The Relationship between Serum VEGF Concentration and Prognosis of Lung Cancer

Seung Ha Park, M.D. and Seung Sei Lee, M.D.

Department of Internal Medicine, Kangbuk Samsung Hospital,
Sungkyunkwan University School of Medicine, Seoul, Korea

Background : VEGF is an important factor for angiogenesis. Although many previous studies have reported an increased serum VEGF concentration in various malignant tumors, there are few studies on the relationship between serum VEGF concentration and its prognosis. This study investigated whether serum VEGF concentration is a prognostic indicator for lung cancer.

Methods : Using the ELISA kit, we measured the serum VEGF concentrations of 86 patients diagnosed with lung cancer on histologic examination. With a cut-off value of 686 pg/mL, the patients were classified as low-concentration (<686 pg/mL, n=58) or high-concentration (≥686 pg/mL, n=28) based on their mean serum VEGF concentration values to compare survival rates, and serum VEGF concentrations for different histologic types and stages.

Results : There was no significant difference in serum VEGF concentration based on stage and histologic type between the two groups. Moreover, there was no significant difference in survival rate between the high-concentration and low-concentration groups (p=0.86).

Conclusion : This study demonstrates that serum VEGF concentration is not associated with the prognosis of lung cancer.

Key Words : VEGF, Angiogenesis, Lung cancer

INTRODUCTION

Angiogenesis plays an important role not only in non-neoplastic diseases such as diabetic retinopathy and arthritis[1, 2], but also in the growth and metastasis of malignant neoplasms[3]. It has been known that such substances as transforming growth factor β (TGF-β), tumor necrosis factor α (TNF-α), platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) are involved in angiogenesis and are secreted by tumor cells, vascular endothelial cells and inflammatory cells[4-6].

VEGF, in particular, increases the vascular permeability such that the plasma protein can easily leak out of the vessels[7, 8], and the permeated plasma protein induces proliferation of tumor cells and vascular endothelial cells by forming fibrin gel[9, 10]. Many studies have reported that VEGF expression is associated with the malignancy of tumors. There have been reports in Korea on the association of VEGF

expression with angiogenic status, malignancy and survival rate in breast cancer[11], stomach cancer[12] and lung cancer[13]. However, the results were not all consistent. Moreover, there was no effect on the patients who did not receive surgery, since most of the studies determined VEGF expression by using the immunochemical staining of the neoplastic tissue. A recent study has reported that serum VEGF (sVEGF) concentration is significantly correlated with intratumoral VEGF and intratumoral microvessel density, stating that sVEGF concentration could be used as a prognostic indicator for angiogenic status of tumors[14]. Accordingly, we have closely examined whether sVEGF concentration can be used as a biological prognostic factor for lung cancer by evaluating the correlation of sVEGF concentration with histologic type, stage, and survival rate in patients with lung cancer.

• Correspondence to : Seung Sei Lee, M.D., Department of Internal Medicine, kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, 108, Pyung-dong, Jongro-ku, Seoul, 110-746, Korea Tel : 82-2-2001-2084, Fax : 82-2-2001-204, E-mail : lss987@samsung.co.kr

MATERIALS AND METHODS

1. Subjects

Eighty-six patients admitted to Kangbuk Samsung hospital in Seoul, Korea between January 1, 2000 to December 31, 2001 and diagnosed with lung cancer on bronchoscopy, percutaneous fine-needle aspiration or sputum cytology were selected for the study. We retrospectively reviewed their medical records to examine their clinical characteristics and survival periods. The mean age was 60.7 years, with 67 males and 19 females participating. Our histopathologic findings showed 65 cases of non-small cell lung cancer 21 cases of small cell lung cancer. Among the non-small cell lung cancer patients, 26 were squamous cell carcinoma, 34 were adenocarcinoma, and 5 were other cases (2 large cell carcinoma, 2 carcinoid tumor, and 1 bronchioloalveolar carcinoma). The stage distribution for non-small lung cancer patients were as follows: 1 Stage I, 1 Stage IIA, 3 Stage IIB, 8 Stage IIIA, 25 Stage IIIB, and 27 Stage IV. On the other hand, two stages were observed for the small cell lung cancer patients: limited stage (6 cases) and extensive stage (15 cases).

