RESEARCH ARTICLE

Overexpression of Platelet-derived Growth Factor-D as a Poor Prognosticator in Endometrial Cancer

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Abstract

Background: Emerging evidence implicates the platelet-derived growth factor-D (PDGF-D) in many types of human solid tumors. We investigated whether PDGF-D plays an important role in endometrial cancer (EC) in relation to clinicopathologic phenotype, angiogenesis, and patient prognosis. Materials and Methods: We analyzed PDGF-D protein expression by Western blotting in twenty-seven human endometrial cancer tissues, and matched normal endometrial controls collected at the third Affiliated hospital of Sun Yat-sen University during 2012-2013 (n=27). Immunohistochemical staining was performed using a human PDGF-D antibody on the endometrial cancer patients collected in the same facility during January 2001 and October 2013 (n=152). Patients were followed from the time of primary surgery in 2001-2013 until death or last follow-up. We correlated the PDGF-D expression levels with clinicopathologic parameters and prognosis in human endometrial cancer patients. Results: Compared with matched normal endometrial cases, PDGF-D was up-regulated in endometrial cancer. Expression of PDGF-D protein, found in 78% of the cases, was associated with nonendometrioid histologic type (p=0.028), FIGO stage III/IV (p=0.039), >50% solid tumor growth (p=0.048), pelvic LN metastasis (p=0.035) and ER and PR negativity (p=0.04 and 0.002). PDGF-D expression was also significantly associated with expression of VEGF-A (p=0.021). In multivariate analysis, PDGF-D expression proved to be an independent prognostic factor in addition to histologic grade and FIGO stage. Patients with high expression levels of PDGF-D had a significantly poorer overall survival rate compared with patients with no expression. Conclusions: PDGF-D expression is frequently up-regulated in endometrial cancer, and is associated with aggressive features and poor prognosis.

Keywords: Endometrial cancer - PDGF-D - clinicopathologic features - prognosis

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Introduction

Endometrial carcinoma is one of the most common gynecologic malignancies in female genital tracts. It accouts for twenty percent to thirty percent female genital tra- ct malignant tumor and threats female health severely (Wang et al., 2012). Many scholars think that the interaction of multiple genes lead to endometrial cancer, but the molecular mechanism of endometrial carcinoma is unknown (Xu et al., 2011). The incidence rate and morbidity rate are on the ascending trend. Therefore, we are urges us to deeply explore the molecular basis for the carcinogenesis, in order to better diagnosis and treatment of endometrial cancer (Wang et al., 2013).

Platelet-derived growth factors (PDGFs) regulate a variety of cellular processes, including cell proliferation, transformation, migration and survival in development and during pathogenesis (Rosenkranz et al., 1999; Jemal et al., 2010). Through activation of two structurally related cell surface receptor tyrosine kinases α-PDGF receptor (PDGFR) and β- PDGFR, PDGF exerts their biological functions (Yu et al., 2003; Jemal et al., 2010). PDGF-D, a newly identified isoformate of PDGFs, is frequently up-regulated in various cancers and plays an important role in tumor growth, angiogenesis and metastasis (Deuel et al., 1987; LaRochelle et al., 2002; Hwang et al., 2003; Ustach et al., 2004; Xu et al., 2005; Wang et al., 2007). PDGF-D primarily interacts with β-PDGFR and activates several down stream signaling cascades, such as β-catenin, notch-1 and nuclear factor-κB (NFκB) signaling, ultimately contributing to tumor development (Deuel et al., 1987; Lokker et al., 2002).

In spite of the discovery of PDGF-D over 10 years ago, the role of PDGF-D is just beginning to be understood. The growing body of literature strongly suggests that PDGFD may function as a key player in the development and progression of human cancers by regulating the processes of cell proliferation, apoptosis, migration, invasion, angiogenesis, and metastasis (Hwang et al., 2003;Wang et al., 2007). In tumor tissue, increased expression of PDGF-D has been found in human prostate, lung, renal, ovarian, brain, and pancreatic cancer (Deuel et al., 1987;

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Ustach et al., 2005; Xu et al., 2005; Kong et al., 2008; Wang et al., 2009). In a mouse model of breast cancer, PDGF-D protein expression increased during tumor progression and returned to normal following regression. In malignant tumors, studies have indicated that PDGF-D signaling contributes to epithelial to mesenchymal (EMT) phenotype which regulates cancer cell (Xu et al., 2005).

