

Original Article

Downregulation of P38 phosphorylation correlates with low-grade differentiation and proliferation of lung squamous cell carcinoma

Yandong Nan^{1*}, Ruijing Chang^{1*}, Hua Jiang¹, Shuanying Yang², Fanguang Jin¹, Yonghong Xie¹

¹Department of Respiration, Tangdu Hospital, Fourth Military Medical University, Xi'an, China; ²Department of Respiratory Medicine, Second Affiliated Hospital, Xi'an Jiaotong University, Xi'an, China. *Equal contributors.

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Abstract: Background: P38MAPK has been investigated as a tumor-related signaling molecule because of its apparent association with tumorigenesis. This study aimed to investigate P38MAPK expression and its role in lung squamous carcinoma (LSCC). Methods: The expression of P38MAPK and phosphorylated P38 (P-P38) in LSCC tissues and cells was examined by Western blot, real-time PCR, and immunohistochemistry. The influence of P38MAPK inhibitor SB203580 on the proliferation of LSCC cells was detected by MTT and flow cytometry. Results: The expression of P-P38 in LSCC tissues and cells was lower than that in cancer-adjacent normal tissues and normal bronchial epithelial cells ($P < 0.05$). In addition, the expression of P-P38 was downregulated in LSCC tissues of poor differentiation, stages III and IV, and with lymph node metastasis compared with the LSCC tissues of well differentiation, stages I and II, and without lymph node metastasis ($P < 0.05$). Moreover, the cell proliferation of LSCC SK-MES-1 cells treated by P38MAPK inhibitor SB203580 significantly increased in a concentration-dependent manner compared with that of SK-MES-1 cells without SB203580 ($P < 0.05$). The inhibition of P38MAPK promoted the transition of the S phase to the G2 phase. Conclusions: P-P38 was poorly expressed in LSCC tissues and cells. Its low expression was correlated with low-grade differentiation, lymph node metastasis, and advanced stage of LSCC. Inhibition of P38MAPK expression could significantly increase the proliferation of LSCC cells by promoting the transition of the S phase to the G2 phase.

Keywords: Lung squamous carcinoma, LSCC, P38MAPK, P-P38, proliferation

Introduction

Lung cancer has become one of the most common malignant tumors seriously threatening human health, especially in China [1]. According to histological classification, lung cancer can be divided into small cell lung cancer, adenocarcinoma, squamous cell carcinoma (SCC), large cell cancer, and other rare types [2]. Lung SCC (LSCC) is still the most common histological type of primary lung cancer in developing countries, although its ratio has decreased, whereas that of adenocarcinoma has increased over the years [3]. Currently, chemotherapy can be used for controlling the growth of LSCC, and such treatment has received certain curative efficacy; however, the side effects and drug resistance of chemotherapeutic drugs have limited its application [4]. Identification of ge-

netic mutations and abnormal copies in LSCC has generated immense impact on the use of targeted inhibitors in clinical settings. However, the results from clinical trials have shown that not all LSCCs are responsive to targeted therapy [5]. Early diagnosis of lung cancer reduces the mortality of patients and improves the cure rate. Thus, identification and validation of diagnostic and prognostic biomarkers are critical to improve the clinical treatment efficacy of LSCC [6].

Proteomics has recently emerged as a powerful technology to identify and screen the differential expression of proteins associated with cancer development and progression [7]. Previously, we used laser capture microdissection technology combining shot-gun proteomics strategy to identify the expression protein pro-

Table 1. Clinicopathological features of lung cancer cases (N=65)

Group	Clinicopathological features	Number (%)
Sex	Male	49 (75.4%)
	Female	16 (24.6%)
Age	<50	23 (35.4%)
	≥50	42 (64.6%)
Smoking	Yes	44 (67.7%)
	No	21 (32.3%)
Pathologic grade	Poorly differentiated	11 (16.9%)
	Moderately differentiated	26 (40%)
	Well-differentiated	28 (43.1%)
Clinical staging	I-II	27 (41.5%)
	III-IV	38 (58.5%)
Lymph metastasis node	Positive	43 (66.2%)
	Negative	22 (33.8%)

files of LSCC; results revealed that five proteins may be used as markers of LSCC: prohibitin (PHB), mitogen-activated protein kinase (MAPK), heat shock protein27 (HSP27), annexin a1 (ANXA1), and high mobility group protein b1 (HMGB1) [8]. The MAPK system is a cluster of serine/threonine protein kinases in cells, and the activated MAPKs participate in a variety of cellular responses including genetic transcription, inducing cell apoptosis, maintaining cell functions, and regulating cell cycle [9]. P38MAPK is a key member of the MAPK family, which regulates multiple biological functions of cells by linking extracellular signals with intracellular signals. P38MAPK was discovered in a pharmacological screen for the identification of compounds that modulate the production of tumor necrosis factor alpha (TNF α) by lipopolysaccharide-stimulated human monocytic cells [10]. A few studies disclosed that abnormal P38MAPK expression and functional changes are associated with the occurrence, development, and migration of malignant tumors [11-13]. However, in terms of its expression level in LSCC tissues, its clinical significance and influence on proliferation

and growth of LSCC remains unclear. We investigated the expression level of P38MAPK in LSCC and assessed its usefulness in discerning lung cancer, as well as evaluated whether it has a certain influence on the proliferation of LSCC.