2. Methods

The patients blood was kept at room temperature for over 30 minutes and was centrifuged at 2000 g for 10 minutes, and then kept at -70℃. The sVEGF concentration was measured by using the ELISA kit (R&D Systems, USA). After diluting the serum to 1:20 and adding 100 μL of diluted conjugate in each well, we added 100 μL of standard, control sample and kept it at room temperature for 1.5 hours before absorbing and rinsing 6 times. We then added an extra 100 μL of substrate, followed by 100 μL of stop solution after 30 minutes at room temperature. By measuring the color development at 450 nm with a microtitre plate reader (Labsystems, Finland) within 30 minutes, we calculated the pg/mL sVEGF concentration. Patients were classified into two groups based on whether their mean sVEGF concentration value was above or below 686 pg/mL: the low-concentration group (<686 pg/mL, n=58) and high-concentration group (≥686 pg/mL, n=28).

3. Statistical Analysis

Statistical analysis was performed using the SPSS program. We used one-way ANOVA tests to compare the values of each group, and the Bonferroni test was employed for post hoc comparisons. Patients' survival rates were tested using the Kaplan & Meier method:. they were determined to be significant at the value of $p<0.05$.

RESULTS

Analysis of the two groups showed that the mean sVEGF concentrations were 341 pg/mL in the low-concentration group and 1,400 pg/mL in the high-concentration group ($p <$ 0.05) (Table 1). There were no significant differences between the two groups in terms of age, sex, histologic type and stages. The high-concentration group did have longer smoking records and higher levels of C-reactive protein with no significant (p=0.43, p=0.44).

The sVEGF concentrations showed no significant difference by stage, but they tended to increase for non-small cell lung cancer patients as the stages progressed (Figure 1). For small cell lung cancer patients, the sVEGF concentration was high in the limited stage compared to the extensive stage, which was not significant (671 pg/mL vs. 579 pg/mL, p=0.72).

Table 1. Comparisons according to the concentration of VEGF in serum

	Low sVEGF (n=58) n (%)	High sVEGF (n=28) n (%)	p-value
Age (year) years	61.9±9.6*	59.3±12.1	NS
Sex			
Male	40 (68.9)	24 (85.7)	NS
Female	18 (31.1)	4 (14.3)	
CRP (mg/dL)	2.76±10.6*	4.48±6.6	NS
Smoking (pack-year)	28.7±25.1*	33.5±31	NS
Histology			
NSCLC			
adeno	21 (36.2)	13 (46.4)	NS
sqcc	19 (32.8)	7 (25)	
others	4 (6.9)	1 (3.6)	
SCLC	14 (24.1)	7 (25)	NS
Stage			
NSCLC			
I	1 (1.7)	0 (0)	NS
IIA	1 (1.7)	0 (0)	
IIB	2 (3.4)	1 (3.6)	
IIIA	5 (8.6)	3 (10.7)	
IIIB	17 (29.2)	8 (28.6)	
IV	18 (31.3)	9 (32.1)	
SCLC			
Limited	4 (6.9)	2 (7.1)	NS
Extensive	10 (17.2)	5 (17.9)	

