RESEARCH COMMUNICATION

Expression of Tiam1 in Lung Cancer and its Clinical Significance

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Abstract

The aim of this study was to ana1yze T-cell lymphoma invasion and metastasis-inducing factor 1 (Tiam1) expression in 1ung cancer patients. A total of 204 patients with lung cancer tissue lesions were enrolled in the present study, along with 40 cases of normal lung tissue and 40 of normal fetal lung tissue. Tiam1 protein expression level was determined using intensity quantitative analysis, for comparison in lung cancer, metastatic, normal lung, and fetal lung tissue. The positive unit (PU) of Tiam1 was 13.5 ± 5.42 in lung cancer, 5.67 ± 1.56 in norma1 epithelial cells, and 5.89 ± 1.45 in fetal lung epithelial cells. The value in the lung cancer tissue was significantly higher than that in the normal lung tissue and the fetal lung tissue (P <0.01). The Tiam1 PU values with lymph node metastasis and without 1ymph node metastasis were 15.2 ± 4.34 and 12.5 ± 4.23, respectively, and the difference was statistically significant (P <0.05). The Tiam1 PU values in different tumor, nodes, metastasis (TNM) stages, III–IV period, and I–II phase were 14.7 ± 4.14 and 11.0 ± 5.34 (P <0.05). A corre1ation was found between Tiam1 expression and the age of patient, tumor size, tumor type, and tumor differentiation. Tiam1 protein expression in the lung tumor tissue is significantly higher than that in the normal lung tissue and fetal lung tissue. Tiam1 expression may be closely related to lung cancer development and metastasis.

Keywords: Lung cancer - tissue microarray - cancer deve1opment - Tiam 1

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Introduction

Tiam1 was first discovered in a proviral insertion mutation study, and isolated and identified in the highly aggressive mice T lymphoma cells variants, also called T-cell lymphoma invasion and metastasis-inducing factor 1 (Tiam1) (Fleming et al., 2000; Engers et al., 2006; Li et al., 2011; Chen et al., 2012). The chemical component protein serves as a guanylic acid transfer factor, which can transform the guanylic acid bisphosphate into guanylic acid triphosphoric acid and promote guanylic acid bisphosphate release and binding to guanylic acid triphosphoric acid. Rac1 protein is activated downstream, thus promoting the occurrence of cytoskeleton rearrangement, and cell migration and mobility. At the same time, Tiam1 might be involved in the regulation of gene expression, cell proliferation, and apoptosis (Fritz et al., 2002; Minard et al., 2004; Adams et al., 2010 McHenry and Vargo-Gogola, 2010). Usually, Tiam1 is expressed only in human brain and testis tissues, and not expressed (or has very low expression) in other normal tissues. Recently, studies found that Tiam1 is highly expressed in lymphoma, melanoma, colorectal cancer, and breast cancer, among others, which may be closely related to tumor metastasis (Walch et al., 2008; Ding et al., 2009; Stebel et al., 2009; Adams et al., 2010; Yang et al., 2010; Hsueh et al., 2011; Zhao et al., 2011). Research shows that in tumor cells, the microtubule, microfilament, and adhesion plaque are destroyed. The stress fiber structure is in serious disorder, with actin occurring together and often appearing as small blob-shaped bodies. Cellular rigidity also declines significantly, cell migration and athletic ability increase, and protuberance and pseudopodium are present in the cell membrane, thus increasing cell invasiveness (Sonoda and Sasaki, 2008; Ferrandina et al., 2009). In the present study, Tiam1 expression in the normal lung tissues and lung cancer tissue was examined by immunohistochemistry to analyze the relationship between Tiam1 expression and metastasis of lung cancer to provide new therapeutic targets for the clinical treatment of cancer.

Materials and Methods

General Data

All clinical specimens were obtained from the Department of Pathology, the First Affiliated Hospital of Zhengzhou University, collected from January 2009 to June 2011. All patients did not receive radiotherapy or chemotherapy before surgery. The tissue group and sample numbers are as follows: normal lung tissue, 40 cases and embryonic lung tissue, 40 cases. Approximately 204 cases had lung cancer lesions, and all patients in the sample were aged 35 to 79 years, with an average of 52 years. Among 204 cases of patients with lung cancer tissue samples, 196 had non-small cell lung cancer, including squamous cell carcinoma (n = 96), adenocarcinoma (n = 54), large cell carcinoma (n=46), and small-cell carcinoma (n=8). All the lung tissue samples were fixed using 10% formalin. They were then paraffin-embedded until they formed 760 tissue chips.

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Immunohistochemistry

The formalin-fixed lung tissues were paraffin-embedded and sectioned coronally with a microtome into 6 μm thick sections. After deparaffinization, the sections were subjected to an antigen retrieval protocol by heating them in 10 mM citrate buffer (pH 6.0) at 100 ºC for 10 min. Potential non-specific binding sites were blocked with 5% normal goat serum in PBS. Then, the sections were incubated with the primary polyclonal antibody rabbit anti-Tiam1 (1: 50; Sigma-Aldrich, USA) at 37 ºC for 1 h and 4 ºC overnight, followed by washing in PBS, incubation with biotinylated secondary antibody (goat anti-rabbit IgG (Maixin-Bio Co., Ltd., China) for 15 min at 37 ºC, and washing in PBS. The sections were further incubated with horseradish peroxidase for 10 min at 37 ºC, washed in PBS, and colored with diaminobenzidine (DAB; Maixin-Bio Co., Ltd., China) at room temperature for 7 min. Finally, the sections were counterstained with hematoxylin for 3 min, dehydrated, rinsed, and coverslipped with glycerin. The sections not incubated with primary antibody served as negative controls.

