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Original Article

Expression of β-catenin and OPG in human non-small cell lung cancer and their clinical significance

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Abstract: The aim of the study was to identify beta chain protein (β-catenin) and osteoprotegerin (OPG) expression levels in non-small cell lung cancer (NSCLC), and to study their clinical significance. The serum OPG levels were concurrently investigated to explore the association between serum levels and clinicopathologic characteristics. β-catenin and OPG immunohistochemical analysis was performed on 101 NSCLC tissues and 24 adjacent normal lung parenchyma of these patients. Chi-squared test was used to compare clinicopathologic characteristics in different groups. 88 serum samples of NSCLC were collected. Enzyme Linked Immunosorbent Assay (ELISA) was used to test the concentrations of the samples. A *P*-value <0.05 was considered to be statistically significant. The expression of β-catenin and OPG was found to be significantly higher in NSCLC than that in adjacent normal lung parenchyma (P<0.001). The level of high β-catenin expression was significantly associated with lymph node metastasis (P = 0.022) and the level of high OPG expression was significantly associated with lymph node metastasis (P<0.001) and clinical stage (P<0.001). β-catenin and OPG protein expression in NSCLC were positively correlated (r = 0.490, P<0.05); High OPG expression in tissues was significantly associated with serum tumor marker concentrations (P<0.05); There was a slight rise in serum OPG level in patients with early stage. In patients with stage IV and recurrence, OPG demonstrated a higher level. The current study demonstrated that the expression of β-catenin and OPG in NSCLC was significantly higher than that in normal lung tissues. β-catenin and OPG expression in the tissues were positively correlated. With the progress of the disease, the serum levels of OPG were elevated. It can be concluded that OPG has certain potential to be a biological marker of NSCLC.

Keywords: Beta-catenin, OPG, Non-small-cell lung carcinoma, immnohistochemistry

Introduction

Lung cancer is the most common cancer worldwide, and has the highest incidence and mortality levels than any other cancer [1]. NSCLC is thought to originate from lung epithelial cells, and comprises diverse histological sub-types including adenocarcinoma, bronchioloalveoar, squamous, anaplastic and large-cell carcinomas [2]. Non-small cell lung cancer (NSCLC) accounts for over 80% of all lung cancer patients [3]. In spite of recent advances in surgical techniques and different strategies of chemotherapy and radiotherapy, the 5-year survival rate of patients with NSCLC is only about 15% [4]. Moreover, due to little symptoms at early stage and the difficulty in making an early diagnosis, most patients with NSCLC are diagnosed at advanced stages. Therefore, it is necessary to identify molecular markers which provide early diagnosis and accurate prognosis prediction.

β-catenin was first found as an adhesion factor. Then it was reported that β-catenin was a multifunctional protein [5]. β-catenin has double function [6]. It can interact with cadherin on the cell membrane, participating in intercellular adhesion. Research had shown that protein complex formed by β-catenin and cadherin played an important role in the cell-cell and cell-matrix relationship [7]. With high β-catenin expression, ability of adhesion between cells was enhanced, then cell invasion and metasta-

sis would not happen easily; On the contrary, with lower β-catenin expression, intercellular adhesion effect was reduced, so cell invasion and metastasis would more likely to happen. Another role of β-catenin is working as an important molecular in classical Wnt signaling pathway [8], regulating cell growth, differentiation and apoptosis [9]. Abnormal accumulation of β-catenin in lung cancer cell is closely related to the occurrence, development, invasion and metastasis of tumor [10].

Wint/β-catenin activation promotes prostate tumor progression in a mouse model [11]. β-catenin is required for prostate development and cooperates with Pten loss to drive invasive carcinoma [12]. In recent years, β-catenin expression in lung cancer has been researched, but research is limited. Whether β-catenin is correlated with pathological characteristics such as tumor stage is controversial.

OPG is an inhibitory receptor of tumor necrosis factor related apoptosis inducing ligand, namely TRAIL [13]. OPG activates integrin, focal adhesion kinase, and Akt signaling in ovarian cancer cells to attenuate TRAIL-induced apoptosis [14]. For a long time, OPG was researched as “bone protection factor”. The researches on OPG in bone metastasis are comparatively mature. Study [15] found that OPG expression in tissues of lung cancer patients with bone metastasis was elevated. Another study [16] found that there were differences in serum OPG levels of colon cancer patients with different clinical stages. With the late stage, the levels of serum OPG rised. According to another study [17], OPG seemed to be a potential colon cancer molecular marker, predicting tumor stage and prognosis. But in other types of cancer, there were not so many researches. Small sample study [15] pointed out OPG expression in tissues was correlated with lymph node metastasis and tumor stage in lung cancer patients. But it still lacks large sample research to verify. In the future, we need to devote more time into the study on OPG which is likely to be a bio-marker predicting the development of lung cancer.