Material and methods

Cell lines and cell cultivation

The LSCC cell line SK-MES-1 was purchased from Shanghai Cell Bank of the Chinese Academy of Sciences. The cell line was cultivated in MEM (Gibco, USA) containing 10% fetal calf serum. Human normal bronchial epithelial cell line BEAS-2B (ATCC) was purchased from Shanghai Baili Biotechnology Company and cultivated in RPMI (Gibco, USA) including fetal calf serum. The culture solutions all contained 1% double antibody and 1% glutamine. All cells were incubated in a cell incubator at 5% CO₂ and 37°C. Cells in the logarithmic growth phase were collected for further experiments.

Medical ethics statement

The institutional review board and medical ethical committee at Fourth Military Medical University (Xi'an, China) approved this study. Informed consent from each patient was obtained before the study.

LSCC patients

A total of 65 cases of LSCC patients (from February 2014 to December 2015) were included in this study (Tangdu Hospital, Fourth Military Medical University, Xi'an, China). All LSCC patients were confirmed by pathological examination. None of the included patients received chemotherapy or radiotherapy before surgery. After removing the tumor mass via surgery, we carefully separated the cancer-adjacent normal tissues (CANTs) from the cancer tissues. The distance between the edge of CANT and tumor tissues was greater than 3 cm to avoid contamination of cancer cells. Clinical characteristics were retrieved from available

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Table 2. Expression levels of P38MAPK and P-P38 in LSCC tissues and CANT

Specimen source	Detection method	Indicator	Number	Gray scale detection	P value
LSCC	Real-time PCR	P38MAPK mRNA	15	16.525 ± 2.287	>0.05
CANT	Real-time PCR	P38MAPK mRNA	15	18.257 ± 3.783	
LSCC	Western Blot	P38MAPK	65	0.71 ± 0.12	>0.05
CANT	Western Blot	P38MAPK	65	0.74 ± 0.09	
LSCC	Western Blot	P-P38	65	0.14 ± 0.03	<0.05
CANT	Western Blot	P-P38	65	0.45 ± 0.04	

LSCC, lung squamous cell carcinoma; CANT, cancer-adjacent normal tissues.

clinical records. The clinicopathological parameters were retrospectively assessed and recorded in detail (**Table 1**).

Real-time PCR

Total RNA was extracted from the tissues of LSCC and CANT according to the Trizol protocol (Invitrogen, USA). After detecting the RNA concentration, cDNA was synthesized using a reverse transcription kit (TAKARA, Japan). Real-time PCR was performed according to the SYBR[®] Prime Ex TaqTM II (Perfect Real Time, TAKARA, Japan) protocol. The housekeeping gene GAPDH served as an internal reference. The primer sequences of P38MAPK (190 bp) for PCR were as follows: forward primer: 5'-GTACCACGATCCTGATGATG-3'; reverse primer 5'-CAGTAGAGTGGGATCAACAG-3'. The sequences of GAPDH (138 bp) were as follows: forward primer: 5'-CTCCTCCACCTTTGACGCTG-3'; reverse primer: 5'-TCCTCTTGCTCTTGCTGG-3'. Fluorescence quantitative PCR results were compared and analyzed by Ct method, and the formula is as follows: Ct=Ct target gene/Ct internal reference gene; $\Delta\Delta Ct = \Delta Ct \text{ LSCC} - \Delta Ct \text{ CANT}$.

Western blot

Total protein was extracted from the tissues of LSCC and CANT according to a previously published method [7]. Protein quantification was performed according to the instructions of the BCA Protein Assay Kit (Beyotime Biotechnology, China). About 80 µg of protein from each sample was diluted with triple volumes of SDS loading buffer. After mixing, the proteins were boiled at 100°C for 10 min. Subsequently, the protein was transferred to polyvinylidene difluoride membranes. The membranes were respectively incubated with anti-P38MAPK (1:1000, Cell Signaling, America) and anti-phosphorylated P38 (P-P38; 1:800, Cell Signaling, USA) at 4°C overnight. They were then incubated with

horseradish peroxidase-conjugated secondary antibody, and positive signals were visualized using the enhanced chemiluminescence method. β-Tubulin served as an internal reference (1:1000, Zhongshan Jinqiao, China), and all experiments were repeated three times.

Immunohistochemistry (IHC)

The expression status of P38MAPK and P-P38 in tissues was tested by IHC (Ultra-sensitive S-P Kit, Zhongshan Jinqiao, China), which was strictly performed according to a previously described method [7]. The dilution of anti-human P38MAPK antibody was 1:200 (Cell Signaling, USA) and anti-human P-P38 was 1:150 (Cell Signaling, USA). PBS was used as the negative control instead of the first antibody. Two experienced pathologists (Li Jun and Zhang M) blindly evaluated the immunostaining of stathmin according to a previously described method [7]. Ten high-magnification fields were randomly selected in each section to assess the expression scores according to the area of staining and staining intensity. The criteria for intensity were as follows: 0, no staining; 1+, mild staining; 2+, moderate staining; and 3+, intense staining. The criteria for area were as follows: 0, no staining of cells in any microscopic fields; 1+, <30% of tissues stained positive; 2+, between 30% and 60% of tissues stained positive; and 3+, >60% of tissues stained positive. The combined score (area of staining + intensity of staining) of ≤ 2 was considered low expression, between 3 and 4 as moderate expression, and between 5 and 6 as high expression [7].