To date, however, there are little reports concerning the expression and function of PDGF-D in human endometrial cancer. Accordingly, in this study we examined PDGF-D protein expression in endometrial cancer specimens and their adjacent non-malignant tissues. The aim of our present study was to investigate PDFG-D expression in endometrial tumors with respect to clinicopathologic phenotype, angiogenesis, vacular invasion by tumor cells, inflammatory markers and patient survival.

Materials and Methods

Patient samples

A retrospective review was conducted on the case records of patients with endometrial carcinoma treated at the Third Affiliated Hospital of Sun Yat-sen University between January 2001 and October 2013. A total of 173 patients diagnosed with endometrial carcinoma and underwent total hysterectomy and bilateral salpingo-oophorectomy, with or without pelvic and para-aortic lymphadenectomy. Of these, 21 cases were excluded due to a lack of substantial information; the remaining 152 cases were included in the current study. All patients were staged according to the 1988 International Federation of Gynecologists and Obstetricians staging system for endometrial cancer. This endometrial cancer histological type, histologic grade, Solid tissue proportion, estrogen receptor, progesterone receptor have previously been reported (Stefansson et al., 2004; Wang et al., 2010). This retrospective study was conducted in compliance with the institutional policy to protect the patients' private information and was approved by the Institutional Review Board of Third Affiliated Hospital of Sun Yat-sen University. Informed consent was obtained from all patients.

Patient follow-up

Patients returned for follow-up appointments at least every 3 months during the first 2 years, and then every 6months thereafter until death. The follow-up duration was calculated from the first day of therapy to the day of death, or to the last examination. The median follow-up time for the patients was 59.37 months (range, 1month-153.5 months). The following endpoints were assessed: overall survival(OS) and progression-free survival (PFS). We calculated OS from the first day of treatment to death, and PFS was calculated from the first day of treatment to the date of disease progression or death from any cause. Written informed consent for participation in the study was obtained from participants. At the end of this study, 78.9% patients still alive.

Western blotting

Endometrial tissue samples were obtained from the Department of Gynecology and Obstetrics, the third Affiliated hospital of Sun yat-sen University during 2012-2013. Fresh tumor tissue was carefully dissected from the surgical specimens and was immediately frozen in liquid nitrogen and stored for later use at -80ºC. Content of tumor cells (by estimated area) was at least 50%, and for the majority >80%. Endometrial tissue were prepared in 1× SDS [loading buffer: 50mM Tris-HCl (pH 6.8), 2% SDS, 10% glycerol], and boiled for 5 min. Total protein was electrophoresed by SDS-PAGE and Western blotting was carried out using antibodies against PDGF-D(AF1159 R&D Systems, USA)and β-actin(Santa Cruz) according to the manufacturers protocols. Blots were exposed to secondary antibodies and visualized using the Super Signal West Pico Chemiluminescent kit (Pierce). For loading control, membranes were stripped and reprobed with anti-β-actin.

Immunohistochecdesmistry

Staining of PDGF-D was performed on 5μm sections of formalin-fixed and paraffin embedded tumors using tissue microarray (TMA) slides. Sections were boiled for 10 minutes at 750W followed by 350W for 15minutes in 10mM citrate buffer and stained with a goat polyclonal PDGF-D antibody (AF1159 R&D Systems, USA). Pretreatment with goat serum diluted 1:4 was conducted before incubation with antibody diluted 1:25 for 1 hour at room temperature (RT) followed by 1:300 diluted Polyclonal rabbit anti-goat IgG-HRP (GP016010/29, Dako) for 1 hour at RT. The peroxidase was localized with diaminobenzi-dine peroxidase (DAB, Dako, GLostrup, Denmark) assubstrate, and sections were counterstained with Dako REAL hematoxylin (Dako).

TMA-slides were evaluated in a standard lightmicroscope (by MM and IMS). Regarding PDGF-D expression, cytoplasmic staining intensity in tumor cells (graded 0-3) and staining area (0: no tumor cells positive; 1: <10%; 2: 10%-50%; 3: >50%) were recorded. A staining index (SI) was calculated as a product of staining intensity and positive area giving a staining index of 0-9(Stefansson et al., 2006). Cases were divided in two subgroups based on the median value (positive cases with SI 1-9 versus negative cases with SI 0). In the survival analysis, the subgroup with strong expression (staining index 9) was shown in addition.

