NOB1 Assumes Signifying Capacity in Lung Cancer Diagnosis.

Jun Yang
The First Affiliated Hospital of Xinxiang Medical University
Hua Zhong
The First Affiliated Hospital of Xinxiang Medical University
Qinghui Yang
The First Affiliated Hospital of Xinxiang Medical University
Jian Yu
The First Affiliated Hospital of Xinxiang Medical University
Cailing Jin
The First Affiliated Hospital of Xinxiang Medical University
Xiaorui Li (dsbosj@yeah.net)
The First Affiliated Hospital of Xinxiang Medical University https://orcid.org/0000-0003-0302-502X

Primary research

Keywords: Lung Cancer, NOB1, Diagnosis

Posted Date: August 3rd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-51778/v1

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 *NOB1* participates in the occurrence and development of various cancers such as human ovarian cancer and colorectal cancer [17-19]. However, the role of *NOB1* in diagnosis of human lung cancer has not been fully investigated.

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

Materials And Methods

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°Cuntil use. Besides, the study protocols were approved by the institutional Ethical Committee, and all patients provided written informed consents.

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 *NOB1* were calculated from the threshold cycle (CT) number using the expression of U6 for normalization.

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 *NOB1* mRNA levels in tumor samples and healthy controls. The Chi-square test was used to analyze the relationship between *NOB1* expression and various clinicopathological characteristics. Receiver operating characteristic (ROC) curves was constructed to determine the diagnostic performance of *NOB1* expression levels in distinguishing patients with lung cancer from the healthy control subjects.

Results

Up-regulated expression of *NOB1* in lung cancer

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

Correlation between *NOB1* expression and clinicopathological characteristics

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

The diagnostic value of *NOB1* in lung cancer

Receiver operating characteristic (ROC) curve was built to investigate the diagnostic value of *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 (**Figure 2**). All the data might demonstrate that *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 bronchosocopy 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 *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. *NOB1* is mainly expressed in the liver, lung and spleen, and downregulated in kidney, prostate, colon and ovarian tissues [10]. Increasing studies indicated that *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, *NOB1* may be involved in the regulation of cell cycle by ZNRD1 structure domain [25-29].

With the deepening of more and more research, *NOB1* is found abnormally expressed in a variety of diseases. Yin et al. found that the *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.

Funding

Not applicable.

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.

Acknowledgements

Not applicable.

References

1. Jemal A, Siegel R, Xu J, Ward E: **Cancer statistics, 2010**. *CA: a cancer journal for clinicians* 2010, **60**(5):277-300.

2. Siegel R, Naishadham D, Jemal A: **Cancer statistics, 2013**. *CA: a cancer journal for clinicians* 2013, **63**(1):11-30.
3. Ulahannan SV, Brahmer JR: **Antiangiogenic agents in combination with chemotherapy in patients with advanced non-small cell lung cancer**. *Cancer investigation* 2011, **29**(4):325-337.
4. Horn L: **Targeted/emerging therapies for metastatic non-small cell lung cancer**. *Journal of the National Comprehensive Cancer Network : JNCCN* 2015, **13**(5 Suppl):676-678.
5. Hirsch FR, Merrick DT, Franklin WA: **Role of biomarkers for early detection of lung cancer and chemoprevention**. *The European respiratory journal* 2002, **19**(6):1151-1158.
6. Jett JR: **Limitations of screening for lung cancer with low-dose spiral computed tomography**. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2005, **11**(13 Pt 2):4988s-4992s.
7. Marchionni L, Wilson RF, Wolff AC, Marinopoulos S, Parmigiani G, Bass EB, Goodman SN: **Systematic review: gene expression profiling assays in early-stage breast cancer**. *Annals of internal medicine* 2008, **148**(5):358-369.
8. van Loon J, van Baardwijk A, Boersma L, Ollers M, Lambin P, De Ruysscher D: **Therapeutic implications of molecular imaging with PET in the combined modality treatment of lung cancer**. *Cancer treatment reviews* 2011, **37**(5):331-343.
9. Liu K, Lin X, Zhou Q, Ma T, Han L, Mao G, Chen J, Yue X, Wang H, Zhang L *et al*: **The associations between two vital GSTs genetic polymorphisms and lung cancer risk in the Chinese population: evidence from 71 studies**. *PloS one* 2014, **9**(7):e102372.
10. Zhang Y, Ni J, Zhou G, Yuan J, Ren W, Shan Y, Tang W, Yu L, Zhao S: **Cloning, expression and characterization of the human NOB1 gene**. *Molecular biology reports* 2005, **32**(3):185-189.
11. Tone Y, Tanahashi N, Tanaka K, Fujimuro M, Yokosawa H, Toh-e A: **Nob1p, a new essential protein, associates with the 26S proteasome of growing saccharomyces cerevisiae cells**. *Gene* 2000, **243**(1-2):37-45.
12. Tone Y, Toh EA: **Nob1p is required for biogenesis of the 26S proteasome and degraded upon its maturation in Saccharomyces cerevisiae**. *Genes & development* 2002, **16**(24):3142-3157.
13. Fatica A, Tollervey D, Dlakic M: **PIN domain of Nob1p is required for D-site cleavage in 20S pre-rRNA**. *RNA* 2004, **10**(11):1698-1701.
14. Lamanna AC, Karbstein K: **Nob1 binds the single-stranded cleavage site D at the 3'-end of 18S rRNA with its PIN domain**. *Proceedings of the National Academy of Sciences of the United States of America* 2009, **106**(34):14259-14264.
15. Hong L, Piao Y, Han Y, Wang J, Zhang X, Du Y, Cao S, Qiao T, Chen Z, Fan D: **Zinc ribbon domain-containing 1 (ZNRD1) mediates multidrug resistance of leukemia cells through regulation of P-glycoprotein and Bcl-2**. *Molecular cancer therapeutics* 2005, **4**(12):1936-1942.
16. Granneman S, Nandineni MR, Baserga SJ: **The putative NTPase Fap7 mediates cytoplasmic 20S pre-rRNA processing through a direct interaction with Rps14**. *Molecular and cellular biology* 2005, **25**(23):10352-10364.

