Prognostic significance of serum osteopontin levels in small-cell lung cancer

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Abstract

Backgroud

Osteopontin (OPN) is involved in the development and metastasis of a variety of tumors. This study explored serum OPN levels in patients with small cell lung cancer (SCLC) and compared them with healthy controls.

Methods

The OPN levels of 96 patients with SCLC before and after first-line chemotherapy were detected by enzyme-linked immunosorbent assay (ELISA) and compared with 60 healthy controls.

Results

The pre-treatment serum OPN levels of patients with SCLC were higher than those of healthy controls (P<0.001). Serum OPN levels were significantly associated with disease stage, tumor size, and lymph node metastasis (P=0.012, 0.034, and 0.037, respectively). Serum OPN levels were significantly decreased after first-line chemotherapy (P=0.019), which was associated with treatment response (P=0.011), but there was no significant difference with pre-treatment OPN levels (P=0.485). Cox proportional hazard analysis showed that serum OPN levels, disease stage, and performance status were independent predictors of overall survival.

Conclusions

Our results suggest that serum OPN levels can be used as biomarkers to predict treatment response and survival in patients with SCLC.

Background

Lung cancer is one of the most common human cancers in the world. According to pathological types, lung cancer can be divided into non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC), which accounts for 13% of the total number of lung cancer [1, 2]. Although SCLC is sensitive to chemotherapy and radiotherapy, the diagnosis is often in advanced stages, resulting in poor prognosis [3]. The median survival of untreated patients was only 2 to 4 months, and the overall 5-year survival rate was approximately 3-8% [4]. To date, platinum and etoposide based chemotherapy remains the first-line treatment, but tumors are prone to recurrence and metastasis leading to poor prognosis [5, 6]. Therefore, a search for novel therapeutic strategies or biomarkers for early detection and prediction of prognosis and treatment responses could lead to better control of this disease.

Osteopontin (OPN) is a multifunctional secreted phosphorylated glycoprotein involved in the regulation of cell adhesion, migration and invasion [7, 8]. Previous studies have shown that many cancers, including pancreatic cancer, colon cancer, breast cancer, and NSCLC, have elevated OPN levels [9-13]. Studies have

shown that overexpression of OPN is closely related to tumor progression and poor prognosis [14, 15]. Although many studies have shown that OPN is associated with prognosis in a variety of cancers, the relationship between OPN expression and clinicopathological features remains unclear in SCLC patients. Therefore, in this study, we first evaluated serum OPN levels in SCLC patients and healthy controls, investigated the clinicopathological features of SCLC patients, and evaluated the treatment responses and overall survival. We sought to provide information regarding OPN as a biomarker to predict treatment responses and overall survival of SCLC patients.

Methods

Patients

In this retrospective case-control study, we obtained serum samples from 96 SCLC patients (66 males and 30 females) who were treated at the Nanjing Brain Hospital from January 2015 to April 2018. All patients were confirmed to be SCLC cases by histology or cytology. Patients who underwent surgery were excluded from the study, and patients who received systemic therapy within 6 months were also excluded. The SCLC patients were staged according to the Veterans Administration of Lung Cancer Study Group (VALSG) staging system [16], and all patients required a measurable disease by computed tomography. Patients were randomized to receive either standard treatment (up to 6 cycles) with etoposide and cisplatin (EP) or etoposide and carboplatin (EC), with or without radiotherapy. The therapeutic dose is adjusted according to the physical condition of each patient. The patient was regularly examined for physical examination, blood chemistry, abdominal ultrasound or chest computed tomography, brain MRI and bone scintigraphy.

In addition, we collected blood samples from 60 healthy controls who were examined at our hospital for health checks and matched with SCLC cases by age and gender.

