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ORIGINAL ARTICLE

Osteopontin expression is associated with platinum-based chemotherapy response and prognosis of patients with advanced non small cell lung cancer

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Summary

Purpose: *To examine the expression of osteopontin (OPN) in patients with advanced non-small cell lung cancer (NSCLC), and to analyze the correlation of the expression level of OPN with response to platinum-based chemotherapy and the prognosis of NSCLC patients.*

Methods: *This retrospective study enrolled 92 patients with advanced NSCLC. All of the patients received platinum-based regimens as first-line chemotherapy. The expression of OPN in advanced NSCLC tissue was detected by immunohistochemistry. The associations between OPN expression with response to chemotherapy and the prognosis were analyzed by SPSS 17.0 statistical software.*

Results: *The positive expression rate of OPN in advanced NSCLC tissue was 36.9% (34/92). The positive expression rate of OPN was higher in patients ≥70 years old (65.2%;15/23) vs those aged <70 years (27.5%;19/69; p<0.05), which was also higher in patients with distant metastasis (51.9%;28/54) than that in patients without metastasis 15.8% (6/38) , the difference with statistically significant (p<0.05). Pearson's correlation analysis results showed that OPN expression was significantly correlated with age (r=0.338, p=0.001) , distant metastasis (r=0.368, p<0.001), curative effect of platinum-based regimen (r=0.403, p<0.001), progression-free survival/PFS (r=-0.486, p<0.001) and overall survival/OS (r=-0.552, p<0.001). Furthermore, the OPN expression was an independent predictor of PFS and OS for patients with advanced NSCLC after receiving platinum-based first-line chemotherapy (p<0.001).*

Conclusion: *OPN expression is an independent predictor of response to platinum-based first-line chemotherapy and of the prognosis of patients with advanced NSCLC.*

Key words: *carcinoma, non-small cell lung, osteopontin, platinum-based chemotherapy, response*

Introduction

NSCLC is a malignant tumor with highest morbidity and mortality. Platinum-based chemotherapy is a standard method as first-line treatment of patients with advanced NSCLC, whose OS ranges about 8-10 months [1]. Although the first-line platinum-based regimens, to a certain extent, can prolong patient survival and improve the quality of life, the overall response is not satisfactory, with 5-year survival rate < 15%, which is closely associated with platinum-resistant advanced NSCLC.

OPN is a protein secreted by bone stromal cell, with bone specificity and rich in sialic acid, which mainly plays a role of bridge between cell and its matrix. Studies in China and abroad indicate that OPN, as a new cytokine, is highly expressed in breast cancer, kidney cancer, esophageal cancer, endometrial cancer and various digestive tract tumors and is closely related to growth, migration, and invasion of tumor cells [2-4]. Philip et al. [5] showed that NSCLC patients with low OPN expression in plasma have obvious survival advan-

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tage after platinum-based chemotherapy.

In this study, immunohistochemistry was used to detect the OPN expression of 92 cases of tissue samples with advanced NSCLC and analyze the correlation between OPN expression level and response to platinum-based first-line chemotherapy and patient prognosis.

Methods

Patients and tissue samples

Ninety two patients with advanced NSCLC treated with chemotherapy in our hospital from March 2007 to August 2011 were selected for this study.

Inclusion criteria

Patients diagnosed with NSCLC after bronchoscopic biopsy or CT-guided FNB of the lung lung; patients with stage III and IV who could not be operated; enough pathological tissue could be provided for immunohistochemistry; first-line chemotherapy with platinum-based regimens; patients should have imaging studies before treatment, and treatment response evaluation should be done after the 2nd and 4th cycle of chemotherapy; patients should have normal blood, liver and kidney function tests and myocardial enzymes before treatment; ECG should be normal; Karnofsky performance status (KPS) score ≥70; expected survival ≥3 months.

Exclusion criteria

Patients with a history of previous malignancy and treated with chemotherapy.

Of the patients 53 (57.6%) were male and 39 (42.4%) female, aged from 37 to 78 years (median 65); 43 patients (46.7%) had squamous cell carcinoma and 49 (53.3%) adenocarcinoma; 38 (41.3%) patients had stage III and 54 (58.7%) stage IV; 20 (21.7%) patients received paclitaxel or docetaxel combined with platinum-based chemotherapy, 50 (54.3%) patients received gemcitabine combined with platinum-based chemotherapy, 9 (9.8%) navelbine combined with platinum-based chemotherapy, 7 (7.6%) pemetrexed combined with platinum-based chemotherapy, and 6 (6.6%) other drugs (vinblastine, etoposide, irinotecan and recombinant human endostatin) combined with platinum-based chemotherapy. Eleven patients (11.9%) received radiotherapy after chemotherapy, 3 (3.7%) had radical disease resection and 26 (28.3%) received epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) targeted therapy. The research was approved by the institutional ethics committee of the First Hospital of Lanzhou University. Written informed consent was obtained from each patient.

