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Elevated Serum Level of Angiopoietin-2 as a Potential Marker for Poor Prognosis in Small Cell Lung Cancer

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Small cell lung cancer (SCLC) is a fast-growing cancer with poor prognosis. Patients with extensive-stage SCLC are generally treated with chemotherapy. Thus, it is essential to identify a predictor of efficacy and prognosis for SCLC. Angiopoietin-2 promotes vascular remodeling and angiogenesis. Increasing evidence reveals that angiopoietin-2 is preferentially expressed in cancer cells, and elevated angiopoietin-2 expression is related to invasive and metastatic phenotypes in various cancers. However, serum angiopoietin-2 level and its prognostic potential in SCLC have not been investigated. The aim of this study was to determine the usefulness of angiopoietin-2 level as a predictor of efficacy and prognosis for SCLC. This study consisted of sixty patients with SCLC. Each patient received four cycles of cisplatin-etoposide chemotherapy, and was followed for 36 months. Serum angiopoietin-2 levels were measured by Enzyme-linked immunosorbent assays. The angiopoietin-2 levels were significantly higher in SCLC patients than those in healthy subjects ($P < 0.001$). The patients were divided into high-level group (32 patients, 2,923.9 ± 294.7 pg/ml) and low-level group (28 patients, 1,789.5 ± 355.1 pg/ml) according to the mean value of the angiopoietin-2 level (2,400 pg/ml). Compared with the patients in the high-level group, the patients in the low-level group showed remarkably survival advantage ($P = 0.002$). During chemotherapy, the patients in the low-level group showed better treatment response than the patients in the high-level group ($P < 0.05$). Therefore, angiopoietin-2 might be useful as a prognostic factor for SCLC and for predicting SCLC response to chemotherapy.

Keywords: angiopoietin-2; chemotherapy; prognosis; serum; small cell lung cancer
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Introduction

Small cell lung cancer (SCLC) is a fast-growing type of cancer, and accounts for nearly 15% of lung cancers globally (Govindan et al. 2006). It is believed that SCLC is derived from neuroendocrine cells (namely APUD cells) (Champaneria et al. 2006), and SCLC spreads more rapidly compared to non-SCLC (Sher et al. 2008). Many cases of SCLCs are diagnosed in an unresectable state, and are treated with combination chemotherapy, but not surgery. Even if a complete response is achieved after chemotherapy, cancer recurrence in a short time may not be avoided. Thus, it is essential to identify a new predictor of efficacy and prognosis for SCLC, and new predictor might be help for optimizing treatment and improving prognosis.

Angiopoietin-2 (Ang-2) is a member of the angiopoietin (Ang) family, which can promote vascular remodeling in concert with vascular endothelial growth factor (Fagiani and Christofori 2013). Therefore, Ang-2 plays an important role in embryonic and postnatal angiogenesis (Maisonpierre et al. 1997). Over the past decades, increasing evidence has revealed that Ang-2 is preferentially expressed in cancer cells (Wong et al. 2000), and elevated Ang-2 expression levels are related to invasive and metastatic phenotypes of several types of cancer (Amo et al. 2004; Hu and Cheng 2009; Helfrich et al. 2009; Li et al. 2015). Previous studies also focused on non-SCLC, and suggested that circulating Ang-2 might be a useful marker for diagnosis and predicting prognosis (Xing et al. 2003; Park et al. 2007; Fawzy et al. 2012; Naumnik et al. 2013).

Therefore, we conducted a prospective observational study to determine the usefulness of serum Ang-2 level as a prognostic factor for SCLC, and to further study clinical significance of Ang-2 in response to chemotherapy.

Materials and Methods

A total of 60 patients with confirmed SCLC in the Affiliated Hospital of Yan'an University recruited into the study between

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January 2008 and December 2011. Inclusion criteria were as follows: (1) Age ≥ 18 years old; (2) Sputum cytology examination and bronchoscopy were performed, and a diagnosis of SCLC was made with definitive pathology; (3) CT scans of the chest, CT scans of the abdomen, CT scans of the brain were performed, and a bone scan was also performed, with sufficient data for TNM staging (Kayser et al. 1987); (4) No patients had received prior anticancer therapy; and (5) All patients were followed for 36 months or until they died. Patients with other types of cancers, other types of respiratory diseases, cardiovascular diseases, hepatobiliary gastrointestinal diseases, urinary system diseases, blood diseases, rheumatological diseases, mental illness and serious infections were excluded.

