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PAK4 expression is associated with the prognosis in non-small cell lung cancer

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Abstract

This study attempted to determine the expression of p21-activated kinase 4 (*PAK4*) in non-small cell lung cancer (NSCLC) tissues and the normal lung tissues. The correlation between *PAK4* expression and prognosis of NSCLC patients was also evaluated in the present study. The expression level of *PAK4* was measured by high-performance liquid chromatography method. Chi-square test was adopted to explore the relationship of *PAK4* expression and clinical features. Kaplan-Meier survival curves were plotted to delineate the overall survival rate of NSCLC patients. Cox regression analysis was performed to evaluate the prognostic significance of *PAK4* expression in NSCLC. The *PAK4* expression in NSCLC tissue samples was significantly higher than that in normal lung tissues ($P<0.001$) and shared significant correlation with Eastern Cooperative Oncology Group score, histological type, and distant metastasis ($P<0.05$). Survival curve revealed that NSCLC patients with high *PAK4* expression had relatively higher mortality than those with low *PAK4* expression ($P = .001$). Cox regression analysis explained that *PAK4* expression was associated with the prognosis of NSCLC patients ($P = .024$; HR, 3.104; 95% CI, 1.164–8.278). In a word, *PAK4* was highly expressed in NSCLC tissues and could act as a prognostic factor for NSCLC patients.

Key words: HPLC, non-small cell lung cancer, *PAK4*, prognosis

1. Introduction

Lung cancer is one of the most frequent malignant diseases and currently is one of the leading causes of cancer-related deaths all over the world.[1–3] The incidence and mortality are increasing every year because of environmental pollution and cigarette abuse.[4] Non-small cell lung cancer (NSCLC) is the capital type of lung cancer, which accounts for more than 85% of lung cancer cases.[5–7] Most NSCLC patients are diagnosed as advanced stage, which is considered to be a crucial reason for short survival of NSCLC patients.[8,9] Treatments for NSCLC patients are mainly surgical resection, chemotherapy, and radiotherapy.[10,11] Despite advances in the treatments, there has been little improvement in the survival of NSCLC patients over the past three decades. And more than 60% patients suffer distant metastasis or local recurrence of NSCLC after treatments.[12] Thus, it is urgently needed to find novel, sensitive biomarkers for early diagnosis and prognosis of NSCLC patients.

The p21-activated kinase (*PAK*) family of serine/threonine kinases are key signaling proteins that play important roles in many cellular processes, including cell signaling, cytoskeletal organization, cell proliferation, migration, and survival.[13–15] Based on homology, six *PAKs* are identified and classified into 2 groups: group I (*PAK1-3*) and group II (*PAK4-6*).[16–18] p21-activated kinase 4 (*PAK4*) is the most divergent member of *PAK* family and locates at 19q13.2-13.3.[19,20] *PAK4* is first identified as an effector protein for Rho GTPase Cdc42.[21] The overexpression of *PAK4* has been identified in various cancers, such as breast cancer, gastric cancer, pancreatic cancer, and ovarian cancer.[22–25] Moreover, a relationship between the overexpression of *PAK4* and poor prognosis of ovarian cancer patients has been proved.[25] Furthermore, previous studies have explored the role of *PAK1* in squamous NSCLC cells and demonstrated that *PAK1* was required for proliferation of squamous NSCLC cells.[26] However, until now, there is no report on the association between *PAK4* and NSCLC.

In this study, we aimed to investigate the expression of *PAK4* in NSCLC and estimate the role of *PAK4* in the prognosis of NSCLC patients.

2. Materials and Methods

2.1. Patients and specimens

The cancer tissue samples were obtained from 75 NSCLC patients in Anqing Hospital Affiliated to Medical University of Anhui. All the patients were diagnosed with NSCLC through histologically and cytologically examinations by 2 experienced pathologists. None of the patients received any antitumor treatments before tissue collection, and they received standard treatments by the same medical team in the hospital. Normal lung tissues (n = 31) as controls were collected from patients who were treated with pulmonectomy for benign pulmonary disease. The tissue specimens used for *PAK4* detection were Ct-guided/bronchoscopic-guided biopsy specimens. The cancer

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All data generated or analyzed during this study are included in this published article (and its supplementary information files).

