Int J Clin Exp Pathol 2016;9(5):5487-5492
www.ijcep.com /ISSN:1936-2625/IJCEP0021320

Original Article

Increased expression of PDGF-BB predicts metastasis and poor prognosis in ovarian cancer patients

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Received December 7, 2015; Accepted March 17, 2016; Epub May 1, 2016; Published May 15, 2016

Abstract: Objectives: Association of PDGF-BB expression with presence, development, metastasis and prognosis of ovarian cancer was investigated. Methods: 102 ovarian cancer-related cases with follow-up information were made into tissue microarrays according to the paired principle of cancer tissues and the adjacent tissues. Subsequently, the expression of PDGF-BB was detected with immunohistochemical analysis and SPSS software was finally utilized to analyze the relationships between experimental data and clinical indicatives. Results: Expression of PDGF-BB in ovarian cancer tissues were notably higher than their adjacent tissues ($P<0.05$) and showed significant positive correlation with lymph node metastasis (LNM) status ($P = 0.002$), latent distant metastasis status ($P = 0.001$) and FIGO histology grade ($P = 0.016$). Moreover, survival analysis indicated that PDGF-BB expression in cancer tissues, serving as an independent correlation factor, was significantly correlated with poor prognosis ($P<0.05$). Conclusions: PDGF-BB is a crucial biomarker that is closely related with prognosis of ovarian cancer and also a potential molecular target for evaluating the prognosis and treatment of ovarian cancer.

Keywords: Ovarian cancer, platelet-derived growth factor-BB (PDGF-BB), prognosis, biomarker, immunohistochemistry

Introduction

Ovarian cancer, one of the most common gynecological malignancies, is rapidly progressing with high rate mortality in women. It has been shown to be represented with increasing estimated new cases and new deaths in recent decades [1]. Despite the currently available therapeutic strategies which include the excision of malignant tissue and combination of radiotherapy and chemotherapy, the overall 5-year survival of ovarian cancer patients still remains lower than 25 percent [2]. The poor prognosis could be attributed to late diagnosis and lack of specific biomarkers to predict tumor progression and prognosis [3]. Therefore, new discoveries of biomarkers for determining the risks of occurrence, progression and metastasis of ovarian cancer are the most important problem in improvement of prognosis.

The family of platelet-derived growth factors (PDGFs) regulates numerous cellular processes, including cell proliferation, transformation, migration and survival in development and during pathogenesis [4]. The increasing body of literature strongly suggests that PDGF-BB may function as a crucial factor in the development and progression of human cancers by regulating the processes of cell proliferation, apoptosis, migration, invasion, angiogenesis, and metastasis [5, 6]. Recently, studies have revealed that the over expression of PDGF-BB is associated with the a lot of tumors, including breast cancer [7], prostate cancer [8], lung cancer [9], gastric cancer [10], and liver cancer [11]. Based on the above studies, PDGF-BB, obviously, plays a significant role in cancer development. However, the expression of the PDGF-BB in ovarian cancer and its significance has not been examined in detail yet.

In the present study, we aimed to evaluate the correction between biological function of PDGF-BB and the progression, migration, and prognosis of ovarian cancer via immunohistochemical method.

Table 1. Expression of PDGF in relation to pathologic and clinical variables

Clinicopathological parameters	N[2]	PDGF expression		χ^2	*P* value
		+	-		
All	102	54	48		
Age (years)				0.177	0.674
<60	68	37	31		
≥60	34	17	17		
Tumor size				0.039	0.844
<7 cm	67	35	32		
≥7 cm	35	19	16		
Lymph node metastasis				9.294	0.002
Absence	56	22	34		
Presence	46	32	14		
Latent distant metastasis				10.176	0.001
Absence	74	32	42		
Presence	28	22	6		
Grade				5.825	0.016
Low	64	28	36		
High	38	26	12		
Bilaterality				2.429	0.119
Unilateral	83	46	37		
Bilateral	19	7	12		

Materials and methods

Source of samples

A total of 102 formalin-fixed, paraffin-embedded ovarian cancer tissues and the adjacent tissues to perform immunohistochemical staining, which were collected from January 2002 to November 2012 at East Hospital, Tongji University. Important clinical data, such as age, tumor size, lymphatic metastasis, latent metastasis, TNM stage, were collected from each patient's medical records. The follow-up time was calculated from the date of surgery to the date of death, or the last known follow-up. Before surgical therapy, none of the patients had received neoadjuvant chemotherapy, radiation therapy, or other related anti-tumor therapies. All ovarian cancer tissue samples in this study were obtained with patients' written informed consent and all experiments have been approved by the ethics committee at local Hospital. Patient characteristics were detailed in **Table 1**.

