of *NOB1* expression using the RNA silencing approach in A549 tumor cells significantly suppressed the proliferation and colony formation ability, and induced tumor apoptosis in vitro. Tumor growth was also suppressed in vivo. Therefore, the above studies showed that although the research about the value of *NOB1* in tumors still in the early stage, *NOB1* is expected to become a new biomarker with the further study and interpretation. And it may provide a new treatment approach for the patients with lung cancer.

In our study, we investigated the expression level of *NOB1* in lung cancer patients and healthy volunteers. The results showed that the level of *NOB1* expression was increased in lung cancer samples compared with the levels in healthy controls. Our data also revealed that the expression of this gene is significant correlation with some clinicopathological features in lung cancer patients, including differentiation and invasion depth. In particular, from the analysis of ROC curves, we found that the expression of *NOB1* could be used as an efficient diagnostic biomarker for lung cancer patients. Based on the present results and the evidence above, we can speculate that the *NOB1* gene may play an important role in lung cancer carcinogenesis and progression.

**Conclusions**

In conclusion, our data offered the convincing evidence for the first time that *NOB1* may contribute to the development and the clinical outcome of lung cancer, and be a valuable diagnostic factor for lung cancer patients. However, the present study has not elucidated the exact molecular mechanisms of *NOB1* acting on lung cancer; therefore, we will need more efforts in the future research.

**List Of Abbreviations**

*NIN1/RPN12 binding protein 1 homolog (NOB1)*

Quantitative real-time polymerase chain reaction (qRT-PCR)

receiver operating characteristics (ROC)

area under ROC curve (AUC)

small cell lung cancer (SCLC)

non-small cell lung cancer (NSCLC)

Nin one binding protein (NOB1p)
computed tomography (CT)

Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of The First Affiliated Hospital of Xinxiang Medical University and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

Consent for publication

We obtaining permission from participants to publish their data.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

J.Y. and H.Z. design of the work; Q.Y. the acquisition, analysis, J.Y. interpretation of data; C.J. and J.Y. the creation of new software used in the work; X.L. have drafted the work or substantively revised it. All authors read and approved the final manuscript.

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**Tables**

**Table 1.** Association between *NOB1* expression and clinicopathological features in lung cancer patients
| Features                        | No. | NOB1 expression | P values |
|--------------------------------|-----|----------------|----------|
|                                | N=97| Low (n=44)     | High (n=53) |
| Age (years)                    |     |                |          |
| <50                            | 36  | 17             | 19       | 0.777 |
| ≥50                            | 61  | 27             | 34       |
| Gender                         |     |                |          |
| Male                           | 43  | 21             | 22       | 0.539 |
| Female                         | 54  | 23             | 31       |
| Tumor size                     |     |                |          |
| <3cm                           | 57  | 26             | 31       | 0.952 |
| ≥3cm                           | 40  | 18             | 22       |
| Differentiation                |     |                |          |
| Poorly differentiation         | 46  | 14             | 32       | 0.005 |
| High differentiation           | 51  | 30             | 21       |
| Invasion depth                 |     |                |          |
| T1 + T2                        | 41  | 25             | 16       | 0.008 |
| T3 + T4                        | 56  | 19             | 37       |
| Lymph node metastasis          |     |                |          |
| Yes                            | 49  | 23             | 26       | 0.752 |
| No                             | 48  | 21             | 27       |
| TNM stage                      |     |                |          |
| I-II                           | 52  | 25             | 27       | 0.564 |
| II-IV                          | 45  | 19             | 26       |

Figures
Figure 1

Expression of NOB1 in lung cancer patients (n=97) and healthy volunteers (n=55). NOB1 expression was significantly higher in patients with lung cancer than that in healthy controls (**, P<0.001).
Figure 2

It showed ROC curve for evaluation of the accuracy of NOB1 expression to discriminate patients with lung cancer from healthy controls.