Expressions of NF-κ B and VEGF in Non - Small Cell Lung Cancer and the Relationships between them and Tumor Angiogenesis*

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ABSTRACT Objective: To investigate the expression of vascular endothelial growth factor (VEGF), NF-κ B and the relationship with tumor angiogenesis in non-small cell lung cancer (NSCLC). Methods: Expressions of NF-κ B P65, VEGF in NSCLC (n=56) and benign tumor tissue (n=20) were detected by immunohistochemical staining. A monoclonal antibody directed against CD34 to identify intratumoral microvessel density (iMVD). Results: The positive expression rates of NF-κ B P65, VEGF in NSNLC tissue were 83.9%, 69.6% respectively with significantly higher than those of benign tumor tissue ($P<0.05$). The expression level of NF-κ B P65 was related with tumor stages, lymphnode involvement, pleural metastasis and smoking of patients ($P<0.05$). The expression level of VEGF was related to lymphnode involvement and pleural metastasis. There was statistically significant correlation between the expression of NF-κB P65 and VEGF, NF-κ B P65 and MVD, VEGF and MVD, respectively ($P<0.05$). Conclusion: The high expressions of NF-κ B P65 and VEGF in NSCLC tissues may be related with tumor invasion and angiogenesis.

Key words: Lung neoplasm; NF-κ B P65; VEGF; Immunohistochemistry

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Introduction

Lung cancer was seen as a rare malignant tumor in the 1950s, but now it is estimated that more than 2.1 million people are diagnosed with lung cancer and 1.1 million people are died of lung cancer each year [1]. Although the surgery and chemoradiotherapy is developing rapidly currently, the overall survival of the patients with lung cancer is still less than 15%, in which, non-small cell lung cancer (NSCLC) accounts for 80%, and NF-κ B plays a key role in the occurrence and development of tumors [2]. NF-κ B target genes can code adhesion molecule, chemotactic factor and others which are essential in the occurrence and development of tumors. VEGF is taken as the strongest known vascularization-promoting factor currently and has a close relationship with non-small cell lung cancer, the purpose of this test is to study the expression of NF-κ B, VEGF in non-small cell lung cancer and to explore their effect in occurrence and development of non-small cell lung cancer and the correlation between them.

1 Materials and Methods

1.1 Specimens and Data

The specimens of the thoracic surgery between October 2009 and October 2010 in PLA hospital 401 were chosen from 56 patients with NSCLC (excluding the following patients: 1. Those who had received the radiotherapy or (and) chemotherapy before the surgery; 2. Those combined with other tumors except for lung cancer, 3. those whose tissues are embedded inadequately in paraffin) in which, 30 patients with squamous carcinoma and 26 patients with adenocarcinoma, (38 males and 18 females, mean age: 55.1 years (39-75 years)). 20 adjacent tissues of NSCLC were selected randomly. The specimens were fixed with 10% neutral formaldehyde solution, dehydrated, made transparent and embedded in paraffin. The histopathological classification and grading were determined by the pathologists from our hospital, and TNM staging was performed with the combination of surgery, imaging examinations (chest CT, craniocerebral MRI (or CT) for each patient, bone sketch, abdominal B ultrasound or PET-CT of the whole body).

1.2 Main Reagents

Rabbit anti-human VEGF monoclonal antibody, rabbit anti-human NF-κ B P65 monoclonal antibody and mouse anti-human CD34 monoclonal antibody were all purchased from Boshide Biotechnology Co., Ltd. Color development kit was purchased from Zhongshan Goldenbridge Biotechnology Co., Ltd.

1.3 Main Methods

The serial sections with the thickness of 5 μm made from paraffin-embedded tissue blocks were used to carry out the HE staining and immunohistochemical staining for which PV-9000 method was used. The steps were as following: dewaxing the sections by the normal procedures, digesting with 3mol/L urea solution, sealing with 3% hydrogen peroxide solution, performing the microwave antigen retrieval in citrate buffer solution, cooling to

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room temperature naturally, sealing with serum, adding the first antibody (diluted by 1: 200), then placing at 4 ℃ overnight, re-warming at 37 ℃, adding the second antibody to make them react at 37 ℃, DAB color developping, staining again with haematoxylin, differentiating with hydrochloric acid in a certain alcohol, carrying out the procedures for dehydration and transparency, sealing with neutral balsam. The first antibody was replaced with PBS as the blank control.

1.4 Results judgement

The result about NF-κ B P65 showing yellow solids precipitation in cytoplasm was positive and about VEGF showing brown-yellow solids precipitation in cytoplasm was positive. Scoring by staining levels: observing by a microscope, 0 for the negative; 1 for the weakly positive (lightly yellow); 2 for the positive (yellow); 3 for the strongly positive (dark brown). Scoring by the percentage of stained cells: assessing the percentage of stained cells counted under 10 high power fields in each section, 0 for the negative (< 10%); 1 for the weakly positive (10%-25%); 2 for the positive (25%-50%); 3 for the strongly positive (> 50%). The judgement was carried out by the sum of the scores of the percentage of the positive cells and staining levels, 0 (the total scores) for the negative, 1-2 for weakly positive, 3-4 for the positive, 5-6 for the strongly positive. The method for the determination of MVD was performed according to Weidner method [3], that is, firstly finding out 3 areas with the highest vascular density under a low power microscope (× 40 times), secondly counting the number of micrangium which were stained brown-yellow by CD34 under a high power microscope (× 200 times), finally the results were shown with the mean number of vessel under 5 fields (× 200 times).