Immunohistochemical staining

The two-step EnVision method has been conducted to perform immunehistochemical experiments. Three magnification visions randomly observed under optical microscope, the number of positive cells in no less than 3 × 100 cells was record, and then calculate the positive rate of positive cells to all cells. The dyeing positive rate was included for the statistical analysis: the positive rate equal or less than 95% was treated as low expression group, otherwise, it was included in high expression group.

Statistical analysis

The expression of PDGF-BB in ovarian cancer and adjacent cancer tissues were compared with paired Wilcoxon test. The association between clinical characteristics of ovarian cancer patients and PDGF-BB expression were using Pearson and Spearman's correlation test. The prognostic of ovarian cancer and PDGF-BB protein expression were using Kaplan-Meier survival analysis and log-rank test for univariate analysis; the significant variables resulted from univariate test were included in the Cox multivariate regression analysis. The *P*-value less than 0.05 was considered statistically significant.

Results

Expression of PDGF-BB significantly increased in ovarian cancer tissues

In order to determine whether PDGF-BB expression is changed in human ovarian cancer, immunohistochemistry staining was exerted in TMA slides to evaluate the PDGF-BB expression in ovarian cancer tissues and paired adjacent non-tumor tissues (**Figure 1**). In training cohort TMA slides containing 102 cases ovarian cancer tissues with paired adjacent non-cancerous tissues, the positive percentage of PDGF-BB expression in ovarian cancer and adjacent non-tumor tissues were 52.9% (54/102) and 6.9% (7/102). There is significantly higher positive expression of PDGF-BB in ovarian cancer compared with the adjacent non-tumor tissues (P<0.001) (**Figure 2**).

Increased PDGF-BB expression correlates with clinicopathological parameters

The correlation between patients' clinical parameters and the expression of PDGF-BB is shown in **Table 1**. It reveals that PDGF-BB expression in the ovarian cancer tissues is sig-

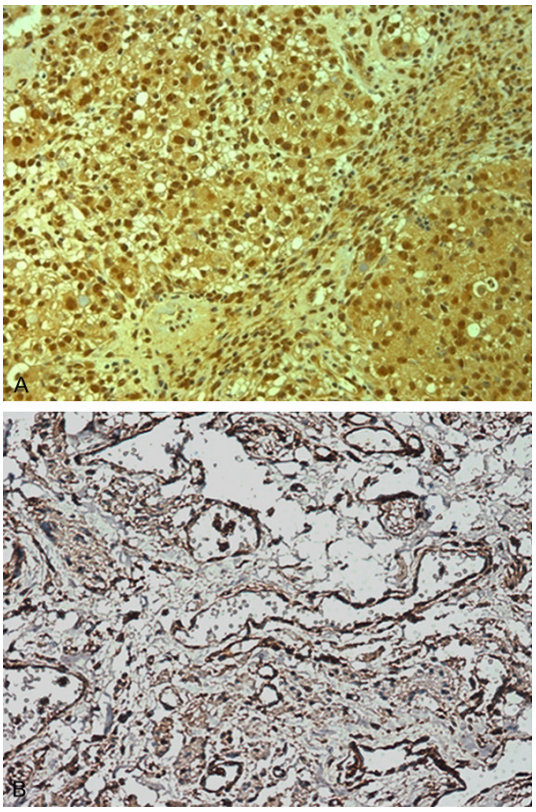

Figure 1. Expression of PDGF-BB of ovarian cancer tissues (A) and tissues adjacent to ovarian cancer (B) studied by immunohistochemistry in tissue microarrays [Original magnification, × 200].

