**Advances in Brief**

**Vascular Endothelial Growth Factor and Platelet-derived Endothelial Cell Growth Factor Are Frequently Coexpressed in Highly Vascularized Human Breast Cancer**

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**Abstract**

Vascular endothelial growth factor (VEGF) and platelet-derived endothelial cell growth factor (PD-ECGF) are known to be angiogenic growth factors in vitro and in vivo. In this study, we have investigated the relationship between VEGF expression and PD-ECGF expression in human breast cancer tissues using immunocytochemical methods.

Of 152 primary breast cancers, 84 (55.3%) and 71 (46.7%) were positive for VEGF and PD-ECGF, respectively. Fifty-three (63.1%) of 84 VEGF-positive tumors had a PD-ECGF-positive phenotype, whereas only 18 (26.5%) of 68 VEGF-negative tumors had a PD-ECGF-positive phenotype. There was a significant correlation between the VEGF expression and PD-ECGF expression ($P < 0.01$). As a single factor, VEGF expression and PD-ECGF expression were significantly associated with an increase in the microvessel density assessed by the immunocytochemical analysis using antifactor VIII-related antigen mAb. Interestingly, in addition, of 53 tumors with more than 100 microvessel counts/mm², 40 (75.5%) had both VEGF- and PD-ECGF-positive phenotypes. It was found that VEGF and PD-ECGF were frequently coexpressed in highly vascularized tumors with high microvessel counts.

It is suggested that VEGF and PD-ECGF might cooperatively function in the neovascularization of human breast cancer.

**Introduction**

In recent investigations, a variety of growth factors and cytokines were characterized as angiogenic growth factors (1, 2). Among them, VEGF and PD-ECGF are thought to be particularly responsible for neovascularization in human solid tumors (3–5). VEGF was first isolated as a selective mitogen for endothelial cells by Ferrera and Henzel (3) and Senger et al. (4). Its receptors, fms-like tyrosine kinase (flt) 1 and kinase insert domain-containing receptor, are demonstrated to be selectively expressed in endothelial cells (6–8). VEGF is now noticed to be involved not only in tumor angiogenesis but also in vasculogenesis (9).

On the other hand, PD-ECGF was first isolated from platelets, and the transfusion of the PD-ECGF gene into ras-transformed NIH 3T3 cells altered more angiogenic cells in the nude mouse model (5). Afterward, PD-ECGF was noted to have dThdPase activity, which is an enzyme involved in nucleic acid metabolism (10).

In a previous series of studies, we have independently indicated the importance of VEGF expression and that of PD-ECGF expression for tumor angiogenesis in human breast cancer (11–13). Thus, in this study, we have examined VEGF expression and PD-ECGF expression in the same series of samples, and investigated the relationship between expressions of these factors and the microvessel density determined by immunocytochemical assay using the antifactor VIII-Ra mAb. This study will show a close association between two different angiogenic growth factor expressions in breast cancer.

**Materials and Methods**

**Patients and Materials.** One hundred fifty-two unselected primary breast cancer tissues with invasive ductal carcinoma were used in this study. All patients received extended, standard, or modified radical mastectomies with full dissection of axillary lymph nodes. Tumor tissues were immediately frozen after removal and stored at $-80^\circ$C until processing. A mouse anti-VEGF mAb, which was estimated to detect all four VEGF species, was used (12). A mouse anti-PD-ECGF mAb was a kind gift from Dr. Ishitsuka (Rosche Institute, Japan; Ref. 13). The antifactor VIII-Ra mAb was obtained from DAKO Japan (Tokyo).

**Immunocytochemistry.** Expressions of VEGF, PD-ECGF, and factor VIII-Ra were assessed in frozen sections using an indirect immunoperoxidase method. As previously described, the expression of VEGF and PD-ECGF was characterized as minus and plus according to its staining intensity (11–13).

Intratumoral microvessel density was evaluated by counting the number of endothelial deposits/mm² in the areas that were considered to be most active for neovascularization. The count was performed at three fields, and the average was calculated (14). These evaluations were done in a completely blinded condition without knowledge of the patients' characteristics.

Histological examinations were performed on slides from paraffin blocks stained with hematoxylin and eosin according to...
Table 1

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$^a$ $\chi^2$ test.

$^b$ NS, not significant.

The criteria of the Japanese Breast Cancer Society, which are based on the UICC criteria. Histological lymphatic invasion and vascular invasion of tumor cells were graded as –, 1+, and 2+.

Hormone Receptors. Estrogen and progesterone receptors were measured according to the dextran-coated charcoal method using $[^{3}H]17$-estradiol, and tumor with more than 5 fmol/mg protein was designated as positive.

Results

Of 152 breast cancer tissues, 84 (55.3%) and 71 (46.7%) were VEGF positive and PD-ECGF positive, respectively. Table 1 gives the background characteristics of various clinicopathological factors. There was no statistical correlation between VEGF expression, or PD-ECGF expression, and menopausal status, tumor size, number of metastatic nodes, hormone receptor status, histological lymphatic invasion, and histological vascular invasion. With regard to the relationship with microvessel density, VEGF expression and PD-ECGF expression were significantly correlated with the increment of the microvessel density ($P < 0.01$ and $P < 0.01$, respectively).