Evaluation of therapy responses

All patients received 6 cycles of randomized treatment, including EP or EC plus or without radiotherapy, and were followed up regularly. Tumor response was measured 4 to 6 weeks after completion of all treatments using the criteria for assessment of solid tumor criteria, including complete response (CR, no residual tumor lesions), partial response (PR, less than one-third within the tumor lesion), Stable disease (SD, no change in tumor lesions) and progressive disease (PD, increased tumor size) [17].

These patients were followed up regularly and the last follow-up was July 1, 2019. We then calculated the overall survival (OS) for each patient, defined as the duration from the diagnosis of the disease to death or the patient's last visit. According to the recurrence site, recurrence is divided into local recurrence or distant metastasis.

Enzyme linked immunosorbent assay (ELISA)

After the diagnosis, blood samples are taken from the patient prior to treatment. After 6 cycles of chemotherapy, serum samples were collected again. The samples were centrifuged at 1,500 ×g for 10 min, serum was collected and stored at -80 °C until analysis. Serum OPN levels were determined using an anti-OPN monoclonal antibody enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, MN, USA) with a cut-off value of OPN: MDD (median detection density) of 0.22 ng/ml.

Statistical analysis

All statistical analyses were performed using SPSS v13.0 software (SPSS Inc., Chicago, Illinois, USA). Serum OPN levels were summarized as mean ± standard deviation. Differences between the two groups were analyzed by Student's t test. The Kaplan-Meier curve was used to plot the overall survival, and the log-rank test was used to generate p-values that were layered by OPN level. Cox regression model was used to analyze independent predictors of different clinical pathological features and serum OPN levels on overall survival. The receiver operating characteristic curve (ROC) was used to analyze the cut-off value of serum OPN in SCLC patients and healthy controls, while evaluating the area under the curve (AUC). P<0.05 was considered statistically significant.

Results

Patients' characteristics

Of the 96 patients with SCLC, 66 were male and 30 were female, with a median age of 55 years. Fifty-eight patients had a history of smoking, 38 had no history of smoking, 28 had limited SCLC, 58 had extensive SCLC, and 70 had lymph node metastasis. Most patients (n=80) received EP chemotherapy and radiotherapy (n=86). After treatment, 78 cases were CR or PR, and 18 cases were SD or even PD (**Table 1**). The median follow-up time was 12 months (2-30 months), the median OS was 11 months, the limited-stage SCLC was 14 months, and the extensive SCLC was 8.5 months.

Upregulation of serum OPN level in SCLC patients

The pre-treatment serum OPN levels in SCLC group were (72.07±19.09) ng/ml, compared with (36.06±5.48) ng/ml in the 60 healthy controls, indicating that the pre-treatment serum OPN levels were significantly higher in SCLC patients than those of the healthy controls (P=0.000, **Fig. 1A**). However, the levels of serum OPN were downregulated after treating the patients with chemoradiotherapy (72.07±19.09 pg/ml vs. 61.69±10.42 ng/ml, P=0.019, **Fig. 1B**).

Association of pretreatment levels of serum OPN with clinicopathological features from SCLC patients

We plotted the ROC curve to assess the cut-off value of pre-treatment serum OPN levels and found that 38 ng/ml is the cut-off value for serum OPN levels. We then analyzed the relationship between OPN expression and clinicopathological features in patients with SCLC. Our data showed that serum OPN levels were closely related to disease stage (P=0.012), tumor size (P=0.034), and lymph node metastasis (P=0.037) (**Table 2**).

Association of serum OPN levels with treatment response

The objective response rate (CR+PR) after chemotherapy and radiotherapy was 81.2% (78 of 96 cases), and the non-response rate (SD+PD) was 18.8% (18 of 96 cases). We then correlated serum OPN levels with treatment response and found that serum OPN levels were significantly associated with treatment response after treatment (*P*=0.011, **Fig. 2A**); however, pretreatment serum OPN levels were not significantly associated with treatment response (*P*=0.485, **Fig. 2B**).