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tissue specimens were with tumor contents more than 60%. The case and controls were matched in age and gender. The present study was approved by Ethics Committee of Anqing Hospital Affiliated to Medical University of Anhui and patients all signed the informed consents in advance.

All the patients enrolled in a 5-year follow-up investigation. The patients whose deaths were not related to NSCLC would be excluded from our study. The preoperative clinical characteristics of the NSCLC patients were collected from their medical records. There were 38 males and 37 females, and their mean age was 63.25 ± 7.63 years. Forty-two patients had smoking history. After tissue collection, 32 patients received radiotherapy or chemotherapy, whereas 43 patients were treated by surgery. According to tumor node metastasis staging, 51 patients diagnosed with stages I–II, and the rest 24 patients were confirmed with stages III–IV. Histological examinations demonstrated that 41 patients were diagnosed with adenocarcinoma, and 34 patients were diagnosed with squamous cell carcinoma. Distant metastasis was observed in 36 patients. The NSCLC patients' general well-being and activities of daily life were quantified by Eastern Cooperative Oncology Group (ECOG) score. The scoring system runs from 0 to 5, with 0 denoting perfect health and 5 death. The patients with ECOG score no more than 2 means that they could take care themselves. Among the included NSCLC patients, 29 cases exhibited ECOG scores no more than 2, while 46 patients had ECOG score more than 2. The detailed clinical information of the NSCLC patients was summarized in Table 1.

2.2. High-performance liquid chromatography

The high-performance liquid chromatography method was adopted to detect the protein expression level of *PAK4*. Five-gram tissue sample (prier to treatments) was weighed and homogenized with the addition of 20-mL methyl cyanide. Then, the homogenate was centrifuged at 4000 r/min for 15 minutes. Supernatant was collected and then dried by a rotary evaporator, followed by resolution with 1-mL mobile phase. Kromasil C_{18} column (250 × 4.6 mm, 5 μm) was used, and the column temperature was maintained at 30 °C. The mobile phase consisted of methyl cyanide and water (pH = 2.5) (v:v = 83:17). The flow rate was 1 mL/min, and the detection wavelength was 280 nm. *PAK4* protein of 0.05 g was weighed and dissolved in a 10-mL volumetric flask by mobile phase and prepared for standard solution. Every sample had 3 repetitions, and the final result was average value of the 3.

2.3. Statistical analysis

All data were analyzed by SPSS 18.0 (SPSS Inc, IL). The relationship between *PAK4* expression and clinical characteristics was analyzed by Chi-square test. Overall survival of NSCLC patients was described by Kaplan-Meier method. The Cox regression was conducted to evaluate the relevance between *PAK4* expression and prognosis of NSCLC patients. $P < .05$ was considered as statistically significant.

3. Results

3.1. Increased expression of PAK4 in NSCLC samples

The expression of *PAK4* was firstly examined by high-performance liquid chromatography in 75 NSCLC samples and 31 normal lung tissue samples. The concentration of *PAK4* in NSCLC tissues was 69.03 ± 12.71, while that in the normal lung tissues was 24.84 ± 6.15. The expression level of *PAK4* was significantly higher in NSCLC than that in the normal lung tissues (Fig. 1; $P < .001$), indicating that abnormal *PAK4* expression might be associated with NSCLC pathogenesis.

3.2. The association of PAK4 expression with clinicopathological characteristics of NSCLC patients

To further address the association between *PAK4* expression and NSCLC, several clinical factors of NSCLC were analyzed. All NSCLC samples were manually divided into 2 groups: the high expression group with the *PAK4* concentration of more than 76.01, and the others belonged to the low group. The statistical analysis revealed that high expression of *PAK4* was tightly related to ECOG score, histological type, and distant metastasis (Table 1; $P < .05$). However, there was no statistical

Table 1

Relationship between *PAK4* expression and clinical characteristics.