Sixty healthy controls matched for age, gender and smoking history were randomly selected from the employee list of the Affiliated Hospital of Yan'an University. All patients and controls signed written informed consents. The study was approved by the ethics committees of the Affiliated Hospital of Yan'an University.

In the patient group, demographic data, smoking history and medical examination reports were obtained from medical records. In the control group, necessary information were collected using questionnaire.

All patients were treated with four cycles of cisplatin-etoposide chemotherapy. Each cycle contained 21 days and used cisplatin at a dose of 80 mg/m^2 on day 1 and etoposide 80 mg/m^2 on days 1, 2, and 3. The response to therapy was estimated according to the World Health Organization (WHO) criteria.

Blood samples were taken from each patient before anti-tumor therapy and after four cycles of chemotherapy. And blood samples were also obtained from controls. The blood samples were centrifuged and stored at −80°C for later assay.

The levels of serum Ang-2 were measured by Enzyme-linked immunosorbent assays (ELISA) according to manufacturer's instructions (Quantikine; R&D Systems, Minneapolis, MN). All samples were duplicately measured, and the mean levels were calculated.

The patients were stratified by several prognostic parameters, such as gender, age, weight loss 10%, clinical stage, onset of anemia and hypoalbuminemia, and subgroup analyses were performed. Anemia diagnosis in men was based on hemoglobin concentrations of less than 130 to 140 g/L while in women it must be less than 120 to 130 g/L (Janz et al. 2013). Hypoalbuminemia diagnosis was based on albumin of less than 35 g/L.

All statistical analyses were conducted using SPSS version 17.0 (SPSS, Chicago, IL, USA). Statistical differences were separately assessed using independent sample t-test in continuous variables and chi-square test in categorical variables. The Kaplan-Meier method was used to estimate survival. P values < 0.05 were considered to be statistical significance.

Results

As shown in Table 1, 60 SCLC patients and 60 healthy controls were enrolled in the study. There were no significant differences between the patients and the controls in age, gender, race, body mass index (BMI) and smoking history (P > 0.05). Compared with the controls, a significantly elevated Ang-2 level was found in the patients (P < 0.001).

As shown in Table 2, several prognostic parameters had no significant effect on serum Ang-2 levels (P > 0.05). Therefore, serum levels of Ang-2 might be an independent prognostic factor of SCLC.

During chemotherapy, "partial response" occurred in 23 patients, "stable disease" in 18 patients and "progressive disease" in 19 patients. But in each "response to chemotherapy" subgroup, the serum Ang-2 level did not change after chemotherapy compared with the serum level at admission (P > 0.05) (Table 3).

The patients were also divided into the high-level group and the low-level group according to the mean value of the serum Ang-2 level at admission (2,400 pg/ml). The serum Ang-2 levels were 2,923.9 ± 294.7 pg/ml in the high-level group (32 patients) and 1,789.5 ± 355.1 pg/ml in the low-level group (28 patients), respectively. Importantly, the serum Ang-2 level in the low-level group was still higher than that in healthy subjects (P < 0.001). During long-term follow-up, there were more "partial response" patients in the low-level group (P = 0.023) and more "progressive disease" patients in the high-level group (P = 0.031) (Table 4).

Table 1. Characteristics of participants at admission.

	SCLC group	Control group	P value
No. included (n)	60	60	
Age (yrs, mean ± SD)	60.4 ± 5.4	59.9 ± 6.0	0.689
Gender (n)			
Male	48	45	0.512
Female	12	15	
Race (n)			
Han	55	57	0.464
Other	5	3	
BMI (kg/m^2, mean ± SD)	20.8 ± 1.8	21.0 ± 1.7	0.507
Smoking status (n)			
Neversmoker	9	13	0.345
Smoker	51	47	
Angiopoietin-2 (pg/ml, mean ± SD)	2,394.5 ± 655.0	928.2 ± 384.1	< 0.001

SD, standard deviation; BMI, body mass index; SCLC, small cell lung cancer.