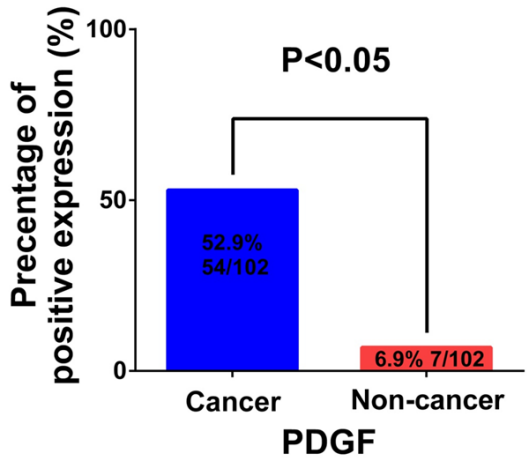

Figure 2. Expression of PDGF-BB in ovarian cancer tissues compared to its adjacent tissues. Results show that there was significant difference between the groups which were statistically evaluated by chi-square test.

nificantly correlated with lymph node metastasis (LNM) status (χ^2 = 9.294, P = 0.002), latent

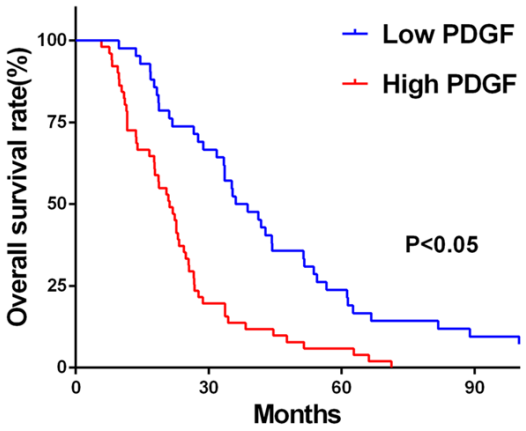

Figure 3. Kaplan-Meier survival analysis for a univariate survival analysis.

distant metastasis status (χ^2 = 10.176, P = 0.001) and FIGO histology grade (χ^2 = 5.825, P = 0.016). However, we did not find significant correlation between PDGF-BB expression with other clinicopathologic features, including patient's age, tumor size, tumor location (P>0.05 for all). Therefore, our data demonstrate that higher PDGF-BB expression in ovarian cancer tissues is positively correlated with tumor metastasis and cancer progression, suggesting that PDGF-BB expression is involved in the progression of human ovarian cancer.

Increased PDGF-BB correlates with poor patient survival

In order to further investigate the prognostic significance of PDGF-BB expression in human ovarian cancer, we performed the log-rank survival analysis according to the PDGF-BB expression level in cancer tissues and collected survival data. The survival analysis demonstrates that the overall survival rate of the subgroup with negative expression is significantly better than that the subgroup with positive PDGF-BB expression (P<0.05, **Figure 3**).

PDGF-BB serves as an independent molecular prognostic indicator for ovarian cancer

Moreover, univariate and multivariate analyses were conducted to confirm the possibility of PDGF-BB used as an independent risk factor for poor prognosis in the 102 cases of ovarian cancer. Univariate Cox regression analyses showed that PDGF-BB expression was significantly associated with OS (**Table 2**). Further-

Table 2. Analysis of independent correlation factors of colorectal cancer prognosis with Cox multivariate regression analysis

Factors	B	S.E.	Ward	Sig.	Exp (B)	95% CI for Exp (B)	
						Lower	Upper
PDGF expression	-0.791	0.238	11.032	0.001	0.454	0.284	0.723
Age	-0.376	0.245	2.355	0.125	0.686	0.425	1.110
Tumor size	-0.134	0.229	0.341	0.559	0.875	0.559	1.370
Lymph node metastasis	-0.623	0.345	2.681	0.077	0.439	0.223	1.086
Latent distant metastasis	-0.772	0.405	3.629	0.057	0.462	0.209	1.023
Grade	-0.850	0.454	3.500	0.061	0.427	0.175	1.041

more, a multivariate Cox regression analysis confirmed PDGF-BB expression as independent predictors of the OS in ovarian cancer patients (**Table 2**).

