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Original Article

Expression of nerve growth factor and tyrosine kinase A in non-small cell lung cancer

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Abstract: The aim of the present study was to investigate the function of nerve growth factor (NGF) and tyrosine kinase A (TrkA) expression in non-small cell lung cancer (NSCLC). A lot of studies showed that NGF is overexpressed not only in nervous system, but also in several types of cancers. However, the role of NGF and TrkA in NSCLC remains unclear. Using immunohistochemical staining, the expression of NGF and TrkA was evaluated in 120 NSCLC tissues and 20 matched adjacent lung tissues. The result showed that the expression of NGF and TrkA in NSCLC tissues was higher than that in the adjacent lung tissues and it was significantly correlated with the tumor differentiation, lymphatic metastasis and TNM-staging ($P<0.05$). However, there was no significant difference among age, sex, histopathological type and tumor size ($P>0.05$). The result also showed NGF expression was positively correlated with TrkA expression ($P<0.01$). The increased expression of NGF and TrkA in NSCLC suggested that they may play an important role in the tumorigenesis of NSCLC. It also showed that NGF and TrkA are likely to be not only satisfactory biomarkers for predicting the prognosis of patients but also to be new targets for therapy in NSCLC. The detection of NGF and TrkA plays an important role in prognosis and treatment of NSCLC.

Keywords: Non-small cell lung cancer, nerve growth factor (NGF), tyrosine kinase A (TrkA), immunohistochemical staining

Introduction

Lung cancer is the most common cause of cancer-related mortality worldwide, of which non-small cell lung cancer (NSCLC) is the most common type. A study of cancer statistics in 2011 reported that the overall 5-year survival rate of lung cancer patients was ~16% [1]. Non-small cell lung cancer (NSCLC), of which squamous cell carcinoma and adenocarcinoma account for the vast majority of cases, represents almost 80% of primary lung cancer cases [2]. Prognosis may be improved with a focus on exploring the specific molecular biomarkers that are involved in the tumorigenesis and progress of NSCLC.

The nerve growth factor (NGF) is a growth factor that belongs to the neurotrophin family [3]. NGF has two structurally different receptors, the p75 neurotrophin receptor (p75NTR) and the tropomyosin-related kinase A (TrkA). Interaction of NGF with its receptors regulates the NGF functions as a signaling molecule by binding with these two known receptor: the common p75NTR binds all of the neurotrophins with almost equal affinity, whereas the specific tyrosine kinase receptors TrkA binds the NGF [4, 5]. Recent studies have shown the presence of NGF and its receptors in variety of human tissues other than in the nervous system alone, and their overexpression may promote the proliferation, growth and invasion in several types of cancer, such as neuroblastoma, breast carcinoma, colon cancer and oral carcinoma [6-9].

In this study we used the immunohistochemical staining method to analyze the expression of NGF and TrkA in non-small cell lung cancer (NSCLC) tissues. Moreover, the relationship between the expression of NGF and TrkA and clinical pathological features was also investigated.

Materials and methods

Clinical samples

A total of 120 formalin-fixed, paraffin-embedded NSCLC tissues and 20 matched tumor

