# Expression of vasohibin as a novel endothelium-derived angiogenesis inhibitor in endometrial cancer

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**We have previously reported on vasohibin as a novel endothelium-derived vascular endothelial growth factor (VEGF)–inducible inhibitor of angiogenesis. The aim of our present study was to define the role of vasohibin in endometrioid endometrial adenocarcinoma. We collected 78 sections of endometrial carcinoma for assessment using immunohistochemistry. Twenty-seven were well differentiated (G1), 25 were moderately differentiated (G2), and 26 were poorly differentiated endometrioid adenocarcinomas (G3). We also included 12 sections of normal cyclic endometria, six of which were in the proliferative phase and six were in the secretory phase. We investigated the expression of vasohibin, and compared it to VEGF receptor-2 (VEGFR-2: KDR/flk-1), CD34, Ki-67, VEGF-A, and D2-40 (as a lymphatic vessel marker). We assessed the ratio of vasohibin- and VEGFR-2-positive vessels in the stroma of endometrial carcinoma. Immunohistochemical assessment was classified as negative or positive based on staining intensity. Vasohibin was selectively expressed on vascular endothelial cells in both cyclic endometria and endometrial carcinomas. Vasohibin was highly expressed in the normal functional endmetrium of the secretory phase, especially in the spiral artery, and was highly expressed in all grades of endometrioid adenocarcinomas. The stromal endothelial cells in G3 expressed vasohibin and VEGFR-2 more frequently than these in G1. In endometrioid adenocarcinomas, there was a significant correlation between the expression percentage of vasohibin and that of VEGFR-2 ($P < 0.0001$, $r^2 = 0.591$). This is the first study to elucidate the correlation between expression of vasohibin in the stromal endothelial cells and that of VEGFR-2 in human carcinomas. (*Cancer Sci* 2008; 99: 914–919)**

Endometrial carcinoma is one of the most common gynecologic malignancies in women worldwide, and its incidence, especially that of endometrioid endometrial carcinoma, has recently increased.[1] The morbidity of endometrial cancer is rapidly increasing in Japan. In order to predict the behavior of aggressive tumors, various factors and/or phenomena associated with endometrial cancer have been studied extensively. It is well recognized that angiogenesis, the process of formation of new vessels, is requisite for tumor growth and enables hematogenous spread of tumor cells throughout the body. Several studies have documented the association between the microvessel density (MVD) and/or the extent of endothelial proliferation and tumor stage, as well as recurrence of endometrial cancer.[2–7] Angiogenesis is determined by the local balance between angiogenic stimulators and inhibitors. The expression of various angiogenesis stimulators, such as vascular endothelial growth factors (VEGFs), angiopoietins, and thymidine phosphorylase, has been described in endometrial cancer.[8–12] However, the significance of endogenous angiogenesis inhibitors in endometrial cancer is poorly documented.

We recently isolated a novel angiogenesis inhibitor, vasohibin, which is specifically expressed in endothelial cells (ECs). Its basal expression in quiescent ECs is low, but it is induced in response to angiogenic stimuli, such as VEGF-A and fibroblast growth factor (FGF)-2, and inhibits angiogenesis in an autocrine manner.[13,14] We therefore propose that vasohibin inhibits angiogenesis as a negative feedback regulator. Among the VEGF family members, VEGF-A is the most important factor for angiogenesis, and most of the VEGF-A-mediated signals for angiogenesis are transduced via VEGF receptor-2 (VEGFR-2).[15] We observed that the VEGF-A-mediated induction of vasohibin was preferentially mediated via the VEGFR-2 signaling pathway.[16]

In the present study, we aimed to elucidate the significance of vasohibin in human endometrium and its disorder(s). We also studied MVD and lymphatic vessel density (LVD). Physiological periodic angiogenesis is observed in functional endometria. We therefore enrolled functional endometria and endometrioid adenocarcinoma, as endometriotic-type endometrial adenocarcinoma, and compared the expression of vasohibin and VEGFR-2. Our analysis revealed a significantly positive correlation between vasohibin and VEGFR-2 in endometrial cancer. This is the first study to profile the expression of vasohibin, a negative feedback regulator of angiogenesis, in gynecologic malignancy.

