Original Article

Expressions of Osteopontin (OPN), αvβ3 and Pim-1 Associated with Poor Prognosis in Non-small Cell Lung Cancer (NSCLC)

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ABSTRACT

Objective: To examine the expressions of osteopontin (OPN), $\alpha_v\beta_3$ and Pim-1 in non-small cell lung cancer (NSCLC), and investigate their potential pathogenic roles in the development of NSCLC.

Methods: Immunohistochemistry was used to examine the expressions of OPN, $\alpha_v\beta_3$ and Pim-1 in cohort (136 cases) of NSCLC samples and their adjacent normal lung tissue specimens. Statistical analysis was performed to evaluate the relationships among expressions of OPN, $\alpha_v\beta_3$ and Pim-1 and their associations with patients clinico-pathological parameters.

Results: The expressions of OPN and Pim-1 were predominantly observed in cytoplasm. The expression of $\alpha_v\beta_3$ was mostly detected in cytoplasm and/or membrane. In NSCLC samples, the positive rates of OPN, $\alpha_v\beta_3$ and Pim-1 expressions were 68.4% (93/136), 77.2% (105/136) and 57.4% (78/136), respectively. In normal lung tissues, in contrast, the positive rates of OPN, $\alpha_v\beta_3$ and Pim-1 were 24.0% (12/50), 26.0% (13/50) and 16.0% (8/50), respectively. There were significant differences of the positive expression rates of OPN, $\alpha_v\beta_3$ and Pim-1 between NSCLCs samples and normal lung tissues ($P<0.01$). In addition, the positive expression of OPN, $\alpha_v\beta_3$ and Pim-1 in NSCLCs samples was significantly associated with increased pathological grade, lymph node metastasis and advanced clinical stage ($P<0.01$), and they were independent of other clinicopathological parameters ($P>0.05$). Furthermore, a significantly positive correlation between the expression of OPN and $\alpha_v\beta_3$ (r=0.38, $P<0.01$), OPN and Pim-1 (r=0.37, $P<0.01$), or $\alpha_v\beta_3$ and Pim-1 (r=0.20, $P<0.05$) was evaluated in our NSCLC cohort.

Conclusion: OPN, $\alpha_v\beta_3$ and Pim-1 proteins are frequently overexpressed in NSCLC, and they may play important roles in the development and/or progression of NSCLC.

Key words: NSCLC; OPN; $\alpha_v\beta_3$; Pim-1; Immunohistochemistry

INTRODUCTION

Primary bronchogenic carcinomas have the highest mortality rate of malignant tumors in the world. Among them, non-small cell lung cancer (NSCLC) accounts for 85%, most patients who have been already clinically diagnosed are in middle-advanced stage, and the 5-year survival rate is very low[1]. Thus, early diagnosis and early treatment are particularly important to improve the survival rate. It is now considered that various pathogenic factors were involved in the evolution of NSCLC.

Osteopontin (OPN) is a multifunctional secrete phosphorylated glycoprotein, and it can promote cell chemotaxis, adhesion and migration, which mediate invasion and metastasis of tumor cells, and it is closely related to the occurrence, development, metastasis and prognosis of a variety of cancers[2,3]. Recent studies have indicated that OPN is involved in NSCLC progression and metastasis through interaction with its receptor, alpha$_v$beta3 ($\alpha_v\beta_3$) integrin, and overexpression of OPN is associated with progression and poor prognosis of NSCLC[2,3]. $\alpha_v\beta_3$ is a peptide of heparin binding factor and is an important molecule of integrin family. $\alpha_v\beta_3$ can mediate adhesion of cell to cell and cell to matrix, regulate intracellular signaling pathways, and induce the activation of protein dissolving enzymes, thereby contributing to extracellular matrix and basement membrane degradation, and promoting invasion and migration migration of tumor cells[4]. Previous study

found that $\alpha v\beta 3$ is detectable in a variety of tumors, and is closely associated with the tumorigenesis and the degree of malignancy of tumor[5]. Other factors which are closely related with genes of cell cycle regulation and proliferation, including Pim-1 (coded by *pim-1* oncogene)，are involved in tumorigenesis[6]. It has been reported that OPN acts through $\alpha v\beta 3$ integrin, which in turn activates the FAK, PI3K, Akt, ERK, NF-κB and Pim-1 pathways, contributing to the migration of lung cancer cells[6]. Taken together, these results suggest OPN mediates migration in human lung cancer cells via the $\alpha v\beta 3$ integrin, FAK, PI3K, Akt, ERK, NF-κB and Pim-1 signaling pathways. Therefore, we chose three factors of signaling pathways, OPN, $\alpha v\beta 3$ integrin and Pim-1 in this study. To date, however, the expression dynamics of OPN, $\alpha v\beta 3$ and Pim-1 in NSCLCs, and their potential biological roles in tumorigenesis of NSCLC have not been elucidated.