Characteristics	Case no.	Expression		χ^2	*P* value
		High	Low		
Sex					
Male	38	25	13	0.455	.500
Female	37	27	10		
Smoking					
Yes	42	30	12	0.197	.657
No	33	22	11		
Treatment					
Radio- or chemotherapy	32	24	8	0.843	.359
Surgery	43	28	15		
ECOG score					
≦ 2	29	16	13	4.459	.035
> 2	46	36	10		
TNM stage					
I–II	51	29	21		.155
III–IV	24	18	6		
Histological type					
Adenocarcinoma	41	33	8	5.292	.021
Squamous cell carcinoma	34	19	15		
Distant metastasis					
Yes	36	29	7	4.101	.043
No	39	23	16		

ECOG = Eastern Cooperative Oncology Group, *PAK4* = p21-activated kinase 4, TNM = tumor node metastasis.

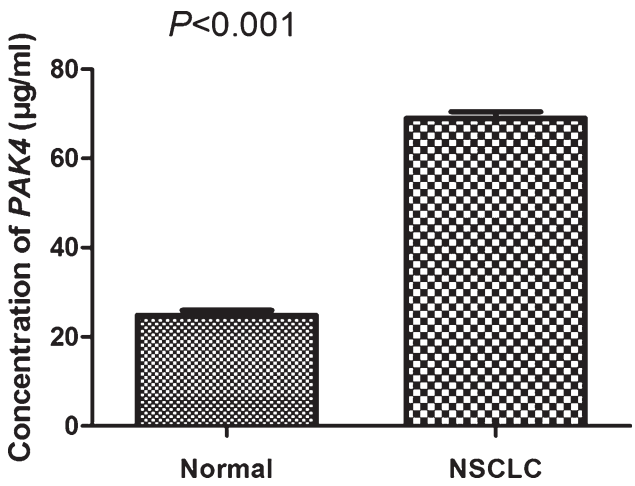

Figure 1. *PAK4* expression in NSCLC tissues and the normal tissues was assayed by HPLC, respectively. The results were presented as mean ± SD. *PAK4* was highly expressed in NSCLC tissues compared to the normal lung tissues ($P < .001$). HPLC = high-performance liquid chromatography, NSCLC = non-small cell lung cancer, *PAK4* = p21-activated kinase 4, SD = XXX.

significance between *PAK4* expression and sex, smoking, treatment, or tumor node metastasis stage (Table 1; $P > .05$).

3.3. Upregulation of PAK4-predicted unfavorable prognosis of NSCLC patients

Kaplan-Meier analysis was adopted to evaluate the effects of *PAK4* expression on the overall survival of NSCLC patients. The survival curves displayed that the NSCLC patients with high expression of *PAK4* had shorter overall survival time than those with low *PAK4* expression (Fig. 2; $P = .001$). The overall 5-year survival rate of patients with high *PAK4* expression was 30.8% (16 out of 52), compared with 73.9% (17 out of 23) of patients with low *PAK4* expression. Multivariate analysis demonstrated that *PAK4* expression shared significant relevance with prognosis of NSCLC patients (Table 2, $P = .024$; HR, 3.104; 95% CI, 1.164–8.278), suggesting that *PAK4* could be a candidate factor for prognosis of NSCLC patients.

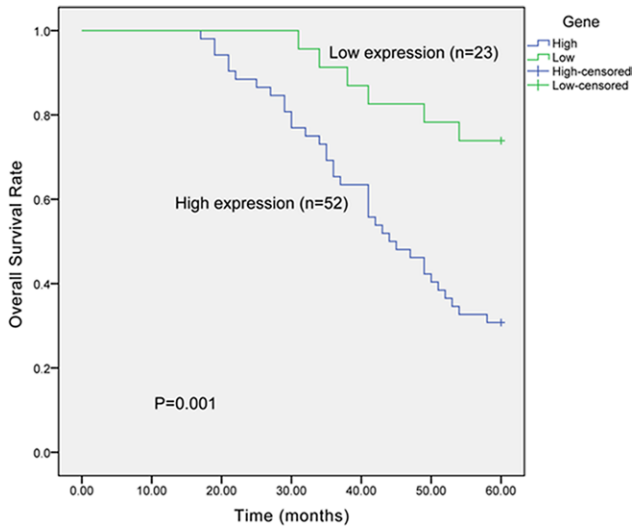

Figure 2. Kaplan-Meier curves showed the influence of *PAK4* expression on overall survival of NSCLC patients. The overall survival rate of NSCLC patients with high *PAK4* expression was significantly lower than those with low *PAK4* expression ($P = .001$). NSCLC = non-small cell lung cancer, *PAK4* = p21-activated kinase 4.