MTT

Cells at the logarithmic growth phase were collected, diluted to a concentration of 5×10^3 cells/ml, and seeded in 96 well plates. After the cells adhered, the medium was discarded and

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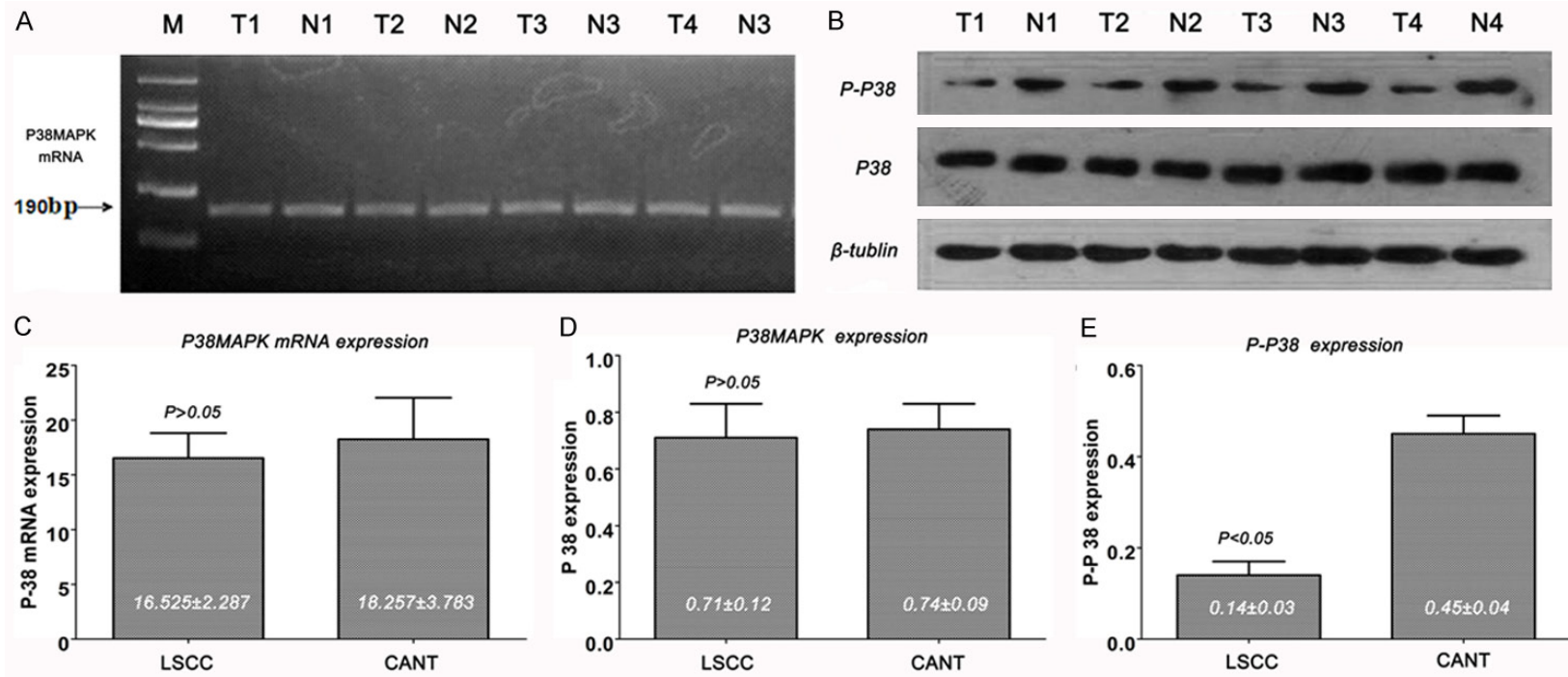


Figure 1. Expression levels of P38MAPK and P-P38 in LSCC tissues and CANT. A. The amplification product size of P38MAPK gene was 190 bp, the expression of P38MAPK mRNA did not show obvious difference between in LSCC and in CANT ($P > 0.05$); B. The expression of P-P38 in LSCC tissues was significantly lower than that in CANT ($P < 0.05$); C. The expression of P38MAPK mRNA in LSCC tissues was not different from CANT ($P > 0.05$); D. There was no significant difference in the expression of P38MAPK between LSCC tissues and CANT ($P > 0.05$); E. Obviously, the expression of P-P38 in LSCC was lower than that in CANT ($P < 0.05$); LSCC, lung squamous cell carcinoma; CANT, cancer-adjacent normal tissues.

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Table 3. Relationship between P-P38 protein expression and clinicopathological features in LSCC tissues (N=65)

Clinicopathological features	n	P-P38/ β -tubulin (Western blot)			P-P38 (IHC)			χ^2 value	P value
		Gray scale detection	T value	P value	Low (%)	Moderate (%)	High (%)		
Differentiation									
Well	28	0.18 \pm 0.07			5 (17.9%)	13 (46.4%)	10 (35.7%)	11.25	<0.05
Moderately	26	0.15 \pm 0.04	3.10	<0.05	4 (15.4%)	14 (53.8%)	8 (30.8%)		
Poorly	11	0.14 \pm 0.05			7 (63.6%)	2 (18.2%)	2 (18.2%)		
Clinical stage									
I-II	27	0.17 \pm 0.05	3.09	<0.05	3 (11.1%)	11 (40.7%)	13 (48.1%)	8.11	<0.05
III-IV	38	0.12 \pm 0.08			13 (34.2%)	18 (47.4%)	7 (18.4%)		
Lymph node metastasis									
Yes	43	0.16 \pm 0.07	1.61	>0.05	13 (30.2%)	24 (55.8%)	6 (14%)	16.88	<0.05
No	22	0.14 \pm 0.03			3 (13.6%)	5 (22.7%)	14 (63.6%)		

washed with D-Hank's solution. An inhibitor of P38MAPK, namely, SB203580 (40, 20, 10, 5, and 1 μ M; Beyotime Biotechnology, China), was added to each column including the blank control. Each well was added with 20 μ L of 5 mg/mL MTT solution, and the cells were cultured further for 4 h. After removing the supernatant fluid carefully, each well was added with 150 μ L of DMSO (Sigma, USA) and the mixture was fully dissolved. The absorbance of each well was determined by measuring the wavelength at 490 nm on a microplate reader.

