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Original Article

Tumor OPG expression is associated with prognosis of cervical cancer in Chinese patients underwent surgical treatment

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Abstract: Aim: Osteoprotegerin (OPG) is associated with breast cancer in women; however, its role in cervical cancer remains unknown. This study was to investigate the association between tumor OPG expression and the prognosis in cervical cancer patients. Methods: A total of 218 patients with primary cervical cancer were enrolled in this study. All patients underwent surgical treatment as primary treatment and the tumor was obtained. The OPG Immunohistochemistry in tumor samples was performed. Results: We found that tumor OPG expression is significantly associated with tumor stage, tumor grade, metastasis and invasion (all P<0.001). Kaplan-Meier survival curves showed that cervical cancer patients with high tumor OPG levels had shorter overall survival period than the patients with low OPG levels). The univariate and multivariate Cox proportional hazard regression analyses identified tumor OPG expression in cancer as prognostic factors of cervical cancer. Conclusion: The findings of our study imply that cancer OPG expression may be used as a molecular marker for prognosis of cervical cancer patients.

Keywords: Osteoprotegerin, cervical cancer, prognosis

Introduction

Cervical cancer is one of the most commonly diagnosed cancers. Despite of the recent progress in diagnosis and effective treatment, the clinical outcome of cervical cancer patients remains poor [1, 2]. Therefore, the identification of potential markers for the early diagnosis and treatment is important to improve the prognosis of cervical cancer [3].

Osteoprotegerin (OPG) is a secreted member of the tumor necrosis factor (TNF) receptor family that has been well characterized as a negative regulator of bone remodeling [4]. OPG is also expressed in human breast cancer tissues and cell lines. The *in vitro* studies suggest that OPG exerts tumor-promoting effects by binding to TNF-related apoptosis inducing ligand (TRAIL) and OPG knockdown reduced invasion of breast cancer cells [5].

To date, the assoiction between tumor OPG expression status and the clinical features and prognosis of cervical cancer remains unknown. In this study, we found that the OPG expression level in tumor tissue is related to higher rate of metastasis and poorer prognosis, suggesting OPG may be used as a monitor for the disease development and prognosis predictor in cervical cancer patients.

Materials and methods

Ethics statement

This study was reviewed and approved by the Ethics Committee of Jinan Fourth People Hospital (JN-2008b-3). The written informed consent was obtained from every subject.

Patient enrollment and follow-up

A total of 218 patients (age 27-80 years) with primary cervical cancer were enrolled in this study. All patients had been histologically diagnosed by the endoscopy biopsy and the diagnosis was confirmed by the postoperative biopsy diagnosis. Patients were staged clinically, according to The International Federation of Gy-

