The diagnostic performance of lysine(K)-specific demethylase 6B (KDM6B) in non-small cell lung cancer

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ABSTRACT

Objective: Accumulating evidence show that histone demethylases play important roles in various types of cancers, including non-small cell lung cancer (NSCLC). In the current study, we evaluated the diagnostic value of lysine(K)-specific demethylase 6B (KDM6B) in NSCLC.

Methods: Serum KDM6B expression levels of 115 NSCLC patients and 88 healthy volunteers were detected by reverse transcription quantitative real-time polymerase chain reaction (qRT-PCR). The relationship between KDM6B and clinical characteristics was assessed by chi-square test. Receiver operating characteristic (ROC) analysis was applied to evaluate diagnostic efficacy.

Results: Serum KDM6B was significantly lower in NSCLC patients than that in healthy controls (p < .001). Moreover, low KDM6B expression was significantly associated with the high clinical stage (p = .028) and positive lymph node metastasis (p = .031). Besides, we found that the expression of KDM6B mRNA was also significantly different among healthy controls, NSCLC early stage and later stage patients (p < .05). ROC curve indicated that KDM6B could serve as a diagnostic marker for NSCLC with the cut-off value of 0.955. The AUC was 0.897 with a sensitivity of 79.5% and specificity of 84.3%.

Conclusion: Down-regulation of KDM6B is significantly associated with aggressive progression of NSCLC and KDM6B may be a tool of early detection of NSCLC.

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Introduction

Non-small cell lung cancer (NSCLC) is the most common type of lung cancer with high morbidity and mortality in the world [1–3]. What is worse, the occurrence rate of NSCLC is increasing in recent years [4]. Although great progress has been made in NSCLC treatments, the clinical outcomes of the patients, especially those with advanced NSCLC, remain poor [5]. Unfortunately, more than 65% of the patients with NSCLC are diagnosed in advanced stages with locally advanced or metastasis [6]. Therefore, markers for early detection of NSCLC are vitally important for the prognosis of the patients. In previous studies, several molecular markers were identified for NSCLC detection, including epidermal growth factor receptor, TP53, carcinoembryonic antigen (CEA), and cancer antigen-125 (CA125) [7–9]. However, due to the low sensitivity and specificity, the clinical application of these markers is limited [10,11]. Therefore, novel biomarkers with high sensitivity and specificity are urgently needed for the early diagnosis of NSCLC, which may also improve the treatments of cancer.

Histone methylation, as a mechanism of epigenetic regulation, plays important roles in the development of several diseases, as well as cancers [12]. Accumulating evidence have proved that histone methyltransferases and demethylases are significantly associated with various cells and tumor progression, including cell proliferation, invasion and metastasis [13–15]. It was reported that the genes associated with histone methylation could serve as diagnostic and prognostic markers for cancer [16]. Lysine(K)-specific demethylase 6B (KDM6B, also known as JMJD3), an H3K27 demethylase, is a histone lysine demethylase. KDM6B plays importantly functional roles in tumor progression. A study carried out by Tokunaga et al. proved that KDM6B could regulate colorectal cancer cell line progression, moreover, its expression level could indicate the outcomes of the patients, which may act as a prognostic marker in the patients [17]. In the study of Ene et al., KDM6B was proved to involve in the gliomagenesis via regulating the p53 pathway [18]. The effects of KDM6B on NSCLC cell lines were also investigated in the previous studies, suggesting KDM6B can promote cell apoptosis [19]. However, the diagnostic value of KDM6B in NSCLC had been rarely reported in the previous studies.

In the present study, we aimed to evaluate the diagnostic significance of KDM6B in NSCLC. Patients pathologically diagnosed with NSCLC and healthy individuals were enrolled in the study. We compared the expression level of KDM6B...
between the participants and evaluated the diagnostic value of the gene. The present study may exploit a novel diagnostic marker for NSCLC, which may be helpful for cancer detection and therapy.

Methods and materials

Patients and samples

This study was approved by the Ethics Committee of the Hospital. Informed consent was obtained from each participant.

The study was scheduled in Shanghai East Hospital, Tongji University School of Medicine. One hundred fifteen patients pathologically diagnosed with NSCLC were finally enrolled, including 73 with squamous carcinoma and 42 with adenocarcinoma. The control group included 88 healthy volunteers. None of the control patients had formerly been diagnosed with any malignancy. Blood specimens were collected from all the participants in the morning after fasting for 8–10 h.

About 5 ml peripheral blood was collected in PAXgene Blood RNA Tubes (Qiagen, PreAnalytiX, Hombrechtikon, Switzerland) specifically designed for the collection and stabilization of cellular RNA from whole blood. Serum samples were obtained by being centrifuged at 2500 × g for 20 min. The serum samples were stored at -80°C until RNA extraction, which was performed within 6 months of collection.