Flow cytometry

Cells at the logarithmic growth phase were collected and washed twice with D-Hank's solution. Subsequently, cells were digested with trypsin and then added to the MEM mixture. The cells were centrifuged at 4°C for 5 min, and the precipitates were collected. The cells were then fixed with precooled PBS, and 500 μ L of propidium iodide staining solution was added to each tube. The fluorescence was detected by flow cytometry to measure the cell cycle DNA content. The proliferation index was calculated using the following formula: $PI = (S+G2)/(G1+S+G2)$.

Statistical analysis

The protein expression of P38MAPK and P-P38 in tissues was analyzed using the Chi-square test. The mRNA expression of P38MAPK in different groups was compared using student's *t*-test (matched samples), one-way ANOVA (single factor analysis), and Kruskal-Wallis test

(nonparametric analysis). Statistical analysis was implemented using SPSS 19.0 (SPSS, Chicago, USA) software. Statistical significance was set at $P < 0.05$.

Results

Comparison of P38MAPK mRNA expression level between LSCC tissues and CANT via real-time PCR

The mRNA level of P38MAPK was detected by real-time PCR in 15 matched tissues of LSCC and CANT. As shown in **Table 2**, the relative expression of P38MAPK mRNA in LSCC was 16.525 ± 2.287 and 18.257 ± 3.783 in CANT. Although the expression of P38MAPK mRNA in LSCC tissues was slightly higher than that in cancer-adjacent normal tissues, the difference was not statistically significant ($P > 0.05$; **Figure 1A and 1C**).

Comparison of P38MAPK and P-P38 protein expression between LSCC tissues and CANT by Western blot

The protein expression levels of P38MAPK and P-P38 were detected by Western blot in 65 matched LSCC tissues and CANTs. As shown in **Table 2**, the gray values of P38MAPK/ β -tubulin in LSCC tissues and CANTs were 0.71 ± 0.12 and 0.74 ± 0.09 , respectively, showing no statistical difference ($P > 0.05$; **Figure 1D**). However, the gray value of P-P38/ β -tubulin in LSCC tissues (0.14 ± 0.03) was lower than that in CANTs (0.45 ± 0.04), which indicated that the expression of P-P38 in LSCC was downregulated ($P < 0.05$; **Figure 1B and 1E**).

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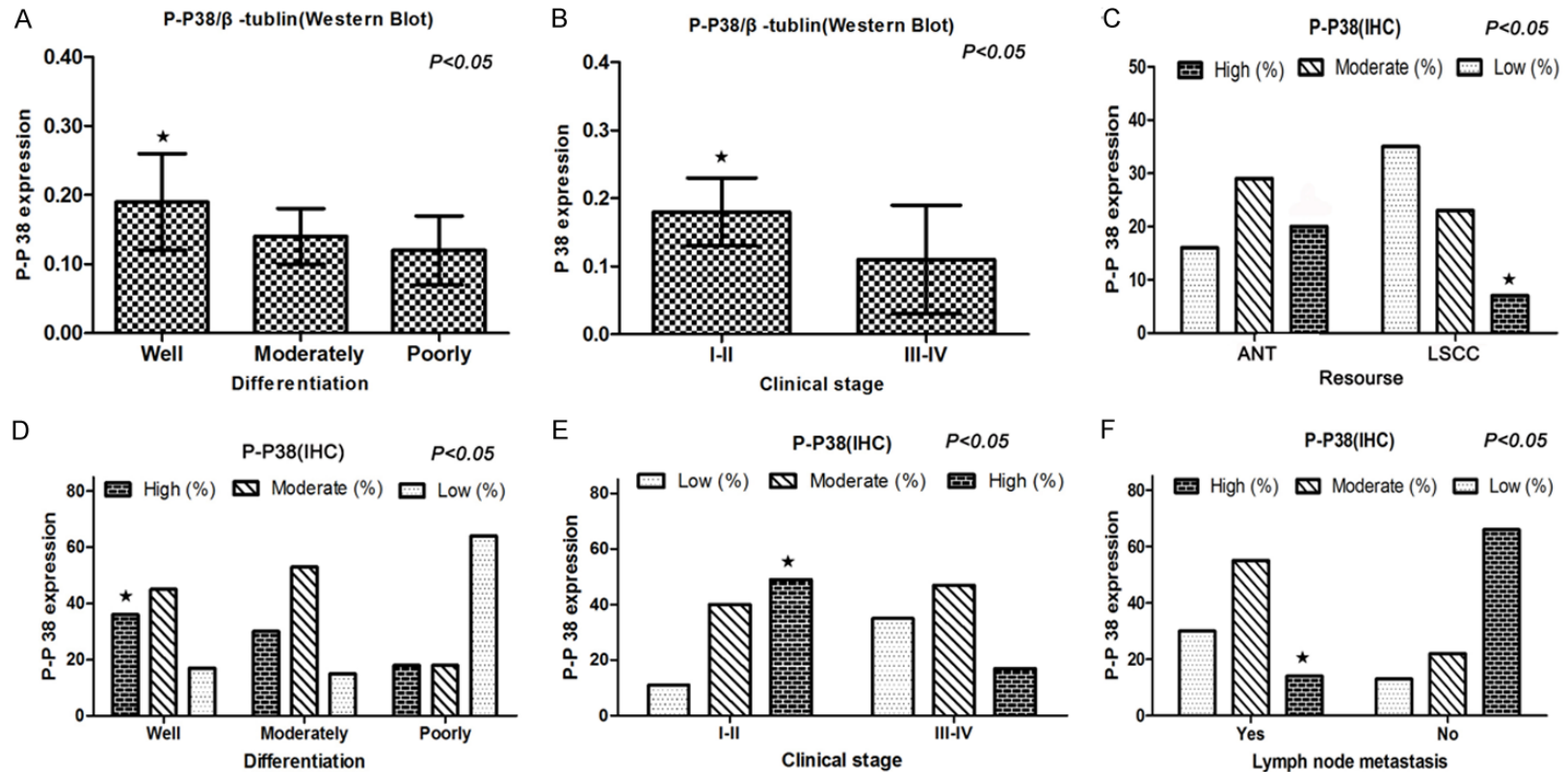