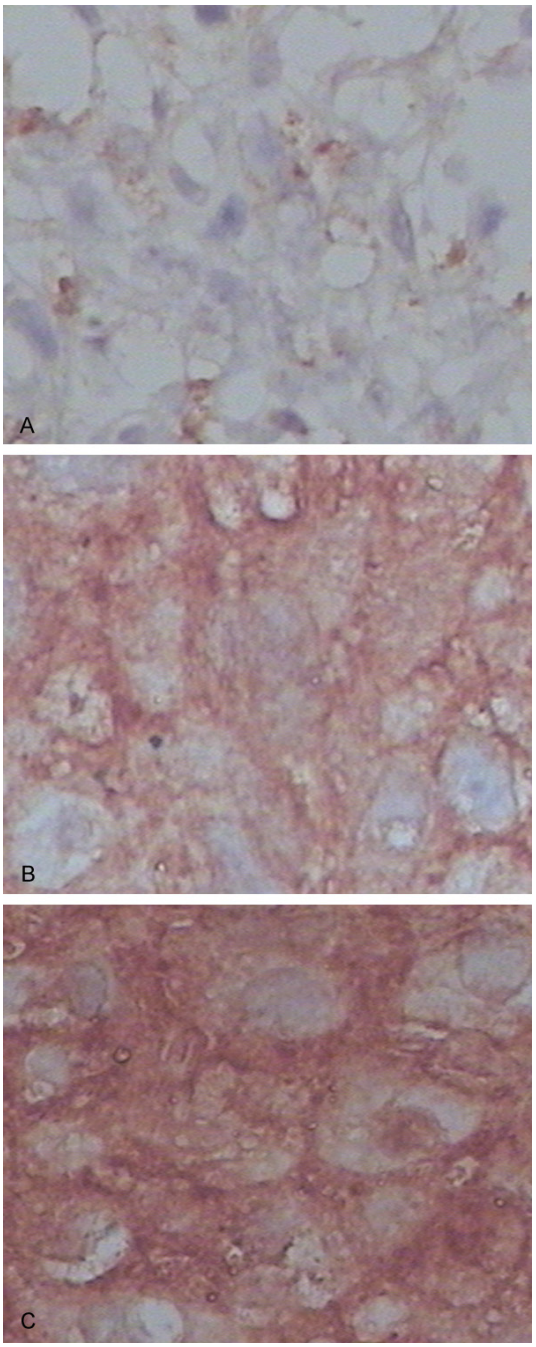

Figure 1. The OPG expression in tumor samples from patients by immunohistochemistry. (A) Negative control; (B) Low OPG expression; (C) High OPG expression (magnitude 200 time). The OPG expression in non-cancerous tissue (A) and tumor tissues (B and C). Tumor tissue had a markedly higher OPG expression than normal tissue. (B) shows the low OPG expression and (C) shows the high OPG expression in tumor.

necology and Obstetrics [6] staging (AJCC 2002, 6th edition) [6]. All patients underwent surgical treatment (Hysterectomy) as primary treatment. Those who had received chemotherapy, hormonotherapy, targeted therapy, or radiotherapy were excluded from this study. After surgery, patients were followed up every 3 months for the first three years and thereafter every 6 months for the fourth and fifth years. Clinical information including Histopathology type, tumor size, FIGO stage, tumor grade, lymph node (LN) metastasis and stromal invasion were acquired from patient's medical charts. All patients were followed for 5 years. Overall survival was defined from surgery to death or last follow-up.

OPG immunohistochemistry

To determine tumor OPG expression, paired cervical cancer tissues and adjacent normal tissues were obtained from each patient during surgical procedure. The samples were embedded with paraffin and sliced for Immunohistochemistry. After Antigen retrieval and deactivation of endogenous peroxidase, The slides were incubated overnight with Anti-OPG antibody (Millipore Corporation, USA) at a 1:150 dilution at 4 degree. The immune reaction was revealed with 0.06 mmol/liter diaminobenzidine (DAB-Dako, DakoCytomation, Carpinteria, CA) and 2 mmol/liter hydrogen peroxide. The degree of immunostaining was scored independently by two observers according to the following criteria: 0 = no staining, 1+ = weak staining, 2+ = moderate staining, and 3+ = strong staining [7]. Those with 0 or 1 staining scores were assigned as low expression and those with 2 or 3 as high expression [7].

Statistical analysis

All statistical analyses were performed by SPSS 16.0 software (SPSS, Chicago, IL). The data were expressed as mean ± standard deviation [8]. Correlations of tissue OPG levels and clinicopathological parameters were calculated by using the Mann-Whitney U test for continuous variables and the χ^2 test for categorical data. The Kaplan-Meier method and log-rank test were used for survival analysis. The prognostic value of serum and cancer tissue OPG levels was evaluated using univariate and multivariate Cox models. All significant parameters in the univariate analysis were entered into a multivariate model. For all statistical analyses, all *P* values were two-tailed, and $P<0.05$ was considered to be statistical significance.

