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

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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

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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%)