Figure 2. Correlation between the expressions of P38MAPK and P-P38 and clinicopathological features of LSCC. A. Western Blot showed that the expression of P-P38 in well differentiated LSCC cells was significantly higher than that in moderately and poorly differentiated LSCC ($P < 0.05$); B. Western Blot showed that the expression level of P-P38 protein in LSCC tissues of stage I-II was significantly higher than that in stage III-IV ($P < 0.05$); C. IHC analysis showed that P-P38 protein expression in LSCC tissues displayed a significant down-regulation compared with the CANT ($P < 0.05$); D. IHC analysis showed that well-differentiated LSCC tissues showed a higher expression than in moderately and poorly differentiated LSCC ($P < 0.05$); E. IHC analysis showed that increased P-P38 protein was observed in LSCC of stage I-II compared with stage III-IV ($P < 0.05$); F. IHC analysis showed LSCC tissues with lymph node metastasis had a lower expression of P-P38, as compared with those without lymph node metastasis ($P < 0.05$); LSCC, lung squamous cell carcinoma; CANT, cancer-adjacent normal tissues; IHC, immunohistochemistry.

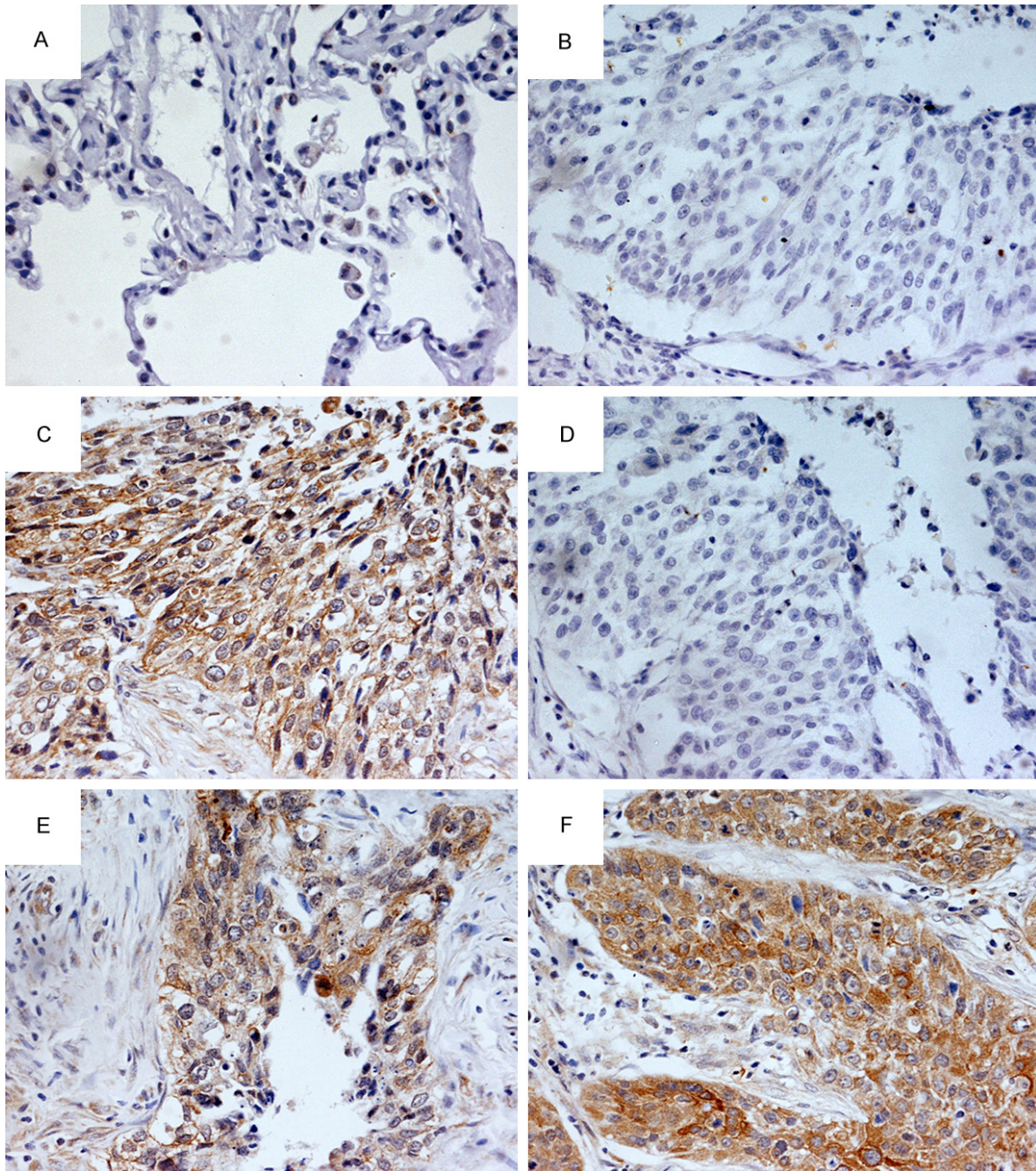


