Available online at www.sciencedirect.com

ScienceDirect

EJSO

the Journal of Cancer Surgery

EJSO 40 (2014) 1136–1142

www.ejso.com

Imbalance between vascular endothelial growth factor and endostatin correlates with the prognosis of operable non-small cell lung cancer

Y. Hu [a], M.-m. Hu [a], G.-l. Shi [b], Y. Han [c], B.-l. Li [a,*]

[a] *Department of General Medicine, Beijing Chest Hospital, Capital Medical University & Beijing Tuberculosis and Thoracic Tumor Research Institute, 101149, China*

[b] *Department of Clinical Laboratory, Beijing Chest Hospital, Capital Medical University & Beijing Tuberculosis and Thoracic Tumor Research Institute, China*

[c] *Department of Thoracic Surgery, Beijing Chest Hospital, Capital Medical University & Beijing Tuberculosis and Thoracic Tumor Research Institute, China*

Accepted 22 May 2014

Available online 26 June 2014

Abstract

Background: Angiogenesis is regulated by a balance of pro-angiogenic and anti-angiogenic factors. Vascular endothelial growth factor (VEGF) and endostatin respectively represents a frequent component of inducers and inhibitors in the process of angiogenesis. The ratio of VEGF/endostatin may reflect the balance of angiogenic switch. This study aimed to determine whether an imbalance between VEGF/endostatin exists in operable non-small cell lung cancer (NSCLC) patients and to assess the correlation, if any, between the imbalance and the prognosis.

Methods: Preoperative serum levels of VEGF and endostatin were simultaneously determined by quantitiative enzyme-linked immunosorbent assay (ELISA) and the ratio of them was calculated among 98 NSCLC patients and 51 healthy controls. The relationship between these factors and clinicopathological features, including prognosis, was examined.

Results: The ratio of VEGF/endostatin levels was significantly higher in operable NSCLC patients [median, 10.4; interquartile range (IQR), 5.9–19.8] than in normal controls [median, 5.1; IQR, 3.3–9.7] ($P = 0.002$). While the ratio in patients who were still alive for more than 60 months was 8.3 (IQR, 4.3–17.9), the ratio in those who died was 12.9 (IQR, 8.0–22.1) ($p = 0.017$). In subgroup analysis of patients with pathological stage N0, there was a statistically significant increase of the survival time in the group with a lower ratio than in the group with a higher ratio ($p = 0.032$). Multivariate analysis confirmed that the VEGF/endostatin ratio was an independent prognostic factor ($p = 0.018$).

Conclusion: There was an imbalance between VEGF and endostatin in serum of operable NSCLC patients. The imbalance correlated with the prognosis of operable NSCLC.

Keywords: Imbalance; Non-small cell lung cancer; Prognosis; Vascular endothelial growth factor; Endostatin

Introduction

Lung cancer is the most common cause of death from cancer worldwide, estimated to be responsible for 19.4% (1.59 million) of all cancer deaths in 2012.[1] Despite diagnostic and therapeutic advances, the survival rate of lung cancer patients is still low (10% after 5 years), and has not changed over the period analyzed in the EUROCARE-4 study.[2] Non-small cell lung cancer (NSCLC) is the most common histological type of lung cancer, accounting for approximately 80% of lung cancers.[3]

In the 1970s, Folkman postulated that tumor growth and metastasis are dependent on angiogenesis.[4] And now the importance of angiogenesis for the development of solid tumors is well recognized. It is acknowledged that angiogenesis is regulated by both promoters and inhibitors. When the stimulators accumulate in excess of inhibitors within

* Corresponding author. Tel.: +86 10 89509309.
E-mail address: libaolan1109@163.com (B.-l. Li).

http://dx.doi.org/10.1016/j.ejso.2014.05.014

an angiogenic tumor, the balance between the inducers and inhibitors is interrupted[5] and the "angiogenic switch" would be activated which progressively enhance the intensity of angiogenesis.[6] A variety pro-angiogenic factors have been described including vascular endothelial growth factor (VEGF).[7] An increasing number of anti-angiogenic factors such as endostatin and angiostatin also have been identified.[8]

