EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR IN PRIMARY NON-SMALL CELL LUNG CARCINOMA

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ABSTRACT

Objective: Tumor growth depends on angiogenesis. The aim of this paper is to clarify the relationship between the expression of vascular endothelial growth factor (VEGF)/vascular permeability factor (VPF) and the angiogenesis, or growth, or invasion and prognostic value in Non-Small Cell Lung Cancer (NSCLC). Methods: Microvessel quantification and expression of VEGF was performed immunohistochemically, using multiclonal antibodies against endothelial protein factor VIII-related antigen (F-VIII antigen, or factor VIII) for evaluating the angiogenesis; against VEGF antigen for the expression of VEGF. Results: A total of 53 patients with NSCLC after the radical resection were evaluated. The patients with high and low expression of VEGF were 33 and 20, respectively. A significant higher microvessel density (MVD) was observed in the tumors with high expression of VEGF compared with the tumors with low expression of it (12.17±2.57/mm^2 vs 6.01±1.161/mm^2, rank sum test, P<0.01). There were 29 patients with lymphonodes metastasis in the high expression VEGF/VPF (29/33, 87.88%) group, and 9 patients in the low (9/20, 45%) group. There was good correlation between MVD and expression of VEGF (chi-square tests, P<0.001). The overall 5 years survival for 53 patients was 20.75±5.78%; that of the high expression of the VEGF group was 3.03±2.98%; that of the low group was 36.36±13.94%, by Log rank test, P=0.0001. The difference between them had a high significance. There was good correlation between the survival and the expression of VEGF. By the COX's proportional hazard model analysis, the expression of VEGF and MVD was considered to be an independent marker of the prognosis in non-small cell lung cancer. Conclusion: the expression of VEGF has a significant correlation with MVD, growth, invasion, and lymph node metastasis. The increasing of the node metastasis and the size of tumor accompanied the increasing of VEGF/VPF. The cancer patient with higher VEGF and MVD expression might have worse prognosis. By multivariate analysis, VEGF/VPF or MVD may be taken as the independent predictors of prognosis for NSCLC.

Key words: VEGF/VPF, angiogenesis, NSCLC, F-VIII antigen, Lymph node metastasis, Prognosis

Tumor growth is dependent upon angiogenesis if it develops from minimal size such as 1 mm^3 to a bigger size.[1] The mechanism by which tumors induce angiogenesis have received considerable attention in recent years. One of the tumor-secreted angiogenesis factors, vascular endothelial growth factor (VEGF), appears to play an important role in tumor angiogenesis.[2,3] VEGF expression has been detected in several human tumors, including glioblastoma,[4,5] ovarian,[6] breast,[7] colorectal,[8] kidney,[9] bladder[10] and gastric carcinomas.[11] Blocking with anti-VEGF antibodies has been shown to inhibit the growth of some xenografted tumors.[12]

We examined the expression of VEGF in human non-small cell lung carcinoma, and quantified tumor vasculature. We also investigated whether there was any association between the expression of VEGF and clinical prognosis in human non-small cell lung carcinomas.

MATERIALS AND METHODS

Patients

The study analyzed 53 patients who had undergone lung resections for non-small cell lung carcinoma between May 1987 and May 1992 at the Department of Surgery, School of Oncology, Beijing Medical University, Beijing. Classification of the tumors was performed according to the general rules for lung cancer.[13] No patients had not received either chemotherapy or radiation therapy before surgery. Of the patients, 20 (38%) were in T_1 or T_2 stages, 33 (62%) were in T_3 stage.

Immunohistochemical Staining

Expression of VEGF and F8 (factor VIII) was determined on formalin-fixed and paraffin-embedded tumor samples by an indirect immunoperoxidase

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method. Antibodies used were a rabbit polyclonal antibody at a 1:200 dilution for VEGF, a rabbit polyclonal antibody at a 1:50 dilution for F8. In negative controls, all reagents except the primary antibody were used. The avidin-biotin immunoperoxidas complex technique was used for staining. 3–5 μm thick sections, mounted on glass slides, were parafinized in xylene and processed in decreasing strength of ethanol. They were then incubated in 3% hydrogen peroxide for 5 minutes. After incubation in 10% normal bovine serum for 5 minutes, each slide was incubated overnight with the relevant antibody at the dilution described. Biotinylated goat antimouse immunoglobulin and antirabbit immunoglobulin were used as secondary antibodies. Peroxidase-conjugated avidin was used at a dilution of 1:500. Slides were counterstanined with hematoxylin. Evaluation of immunostaining and vessel counting VEGF/VPF expression was performed according to Tsao's standard.[14] Slides were examined under low power (×40) to identify the region with the highest VEGF staining and that of highest vessel density. Intensity of staining for VEGF was graded on a scale of 0 to 3+, with 0 representing no detectable staining and 3+ representing the strongest staining examined under a microscope (×200). Factor VIII was detected according to Weidner's standard.[15] For vessel counting, a (×200) field in each of the five most vascular areas was counted, and the average counts were recorded. Characterization of VEGF expression and vessel counting were performed by two investigators who had no knowledge of the patients other clinicopathologic features or clinical outcomes.

Statistical Analysis

Correlation between vessel counts and intensity of VEGF, and lymphonode metastasis were analyuzed using rank sum test and log rank tests. Lymphonode metastasis and size of tumors were compared with the expression of VEGF in the tumor cells and checked by fourfold table x^2 tests. Survival curves were plotted according to the Kaplan-Meier method and their statistical differences were analyzed by the generalized Wilcoxon test. The influence of various clinicopathologic factors, including vessel counts, on survival was assessed by the Cox proportional hazards model. For all statistical analyses, SPSS system for personal computers was used, with significance defined as a P value less than 0.05.