Statistical methods

Statistical analyses were performed by the PASW statistical software package version 17 (SPSS Inc., Chicago, IL). Associations between different categorical variables assessed by Pearson's chi-square test. An association was considered significant if a p-value <0.05 was obtained. Overall survival analysis was performed using the Kaplan-Meier method (log-rank significance test). PDGF-D, together with standard these variables could be incorporated in the Cox' proportional hazards regression model (likelihood ratio significance test).

Results

PDGF-D protein expression in the endometrial cancer

Table1. PDGF-D Protein Expression Correlation with Clinicopathological Variables and Molecular Markers Among 152 Endometrial Cancers

Variable	PPDGF-DSI 0N(%) N=33	PDGF-DSI1 -9N(%) N=119	*p* value[a]
Age			
<50	11(33%)	26 (22%)	0.38
50-70	19 (58%)	82(69%)	
>70	3(9%)	11(9%)	
Type			
Endometrioid	24(73%)	105(88%)	0.028
Nonendometrioid	9(27%)	14(12%)	
Grade			
Grade 1 and 2	23(70%)	92(77%)	0.26
Grade 3	10(30%)	27(23%)	
Solid proportion			
<50%	28(85%)	80(67%)	0.048
≥50%	5(15%)	39(33%)	
FIGO Stage[b]			
I/II	30(91%)	88(74%)	0.039
III/IV	3(9%)	31(26%)	
Pelvic LNs[c]			
Negative	32(97%)	98(82%)	0.035
Positive	1 (3%)	21(18%)	
ER[d]			
Negative	13(43%)	27(24%)	0.04
Positive	17 (57%)	84(76%)	
PR[e]			
Negative	18(58%)	32(29%)	0.002
Positive	13(42%)	78(71%)	
VEGF-A			
Weak	16(48%)	34(29%)	0.03
Strong	17(52%)	85(71%)	

*[a]*p* value from χ^2 test. [b]FIGO stage: according to 1998 criteria. [c]LNs=lymph nodes. [d]ER: missing data in 11cases. [e]PR: missing data in 11 cases

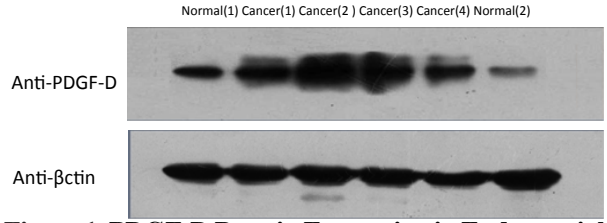


Figure 1. PDGF-D Protein Expression in Endometrial Cancer and Normal Endometrial by Western Blotting

PDGF-D protein expression was increased in the endometrial cancer tissue compared to the matched normal endometrial controls by Western blotting (Figure 1).

In total, 119 (78%) of the endometrial cancers were positive for PDGF-D cytoplasmic protein expression, while 33(22%) of the cases were negative (SI=0). Twenty-three endometrial cancers (15%) had a very strong cytoplasmic expression of PDGF-D (SI=9) (Figure 2).

PDGF-D expression associations with Clinicopathologic features

Table 1 summarized the main results of PDGF-D expression associations with Clinicopathologic features. PDGF-D protein expression showed a significant association with aggressive features nonendometrioid histologic type (p=0.028), FIGO stage III/IV (p=0.039), >50% solid tumor growth (p=0.048) and Pelvic LNs metastasis (p=0.035) but not with histological grade.

Table 2. Associations between PDGF-D Expression and Recurrence Spread among 152 Endometrial Cancers

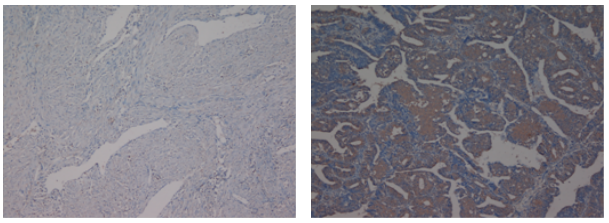
Variable	PDGF-DSI 0N(%) N=33	PDGF-DSI 1-9N(%) N=119	*p* value[a]
Recurrent disease			
No tumor recurrence	29(88%)	97(82%)	0.73
Vaginal cuff	2 (6%)	9 (7%)	
Pelvic lymph nodes	1 (3%)	7 (6%)	
Distant metastasis	1 (3%)	6 (5%)	

*[a]*p* value from Pearson's χ^2 test.