Table 1. The baseline clinical characteristics of all enrolled patients based on OPG expression levels

Numbers	High (n = 98)	Low (n = 120)	X^2	*P* value
Age (years)				
≤40	40	62	2.55	0.134
>40	58	58		
Histopathology				
Squamous cell carcinoma	60	81	0.93	0.335
Adenocarcinoma	38	39		
FIGO stage				
IB	36	99	47.1	<0.001
>IB	62	21		
Grade				
G1 + G2	44	85	15.0	<0.001
G3	54	35		
LN metastasis				
Negative	38	88	26.4	<0.001
Positive	60	32		
Stromal invasion				
≤2/3	32	87	33.1	<0.001
>2/3	64	33		

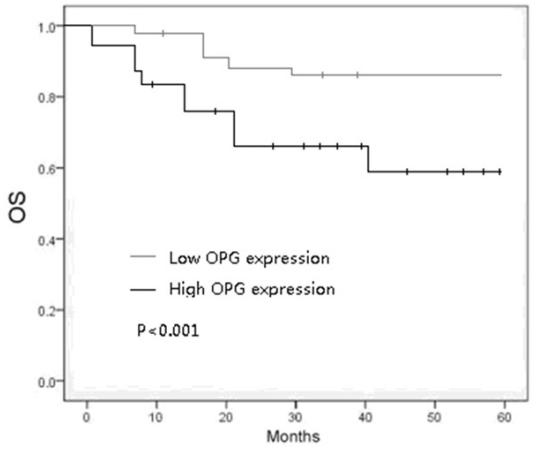

Figure 2. Kaplan-Meier curves of cervical cancer patients with low versus high expression of OPG. The curves showed that cervical cancer patients with high tumor OPG levels had shorter OS period than the patients with low OPG levels (16.8±3.1 vs. 22.5±2.5, month, P = 0.018).

Results

The representative figures of OPG staining in cervical cancer tissue are shown in **Figure 1**. The OPG was predominantly expressed in the cytoplasm. **Table 1** summarizes the baseline clinical characteristics of all enrolled patients. We analyses the clinical features based on the OPG expression levels. A total of 98 patients had high OPG expression while 120 had low OPG expression in tumor samples. We found that the high tumor OPG expression tended to be positively correlated with high FIGO stage, higher tumor grade, presence of LN metastasis and deeper stromal invasion (all P<0.001, **Table 1**).

A total of 22 patients were lost contact during the follow-up period and 196 patients (86 in high OPG group and 110 in low OPG group) completed the follow-up study. The 5-year cumulative survival rates of patients with high and low OPG levels were 85.6% and 58.9%, respectively (log-rank test, P<0.001, **Figure 2**). To investigate the prognostic value of OPG levels, we assessed the association between OPG levels and patient survival using Kaplan-Meier survival curves. The curves showed that cervical cancer patients with high tumor OPG levels had shorter OS period than the patients with low OPG levels (16.8±3.1 vs. 22.5±2.5, month, P = 0.018, **Figure 2**).

In order to identify the independent prognostic value of clinical variable in cervical cancer patients, we performed the univariate and multivariate Cox proportional hazard regression analyses including age, sex, smoking status, FIGO stage, grade, lymph node metastasis, stromal invasion, and OPG level. We observed that FIGO stage, grade, lymph node metastasis, stromal invasion, and OPG level were selected as predictors for prognosis of cervical patients by univariate analyses. In multivariate analyses, all statistically significant markers from univariate analyses were identified as prognostic factors of cervical cancer (**Table 2**).

Discussion

In this study, we investigated the possible association between the tumor OPG expression and the clinical features of cervical cancer patients. We found that the high tumor OPG expression are more common in patients with high FIGO stage, higher tumor grade, presence of LN metastasis and deeper stromal invasion (all P< 0.001). More importantly, our prognostic study reveals that OPG expression level is associated with the overall survival in patients receiving surgical treatment. Patients with high OPG levels had lower 5-year cumulative survival rates

Table 2. The univariate and multivariate analyses for prognostic factors of cervical cancer

Variables	Univariate			Multivariate		
	Adjusted HR	95% CI	Adjusted P	Adjusted HR	95% CI	Adjusted P
			P			P
FIGO stage (IB vs. >IB)	1.65	1.87-3.46	0.045	1.34	1.97-3.46	0.014
Grade (G3 vs. G1 + G2)	1.88	1.23-4.23	0.023	1.66	1.23-3.08	0.015
Lymph node metastasis (positive vs. negative)	2.67	2.03-5.15	<0.001	2.82	2.18-4.87	<0.001
Stromal invasion (>2/3 vs. ≤2/3)	2.04	1.67-3.56	0.013	1.98	1.27-2.90	0.01
OPG level (high vs. low)	2.45	1.98-4.29	0.009	1.83	1.35-3.35	0.012

HR, Hazard ratio; 95% CI, Confidence Intervals.

compared to those with low OPG expression level.

