Advances in Brief

Clinical Implication of Expression of Platelet-derived Endothelial Cell Growth Factor (PD-ECGF) in Metastatic Lesions of Uterine Cervical Cancers

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Abstract

The platelet-derived endothelial cell growth factor (PD-ECGF) level was significantly ($P < 0.05$) increased in 8 of 40 metastatic lymph node lesions of uterine cervical cancers. The prognosis of the eight patients with high PD-ECGF (>10,000 pg/mg protein) in metastatic lymph node lesions was extremely poor. On the other hand, the 24-month survival rate of the 32 patients with low PD-ECGF (<10,000 pg/mg protein) in metastatic lymph node lesions was 75%. This indicates that PD-ECGF may contribute to the advancement of metastatic lesions, and that the PD-ECGF level in metastatic lesions may be a prognostic indicator.

Introduction

PD-ECGF[2] was cloned as a novel angiogenic factor (M_r 45,000 polypeptide) from human platelet (1), and thereafter was completely identified with TP (2, 3). PD-ECGF/TP does not stimulate the growth of endothelial cells but the chemotaxis of them and induces angiogenesis *in vivo* with the activation of TP as an enzyme (4, 5).

PD-ECGF action on the advancement of female genital tract diseases can be gleaned from the following: PD-ECGF was up-regulated in normal uterine endometrium after ovulation (6), although steroidal regulation of PD-ECGF was deficient in ovarian endometriosis during the menstrual cycle (7). In uterine endometrial cancers, PD-ECGF was dominantly expressed in interstitial cells and contributed to myometrial invasion of the cancer cells and tumor growth in the early stage (8). In ovarian cancers, PD-ECGF was remarkably highly expressed in some ovarian cancers; however, its levels did not correlate with patients' prognoses (9). In squamous cell carcinoma of the uterine cervix, PD-ECGF was dominantly expressed in interstitial cells, and its levels correlated with microvessel density and patients' prognoses (10).

Among female genital tract cancers, PD-ECGF in the primary tumor of uterine cervical cancers is recognized as a prognostic indicator. On the other hand, although the presence of lymph node metastasis, recognized as the most common metastatic lesion, is critical to patients' prognoses (11–14), there is as yet no prognostic indicator for lymph node-positive patients in uterine cervical cancers. This status prompted us to investigate the clinical significance of PD-ECGF expression in metastatic lymph nodes of uterine cervical cancers.

Materials and Methods

Patients. Consent for the following studies was obtained from all of the patients and the Research Committee for Human Subjects, Gifu University School of Medicine. Forty patients, ages 42–68 years, underwent curative resection for cervical cancer stage IIb with metastatic lymph nodes and were given a 24-month survival rate at the Department of Obstetrics and Gynecology, Gifu University School of Medicine, between March 1994 and December 1996. None of the patients had received any preoperative therapy. A part of each tissue of the uterine cervical cancers was obtained immediately after hysterectomy and was snap-frozen in liquid nitrogen to determine the levels of PD-ECGF, and a neighboring part of the tissues was submitted for histopathological study. The clinical stage of uterine cervical cancers was determined by International Federation of Obstetrics and Gynecology (FIGO) classification (15).

Immunohistochemistry. For formalin-fixed paraffin-embedded tissues, 4-μm sections were cut with a microtome and dried overnight at 37°C on a silanized slide (Dako, Carpinteria). Samples were deparaffinized in xylene at room temperature for 80 min and washed with a graded ethanol/water mixture and then with distilled water. The samples for PD-ECGF antigen were soaked in a phosphate buffer (PBS), and then autoclaved at 121°C for 10 min, and those for factor VIII-related antigen were treated with 0.3 μg/ml trypsin in PBS at room temperature for 20 min. The protocol for a DAKO LSAB2 kit, peroxidase (Dako) was followed for each sample. In the described procedures, mouse antihuman PD-ECGF antigen 654–1 (10 μg/ml, Nippon Roche, Kamakura, Japan; Ref. 16), and rabbit anti-factor VIII-related antigen (Zymed, San Francisco, CA) were used at dilutions of 1:100 and 1:2, respectively, as the first antibodies. The addition of the first antibody, mouse antihuman PD-ECGF antigen 654–1 or rabbit anti-factor VIII-related antigen, was omitted in the protocols for negative controls of PD-ECGF or factor VIII-related antigen, respectively. Vascular density was evaluated by way of microvessel counting (17).