Table 3. Multivariate Survival Analysis (Cox' Proportional Hazards Regression Model) of Clinicopathologic Variables and PDGF-D Expression in Patients with Endometrial Cancer (n=152)

Variables	Categories	HR[a]	95% CI[b]	*p* value
PDGF-D	Negative	1		
	Weak/moderate	1.2	0.7-1.9	
	Strong	3.8	1.5-10.3	0.028
Histologic type	Endometrioid	1		
	Nonendometrioid	1.07	0.60-1.93	NS
Histologic grade	Grade 1 and 2	1		
	Grade 3	4.53	2.46-8.32	<0.001
FIGO stage	I/II	1		
	III/IV	2.8	1.6-4.9	<0.001

*All statistical tests were two-sided. Significance level: p<0.05. [a]HR=hazard ratio. [b]CI=confidence interval



(A) (B)

Figure 2. PDGF-D protein expression: Immunohistochemical Staining Showing no Expression and Strong of PDGF-D in Endometrial Cancer (magnification ×400). A). no expression of PDGF-D in endometrial cancer; **B)** strong expression of PDGF-D in endometrial cancer

PDGF-D expression associations with molecular markers

Strong PDGF-D expression was significantly associated with ER and PR negative tumors (p=0.04 and 0.002). There was a significant association between PDGF-D staining and VEGF-A expression (p=0.021). (Table 1)

PDGF-D expression associations with tumor recurrence

Twenty-six of 152 patients (17.1%) showed recurrence of their primary endometrial cancer during the follow-up period. Regarding the site of recurrent tumors, 42.3% in vaginal cuff, 30.8% in pelvic lymph nodes, 26.9% represented distant metastases, 126 cases did not show any spread of the disease. Nine recurrent endometrial cancers had a very strong cytoplasmic expression of PDGF-D (SI=9). Strong PDGF-D expression in the primary tumor was significantly associated with tumor recurrence (Table 2).

PDGF-D expression associations with OS

PDGF-D expression was an independent prognostic

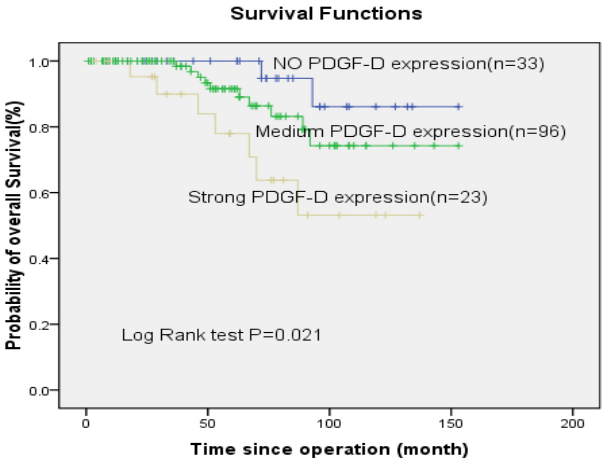


Figure 3. Overall Survival Analysis of 152 EC Patients Stratified by PDGF-D Expression (no PDGF-D Expression: SI=0; Medium PDGF-D Expression: SI=1-8; Strong PDGF-D Expression: SI=9). All statistical tests were two-sided. Significance level: $p<0.05$

marker for poor OS, with hazard ratio (HR) of 3.8, $p=0.028$. Histologic grade (HR 4.53, $p<0.001$), and FIGO stage (HR 2.8, $p<0.001$) were independent prognostic factors in addition, whereas histologic type was not (HR 1.07, NS) (Table 3).

Strong PDGF-D staining was associated with the worse survival. Absence of PDGF-D staining was associated with the best survival. Cases with medium staining index (SI 2-6) showed an intermediate survival, whereas the subgroup of patients showing strong PDGF-D expression (staining index9) was associated with the poorest outcome (Figure 3).

Discussion

PDGF-D has been at the forefront of research efforts to investigate the mechanism of PDGF-D in various solid tumors. To date, little report has associated the PDGF-D with EC. Our investigation compares primary human EC tissues with matched normal (adjacent) endometrial tissues, and revealed nearly uniform increase of PDGF-D expression in the malignant samples. We demonstrate that PDGF-D was up-regulated in endometrial cancer similar with lung, renal, ovarian, brain, and pancreatic cancer (Deuel et al., 1987; Ustach et al., 2005; Xu et al., 2005; Kong et al., 2008; Wang et al., 2009).