Figure 3. The IHC staining of P38MAPK and P-P38 in LSCC tissues and CANT (IHC×400). A. Low staining of P-P38 in normal lung tissues; B. Low staining of P38MAPK in well differentiated LSCC; C. High staining of P38MAPK in poorly differentiated LSCC; D. Low staining of P-P38 in poorly differentiated LSCC; E. Moderate staining of P-P38 in moderately differentiated LSCC; F. High staining of P-P38 in well differentiated LSCC; LSCC, lung squamous cell carcinoma.

Relationship between the expression levels of P38MAPK and P-P38 and clinicopathological parameters of LSCC (Western blot)

The expression of P38MAPK in LSCC was not correlated with the clinicopathological features of LSCC ($P > 0.05$). Interestingly, as shown in

Table 3, the expression of P-P38 in well-differentiated LSCC tissues (0.18 ± 0.07) was higher than that in moderately (0.15 ± 0.04) and poorly differentiated LSCC tissues (0.14 ± 0.05 ; $P < 0.05$; **Figure 2A**). Meanwhile, the expression level of P-P38 protein in LSCC tissues of stages I and II (0.17 ± 0.05) was significantly higher

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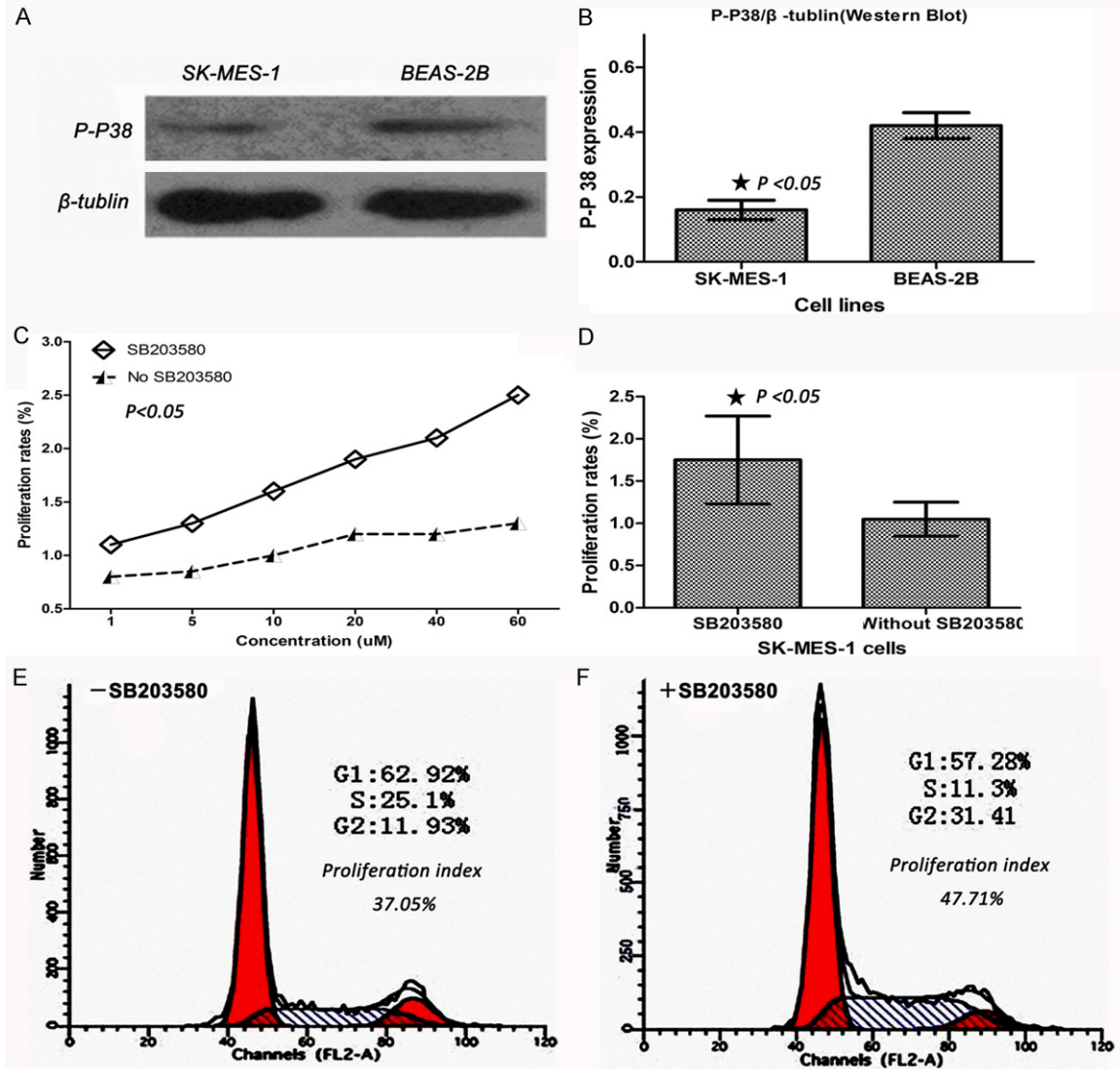