Table 2. Serum angiopoietin-2 levels of small cell lung cancer patients with several prognostic parameters.

	No. of patients (n)	Angiopoietin-2 (pg/ml, mean ± SD)	P value
Gender			
Male	48	2,312.2 ± 627.9	0.051
Female	12	2,723.6 ± 685.0	
Age			
< 60 yrs	24	2,471.7 ± 561.2	0.461
≥ 60 yrs	36	2,343.1 ± 713.8	
Weight loss 10%			
Positive	43	2,383.6 ± 691.6	0.839
Negative	17	2,422.1 ± 570.6	
Clinical stage			
I-II	45	2,322.6 ± 681.0	0.142
III-IV	15	2,610.1 ± 533.0	
Anemia[a]			
Positive	44	2,450.3 ± 644.3	0.277
Negative	16	2,241.0 ± 680.8	
Hypoalbuminemia[b]			
Positive	37	2,433.7 ± 650.1	0.561
Negative	23	2,331.5 ± 672.6	

SD, standard deviation.
[a]Anemia diagnosis in men was based on a hemoglobin of less than 130 to 140 g/L while in women it must be less than 120 to 130 g/L.
[b]Hypoalbuminemia diagnosis was based on an albumin of less than 35 g/L.

Table 3. Serum angiopoietin-2 levels of small cell lung cancer patients before and after chemotherapy with different chemotherapy response.

Chemotherapy response	No. of patients (n)	Angiopoietin-2 before chemotherapy (pg/ml, mean ± SD)	Angiopoietin-2 after chemotherapy (pg/ml, mean ± SD)	P value
Partial response	23	2,241.1 ± 664.0	2,027.1 ± 518.2	0.338
Stable disease	18	2,495.4 ± 719.9	2,326.6 ± 548.0	0.434
Progressive disease	19	2,484.5 ± 573.8	2,502.5 ± 631.6	0.927

SD, standard deviation.

As shown in Fig. 1, there was remarkably survival advantage in the low-level group compared with the high-level group (P = 0.002).

Discussion

Naumnik et al. (2009) conducted a study focusing on non-SCLC and suggested that Ang-2 concentration had no clinical significance in the prognosis of the survival time in lung cancer and could not be used as a predictor of response to the chemotherapy. As far as we know, no previous study had investigated such topic on SCLC. Therefore, we performed the study and tried to reveal the association between the serum Ang-2 level at admission and the prognosis of SCLC patients with combination chemotherapy for the first time.

Based on the results in the study, the serum Ang-2 level was significantly elevated in the patients with SCLC (Table 1). Furthermore, the groups in our study were not only well matched in several demographic parameters (mainly age, gender, race and BMI), but they were also equivalent for smoking history. This arrangement might avoid bias and gave us more reliable results, because smoking had a potential ability to affect the serum level of Ang-2 (Kanazawa et al. 2009).

In Table 4, "partial response" occurred more frequently in the patients with the low Ang-2 level at admission. Conversely, the high level patients were prone to "progressive disease" during combination chemotherapy. This result might partially explain the survival advantage in the patients with the low serum Ang-2 level compared with the patients with the high serum Ang-2 level (Fig. 1). However, a potential mechanism of the relationship between the serum Ang-2 level and the response to chemotherapy requires further investigation.