Prognostic value of serum OPN for SCLC patients

We performed univariate and multivariate analyses to predict prognostic factors for OS in these patients. Our univariate analysis data showed that performance status, disease stage, and post-treatment serum OPN levels were prognostic factors for OS, and our multivariate analysis showed that performance status, disease stage, and post-treatment serum OPN levels were all prognostic predictors for OS of these patients. However, pre-treatment serum OPN levels were not associated with OS in these patients (**Table 3**).

We also used Kaplan-Meier curves and log-rank tests to correlate post-treatment serum OPN levels with OS in these SCLC patients and found that the reduced post-treatment level of serum OPN was associated with better OS of these SCLC patients (**Fig. 3**).

Discussion

SCLC remains a deadly disease, even as early as possible in the discovery and improvement of treatment options [18]. This study found that serum OPN levels in patients with SCLC were higher than in healthy controls. Therefore, serum OPN levels can be used as an effective biomarker to assess treatment response and prognosis in SCLC patients.

OPN is a secreted phosphorylated glycoprotein with a variety of biological activities [7, 8]. At the same time, OPN induces immune cells to return to their origin, thereby promoting the invasion and metastasis of tumor cells [19]. At present, many studies have shown that the abnormal expression of OPN is closely related to the occurrence and development of liver cancer, colon cancer and some gynecological malignancies. The mechanism may be that OPN promotes the formation of new blood vessels, chemotactic transfer of cytokines, adhesion and extracellular matrix, while inhibiting apoptosis in varying degrees [20].

OPN expression is an independent predictor of platinum-based first-line chemotherapy response and prognosis in patients with advanced NSCLC [21]. Patients with advanced NSCLC have low serum OPN levels and have a higher survival rate after chemotherapy than those with high OPN levels [22]. In SCLC, OPN reduces cisplatin-induced apoptosis and induces chemoresistance [23]. In our current study, we confirmed that serum OPN levels were higher in SCLC patients than in healthy controls. Our data also demonstrate that serum OPN can predict treatment response in patients with SCLC. All of these studies

demonstrate the effect of OPN expression on cancer progression and therapeutic response. Therefore, further research is needed. Overexpression of OPN in tumor tissues may be due to rapid growth of tumor cells, lack of proper blood supply, and death of tumor cells by induction of apoptosis or necrosis, leading to up-regulation of apoptosis-related proteins.

Previous studies have speculated that OPN secretion is associated with tumor cell proliferation [24]. In our study, we found that serum OPN levels were associated with VALSG stage, tumor size, and SCLC lymph node metastasis, suggesting that elevated serum OPN levels may be derived from tumor cells. In addition, our current data demonstrated that serum level of OPN was able to predict OS of SCLC patients, although proper detection of OPN levels in SCLC has not yet been established.

Conclusions

In summary, our study revealed the predictive value of serum OPN levels in patients with SCLC. However, before OPN is used as a prognostic and predictive indicator for SCLC, a large sample of multicenter studies is needed to validate our current data.

Abbreviations

OPN: Osteopontin; SCLC: Small cell lung cancer; ELISA: Enzyme-linked immunosorbent assay; NSCLC: Non-small cell lung cancer; EP: Etoposide and cisplatin; EC: Etoposide and carboplatin; CR: Complete response; PR: Partial response; SD: Stable disease; PD: Progressive disease; OS: Overall survival (OS)

Declarations

Authors' contributions

CHX carried out most of the experiment and writing this manuscript; YCW and QY did the ELISA; CZC, QZ, LL and WW collected data; RSY helped the design and all through the research. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was reviewed and approved by the Ethics Committee of Nanjing Brain Hospital. Informed consent was waived because this was a retrospectively study. We obtained patient data from the Medical Records and Statistics Room. We analysed the data anonymously. The use of the raw data was permitted by the Ethics Committee of Nanjing Brain Hospital.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1 The characteristics of SCLC patients and healthy controls.