Figure 4. Expression of P38MAPK in LSCC cell line and its role in cell cycle regulation and proliferation of LSCC cells. A, B. The expression of P-P38 in LSCC SK-MES-1 cells was significantly lower than that in normal human bronchial epithelial BEAS-2B cells ($P < 0.05$); C, D. Compared with SK-MES-1 cells without containing P38 specific inhibitor SB203580, the proliferation ability of K-MES-1 cells treated by SB203580 was promoted, and this proliferation capacity was positively correlated with SB203580 concentration ($P < 0.05$). E, F. The down-regulation of P38 protein promoted the transition of S phase to G2 phase of LSCC SK-MES-1 cells, which meant the down-regulation of P38 protein speeded up the proliferation of LSCC SK-MES-1 cells ($P < 0.05$).

than that in stages III and IV (0.12 ± 0.08 ; $P < 0.05$; **Figure 2B**). However, the expression of P-P38 protein was not related to the gender, age, smoking, and lymphatic metastasis of LSCC patients ($P > 0.05$; **Table 3**).

Comparison of P38MAPK and P-P38 protein expression levels between LSCC tissues and CANTs by IHC

The results from IHC showed that P38MAPK protein was expressed in the cytoplasm and

nucleus of LSCC cells, especially in the cytoplasm. P-P38 protein was mainly located in the nucleus. The nucleus of LSCC cells was faintly stained but deeply stained in normal lung cells (**Figure 3A-F**). The expression of P38MAPK in LSCC and CANT did not show a difference ($P < 0.05$), and its expression did not display any relationships with the clinicopathological features of LSCC ($P > 0.05$). Interestingly, the expression of P-P38 protein in LSCC tissues was significantly downregulated (16/65, 24.6%)

compared with that in CANT (35/65, 58.5%; $P < 0.05$; **Figure 2C**).

Relationship between the expression levels of P38MAPK and P-P38 and clinicopathological parameters of LSCC via IHC

IHC analysis revealed that well-differentiated LSCC tissues showed a higher expression of P-P38 (10/28, 35.7%) than moderately (8/26, 30.8%) and poorly differentiated LSCC (2/11, 18.2%; $P < 0.05$; **Figure 2D**). In addition, increased P-P38 protein was observed in LSCC tissues of stages I and II (13/27, 48.1%) compared with stages III and IV (7/38, 18.4%; $P < 0.05$; **Figure 2E**). Moreover, LSCC tissues with lymph node metastasis (6/43, 14%) poorly expressed P-P38 compared with those without lymph node metastasis (14/22, 63.6%; $P < 0.05$; **Figure 2F**).

Expression of P-P38 protein in LSCC SK-MES-1 and normal bronchial epithelial BEAS-2B cells

The expression of P-P38 in SK-MES-1 and BEAS-2B cells was detected by Western blot. Gray scale analysis showed that the expression levels of P-P38 in SK-MES-1 and BEAS-2B cells were 0.16 ± 0.03 and 0.42 ± 0.04 , respectively. The results suggested that the expression of P-P38 in SK-MES-1 cells was significantly lower than that in BEAS-2B cells ($P < 0.05$; **Figure 4A** and **4B**).

Effect of P38 specific inhibitor SB203580 treatment on the proliferation of LSCC SK-MES-1 cells

SK-MES-1 cells were divided into two groups: intervention group (with SB203580) and control group (without SB203580). Each well of the intervention group was supplemented with cell culture solution containing different concentrations of SB203580 (1, 5, 10, 20, 40, and 60 μM). Meanwhile, cell culture solution without SB203580 was added to each well of the control group. The research results showed that the cell proliferation ability of SK-MES-1 cells containing SB203580 (1.75 ± 0.52) was increased markedly compared with that without SB203580 (1.05 ± 0.20) ($P < 0.05$). The proliferation ability of SK-MES-1 cells gradually increased with the increase in the SB203580 concentration (**Figure 4C** and **4D**).

Influence of P38 specific inhibitor SB203580 on the cell cycle regulation of LSCC SK-MES-1 cells

As shown in **Figure 4E** and **4F**, compared with the cells of the control group (without SB203580), the percentage of cells in the S phase in LSCC SK-MES-1 cells treated by SB203580 decreased (11.3% in the intervention group versus 25.1% in the control group), but the proportion of cells in the G2 phase (32.41% in the intervention group versus 11.93% in the control group) was significantly upregulated. These results indicated that the downregulation of P38 protein promoted the cell transformation from S phase to G2 phase in LSCC SK-MES-1 cells. Further tests showed that the cell proliferation index of the intervention group was 47.71%, whereas that of the control group was 37.05%. Thus, the downregulation of P38 protein promoted the cell proliferation level of LSCC SK-MES-1 cells.

Discussion

MAPKs are a highly conserved family of serine/threonine protein kinases involved in a variety of fundamental cellular processes such as proliferation, differentiation, motility, stress response, apoptosis, and survival [10, 14]. The first member of the P38MAPK family was independently identified by four research groups as a 38 kDa protein (p38) that was rapidly phosphorylated on tyrosine in response to lipopolysaccharide stimulation [14]. In recent years, the p38MAPK signaling pathway was found to play an important role in cell invasion and metastasis of malignant tumors. A series of studies suggested that the signaling regulation of P38MAPK is involved in various cancers, including prostate cancer, breast cancer, bladder cancer, liver cancer, lung cancer, transformed follicular lymphoma, and leukemia [15]. In addition, P38MAPK has been implicated in a wide range of complex biological processes, such as cell proliferation, cell differentiation, cell death, cell migration, and invasion. In particular, dysregulation of P38MAPK in cancer patients is associated with advanced stages of the disease and short survival [15]. A previous study showed that P38MAPK is usually associated with stress responses, growth arrest, and apoptosis of cells; it is activated in human lung cancer samples, thereby suggesting an additional role for this pathway in lung cancer cell

growth or transformation [16]. P38MAPK may be associated with the cell cycle and apoptosis of lung adenocarcinoma, but whether it is correlated with the occurrence and development of LSCC remains unclear. This study was conducted to investigate the relationship between P38MAPK expression and the clinical pathological characteristics of LSCC, as well as disclose the possible mechanisms by which p38MAPK acts on LSCC.