## Materials and Methods

**Tissue specimens and clinical data.** Seventy-eight endometrioid endometrial carcinomas (27 well differentiated, 25 moderately differentiated, 26 poorly differentiated; 50 stage I, 3 stage II, 20 stage III, 5 stage IV) were retrieved from the surgical pathology files of Tohoku University Hospital, Sendai, Japan. The average age of the patients was $55.6 \pm 10.7$ years. The protocol for this study was approved by the Ethics Committee at Tohoku University School of Medicine (Sendai, Japan). Each patient provided written informed consent before her surgery. None of the patients examined had received irradiation, hormonal therapy, or chemotherapy prior to surgery. The clinicopathological findings of the patients, including age, histology, stage, grade, and preoperative therapy was retrieved by extensive review of the charts. A standard primary treatment for endometrial carcinoma at Tohoku University Hospital was surgery consisting of total abdominal hysterectomy, salpingo-oopholectomy, pelvic and/or para-aortic lymphadenectomy, and peritoneal washing cytology. The lesions were classified according to the Histological Typing of Female Genital Tract Tumors by the World Health Organization, and staged according to the International Federation of Gynecology and Obstetrics system.[17,18] Patients with subtypes other than endometrioid or

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with second primary carcinoma were excluded from this series, because the endometrioid type and the others are referred to as type 1 and type 2 endometrial cancer are considered to originate from different mechanisms and exhibit different clinical behaviors.[17]

We also examined 12 sections of normal cyclic endometria derived from surgically resected specimens for benign uterine diseases, six of which were in the proliferative phase and six of which were in the secretory phase. The average age of these patients was 38.7 ± 4.9 and 40.3 ± 6.1 years, respectively. All specimens were routinely processed (i.e. 10% formalin fixed for 24–48 h), paraffin embedded, and thin sectioned (3 μm).

**Immunohistochemical staining and scoring of immunoreactivity.** We performed immunohistochemical staining for vasohibin, VEGFR-2, CD34 as a marker for vascular endothelial cells, and D2-40 as a lymphatic vessel marker. Ki-67 and VEGF-A were investigated in endometrial carcinoma cells. Paraffin-embedded tissue sections from human endometrial cancers were deparaffinized, rehydrated, and incubated with 3% $H_2O_2$ for 10 min to block endogenous peroxidase activity. Sections were incubated for 30 min at room temperature (RT) in a blocking solution of 10% goat serum (Nichirei Biosciences, Tokyo, Japan), and then stained for 12 h at 4°C with primary antibodies, followed by staining for 30 min at RT with secondary antibodies. The primary antibodies were all mouse monoclonal antibodies and were used as follows: 2 μg/mL antihuman vasohibin monoclonal antibody, anti-VEGFR-2 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) diluted 1:100, anti-CD34 (Dako, Copenhagen, Denmark) diluted at 1:200, Ki-67 (Dako) diluted 1:100, anti-D2-40 (Dako) diluted 1:100, and anti-VEGF-A (Lab Vision, Fremont, CA, USA) diluted 1:100. We have previously described a mouse monoclonal antibody against a synthetic peptide corresponding to the 286–299 amino acid sequence of vasohibin.[13] The positive control slide for CD34 antigen was prepared from paraffin-fixed breast cancer tissue that was known to contain a high microvessel density. Nuclei were counterstained with hematoxylin.

