RESEARCH ARTICLE

Utility of VEGF and sVEGFR-1 in Bronchoalveolar Lavage Fluid for Differential Diagnosis of Primary Lung Cancer

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Abstract

   Published data have shown that the levels of vascular endothelial growth factor (VEGF) and soluble VEGF receptor-1 (sVEGFR-1) in plasma and pleural effusion might be usefulness for lung cancer diagnosis. Here, we performed a prospective study to investigate the utility of VEGF and sVEGFR-1 in bronchoalveolar lavage fluid (BALF) for differential diagnosis of primary lung cancer. A total of 56 patients with solitary pulmonary massed by chest radiograph or CT screening were enrolled in this study. BALF and plasma samples were obtained from all patients and analyzed for VEGF and sVEGFR-1 using a commercially available sandwich ELISA kit. The results showed that the levels of VEGF in BALF were significantly higher in patients with a malignant pulmonary mass compared with patients with a benign mass ($P < 0.001$). However, no significant difference of sVEGFR-1 in BALF was found between malignant and non-malignant groups ($P = 0.43$). With a cut-off value of 214 pg/ml, VEGF showed a sensitivity and specificity of 81.8% and 84.2%, respectively, in predicting the malignant nature of a solitary pulmonary mass. Our study suggests that VEGF is significantly increased in BALF among patients with lung cancer than in benign diseases. Measurement of VEGF in BALF might be helpful for differential diagnosis of primary lung cancer.

Keywords: Lung cancer - Bronchoalveolar lavage fluid - VEGF - sVEGFR-1

Introduction

   Lung cancer is currently the most frequently occurring cancer and one of the leading causes of cancer-related death in the world (Cao et al., 2011). A major factor in the high mortality of lung cancer patients is the presence of metastatic tumors in approximately two-thirds of patients at the time of diagnosis (Wingo et al., 1999). Published data indicated that detection of lung cancer at earlier stages could potentially increase survival rates by 10- to 50-fold (Wingo et al., 1999). However, lung cancer screening by chest X-ray and sputum cytology have proven ineffective in increasing patient survival (Ellis et al., 2001; Marcus, 2011), leading to the search for more sensitive and specific tests. Nowadays, fiberoptic bronchoscopy is the most commonly used method for diagnosing lung cancer (Karahalli et al., 2001; Mazzone et al., 2002). Patients suspected of having lung cancer often undergo fiberscopic examination and bronchoalveolar lavage fluid (BALF) was used as a clinical marker for lung cancer diagnosis has been described (Charalabopoulos et al., 2007; Emad et al., 2008).

   Angiogenesis is a complex process regulated by several growth factors among which vascular endothelial growth factor (VEGF) plays an important role (Ferrara et al., 1997; Ferrara, 2002). A VEGF-specific tyrosine kinase receptor, VEGF receptor-1 (VEGFR-1/Flt-1), has been found in the tumor vessels and a soluble form of VEGFR-1 (sVEGFR-1) has been detected in the circulation (Fiorelli et al., 2013). Evidence from clinical data has shown that the levels of VEGF and sVEGFR-1 in plasma and pleural effusion (PE) might be usefulness for lung cancer diagnosis (Kishiro et al., 2002; Hooper et al., 2012; Fiorelli et al., 2013). In our previous studies, we also found a link between VEGF expression and the risk and clinical characteristics of lung cancer (Sun et al., 2013). Based on the important role of VEGF and sVEGFR-1 in carcinogenesis, we performed a prospective study to investigate the usefulness of such markers in BALF for differential diagnosis of primary lung cancer.

Materials and Methods

Patients
   A total of 56 patients who were found solitary pulmonary mass by chest radiograph or CT screening at the Affiliated Hospital of Ningbo University between February 2011 and July 2012 were enrolled in this study. All patients had histological confirmed and were excluded if they had received preoperative chemotherapy or radiotherapy. Information regarding patient characteristics was based on patient records and registries. Approval for

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Table 1. The Characteristics of the Patients

	Malignant group	Benign group
Age, years	55.4±8.4	48.1±9.2
Gender		
Male	28	12
Female	9	7
Smoking status		
Smokers	26	10
Non-smokers	11	9
Pack/years	38.5±4.4	27.1±5.2
Histological type		
Squamous cell carcinoma	19	
Adenocarcinoma	15	
Small-cell lung cancer	3	
Stage of cancer		
I	23	
II	9	
III	5	

this study was obtained from the local ethics committee, and informed consent was obtained from all participating subjects.