17. Lin Y, Peng S, Yu H, Teng H, Cui M: **RNAi-mediated downregulation of NOB1 suppresses the growth and colony-formation ability of human ovarian cancer cells**. *Med Oncol* 2012, **29**(1):311-317.
18. He XW, Tao F, Luo SS, Yu XK: **[Effect of lentivirus-mediated NOB1 gene silencing by RNA interference on proliferation and apoptosis of human colon cancer cells]**. *Zhonghua wei chang wai ke za zhi = Chinese journal of gastrointestinal surgery* 2012, **15**(11):1182-1186.
19. Wu DP, He XW: **[Expression of NOB1 and its significance in colorectal cancer]**. *Nan fang yi ke da xue xue bao = Journal of Southern Medical University* 2012, **32**(3):420-422.
20. Wood DE, Kazerooni E, Baum SL, Dransfield MT, Eapen GA, Ettinger DS, Hou L, Jackman DM, Klippenstein D, Kumar R *et al*: **Lung cancer screening, version 1.2015: featured updates to the NCCN guidelines**. *Journal of the National Comprehensive Cancer Network : JNCCN* 2015, **13**(1):23-34; quiz 34.
21. Howlader N, Mariotto AB, Woloshin S, Schwartz LM: **Providing clinicians and patients with actual prognosis: cancer in the context of competing causes of death**. *Journal of the National Cancer Institute Monographs* 2014, **2014**(49):255-264.
22. Henschke CI, Yankelevitz DF, Libby DM, Pasmantier MW, Smith JP, Miettinen OS: **Survival of patients with stage I lung cancer detected on CT screening**. *The New England journal of medicine* 2006, **355**(17):1763-1771.
23. McWilliams AM, Mayo JR, Ahn MI, MacDonald SL, Lam SC: **Lung cancer screening using multi-slice thin-section computed tomography and autofluorescence bronchoscopy**. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer* 2006, **1**(1):61-68.
24. Goldstraw P, Crowley J, Chansky K, Giroux DJ, Groome PA, Rami-Porta R, Postmus PE, Rusch V, Sobin L: **The IASLC Lung Cancer Staging Project: proposals for the revision of the TNM stage groupings in the forthcoming (seventh) edition of the TNM Classification of malignant tumours**. *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer* 2007, **2**(8):706-714.
25. Chen P, Hochstrasser M: **Autocatalytic subunit processing couples active site formation in the 20S proteasome to completion of assembly**. *Cell* 1996, **86**(6):961-972.
26. Ramos PC, Hockendorff J, Johnson ES, Varshavsky A, Dohmen RJ: **Ump1p is required for proper maturation of the 20S proteasome and becomes its substrate upon completion of the assembly**. *Cell* 1998, **92**(4):489-499.
27. Ferreira-Cerca S, Poll G, Gleizes PE, Tschochner H, Milkereit P: **Roles of eukaryotic ribosomal proteins in maturation and transport of pre-18S rRNA and ribosome function**. *Molecular cell* 2005, **20**(2):263-275.
28. Ferreira-Cerca S, Poll G, Kuhn H, Neueder A, Jakob S, Tschochner H, Milkereit P: **Analysis of the in vivo assembly pathway of eukaryotic 40S ribosomal proteins**. *Molecular cell* 2007, **28**(3):446-457.
29. Hong L, Chen Z, Zhang X, Xia L, Han Z, Lu Y, Jin H, Song J, Qiao T, Fan D: **Zinc ribbon domain containing 1 protein: modulator of multidrug resistance, tumorigenesis and cell cycle**. *Experimental*

oncology 2006, **28**(4):258-262.