*, Values are mean±SD.
NS, not significant, Low sVEGF, sVEGF<686 pg/mL; High sVEGF, sVEGF≥686 pg/mL; CRP, C-reactive protein; NSCLC, non small cell lung cancer; adeno, adenocarcinoma; sqcc, squamous cell carcinoma; SCLC, small cell lung cancer; others, contain large cell carcinoma, carcinoid tumor and bronchioloalveolar carcinoma.
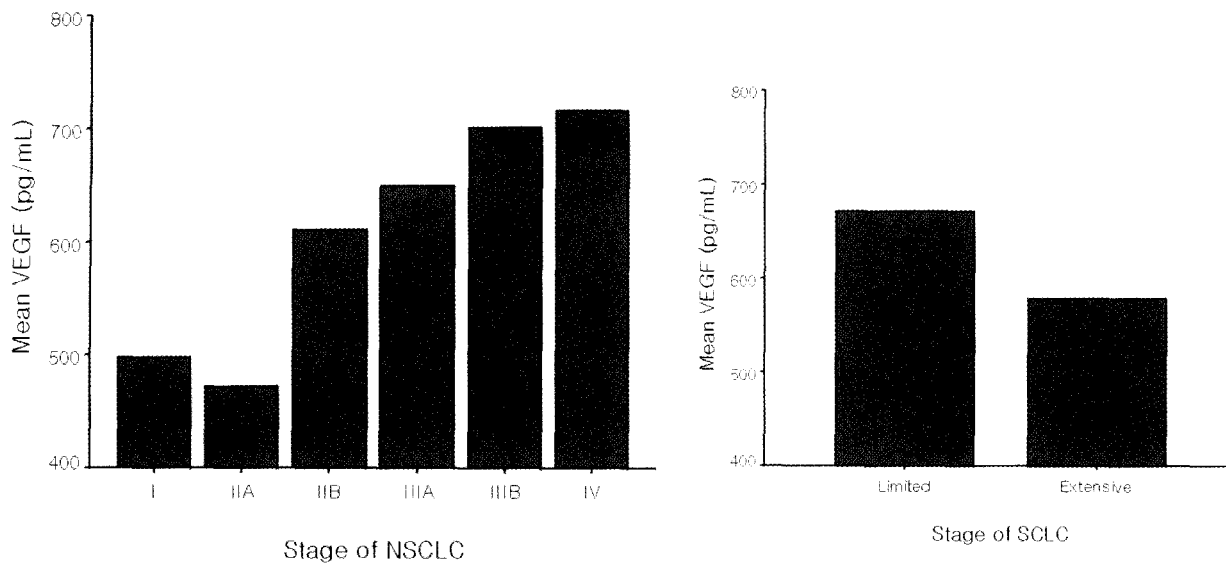
Figure 1. Mean concentrations of VEGF according to stage of non-small cell lung cancer (NSCLC, left) and small cell lung cancers (SCLC, right). All p-values between separate groups were not significant.
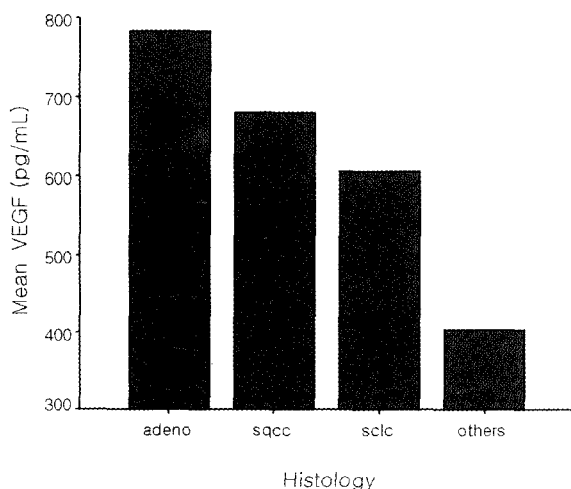
Figure 2. Mean concentration of VEGF according to histology. All p-values between separate groups were not significant. adeno, adenocarcinoma; sqcc, squamous cell carcinoma; sclc, small cell lung cancer; others, contain large cell carcinoma, carcinoid tumor and bronchioloalveolar carcinoma.

While mean sVEGF concentration was 782 pg/mL in adenocarcinoma, 680 pg/mL in squamous cell carcinoma, and 605 pg/mL in small cell lung cancer, with adenocarcinoma being the highest, there was no significant difference within each histologic type (Figure 2). Overall, the median survival period for the 86 patients was 13.8 months, with 55% surviving for more than one year. The median period of survival for the low-concentration group was 13.9 months and 13.3 months

for the high-concentration group, showing nosignificant difference between the two groups (p=0.86) (Figure 3).

Duration (Months)
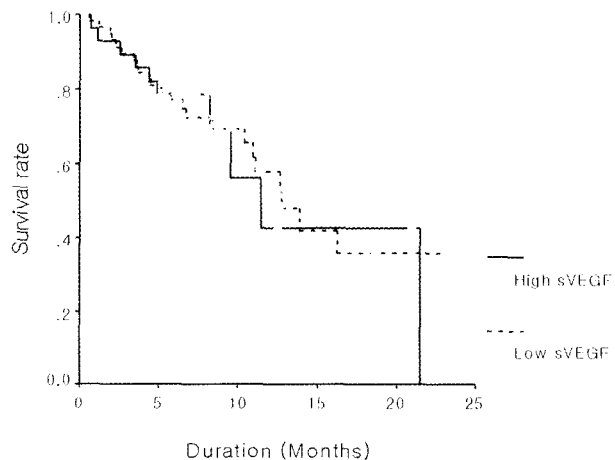
Figure 3. Overall survival curve according to concentration of VEGF in serum (p=0.86). Low sVEGF, sVEGF<686 pg/mL; High sVEGF, sVEGF≥686 pg/mL.

