Circulating Pigment Epithelium-derived Factor (PEDF) Is Associated with Pathological Grade of Prostate Cancer

HISAMITSU IDE1, SHO-ICHI YAMAGISHI2, YAN LU1, KENTARO SAKAMAKI3, AKIKO NAKAJIMA1, AKIRA HORIUCHI1, KOSUKE KITAMURA1, SCHIN-ICHI HISASUE4, SATOSU MUTO1, RAIZO YAMAGUCHI1 and SHIGEO HORIE5

1Department of Urology, Teikyo University School of Medicine, Tokyo, Japan; 2Department of Pathophysiology and Therapeutics of Diabetic, Vascular Complications, Kurume University School of Medicine, Kurume, Japan; 3Department of Biostatistics and Epidemiology, Yokohama City University School of Medicine, Yokohama, Japan; 4Department of Urology, Juntendo University, Graduate School of Medicine, Tokyo, Japan

Abstract. Background/Aim: Pigment epithelium-derived factor (PEDF) plays a protective role against oxidative stress. Levels of circulating PEDF have not been examined in patients with prostate cancer. We examined whether PEDF can be used to predict the clinical features of prostate cancer prior to therapy. Materials and Methods: Two hundred patients with an abnormal serum level of prostate-specific antigen (PSA) who underwent biopsy between 2008 and 2011 were identified for retrospective analysis. We determined the relationship of the PEDF level to clinical parameters of prostate cancer. We measured levels of PEDF and 8-hydroxy-2'-deoxyguanosine (8-OHdG) in all 200 patients, 100 of whom had histologically-confirmed prostate cancer at the outset. We also investigated the PEDF expression in prostate cancer tissues by immunohistochemistry. Results: The PEDF level was significantly higher in patients with prostate cancer than in those without. Statistical analysis confirmed that PEDF was significant, positively associated with pathological grading (Gleason score). However, PEDF expression was only detected in few prostate cancer cells by immunohistochemistry. Levels of the oxidative marker, 8-OHdG, in patients with prostate cancer are higher than in those without cancer. Conclusion: Preoperative PEDF measurement in patients with prostate cancer may provide clinically relevant information regarding the pathological grade of tumor.

Correspondence to: Hisamitsu Ide, MD, Ph.D., Department of Urology, Teikyo University School of Medicine, 2-11-1, Kaga, Itabashi-ku, Tokyo 173-8605, Japan. Tel: +81 339642497, Fax: +81 339648934, e-mail: ihisamit@med.teikyo-u.ac.jp

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PEDF expression is often decreased or absent in human prostate cancer cells compared to adjacent normal gland (5, 14). These findings indicate that PEDF could play an important role in regulating the normal prostate, and that its loss in prostate tumors could contribute to tumor progression. We, therefore, examined the plasma PEDF level both in normal prostate and patients with prostate cancer to determine if PEDF predicts clinical features of prostate cancer prior to therapy. Contrary to what we expected, PEDF levels in patients with prostate cancer were significantly higher than in those without, and were significantly associated with pathological grading (Gleason score). We discuss the possible anti-inflammatory and antitumor properties of PEDF, which may support these results.

Materials and Methods

Patients. We retrospectively analyzed the medical records of all patients with prostate cancer who underwent prostate biopsy at Teikyo University School of Medicine Hospital from January 2008 through November 2011. One hundred individuals with adenocarcinoma of the prostate and one hundred patients in whom prostate cancer was not detected by needle biopsy were enrolled in this study. All patients gave their written informed consent, and approval was obtained from the hospital Research Ethics Board (Approval number: 13-121). Histopathological evaluation by prostate biopsy of all patients was undertaken and the Gleason score was 6 in 29 cases, 7 in 29, 8 in 21, 9 in 17 and 10 in four cases. None of the patients had had any treatment for prostate cancer prior to this study.