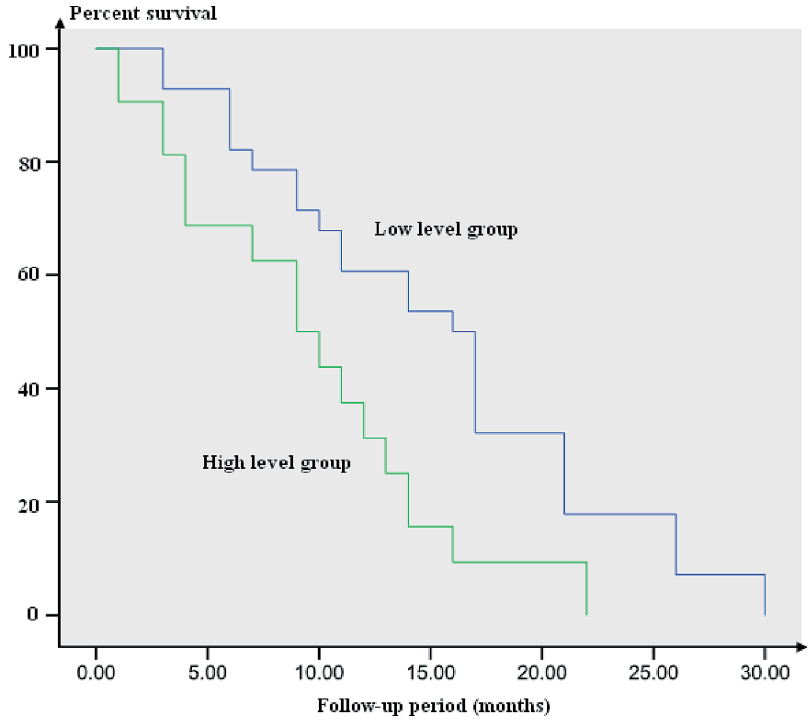

Fig. 1. Survival analysis of small cell lung cancer in relation to serum angiopoietin-2 levels (log rank P value = 0.002).

Table 4. Different chemotherapy responses of small cell lung cancer patients in the high angiopoietin-2 group and the low angiopoietin-2 group.

Group	High level group (≥ 2,400 pg/ml)	Low level group (< 2,400 pg/ml)	P value
Total (n)	32	28	
Partial response (n)	8	15	0.023
Stable disease (n)	10	8	0.821
Progressive disease (n)	14	5	0.031

In the study, the patients were divided into the high-level group and the low-level group according to the mean value of the Ang-2 level, but not the cutoff value of the Ang-2 level. This might underestimate the association between the Ang-2 level and the disease prognosis, but we still obtained positive results. The reason why we did not use the cutoff value was that all patients had died at the end of the follow-up period.

Two previous studies focused on non-SCLC determined the serum Ang-2 by ELISA technique (Park et al. 2007; Naumnik et al. 2009), and Park et al. (2007) also analyzed SCLC patients using the same technique. These studies revealed that the Ang-2 concentrations were higher in patients with lung cancer (non-SCLC or SCLC) than in healthy people. But the previous studies did not study the usefulness of serum Ang-2 level as a prognostic factor for SCLC. To our knowledge, this is the first published article focusing on this topic. Using similar ELISA Kit, we have shown similar serum Ang-2 concentrations compared with the studies mentioned above, suggesting the validity of our measurement of the serum Ang-2.

Pathological staging is usually more accurate than clinical staging on judgment of tumor progress. But we failed to collect enough data for pathological staging, which might be one of the limitations in the study. Another limitation was the small sample size due to low incidence of SCLC. Thirdly, Ang is part of vascular growth factor family, which consists of Ang-1, Ang-2 and other factors. Ang-1 promotes vessel maturation, adhesion and survival (Suri et al. 1996), but Ang-2 is partly responsible for cell death and disrupts vascularization (Maisonpierre et al. 1997). Both factors are competitive ligands for tyrosine kinase (Tie-2), which is a receptor discovered on vessel endothelial cells, and form a dynamic balance system for regulation of angiogenesis (Suri et al. 1996; Davis et al. 1996; Maisonpierre et al. 1997). Previous studies had performed to reveal the significances of Ang-1 and Tie-2 on many types of cancer (Caine et al. 2003; Niedźwiecki et al. 2006; Park et al. 2009). Our study only focused on Ang-2, but not included Ang-1 and Tie-2. Therefore, population-based study should be done to determine these issues in the future.

In conclusion, our study provides the evidence that serum Ang-2 might be a useful and noninvasive prognostic factor for SCLC. Furthermore, serum Ang-2 levels may be helpful for predicting SCLC response to chemotherapy.

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Conflict of Interest

The authors declare no conflict of interest.

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