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Research Article

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Forkhead box 1 expression is upregulated in non-small cell lung cancer and correlates with pathological parameters

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Abstract: *Objectives*: As the member of the Fox family of transcription factors, Forkhead box M1 (FoxM1) is known to be critical in pathogenesis and development of many solid tumors. However, the clinical value and expression pattern in non-small cell lung cancer (NSCLC) is still poorly understood. *Methods*: In this study, real-time PCR was mainly applied to examine the gene expression levels of FoxM1 in 120 pairs of clinical NSCLC tissues, which were classified into different groups according to smoking status, lymph node metastasis, and tumor grade. By utilizing the online Kaplan-Meier plotter, overall survival analysis was performed to study the correlation between FoxM1 expression and prognosis of lung cancer (LC) patients. Afterwards, the correlation of FoxM1 gene expression and the clinical pathological parameters was examined by κ^2 test in these 120 NSCLC patients. *Results*: FoxM1 was found to be aberrantly upregulated in NSCLC patients, and its overexpression was correlated to groups designated as smokers, cases of positive lymph node metastasis and cases of advanced tumor grades. Online survival analysis showed that high expression of FoxM1 predicted shorter overall survival of NSCLC patients. Additionally, FoxM1 upregulation was statistically correlated with positive smoking history, lymph node metastasis and higher tumor grades. *Conclusion*: FoxM1 is overexpressed in cancerous tissues and is associated with the poor prognosis of NSCLC patients. Our results provide insights into the utility of FoxM1 as an important biomarker and prognostic factor for NSCLC.

Keywords: FoxM1, upregulation, NSCLC, prognosis

1 Introduction

Lung cancer (LC) ranks as the top leading cause of cancer-related deaths worldwide. Deaths caused by LC are equal to that of the other four most deadly tumors combined (breast, colon, prostate, and pancreas) [1,2]. Because few symptoms are observed at early stages, LC patients are usually diagnosed at the later stages, when organ metastasis might have occurred [3]. Non-small cell lung cancer (NSCLC) is the most common type of LC, accounting for 80%-85% of all LC patients. Lymph node (LN) status is a major determinant of stage and survival in NSCLC patients. Most NSCLC patients are in an advanced stage at the time of diagnosis, along with LN and distant metastasis [4]. The 5-year survival rate for NSCLC remains as low as 15%, and the prognosis has not been largely improved over the last decade [5].

Forkhead box M1 (FoxM1), a member of the Fox family of transcriptional factors, belongs to a group of evolutionarily conserved transcriptional factors that are characterized by the presence of a DNA-binding domain called the Forkhead box [6,7]. FoxM1 is found to be expressed in actively dividing cells [8,9], and is critical for cell cycle progression by regulating G1/S and G2/M phase transitions and ensuring the proper execution of mitotic cell division [6,7,10]. In addition, FoxM1 is also involved in DNA damage and apoptotic pathways, suggesting a role in multidrug resistance [11,12]. It has been found to be overexpressed in multiple human cancers, including liver, breast, lung and bladder cancer, clear cell renal cell carcinoma and early stage cervical cancer. Also,

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abnormally highly expression of FoxM1 has been shown to be associated with poor prognosis and is considered to be an independent predictor of poor survival in many solid cancers. In NSCLC, it has been reported that FoxM1 is associated with poor prognosis of NSCLC patients by enhancing tumor metastasis [13]. In addition, it has been shown that FoxM1 overexpression correlated with the short survival of patients using immunohistochemistry analysis of 69 cases of squamous cell carcinoma specimens [14].

Although previously studies have demonstrated the important clinical significance of FoxM1 in NSCLC, large scale population study is still missing. Here, we evaluated the expression and clinical value of FoxM1 in a total of 120 pairs of NSCLC tissues and matched non-cancerous normal lung tissues in order to further disscuss the importance of FoxM1 expression in NSCLC.

2 Materials and methods

2.1 Patients and tissues

A total of 120 confirmed NSCLC patients were consecutively recruited between January 2012 and January 2016 for the treatment of non-small cell lung cancer (NSCLC) at Hangzhou Third Hospital. All surgical tissues were examined by a pathologist and final surgical pathology reports were obtained and recorded. The written consent to use their samples was obtained from all the patients.