RNA extraction and quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from serum using TRIzol LS reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instructions. RNA quantity and quality were assessed with NanoDrop 2000 (Thermo Scientific, Wilmington, DE, USA). cDNA was generated by reverse transcription of 100 ng RNA from each sample in triplicate, using PrimeScript RT kit (Tahara, Dalian, China), according to the manufacturer’s instructions. The resulting cDNA was pooled and amplified by qRT-PCR, which was performed with SYBR green I Master Mix kit (Invitrogen). GAPDH served as internal control and the relative expression of KDM6B was calculated by 2^−ΔΔCt method. The primer sequences were as followed: KDM6B forward: 5′-TGGTCTGTGTTACCCACTG-3′; reverse: 5′-CGAC AATGACTCGCCCTCTCG-3′; GAPDH forward: 5′-TGCACCACCAAGTGTTACAC-3′; reverse: 5′-GGCATGCACGTGTTGTCATGAG-3′.

The protein level of KDM6B was evaluated by Western blot according to the standard operation. The primary antibody was anti-JMJD3 (ab38113, Abcam, Cambridge, UK) and the protein levels were detected by ECL Prime Western Blotting Detection System (Amersham/GE, USA).

Statistical analysis

All statistical analyses were carried out in SPSS 18.0 software (SPSS, Chicago, IL, USA). Graphs were plotted by GraphPad Prism 5 (GraphPad, San Diego, CA, USA). The continuous data were presented as mean ± SD and compared between two groups by Student’s t test. Chi-square test was used to analyze the association between KDM6B expression and clinicopathologic features. ROC curve was applied to evaluate the diagnostic value of KDM6B in NSCLC. p values less than .05 was considered statistically significant.

Results

Serum KDM6B expression in NSCLC patients

QRT-PCR was used to detect the relative expression of KDM6B in collected serum specimens. The results showed that serum KDM6B expression was significantly decreased in NSCLC, compared with healthy control (p < .001) (Figure 1). The results of Western blot suggested that the protein level of KDM6B was markedly down-regulated in NSCLC patients, compared with healthy control, which was in accordance with the results of mRNA level.

In addition, we also analyzed the expression differences of KDM6B mRNA among NSCLC patients according to different age, gender, clinical stage, and lymph node metastasis. As displayed in Figure 2, KDM6B expression was not significantly influenced by patients’ age (p = .303) or gender (p = .474), but clinical stage (p = .009) and lymph node metastasis (p = .004) showed close association with KDM6B mRNA levels in NSCLC patients.

The association of KDM6B expression with clinicopathologic features of NSCLC

In order to evaluate the relationship between KDM6B expression and clinicopathologic variables, the patients were divided into two groups according to their average expression of KDM6B. Sixty-two patients were classified as low-KDM6B group and 53 patients were classified in high-KDM6B group. Chi-square test was carried out to assess the association between KDM6B expression levels and different clinicopathologic factors. The result showed that low KDM6B expression was significantly correlated with high clinical stage (p = .028) and positive lymph node metastasis (p = .031). In addition,
**Figure 2.** The comparison of KDM6B mRNA levels among NSCLC patients based on their age (A), gender (B), clinical stage (C) and lymph node metastasis (D). *p < .05 and **p < .01 indicated the significant difference between the compared two.

**Table 1.** Association between KDM6B expression and clinicopathological characteristics of NSCLC patients.

| Characteristics                              | No. of cases | KDM6B expression | χ² | p Values |
|----------------------------------------------|--------------|------------------|----|----------|
|                                              | N = 115      |                  |    |          |
| Age (years)                                  |              |                  |    |          |
| <50                                          | 47           | Low (N = 62)     | 0.260 | .610    |
| ≥50                                          | 68           | High (N = 53)    | 0.260 | .610    |
| Gender                                       |              |                  |    |          |
| Male                                         | 86           | Low (N = 44)     | 1.308 | .308    |
| Female                                       | 29           | High (N = 18)    | 1.308 | .308    |
| Smoking index                                |              |                  |    |          |
| <400                                         | 24           | Low (N = 13)     | 0.001 | .978    |
| ≥400                                         | 91           | High (N = 49)    | 0.001 | .978    |
| Histological type                            |              |                  |    |          |
| Squamous cell carcinoma                      | 73           | Low (N = 41)     | 0.408 | .523    |
| Adenocarcinoma                               | 42           | High (N = 21)    | 0.408 | .523    |
| Differentiation                              |              |                  |    |          |
| Well and moderate                            | 81           | Low (N = 43)     | 0.075 | .784    |
| Poor                                         | 34           | High (N = 19)    | 0.075 | .784    |
| Clinical stage                               |              |                  |    |          |
| I–II                                         | 77           | Low (N = 36)     | 4.408 | .028    |
| III–IV                                       | 38           | High (N = 26)    | 4.408 | .028    |
| Lymph nodes metastasis (N)                   |              |                  |    |          |
| N0                                           | 68           | Low (N = 31)     | 4.641 | .031    |
| N1 + N2                                      | 47           | High (N = 31)    | 4.641 | .031    |
| Tumor size (cm)                              |              |                  |    |          |
| <3                                          | 74           | Low (N = 35)     | 3.656 | .056    |
| ≥3                                          | 41           | High (N = 27)    | 3.656 | .056    |
there was no marked relationship between KDM6B expression and other clinical features, such as age, gender, smoking index, histological type, differentiation, or tumor size (p > 0.05 for all) (Table 1).