In the present study, immunohistochemistry was used to examine the expression dynamics of OPN, $\alpha v\beta 3$ and Pim-1 in cohort (136 cases) of NSCLC samples and their adjacent normal lung tissue specimens. Next, the potential correlations among the 3 protein expressions and their associations with NSCLC patients' clinico-pathological features were evaluated.

MATERIALS AND METHODS

Clinical Data

In this study, specimens were obtained from archived paraffin-embedded tissue sections of 136 patients with NSCLC at the Third Affiliated Hospital, Sun Yat-sen University, Guangzhou from January 1st, 2009 to December 31st, 2010. A group of 50 normal lung tissue cases was conducted as control. In this cohort of NSCLC patients, 97 were men and 39 were women, with a median age of 60 years (range 30–82 years). According to the World Health Organization criteria of lung cancer published in 2004[7], NSCLC patients were classified as follows: adenocarcinoma 72 cases, squamous cell carcinoma 40 cases, and other types 24 cases; well and moderately differentiated carcinoma 88 cases, and poorly differentiated carcinoma 48 cases. According to the staging standard of the TNM system of International Association for the Study of Lung Cancer[8], the NSCLC patients were classified as stage I/II 96 cases, and stage III/IV 40 cases. In 136 NSCLC cases of the present study, 35 had lymph node metastasis.

Protein Expression of OPN, $\alpha v\beta 3$ and Pim-1 in NSCLC

Immunohistochemical technique using streptavidin-peroxidase (SP) was employed for OPN, $\alpha v\beta 3$ and *Pim-1* detection. Mouse-anti-human monoclonal antibodies of OPN, $\alpha v\beta 3$, Pim-1 and SP kit were purchased from Maxim biological and technical company, Fuzhou, China; ready-to-use. All the sections were routinely

deparaffinized and rehydrated, then the sections were rinsed in phosphate-buffered saline (PBS, pH=7.4), subsequently were treated for antigen retrieval. Sections were treated in EDTA buffer (pH=8.0) in an autoclave sterilizer. After cooling at room temperature for 20 min, the sections were rinsed in PBS, then immersed in 3% H_2O_2 for 15 min to block the endogenous enzymes. After being rinsed in PBS, the sections were incubated with normal goat serum at 37°C for 15 min to block nonspecific antibodies. After interaction with OPN antibody, $\alpha v\beta 3$ and Pim-1 antibodies (monoclonal antibodies), the sections were rinsed in PBS, then incubated with biotinylated secondary antibodies and rinsed in PBS again. After interaction with streptavidin-horseradish peroxidase (HRP) and being rinsed in PBS, the sections were visualized by reaction with 3,3'-diami-nobenzidine and counterstained with hematoxylin. They were then dehydrated, transparentized and covered with coverslips and sealed with neutral gum. PBS substituting the primary antibody was used as negative control.

Judgment of Positive Result of OPN, $\alpha v\beta 3$ and Pim-1

The judgment that whether the tumor and the normal tissues were positive or not was performed by two pathology doctors. The positive expression of OPN was mostly in cytoplasm with brown-yellow staining, $\alpha v\beta 3$ was mostly in cytoplasm and membrane with brown-yellow staining, and Pim-1 was mostly in cytoplasm with brown-yellow staining. A tumor or normal tissue in which more than 10% of cells were stained with those antibodies was recognized as being positive; while less than 10% was recognized as being negative.

Statistical Analysis

Data were analyzed with computer aided SPSS 13.0 statistical software (SPSS Inc., Chicago, IL, USA). Chi-square test was used in comparing the expressions of OPN, $\alpha v\beta 3$ and Pim-1 in NSCLC samples and in normal lung tissue specimens. The associations between the expressions of OPN, $\alpha v\beta 3$ and Pim-1 and NSCLC patients' clinicopathological parameters were also evaluated by the Chi-square test. The correlation between two variables was evaluated by the Spearman rank correlation test. A value of $P<0.05$ was considered statistically significant.

RESULTS

Relationship between Protein Expression of OPN and Clinicopathological Parameters in NSCLC

The expression of OPN in NSCLC tissues was predominantly a cytoplasmic pattern (Figure 1 A, B). In our study, 93 (68.4%) of 136 NSCLC cases showed positive expression of OPN, while the positive rate of OPN in normal lung tissues was 24.0% (12/50). There