Enzyme Immunoassay for Determination of Human PD-ECGF Antigen. All of the steps were carried out at 4°C. Tissues (wet weight: 10–20 mg) were homogenized in HG buffer [5 mM Tris-HCl (pH 7.4), 5 mM NaCl, 1 mM CaCl$_2$, 2 mM ethyleneglycol-bis-[β-aminoethyl ether]-*N,N,N′,N′*-tetraacetic acid, 1 mM MgCl$_2$, 2 mM dithiothreitol (DTT), 25 μg/ml aprotinin, and 25 μg/ml leupeptin) with a Polytron homogenizer (Kinematics, Luzern, Switzerland). This suspension was centrifuged in a microfuge at 12,000 rpm for 3 min to obtain the supernatant. The protein concentration of samples was measured by the method of Bradford (18) to standardize PD-ECGF antigen levels.

PD-ECGF antigen levels in the samples were determined by the sandwich enzyme immunoassay described by Nishida *et al.* (16). The levels of PD-ECGF were standardized with corresponding cellular protein concentrations.

Statistics. Survival curves were calculated using the Kaplan-Meier method and analyzed by the log-rank test. PD-ECGF levels were measured from three parts of the same tissue in triplicate. Statistical analysis was performed with Student's *t* test. Differences were considered significant when $P < 0.05$.

Results

There was a significant correlation between PD-ECGF levels and microvessel counts in the primary and metastatic lesions as shown in Fig. 1 ($Y = 18.332 + 0.004x$; $r = 0.687$; $P < 0.01$).