Bronchoalveolar lavage

BAL was performed during fiberoptic bronchoscopy after premedication with atropine (0.5 mg im) and 2% lidocaine upper respiratory tract anesthesia. The lavage was done before brushing or biopsies. The bronchus with mass was washed with 37°C sterile physiological saline and the fluid was gently withdrawn into a siliconized container placed in iced water. The chilled lavage fluid was filtered through a nylon filter to remove mucus and centrifuged at 3,000 rpm for 10-min. The cell pellets were separated from the supernatants and stored at -80° C.

Measurements of VEGF and sVEGFR-1

The levels of VEGF (pg/ml) and sVEGFR-1 (pg/ml) were measured using Quantikine sandwich enzyme linked immunosorbent assays (ELISA; R&D systems, Minneapolis, MN, USA). The assays were conducted according to the manufacturer's guidelines. Each sample was analyzed in duplicate, with dilutions as appropriate, and samples were analyzed in batches to minimize interassay variability. The minimum detectable levels of VEGF and sVEGFR-1 were 9 pg/ml and 3.5 pg/ml, respectively.

Statistical analysis

The results are presented as mean ± SEM for all variables that were normally distributed and as median (interquartile range) when not normally distributed. Comparison between different groups was done using the non-parametric Mann-Whitney U-test. Correlations between measured VEGF and sVEGFR-1 were performed with Spearman's rank correlation. Receiver operating characteristics (ROC) analysis was performed to evaluate the threshold value of variables in differentiating malignant from benign pulmonary mass. The optimum cut-off point was determined as the value of the parameter that maximized the sum of specificity and sensitivity. A probability value of < 0.05 was considered statistically significant. The analyses were conducted using SPSS version 13.0 (SPSS, Chicago, IL, USA).
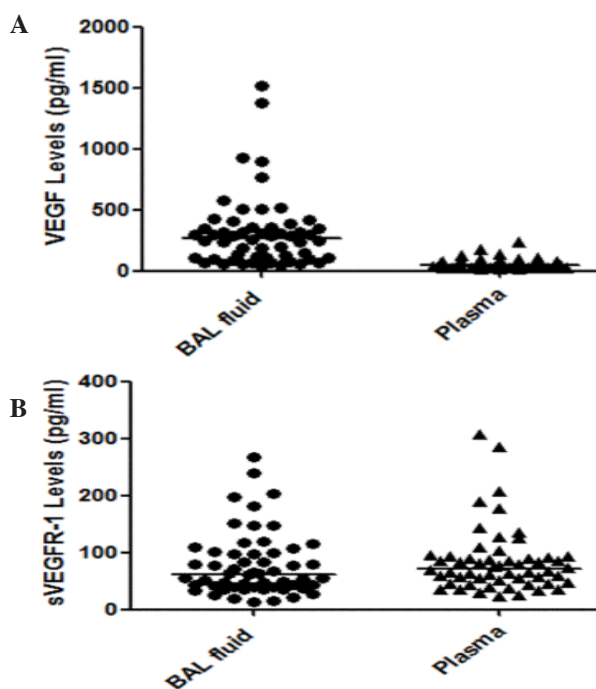
Figure 1. Comparison of VEGF and sVEGFR-1 Levels Between Bronchoalveolar Lavage (BAL) Fluid and Plasma. The levels of VEGF were significantly higher in BAL fluid than those measured in plasma ($P < 0.001$, Mann–Whitney test) (A); no significant difference of sVEGFR-1 levels was found between BAL fluid and plasma ($P = 0.30$, Mann–Whitney test) (B). Horizontal lines represent the median values

Results

Patients characteristics

Basic characteristics for patients are summarized in Table 1. There were 37 lung cancer patients (28 males and 9 females; age: 55.4 ± 8.4 years) and 19 patients with noncancerous diseases (12 males and 7 females; age: 48.1 ± 9.2 years). The pathologic types included 19 squamous cell carcinomas, 15 adenocarcinomas, and 3 small cell carcinomas. According to the TNM clinical staging, 23 patients were at stage I, 9 at stage II, and 5 at stage III.