VEGF and endostatin are two factors which have been reported in more detail about their roles in the angiogenesis. VEGF, which is also known as vascular permeability factor (VPF), is the most powerful angiogenic factor known to date with direct effects on endothelial cell proliferation, migration and tubule formation under physiologic and pathologic conditions.[9] It acts through binding to VEGF receptor (VEGFR) generating a cascade of intracellular signaling, leading to activation of transcription factors in the nucleus that ultimately lead to new vessel formation.[10]

Endostatin, a strong endogenous inhibitor of angiogenesis which was discovered as a 20 kDa internal fragment of the carboxy terminus of collagen XVIII, was reported to be identified in a matrix protein in 1994,[11] and was proved to be an endogenous angiogenesis inhibitor and a tumor suppressor in 2005.[12] Endostatin which is the most rigorously studied one of the endogenous angiogenesis inhibitors inhibits endothelial cell proliferation, migration/invasion, tubule formation and increases apoptosis of malignant cells.[13]

Our overall hypothesis was that there is an imbalance of angiogenic factors in NSCLC patients since the balance of the inhibitors and inducers controls the angiogenic switch. Although there are many promoters and inhibitors involved in NSCLC angiogenesis, VEGF and endostatin might preliminarily represent the two categories respectively. This is a retrospective cohort study designed to determine whether an imbalance between VEGF and endostatin exists in operable NSCLC patients and evaluate the possible prognostic correlation of such imbalance.

Patients and methods

Subjects

Ninety-eight cases with NSCLC were enrolled in this study. Surgeries for radical resection of the tumors were performed consecutively for each of the patients in the Department of Thoracic Surgery, Beijing Chest Hospital, Capital Medical University, between February 2007 and July 2008. Inclusion criteria consisted of histologically or cytologically proven NSCLC and all patients were previously untreated with chemotherapy or radiotherapy. The pathological surgical staging Ia–IIIa was done according to the 7th edition TNM staging classification in lung cancer. Simultaneously, 51 healthy volunteers were enrolled as the control group, brief history and physical examinations including the routine blood tests, electrocardiogram (ECG), chest X-ray, abdominal and pelvic ultrasound, etc. confirmed that they were healthy. This study was approved by the ethic committee of the Beijing Chest Hospital. Informed consent was obtained from each patient and healthy control.

Serum collection and measurements

Five milliliters of peripheral venous blood was obtained before surgery, then centrifuged at 1000 *g* for 15 min in a refrigerated centrifuge to get serum aliquots and stored at −80 °C until further assay. The blood samples from control subjects were processed similarly. Blood samples were taken from the female subjects during non-menstrual period. The concentration of VEGF and endostatin in serum preoperatively were determined by a quantitative sandwich enzyme-linked immunosorbent assay (ELISA). The levels of serum VEGF and endostatin were determined according to the manufacturer's instructions of VEGF ELISA kit (Jingmei Biotech Co., Ltd, Beijing, China) and endostatin ELISA kit (R&D Systems, Minneapolis, MN, USA). All determinations were made in duplicate.

Statistical analysis

Statistical analyses were performed using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). All data were expressed as medians, with the 25th percentile and 75th percentile, due to skewed distribution examined by Kolmogorov–Smirnov test. Nonparametric statistical analyses were performed using Mann–Whitney *U* test determining the differences between two independent groups and Kruskal–Wallis test for determining the differences among more than two groups.

Survival time was defined from the date of surgery to death or the date of the last follow-up. None of the patients died within 60 days after operation. Among the 98 patients, 1 patient could not be contacted soon after surgery and 2 patients were lost to follow-up in the third year after the operation. The median follow-up time was 55.7 months (range, 3.2–80.1 months).

The impact of the ratio VEGF/endostatin on survival in the subgroup analysis was assessed with the Kaplan–Meier method and compared by the log rank test. To assess the independent value of different variables on survival, multivariate analysis was performed using the Cox proportional hazards model. Variables with p value <0.05 in univariate analysis were entered into the Cox regression analysis. In the study, $P < 0.05$ was considered statistically significant.

Results

Clinicopathological characteristics

The median age of the patients was 62 years (range, 36–84 years), 82.7% were males. The majority (53.1%)

of tumors were squamous cell carcinoma. Among these 98 patients, pathological tumor stage included 43 (43.9%) cases at stage I, 22 (22.4%) at stage II, and 33 (33.7%) at stage III. The most frequently seen histological grade was moderately differentiated (83.7%) type of the tumor. Of the total cases, 74 (75.5%) underwent lobectomy, while 20 (20.4%) and 4 (4.1%) received pneumonectomy and sublobectomy (wedge resection or segmentectomy), respectively. In the control group, the median age was 60.5 years (range, 55–64 years), 72.5% of them were male. There were no significant differences in age and gender between the two groups (Table 1).