Enrico et al [16] also found that β-catenin could interact with T cell factor (TCF) to raise the expression of OPG. In histology, the relationship between β-catenin and OPG expression has not yet been studied. In our study, we investigated β-catenin and OPG expression levels in NSCLC tissues to study their mutual relationship and clinical significance. At the same time, the serum OPG levels were investigated and the potential of OPG as a molecular marker of NSCLC patients was explored.

Materials and methods

Patients and samples

The samples of 101 patients with clinical stage I-III NSCLC were collected from Zhongnan Hospital of Wuhan University between September 2013 and December 2014. Among the 101 patients, only 24 cases were NSCLC combined with normal adjacent lung parenchyma. In these 101 NSCLC cases, there were males and females with age ranging from 37 to 76 years. According to the Union for International Cancer Control-American Joint Committee on Cancer (UICC/AJCC, seventh edition), there were 65 patients with stage I-II, 36 patients with stage III. None of the patients received radiotherapy or chemotherapy before curative resection. OPG serum concentrations of 23 healthy individuals, and 65 patients with non-small cell lung cancer were assayed by ELISA according to the manufacturer’s instructions. According to UICC/AJCC, the patients with NSCLC lung cancer included 21 patients with stage I-II, 22 patients with stage III, 22 patients with stage IV or recurrence. In the patients with stage IV or recurrence, only 2 had overt bone metastasis. The study was approved by the ethics committee of the Zhongnan Hospital of Wuhan University and informed consent was obtained from each patient undergoing surgery.

Immunohistochemistry

Collected 125 tissue samples of postoperative patients with non-small cell lung cancer were placed in 4°C refrigerator after being soaked with 4% paraformaldehyde. After 24 h to 48 h, we used paraffin embedding to treat the tissues. β-catenin and OPG expression was analyzed immunohistochemically on 4 micrometer-thick, formalin-fixed, paraffin-embedded specimen sections. Sections were deparaffinized in xylene and dehydrated in ethanol, and treated with a solution of peroxidase-blocking reagent (Dako, Glostrup, Denmark) to exhaust endogenous peroxidase activity. β-catenin and OPG

proteins were respectively used ethylene diamine tetraacetic acid (EDTA) microwave repair, EDTA repair with high temperature and high pressure to repair antigen hidden inside the tissue due to formalin fixation. Then paraffin sections were treated with 3% hydrogen peroxide solution under dark light and were treated at room temperature for 10 min. The sections were washed three times with PBS buffer, each time 5 min. To inhibit non-specific antigen-antibody reactions possible in immunohistochemical staining, protein blocker (Research Genetics, Huntsville, AL, USA) was used for 5 min and the sections were washed thoroughly with PBS buffer. Then the sections were incubated overnight with the primary antibodies against β-catenin (1:100; rabbit polyclonal antibody, ab32572, Abcam, UK) and OPG (1:20; rabbit polyclonal antibody, AB21015a, Bio-engineering Limited Company, Shanghai, China) at 4°C. After a PBS wash, sections were incubated with the goat anti-rabbit secondary antibody (1:200; BA1003, Boster Bio-engineering Limited Company, Wuhan, China) for 20 min at room temperature, followed by further washing and a complex of avidin with horseradish peroxidase was then applied for 20 min at room temperature. Finally, the sections were stained with 3, 3'-diaminobenzidine (DAB, Sigma-Aldrich, St Louis, MO, USA), then they were counterstained with hematoxylin and mounted. In each immunohistochemistry run, the positive section provided by reagent manufactory served as positive control and omission of the primary antibody served as negative control.

Immunohistochemical staining evaluation

Staining evaluation of β-catenin and OPG expression was carried out with bright-field light microscopy independently by two experienced pathologists who had no knowledge of the clinicopathologic information. Moderate or above grade β-catenin staining and staining area equal to or greater than 50% was evaluated to be high expression, and others were evaluated to be lower expression. OPG protein expression levels were classified semiquantitatively combining the proportion of positive of cancer cells (0, none; 1, <10%; 2, 10-50%; 3, 51-80%; 4, >80%) and staining intensity (0, negative; 1, weak; 2, moderate and 3, strong). The final scores were summed for percentage and intensity of positive cells. Scores of 0-3 were defined as "negative expression" (-), scores 4-5 as "weakly positive expression" (+), and scores of 6-7 as "strongly positive expression" (++). In addition, all scores were divided into two groups: low expression (scores 0-5) and high expression (scores 6-7) in NSCLC samples.