DISCUSSION

Many studies have reported that VEGF expression is evidently involved in the angiogenesis of tumor cells. VEGF expression has also been associated with malignancy as well

as the survival rate of some malignant tumors[15, 16]. In addition, some animal experimental models have shown that angiogenesis and tumor cell growth increased with VEGF administration[17, 18] while injecting anti-VEGF antibodies[19]. Inhibited tumor growth. In a study on patients with lung cancer, Tamura et al.[14] reported that sVEGF concentration was higher in patients with lung cancer than in the normal controls and was correlated with intratumoral VEGF, stating that sVEGF concentration is an indicator for angiogenic status of tumor cells. In another study, sVEGF concentration decreased significantly after chemotherapy in a response group while no-response group with lung cancer exhibited no change[20]. However, in this study, sVEGF concentration displayed a tendency to increase with stage progression, while it registered no significant difference in each of the stages. At the same time in the survival rates between the low-concentration and high-concentration groups were relatively similar. The mean sVEGF concentration for of the patients was 686 pg/mL higher than other previous studies, as 81% of the patients were above stage IIIB.

The association of VEGF expression with angiogenic status and prognosis of lung cancer has been controversial. Some studies reported that VEGF correlated with angiogenesis but didnot associated with neoplastic proliferation in squamous cell carcinoma[21], whereas increased VEGF expression was associated with high angiogenic status and poor prognosis in adenocarcinoma[22]. This study revealed that sVEGF concentration was high in adenocarcinoma in comparison with other histologic types, if not significant, which was similar to the study by Imoto et al.[23] that reported VEGF expression to be higher in adenocarcinoma than in squamous cell cacinoma. In a study on patients with small cell lung cancer, Salven et al.[24] reported that prognosis was poor for patients with high sVEGF concentration or at the extensive stage prior to anti-cancer chemotherapy positing that sVEGF as well as stage was attributable to prognostic indication. However, our study showed that sVEGF concentration was relatively high for the limited stage, indicating that there was no correlation between sVEGF concentration and stage in small cell lung cancer patients.

These discrepancies are assumed to be due to the following reasons. First, angiogenesis is caused by interaction between various activators and inhibitors for angiogenesis, other than VEGF. Second, VEGF acts as an autocrine or paracrine growth factor, rather than an endocrine growth factor in malignant tumors therefore, sVEGF concentration is not an indicator for tumor growth and angiogenesis. Third, sVEGF concentration may be affected by leukocytes and C-reactive protein in such diseases as infection and chronic inflammation. Fourth, since 81% of the patients were over

stage IIIB in this study, the survival rate rather than sVEGF concentration was greatly affected by the patient's nutritional status, presence of accompanied diseases and remote metastasis.

Based on previous reports that VEGF expression correlates with the prognosis of lung cancer, we examined the correlation of sVEGF with stage, histologic type, prognosis and survival rate. However, we found that there was no correlation between them. Hence, we conclude that sVEGF concentration is not associated with the prognosis of progressive lung cancers, but further studies are needed for complete verification.

REFERENCES

1) Folkman J. How is blood vessel growth regulated in normal and neoplastic tissue?-G.H.A. Clowes memorial award lecture. Cancer Res 46:467-473, 1986

2) Hobson B, Denekamp J. Endothelial proliferation in tumors and normal tissues: Continuous labelling studies. Br J Cancer 49:405-413, 1984

3) Gimbrone MA, Cotran RS, Leapman SB, Folkman J. Tumor growth and neovascularization: An experimental model using the rabbit cornea. J Natl Cancer 52:413-427, 1974

4) Folkman J, Klagbrun M. Angiogenic factors. Science 235:442-447, 1987

5) Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N. Vascular endothelial growth factor is secreted angiogenic mitogen related to PDGF. Science 246:1306-1309, 1989

6) Anan K, Morisaki T, Katano M, Ikubo A, Kitsuki H, Uchiyama A, Kuroki S, Tanaka M, Torisu M. Vascular endothelial growth factor and platelet-derived growth factor are potential angiogenic and metastatic factors in human breast cancer. Surgery 119:333-339, 1996

7) Esser S, Wolburg K, Wolburg H, Breier G, Kurzchalia T, Risau W. Vascular endothelial growth factor induces endothelial fenestrations in vitro. J Cell Biol 140:947-959, 1998

