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The role of PTPN13 in invasion and metastasis of lung squamous cell carcinoma

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ABSTRACT

Objectives: PTPN13 is a new candidate tumor-suppressing gene. To investigate the PTPN13 expression and its potential function in the invasion and metastasis of lung squamous cell carcinoma (LSCC), we performed this study in 91 primary LSCC tissues and the adjacent non-cancerous tissues.
Methods: The mRNA expression of PTPN13 and FAK was quantitated by reverse transcription polymerase chain reaction. The protein expression of PTPN13, focal adhesion kinase (FAK) and phosphorylated FAK (P-FAK) was evaluated using immunohistochemical staining and western blotting. The association among PTPN13 expression, FAK expression and the clinicopathological parameters were analyzed.
Results: PTPN13 expression was down-regulated in LSCC, and was negatively correlated with the cancer grade and stage. FAK mRNA, as well as FAK protein level was elevated in LSCC tissues. P-FAK level, also found increased, had no association with FAK mRNA and FAK protein expression, but had a negative correlation with the PTPN13 expression. P-FAK level had a significant positive correlation with the TNM classification.
Conclusion: The over-expression of FAK and increased FAK phosphorylation plays an important role in the invasion and metastasis of LSCC.

Introduction

Lung cancer is the most common cause of cancer-related death in human being worldwide. Currently lung cancer is mainly treated with surgery; however, the therapeutic efficacy is disappointing once metastasized. Despite advances in early detection and standard treatment, lung cancer is often diagnosed at an advanced stage, and patients often have poor prognosis and a low survival rate (Minna et al., 2002; Mountain, 2000). Therefore, a better understanding of the molecular origins of the disease will contribute to improve therapeutic treatment of patients with lung cancer. Investigation of the lung cancer related genes is particularly important. In 2009, Wrage et al. found that chromosome deletion of 4q in the bone marrow or brain metastasized lung cancer cases is significantly higher than that in the non-metastasized cases. They then confirmed that 4q deletion is closely concerned with the lung cancer metastasis via fluorescence in situ hybridization (FISH), comparative genomic hybridization (CGH) and gene chip (Wrage et al., 2009). But which genes are specifically involved is still unclear. Recently, we used single nucleotide polymorphism (SNP) 6.0 gene chip to analyses genome wide molecular genetics information and to screen lung squamous cell carcinomas (LSCC) related genes and chromosome segments. A significant decrease of copy number variation was found on chromosome 4. We unveiled more genes and chromosome segments with high frequent loss of heterozygosity (LOH) in LSCCs with lymph nodes metastasis than in those without lymph nodes metastasis. Moreover, we located the high frequent LOH on chromosome 4q, especially on 4q18–32. We thus hypothesized there must be some tumor-suppressing gene on the 4q18–32 (results to be reported).

PTPN13 gene, located at 4q21.3, is a newly found candidate tumor-suppressing gene. It has been found frequently mutated or down-regulated in colorectal, liver, and head and neck carcinomas. Whether this gene is related with the development of lung cancer remains unknown.

Focal adhesion kinase (FAK) is a member of the non-receptor protein tyrosine kinases (NRPTKs) family in cytoplasm. The over-expression of FAK and increased FAK phosphorylation can initiate multiple downstream signaling pathways. In particular, regulation of cell migration by

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integrin signaling through FAK is well established, which contributes to pathogenesis of cancer (Zhao and Guan, 2011). Most of the tumor-suppressing genes function via inhibiting the phosphorylation of FAK and thus suppress the cancer cell invasion and metastasis. Whether PTPN13 gene has the same function and whether it works in the same way of inhibiting the phosphorylation of FAK are of great interest.

In this study, we investigated the PTPN13 and FAK mRNA expression in 91 primary lung squamous cell carcinomas (LSCC) specimens using quantitative reverse transcription polymerase chain reaction (RT-PCR). The protein expression of PTPN13, FAK and phosphorylated FAK (P-FAK) was detected using western blotting analysis. By statistically analyzing the gene and protein expressions of PTPN13 and FAK in LSCCs of different stage, we found strong evidences that can support the identification of PTPN13 as candidate tumor suppressor gene in LSCC.