Variables	SCLC patients	Healthy controls	*P*
Subject, No	96	60	
Median (range)	55 (36-75)	56 (38-74)	0.618
Male/Female	66/30	40/20	0.786
Smoking history			
Ever smoker	58	32	0.384
Never smoker	38	28	
Performance status			
0-1	56		
2-3	40		
Disease stage			
Limited	28		
Extended	58		
Tumor size (cm)			
≥3	76		
<3	20		
Lymph node metastasis			
N0	26		
N1-3	70		
Chemotherapy			
EP	80		
EC	16		
Radiotherapy sequence			
Concurrent	43		
After-chemotherapy	40		
None	13		
Responses			
CR + PR	78		
SD + PD	18		

EP, etoposide+platinum; EC, etoposide+cisplatin; CR, complete response; PR, partial response; PD, progressive disease; SD, stable disease.

Table 2 Association between serum OPN levels and characteristical features in SCLC patients.

Features	Number	OPN level (ng/ml)	*P*
Age, yr			0.954
≥60	54	73.78±16.55	
<60	42	73.43±17.90	
Gender			0.317
Male	66	75.38±16.44	
Female	30	68.38±18.19	
Smoking history			0.077
Ever smoker	58	68.32±20.11	
Never smoker	38	78.94±11.13	
Performance status			0.174
0-1	56	70.02±17.39	
2-3	40	78.27±15.55	
Disease stage			0.012
Limited	28	66.32±19.64	
Extended	58	80.94±9.41	
Tumor size (cm)			0.034
≥3	76	77.96±14.31	
<3	20	60.63±18.18	
Lymph node metastasis			0.037
N0	26	64.08±21.85	
N1-3	70	79.40±9.72	
Responses			0.263
CR + PR	78	71.15±18.33	
SD + PD	18	78.01±13.74	

OPN, osteopontin; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease.

Table 3 Univariate and multivariate Cox analysis of variables considered for OS of SCLC patients.

Characteristics	Univariate			Multivariate		
	HR	95% CI	*P*	HR	95% CI	*P*
Gender (Male vs. Female)	3.563	0.496-25.582	0.206	2.264	0.382-13.404	0.368
Age (<60 vs. ≥60)	0.819	0.140-4.806	0.825	2.091	0.514-8.502	0.302
Disease stage (Limited vs. Extended)	4.277	1.233-14.835	0.022	3.429	1.096-10.726	0.034
Lymph node metastasis (N_0 vs. N_{1-3})	0.585	0.157-2.182	0.424	2.358	0.606-9.181	0.216
PS (0–1 vs. 2–3)	4.820	1.196-19.424	0.027	4.004	1.281-12.513	0.017
Smoking history (Ever vs. Never)	1.719	0.551-5.365	0.351	0.649	0.218-1.933	0.438
Pre-OPN (Negative vs. Positive)	2.662	0.908-7.807	0.075	0.305	0.091-1.106	0.053
After-OPN (Negative vs. Positive)	4.936	1.793-13.586	0.002	6.114	1.661-22.510	0.006

CI, confidence interval; HR, hazard ratio; PS, performance status; OPN, osteopontin; OS, overall survival.