Immunohistochemical staining for PD-ECGF in a representative case of large cell nonkeratinizing squamous cell carcinoma is shown in Fig. 2. PD-ECGF was distributed in the surrounding interstitium near the cancer cells and in the cytoplasm and nuclear compartments of the cancer cells. In 8 of the 40 cases, stronger staining for PD-ECGF was found in the cancer cells or interstitial cells of the metastatic lesion than in the primary lesion (Table 1). The PD-ECGF-increased cases identified by immunohistochemical staining (Table 1) were consistent with those identified by the sandwich immunoassay [Fig. 3 (*cases 2, 7, 11, 19, 25, 29, 31,* and *36*)].
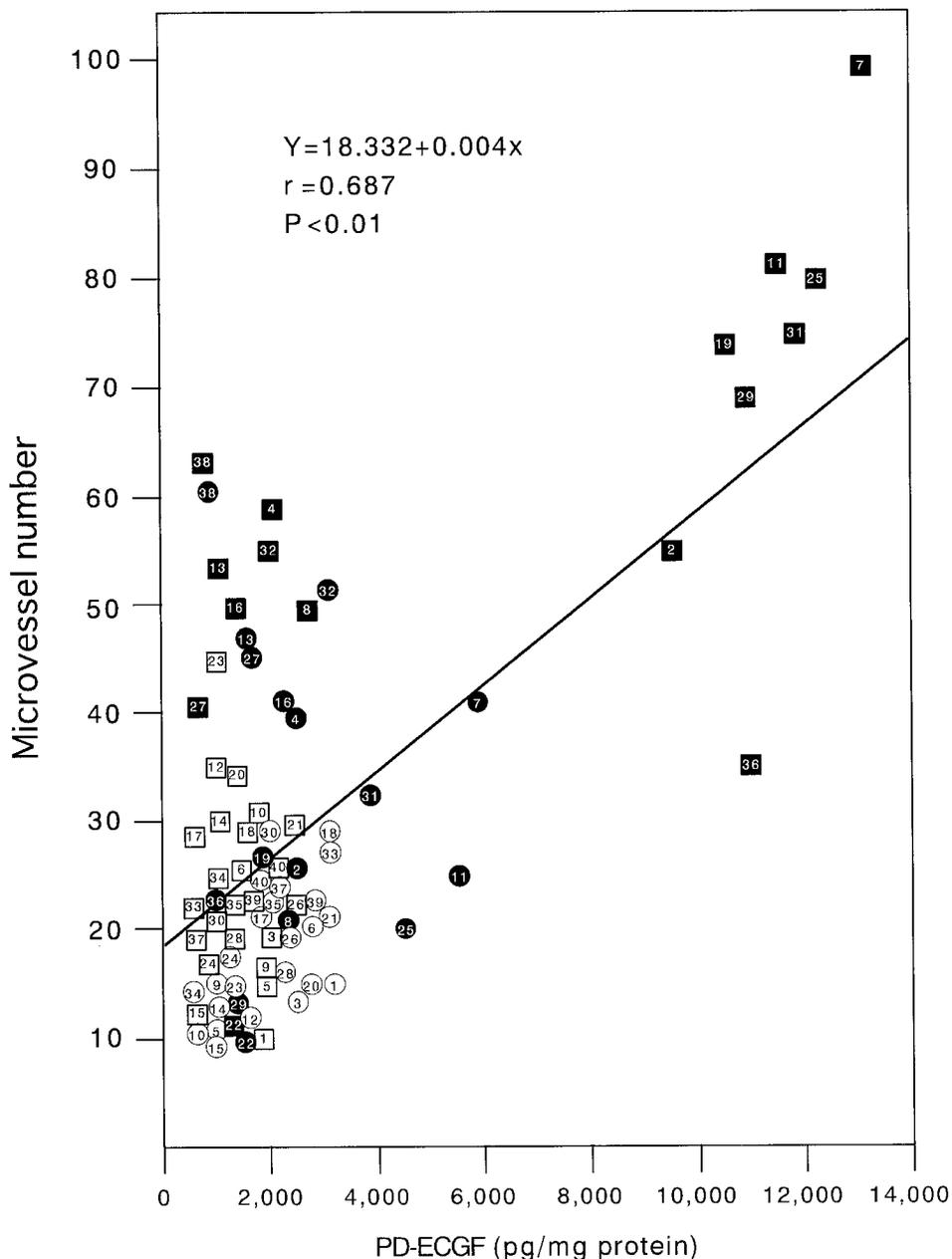
Fig. 1. Correlation between PD-ECGF level and microvessel count. PD-ECGF antigen levels in the samples were determined by sandwich enzyme immunoassay (16). Vascular density was evaluated by way of microvessel counting (17). In the primary lesions: *number in* ○, living case; *number in* ●, deceased case. In the metastatic lesions: *number in* □, living case; *number in* ■, deceased case.

The PD-ECGF level in the primary tumors was less than approximately 6,000 pg/mg protein (Fig. 3). On the other hand, the PD-ECGF level in 8 of 40 metastatic lesions of uterine cervical cancers was remarkably ($P < 0.05$) increased up to approximately 10,000 pg/mg protein, whereas the level in the other 32 lesions was not altered (Fig. 3). The prognosis of the eight patients with high PD-ECGF (>10,000 pg/mg protein) in the metastatic lesions was extremely poor, whereas the 24-month survival rate of the other 32 patients with low PD-ECGF (<10,000 pg/mg protein) in metastatic lesions was 75% (Fig. 4).