Analysis of OPG in serum

We collected 3 ml peripheral venous blood in each patients; After natural solidification and 3000 r/min centrifugal, we obtained supernatant, and then placed supernatant in -80°C refrigerator. ELISA assay was used to detect the concentrations of serum OPG, and it was carried out in accordance with the instructions (EK0480, Boster Bio-engineering Limited Company, Wuhan, China). Then we added 100 μl standard 6000 pg/ml, 3000 pg/ml, 1500 pg/ml, 750 pg/ml, 375 pg/ml, 187.5 pg/ml, 93.8 pg/ml samples to 7 holes respectively, and added sample diluent to another hole as zero hole. The rest holes were added with 100 μl diluent samples. With enzyme label plate covered, samples reacted for 90 min at 37°C. The liquid inside the enzyme label plate was washed with automatic washing machine, or shaken off, and then tapped on the blotting paper. Without wash, 0.1 ml OPG antibody working liquid was added in each hole. Each hole reacted for 60 min at 37°C. Then each hole was washed with 0.01 M TBS for 1 min. Prepared 0.1 ml ABC working liquid was added in each hole, reacting for 30 min at 37°C. 0.01 M TBS was used to wash the hole for five times, each time 1 to 2 min. All holes were added with 90 μl TMB substrate which had been balanced at 37°C for 30 min. Then each hole reacted for 25 min to 30 min at 37°C without light. In the end, each hole was added with 0.1 ml TMB terminated liquid, with the same order as adding TMB substrate. Then blue immediately turn into yellow. We drawed the standard curve according to the standard samples. With the standard curve, sample concentrations were figured out. The preliminary experimental results showed that the samples did not need any dilution, so the calculated value was the actual concentration of the sample.

Statistical analyses

All statistical analyses were performed by SPSS software version 21.0 (SPSS Inc., Chicago, IL, USA). The association of clinicopathologic char-