Circulating VEGF level, endostatin concentration and the ratio of VEGF/endostatin

The concentration of serum VEGF was significantly higher in operable NSCLC patients [median, 802.5 pg/mL (IQR, 501.4–1136.4 pg/mL)] than in healthy controls [median, 367.3 pg/mL (IQR, 209.9–557.7 pg/mL)] ($P < 0.0001$) (Fig. 1A). Similarly, serum level of endostatin in lung cancer patients [median, 69.0 ng/mL (IQR, 53.3–85.8 ng/mL)] was significantly higher than that of the control volunteers [median 52.1 ng/mL (IQR, 45.5–83.1 ng/mL)] ($P = 0.016$) (Fig. 1B). No significant correlation was found between VEGF and endostatin levels in both NSCLC patients ($r = 0.146$, $p = 0.151$) and control cases ($r = 0.125$, $p = 0.383$). The ratio of VEGF/endostatin levels was significantly higher in operable NSCLC patients [median, 10.4 (IQR, 5.9–19.8)] than in healthy controls [median, 5.1 (IQR, 3.39–9.75)] ($P = 0.002$) (Fig. 1C).

Table 1
Clinical characteristic of the NSCLC patients.

Characteristics	*N* (%)		*P*
	Patients	Controls	
Total	98	51	
Age (years)			
Median (min, max)	62 (36–84)	60.5 (55–64)	0.272
Gender			
Male	81 (82.7)	37 (72.5)	0.149
Female	17 (17.3)	14 (27.5)	
Smoking status			
Current or ex-smoker	72 (73.5)		
Non-smoker	26 (26.5)		
Postsurgical stage			
I	43 (43.9)		
II	22 (22.4)		
IIIA	33 (33.7)		
Node invasion			
N0	67 (68.4)		
N1–2	31 (31.6)		
Histology type			
Adenocarcinoma	37 (37.8)		
Squamous	52 (53.1)		
Others	9 (9.2)		
Differentiation			
Well/moderate	83 (84.7)		
Poor	15 (15.3)		
Type of resection			
Lobectomy/sublobectomy	78 (79.6)		
Pneumonectomy	20 (20.4)		

NSCLC, non-small lung cancer.

Clinicopathological characteristics and VEGF/endostatin level

The correlation between clinicopathological characteristics and serum level of VEGF, endostatin and the VEGF/endostatin ratio was summarized (Table 2). Circulating VEGF did not demonstrate any significant relationship with patients' clinicopathological features. Serum endostatin was only significantly higher in patients with poorly differentiated cancer than in those with well or moderately differentiated cancer ($p = 0.014$).

Survival analysis

The survival analysis of the 95 patients who could be followed up to the end of the study revealed that 46 of them (48.4%) were still alive with the median follow-up time: 74.1 months (range, 62.1–80.1 months). The median survival time among the patients who died was 16.7 months (range, 3.2–57.2 months). The survival time of the survivors was significantly longer than that of the non-survivors ($p < 0.0001$).

The serum level of VEGF in the patients still alive was significantly lower than that in those who died [median, 687.0 pg/mL (IQR, 381.5–932.05 pg/mL) vs. 879.1 pg/mL (IQR, 529.7–1254.6 pg/mL), respectively] ($p = 0.049$) (Fig. 2A). The median level of the VEGF/endostatin ratio of the two groups were 8.3 (IQR, 4.3–17.9) vs. 12.9 (IQR, 8.0–22.1) respectively, the difference was also significant ($p = 0.017$) (Fig. 2B). The median serum levels of endostatin were 68.5 ng/mL (IQR, 53.4–97.5 ng/mL) and 69.8 ng/mL (IQR, 52.1–80.5 ng/mL) respectively, no significant difference was found between them ($p = 0.396$) (Fig. 2C).

The analysis of the impact of VEGF/endostatin ratio on the survival in the subgroup of patients with pathological stage N0 showed that, there was a significant increase of the survival time in the group with lower ratio than in the group with higher ratio ($p = 0.032$) (median, 63.53, 55.67 months, respectively) (Fig. 3).