In this study, we first explored the expression pattern of P38MAPK and P-P38 in LSCC and CANT. When we compared LSCC with CANT, we found that P38MAPK did not show an expression difference, whether at the protein or mRNA level. However, the expression of P-P38 in LSCC was significantly lower than that in CANT, which indicated that the low expression of P-P38 may be associated with the occurrence and development of LSCC. Previous studies showed that P38MAPK belongs to the tyrosine kinase family and exerts different activities and functions after phosphorylation [10, 14-16]. In LSCC, no difference was found in the expression of the P38MAPK gene and protein between LSCC and CANT, but the significant decrease in the expression of P-P38 after protein modification possibly indicated that P-P38 was involved in the growth regulation of LSCC. In addition, IHC revealed that P38MAPK and P-P38 were expressed in the cytoplasm, but P-P38 was mainly located in the nucleus of LSCC cells. A series of studies showed that P38MAPK, as a kind of protein kinase, exists in the cytoplasm. After being stimulated by an external stimulus, P38MAPK activates the MAPK signaling pathway, translates into its activated form of P-P38, translocates into the nucleus of cells, and exerts important biological effects [9, 10, 14-16]. In our study, we found that the expression of P-P38 in poorly differentiated LSCC was significantly lower than that in the well-differentiated group, and its expression was negatively correlated with the clinical stage of LSCC. Using IHC to test the expression of P-P38 in the tissue level, we further noted that the LSCC tissues with lymph node metastasis demonstrated poor expression of P-P38 as compared with those without lymph node metastasis. Based on the above study, we suggest that the downregulation of P-P38 may be correlated with the occurrence and growth of LSCC, and the poor expression of P-P38 may be used as a indicator

for estimating the prognosis and monitoring treatment of LSCC.

The investigation showed that signaling regulation of the P38MAPK pathway was correlated with the inhibition of tumor cell proliferation. This process occurred at various monitoring points of the cell cycle and affected the switching of G0, G1/S, and G2/S [17]. We found that P-P38 expression in LSCC SK-MES-1 was significantly lower than normal bronchial epithelial BEAS-2B, indicating that the downregulation of P-P38 was likely related to the occurrence and development of LSCC. In addition, after adding the P38MAPK inhibitor SB203580, the proliferation of LSCC SK-MES-1 cells was significantly enhanced, and the proliferation ability of the cancer cells increased with increasing SB203580 concentration. This finding indicates that the proliferation rate of LSCC cells increased with decreasing levels of P38MAPK expression. Compared with the control group (not adding SB203580) SK-MES-1 cells, LSCC SK-MES-1 cells treated by SB203580 evidently displayed that the percentage of cells in S phase was lower but the percentage of cells in G2 phase increased. Thus, the downregulation of P38 protein in LSCC SK-MES-1 cells could promote the cell transition of the S phase to the G2 phase. Research shows that P38MAPK regulates the cell cycle through a variety of mechanisms. When P38 is activated, it prevents cells from entering the G2/M checkpoint and inhibits cell proliferation by downregulating key proteins in the G2/M phase, such as Cyclin B1, Cdc2, and Cdc25C [18, 19]. The intervention of the P38MAPK signaling pathways may be used for developing new molecular targeted therapy, and inhibitors of P38 will undoubtedly be one of the next groups of drugs developed for the treatment of different solid tumors [15]. In conclusion, the low expression P-P38 may serve as a useful indicator for estimating the malignant degree of LSCC and may contribute to the early diagnosis of LSCC and monitoring of metastasis and recurrence. In addition, the correlation of the downregulation of P-P38 with LSCC and the reduced proliferation of LSCC caused by the inhibition of P38 may present novel ideas for developing targeted therapies for treating LSCC.

This study had several limitations. First, it was conducted in a single institute in China, which

may not represent the features of a wide population. Second, the study did not sufficiently assess the difference of P-P38 level among different LSCC cell lines. Therefore, large numbers of lung cancer subjects are required for prospective studies and further studies that are warranted to investigate the potential mechanism of P-P38 in lung cancer. Additional studies to evaluate the differential requirement of the P38MAPK pathway for survival and metastasis of LSCC cells are necessary to fill the gaps that exist in our current understanding of the functional consequences of P38MAPK inhibition with respect to solid tumor biology.

Conclusion

P-P38 was lowly expressed in LSCC tissues and cells. Its low expression was correlated with low-grade differentiation, advanced stage, and lymph node metastasis of LSCC. In addition, the inhibition of P38MAPK significantly increased the proliferation of LSCC cells by promoting the cell transition of the S phase to the G2 phase.

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Disclosure of conflict of interest

None.

Address correspondence to: Drs. Faguang Jin and Yonghong Xie, Department of Respiration, Tangdu Hospital, Fourth Military Medical University, No. 569, Xinsi Road, Baqiao District, Xi'an 710038, China. Tel: +86 15929999191; E-mail: jinfag@fmmu.edu.cn (FGJ); xieyh65@fmmu.edu.cn (YHX)

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