2.2 Reverse transcription (RT) and Real-Time PCR

Total RNA was isolated from tissues using TRIzol reagent (Life Technologies). First-strand cDNA was synthesized from 2mg of total RNA using reverse transcriptase (Promega). Real-time PCR analysis of FOXM1 expression was carried out using 1ml of cDNA and the SYBR Green Master Mix (Takara), as recommended by the manufacturer. ACTIN was used as an internal control. PCR products were run in triplicate for target and internal control genes.

2.3 Statistical analysis

The two-tailed χ^2 test was used to determine the significance of differences between FOXM1 expression and clinicopathological features. Kaplan-Meier survival curves were obtained using Kaplan-Meier plotter (http://kmplot.com/analysis/). $P < 0.05$ was considered as significant.

3 Results

3.1 Overexpression of FoxM1 in NSCLC is associated with smoking status, LN metastasis and tumor grade

Firstly, we explored the gene expression of FoxM1 in a total of 120 pairs of NSCLC tissues by real-time PCR. According to the previous studies, FoxM1 is found to be highly expressed in tumoral tissues including NSCLC [13]. The current study also showed that FoxM1 was significantly highly expressed ($P < 0.01$) in NSCLC tissues than in paired normal lung tissues (**Figure 1A**). To investigate whether the increased expression of FoxM1 was associated with some prognostic factors, the collected NSCLC cases were divided into different groups according to smoking history, LN metastasis (LM), and tumor grade. As shown in **Figure 1B-D**, higher expression of FoxM1 was correlated with smoking history ($P < 0.05$), LN metastasis ($P < 0.05$) and higher tumor grades ($P < 0.05$), suggesting the important clinical value of FoxM1 in NSCLC prognosis. The histological classification of NSCLC was based on the 2004 WHO criteria. These patients did not receive any radiotherapy or chemotherapy.

3.2 Elevated FoxM1 expression predicts shorter overall survival time of NSCLC patients

Next, we utilized the online Kaplan-Meier Plotter (http://kmplot.com/analysis/) to analyze the overall patient survival time and to determine the correlation between FoxM1 expression and prognosis of LC patients. The subgroup analysis of non-smoking ($n = 247$), smoking ($n = 970$), adenocarcinoma (ADC, $n = 866$) and squamous cell carcinoma (SCC, $n = 675$) were shown respectively in **Figure 2A-D**. From this independent analysis, we believe that high expression of FoxM1 predicted shorter overall survival of NSCLC patients, especially in those smoking and ADC patients. These findings strongly suggest that FoxM1 was an independent and significant predictor of shorter survival times for NSCLC patients.