Figures

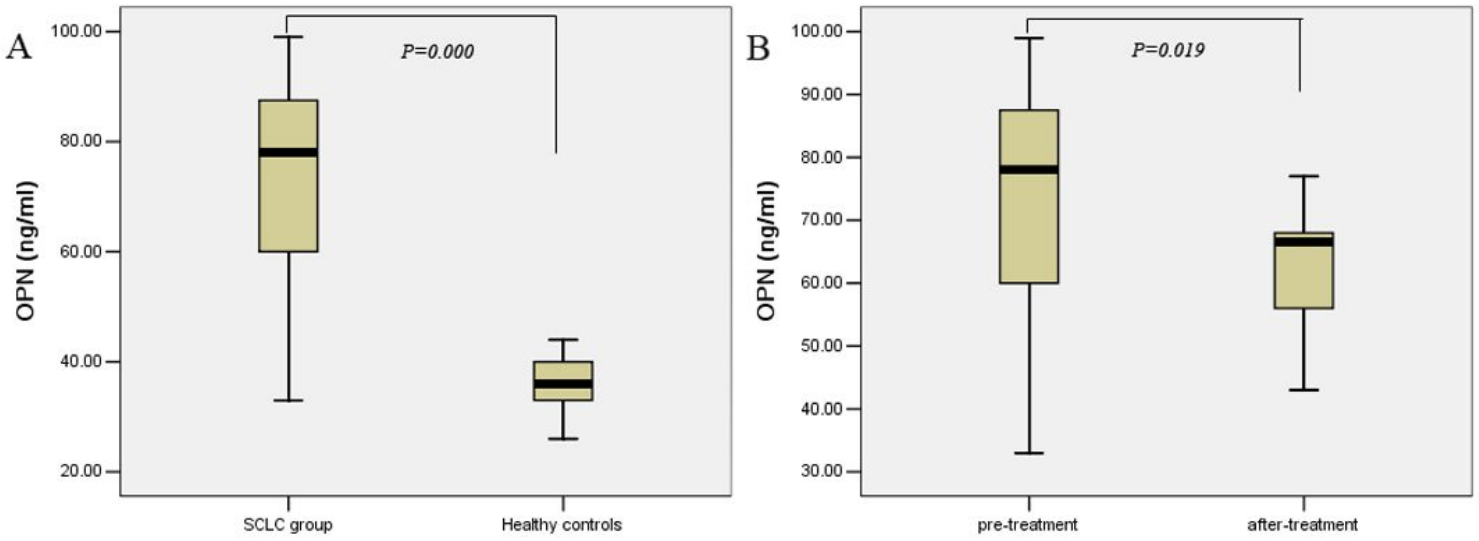

Figure 1

The serum levels of OPN in SCLC patients vs. healthy controls or pre-and post-treatment. (A) Patients with SCLC had higher serum OPN level than that of healthy controls (P=0.000). (B) Association of pre- and post-treatment levels of serum OPN in SCLC patients (P=0.019).