8) Roberts WG, Palade GE. Neovasculature induced by vascular endothelial growth factor is fenestrated. Cancer Res 57:765-772, 1997

9) Dvorak AM, Kohn S, Morgan ES, Fox P, Nagy JA, Dvorak HF. The vesiculo-vacuolar organelle (VVO): a distinct endothelial cell structure that provides a transcellar pathway for macromolecular extravasation. J Leukocyte Biol 59:100-115, 1996

10) Dvorak HF, Nagy JA, Berse B, Brown LF, Yeo KT, Yeo TK, Dvorak AM, Vandewater L, Sioussat TM, Senger DR. Vascular permeability factor, fibrin and the pathogenesis of tumor stroma formation. Ann N.Y. Acade. Sci 667:110-111, 1992

11) Jang JE, Choi SY, Park CH, Ko YH, Kim KH, Jeong HS, Ahn KS, Yang JH, Nam KS, Yang JH, Nam SJ, Yoon SS. Correlation between VEGF expression and prognostic factors in breast cancer. J Korean Cancer Assoc 31:483-491, 1999

12) Im SA, Lee SN, Kim SS. Tumor angiogenesis as a predictor of

prognosis in gastric carcinoma. *J Korean Cancer Assoc 29:640-647, 1997*

13) Kim YS, Shin DH, Kim YB, Chung KY. *Correlation of neovascularization, lymph node metastasis and p53 expression in squamous cell carcinoma of lung. J Korean Cancer Assoc 28:622-631, 1996*

14) Tamura M, Ohta Y, Kajita T, Kimura K, Go T, Oda M, Nakamura H, Watanabe G. *Plasma VEGF concentration can predict the tumor angiogenic capacity in non-small cell lung cancer. Oncology Report 8:1097-1102, 2001*

15) Senger DR, Galli SJ, Dvorak AM, Peruzzi CA, Harvey VS, Dvorak HF. *Tumor cell secretes a vascular permeability factors that promotes accumulation of ascites fluid. Science 219:983-985, 1983*

16) Senger DR, Van De Water L, Brown LF. *Vascular permeability factor(VPF, VEGF) in tumor biology. Cancer Metastasis Rev 12:303-324, 1993*

17) Ferrara N, Winer J, Burton T, Rowland A, Siegel M, Phillips HS, Terrel T, Keller GA, Levinson AD. *Expression of vascular endothelial growth factor does not promote transformation but confers a growth advantage in vivo to Chinese-hamster ovary cells. J Clin Invest 91:160-170, 1993*

18) Zhang HT, Craft P, Scott PA, Ziche M, Weich HA, Harris AL, Bicknell R. *Enhancement of tumor growth and vascular density by transfection of vascular endothelial cell growth factor into MCF-7*

human breast-carcinoma cell. *J Natl Cancer Inst 87:213-219, 1995*

19) Kim KJ, Li B, Winer J, Amanini M, Gillett N, Phillips HS, Ferrara N. *Inhibition of vascular-endothelial-growth-factor-induced angiogenesis suppresses tumor growth in vivo. Nature(Lond) 362:841-844, 1993*

20) Kido Y. *Vascular endothelial growth factor(VEGF) serum concentration changes during chemotherapy in patients with lung cancer. Kurume Medical Journal 48:43-47, 2001*

21) Mattern J, Koomagi R, Volm M. *Association of vascular endothelial growth factor expression with intramural microvessel density and tumor cell proliferation in human epidermoid lung carcinoma. Br J Cancer 73:931-934, 1996*

22) Kawaguchi T, Yamamoto S, Kucoh S, Goto K, Wakasa K, Sakurai M. *Tumor angiogenesis as a major prognostic factor in stage I lung adenocarcinoma. Anti-Cancer Res 17:3743-3746, 1997*

23) Imoto H, Osaki T, Taga S, Ohgami A, Ichiyoshi Y, Yasumoto K. *Vascular endothelial growth factor expression in non-small-cell lung cancer: Prognostic significance in squamous cell carcinoma. J Thorac Cardiovasc Surg 15:1007-1014, 1998*

24) Salven P, Ruotsalainen T, Mattson K, Joensuu H. *High pretreatment serum level of vascular endothelial growth factor(VEGF) is associated with poor outcome in small-cell lung cancer. Intern J Cancer 79:144-146, 1998*