Immunohistochemical staining

All the postoperative pathological specimens were fixed with formaldehyde, embedded in paraffin and cut in sections 4μm thick; the detection of OPN expression was performed according to the streptavidin-peroxidase (S-P) steps of immunohistochemistry. The immunohistochemical kit was purchased from Shanghai Dingjie Biological Technology Co., Ltd; NSCLC tissue sections were conventionally dewaxed and placed in citrate buffer (pH=0.6) for the antigen retrieval under high pressure and at high temperature. H_2O_2 3% was added in the solution, which was incubated at room temperature for 5 min. Phosphate buffer saline (PBS) rinse solution was used to repeatedly wash the sample and fetal calf serum was added for blocking. After the addition of human-mouse anti-human OPN monoclonal antibody (Fuzhou Maixin Bio, China; dilution: 1:200), the sample was incubated at 4°C. On the 2nd day, 50 μl S-P solution were added and the sample was left at room temperature for 5 min. Next, PBS was used to wash. Later, DAB coloration was applied. The sample was then processed with hematoxylin counterstaining, rinsed by distilled water, and mounted by neutral gum. For each staining, the known positive sections were used as positive controls, and PBS was used to replace the primary antibody as negative controls.

Evaluation of immunohistochemical staining results

The prepared immunohistochemical sections were evaluated by 2 pathologists in our hospital with the single-blind method, and if dispute appeared, it was determined by one senior pathology expert. The proportion of the stained cells and the extent of the staining were used as criteria of evaluation. Staining was graded as follows: 0 for no staining; 1 for pale yellow staining; 2 for tan staining; 3 for sepia staining. The proportion of positive cells was graded as 0-100%. Positive expression of OPN cell nucleus refers to the product staining degree and the proportion of positive cells, and this should be >0.

Assessments

Tumor response (according to RECIST) included short-term and long-term efficacy of chemotherapy. The short-term efficacy was divided as complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD).The long-term efficacy was evaluated with respect to PFS and OS, where PFS referred to the time from starting treatment until tumor progression or death and OS referred to the time from starting treatment until death from any cause.

Statistics

SPSS 17.0 software (SPSS Inc., Chicago, IL) was used for statistical analysis of the data. Difference between groups was assessed using chi-square test or Fisher's exact test; correlation of different factors was

performed using Pearson's correlation analysis method; logistic regression model was used to perform multiple factors analysis, with backward step by step; Kaplan-Meier method was used for survival analysis and to calculate PFS and OS. Log-rank test and Cox regression model were used for multiple factor analysis. All the statistical tests were two-sided, with alpha=0.05, and p <0.05 was considered as statistically significant.

Results

OPN expression in advanced NSCLC tissue and its relationship with clinical features

According to the immunohistochemical staining, OPN positive protein staining was limited in the nucleus of tumor cells (Figures 1,2).

The positive expression of OPN was 36.9% (34/92). The positive OPN expression in patients <70 years old was 27.5% (19/69), and 65.2% (15/23) in those ≥70 years old (p<0.05). The positive expression of OPN of patients with distant metastasis was 51.9% (28/54), significantly higher than the positive expression of OPN (15.8% ; 6/38) of patients without distant metastasis (p<0.05). Correlation analysis results showed that OPN expression was significantly correlated with age (r=0.338, p=0.001) and distant metastasis (r=0.368, p<0.001) of advanced NSCLC patients, but not correlated with gender, pathological type, grade of differentiation and chemotherapy regimen (p >0.05) (Table 1).

Relationship between OPN expression and short-term therapeutic effect of advanced NSCLC patients

Pearson's correlation analysis results showed that OPN expression was significantly correlated with the response to platinum-based regimen (r=0.403, p<0.001). After correcting for gender, age, pathological type, grade of differentiation and metastasis, logistic regression analysis indicated that OPN expression (p=0.005) and grade of differentiation (p=0.000) were independent predictors of response to first-line platinum-based chemotherapy (Table 2). The response of advanced NSCLC patients with positive expression of OPN and poor differentiation treated with platinum-based chemotherapy was obviously worse than that of patients with negative expression of OPN and good differentiation (p<0.05).