OPG is a glycoprotein that has multifaceted role and is associated with several cancer malignancies like that of bladder carcinoma, gastric carcinoma, prostate cancer, multiple myeloma and breast cancer [9]. Previous studies showed that human breast cancer cells secrete high levels of the cytokine OPG compared to primary human mammary epithelial cells. High expression of OPG was also detected in human breast cancer tissue samples compared to the uninvolved tissue from the same patient. In vitro studies suggest that OPG exerts tumor-promoting effects by binding to TNF-related apoptosis inducing ligand (TRAIL), thereby preventing induction of apoptosis [5]. In ovarian cancer, exogenous OPG protected from TRAIL-induced apoptosis in a TRAIL binding-independent manner. Moreover, OPG-mediated activation of integrin/FAK signaling resulted in the activation of Akt. Inhibition of both integrin/FAK and Akt signaling significantly inhibited OPG-mediated attenuation of TRAIL-induced apoptosis [10]. Up-regulated OPG expression was detected in Non-small-cell lung carcinoma (NSCLC) cell lines and in tumor tissues with bone metastasis and he increased expression of OPG correlated with tumor stage, lymph node metastasis, and distant metastasis [11]. In bladder urothelial carcinoma (UC), OPG expression was significantly different among histological grades, being higher in low-grade UCs and was inversely correlated with the presence of lymphovascular invasion (LVI). TRAIL and OPG expression in bladder urothelial carcinoma: correlation with clinicopathological parameters and prognosis [12]. In our study, we found that the OPG expression in tumor is correlated to the major clinical feature of cervical cancer, including FIGO stage, tumor grade, metastasis and invasion. Our date suggest that tumor OPG expression may reflect the clinical characteristics of cervical cancer. Previous research has shown that OPG is actively involved in the tumor progression by aiding in angiogenesis [13] and OPG deficient mice exhibited vascular calcification thus highlighting the involvement of OPG in the active and intricate vascular system [14]. RANKL/OPG expressions were associated with tumor stage, lymph node metastasis, and distant metastasis, suggesting that the metastasis of NSCLC cells from primary sites to secondary sites (i.e., regional lymph nodes or distant organs) depends on the level of RANKL/OPG expression. Knockdown of OPG expression reduced metastasis formation in chick tissues both from a primary tumor and after direct introduction of cells by intravenous injection. OPG knockdown cells express lower levels of Matrix Metalloproteinase-2 and have a reduced ability to invade through a collagen matrix. This would suggest that OPG can exert an autocrine effect and promote breast cancer cell invasion and metastasis [15]. We postulate that the OPG the pro-angiogenesis and direct invasion prosperity of OPG attribute the tumor metastasis and invasion.

The prognostic role of OPG in cancer was also reported. Using Kaplan-Meier survival analysis, lower expression levels of OPG were found to be associated with significantly better overall

patient survival in our cohort [16]. In this study, we found that the OPG is associated with the overall survival in patients receiving surgical treatment. Patients with high OPG levels had lower 5-year cumulative survival rates compared to those with low OPG expression level. Our finding is consistent with that of Owen et al., suggesting that OPG expression in tumor can predict the prognostic in patients with cervical cancer.

OPG expression was related to small tumor size, node negativity, and low Ki-67. There was no significant difference in clinicopathologic features between tumors with RANK and those without RANK. RANK expression was significantly associated with poor disease-free survival in univariate analysis and multivariate analysis. RANKL expression was associated with improved skeletal disease-free survival in multivariate analysis [17].

Several libations should be addressed in this study. Firstly, the sample size is relatively small and only Chinese patients were enrolled. Secondly, we did not perform in vitro study to explore the molecular mechanism under which OPG affect the cancer cell biological prosperity. Thirdly, we did not detect expressions of the other members of RANKL/RANK/OPG System.

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Disclosure of conflict of interest

None.

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