Three investigators (A.T., H.T., M.K.) independently evaluated the immunohistochemical staining of the tissue sections. They were blinded to the clinical course of the patients and the average of the numbers counted by the three investigators was adopted for subsequent analysis. We carefully selected for investigation areas where cancer cells came into contact with or invaded into the stroma. First, microvessels were counted by searching for CD34-positive signals after scanning the immunostained section at low magnification. The areas with the greatest number of distinctly highlighted microvessels were selected. Any cell clusters with CD34-positive signals were regarded as a single countable microvessel, regardless of whether a lumen was visible or not. Unstained lumina were considered artifacts even if they contained blood or tumor cells. Microvessel density (MVD) was assessed by light microscopy in areas of invasive tumor containing the highest numbers of capillaries and small venules per area (neovascular hot spots) according to the original method.[19] In endometrioid adenocarcinoma, the ratio of stroma per total area (as neovascular hot spots) decreased significantly with poorer histological differentiation. Thus, we measured the ratio of stroma per total area using National Institute of Health imaging (200 × magnification hot spot picture captured with Nikon imaging) and revised the true vessel counts per 1 $mm^2$ of stroma (microvessel density) for each case.

Investigation of lymphatic vessel density (LVD) was performed using the same procedure described as above, by searching for D2-40-positive signals.

Next, immunostaining for vasohibin and VEGFR-2 was evaluated in serial thin sections. Positive immunoreactive signals for vasohibin and VEGFR-2 in the CD34-positive microvessels were counted and calculated as positive ratios of vasohibin and VEGFR-2 in microvessels. Evaluation of Ki-67 immunoreactivity was performed at high-power field (400X) and used as a marker of cell proliferation. More than 500 tumor cells from each of three different representative fields were counted and the percentage of the number of positively stained nuclei relative to the total numbers of cells were determined as a labeling index (LI). The protein expression of selected angiogenic factor (VEGF-A) was examined by immunohistochemistry using an established antibody. For this marker, cytoplasmic staining intensity and the proportion of positive tumor cells were recorded and a staining index (values of 0–9) was calculated as the product of staining intensity (0–3) and the area of positive staining (1, <10%; 2, 10–50%; 3, > 50%).[20]

**Statistical analysis.** Statistical analysis, such as the Student's *t*-test and Peason's correlation coefficient test, were performed using StatView (version 4.5; SAS Institute Inc., Cary, NC, US). The results were considered significant when the *P*-values were <0.05.

## Results

**Microvessel density of endometrioid adenocarcinoma.** CD34-positive microvessel density (counts per $mm^2$) were 41.1 ± 3.1, 36.9 ± 2.3, and 29.7 ± 1.7 in G1, G2, and G3, respectively; 35 ± 2.70 in the proliferative phase; and 36.3 ± 1.83 in the secretory phase (Figs 1 and 2). We measured the ratio of the stromal area per total hot spot area in the location with the greatest number of distinctly highlighted microvessels. In G1, the ratio was 32.7% ± 0.24; in G2, 27.5% ± 0.19; in G3, 5.8% ± 0.05; 73.2% ± 2.70 in the proliferative phase; and 70.0% ± 1.83 in the secretory phase. The ratio of the stromal area per total area of G3 was significantly lower than that of G1 and G2. CD34-positive microvessel density (counts per $mm^2$), as revised by the ratio of the stroma per total area, was 141.65 ± 13.2 in G1, 168.62 ± 19.38 in G2, and 788.94 ± 105.8 in G3, respectively; 48.71 ± 2.70 in the proliferative phase; and 52.45 ± 1.83 in the secretory phase. The MVD of G3 was significantly higher than the MVD of G1 and G2 (Fig. 3a).

**Lymphatic vessel density of endometrioid adenocarcinoma.** D2-40 positive lymphatic vessel density (counts per $mm^2$) was 4.73 ± 0.70 in G1, 8.83 ± 2.24 in G2, and 2.88 ± 0.54 in G3, respectively; 1.80 ± 0.20 in the proliferative phase; and 6.10 ± 0.50 in the secretory phase (Figs 1 and 2). D2-40 positive lymphatic vessel density (counts per $mm^2$), as revised by the ratio of stroma per total area, was 15.90 ± 2.30 in G1, 9.47 ± 1.95 in G2, and 91.01 ± 23.06 in G3, respectively; 2.53 ± 0.36 in the proliferative phase; and 8.83 ± 0.82 in the secretory phase. The LVD of G3 was significantly higher than the LVD of G1 and G2 (Fig. 3b).