Relationship between OPN expression and long-term therapeutic effect of advanced NSCLC patients

Pearson's correlation analysis showed that

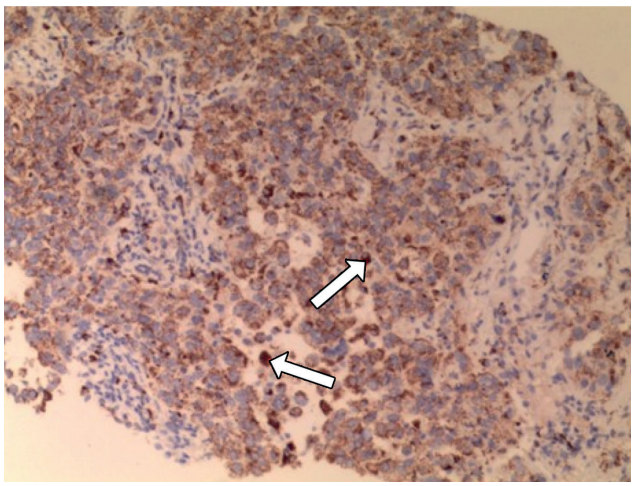

Figure 1. Expression of OPN in lung squamous cell carcinoma (arrows, ×200).

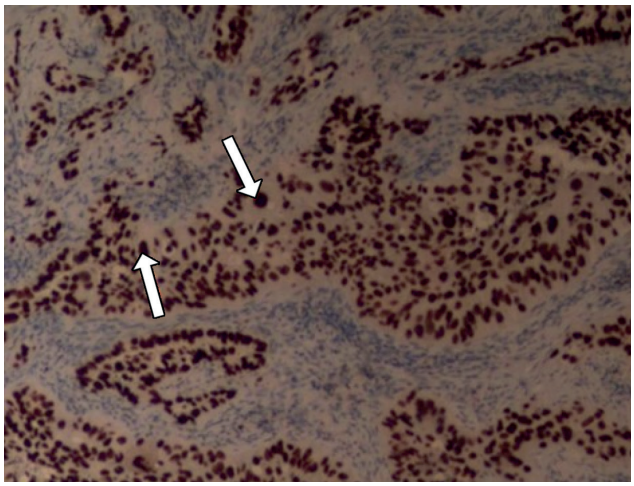

Figure 2. Expression of OPN in lung adenocarcinoma (arrows, ×200).

OPN expression was significantly correlated with PFS (r=-0.486, p<0.001) and OS (r=-0.552, p<0.001). Kaplan-Meier survival analysis showed that the mean PFS of patients with negative expression of OPN was 6.38±0.64 months (95% CI: 5.12-7.63), and the mean OS was 20.91±0.49 months. The mean PFS of patients with positive expression of OPN was 3.69±0.25 months and the mean OS was 11.03±0.18 months. PFS and OS of patients with negative OPN expression were significantly higher than that of patients with positive expression of OPN (p<0.001; Figure 3). After correcting for gender, age, pathological type, grade of differentiation, metastasis and chemotherapy regimens, Cox multivariate analysis indicated that OPN expression was an independent predictor of PFS and OS for patients with advanced NSCLC after receiving platinum-based first-line chemotherapy (p<0.001), meaning that the PFS and OS of advanced NSCLC patients with negative OPN expression were sig-

Table 1. Relationship between expression of OPN and clinical characteristics of NSCLC patients

Clinical characteristics	*Cases N*	*OPN positive expression rate N (%)*	x^2	*p-value*
Gender			0.481	0.488
Male	53	18 (34.0)		
Female	39	16 (41.0)		
Age/years			10.513	0.001
< 70	69	19 (27.5)		
≥ 70	23	15 (65.2)		
Pathologic type			0.149	0.7
Squamous cell	43	15 (34.9)		
Adenocarcinoma	49	19 (38.8)		
Differentiation			2.731	0.255
Low	4	1 (25.0)		
Moderate	62	20 (32.3)		
High	26	13 (50.0)		
Smoking history			0.000	0.992
Yes	65	24 (36.9)		
No	27	10 (37.0)		
Metastasis			12.450	<0.001
M0	38	6 (15.8)		
M1	54	28 (51.9)		
First-line chemotherapy with platinum-based regimens including:			7.735	0.102
Taxanes	20	6 (30.0)		
Gemcitabine	50	18 (36.0)		
Navelbine	9	4 (44.4)		
Pemetrexed	7	1 (14.3)		
Others	6	5 (83.3)		
Response			16.059	<0.001
PR	33	6 (18.2)		
SD	40	14 (35.0)		
PD	19	14 (73.7)		