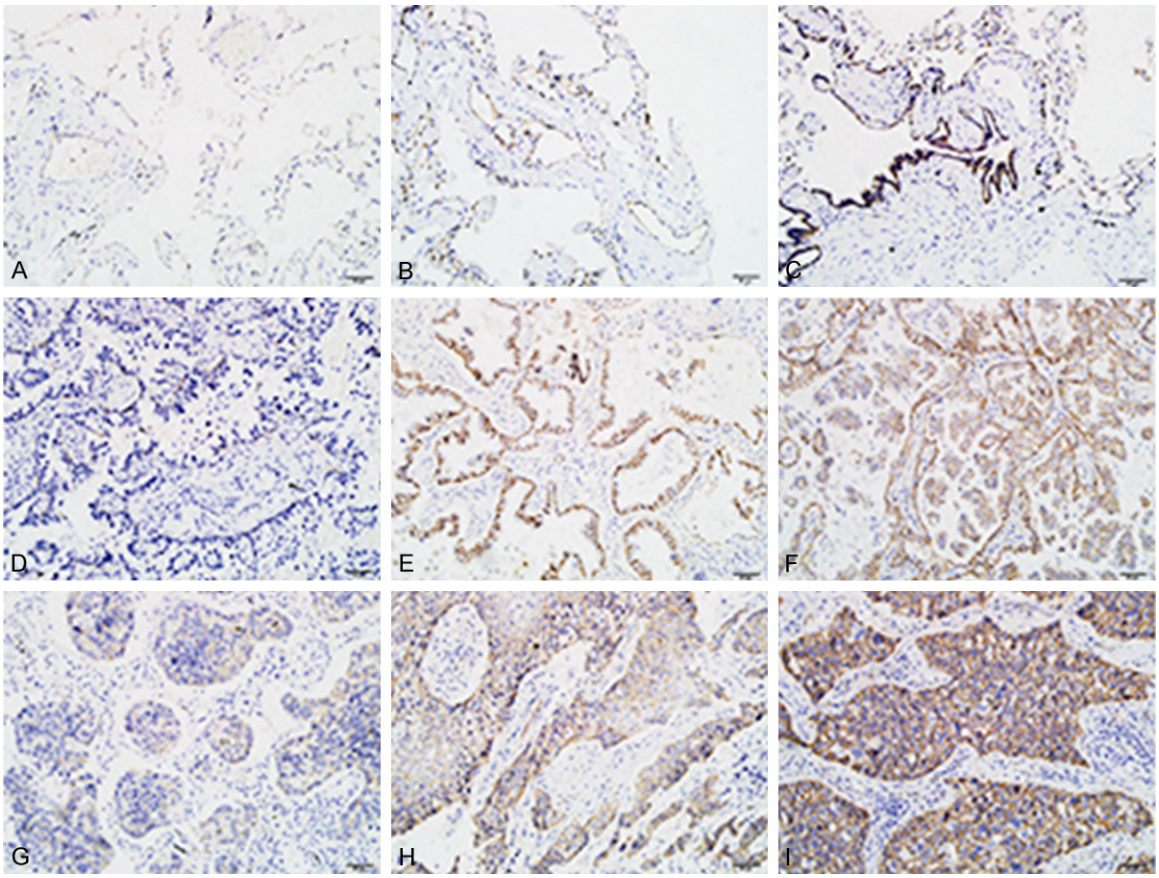

Figure 1. Expression of β-catenin by immunohistochemistry in normal lung tissues and NSCLC (magnification ×200). The expression of β-catenin was significantly elevated in NSCLC compared with normal lung parenchyma (P<0.001). A-C were the negative, weak, strong expression in normal lung tissues respectively. D-F were the negative, weak, strong expressions in adenocarcinomas respectively. G-I were the negative, weak, strong expressions in squamous cell carcinomas respectively. Representative results are shown.

acteristics with β-catenin and OPG expression status was analyzed by the Pearson's χ^2 test or Fisher's exact test for categorical variables. OPG serum levels were performed by Graphpad software version 5.1. Unpaired t test or single factor variance analysis was used to perform the statistical analyses. Measurement data was shown with x±s; A *P*-value <0.05 was considered to be statistically significant.

Results

β-catenin and OPG are highly expressed in NSCLC

We measured subcellular localization and the protein levels of β-catenin and OPG in 101 paraffin-embedded NSCLC samples and 24 paraffin-embedded normal lung tissues by immunohistochemical staining (**Figures 1**, **2**). The expression of β-catenin was mainly observed in plasmalemma and cytoplasm. High β-catenin expression was detected in 40 (39.6%) of 101 NSCLC samples, compared with only 3 (12.5%) of the 24 matched normal lung tissue samples. The expression of OPG was mainly observed in cytoplasm. High OPG expression was detected in 43 (42.6%) samples, compared with none of 24 match normal lung tissue samples. The expression of β-catenin and OPG was significantly elevated in NSCLC compared with normal lung parenchyma (P<0.001, **Table 1**).

Relationship between β-catenin/OPG expression and clinicopathological parameters of NSCLC patients

The relationship between β-catenin/OPG expression and clinicopathological characteristics in patients with NSCLC were summarized