Table 2

Multivariate analyses for clinical factors and *PAK4* expression in patients with NSCLC.

Characteristics	*P* value	HR (95% CI)
Sex	.533	0.810 (0.418–1.571)
Smoking	.396	0.749 (0.385–1.459)
Treatment	.228	0.662 (0.338–1.296
Histological type	.647	1.197 (0.555–2.581)
Distant metastasis	.653	1.168 (0.593–2.302)
PAK4 expression	.024	3.104 (1.164–8.278)

CI = XXX, HR = XXX, NSCLC = non-small cell lung cancer, *PAK4* = p21-activated kinase 4.

4. Discussion

NSCLC is the most frequent lung cancer, and its most common 3 types are adenocarcinoma, squamous cell carcinoma, and large cell anaplastic carcinoma. Although there are several therapies, including surgery, radiotherapy, and chemotherapy, most NSCLC patients experience recurrence because of the poor sensitivity to those treatments. The 5-year survival rate of NSCLC patients is low, and the prognosis is dismal.[27] Therefore, identification of novel molecular markers, which could improve the diagnosis and prognosis stratification and serve as possible therapy targets, is of great importance. In addition, several clinicopathological factors have been found to relate with the risk of lung cancer, such as sex, tumor stage and vascular invasion. However, the accuracy of the markers is not sufficient, so finding a novel and specific targeted gene for therapy and prognosis of NSCLC patients is necessary.

Recently, the research on *PAKs* has become a hotpot in several fields, including tumor occurrence, development, and metastasis. *PAK4*, as an important member of *PAK* family, develops a variety of biological effects, such as regulation of cell cycles, cell growth, and cell apoptosis. Siu et al[25] showed that *PAK4* regulated the cell proliferation in ovarian cancer. Moreover, it has been reported that *PAK4* participated in signal pathways of tumor cells, functioning on proliferation, invasion, and metastasis of tumor cells. Thus, *PAK4* might be a novel target for therapy and prognosis of tumors. Precious studies have demonstrated that *PAK4* was overexpressed in a variety of cancer cells, and knockdown of *PAK4* reduced the proliferation of various cancer cells. For instance, Kimmelman et al[24] have confirmed that *PAK4* was overexpressed in pancreas cancer. Meanwhile, Wong et al[14] have suggested that knockdown of *PAK4* reduced proliferation and migration of tumoral cells in breast cancer. Minden[13] proved that overexpression of *PAK4* was observed in breast cancer. However, we would like to know the role of *PAK4* in NSCLC.

The results of our study were in accordance with the above observations. We found that the expression of *PAK4* in NSCLC tissues was higher than that in the normal lung tissues, suggesting that *PAK4* could be a predictive marker for the therapy and prognosis of NSCLC. To our knowledge, this is the first time to demonstrate the prognostic role of *PAK4* in NSCLC. In the present study, Chi-square test revealed that high expression of *PAK4* was related to ECOG score, histological type, and distant metastasis. ECOG score is frequently used to quantify cancer patients' general well-being and activities of daily life, runs from 1 to 5. High scores predict poor living status. Among the included NSCLC patients, 69.2% patients with high expression of *PAK4* exhibited ECOG score more than 2, while only 43.5% patients with low *PAK4* expression high expression had ECOG score more than 5.

High expression of *PAK4* predicted poor living status of NSCLC patients. The following survival outcome proved that the overall survival rate of patients with high *PAK4* expression was significantly lower than those with low *PAK4* expression. Multivariate analysis delineated that overexpression of *PAK4* was correlated with poor prognosis of NSCLC patients after adjusted to the potential confusing factors, indicating that *PAK4* might be an independent prognostic factor for NSCLC patients.