The diagnostic value of KDM6B expression for NSCLC patients

To evaluate the diagnostic value of serum KDM6B mRNA level in NSCLC patients, we performed ROC curve analysis. Analysis results indicated that KDM6B could distinguish the NSCLC patients from healthy individuals with the optimal cut-off value of 0.955. The area under the ROC curve (AUC) was 0.897, with the sensitivity of 79.5% and the specificity of 84.3% (Figure 3).

Moreover, we explored the expression differences of serum KDM6B mRNA among healthy controls, NSCLC early stage (stage I–II) and later stage patients (stage III–IV), the results indicated the difference was significant between any compared two groups (p < 0.05, Figure 4). So, KDM6B might be a tool of early detection of NSCLC.

Discussion

NSCLC is one of the most lethal and aggressive neoplasms. The low-survival rate of cancer may be contributed to the poor early detection and the lack of effective treatments for advanced stage [20]. The novel biomarkers with high sensitivity and specificity for early diagnosis of NSCLC may significantly improve the outcomes of the patients. In the present study, we investigated the diagnostic value of KDM6B in NSCLC. Analysis results indicated that KDM6B may be a potential biomarker for early detection of NSCLC.

Histone methylation plays a key role in the regulation of gene expression during the cell cycle and its abnormality may be related to tumor occurrence [21]. It was reported that site-specific histone methyltransferases and demethylases could regulate histone methylation, which is a reversible process [22]. Abnormal expression or mutation of histone methyltransferases and demethylases related genes were frequently associated with cancer progression [23]. KDM6B encoding an enzyme which can catalyze histone H3K27me3 demethylation. Abnormal H3K27me3 methylation was reported to be correlated with several cancers, including melanoma, colon, gastric, stomach, ovarian, breast and kidney cancers [24,25]. Thus, KDM6B may also involve in tumor progression.

In this study, we investigated the clinical significance of KDM6B in NSCLC. We firstly detected KDM6B expression levels in the NSCLC serum samples. Our results showed that serum KDM6B expression levels were significantly lower in NSCLC patients compared with healthy controls. Moreover, the down-regulated level of KDM6B was significantly correlated with high clinical stage and positive lymph nodes metastasis. Ma et al. had reported that KDM6B was a tumor suppressor gene in NSCLC, which can promote cell apoptosis [19]. The conclusion supported our results. However, some studies hold different opinions. Tian et al. had proved that the decreased expression level of KDM6B was significantly associated with high TNM stage and positive lymph node metastasis, however, the cell experiments indicated that KDM6B can promote NSCLC cell growth, inhibit cell apoptosis and had no effects on cell migration [26]. The differences may reveal that KDM6B can influence the progression of the NSCLC by different pathways. In addition, the effects of KDM6B on NSCLC cell lines were needed to be identified in the next study.

Various biomarkers are confirmed to be used in the diagnosis of NSCLC, of which the most widely used are CEA and CK19 [27]. However, the low sensitivity and specificity limit...
their clinical application, especially for the detection of early NSCLC. Therefore, the novel diagnostic markers with high sensitivity and specificity for early detection of NSCLC were urgently needed. In the present study, we evaluated the diagnostic value of KDM6B in patients with NSCLC. ROC curve indicated that KDM6B could distinguish patients with NSCLC from healthy individuals, with high sensitivity and specificity. In the previous studies, KDM6B was reported to be markedly correlated with various tumor progression, such as pancreatic cancer, colon cancer, Hodgkin's Lymphoma, clear cell renal cell carcinoma [28–31]. The tumor progression gene may be effective for early detection of NSCLC, which can significantly improve the outcomes of the patients.

Although we proved the diagnostic value of KDM6B in NSCLC, there were still several limitations in the present study. Firstly, the number of patients collected in the study was small and further studies with larger numbers of patients are needed to validate the results. Secondly, the patients, in this study, all came from one hospital and the study results may differ according to the techniques used. Therefore, multi-center studies are needed to confirm the diagnostic value of KDM6B in NSCLC. In addition, the mechanisms for KDM6B regulating tumor progression of NSCLC were needed to be identified further. Given the mentioned limitations, the application of serum KDM6B for early diagnosis of NSCLC might cause diagnostic errors. The final diagnosis of NSCLC might be employed as an auxiliary tool for NSCLC screening. The combined application of KDM6B with other indicators may have the ability to improve the early diagnosis rate of NSCLC.

In conclusion, serum KDM6B mRNA level is markedly lower in patients with NSCLC than that in the healthy controls. Moreover, the decreased level is significantly associated with aggressive clinical characteristics in NSCLC. KDM6B may be applied for early screening of NSCLC, which may markedly improve the management of cancer.

**Disclosure statement**

We declare that we do not have any conflict of interest.

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