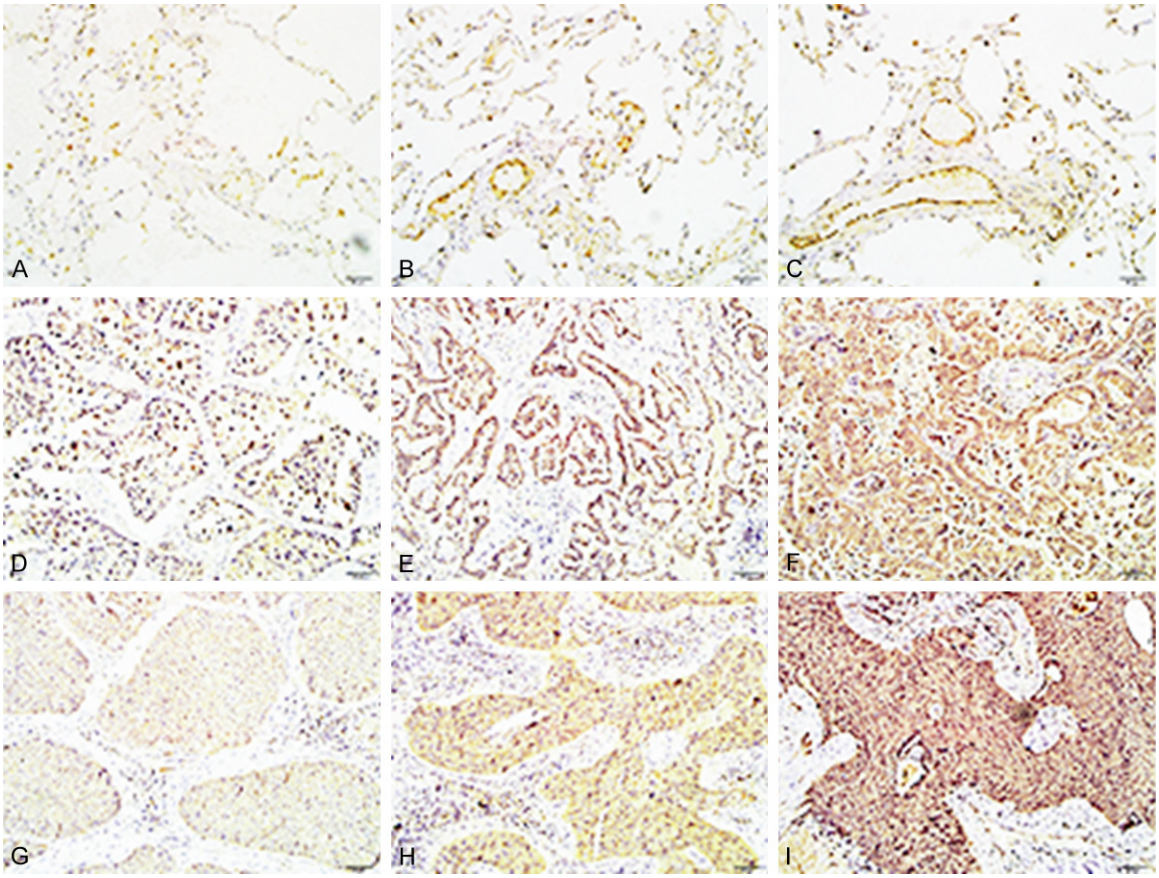

Figure 2. Expression of OPG by immunohistochemistry in normal lung tissues and NSCLC (magnification ×200). The expression of OPG was significantly elevated in NSCLC compared with normal lung parenchyma (P<0.001). A-C were the negative, weak, weak expression in normal lung tissues respectively. D-F were the negative, weak, strong expressions in adenocarcinomas respectively. G-I were the negative, weak, strong expressions in squamous cell carcinomas respectively. Representative results are shown.

Table 1. Summary of immunohistochemical expression of β-catenin and OPG in NSCLC tissues and normal tissues of patients

Group	β-catenin staining			*P* value	OPG staining			*P* value
	- (%)	+ (%)	++ (%)		- (%)	+ (%)	++ (%)	
Normal	16 (66.7)	5 (20.8)	3 (12.5)	<0.001	19 (79.2)	5 (20.8)	0	<0.001
Cancer	5 (5.0)	56 (55.4)	40 (39.6)		6 (6.0)	52 (51.5)	43 (42.6)	

in **Table 2**. The high expression of β-catenin was significantly more prevalent in lymph node metastasis-positive than lymph node metastasis-negative cases (52.3% vs. 29.8%, respectively; P = 0.022). High expression of OPG was significantly more prevalent in lymph node metastasis-positive than lymph node metastasis-negative cases (61.45% vs. 28.1%, respectively; P<0.001), and NSCLC samples with high-expression of OPG was significantly correlated with poor clinical stage (24.6% vs. 75.0%, respectively; P<0.001). Statistical analysis revealed no significant correlations between β-catenin/OPG expression and age, gender, histological type and tumor size.

Correlation between the β-catenin and OPG expression in tissue samples

According to β-catenin and OPG protein expression in non-small cell lung cancer, β-catenin and OPG protein expression in NSCLC in tissues were positively correlated (r = 0.490, P<0.05, **Table 3**).

Table 2. NSCLC patients' characteristics and β-catenin/OPG expression

Characteristics	Number of patients	β-catenin			*P* value	OPG			*P* value
		-	+	Positiverate (%)		-	+	Negative rate (%)	
NSCLC	101								
Age					0.835				0.820
>60 years	48	28	20	41.7		27	21	43.8	
≤60 years	53	32	21	39.6		31	22	41.5	
Gender					0.880				0.648
Female	42	25	17	40.5		23	19	45.2	
Male	59	36	23	39.0		35	24	40.7	
Histology					0.794				0.961
SCC	42	26	16	38.1		24	18	41.9	
ADC	59	35	24	40.7		34	25	42.4	
Pathologic differentiation					0.630				0.990
Moderate/High	61	38	23	37.7		35	26	42.6	
Low	40	23	17	42.5		23	17	42.5	
Tumor size					0.537				0.874
>5 cm	26	18	8	30.8		16	10	38.5	
3~5 cm	53	31	22	41.5		30	23	43.4	
<3 cm	22	12	10	45.5		12	10	45.5	
LN Metastasis					0.022				<0.001
Positive	44	21	23	52.3		17	27	61.3	
Negative	57	40	17	29.8		41	16	28.1	
TNM stage					0.112				<0.001
I-II	65	43	22	33.8		49	16	24.6	
III	36	18	18	50.0		9	27	75.0	

SCC, squamous cell carcinoma; ADC, adenocarcinoma; LN, lymph node.