Approximately 78% of endometrial cancer tissues in the present study were positive for PDGF-D expression. Emerging evidence implicates the platelet-derived growth factor-D plays an important role in endometrial cancer. The role of PDGF-D as a tumor supporter has been suggested by many other authors. PDGF-D is highly expressed in human pancreatic adenocarcinoma specimens, in chronic pancreatitis associated with pancreatic adenocarcinoma, and in different human pancreatic cancer cell lines, suggesting that PDGF-D could be important in human pancreatic cancer progression (Deuel et al., 1987).

A number of clinicopathologic characteristics predict clinical outcome in EC, including stage, grade, and histology. Additionally, depth of myometrial invasion, cytology, lymphovascular space invasion (LVSI), and pelvic lymph node status predict clinical behavior and direct adjuvant treatment options (Creasman et al., 1987; Xiao et al., 2010; Wan et al., 2012). In this study, we demonstrated that PDGF-D expression was associated with aggressive features, including the nonendometrioid histologic type, high stage, Pelvic LNs metastasis and solid tumor growth in endometrial carcinoma. Ustach et al found that PDGF-D expression greatly accelerates prostate carcinoma tumor growth and enhances prostate carcinoma cell interaction with the surrounding stromal layers in a severe combined immunodeficient (SCID) mouse model, suggesting the potential oncogenic activity of PDGF-D in human prostate cancer progression(Xu et al., 2005). Moreover, Xu et al. reported that PDGF-D over-production in renal cancer SN12-C cells increased the proliferation and migration of cells in vitro and improved perivascular cell coverage in vitro. Furthermore, blocking PDGF-D/PDGFR signaling inhibited survival and mitogenic pathways in the glioblastoma cell lines and prevented glioma formation in a nude mouse xenograft model.

PDGF-D expression has been associated with ER negative, PR negative. PDGF-D expression was significantly associated with expression of VEGF-A .Further, PDGF-D was associated with vascular invasion (tumor cells invading lymphatic or blood vessels). Importantly, these results support a hypothesis for the expression of PDGF-D as a carcinogenetic event in EC, leading to EMT activity and proliferation (Aas et al., 1996).

The gene expression levels in early stage cancer may reflect patients with poorer clinical outcomes, as there is quite a heterogeneity in prognoses (Creasman et al., 1987). However, the addition of other known prognostic clinicopathologic characteristic strengthens the predictive value of PDGF-D expression in EC. We demonstrated that high PDGF-D expression in patients with Histologic grade and FIGO stage, all prognostic factors are indicative of poorer clinical outcomes in endometrial carcinoma. Comparison of PDGF-D expression levels between with grade 1 and 2 patients and grade 3 patients in human endometrial cancer did not yield a statistically significant difference, likely due to the small number of tissues used in this study.

Despite these findings, an evaluation of PDGF-D expression levels linked to survival and recurrence in a higher-powered study would be of considerable value in this setting. Nonetheless, the role of PDGF-D as a potential prognostic marker is supported by prior reports in pancreatic cancer (Xu et al., 2005), where high PDGF-D expression in tumor endothelium is associated with recurrence and metastases.

Finally, the data presented here demonstrate that PDGF-D expression predicts poor prognosis since cases with strong staining showed a decreased survival compared to those with no staining as demonstrated by multivariate analysis. Overexpression of PDGF-D is an independent poor prognostic marker in Endometrial Cancer.

In conclusion, to date, a number of studies

have suggested a role for PDGF-D in endometrial carcinogenesis. Despite the limited literature associating PDGF-D with endometrial carcinogenesis, this field deserves further study, especially in light of the inadequate treatment options which currently exist for women with advanced and recurrent EC. Our data demonstrate that PDGF-D expression is up-regulated in endometrial cancer. High expression levels of PDGF-D correlating with clinicopathologic factors including histologic grade and FIGO stage which predict poor prognosis. Our studies suggest that PDGF-D plays an important role in EC as a tumor supporter and may be a candidate as a novel biomarker and suggest the importance of further studies to target the PDGF-D as novel targeted therapy.

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