For abbreviations see text

Table 2. Comparison of variables related to response to platinum-based first-line chemotherapy by univariate logistic regression analysis

Variables	*Regression coefficient β*	*Standard error*	*Wald*	*p-value*	*Exp (B)*	*95%CI*
Gender	0.330	0.734	0.202	0.653	1.390	0.330-5.86
Age	0.029	0.831	0.001	0.972	1.030	0.202-5.251
Pathologic type	-0.890	0.790	1.270	0.260	0.411	0.087-1.931
Differentiation	3.544	0.843	17.658	0.000	34.604	6.626-180.719
Metastasis	-0.164	0.860	0.036	0.849	0.849	0.157-4.583
OPN	2.522	0.890	8.037	0.005	12.457	2.178-71.243

Table 3. Cox multivariate regression analysis of factors related to disease-specific survival of NSCLC patients

Factors	*Regression coefficient β*	*Standard error*	*Wald*	*p-value*	*Exp (B)*	*95%CI*
PFS						
Gender	-0.127	0.224	0.322	0.570	0.881	0.568-1.366
Age	-0.223	0.288	0.602	0.438	0.800	0.455-1.406
Pathologic type	0.286	0.224	1.634	0.201	1.331	0.859-2.064
Differentiation	0.165	0.216	0.587	0.444	1.180	0.773-1.801
Metastasis	0.273	0.229	1.422	0.233	1.314	0.839-2.058
Chemotherapeutic regimen	-0.321	0.255	1.582	0.209	0.726	0.44-1.196
OPN	1.094	0.247	19.597	<0.001	2.985	1.839-4.844
OS						
Gender	-0.378	0.271	1.940	0.164	0.686	0.403-1.166
Age	-0.397	0.313	1.610	0.204	0.672	0.364-1.241
Pathologic type	-1.806	0.308	34.316	<0.001	0.164	0.09-0.301
Differentiation	0.382	0.241	2.515	0.113	1.465	0.914-2.349
Metastasis	0.174	0.270	0.415	0.519	1.190	0.701-2.02
Chemotherapeutic regimen	-0.745	0.289	6.635	0.010	0.475	0.269-0.837
EGFR-TKI treatment	-0.477	0.240	3.953	0.047	0.620	0.388-0.993
OPN	2.486	0.344	52.106	<0.001	12.010	6.115-23.587