30. Yin J, Wang J, Jiang Y, Wang L, Wu H, Liu H: **Downregulation of NOB1 inhibits proliferation and promotes apoptosis in human oral squamous cell carcinoma**. *Oncology reports* 2015, **34**(6):3077-3087.
31. Zhang X, Zhang D, Qu F, Hong Y, Cao J, Pan X, Li L, Huang Y, Huang H, Yin L *et al*: **Knockdown of NOB1 expression inhibits the malignant transformation of human prostate cancer cells**. *Molecular and cellular biochemistry* 2014, **396**(1-2):1-8.
32. Li Y, Ma C, Qian M, Wen Z, Jing H, Qian D: **Downregulation of NOB1 suppresses the proliferation and tumor growth of non-small cell lung cancer in vitro and in vivo**. *Oncology reports* 2014, **31**(3):1271-1276.

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

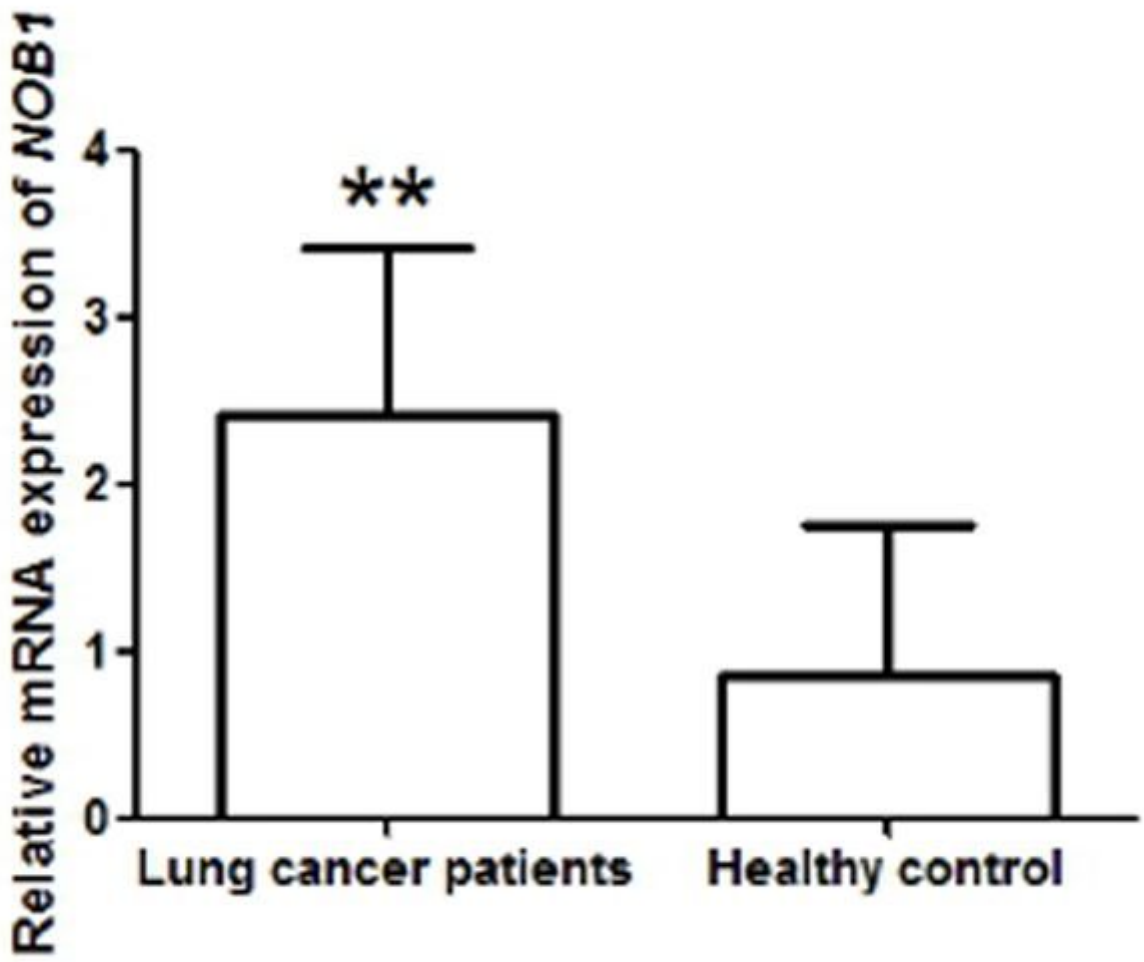

Figure 1

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).