Immunoaassay.Venous blood plasma samples were collected from each patient before biopsy. PEDF measurements were performed with a competitive enzyme-linked immunosorbent assay (ELISA) as described previously (15). Levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG) were also measured by ELISA (Japan Institute for the Control of Aging, Shizuoka, Japan). Prostate-specific antigen (PSA) levels were measured by chemiluminescence enzyme immunoassay with a Lumipulse kit (Fujirebio, Tokyo, Japan). Testosterone levels were measured using the Architect testosterone kit (Abbott Japan, Tokyo, Japan). Limit of quantification of PDEF, 8-OHdG, PSA and testosterone is 0.1 μg/ml, 0.03 ng/ml, 0.01 ng/ml, 4.3 ng/dl, respectively.

Immunohistochemistry. For immunohistochemistry, serial 4-μm-thick sections of prostate cancer tissues samples were deparaffinized in three changes of xylene and rehydrated through a 100% to 70% descending series of ethanol. They were then immersed in citrate buffer (pH 6.0) in autoclaves for 5 min, and placed in 3% H2O2 in methanol for 10 min at room temperature to block endogenous peroxidase activity. After the blocking of non-specific protein binding by incubation for 30 min to 1 h with 5% goat serum, the whole tissue sections were incubated with monoclonal mouse antibody to PEDF (at a concentration of 10 μg/ml; Millipore, Massachusetts, USA) overnight at 4°C. Subsequently, sections were processed for immunohistochemistry using DakoCytomation EnVision+ System (Dako, California, USA). We stained tissue sections of kidney with monoclonal mouse antibody to PEDF as a positive control. Specificity of staining was confirmed by replacing the primary antibody with PBS containing 1% preimmunized rabbit serum and by blocking positive staining by excess peptide of PEDF added to the solution of primary antibody.

Results

The characteristics of the study population are shown in Table I. Univariate analysis revealed statistically significant differences in age (p<0.001), PSA (p=0.001), PEDF (p=0.01), creatinine (p=0.036) and testosterone (p=0.007) in patients with and without prostate cancer (Table I). A broad range of levels of circulating PEDF was found, ranging from 5.24 to 36.9 μg/ml. As Figure 1A shows, there was a statistically significant difference in PEDF level between patients with prostate cancer and those without cancer, with higher levels in the former. The mean±SD PEDF level was 12.93±3.75 in those without cancer (based on needle biopsy) and 14.61±5.27 in patients with prostate cancer, with a statistically significant difference (p=0.01). The mean PEDF in patients with tumors graded as Gleason score 6, 7, 8, 9 and 10 were 13.9±4.14, 13.69±4.71, 14.93±6.99, 15.92±5.54 and 19.35±2.6 μg/ml, respectively, showing a positive correlation between the Gleason score and the PEDF value. A one-way ANOVA revealed a significant positive correlation of PEDF with the Gleason score (p=0.014) (Figure 1B). The PEDF level positively correlated with the BMI in cases without cancer, but not in those with prostate cancer (Figure 2).

Table I. Baseline characteristics of the patients enrolled in this study.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls (n=100)</th>
<th>Patients with prostate cancer (n=100)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age, years (range)</td>
<td>68.15 (38-90)</td>
<td>73.58 (42-90)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PEDF (μg/ml)</td>
<td>12.93±3.8</td>
<td>14.61±5.3</td>
<td>0.01</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>0.53±1.6</td>
<td>0.21±0.5</td>
<td>0.08</td>
</tr>
<tr>
<td>PSA (ng/ml)</td>
<td>8.2±10.0</td>
<td>44.45±106.1</td>
<td>0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.73±3.3</td>
<td>23.68±2.9</td>
<td>0.07</td>
</tr>
<tr>
<td>Testosterone (ng/dl)</td>
<td>458.8±187</td>
<td>529.1±171.9</td>
<td>0.007</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.87±0.2</td>
<td>1.1±1.1</td>
<td>0.036</td>
</tr>
</tbody>
</table>

PEDF, Pigment epithelium-derived factor; CRP, C-reactive protein; PSA, prostate-specific antigen; BMI, body-mass index. Data are the mean±SD, except for age.
To determine the expression of PEDF in prostate, we used mouse a monoclonal antibody against human PEDF for immunohistochemistry. Reactivity, shown as brown color in cancerous glands, was detected in few prostate tissues which were obtained by radical prostatectomy (Figure 3). The PEDF staining was heterogeneous and predominately cytoplasmic in prostate epithelial and cancer cells. Out of the five specimens of prostate cancer studied, we clearly detected positive staining of PEDF in one sample.