**Vasohibin expression in microvessels in endometrial tissues.** The positive ratios of vasohibin in microvessels were 31.4% and 43.1% in the proliferative and secretory phases, respectively (Fig. 2). The positive ratios were significantly different between the two phases (Fig. 4a). In addition, the endothelium of the spiral arteries characteristically exhibited positivity in the secretory phase; the positive ratio of vasohibin was 95.4% and the positive ratio of VEGFR-2 was 87.4%. (Fig. 2).

The positive ratio of vasohibin in microvessels was 56.6% ± 12.8 in the endometrioid adenocarcinoma cases examined. Interestingly, the positive ratios differed according to grade: 52.5 ± 13.9 in G1 (well differentiated), 57.8% ± 13.5 in G2 (moderately differentiated), and 59.9% ± 10.0 in G3 (poorly differentiated) (Fig. 1). The positive ratio of vasohibin in microvessels in G3 was significantly higher than that in G1 (Fig. 4a). Positive ratios of vasohibin in microvessels in each histlogical grade were significantly higher than that of the proliferative phase ($P < 0.05$). Positive ratios were compared in clinical stages: 56.2% ± 11.2 in stage 1, 61.9% ± 15.5 in stage 2,

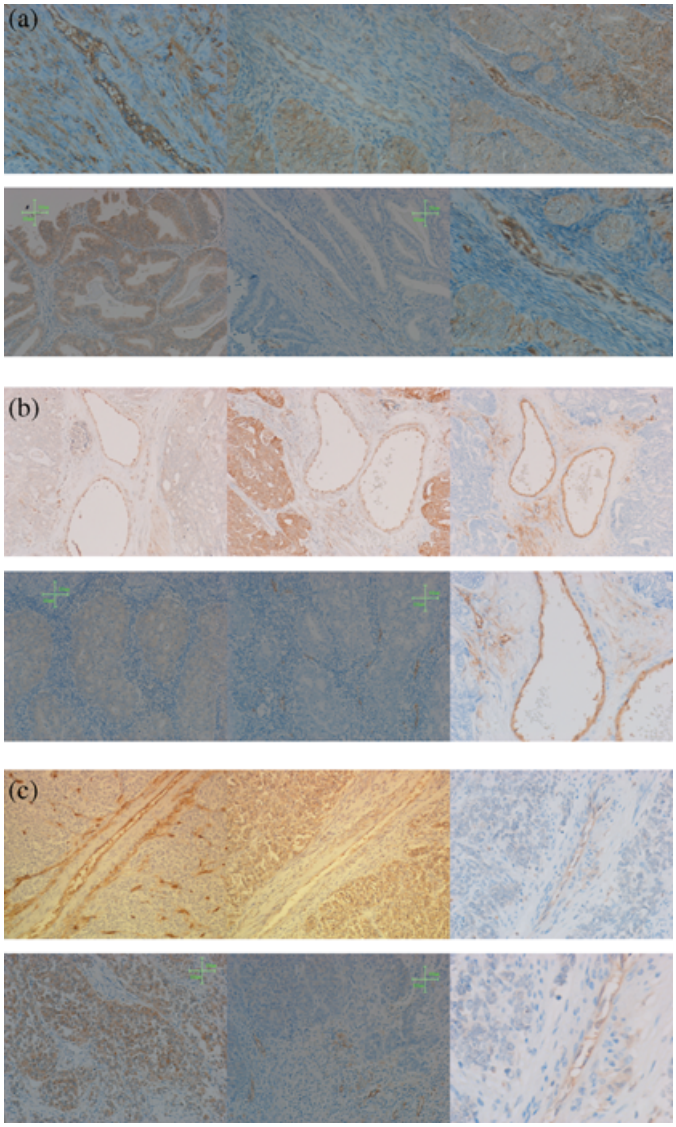


**Fig. 1.** (a) Immunohistochemistry in well-differentiated endometrioid adenocarcinomas. All sections stained positively for CD34 (upper left, original magnification 200×), vascular endothelial growth factor receptor-2 (VEGFR-2) (upper middle, original magnification 200×), vasohibin (upper right, original magnification 200× and lower right, original magnification 400×), and vascular endothelial growth factor-alpha (VEGF-A) (lower left, original magnification 200×). D2-40 (lower middle, original magnification 200×). (b) Immunohistochemistry in moderately differentiated adenocarcinoma. All sections stained positively as for (a). (c) Immunohistochemistry in poorly differentiated adenocarcinoma. All sections stained positively as for (a).