Discussion

The patient prognosis in colorectal carcinoma (19) and oral and oropharyngeal carcinomas (20) with high PD-ECGF associated with more extensive angiogenesis was poor. PD-ECGF is dominantly expressed in deeply invasive bladder carcinomas, which indicates that PD-ECGF is a tumor-advancing factor mainly via angiogenic activity (21). PD-ECGF expression is associated with a poor prognosis in patients with ductal adenocarcinoma of the pancreas and enhances the abilities of tumor invasion and metastasis through its angiogenic properties (22). Furthermore, elevated PD-ECGF expression predicted early recurrence in bladder carcinoma and was associated with a greater possibility of recurrence and disease progression (23). In gastric carcinoma, PD-ECGF expression is closely associated with the promotion of angiogenesis and hepatic and lymph node metastases

Table 1 *Alteration in immunohistochemical staining for PD-ECGF from the primary to metastatic lesion of uterine cervical cancers*

Comparatively increased staining for PD-ECGF of metastatic lesion to that in the primary lesion was recognized as "increased." The PD-ECGF-increased cases by immunohistochemical staining were consistent with those by the sandwich immunoassay.

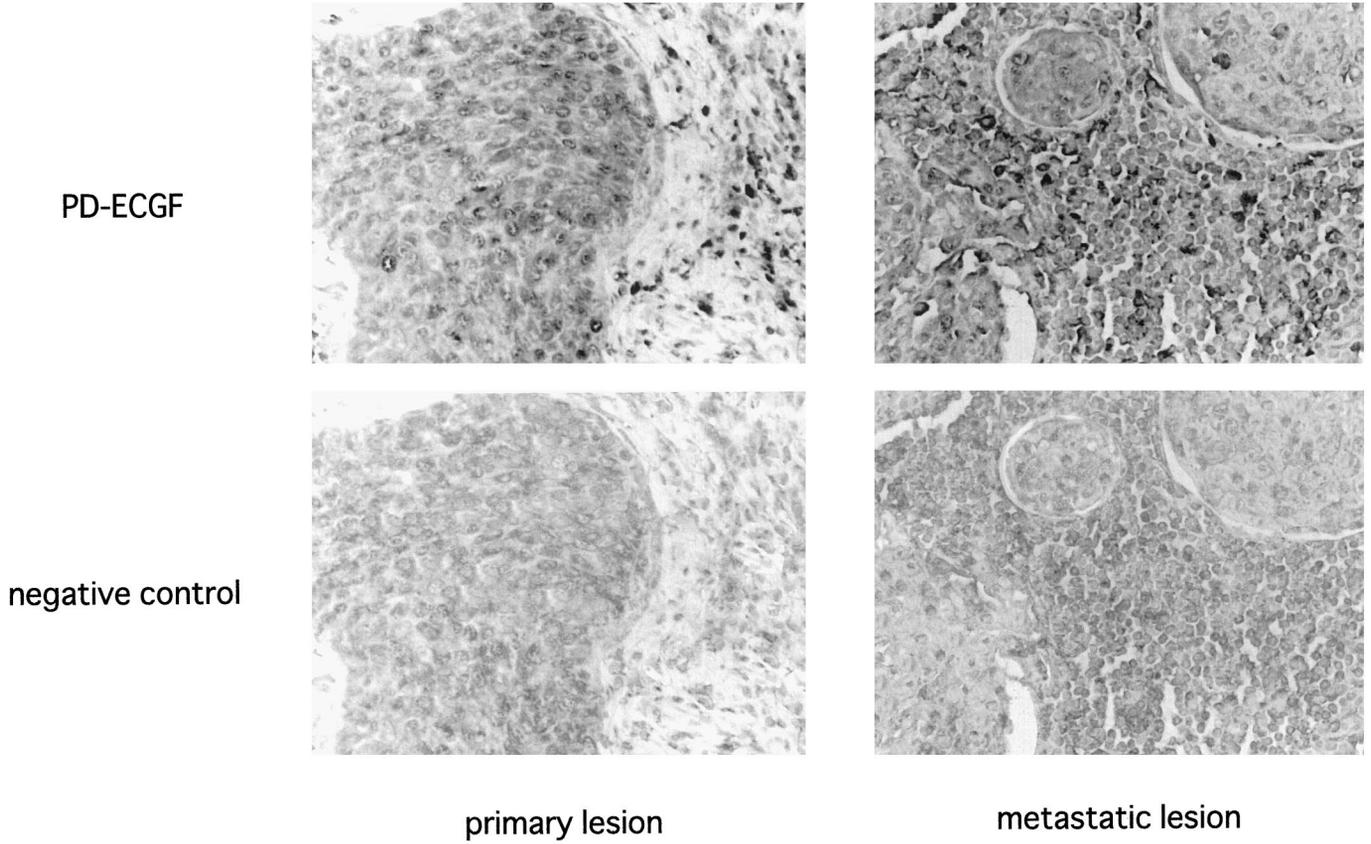| Cancer cells	Interstitial cells	Case no.
No change	No change	32
No change	Increase	6
Increase	Increase	2

