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Original Article

OTUD7B and NIK expression in non-small cell lung cancer: Association with clinicopathological features and prognostic implications

Boxiang Zhang[a], Huangzhen Wang[a], Litao Yang[a], Yiwen Zhang[a], Peili Wang[a], Guanghong Huang[a], Jie Zheng[b], Hong Ren[a,*], Sida Qin[a,*]

[a] Department Two of Thoracic Surgery, the First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, 710061, PR China
[b] Clinical Research Center,the First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, 710061, PR China

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ABSTRACT

Purpose: To investigate the correlation among OTUD7B and NIK expression and the clinicopathological characteristics in NSCLC patients.
Methods: One hundred and twenty patients were involved in this study. We detected OTUD7B and NIK expression by immunohistochemistry and analyzed their correlation with clinicopathological data.
Results: The expression of OTUD7B and NIK were negatively correlated in NSCLC tumor samples ($r_s = -0.421$, $P < 0.001$). The higher expression of OTUD7B was associated with smaller tumor size($P = 0.018$), less lymph node metastasis ($P = 0.012$) and earlier TNM stage($P = 0.039$), while the higher expression of NIK was only related to more lymph node metastasis($P = 0.031$) and later TNM stage($P = 0.011$). MMP-9 was negatively correlated with OTUD7B and positively correlated with NIK. In addition, the high expression of OTUD7B was associated with good prognosis of NSCLC patients (log-rank = 6.714, $P = 0.0096$), and a high OTUD7B/low NIK index can predict an even better prognosis (log-rank = 11.794, $P = 0.0006$). Moreover, the multivariate Cox regression analysis showed that OTUD7B rather than NIK is an independent marker of overall survival in NSCLC patients(HR = 1.602, 95% CI 1.009-2.544, $P = 0.046$).
Conclusions: OTUD7B and NIK may play important roles in the development of lung cancer. The combination of OTUD7B and NIK expression may be a good index for predicting the prognosis of NSCLC.

1. Introduction

Lung cancer, accounting for about 13% of total cancer diagnoses, is the leading cause of male cancer deaths and the second leading cause of female cancer deaths world-wide in 2012 [1]. Non–Small-Cell Lung Cancer (NSCLC) accounts for nearly 80% of all lung cancers [2]. In spite of the rapid development of diagnostic and therapeutic technologies for NSCLC, its outcome remains poor [3,4]. It is because most patients are diagnosed at the advanced stage due to the vague symptoms and the lack of a specific target for this disease at its earlier stage [5,6]. Five-year survival rate of the advanced NSCLC patients is still less than 20% [7]. Therefore, new targets are required to diagnose NSCLC at the early stage and evaluate the prognosis of patients.

NF-κB signaling pathway regulates a variety of cellular functions, such as cell proliferation, apoptosis, metastasis, angiogenesis and so on [8,9]. It has been suggested that NF-κB pathway plays a vital role in the development of NSCLC [10]. NF-kappaB-inducing kinase (NIK), a serine/threonine protein kinase, is a central signaling component of non-canonical NF-κB pathway. NIK mediated phosphorylation of IKKα and subsequent partial proteasomal degradation of p100 into p52. Finally, p52 translocates into the nucleus to activate non-canonical NF-κB signaling [11,12]. The expression of NIK is kept low in normal conditions but is prominently elevated in response to stimulation by specific receptors [13]. In recent years, many reports indicated that the expression of NIK is increased in many cancers, such as melanoma, pancreatic cancer, lung cancer, and it is so closely related to the invasion and metastasis [10,14,15]. For this reason, NIK might be used as a new generation of therapeutic targets for tumor treatment.

Recent Studies have demonstrated that NF-κB is down-regulated by overexpression of deubiquitinating enzyme A20 [16]. OTU domain-containing 7 B (OTUD7B) is a member of A20 family and has been known to encode deubiquitinating enzyme, which

* Corresponding authors at: No. 277 West Yanta Road, Xi'an, Shaanxi 710061, PR China.
E-mail addresses: renhongs2000@gmail.com (H. Ren), sida.qin@yahoo.com (S. Qin).

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participates into inflammation, scar formation and hypoxia regulation [17–19]. However, the role of OTUD7B in the development of cancer, especially NSCLC, remains to be fully elucidated.

In our study, we detected the expression of OTUD7B and NIK in 120 lung cancer samples and 63 paired normal lung tissue by immunohistochemistry (IHC), analyzed their correlation with clinicopathological data and evaluated the prognosis of NSCLC patients. Here we demonstrate that OTUD7B and NIK may play an important role in the development of lung cancer and OTUD7B+/NIK- is a good index for predicting the prognosis of NSCLC.

2. Material and methods

2.1. Patients

All tissue samples were obtained from 120 NSCLC patients who undergone curative resections between March 2006 and September 2009 at the First Affiliated Hospital of Xi'an Jiaotong University(Xi'an, China). The including criteria of the study were as following: (1) All NSCLC tissues were histologically confirmed, (2) None of the patients had distant metastasis or received anticancer therapies before the operation, (3) None of the patients had the serious complications or other malignant diseases. Tumor stage was classified according to the tumor-node-metastasis (TNM) of the International Association for the Study of Lung Cancer (7th Edition). The adenocarcinoma cases were classified according to 2015 WHO Histological Classification of Tumors of the Lung, Pleura, Thymus, and Heart (4th edition). Overall survival (OS) was defined as from the time of lung resection to the time of the patients' death or last follow-up. The last follow-up date was on 3 April 2015. The median follow-up period was 54 months (4–94 month). The main cause of death was NSCLC recurrence. The study was approved by the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University, based on the patients' informed written consent for the usage of the biologic material.