The rapid and uncontrolled growth of cancer cells is primarily due to the development of apoptotic resistance and enhanced cell-cycle progression.[28] Studies have demonstrated that *PAK4* can regulate the cell-cycle progression of cancer cells through several signal pathways. Tyagi et al[29] have illustrated that *PAK4* regulated the proliferation and survival of pancreatic cancer through the NF-κB pathway. Fu et al[30] have identified that *PAK4* functioned on cisplatin resistance in gastric cancer cells by PI3K/Akt- and MEK/ERK-dependent pathways. Besides, Siu et al[25] have confirmed that *PAK4* induced ovarian cancer cell proliferation via *Pak4*/c-Src/EGFR pathway. Based on the previous reports, we can further investigate the mechanism of *PAK4* on the progression of NSCLC and provide novel possible therapy for the diagnosis and treatment of NSCLC patients.

Despite the encouraging results, some limitations in current study should be stated. Firstly, the sample size was relatively small that might influence the statistical power of our results. Secondly, the molecular mechanisms of *PAK4* in NSCLC had not been explored in our study. Finally, the association between *PAK4* expression and other prognostic and diagnostic markers in NSCLC was not analyzed. The combined application of *PAK4* with other biomarkers might improve the clinical values of these parameters. Therefore, further well-designed studies with larger sample size are required to address the above issues.

Generally speaking, *PAK4* was significantly overexpressed in NSCLC, and statistical significance was found between *PAK4* expression and clinicopathological features. Most importantly, *PAK4* could serve as a novel independent biomarker in NSCLC patients.

References

[1] Jin T, Jin J, Li X, et al. Prognostic implications of ezrin and phosphorylated ezrin expression in non-small cell lung cancer. BMC Cancer. 2014;14:191.

[2] Yang YR, Zang SZ, Zhong CL, et al. Increased expression of the lncRNA PVT1 promotes tumorigenesis in non-small cell lung cancer. Int J Clin Exp Path. 2014;7:6929–35.

[3] Nii K, Tokunaga Y, Liu D, et al. Overexpression of G protein-coupled receptor 87 correlates with poorer tumor differentiation and higher tumor proliferation in non-small-cell lung cancer. Mol Clin Oncol. 2014;2:539–44.

[4] Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2006. CA Cancer J Clin. 2006;56:106–30.

[5] Zhou H, Wu A, Fu W, et al. Significance of semaphorin-3A and MMP-14 protein expression in non-small cell lung cancer. Oncol lett. 2014;7:1395–400.

[6] Jiang Y, Wang C, Lv B, et al. Expression level of serum human epididymis 4 and its prognostic significance in human non-small cell lung cancer. Int J Clin Exp Med. 2014;7:5568–72.

[7] Guo S, Yan F, Xu J, et al. Identification and validation of the methylation biomarkers of non-small cell lung cancer (NSCLC). Clin Epigenetics. 2015;7:3.

[8] Chen C, Zhao Z, Liu Y, et al. microRNA-99a is downregulated and promotes proliferation, migration and invasion in non-small cell lung cancer A549 and H1299 cells. Oncol Lett. 2015;9:1128–34.

[9] Raungrut P, Wongkotsila A, Lirdprapamongkol K, et al. Prognostic significance of 14-3-3gamma overexpression in advanced non-small cell lung cancer. Asian Pac J Cancer Prev. 2014;15:3513–8.

[10] Yan B, Zhang W, Jiang LY, et al. Reduced E-cadherin expression is a prognostic biomarker of non-small cell lung cancer: a meta-analysis based on 2395 subjects. Int J Clin Exp Med. 2014;7:4352–6.

[11] Liu B, Xu K, Jiang Y, et al. Aberrant expression of Per1, Per2 and Per3 and their prognostic relevance in non-small cell lung cancer. Int J Clin Exp Path. 2014;7:7863–71.