Of 84 VEGF-positive tumors, 53 (63.1%) were PD-ECGF positive, whereas only 18 (26.5%) of 68 VEGF-negative tumors were positive for PD-ECGF (Table 2). There was a close association between VEGF expression and PD-ECGF expression ($\chi^2 = 18.81, P < 0.01$). Of 152 tumors, 103 (67.8%) showed an accordance between the status of VEGF expression and PD-ECGF expression.

In addition, an analysis using the combination of VEGF and PD-ECGF status showed that 40 (75.5%) of 53 VEGF-positive and PD-ECGF-positive tumors were highly vascularized tumors with more than 100 microvessel counts/mm². On the other hand, of 50 VEGF-negative and PD-ECGF-negative tumors, 19 (38.0%) and 24 (48.0%) were poorly (less than 50 microvessel counts/mm²) and moderately (51–100 microvessel counts/mm²) vascularized tumors, respectively. The $\chi^2$ test demonstrated a significant correlation between the VEGF/PD-ECGF status and the microvessel density (Table 3).

Discussion

In this study, a close correlation between the VEGF expression and PD-ECGF expression was found. As far as we know, no basic data indicating a close interaction between VEGF and PD-ECGF were reported. Although these two factors were isolated as endothelial growth factors, their characteristics seem to be different. VEGF is a specific and potent mitogen for endothelial cells. In particular, two smaller size species, VEGF121 and VEGF165, out of four VEGF species, are secreted proteins, although two larger size species, VEGF189 and
VEGF206, are bound to heparin-containing proteoglycans in the cell surface (15). VEGF exerts its angiogenic activity, particularly mitogenic activity, through tyrosine kinase-type cell membrane receptors, flt-1 and kinase insert domain-containing receptor, which are selectively expressed in endothelial cells (6–8). On the other hand, PD-ECGF was also identified as an angiogenic growth factor from platelets; however, afterward, PD-ECGF was demonstrated to be identical to dThdPase in the cDNA sequence (10, 16). Moghaddam and Bicknell (17) confirmed that the PD-ECGF’s effect on the uptake of thymidine by endothelial cells was due to modulation of cellular thymidine pools. Recently, Haraguchi et al. (18) found that 2-deoxy-o-ribose, a degradation product of thymidine caused by PD-ECGF/dThdPase, had an angiogenic property, particularly chemotactic activity, both in vitro and in the chorioallantoic membrane assay. Therefore, the angiogenic activity of PD-ECGF is thought to be due to the metabolites of thymidine caused by PD-ECGF/dThdPase.

Although the switch-on mechanism of the angiogenesis phenotype, including induction of VEGF and PD-ECGF, has been widely investigated, many still remain unseen. Hypoxia is noted to be a potent inducer of VEGF expression; however, it seems evident that VEGF is also continuously expressed in cultured tumor cells under usual conditions (11). In addition, the effect of hypoxia seems to be unlikely for other types of angiogenic growth factors including PD-ECGF. On the other hand, several kinds of growth factors or cytokines, including tumor necrosis factor α, interleukin 1, and IFN-γ, were reported to induce the expression of PD-ECGF/dThdPase (19). However, there are few data on the induction of VEGF expression by these factors. In our preliminary study, no marked induction of VEGF expression by these factors has been detected. Some unknown mechanism should be addressed in order to account for the frequent coexpression between VEGF and PD-ECGF in human breast cancer. In recent investigations, the potentiation of protein kinase C induction of VEGF by mutant p53 was indicated (20).

Clinical studies investigating the prognostic value of angiogenesis have confirmed that the microvessel density is a potent and independent prognostic indicator in a variety of tumors, including breast cancer (14, 21–26). In the present study, we found that VEGF and PD-ECGF were frequently coexpressed in highly vascularized breast tumors with more than 100 microvessel counts. This simply seems to suggest that VEGF and PD-ECGF cooperatively function for the promotion of angiogenesis in human breast cancer. Because of the difference in the activity of these factors for the endothelium, this cooperation hypothesis between PD-ECGF and VEGF seems to be interesting.

On the other hand, in recent reports, it was documented that wild-type p53 inhibited angiogenesis through regulation of thrombospondin-1, which is a potent inhibitor of endothelium, in fibroblasts (27). In these cases, a putative endogenous inhibitor of angiogenesis may have to be down-regulated before an increased number of microvessels. In addition, O’Reilly et al. (28) found a novel endogenous angiogenesis inhibitor, angiotatin. This inhibitor is produced by tumor cells and is present in the circulating level in mice. In addition to the expression of positive angiogenesis regulators, the role of negative angiogenesis regulators, and the balance between positive and negative regulators should also be studied in human tumors.

Several angiogenesis approaches are now being applied for cancer treatment. Low-molecular-weight compounds with antiangiogenic activity are being studied in clinical trials. In in vivo experiments, it was underscored that anti-VEGF mAb markedly suppressed tumor growth of xenografts in nude mice (29, 30). In addition, an anticancer agent, 5'-deoxy-5-fluorouridine, which is converted to 5-fluorouracil by PD-ECGF/dThdPase, has been shown to be effective for breast cancer patients. Regulation of VEGF and PD-ECGF activity may have to be an important target of antiangiogenesis treatment.

### References


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