Materials and methods

Specimen sources

Cancerous tissues (CT) and the adjacent non-cancerous tissues (ANCT) were collected from 91 primary LSCC patients who underwent resection at Department of Chest Surgery of Tangdu Hospital, Xi'an, China, during the period from January 2008 to July 2010. Among the 91 patients, 58 male and 33 females, aging from 47 to 72 years old, and the median age was 64. None of these cases underwent chemotherapy, radiotherapy or immunotherapy before surgery. According to the 7th Edition of Union for International Cancer Control (UICC) TNM Classification of Malignant Tumours (Sobin et al., 2009), 29 were T1N0M0, 23 were T2N0M0 (10 T2aN0M0, 13 T2bN0M0), 9 were T2N1M0 (5 T2aN1M0, 4 T2bN1M0), 15 were T3N0M0, 12 were T3N1M0, 3 were T3N2M0, Routine H&E (hematoxylin–eosin) stained tissue sections from all specimens were reviewed to confirm the diagnosis of LSCC and graded using WHO grading system (Damjanov and Fan, 2007) by two pathologists. Among the 91 cancerous tissues, 30 were well differentiated (low grade), 46 were moderately differentiated (intermediate grade) and 15 were poorly differentiated (high grade). The study was approved by the Ethics Committee of the Tangdu Hospital. Informed consent was obtained from all patients for the subsequent use of their excess pathological specimens.

Immunohistochemistry

All the specimens were fixed with 4% paraformaldehyde and embedded with paraffin. Sections 4 μm thick were cut and transferred to glass slides coated with 100 g/L polylysine. The immunohistochemical staining was performed as follows. Anti-PTPN13 (AC21) and anti-P-FAK Tyr 397 were purchased from AbCam (Cambridge, UK). Anti-FAK (H-1) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The primary antibodies were diluted in PBS: Anti-PTPN13, 1:50; Anti-FAK, 1:100; and anti-P-FAK Tyr 397, 1:50. An immunohisto-chemical (IHC) analysis was performed on paraffin-embedded sections. The sections were autoclaved

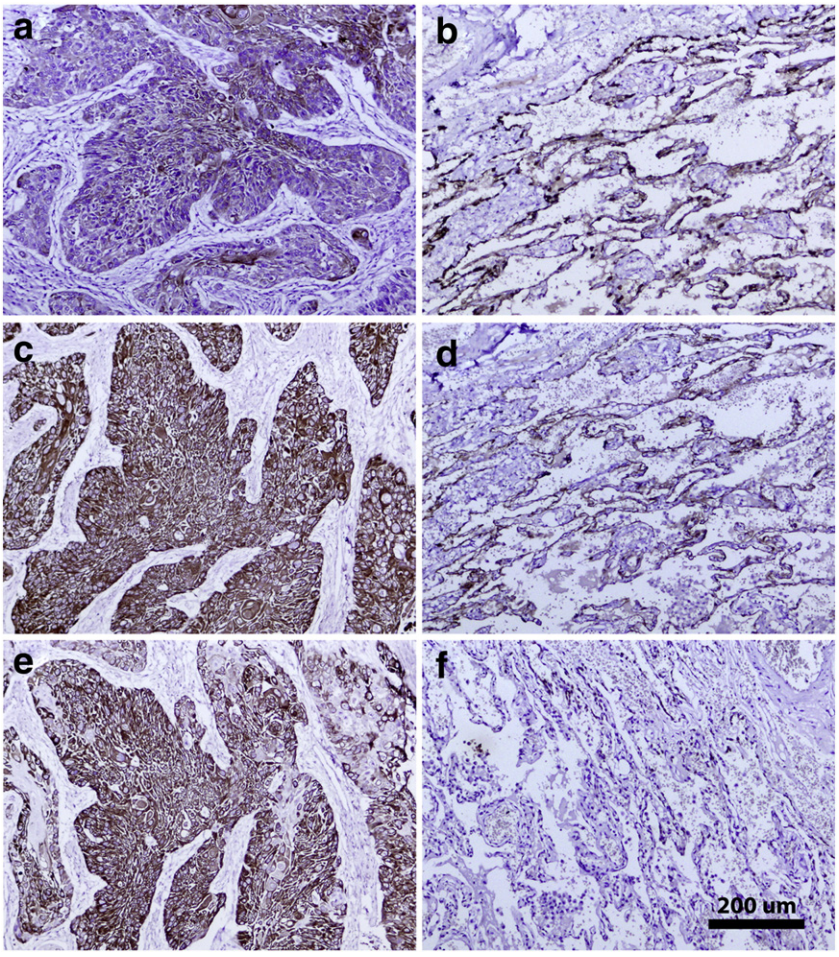

Fig. 1. Immunohistochemical staining of a lung squamous cell carcinoma (a, c and e) and the adjacent non-cancerous tissue (b, d and f). PTPN13 expression in the cancerous tissue (a) was much lower than in the non-cancerous tissue (b). Little detectable FAK (d) and P-FAK (f) expression was evident in the non-cancerous tissue, while intense FAK (c) and P-FAK (e) staining was demonstrated in the cancerous tissue.