VEGF and sVEGFR-1 levels

The BAL fluid of VEGF was significantly greater than those measured in plasma (270.3 [106.6- 356.3] pg/ml versus 45.5 [31.8-67.0] pg/ml, $P < 0.001$; Figure 1A). No significant difference of sVEGFR-1 levels was found between BALF and plasma (62.3 [39.9-101.4] pg/ml versus 72.2 [48.1-92.7] pg/ml, P = 0.30; Figure 1B).

Moreover, BAL VEGF was significantly higher in patients with a malignant pulmonary mass compared with patients with a benign mass (308.9 [240.1-418.9] pg/ml versus 94.6 [61.7-182.4] pg/ml, $P < 0.001$; Figure 2A). However, there was no significant difference in BAL sVEGFR-1 between malignant and nonmalignant groups (54.3 [38.5-99.9] pg/ml versus 66.5 [42.2-107.2] pg/ml, respectively, $P = 0.43$; Figure 2B).

Correlation of VEGF and sVEGFR-1 in plasma and BALF

We further evaluated the correlation of VEGF and sVEGFR-1 in plasma and BALF by the Spearman's rank correlation test analyses. There was no significant
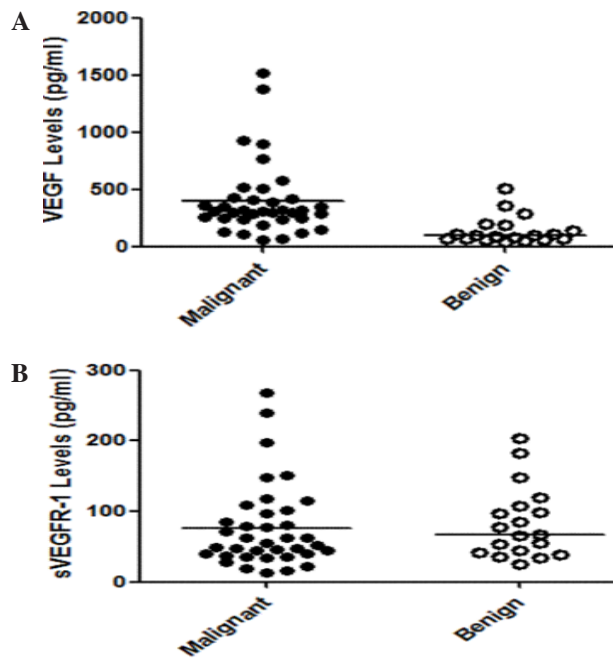
Figure 2. Comparison of VEGF and sVEGFR-1 Levels in Bronchoalveolar Lavage Fluid Between Benign and Malignant Groups. The levels of VEGF were significantly higher in lung cancer patients than those in benign diseases ($P < 0.001$, Mann–Whitney test) (A); no significant difference of sVEGFR-1 levels was found between malignant and nonmalignant groups ($P = 0.43$, Mann–Whitney test) (B). Horizontal lines represent the median values

correlation between VEGF and sVEGFR-1 within either the plasma ($r = 0.041$, $P = 0.764$) or BAL fluid ($r = 0.129$, $P = 0.343$).

ROC analysis and cut-off value of BAL VEGF

Using logistic regression models, we calculated sensitivity and specificity of VEGF to predict malignant pulmonary mass for possible threshold value. The diagnostic threshold afforded by the ROC analysis for VEGF was 214 pg/ml. The area under the ROC was 0.855 (Figure 3). With a threshold value of 214 pg/ml, VEGF had a sensitivity of 81.8%, a specificity of 84.2%, a positive predictive value of 90.9%, and a negative predictive value of 69.6%.

Discussion

The solitary pulmonary mass is a common and challenging clinical problem. It is essential to distinguish malignancy from benign mass, because malignancy should be identified and resected promptly to improve patients' life quality and survive, but also avoid a benign mass being unnecessary resected. Patients suspected of having lung cancer often undergo fiberscopic examination and bronchial washing are traditionally used. Published data has shown that detection of biomarkers in BALF might serve as an important adjunct to bronchoscopy in differential diagnosis of lung cancer (Cremades et al., 1998; Ohta et al., 2002; Bugdayci et al., 2006; Domagała-Kulawik et al., 2006; Charalabopoulos et al., 2007; Emad et al., 2008).