Discussion

In this study, we evaluated the expression level of PDGF-BB in the ovarian cancer tissues and the paired adjacent tissues by immunohistochemical techniques. Our results demonstrated that the expression level of PDGF-BB in the cancer tissues was significantly higher than that in the adjacent tissues. Then, the Spearman's rank-order correlation analysis showed a positive correlation between PDGF-BB level and ovarian cancer progression. Moreover, the univariate survival analysis including the Kaplan-Meier survival analysis and Log-rank statistical test revealed that ovarian cancer patients with negative PDGF-BB expression in cancer tissues have a significantly higher survival time than those with positive PDGF-BB expression in cancer tissues. Cox multivariate regression analysis tested all the variables with statistical significance in the univariate survival analysis, and showed that PDGF-BB expression was the independent correlation variables for the ovarian cancer prognosis.

The pathogenesis of tumor is a complex process of multiple factors, multiple steps and many stages [12, 13]. But the invasion and metastasis of tumor cells are the main causes for cancer treatment failure among these factors [14]. The invasion and metastasis of ovarian cancer involves a series of events such as detachment from primary tumor, invasion into adjacent tissues of the initial sites, penetration into blood vessels and lymphatics, arriving at the distant sites, and the formation of new lesion along with neoangiogenesis [15].

Platelet-derived growth factor BB (PDGF-BB) is an important growth factor that is involved in the regulation of cell growth and division, including cell proliferation, differentiation, survival, transformation [16]. In addition, it plays a significant role in angiogenesis [17]. It is reported that the major role of PDGF-BB in the regulation of angiogenesis is control of pericyte function. PDGF-BB has been shown to upregulate VEGF in vascular smooth muscle cells, which in turn, enhances endothelial cell survival [18, 19]. One of the most obvious characteristics of VEGF is its ability to induce vascular permeability [20]. This enhanced permeability leads to subsequent fibrin deposition in the extracellular matrix that can then serve as a scaffold for migrating endothelial cells.

In addition to these, to date, several reports have shown that PDGF-BB are frequently produced by tumor cells and may affect tumor growth and dissemination in several different ways [21]; thus, PDGF-BB may further be involved in the recruitment of tumor fibroblasts and pericytes [22, 23]. Tumor fibroblasts may function, in turn, to produce factors that directly act on tumor cells to promote their proliferation and migration. Moreover, tumor fibroblasts may also secrete angiogenic factors that help to sustain tumor angiogenesis and promote metastasis of tumor cells [24]. What's more, the PDGF-BB and its receptor engage several signaling pathways, such as PI3K, RAS-MAPK, and PLC, which are involved in multiple cellular processes, including carcinogenesis [25]. AKT/PKB is a kind of the serine/threonine kinase effectors of PI3K signaling [26], PDGF-BB can active PI3K pathway and then promote actin reorganization, stimulate cell growth and inhibit apoptosis [27]. It is reported that PDGF-BB can activate STAT proteins in many solid cancers and blood malignancies [28]. Collectively, PDGF-BB is shown to function as an important regulator in the pathologic process of ovarian cancer. It's of great clinical value to further elu-

cidate the specific mechanism of PDGF-BB in ovarian cancer.

In conclusion, our study show positive PDGF-BB expression is significantly associated with lymph node metastasis, latent distant metastasis status, FIGO histology grade and shorter survival time in ovarian cancer. This implies that PDGF-BB can be a good prognostic marker of cancer progression in early-stage ovarian cancer. Our findings also demonstrate that PDGF-BB may be a potential therapeutic target in patients with ovarian cancer.

Acknowledgements

This research was supported in part by the National Nature Science Foundation of China (81573008), National Nature Science Foundation of China (81201798), the Fund of Pudong Health Bureau of Shanghai (PWRd2014-01) and the Project of Key Disciplines Group Construction of Pudong Health Bureau of Shanghai (PWZxq2014-04).

Disclosure of conflict of interest

None.

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