Table 3. Correlation between β-catenin and OPG expression in NSCLC tissues

		β-catenin		r value	*P* value
		+	-		
OPG	+	29	14	0.490	<0.05
	-	11	47		

Table 4. Serum tumor marker levels and OPG expression in tissues

Variables	n	OPG expression		*P* value
		Low	High	
Tumor markers				
Abnormal	36	17 (47.2)	19 (52.8)	0.045
Normal	37	26 (70.3)	11 (29.7)	
CEA				
>5	25	10 (40.0)	15 (60.0)	0.069
<5	55	34 (61.8)	21 (38.2)	
CA125, CA199				
Normal	48	28 (58.3)	20 (41.7)	0.406
Abnormal	16	6 (37.5)	10 (62.5)	

Relationship between OPG expression in tissue samples and tumor marker serum levels in NSCLC patients

We collected the information of preoperative tumor markers in the 101 patients. Statistical results showed that there was a difference between normal tumor markers group and abnormal group, and the high expression of OPG was associated with abnormality in tumor marker levels (P<0.05, **Table 4**). But in the presence of single abnormality in CEA or CA125/CA199, there was no statistical difference between the OPG expression and serum tumor marker levels (P>0.05).

Relationship between the serum OPG concentrations and clinicopathological parameters of NSCLC patients

To better understand the expression of OPG in patients with NSCLC, we assessed OPG concentration in serum from healthy individuals,

Table 5. NSCLC patients' characteristics and serum OPG levels

Characteristics	No. of patients (%)	OPG levels x±s (pg/ml)	t value	*P* value
Age				
>60 years	30	148.6±16.71	0.4817	0.6317
≤ 60 years	35	159.1±14.25		
Gender				
Male	39	146.4±16.27	0.8895	0.3771
Female	26	166.0±11.62		
Tumor size (diameter)				
>5 cm	23	164.4±12.37	0.6893	0.4931
≤ 5 cm	42	148.7±15.34		
Histology				
ADC	30	141.9±19.23	1.328	0.1889
SCC	35	170.7±11.54		
Pathologic differentiation				
Moderate or high	34	158.2±14.64	0.3765	0.7078
Low	31	149.9±16.22		
Lymph node metastasis				
Positive	34	158.7±16.35	0.6499	0.5181
Negative	31	144.0±15.50		
TNM stage				
I-II	21	86.78±12.73		<0.0001
III	22	152.9±10.61		
IV or recurrence	22	220.0±19.49		

patients with lung adenomas, and patients with various stages of NSCLC lung cancers (**Table 5**). There was no significant difference in age between healthy individuals and cancer patients. The mean serum concentration in healthy individuals was 93.45 pg/ml; In patients with lung cancer, serum OPG levels were found to be in the range between 90 and 300 pg/ml. We observed a significant increase in serum OPG concentrations in patients with stage III and IV cancer compared with healthy individuals (P<0.001, respectively, **Figure 3**). Moreover, a significant increase in OPG serum levels was observed in patients with metastatic disease compared with patients with locally advanced disease (UICC stage IV or recurrence versus UICC stage III; P = 0.0043). The majority of the stage IV patients had brain metastases, only two patients had bone metastases, ruling out that bone metastasis or recurrence might be the underlying cause for elevated OPG serum levels. Additionally, we observed a trend between OPG serum level and lung adenomas, although this was not statistically significant. Results also showed that there was no significant correlation between OPG serum levels and lymph node metastasis and OPG serum levels did not relate with the serum tumor marker levels.

Discussion

The study on β-catenin expression in cancer is controversial. Recent researches demonstrate β-catenin has played important roles in several aspects. Wnt/β-catenin signaling pathway participates in the process of mammalian neural development, also in the adjustment of the cerebral cortex and the number of neural stem cells [18]. Mbalaviele et al thought β-catenin induced the differentiation from mesenchymal stem cell to osteoblast by enhancing BMP-2 reply of mesenchymal stem cell [19]. Glass D.A found that Wnt/β-catenin signaling pathway started OPG gene promoter in osteoblasts through the β-catenin stimulus. OPG, as an inhibiting osteoclast differentiation factor, suggested that Wnt/β-catenin pathway promoted osteoblast differentiation and inhibited osteoclast differentiation at the same time [20]. Kieslinger et al found that Wnt/β-catenin pathway could inhibit receptor activator of NF-κB/ligand of receptor activator of NF-κB signals by inducing the release of OPG through EBF-2 in osteoblast, thus reducing the production of osteoclasts [21].