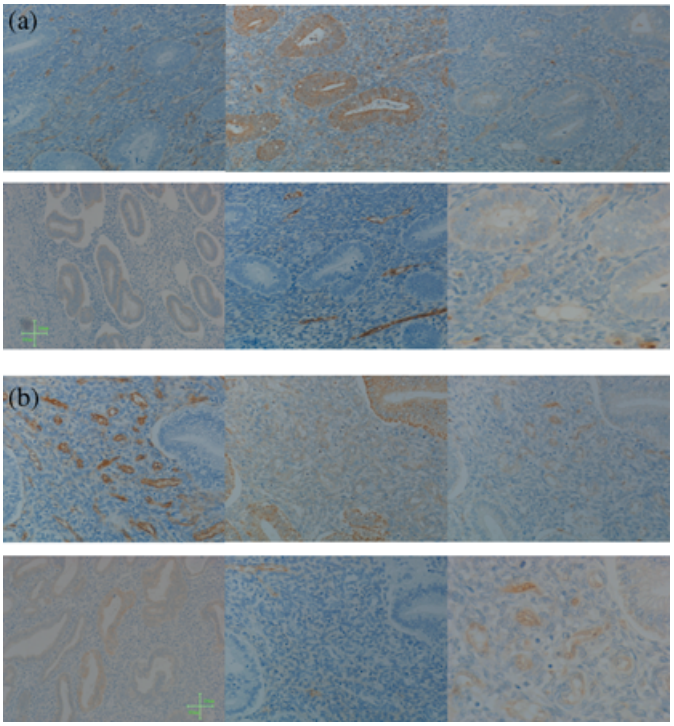


**Fig. 2.** (a) Immunohistochemistry in proliferative phase of cyclic endometria. All sections stained positively for CD34 (upper left, original magnification 200×), vascular endothelial growth factor receptor-2 (VEGFR-2) (upper middle, original magnification 200×), vasohibin (upper right, original magnification 200× and lower right, original magnification 400×), and vascular endothelial growth factor-alpha (VEGF-A) (lower left, original magnification 200×). D2-40 (lower middle, original magnification 200×). (b) Immunohistochemistry in secretory phase of cyclic endometria. All sections stained positively as for (a). Immunopositivity was significantly different between the two phases for vasohibin and VEGFR-2. The endothelium of the spiral arteries exhibited characteristic positivities in the secretory phase.

56.7% ± 12.9 in stage 3, and 57.7% ± 21.3 in stage 4. There was no significant difference between each group.

**VEGFR-2 expression in microvessels in endometrial tissues.** The VEGFR-2 positive ratio of the microvessels was 8.6% ± 1.0 and 22.5% ± 3.0 in the proliferative and secretory phases, respectively (Fig. 2). VEGFR-2 positive vessel ratio in the proliferative phase was significantly higher than in the secretory phase (Fig. 4b).

VEGFR-2 positive ratios in G1, 2, and G3 were 22.3% ± 13.0, 39.8% ± 12.8, and 47.6% ± 11.5, respectively (Fig. 1). The VEGFR-2 positive ratio in G2 was significantly higher than in G1 and cyclic endometria. The VEGFR-2 positive ratio in G3 was significantly higher than those of G1 and G2 (Fig. 4b). Positive ratios were compared between clinical stages: 32.8% ± 15.4% in stage 1, 33.9% ± 19.0 in stage 2, 37.5% ± 15.1 in stage 3, and 46.4% ± 16.8 in stage 4. There was no significant difference between each group.