The prognostic variables were determined by Cox proportional hazard regression analysis. In the univariate analysis, postsurgical TNM stage ($p < 0.0001$), ratio of VEGF/endostatin ($p = 0.018$) and the type of resection ($p = 0.002$) were significantly associated with survival time. The variables with $P < 0.05$ were entered into the multivariate analysis and the result showed that postsurgical stage ($P < 0.0001$), ratio of VEGF/endostatin ($P = 0.045$) and the type of resection ($P = 0.001$) were the independent variables correlated with the prognosis of operable NSCLC (Table 3).

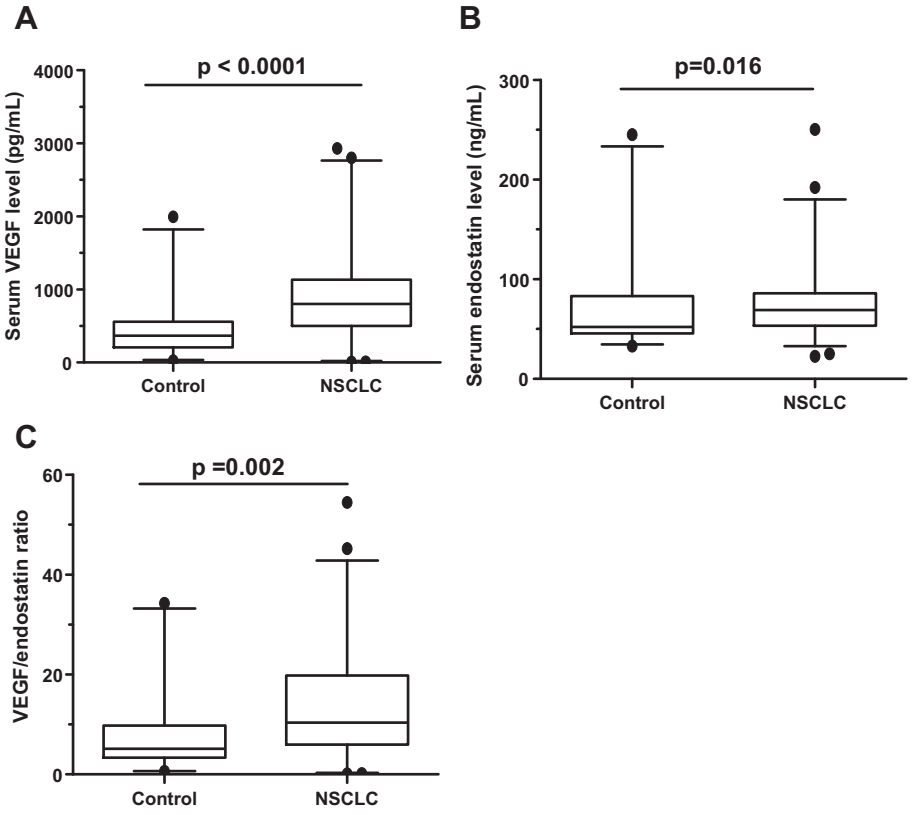

Figure 1. Circulating level of VEGF, endostatin and VEGF/endostatin ratio between the normal control and the patients with NSCLC. A, B, and C figures respectively illustrated the level of serum VEGF and endostatin and VEGF/endostatin of patients with NSCLC. The line inside each box represents the median. The bottom and top limits of each box represents the 5th and 95th percentile, respectively. The black dots represented outliers.

Discussion

The aim of this study was to analyze whether there was an imbalance of the angiogenic inducer and inhibitor in patients with operable NSCLC, and to study the correlation between the imbalance and the prognosis.

We hypothesized that there might be an imbalance between VEGF and endostatin, which represent the important

Table 2
Association between clinical characteristics and the level of VEGF, endostatin and VEGF/E dostatin ratio.