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Fig. 2. Immunohistochemical staining for PD-ECGF in primary and metastatic lymph node lesions of uterine cervical cancers. A case of large cell nonkeratinizing squamous cell carcinoma of the uterine cervix, metastatic lesion in cardinal lymph nodes. The addition of the first antibody—mouse antihuman PD-ECGF antigen 654–1 or rabbit anti-factor VIII-related antigen—was omitted in the protocols for negative controls of PD-ECGF or factor VIII-related antigen, respectively. ×200.
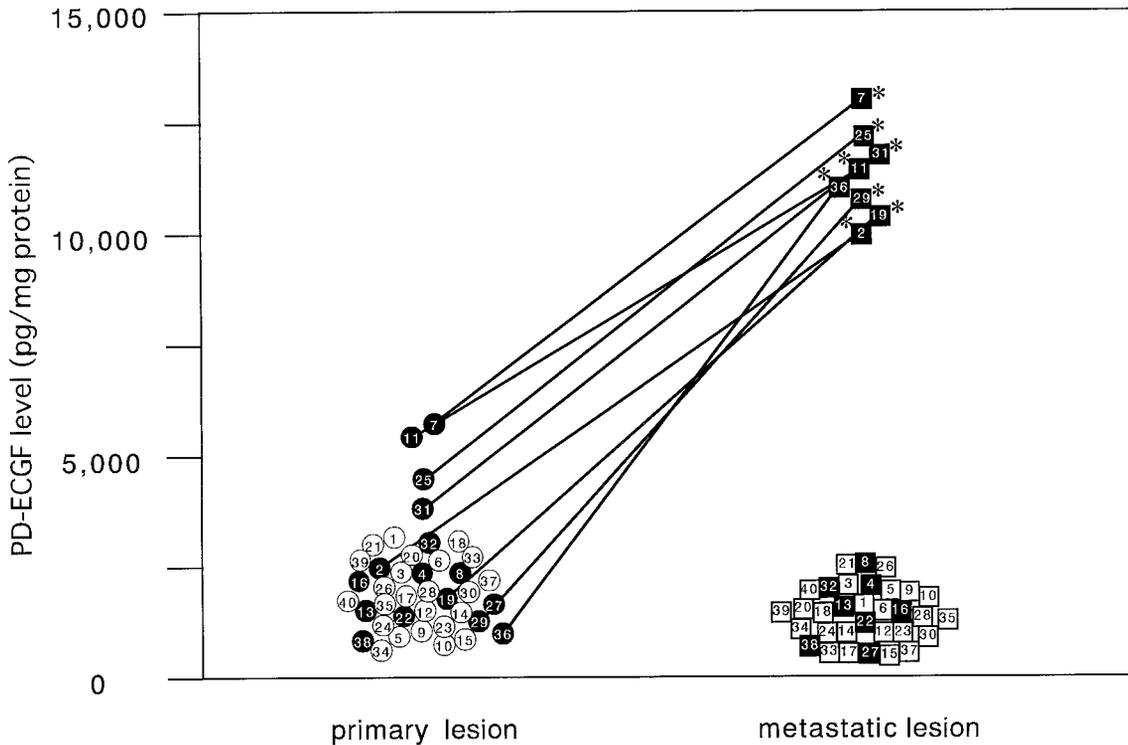