Our study demonstrated that high β-catenin expression was significantly associated with lymph node metastasis. But there was no significant correlation between β-catenin expression and other characteristics. We had not identified a relationship between the β-catenin expression and the tumor stage. But we observed a trend between β-catenin expression and stage, although this was not statistically significant. This may be caused by the limited sample size or staining evaluation without separate analysis.

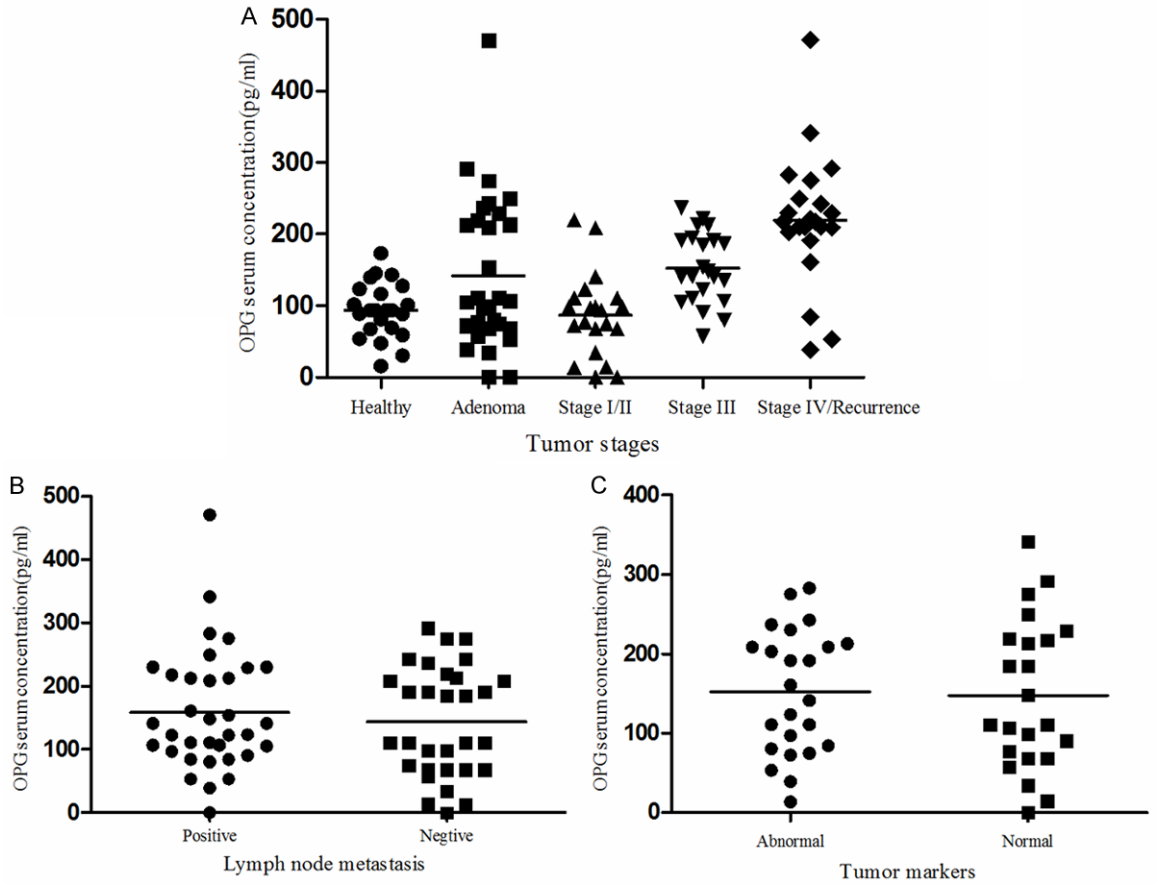

Figure 3. Serum OPG concentration in NSCLC. Serum OPG concentration is increased in patients with late-stage lung cancer. The correlation of serum OPG levels with the lymph node metastasis and abnormal serum tumor marker levels had not been observed. A. OPG serum levels in 23 healthy individuals, 30 patients with lung adenomas, and 65 patients with different stages of lung cancers (21 UICC I/ II, 22 UICC III, 22 UICC IV and recurrence) and mean serum levels. B. Serum OPG concentrations of lymph node metastasis-positive group and lymph node metastasis-negative group. C. Serum OPG concentrations of abnormal tumor marker group and normal tumor marker group. Representative results are shown.