Quantitative analysis

Images of immunohistochemical stains were taken using a Nikon microscope, and images at 40 times magnification were captured using software. Then, the positive images were randomly selected, with 20 positive areas taken at each sample. The gray area of every cell was evaluated using the grayscale analysis software Imagepro Plus. The background gray was also analyzed, and the average was obtained, respectively. The positive unit (PU) area of each cell was calculated according to the reported method (Ferrandina et al., 2009). The average PU of 20 cells in each sample was the sample PU value.

Statistical analysis

All analyses were conducted using the statistical software SPSS 13.0. $P < 0.05$ was considered significant. First, each sample was analyzed by variance consistency. If the variance consistency was good, then multiple comparison analysis was performed using one set of standard deviations. If the variance consistency was not good, a comparative analysis between groups was performed using Dunnett's T3 test. A comparison of the two samples was analyzed by t-test, and $P < 0.05$ was considered significant.

Results

Tiam1 protein expression

Tiam1 protein was expressed in the cytoplasm of the lung epithelial cells, and yellowish-brown staining by DAB was shown. Tiam1 protein expression level in the lung carcinoma was significantly higher than in the normal lung tissue epithelial cells and embryo lung tissue epithelial cells. No significant difference in Tiam1 protein expression was found between the normal lung tissue epithelial cells and the embryo lung tissue epithelial cells (P>0.05). The PU value of Tiam1 in non-small-cell lung cancer tissue was significantly higher than those of the normal lung tissue epithelial cells and embryo lung epithelial cells (P< 0.05). Moreover, the PU value of Tiam1 in the small-cell lung cancer tissue was significantly higher than those of the normal lung tissue epithelial cells and embryo lung tissue epithelial cells (P< 0.05).

Table 1. PU Value of Tiam1 in Normal, Embryo Lung Tissue Epithelial Cells and Lung Lesions Tissue Cancer Cells

Group	Cases	Tiam1
Normal lung tissue epithelial cells	40	5.67±1.56
Embryo lung tissue epithelial cells	40	5.89±1.45
Lung cancer cells		
Squamous-cell carcinoma	96	12.5±5.22
Adenocarcinoma	54	13.3±4.13
Large cell carcinoma	46	10.7±2.73
Small cell carcinoma	8	10.2±2.76

Table 2. The Relationship of Lung Cancer Tiam1 PU Value and Clinical Pathological Features

Item	n	PU value	t value	P value
Age			0.775	0.44
<60	120	12.21±4.12		
>60	84	12.93±5.26		
Gender			0.852	0.396
Male	146	12.75±4.77		
Female	58	11.89±4.22		
General types			1.449	0.15
Peripheral	88	13.27±4.12		
Central	116	11.94±4.93		
SCC Differentiation	96		1.234	0.223
Well-differentiated	56	14.43±6.03		
Low-differentiation	40	12.08±5.01		
Adenocarcinoma	54		0.464	0.646
Well-differentiated	34	13.57±4.01		
Low differentiation	20	12.91±4.41		
Lymph node metastasis			3.674	0
Yes	88	14.33±4.66		
No	116	11.13±4.11		
TMN stage			2.72	0.0008
I-II	168	11.95±4.63		
III-IV	36	15.11±3.61		
Lesions			0.212	0.836
Primary focal	88	16.63±3.97		
Lymph node metastases	88	16.16±4.43		

The PU value of Tiam1 in the primary lesion of lung cancer patients with lymph node metastasis was significantly higher than that without lymph node metastasis (P<0.05). The PU value of primary lung cancer lesions tissue was the same as that of lymph node metastasis of lesions (P>0.05, Table 1).

According to the different TNM stages, the PU value of Tiam1 in the III–IV and I–II stages were 14.71 +/- 4.14 and 10.95 +/- 5.34, respectively ($P < 0.05$). The results indicated that the Tiam1 expression level had no correlation with the age of the patient, tumor size, tumor tissue type, and degree of tumor differentiation (Table 2).

Discussion

Tiam1 is highly expressed in lymphoma, melanoma, colorectal cancer, and breast cancer, among others, which

may be closely related to tumor metastasis (Walch et al., 2008; Ding et al., 2009; Stebel et al., 2009; Yang et al., 2010; Hsueh et al., 2011; Zhao et al., 2011). When "antisense" nucleotide was used to inhibit the expression of Tiam1, the metastasis of tumor was significantly reduced (Hou et al., 2004; Liu et al., 2006). The semi-quantitative method confirmed that the high expression of Tiam1 may be relevant to the metastasis and prognosis of small-cell lung cancer (Buchanan et al., 2000).

At present, Tiam1 is investigated by immunohistochemistry, the current study used the positive unit to analyze the cell expression level, and the results showed that the Tiam1 protein expression level in lung carcinoma was significantly higher than that in the normal lung tissue epithelial cells and embryo lung tissue epithelial cells ($P< 0.05$). The results indicated that the Tiam1 expression level had no correlation with the age of the patient, tumor size, tumor tissue types, and degree of tumor differentiation.

In conclusion, high Tiam1 protein expression level may be related to the occurrence, development, and metastasis of lung cancer, which provides new therapeutic targets.

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