1.5 Statistical Analysis

SPSS 17.0 system was applied for statistical analysis, and X^2 test was used for grading and comparison between the experimental group and control group, within the experimental groups. P <0.05 was considered statistically significant.

2 Results

2.1 Expression of NF-κ B P65 and VEGF in the tissues of non-small cell lung cancer and its adjacent tissues

The positive expressions of NF-κ B P65 protein and VEGF protein were mainly localized in cytoplasm (see Fig.1 and Fig.2 respectively); The positive expression rates of NF-κ B P65 and VEGF in non-small cell lung cancer were 83.9% (47/56) and 80% (45/56) respectively and those in normal tissue were 20 % (4/20) and 30 % (6/20). The positive rates of NF-κ B P65 and VEGF in non-small cell lung cancer were both higher than those in the adjacent tissues with a statistically significant difference (P < 0.05).

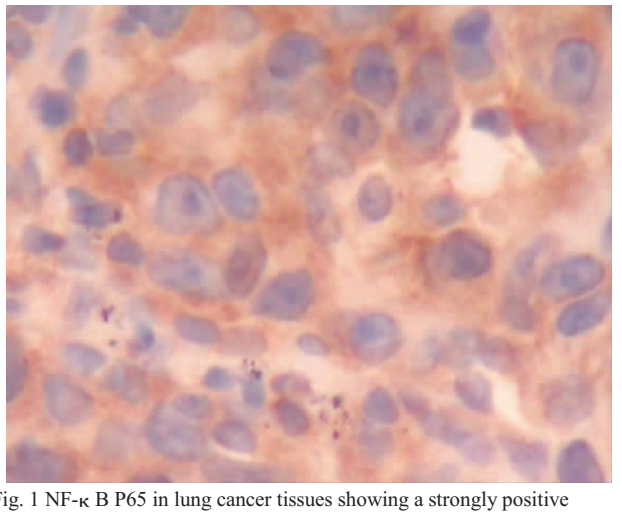

Fig. 1 NF-κ B P65 in lung cancer tissues showing a strongly positive expression × 400

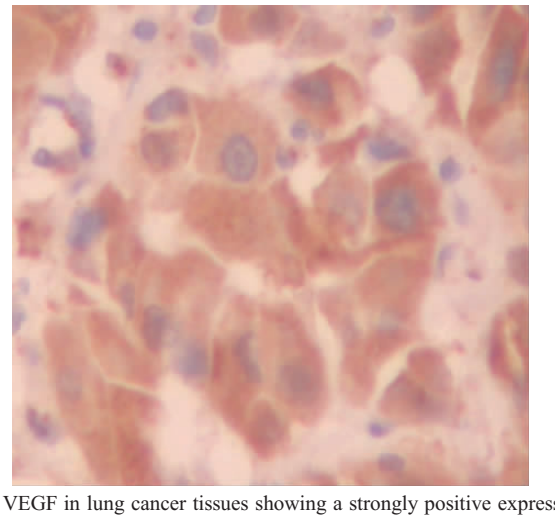

Fig.2 VEGF in lung cancer tissues showing a strongly positive expression × 400

2.2 Relationship between the expression of NF-κ B P65 and VEGF and the clinical pathology of non-small cell lung cancer

There was no relationship between the expression of NF-κ B P65 in NSCLC tissues and the patient' age, gender and tissues type (P > 0.05), but there was relationship between the expression of NF-κ B P65 in NSCLC tissues and NSCLC' TNM staging, lymph node metastasis, pleural metastasis and smoking history (P < 0.05). There was no relationship between the expression of VEGF wasand the patient' age, gender, tissues type, TNM staging and smoking history (P > 0.05), but there was relationship between lymph node metastasis and pleural metastasis (P < 0.05), (Table 1).

2.3 Correlation Analysis on NF-κ B P65, VEGF and MVD

MVD scores of the positive expression of NF-κ B P65 and VEGF in lung tissues were significantly higher than those of their negative expression in lung cancer tissues with a significant difference and correlation (Table 2).