Research showed that patients with negative expression of membranous β-catenin had a trend towards shorter survival than those with positive expression. In contrast to previous studies, they found that increased expression of either cytoplasmic or nuclear β-catenin was strongly associated with poor prognosis and was an independent prognosticator for overall survival [10]. A study demonstrated overexpression of β-catenin was more often detected in patients with stage III and IV than in those with stage I, and II in ovarian serous carcinomas. No significant relationship was found between expression of β-catenin and pathological grade. Positive expression of β-catenin related to lower survival rate [22]. A research on esophageal squamous cell found the aberrant β-catenin expression could be an adverse underlying factor in carcinogenesis and progression [23]. A research on colorectal carcinoma (CRC) patients demonstrated expression of β-catenin was significantly correlated with tumor location, differentiation, lymph node metastasis. Expression of β-catenin was associated with favorable clinicopathologic variables and it was a clinically significant prognostic indicator for CRC patients [24]. A meta-analysis showed that abnormal β-catenin immunohistochemical expression may be associated with tumor progression and could be a predictive factor of poor prognosis in patients with gastric cancer [25]. Another demonstrated cytoplasmic and/or nuclear accumulation of β-catenin, as an independent prognostic factor, significantly associated with poor prognosis and deeper invasion of hepatocellular carcino-

ma (HCC), and could serve as a valuable prognostic predictor for HCC [26].

OPG, which is a member of TRAIL family, has been implicated in many biological processes. OPG is known to be associated with aggressiveness in several cancers through inhibition of apoptosis via neutralization of TNF-related apoptosis inducing ligand [17]. The role of OPG is broad, just like a double-edged sword, it can be used as a "bone protection factor", but also participate in the process of tumor progression. OPG expression in triple-negative breast cancer cells promotes metastasis [27]. Recent studies suggest that OPG also plays a certain role in the formation of chronic kidney disease (CKD). OPG can make the degree of vascular calcification lower and prevent the formation of aneurysm [28]. OPG levels in circulating blood were increased in patients with CKD [29]. OPG can act as a bio-marker for diabetic cardiovascular complications [30]. These are important findings on OPG in other system diseases. In recent years, research has found the correlation between OPG and tumor lymph node metastasis. Whether OPG promotes the occurrence of the tumor or only plays a protective role in the process of tumor progression still remains a question. So more studies are needed to explore the function of OPG deeply.

In the study, we demonstrated that OPG expression was higher in 101 NSCLC samples than in adjacent normal lung tissues. These observations suggested that OPG may function as an oncogene or anti-oncogene and have a potential role in the pathogenesis of NSCLC. In order to further identify the role of OPG in the development and progression of lung cancer, we analyzed the expression of OPG in 101 NSCLC patients and found OPG over-expression was significantly associated with clinical stage ($P<0.001$) and positive lymph node metastasis ($P<0.001$). These results may indicate that OPG plays significant roles in NSCLC progression and metastasis. Over-expression OPG was significantly associated with lymph node metastasis, recurrence and abnormal tumor marker levels and it might be a predictive bio-marker of CRC recurrence and a target for treatment of this disease [17]. Along with the progress of prostate cancer, the serum OPG levels of patients increased. Serum OPG concentrations have been reported to be increased in patients with prostate cancer which progressed after androgen ablation therapy [31], and in patients with colorectal and pancreatic cancers compared with healthy individuals [32].

In our study, there also existed a positive correlation between serum OPG levels of lung cancer with lung cancer stage. Although OPG levels did not rise in the serum of patients with early stage, serum OPG levels of advanced lung cancer patients showed an obvious increase. OPG has direct effects on cells by interacting with syndecans, promoting cell adhesion and migration [33]. In this study, our research provided the first evidence that β-catenin and OPG expression in the tissues were positively correlated. With the progress of the disease, the serum levels of OPG were elevated. But a relationship between the serum OPG levels and lymph node metastasis had not been found and the trend between serum OPG levels and abnormal tumor marker levels did not statistically exist. This may result from the small samples. So a large sample study should be performed to verify the relationship. At the same time, further investigations are needed to fully elucidate the exact molecular mechanisms.

In conclusion, The current study demonstrated that the expression of β-catenin and OPG in NSCLC was significantly increased in NSCLC. Because of the limited sample size of patients in our study, further studies would be needed to verify the role of OPG as a convictive clinical predictor for the prognosis of NSCLC patients and find the new function of OPG in the tumorigenesis.

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Disclosure of conflict of interest

None.

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