RESULTS

Correlation between Expression of VEGF and Microvessel Density (MVD)

Of our 53 patients, 33 had high expression of VEGF and 20 had low expression. MVD in the high expression of VEGF was 12±2.6/mm^2, ranged 6.2–19.1/mm^2, whereas in the low expression of VEGF it was 6.0±1.2/mm^2 (P<0.01, by rank sum tests.), which suggests a significant correlation between expression of VEGF and MVD. The MVD increase significantly the VEGF expression increase.

Correlation between Expression of VEGF and T Stage of Tumor, and Lymphonode Metastasis

Of the 53 patients, 38 had lymphonode metastasis, and 29 had high expression of VEGF among them (29/38, 76%); whereas 15 patients had no lymphonode metastasis, among them, only 4 had high expression of VEGF (4/15, 27%), P<0.001 (Table 1). Their difference was significant. Of the 20 patients in T_1 and T_2 stages, 7 had high expression of VEGF, (7/20, 35%); whereas, in the 33 patients with T_3 stage, 26 had high expression of VEGF (26/33, 79%). (X^2=10.162) P<0.01 (Table 1). The difference was also significant. The results suggest that expression of VEGF is closely related to lymphonode metastasis, and the size of tumor. When the T stage of tumor was hgher, and lymphonode metastasis increased there was an increase of VEGF in the tumor cells.

Table 1. Correlation between expression of VEGF and T stage of tumor and lymphonode metastasis (LM)

	LM (cases, %)		T stage (cases, %)		Total
	Positive	Negative	T_1+T_2	T_3	
VEGF High expression	29 (23.66)	4 (9.43)	7 (12.45)	26 (14.55)	33
VEGF Low expression	9 (14.34)	11 (5.66)	13 (7.55)	7 (12.45)	20
Total	38	15	20	33	52

X^2 Test P<0.01

Correlation between Expression of VEGF and Survival

Survival rates of the high and low VEGF expression patients were calculated using the Kaplan-Meier method. In the 33 patients with high expression of VEGF, their 5-year survival rate was 3±3%, whereas the 20 cases with the low expression,

36±13%, the difference was very significant, $P<0.0001$ (by Log Rank Test) (Table 2). Survival rates of patients decreased to the accompaniment of the expression rise of VEGF in the tumor cells.

Table 2. Correlation between expression of VEGF and survival

	Total	Died	Lived	5-year-survival (%)
VEGF high expression	33	32	1	3.03
VEGF low expression	20	10	10	50
Total	53	42	11	20.75

X^2 Test $P<0.001$

Correlation between MVD and Survival

According to difference of the MVD, the patients were divided into four groups, MVD<5, 7 patients; MVD 6~10, 20 patients; MVD 11~15, 17 patients; MVD>16, 9 patients. Their 5-year survival rates were 42.9%, 40.0%, 0.0%, 0.0% respectively. Their differences were significant, $P<0.001$ (by Log Rank Test) (Table 3). Survival rates significantly decreased as MVD increased.

Table 3. Correlation between MVD and survival

	Patientt total	Died	Lived	Survival (%)
MVD<5	7	4	3	42.86
MVD6-10	20	12	8	40.00
MVD11-15	17	17	0	0
MVD>16	9	9	0	0
Total	53	42	11	20.75

Log Rank Test $P<0.001$

The Cox Model

The variables, including sex, age, size of tumor (≤ 3 cm, >3 cm), metastasis in lymphonode (N_0, N_1, N_2),[13] MVD, and expression of VEGF, were analyzed by the Cox proportional hazards model (Table 4), the results suggests MVD and VEGF can be independent prognostic factors.

Table 4. Cox proportional hazards model

Risk factor	*P* value
VEGF	0.0286
MVD	0.0034

DISCUSSIOIN

VEGF is a multi-function cytokines.[16] In the present study, we demonstrated that the expression of VEGF appeared to correlate closely with tumor neovascularization in 53 non-small cell lung carcinomas. We also found a strong correlation between non-small cell lung cancer vascularization, and survival, and development of lymphonode metastasis and the size of the tumor.

In a variety of human tumors, such as gastrointestinal carcinoma, elevated expression of VEGF has been found.[4,8,11,17] In support of an important role for VEGF in the induction of vascular stroma in colon carcinomas, administration of a monoclonal antibody to VEGF has been shown to decrease the density of blood vessels and suppress the growth in human colon xenograft. Our studies demonstrated the significant correlation between intensity of VEGF staining and vessel counts. The finding suggested that VEGF appears to play a role in the angiogenesis and progression of non-small cell lung cancer.

Many investigators have demonstrated that tumor vascularization is a significant predictor of survival in a variety of tumors.[15,18,19] Our study explored the prognostic significance of tumor angiogenesis in the patient with non-small cell lung cancer who had undergone curative resection. The Cox model indicated that expression of VEGF and tumor MVD was the most significant and independent prognostic factor among various variables at the period of surgery.

Tumor angiogenesis is a complex multistep process controlled by various factors, including angiogenic stimulators and inhibitors. Various other peptides growth factors, including basic fibroblast growth factor,[20], hepatocyte growth factor,[21] and transforming growth factor VIII,[22] have received considerable attention as angiogenic stimulators. Ascertainment of these factors that are highly relevant to tumor angiogenesis, based on the data of tumor vascular density combined with clinical outcomes like in this kind of study, may have an influence on the future development of new strategies to suppress tumor growth and metastatic development by inhibiting tumor angiogenesis.

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