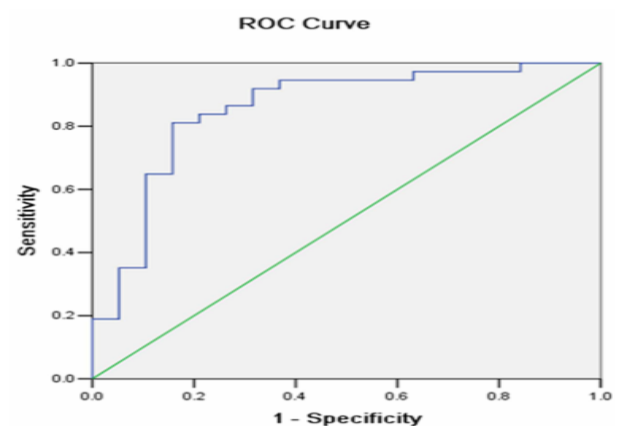In the present study, we conducted a prospective study

Figure 3. Receiver Operating Characteristic (ROC) Curve was Performed to Evaluate the Threshold Value of VEGF in Differentiating Malignant from Benign Pulmonary Mass. VEGF reached a sensitivity of 81.8%, a specificity of 84.2%, a positive predictive value of 90.9%, and a negative predictive value of 69.6% (cut-off value: 214 pg/ml; area under the curve: 0.855)

to investigate whether levels of BAL VEGF and its soluble receptor, sVEGFR-1 could be useful in distinguishing malignant from benign solitary pulmonary mass. We first determined plasma and BAL levels of VEGF in all the patients. The result showed that the levels of plasma VEGF in patients with lung cancer were higher than patients with noncancerous diseases, which agreed with previous studies (Kishiro et al., 2002; Tamura et al., 2002; Swidzińska et al., 2004). Furthermore, our study found VEGF was present in significantly higher levels in BALF than in plasma, suggesting that in this context VEGF was produced locally within airways and that airways production is disproportionate to systemic.

VEGF expression was significantly higher in patients with a malignant pulmonary mass compared with patients with a benign mass, indicating VEGF in BALF might be a good marker for lung cancer diagnosis. ROC analysis was further performed to evaluate the threshold value of VEGF in differentiating malignant from benign pulmonary mass. The result showed that the diagnostic threshold afforded by the ROC analysis for VEGF was 214 pg/ml. The area under the ROC was 0.855. With a threshold value of 214 pg/ml, VEGF had a sensitivity of 81.8%, a specificity of 84.2%, a positive predictive value of 90.9%, and a negative predictive value of 69.6%. These results may provide a new approach with a higher diagnostic value in patients with solitary pulmonary mass discovered by chest radiograph or CT screening. On the other hand, the presence of low VEGF levels in BALF indicated a low probability of malignancy, which might serve to avoid performing an invasive procedure.

A recent study found higher levels of sVEGFR-1 in malignant pleural effusion than in benign PE (Fiorelli et al., 2013). Moreover, another study showed that high levels of sVEGFR-1 in PE were strongly associated with poor outcomes in lung cancer patients (Hooper et al., 2012). However, no significant difference in terms of plasma or BALF sVEGFR-1 levels between malignant and nonmalignant groups was found in our study. The reason might be due to the fact that there was quite difference

between airways and pleural response to the presence of lung cancer cells. At the same time, published studied indicated that high levels of sVEGFR-1 were obtained when the stage of lung cancer was high and the prognosis was worst (Ilhan et al., 2004). More than half of the lung cancer patients in our study were at early stage, which may also contribute to the low levels of sVEGFR-1 in malignant group.

sVEGFR-1, an endogenous VEGF inhibitor, has an important function in the regulation of VEGF mediated activities in vivo (Denizot et al., 2007). It is surprising that we failed to find a correlation between VEGF and sVEGFR-1 in either BALF or plasma, suggesting that there were other receptors of VEGF play a role in lung carcinogenesis. Up to date, three VEGF receptors have been identified: VEGFR-1, VEGFR-2, and VEGFR-3. Each receptor plays a key part in the regulation of tumor angiogenesis (Shibuya, 2011; Wang et al., 2012). Therefore, BAL VEGFR-2 and VEGFR-3 should be measurement in future studies, which may lead to better, comprehensive understanding of the important role of VEGF/VEGFRs pathways in the pathogenesis of lung cancer.

In conclusion, our study demonstrated that the levels of VEGF in BALF could serve as an important adjunct to bronchoscopy in lung cancer differential diagnosis. Measurement of VEGF in BALF might be helpful for differential diagnosis of patients with solitary pulmonary mass but it should be studied in larger groups to elucidate its benefit in clinics.

Acknowledgements

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