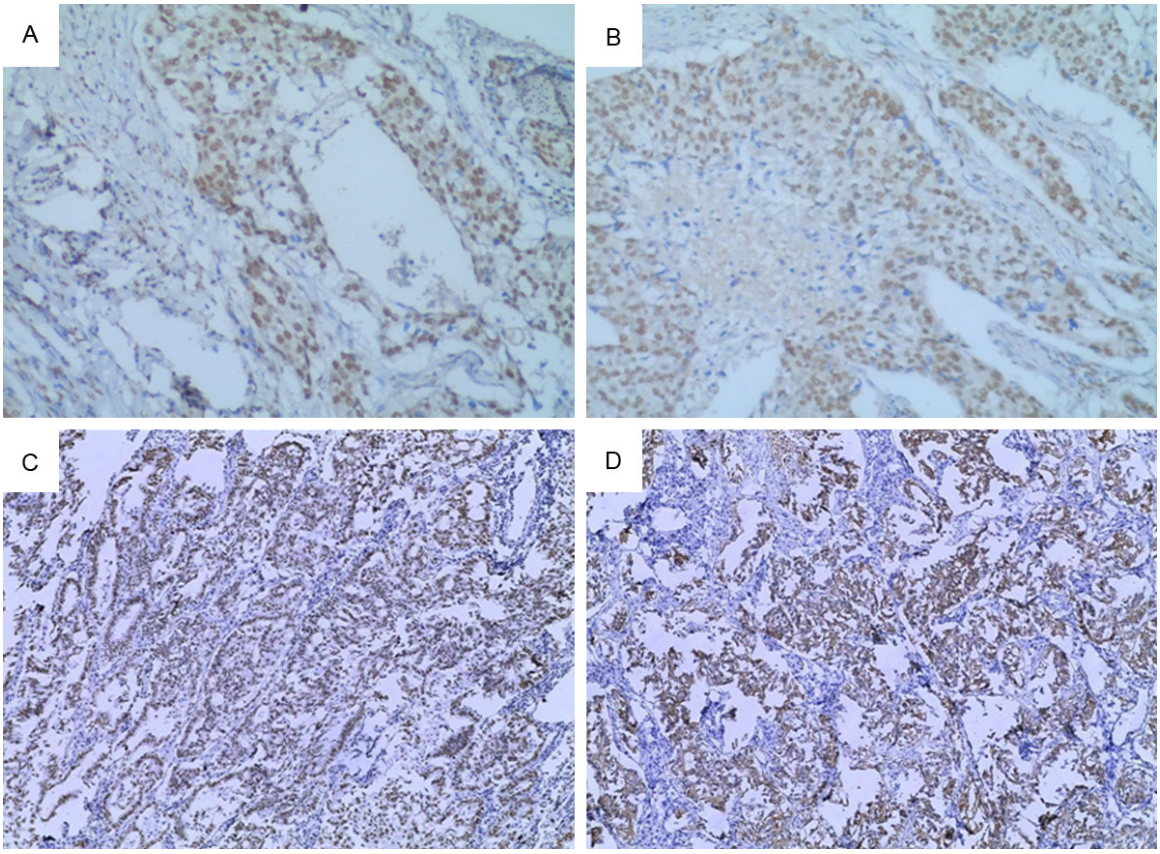

Figure 1. A: NGF protein in the lung squamous cell carcinoma of the organization expression; B: TrkA protein in the lung squamous cell carcinoma of the organization expression; C: NGF protein in lung adenocarcinoma expression in the organization; D: TrkA protein in lung adenocarcinoma expression in the organization.

adjacent lung tissues were obtained between 2011 and 2014 from the Department of Pathology, the First Affiliated Hospital of Jishou University (Jishou, Hunan, China). 105 of these patients were Tujia ethnic group. None of these patients received chemotherapy or radiotherapy prior to surgery. All clinic data, such as, gender, age, pathology classification, TNM stage, tumor cell differentiation, lymph node metastasis, were collected from patients' medical records.

The group was composed of 87 males and 33 females, with a mean age of 58 years old (range from 38 to 75 years old) at the time of the surgery. A summary of the patients' characteristics and the pathological characteristics were presented in details in the results. All 120 cases were independently classified as NSCLC according to the World Health Organization histological typing criteria. Of the 120 patients, 36 patients (30%) were in stage I, 62 patients (51.7%) in stage II, 22 patients (18.3%) in stage III~IV. 72 patients were squamous cell carcinomas, 38 patients were adenocarcinoma, 10 patients were large cell carcinomas.

Immunohistochemical staining

Immunohistochemical studies on NGF and TrkA were performed on formalin-fixed, paraffin-embedded tissue sections obtained from the aforementioned patients with NSCLC. Paraffin-embedded 4-μm-thick serial sections were subjected to paraffin removal and rehydrated through graded alcohol. To block the endogenous peroxidase activity, slides were pretreated with 3% H_2O_2. Tissue sections were then boiled in 0.01 M sodium citrate buffer (pH 6.0) in a 1,000-watt microwave oven for 10 min to retrieve cell antigens. Primary antibodies were diluted to 1:50 for anti-NGF rabbit polyclonal antibody (Cell Signaling Technology, Danvers, MA, USA) and 1:100 for anti-TrkA rabbit polyclonal antibody (Cell Signaling Technology, Danvers, MA, USA). The sections were incubat-

Table 1. Expression of NGF and TrkA in 120 NSCLC tissues and adjacent lung tissues

Groups	Total cases	NGF		TrkA		P
		-	+	-	+	
Lung cancer tissues	120	35	85	26	94	P<0.01
Adjacent lung tissues	20	19	1	18	2	

Table 2. Correlation between the expression of NGF and TrkA and clinicopathological characteristics of 120 cases of NSCLC