Characteristics	Serum VEGF (pg/mL)	*p*	Serum endostatin (ng/mL)	*p*	VEGF/endostatin	*p*
	Median (IQR)		Median (IQR)		Median (IQR)	
Overall level	802.50 (501.43–1136.45)		69.00 (53.35–85.80)		10.40 (5.96–19.82)	
Histology						
Adenocarcinoma	760.50 (500.95–1081.75)	0.659	69.50 (56.20–84.25)	0.193	9.56 (6.69–16.53)	0.420
Squamous	836.10 (525.2–1235.08)		71.10 (53.25–96.28)		11.18 (5.55–21.19)	
others	907.60 (451.90–1495.45)		54.30 (47.95–69.30)		20.11 (6.30–33.69)	
Differentiation						
Poor	861.30 (501.90–1307.30)	0.937	83.50 (69.50–98.30)	0.014	8.95 (5.54–14.47)	0.703
Well/moderate	789.90 (500.00–1123.90)		66.90 (22.60 51.40–81.20)		11.67 (6.09–20.11)	
Lymph node status						
N0	861.30 (563.00–1128.40)	0.176	72.10 (53.40–97.20)	0.170	8.95 (5.59–20.04)	0.622
N1–2	621.80 (425.00–1160.60)		65.40 (52.70–73.20)		11.86 (7.07–18.81)	
Postsurgical stage						
I	857.10 (512.60–1078.00)	0.703	68.40 (51.40–97.20)	0.956	9.93 (5.54–18.79)	0.413
II	712.20 (504.20–1490.80)		76.45 (53.20–93.50)		12.31 (6.09–22.99)	
IIIa	760.50 (436.68–1234.00)		69.50 (54.45–77.63)		10.30 (7.08–18.15)	

VEGF, vascular endothelial growth factor.
IQR, interquartile range.

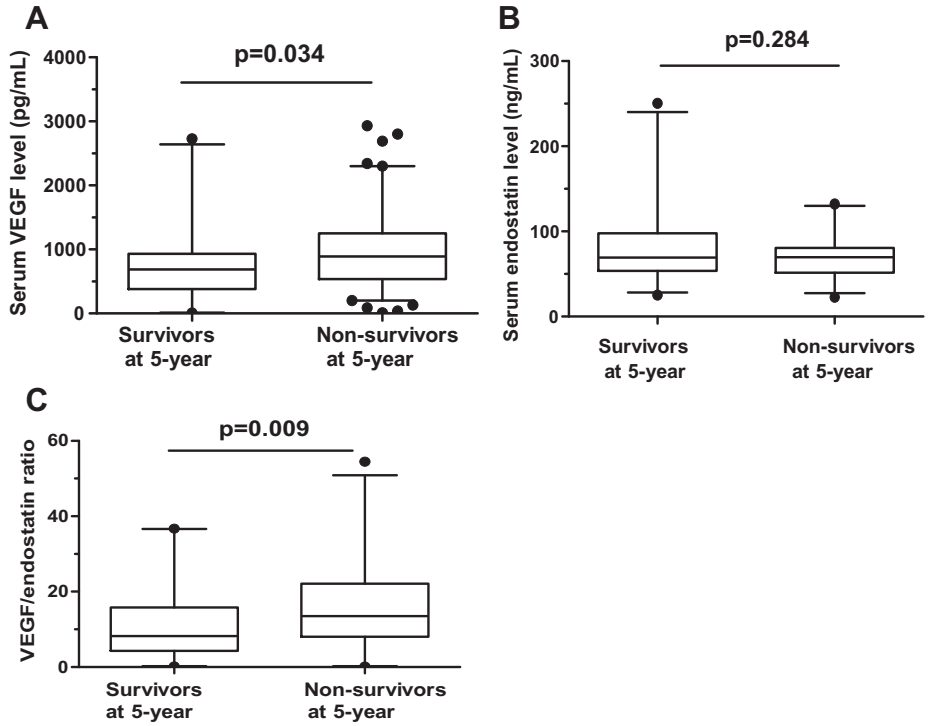

Figure 2. Circulating level of VEGF, endostatin and VEGF/endostatin ratio between survivors and non-survivors at 5-year with NSCLC. A, B, and C figures respectively illustrated the circulating level of serum VEGF and endostatin and VEGF/endostatin of patients with the two groups. The line inside each box represents the median. The bottom and top limits of each box represents the 5th and 95th percentile, respectively. The black dots represented outliers.