For abbreviations see text

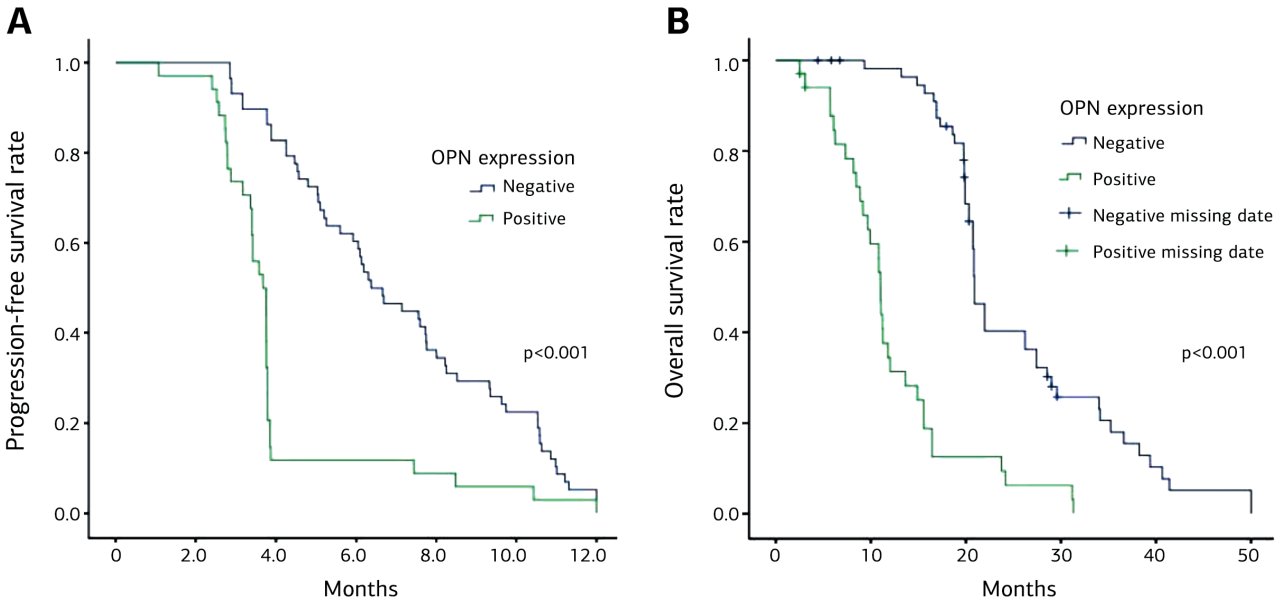

Figure 3. Kaplan-Meier survival curves of patients with advanced NSCLC receiving first-line platinum-based chemotherapy (N=92). **A:** Progression-free survival; **B:** Overall survival.

nificantly longer. After correcting for gender, age, pathological type, grade of differentiation, type of chemotherapy and acceptance of EGFR-TKI targeted therapy or not, we found that pathological type (p<0.001) and acceptance of EGFR-TKI targeted therapy or not (p=0.047) were independent predictors of OS of advanced NSCLC patients. Adenocarcinoma patients who were treated with EGFR-TKI had longer OS (Table 3).

Discussion

Response to therapy is closely related to the prognosis of patients with advanced NSCLC, so searching for predictors of response to chemotherapy has been one of the hot spots in clinical research. The study by Snitcovsky et al. showed the patients with head and neck tumors and low serum OPN level attained better response rates

after receiving radiation and chemotherapy [8]. Advanced NSCLC patients with low serum OPN level have better survival rate than patients with high OPN level after receiving platinum-based chemotherapy [7]. The studies above suggest that OPN level may be closely associated with resistance to platins, implying that OPN may be the ideal marker to predict response to chemotherapy.

OPN, a secreted phosphorylated glycoprotein with thrombin cracking site, $\alpha_9\beta_1/\alpha_4\beta_1$ combined domain and several binding sites of elements such as Ca^{2+}, can produce a variety of biological activities, whose C terminal is coupled with the transmembrane glycoprotein CD44 [9-13]. At the same time, OPN induces the return of immune cells to their sites of origin, which in turn facilitates invasion and metastasis of tumor cells [14-16]. The abnormal expression of OPN in the body has a certain cell specificity and is adjusted and controlled by several factors such as cancer suppressor genes, hormones, proto-oncogenes, carcinogens, and growth factors. At present, many studies show that abnormal expression of OPN is closely related with the occurrence and development of tumors such as liver cancer, breast cancer, colon cancer and some gynecological malignant tumors, whose possible mechanism is that OPN promotes the formation of neovascularization, chemotactic transfer and adhesion of cytokines as well as extracellular matrix degradation, while it inhibits cell apoptosis in different degrees [17-19].

Our results, based on immunohistochemistry, showed that OPN expression varies in advanced NSCLC patients, with positive expression rate of OPN of 36.9%. According to the statistics, patients aged ≥70 years had high positive rate of OPN, but OPN expression was not correlated with smoking, gender, and histological type, which may be due to the small number of cases. This study found that the positive rate of OPN expression of patients with distant metastasis (51.9%) was significantly higher than that of patients without distant metastases (15.8%), showing that tumor cells of advanced NSCLC patients with positive OPN expression were more aggressive, which may be related to OPN combined with intergrin receptors on the surface of cell membrane and the extracellular matrix to promote adhesion and distant metastasis [20]. OPN is an independent predictor of response to chemotherapy. PFS and OS of patients with negative expression of OPN were significantly prolonged, which is similar with the results of the SWOG Study S0003 [7]. Negative OPN expression is predictive of good response in patients with advanced NSCLC, based on the following observations: OPN negative patients had high objective remission rates and they could receive more cycles of chemotherapy, and also both the proliferation and metastatic potential of tumor cells were reduced.

To conclude, OPN expression is significantly correlated with the response of first-line platinum-based chemotherapy, and PFS and OS of patients with advanced NSCLC. As a consequence, the detection of expression of OPN can predict the sensitivity to platins, which can help develop individualized chemotherapy regimens for NSCLC patients.

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