ITEMS	n	NGF		P	TrkA		P
		-	+		-	+	
Gender				0.866			0.941
Males	87	25	62		19	68	
Females	33	10	23		7	26	
Ethnic group				0.005			0.012
Tujia	105	26	79		19	86	
Other	15	9	6		7	8	
Age				0.247			0.912
>65	78	20	58		17	61	
<65	42	15	27		9	33	
Pathology classification				0.896			0.934
Squamous cell carcinomas	72	22	50		15	57	
Adenocarcinoma	38	10	28		9	29	
Large cell carcinomas	10	3	7		2	8	
TNM stage				0.001			0.001
I	36	26	10		16	20	
II	62	6	56		6	56	
III~IV	22	3	19		4	18	
Tumor cell differentiation				0.015			0.001
Well	48	21	27		21	27	
Moderate	48	10	38		3	45	
Poor	24	4	20		2	22	
Lymph node metastasis				0.018			0.003
Yes	68	14	54		8	60	
No	52	21	31		18	34	

ed with the primary antibodies at 4°C overnight. Subsequently, the slides were incubated with goat-anti-rabbit biotinylated secondary antibodies at a concentration of 1:100 for 30 minutes at 37°C and then reacted with streptavidin-peroxidase conjugate for 30 minutes at 37°C. After several further washes with phosphate buffer, slides were treated with diaminobenzidine (DAB) and counterstained with hematoxylin. The sections were dehydrated, mounted and then were observed under a light microscope. Omitting the primary antibody for each protein was used as the negative control, and the sections didn't show any background staining.

Immunohistochemical assessment

Nerve growth factor was expressed diffusely in NSCLC, NGF was expressed in the cytoplasm and staining intensity was graded into two groups: positive (positive cytoplasmic staining more than 10% of NSCLC) [10] and negative (staining less than 10%). TrkA was expressed in the cytoplasm and the membrane of NSCLC tissues and staining intensity was graded into two groups: positive (positive membrane staining in more than 10% of NSCLC or/and intense staining in cytoplasm of NSCLC) and positive expression (staining less than 10%) [10].

Statistical analysis

The data were subject to statistical analysis using the SPSS software package (version 13.0; SPSS, Inc., Chicago, IL, USA). The significant expression of NGF and TrkA in the tissues as well as the correlation between their expression and the clinicopathological parameters were tested by χ^2 test and/or multivariate analysis. The correlation between the expression of NGF and TrkA was tested by Spearman's rake correlation coefficient. P<0.05 was considered to indicate a statistically significant difference.

Results

Expression of NGF and TrkA in the NSCLC and adjacent lung tissues

Among the 120 NSCLC specimens, 85 (70.8%) specimens had positive NGF expression, 94 (78.3%) specimens had positive TrkA expression. The positive expression of NGF mainly located in the nuclei (**Figure 1A** and **1B**). The positive expression rate of NGF (85/120) in NSCLC was significantly higher than that in

Table 3. The relationship between the expression of NGF and TrkA in 120 NSCLC cases

NGF	n	TrkA				P
		-	+	++	+++	
-	35	16	6	7	6	P=0.001
+	85	10	24	24	27	

Table 4. The percent of tumors with different combination of NGF and TrkA staining of 120 cases of NSCLC

NGF	n	TrkA				P
		+/+	-/-	+/-	-/+	
Gender						
Males	87	56	13	6	12	
Females	33	19	3	4	7	P>0.05
Ethnic group						
Tujia	105	71	9	8	15	
Other	15	4	5	2	4	P<0.01
Age						
>65	78	51	10	7	10	
<65	42	24	6	3	9	P>0.05
Pathology classification						
Squamous cell carcinomas	72	47	12	3	10	
Adenocarcinoma	38	22	3	6	7	P>0.05
Large cell carcinomas	10	6	1	1	2	
TNM stage						
I	36	8	14	2	12	
II	62	51	1	5	5	P<0.01
III~IV	22	16	1	3	2	
Tumor cell differentiation						
Well	48	20	14	7	7	
Moderate	48	36	1	2	9	P<0.01
Poor	24	19	1	1	3	
Lymph node metastasis						
Yes	68	50	4	4	10	
No	52	25	12	6	9	P<0.01

adjacent lung tissues (1/20) (P<0.01) (**Table 1**). The positive expression of TrkA mainly located in the cytoplasm (**Figure 1C** and **1D**). The positive expression rate of TrkA (94/120) in NSCLC was significantly higher than that in adjacent lung tissues (2/20) (P<0.01) (**Table 1**).