Fig. 3. Levels of PD-ECGF in primary and metastatic lymph node lesions of uterine cervical cancers. The levels of PD-ECGF were determined by a sandwich enzyme immunoassay. Each level is the mean ± SD of nine determinations. In the primary lesions, alive and deceased cases are numbered in ○ and ●, respectively. In the metastatic lesions, alive and deceased cases are numbered in □ and ■, respectively. *, $P < 0.05$ *versus* each primary lesion
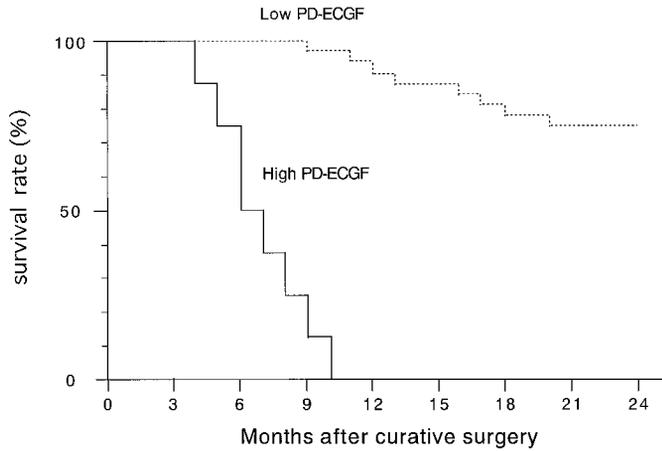
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Fig. 4. Survival rate after curative resection for uterine cervical cancers. Patient prognosis was analyzed with a 24-month survival rate. High PD-ECGF, > 10,000 pg/mg protein, $n = 8$; low PD-ECGF, < 10,000 pg/mg protein, $n = 32$.

(17, 24). However, until now, there has been no analysis of PD-ECGF expression in metastatic lesions of any cancers. The determination of PD-ECGF expression in metastatic lesions and in the corresponding primary tumors should show PD-ECGF function in metastatic lesions. In the present study, positive correlation between PD-ECGF levels and microvessel counts demonstrates that PD-ECGF works as an angiogenic factor.

Of the 32 patients with a low PD-ECGF level in the metastatic lesion, 8 died within the two-year follow-up period. In those eight patients, we analyzed other angiogenic factors in addition to PD-ECGF. Microvessel density in tumors of seven of the eight patients (all except for case 22) was relatively higher than in the other patients with low PD-ECGF values. The levels of basic fibroblast growth factor (bFGF) in primary tumors (bFGFp) and metastatic lesions (bFGFm) of three patients were relatively higher than those seen among primary tumors (25): 117:195 (case 4), 132:174 (case 13), and 214:208 (case 38) pg/mg protein bFGFp:bFGFm. The levels of vascular endothelial growth factor (VEGF) in primary tumors (VEGFp) and metastatic lesions (VEGFm) of three other patients were relatively higher than those found among primary tumors (26): 1,013:1,432 (case 8), 1,217:1,349 (case 16), and 1,494:1,531 (case 32) pg/mg protein VEGFp:VEGFm. In the other two cases (cases 22 and 27), high expressions of angiogenic factors among basic FGF, VEGF, and PD-ECGF were not detected. Therefore, angiogenesis in the tumors of 7 of the 8 patients may have been dominantly activated by angiogenic factors other than PD-ECGF.

PD-ECGF in the primary tumor of uterine cervical cancers is recognized as a prognostic indicator associated with angiogenic activity (10). On the other hand, the presence of lymph node metastasis is critical to patients' prognoses. In the present study, a significant difference in patient prognosis was found between high and low PD-ECGF in metastatic lymph node lesions of uterine cervical cancers. This indicates that PD-ECGF may be essential to the advancement of metastatic lesions, and that the PD-ECGF level in metastatic lesions may be recognized as an even more critical prognostic indicator than the PD-ECGF level in primary lesions.

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