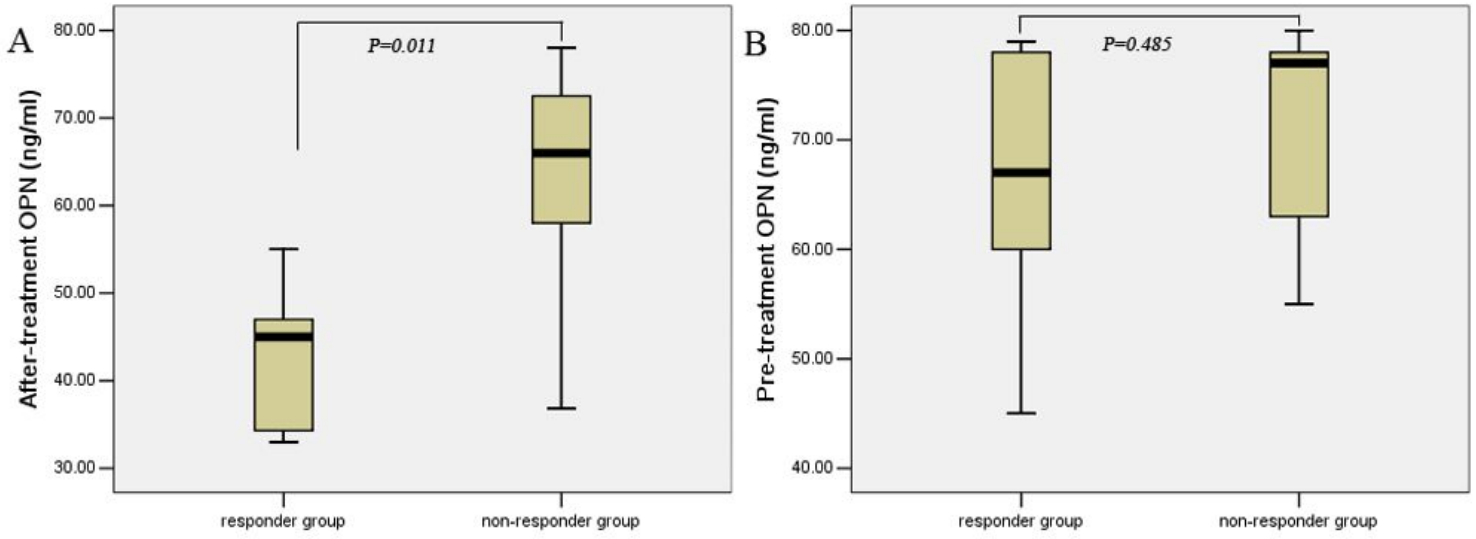

Figure 2

Association of pre- and post-treatment levels of serum OPN in SCLC patients. (A) Association of post-treatment levels of serum OPN in SCLC patients, with responders vs. non-responders (P=0.011). (B) Association of pretreatment levels of serum OPN in SCLC patients with responders vs. non-responders (P=0.485).

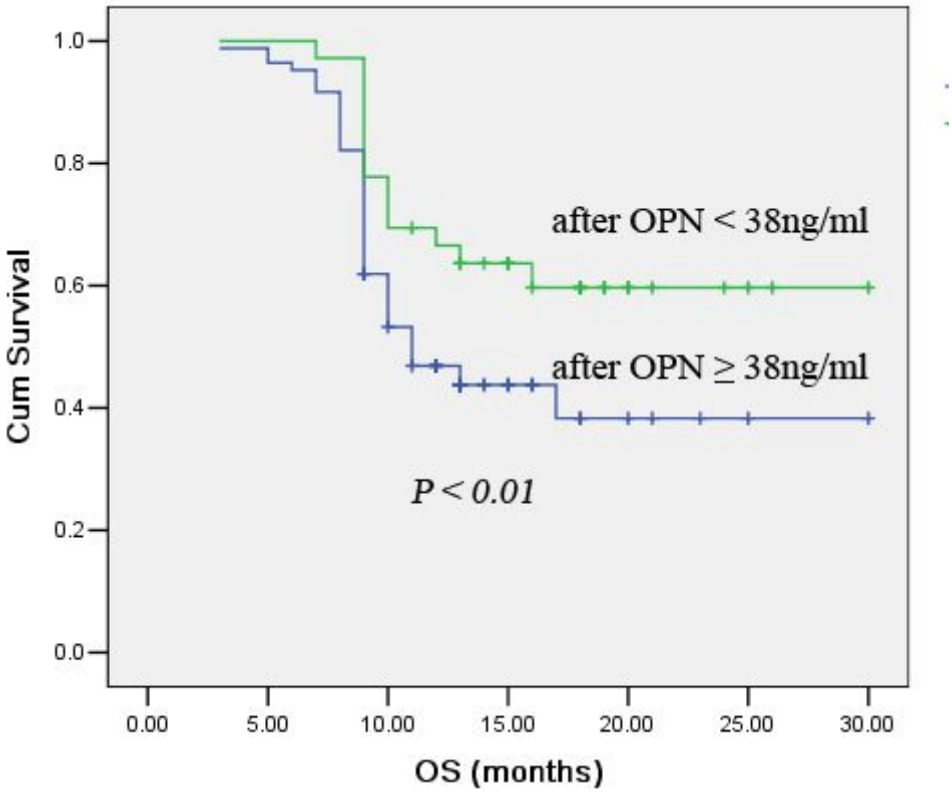

Figure 3

Kaplan–Meier curves stratified by the post-treatment levels of serum OPN. Log-rank test determined that the OS in low OPN group were significantly longer than those in the high OPN group ($P<0.05$).