Correlation between the expression of NGF and TrkA and clinicopathological characteristics of 120 cases of NSCLC

There was no significant association between NGF and TrkA expression and other factors including age, gender and pathology classification (**Table 2**). However, the expression of NGF and TrkA was significantly correlated with the tumor differentiation, lymphatic metastasis and TNM-stage (P<0.05).

Correlation between the expression of NGF and TrkA in NSCLC

The percent of double NGF and TrkA positive expression was 62.5% (75/120). The positive NGF expression was significantly associated with positive TrkA expression in NSCLC tissues (*P*=0.001) (**Tables 3** and **4**).

Discussion

NGF functions mostly by interacting with its two receptors: p75NTR and TrkA receptor. The p75NTR is a low affinity receptor and TrkA is a high affinity receptor. TrkA is distinguishing in the Trk receptors because it functions by autophosphorylating and activating of various signaling cascades [11]. When a high level of TrkA was observed, NGF prompted the growth of tumor cells. When there was little or even no TrkA, the action of NGF to tumor cells did not exist or even exert restraint [12]. Thus NGF and its receptor TrkA has been a couple of the most closely related molecules in the field of cancer. Overexpression of NGF and TrkA has been studied in many types of human malignancies, including neuroblastoma, breast carcinoma, colon cancer, oral carcinoma and hepatocellular carcinoma [6-9, 13]. Most recently, Lu et al. reported that the expression of NGF was remarkably higher in NSCLC tissues in 20 Chinese Han ethnic group [14]. In this study, we did a further study of the correlation, while investigating 120 NSCLC samples with a large portion of Chinese Tujia ethnic group, our results showed that not only NGF but also TrkA expression in NSCLC were significantly higher than tissues adjacent to carcinoma. The study displayed that NGF was strongly expressed in 85 (70.8%) NSCLC tissues and

TrkA was expressed in 94 (78.3%) NSCLC tissues. It is also interesting to note that the expression of NGF and TrkA in NSCLC tissues in Tujia ethnic group was significantly higher than that in other ethnic groups. To our knowledge, this is the first to study the relationship between the expression of NGF and Trk and NSCLC in Tujia ethnic group as an object. Although the specific reason for the difference is not clear and the result remains to be clarified further, our data at least suggested that NGF and TrkA may play an important role in the tumorigenesis of NSCLC. NGF and TrkA express in NSCLC, probably NGF and TrkA activate VEGF which promote tumor growth and metastasis, Ferrara N et al. also think so [15].

We also found that the expression of NGF and TrkA was closely correlated to the prognosis of NSCLC. When the expression of NGF and TrkA in NSCLC was analyzed with the clinicopathological data, it was apparent that NGF and TrkA expressions correlated with positive lymph node metastasis, poorer tumor differentiation, and higher TNM stage. However, the expression of NGF and TrkA was not correlated with age, gender and pathology classification. The results proved that the expression of NGF and TrkA was correlated with the progression of NSCLC and can be used as a new index for evaluating the prognosis of NSCLC. These results are in accordance with the previous studies on the ovarian and prostate cancer [16-18]. The molecular mechanisms of NGF and TrkA that governing the cancer cells growth and progression remain unclear. Through its TrkA receptor, NGF may generate a positive microenvironment for cancer cell survival and proliferation. For example, overexpression of NGF and TrkA may promote angiogenesis in the cancer cells by increasing vascular endothelia growth factor (VEGF) and hypoxia inducible factor-1alpha (HIF-1alpha) [15, 17, 19]. Recently, in vitro and in vivo studies demonstrated that GTx-186, a novel inhibitor with unique kinase inhibitory of Trk family, could inhibit cancer cell and tumor growth [20]. This newly identified chemical might open new therapeutic avenues for the treatment of NSCLC through NGF/TrkA signal transduction pathway. This result, on the other hand, revealed the important role of NGF/TrkA pathway in the progress and prognosis of NSCLC. Thus NGF and its receptor TrkA has been a couple of the most closely related molecules in the field of cancer.

In summary, results of our immunohistochemical study of 120 NSCLC patients suggested that NGF and TrkA are potential markers not only as prognostic factors, but also as therapeutic targets in NSCLC. We hope our study could trigger the interest of NGF or TrkA as potential drug targets and help find new therapy of NSCLC.

Disclosure of conflict of interest

None.

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