2.2. IHC staining

The expression of OTUD7B and NIK was evaluated by immunohistochemical (IHC) analyses in 4-μm sections of the 10% formaldehyde-fixed and paraffin-embedded blocks. The tissue slides were deparaffinized in xylene and rehydrated through a series of graded alcohols. For exhaust endogenous peroxidase activity, the slides were treated with 3% H_2O_2 for 10 min. Then, the slides were immersed in 0.01 M citrate buffer PH6.0 for 3 min in a pressure cooker at 125 °C, then placing them at room temperature for 30 min to cool down. After washing with phosphate buffered saline(PBS), the slides were pre-incubated for 30 min in 10% normal goat serum and then incubated with OTUD7B mouse monoclonal antibody (1:400, Abcam, #ab118387, UK), NIK rabbit monoclonal antibody (1:400, Abcam, # ab191591, UK), Ki-67 mouse monoclonal antibody (1:200, Cell Signaling, #12202, USA) and MMP-9 rabbit monoclonal antibody (1:200, Cell Signaling, #13667, USA) overnight at 4 °C. After washing with PBS, the slides were incubated for 30 min secondary antibody (1:500, anti-mouse/rabbit secondary antibody, Maixin Biotechnology Co. Ltd., Fuzhou) at room temperature. All slides were generated using a diaminobenzidine chromogen solution. The counterstaining was performed with hematoxylin. The slides then were dehydrated, cleared and mounted. Human liver cancer and pancreatic cancer served as positive controls for OTUD7B and NIK, respectively. For negative control, the slides were treated with PBS instead of primary antibody.

Both the intensity and extent of staining were taken into consideration when analyzing the data. The extent of staining was scored from 0% to 100%(1 means 1–25%, 2 means 26–50%, 3 means 51–75%, 4 means 76–100%) and the intensity of staining was scored from 0 to 2(0 means none; 1 means weak to moderate; 2 means strong). The IHC score was determined as high expression (+): score ≥ 3; low expression (−): score ≤ 2.

2.3. Statistical analysis

SPSS 13(SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. OTUD7B and NIK status were calculated for an association with clinicopathological features using the χ^2 and Fisher's exact test. Spearman correlation analysis was used to analyze the correlation of OTUD7B with NIK status. Survival curves were generated using the Kaplan-Meier. Univariate and multivariate analysis were generated using the Cox proportional hazards model. $P < 0.05$ was considered to indicate a statistically significant result.

3. Results

3.1. OTUD7B *and NIK expression in NSCLC*

To illuminate the biological significance of OTUD7B and NIK in NSCLC, we used immunohistochemistry to detect the expression of OTUD7B and NIK protein in 120 NSCLC samples and 63 paired normal lung tissue. Typical OTUD7B immunohistochemical staining was observed in the nucleus and cytoplasm of cells, while NIK staining was identified in the cytoplasm. The results showed that the percentages of high expression of OTUD7B and NIK were 54.2% (65 of 120 patients) and 51.7%(62 of 120 patients) in lung cancer tissues, in contrast to 71.4% (45 of 63 patients) and 23.8%(15 of 63 patients) in normal lung tissue, respectively. (Table 1, Fig. 1).

3.2. Correlations between OTUD7B or NIK protein expression and clinicopathological features

To examine the association between the expression of OTUD7B or NIK and clinicopathological features of NSCLC patients, we compared the OTUD7B and NIK expression in 120 NSCLC specimens with 10 clinicopathological features, such as gender, age, smoking history, tumor differentiation, Histopathology, Tumour size, Lymphatic invasion, TNM Stage, the expression of Ki-67 and MMP-9. (Table 2, Fig. 2). Our results showed that the high expression of OTUD7B displayed a significant negative correlation with tumor size($P = 0.018$), lymphatic invasion($P = 0.012$), stage($P = 0.039$), and MMP-9 expression, while the high expression of NIK was significantly positively correlated with lymphatic invasion ($P = 0.031$), stage ($P = 0.011$) and MMP-9 expression. The higher expression of OTUD7B was more frequently observed in earlier stage tumors with smaller tumor size and less lymph nodes metastasis. In contrast, the higher expression of NIK was more frequently observed in later stage tumors with larger tumor size and more lymph nodes metastasis. However, the OTUD7B and NIK levels were not significantly correlated with gender, age, tumor differentiation, histopathology, smoking, and the expression of Ki-67 ($P > 0.05$, Table 2). Moreover, we classified the adenocarcinoma cases according to 2015 WHO Histological Classification of Tumors of the Lung, Pleura, Thymus, and Heart (4th ed), and there was no significant correlation between histological subtypes and NIK or OTUD7B expression. (Table 3).

3.3. Association between OTUD7B and NIK expression in NSCLC

Both OTUD7B and NIK were highly expressed in 21 specimens, while both of them were low expression in 14 specimens. OTUD7B overexpression was detected in 75.9% (44/58) of NIK low expression specimens. NIK overexpression was detected in