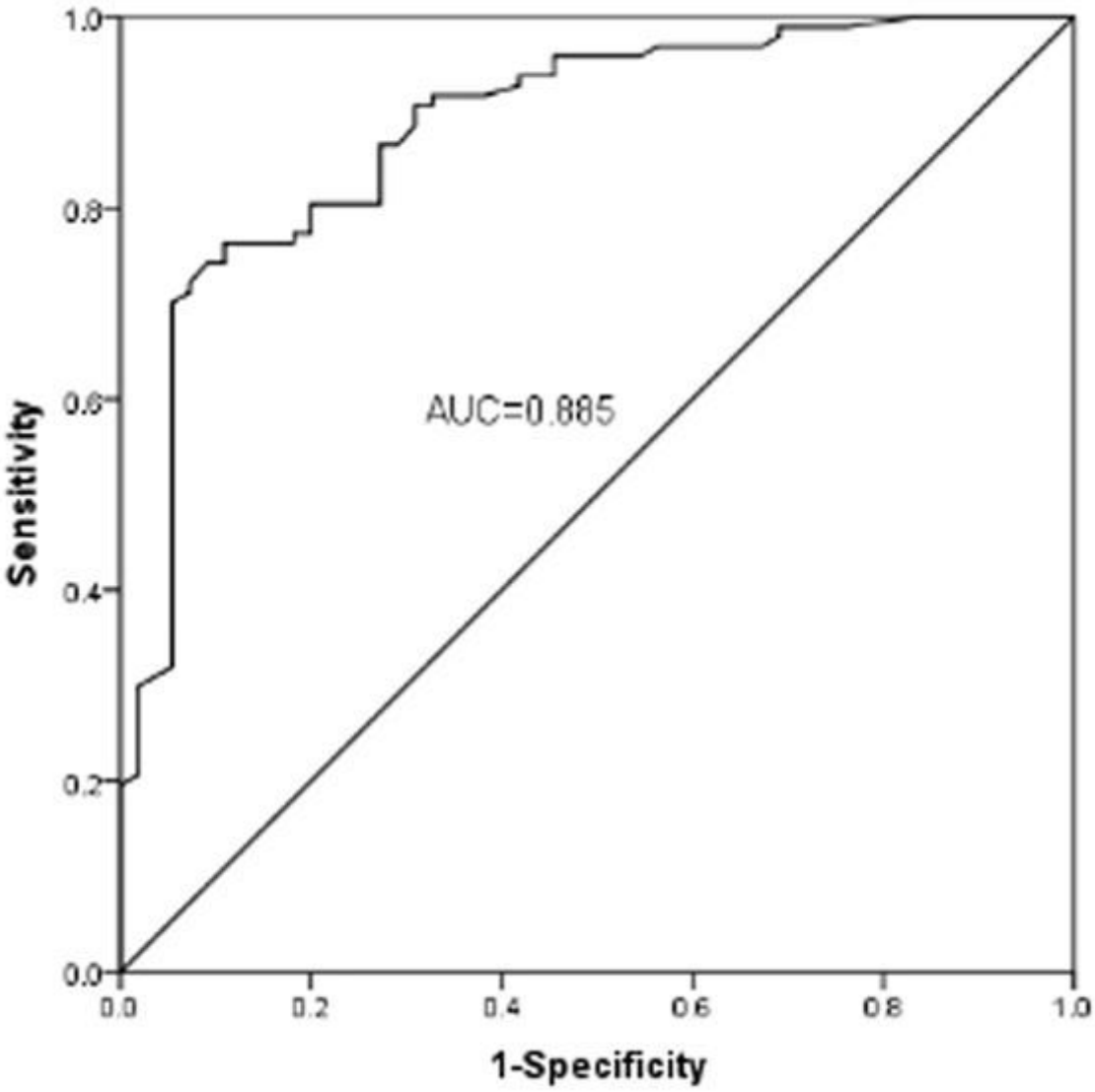

Figure 2

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