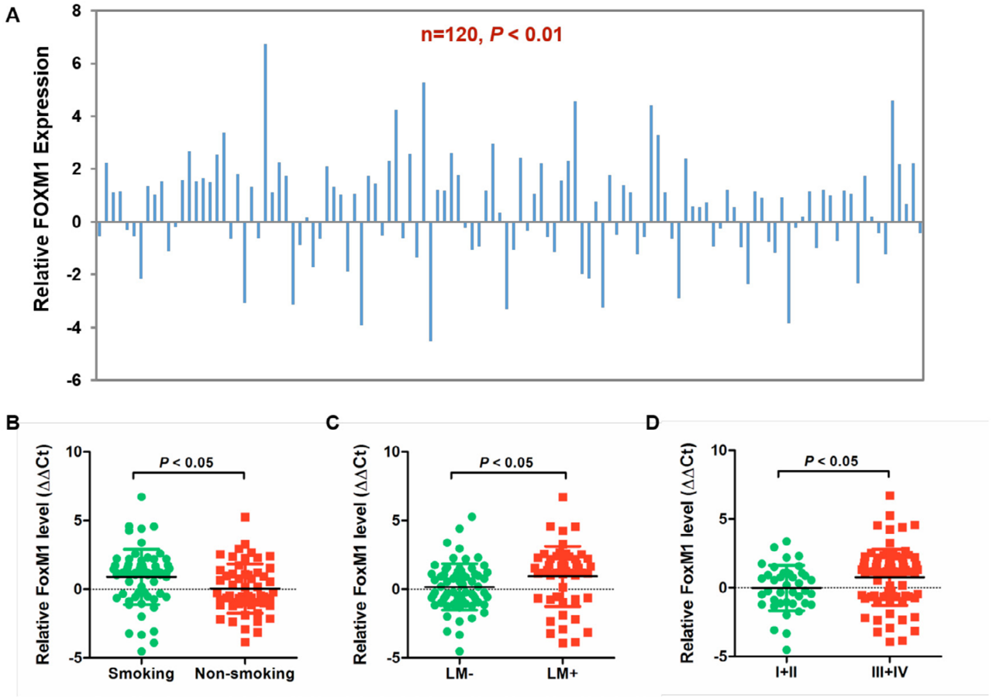

Figure 1 FoxM1 expression in human NSCLC and its association with pathological features. (A) FoxM1 is upregulated in paired NSCLC samples (n = 120, P < 0.01). **(B)** FoxM1 expression is positively correlated with smoking history (P < 0.05). **(C)** FoxM1 expression is positively correlated with lymph node metastasis (LM) (P < 0.05). **(D)** FoxM1 expression is positively correlated with higher tumor grades (I+II vs. III+IV, P < 0.05).

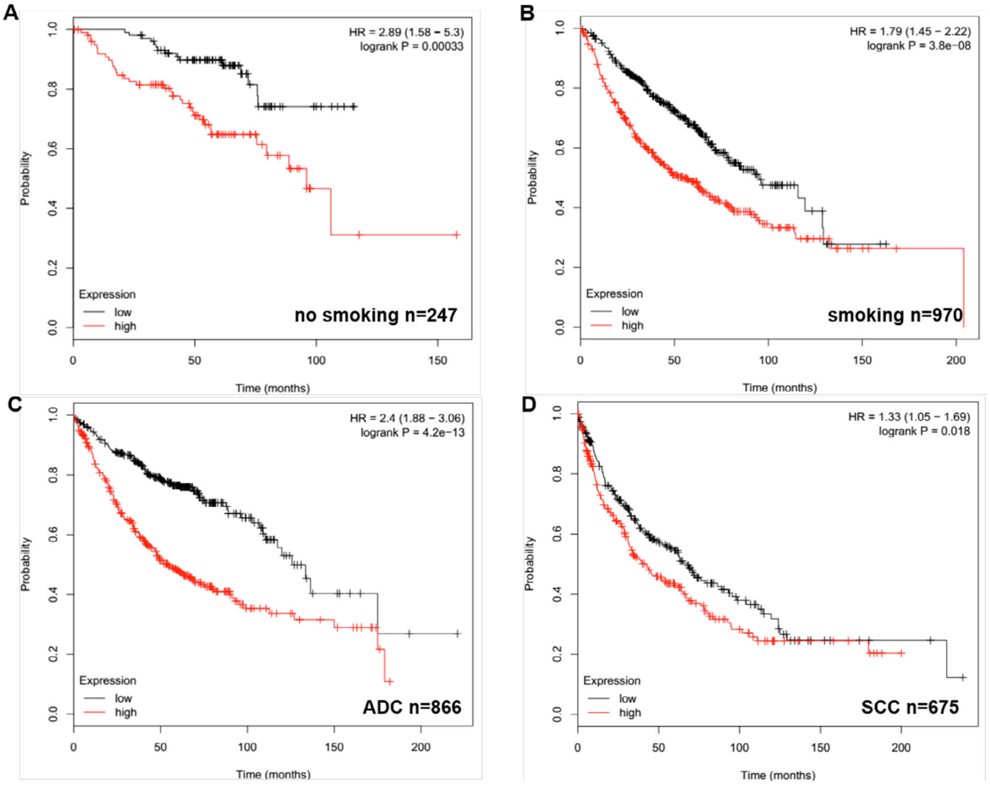

Figure 2 Kaplan-Meier survival analysis of overall survival of LC patients with respect to their level of expression of FoxM1 in no smoking **(A)**, smoking **(B)**, adenocarcinoma **(C)** and squamous cell carcinoma **(D)** populations by the online KM plotter. The log-rank test was used to test the two survival distributions. P < 0.05 was considered to have statistical significance.