3 Discussion

In 1986, Sen and Baltimore detected a nucleoprotein factor called for nuclear factor κ B from B cells extracts for the first

Table 1 Relationship between the expression of NF-κ B P65 and VEGF and the clinical pathology of NSCLC

	NF-κ BP65(+)	P	VEGF(+)	P
Age(year)(n)				
Under55.1(30)	24	0.127	20	0.603
Over55.1(26)	16		19	
Gender				
Female(20)	14	0.546	12	0.242
Male(36)	26		27	
Histology				
S.C. (36)	25	0.659	25	0.965
A.C. (20)	15		14	
Smoking status				
Smoking (38)	32	0.002	28	0.339
Non-smoking (18)	8		11	
TNM stages				
Ⅰ-Ⅱ (30)	18	0.042	22	0.519
Ⅲ-Ⅳ (26)	22		17	
N stage				
N0 (17)	8	0.000	7	0.002
N1 (39)	32		32	
Pleural metastasis				
NO (30)	8	0.000	8	0.000
YES (39)	32		32	

Table 2 Relationship among NF-κ B P65, VEGF and MVD

		MVD		X^2	P 值
		≤ 31.1	≥ 31.1		
VEGF(+)	(+)	13	26	6.250	0.012
	(-)	10	7		
NF-κ B P65	(+)	11	36	9.878	1.002
	(-)	13	26		

time，which can combine specifically with immunoglobulin κ light-chain gene enhancer κ B sequence[4]. NF-κ B is a class of common transcription regulators, and the present studies find that it can regulate the transcription of a variety of cell factors so as to play the role in fighting apoptosis, promoting the proliferation of cells, vasculogenesis and tumor metastasis [5], such as pancreatic cancer, breast cancer, gastric cancer and prostate cancer [6-9]. The results of this study showed that the expression of NF-κ B P65 in NSCLC tissues is higher than that in the adjacent tissues with a statistically significant difference, Jin and other persons find that the expression of NF-κ B P65 in lung cancer tissues is higher than that in the adjacent tissues by using the protein gel electrophoresis and immunohistochemical method [10]. Similar to the results of the study above, it is found in this study that the expressions of NF-κ B P65 in the smoking, TNM stage Ⅲ and Ⅳ and metastasis group are significantly higher than those in non-smoking group, TNM stage Ⅰ, Ⅱ group and non-metastasis group. Zhang et al, report that the high expression of NF-κ B in lung cancer tissues indicates the worse tissue differentiation and higher degree of malignancy [11]. There have been some reports in which it was found that the high expression of NF-κ B P65 in NSCLC indicated the lower survival of NSCLC[12].

VEGF is taken as one of the strongest known vascularization-promoting factors currently, and it can involve in lung cancer invasion and metastasis by promoting angiogenesis [13-14]. This study found that VEGF had no relationship with the patient' age,

gender, tissues type and TNM staging, which was similar with the results of the studies by Fortanini [15]. The previous studies show: after VEGF genes were transfected into the tumor cell lines with low expression level of VEGF and the cell lines were implanted into the body of the nude mice, the tumor cells showed a stronger angiogenesis activity and invasiveness [16]. The results of this study showed that the positive expression rate of VEGF in NSCLC tissues increase significantly and showed a significant positive correlation with MVD, which indicated that VEGF was closely related with tumor angiogenesis and its expression in NSCLC tissues was associated with lymph and pleural metastasis; The micrometastasis of Phase Ⅰ lung cancer were analyzed by Yasuhiko, thus finding that the positive expression rate of VEGF of the primary cancer with lymph node metastasis was 68.6% and that without lymph node metastasis was only 31.0% ($P < 0.05$) . It is indicated that the generation of the new vessels regulated by VEGF has a close relationship with lymph node metastasis [17]. It was found that the capillary permeability increased and the hydrothorax was formed after the strains with low expression level of VEGF, in which VEGF165 cDNA was transfected by Yano et al, were implanted into the chest [18].

NF-κ B plays a central role in forming the tumor vessels and it promotes the formation of the tumor vessels by up-regulating the expression of VEGF [19-20]. This study showed that there was a high correlation between NF-κ B and the expression of VEGF and MVD, which indicated that NF-κ B promotes the formation of the tumor vessels by up-regulating the expression of VEGF. The previous studies find that the up-regulation of the expression of NF-κ B-mediated cyclooxygenase-2 causes the expression of the hypoxia-inducible factor la to produce VEGF, which may be the mechanism of action. However, the further studies are still needed for the interaction of NF-κ B and VEGF in occurrence and development of lung cancer.

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非小细胞肺癌中 NF-κB、VEGF 的表达及其与肿瘤血管形成的研究*

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摘要 目的:探讨核因子-κB(NF-κB)和血管内皮生长因子(VEGF)在非小细胞肺癌中的表达及其与肿瘤血管形成的关系。方法:应用免疫组织化学方法检测 56 例非小细胞肺癌及 20 例癌旁肺组织中的 NF-κB P65、VEGF 的表达,并用抗 CD34 测定肿瘤血管的密度(MVD)。结果:(1)在非小细胞肺癌中 NF-κB P65、VEGF 的表达阳性率分别为 83.9%(47/56)、69.6%(39/56),明显高于癌旁组织(P<0.05);(2)NF-κB P65 的表达在不同的 TNM 分期、淋巴结及胸腔积液、吸烟的分组之间差异有统计学意义,VEGF 的表达在淋巴结及胸膜转移之间差异有统计学意义;(3) NF-κB P65、VEGF、MVD 三者间存在明显相关性。结论:NF-κB、VEGF 异常表达与 NSCLC 的发生、发展及肿瘤血管的形成密切的关系。

关键词:肺肿瘤;NF-κB P65;VEGF;免疫组织化学

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