component of the inducers and inhibitors involved in the angiogenesis. In this study, preoperative serum levels of VEGF and endostatin were examined and compared to 51 healthy controls by ELISA. The serum levels of VEGF and endostatin in operable NSCLC patients were significantly higher than those in control individuals. The result was consistent with the reports of other studies.[14–17] It is very interesting that both angiogenic activators and inhibitors are increased. We determined the ratio of VEGF/endostatin and compared it with the ratio of the healthy control. The ratio was significantly increased in patients with operable NSCLC. A net balance of angiogenic inhibitors over activators would maintain the angiogenic switch in the off position, whereas a shift to an excess of activating stimuli would turn on angiogensis.[18] So we presumed that in the process of the angiogenesis, while the positive angiogenic factors including VEGF were induced to produce more, the negative angiogenic factors including endostatin also increased as a feedback to regulate the angiogenesis balance. But the increase of endostatin may be not proportionate to the increased level of VEGF, the balance would be changed. Then changes in the balance mediate the angiogenic switch which would benefit the progress of the cancer.[19]

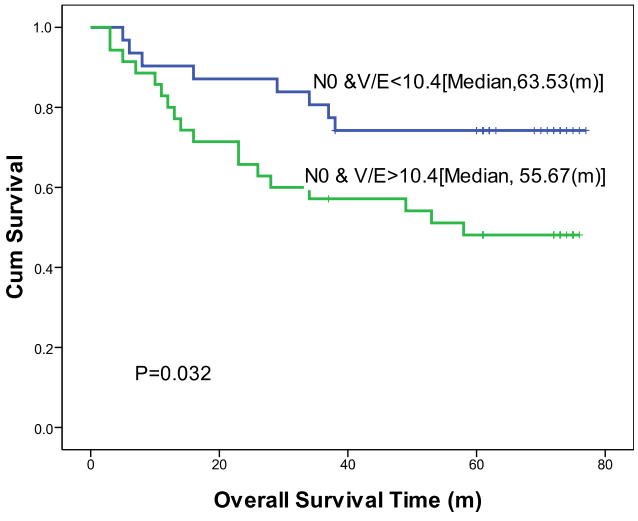

Figure 3. Association between patients survival and VEGF/endostatin ratio. Overall survival stratified by the V/E ratio level in patients with N0 (V/E, the ratio of VEGF/endostatin).

Although it was reported that VEGF was related to endostatin,[20] this study did not find significant correlation between them and it is in accordance with the result that VEGF and endostatin were not linearly correlated reported by Suzuki et al.[16] Regardless the fact that there is no linear relationship between the two factors, the ratio of them might be a valuable variable. The significantly increased VEGF/endostatin ratio in this study might reflect an imbalance between angiogenic inducer and inhibitor.

Results of studies on prognostic value of VEGF and endostatin were not consistent.[21–24] This study did not find the value of endostatin in the prognostic significance, although a systematic review with meta-analysis concluded

Table 3
Analysis with overall survival.[a]

Variables	Univariate analysis			Multivariate analysis		
	HR	95%CI	*p*	HR	95%CI	*p*
Gender						
Male	0.963	0.467–1.985	0.918	Not included in multivariable model		
Female						
Smoking status						
Current or ex-smoke	0.660	0.337–1.291	0.225	Not included in multivariable model		
Non-smoker						
Postsurgical stage						
I	2.312	1.643–3.255	<0.0001	2.245	1.587–3.174	<0.0001
II						
IIIA						
Histology type						
Squamous	1.532	0.931–2.520	0.093	Not included in multivariable model		
Adenocarcinoma						
Others						
Differentiation						
Poor	0.656	0.318–1.352	0.253	Not included in multivariable model		
Well/moderate						
Type of resection						
Lobectomy/sublobectomy	2.622	1.405–4.894	0.002	2.938	1.541–5.601	0.001
Pneumonectomy						
Serum endostatin (ng/ml)	0.991	0.981–1.002	0.099	Not included in multivariable model		
Serum VEGF (pg/ml)	1.000	1.000–1.001	0.070	Not included in multivariable model		
VEGF/endostatin ratio	1.029	1.005–1.054	0.018	1.024	1.000–1.048	0.045

HR, hazard ratio; CI, confidence interval; VEGF, vascular endothelial growth factor.
[a] 97 patients in the analysis. Cox regression analysis.

that endostatin was correlated with the prognosis of NSCLC.[25] In the current study lower VEGF level was found in the patients who lived for more than 5 years compared with the level of those who lived for less than 5 years. But in univariate analysis, the difference did not reach the statistical significance. A meta-analysis reported by Hu et al. demonstrated that high VEGF level was associated with poor outcome in inoperable stage instead of in operable stage.[26] As the inconsistent reports about the correlation of VEGF and endostatin with the prognosis, we assume that it might be not enough to assess only the angiogenic inducers or the inhibitors. In the internal environment, the homeostasis is essential for the normal function and structure.[27] So it is necessary to study the balance of the angiogenic factors.