**VEGF-A expression in endometrial tissues.** VEGF-A expression was detected in the cytoplasm of epithelial cells. The staining index of VEGF-A is shown in Figure 5. The VEGF-A positive ratio of cytoplasmic staining intensity was 0.67 ± 0.49 and 2.33 ± 0.76 in the proliferative and secretory phases, respectively (Fig. 5). In endometrioid adenocarcinomas VEGF-A positive ratios of cytoplasmic staining in grades 1, 2, and 3 were 3.85 ± 0.39, 4.2 ± 0.37, and 5.88 ± 0.37, respectively (Fig. 5). The VEGF-A positive ratio in G3 was significantly higher than in G1 and 2. The VEGF-A positive ratio in the proliferative phase was significantly lower than G1, G2, and G3, respectively, and the VEGF-A positive ratio in secretory phase was significantly lower than G2 and G3, respectively.

**Correlation between vasohibin and VEGFR-2 positive ratios in microvessels, and cell proliferation and expression of angiogenic factor.** A strongly positive correlation was found between vasohibin and VEGFR-2 positive ratios in microvessels in endometrioid adenocarcinomas ($P < 0.0001$, $r^2 = 0.591$) (Fig. 6).

We then analyzed vasohibin and VEGFR-2 positive ratios in comparison to cell proliferation and expression of angiogenic factor. The ratios of both vasohibin- and VEGFR-2-positivity did not correlate significantly to the LI of Ki-67 (data not


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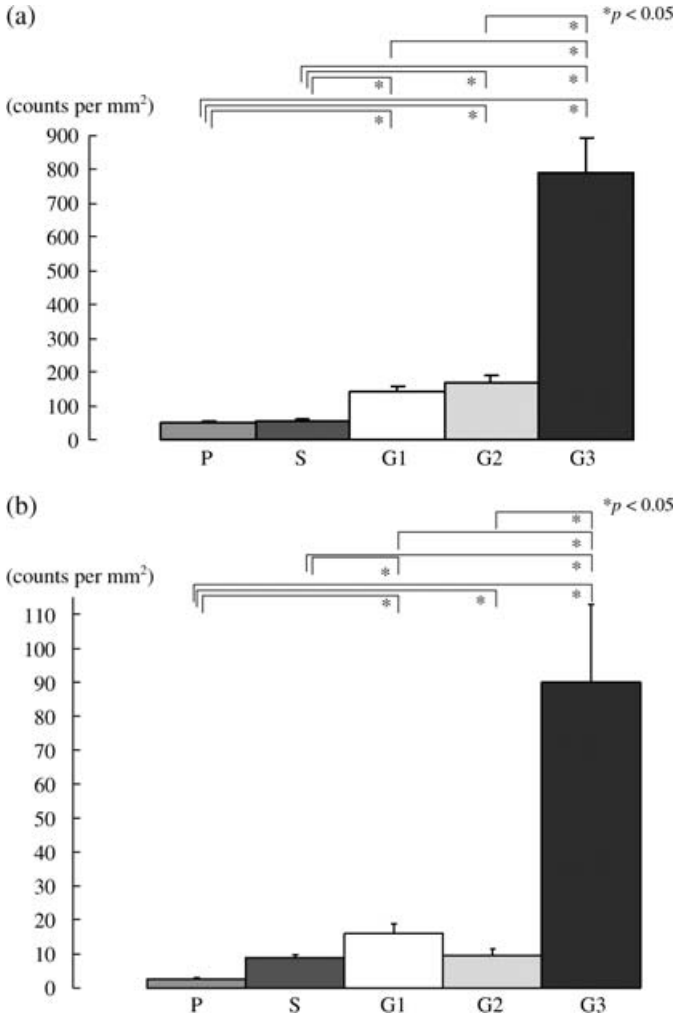


**Fig. 3.** (a) Microvessel density of cyclic endometria and endometrioid adenocarcinoma. Microvessel density of G3 was significantly higher than that of G1 and G2. G1, well-differentiated adenocarcinoma; G2, moderately differentiated adenocarcinoma; G3, poorly differentiated adenocarcinoma, $*P < 0.05$. (b) Lymphatic vessel density of cyclic endometria and endometrioid adenocarcinoma. Lymphatic vessel density of G3 was significantly higher than that of G1 and G2. G1, well-differentiated adenocarcinoma; G2, moderately differentiated adenocarcinoma; G3, poorly differentiated adenocarcinoma, $*P < 0.05$.