Among all bases in nucleic acid, guanine is the most susceptible to oxidative damage which leads to the formation of 8-OHdG. As it is stable and excreted in bodily fluids with DNA repair, 8-OHdG is one of the most commonly used markers for evaluating oxidative damage (16). Figure 4
shows a statistically significant difference in 8-OHdG levels in patients with prostate cancer and in those without, with higher levels in the former. Considering that PEDF is endogenously produced, widely expressed in immune cells such as macrophage, and abundant throughout the body, these results suggest that PEDF was elevated as an antioxidative, antitumor reaction to the presence of prostate cancer cells.

**Discussion**

PEDF is a glycoprotein that belongs to the superfamily of serine protease inhibitors (16). PEDF is reportedly widely expressed in a variety of human body tissues including the brain, spinal cord, eye, plasma, bone, pancreas, heart, lung and prostate (2). Recently, PEDF has been shown to be the most potent inhibitor of angiogenesis (17). Furthermore, PEDF not only inhibits cell damage, but also blocks apoptotic cell death through its antioxidative properties (18). In prostate cancer, PEDF expression is down-regulated compared to normal prostatic epithelial cells. Clinical observation suggests that loss of PEDF expression may be associated with progression toward a metastatic phenotype in prostate cancer (19). Indeed, PEDF inhibited stromal vasculature and epithelial growth in a xenograft tumor model (20). In addition, PEDF expression was inversely correlated with metastasis in both a rat prostate cancer model and poorly-differentiated human prostate cancer, indicating that PEDF has direct effects on prostate tumor cells (19). The mechanisms of antitumor effects of PEDF are not limited to antiangiogenesis, but also include growth-inhibitory action on prostate carcinoma cells through induction of tumor cell apoptosis. This effect is accompanied by increased caspase 3 activation (21).

Recently, PEDF was identified as a biomarker for components of metabolic syndrome (15). In addition, PEDF is elevated in patients with coronary artery disease as a predictor of cardiovascular events (22). Several studies suggest that PEDF is a serum marker of clinical and subclinical inflammation. However, there have been few reports concerning PEDF levels among patients with cancer, especially those with prostate cancer. Our current study found a statistically significant difference in PEDF levels between patients with biopsy-confirmed prostate cancer, and patients in whom biopsy had not detected prostate cancer, with higher levels in the former. However, elevated PEDF values were positively correlated with the Gleason score.
which is a pathological indicator of malignancy. It has been reported that PEDF is suppressed by androgen in the cultured prostate epithelium and increases in the prostate in vivo upon castration (14). Our study measured PEDF values when biopsies were performed, and did not include patients who had received any prior androgen ablation therapy. Patients with high Gleason scores (indicating aggressive malignancy) may have metastasis to bone, lymph nodes, and other sites, with angiogenesis stimulated in the structure surrounding the tumor. In fact, our study found a correlation between PEDF level and BMI in patients without cancer, a correlation undetected in patients with prostate cancer. Elevation of PEDF in blood vessels and macrophage to work as a defensive reaction against the tumor may have manifested an antitumor effect in patients with aggressive prostate cancer (23, 24). Further clinical investigation is needed to clarify the relationship between prostate cancer progression/metastasis and PEDF, as well as the biological function of PEDF and its overall physiological role in the human body.

To our knowledge, our study is the first to show that PEDF is a possible biomarker reflecting tumor activity of prostate cancer and that it may be useful in risk stratification of individual patients with prostate cancer. Further elucidation of the clinical significance of PEDF is required for accurate correlation of its production in cancer with clinical and pathological variables.

Conclusion

Although the precise extent of the contribution of PEDF to prostate cancer progression remains unknown, we postulate that measuring PEDF may yield important information in the diagnosis of prostate cancer and might provide clinically relevant information about the pathological grade of tumor. Information regarding potential tumor progression would be particularly useful in weighing cancer therapy strategies. Our results suggest that PEDF levels may be elevated as a counter-system against prostate cancer angiogenesis and its progression.

References


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