In the present study, the ratio of VEGF/endostatin was significantly higher in operable NSCLC cases than in the normal control. And it correlated with the prognosis of operable NSCLC. In subgroup analysis, patients with N0 presented significantly different prognosis when the ratio was divided by the median. To date, although there are limitations and controversies about the TNM staging,[28] it still acts as the best common method for measuring the prognosis to NSCLC. The present study suggests that the VEGF/endostatin ratio is an independent factor correlated with the prognosis of NSCLC patients and the ratio might supplement the limitation of the TNM staging.

To date, two categories of medication are used in the development of anti-angiogenic therapy, involving the inhibition of angiogenic factors (bevacizumab and ramucirumab act through this pathway [29]) and the application of endogenous angiogenesis inhibitors (endostatin is one of the approved therapy in China [30]). Restoration of pro- and anti-angiogenic balance in tumors may "normalize" tumor vasculature.[31] Patients perhaps should be treated with different types of anti-angiogenic drugs according to the need of the internal balance of angiogenesis, such as reducing the angiogenic inducers or increasing the angiogenic inhibitors, should not be treated with anti-VEGF or endogenous angiogenic inhibitors without careful selections.

Conclusions

This investigation revealed that an imbalance between VEGF and endostatin levels exits in the serum of operable NSCLC patients. This imbalance predicted the prognosis of NSCLC patients. However, whether the ratio of VEGF/endostatin can be a predictor for some treatments or progression/recurrence of the disease should be further explored in the future. The more we understand the angiogenesis in NSCLC, the more effective modalities would be applied to NSCLC patients.

Conflict of interest statement

All of our authors declare that we have no conflicts of interest concerning this article.

Acknowledgments

We thank Yun-wen Wang in the Division of Medical Records of Beijing Chest Hospital, Capital Medical University, for her help in following up the patients. And many thanks to Chang-xing Song and Chang-li Rong in the Department of Clinical Laboratory of Beijing Chest Hospital, Capital Medical University, for their help in laboratory work. We also thank Prof. Getu Zhaori for his reading and revision of this manuscript.