3.3 The correlation between FoxM1 expression and pathological parameters of NSCLC patients

Finally, we performed k^2 test to study the correlation between FoxM1 gene expression and the clinical pathological parameters in these 120 NSCLC patients. The results are shown in **Table 1**. These results clearly showed that elevated FoxM1 expression was positively correlated with smoking status ($P < 0.05$), LN metastasis ($P < 0.05$) and higher tumor grade ($P < 0.05$). This was consistent with the above real-time PCR data (**Figure 1B-D**).

4 Discussion

In the present study, we accessed the expression pattern and clinical significance of FoxM1 in a total of 120 pairs of NSCLC tissues. We demonstrated that FoxM1 was highly expressed in NSCLC tissues, and its high expression was correlated with smoking history, LN metastasis and advanced tumor grade. This correlation was supported by the statistical k^2 test undertaken in this study. Furthermore, when applying an independent online database called Kaplan-Meier plotter, we found that altered expression of FoxM1 in different patient groups or subtypes of LC was predictive of poorer survival times for these patients. Collectively, these evidences suggested that abnormal upregulation of FoxM1 in cancerous tissues is strongly associated with a poor prognosis for NSCLC patients.

The association of FoxM1 expression with poor prognosis has been reported by other groups in many human cancers, including LC [13-15]. It has been found that FoxM1 expression was related with clinical factors and functioned as a critical predictor in prognosis in 69 cases of SCC patients, but not in ADC [14]. Recently, Liu and his colleagues [15] demonstrated that FoxM1 could serve as an independent factor for prognosis in 68 NSCLC patients, including 37 ADC and 19 SCC. However, the relationship between FoxM1 level and other clinical parameters was not found to be correlated with each other

Table 1. Correlation between FOXM1 expression and clinicopathological features.

Clinical characteristics	Non-increased (DDCt< = 1) n = 62	Increased (DDCt > 1) n = 58
Sex		
Male	40	34
Female	22	24
		P = 0.5068
Age		
< 60	16	21
≥ 60	46	37
		P = 0.2176
Smoking History		
Smoking	23	42
Non-smoking	39	16
		P = 0.0001
Tumor Type		
Squamous cell lung carcinoma	38	43
Lung adenocarcinoma	15	10
Large-cell lung carcinoma	9	5
		P = 0.3134
Tumor Grade		
I	3	3
II	27	7
III	16	10
IV	16	38
		P < 0.0001
Lymph Node Metastasis		
-	46	21
+	16	37
		P < 0.0001

[15], which raises questions about how tumor specimens were obtained and how IHC results were analyzed. These paradoxical results may due to their different study populations, small sample sizes and varied quality of clinical data. In our study, we took advantage of our large sample population including 120 pairs of NSCLC tissues, and concluded from our data that FoxM1 was indeed upregulated and that this elevated expression correlated with poor prognosis of these NSCLC patients. We found that FoxM1 expression in tumoral tissues was positively associated with several pathological factors, including smoking history, LN metastasis and clinical stage. These results indicated that FoxM1 was related with NSCLC progression and prognosis. However, putative underlying mechanisms need to be further explored.

Given that FoxM1 plays a role as a crucial regulator for the G1/S and G2/M transition, and M phase progression, some studies have raised the possibility of FoxM1 expression as a biomarker to predict the clinical outcome of NSCLC patients. In addition, forced expression of FoxM1 was found to have the potential to increase the migratory and invasive abilities of NSCLC cells [13]. This information indicated that FoxM1 is also critical for the invasiveness of malignant NSCLC cells, and this effect was at least, in part through epithelial-mesenchymal transition (EMT) modulation. Hence, our findings that FoxM1 expression correlated with LN metastasis just provided another clue supporting its role in metastasis of NSCLC cells.

In summary, the results presented here confirmed that FoxM1 was highly expressed in NSCLC tissues, and its elevated expression levels correlated with smoking status, LN metastasis and tumor grade of these 120 NSCLC patients. Survival analysis showed that FoxM1 was an independent and significant predictor of poorer survival time for NSCLC patients. Therefore, our observation makes FoxM1 an attractive target of NSCLC therapy, and it could be used as a predictive biomarker for prognosis.

Conflict of interest: Authors declare nothing to disclose.

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