[12] Jassem J, Skokowski J, Dziadziuszko R, et al. Results of surgical treatment of non-small cell lung cancer: validation of the new postoperative pathologic TNM classification. J Thorac Cardiovasc Surg. 2000;119:1141–6.

[13] Minden A. The pak4 protein kinase in breast cancer. ISRN Oncol. 2012;2012:694201.

[14] Wong LE, Chen N, Karantza V, et al. The Pak4 protein kinase is required for oncogenic transformation of MDA-MB-231 breast cancer cells. Oncogenesis. 2013;2:e50.

[15] Eswaran J, Soundararajan M, Kumar R, et al. UnPAKing the class differences among p21-activated kinases. Trends Biochem Sci. 2008;33:394–403.

[16] Zhang H, Li Z, Viklund EK, et al. P21-activated kinase 4 interacts with integrin alpha v beta 5 and regulates alpha v beta 5-mediated cell migration. J Cell Biol. 2002;158:1287–97.

[17] Tabusa H, Brooks T, Massey AJ. Knockdown of PAK4 or PAK1 inhibits the proliferation of mutant KRAS colon cancer cells independently of RAF/MEK/ERK and PI3K/AKT signaling. Mol Cancer Res. 2013;11:109–21.

[18] Law SH, Sargent TD. Maternal pak4 expression is required for primitive myelopoiesis in zebrafish. Mech Dev. 2013;130:181–94.

[19] Zhang HJ, Siu MK, Yeung MC, et al. Overexpressed PAK4 promotes proliferation, migration and invasion of choriocarcinoma. Carcinogenesis. 2011;32:765–71.

[20] Lu Y, Pan ZZ, Devaux Y, et al. p21-activated protein kinase 4 (PAK4) interacts with the keratinocyte growth factor receptor and participates in keratinocyte growth factor-mediated inhibition of oxidant-induced cell death. J Biol Chem. 2003;278:10374–80.

[21] Callow MG, Clairvoyant F, Zhu S, et al. Requirement for PAK4 in the anchorage-independent growth of human cancer cell lines. J Biol Chem. 2002;277:550–8.

[22] Liu Y, Chen N, Cui X, et al. The protein kinase Pak4 disrupts mammary acinar architecture and promotes mammary tumorigenesis. Oncogene. 2010;29:5883–94.

[23] Ahn HK, Jang J, Lee J, et al. P21-activated kinase 4 overexpression in metastatic gastric cancer patients. Transl Oncol. 2011;4:345–9.

[24] Kimmelman AC, Hezel AF, Aguirre AJ, et al. Genomic alterations link Rho family of GTPases to the highly invasive phenotype of pancreas cancer. Proc Natl Acad Sci USA. 2008;105:19372–7.

[25] Siu MK, Chan HY, Kong DS, et al. p21-activated kinase 4 regulates ovarian cancer cell proliferation, migration, and invasion and contributes to poor prognosis in patients. Proc Natl Acad Sci USA. 2010;107:18622–7.

[26] Ong CC, Jubb AM, Haverty PM, et al. Targeting p21-activated kinase 1 (PAK1) to induce apoptosis of tumor cells. Proc Natl Acad Sci USA. 2011;108:7177–82.

[27] Yin QW, Sun XF, Yang GT, et al. Increased expression of microRNA-150 is associated with poor prognosis in non-small cell lung cancer. Int J Clin Exp Path. 2015;8:842–6.

[28] Arora S, Bhardwaj A, Srivastava SK, et al. Honokiol arrests cell cycle, induces apoptosis, and potentiates the cytotoxic effect of gemcitabine in human pancreatic cancer cells. PLoS One. 2011;6:e21573.

[29] Tyagi N, Bhardwaj A, Singh AP, et al. p-21 activated kinase 4 promotes proliferation and survival of pancreatic cancer cells through AKT- and ERK-dependent activation of NF-kappaB pathway. Oncotarget. 2014;5:8778–89.

[30] Fu X, Feng J, Zeng D, et al. PAK4 confers cisplatin resistance in gastric cancer cells via PI3K/Akt- and MEK/ERK-dependent pathways. Biosci Rep. 2014;34:e00094.