References

1. *GLOBOCAN 2012: estimated cancer incidence, mortality and prevalence worldwide in 2012*. http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx.
2. Berrino F, De Angelis R, Sant M, et al. Survival for eight major cancers and all cancers combined for European adults diagnosed in 1995–99: results of the EUROCARE-4 study. *Lancet Oncol* 2007;**8**:773–83.
3. Scagliotti GV, De Marinis F, Rinaldi M, et al. The role of histology with common first-line regimens for advanced non-small cell lung cancer: a brief report of the retrospective analysis of a three-arm randomized trial. *J Thorac Oncol* 2009;**4**:1568–71.
4. Folkman J. Tumor angiogenesis: therapeutic implications. *N Engl J Med* 1971;**285**:1182–6.
5. O'Reilly MS, Holmgren L, Shing Y, et al. Angiostatin: a circulating endothelial cell inhibitor that suppresses angiogenesis and tumor growth. *Cold Spring Harb Symp Quant Biol* 1994;**59**:471–82.
6. Rak JW, St Croix BD, Kerbel RS. Consequences of angiogenesis for tumor progression, metastasis and cancer therapy. *Anticancer Drugs* 1995;**6**:3–18.
7. Veikkola T, Alitalo K. VEGFs, receptors and angiogenesis. *Semin Cancer Biol* 1999;**9**:211–20.
8. Sim BK, MacDonald NJ, Gubish ER. Angiostatin and endostatin: endogenous inhibitors of tumor growth. *Cancer Metastasis Rev* 2000;**19**:181–90.
9. Kerbel RS. Tumor angiogenesis. *N Engl J Med* 2008;**358**:2039–49.
10. Bremnes RM, Camps C, Sirera R. Angiogenesis in non-small cell lung cancer: the prognostic impact of neoangiogenesis and the cytokines VEGF and bFGF in tumours and blood. *Lung Cancer* 2006;**51**:143–58.
11. O'Reilly MS, Boehm T, Shing Y, et al. Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. *Cell* 1997;**88**:277–85.
12. Sund M, Hamano Y, Sugimoto H, et al. Function of endogenous inhibitors of angiogenesis as endothelium-specific tumor suppressors. *Proc Natl Acad Sci U S A* 2005;**102**:2934–9.
13. Abdollahi A, Hlatky L, Huber PE. Endostatin: the logic of antiangiogenic therapy. *Drug Resist Updat* 2005;**8**:59–74.
14. Carrillo-de Santa Pau E, Carrillo Arias F, Caso Pelaez E, et al. Vascular endothelial growth factor (VEGF) serum levels are associated with survival in early stages of lung cancer patients. *Cancer Invest* 2010;**28**:393–8.
15. Laack E, Köhler A, Kugler C, et al. Pretreatment serum levels of matrix metalloproteinase-9 and vascular endothelial growth factor in non-small-cell lung cancer. *Ann Oncol* 2002;**13**:1550–7.
16. Suzuki M, Iizasa T, Ko E, et al. Serum endostatin correlates with progression and prognosis of non-small cell lung cancer. *Lung Cancer* 2002;**35**:29–34.
17. Yamamoto Y, Toi M, Kondo S, et al. Concentrations of vascular endothelial growth factor in the sera of normal controls and cancer patients. *Clin Cancer Res* 1996;**2**:821–6.
18. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* 1996;**86**:353–64.
19. Bouck N, Stellmach V, Hsu SC. How tumors become angiogenic. *Adv Cancer Res* 1996;**69**:135–74.
20. Zhao J, Yan F, Ju H. Correlation between serum vascular endothelial growth factor and endostatin levels in patients with breast cancer. *Cancer Lett* 2004;**204**:87–95.
21. Iizasa T, Chang H, Suzuki M, et al. Overexpression of collagen XVIII is associated with poor outcome and elevated levels of circulating serum endostatin in non-small cell lung cancer. *Clin Cancer Res* 2004;**10**:5361–6.
22. Edelman MJ, Hodgson L, Wang X, et al. Serum vascular endothelial growth factor and COX-2/5-LOX inhibition in advanced non-small cell lung cancer and leukemia group B 150304. *J Thorac Oncol* 2011;**6**:1902–6.
23. Laack E, Scheffler A, Burkholder I, et al. Pretreatment vascular endothelial growth factor (VEGF) and matrix metalloproteinase-9 (MMP-9) serum levels in patients with metastatic non-small cell lung cancer (NSCLC). *Lung Cancer* 2005;**50**:51–8.
24. Liao M, Wang H, Lin Z, Feng J, Zhu D. Vascular endothelial growth factor and other biological predictors related to the postoperative survival rate on non-small cell lung cancer. *Lung Cancer* 2001;**33**:125–32.
25. Zhan P, Wang J, Lv XJ, et al. Prognostic value of vascular endothelial growth factor expression in patients with lung cancer: a systematic review with meta-analysis. *J Thorac Oncol* 2009;**4**:1094–103.
26. Hu P, Liu W, Wang L, Yang M, Du J. High circulating VEGF level predicts poor overall survival in lung cancer. *J Cancer Res Clin Oncol* 2013;**139**:1157–67.
27. Folkman J. The role of angiogenesis in tumor growth. *Semin Cancer Biol* 1992;**3**:65–71.
28. Motta G, Nahum MA, Testa T, Spinelli E. TNM staging system of lung carcinoma: historical notes, limitations and controversies. *Ann Ital Chir* 1995;**66**:425–32.
29. Aggarwal C, Somaiah N, Simon G. Antiangiogenic agents in the management of non-small cell lung cancer: where do we stand now and where are we headed? *Cancer Biol Ther* 2012;**13**:247–63.
30. Wang J, Sun Y, Liu Y, et al. Results of randomized, multicenter, double-blind phase III trial of rh-endostatin (YH-16) in treatment of advanced non-small cell lung cancer patients. *Zhongguo Fei Ai Za Zhi* 2005;**8**:283–90.
31. Fukumura D, Jain RK. Tumor microvasculature and microenvironment: targets for anti-angiogenesis and normalization. *Microvasc Res* 2007;**74**:72–84.