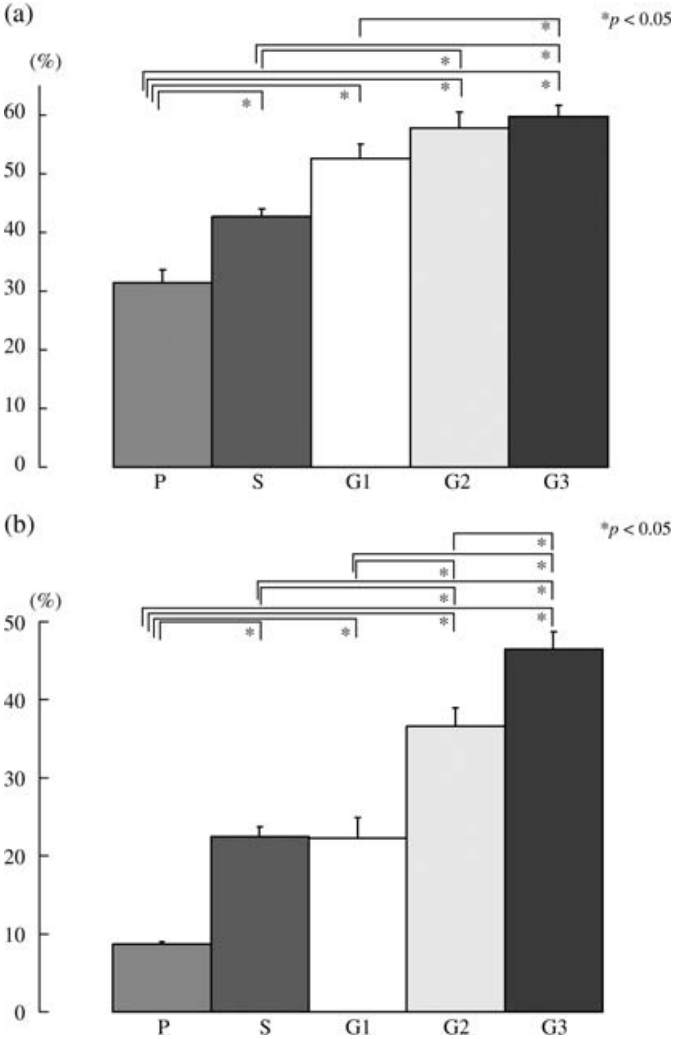


**Fig. 4.** (a) Proportion of vasohibin/CD34-positive vessels in cyclic endometria and endometrioid adenocarcinoma. Vasohibin-immunopositivity in microvessels in G3 was significantly higher than that in G1. S, secretory phase; P, proliferative phase; G1, well-differentiated adenocarcinoma; G2, moderately differentiated adenocarcinoma; G3, poorly differentiated adenocarcinoma, $*P < 0.05$. (b) Proportion of vascular endothelial growth factor receptor-2 (VEGFR-2)/CD34-positive vessels in cyclic endometria and endometrioid adenocarcinoma. VEGFR-2-immunopositivity of vessels in the proliferative phase was significantly higher than in the secretory phase. S, secretory phase; P, proliferative phase; G1, well-differentiated adenocarcinoma; G2, moderately differentiated adenocarcinoma; G3, poorly differentiated adenocarcinoma, $*P < 0.05$.

shown). No significant correlation was observed between the vasohibin- and VEGFR-2-positive ratios and VEGF-A expression in endometrioid adenocarcinoma (data not shown).

## Discussion

Here we examined the vascular density of endometrial cancer and compared it with that of normal endometrium. Some reports have previously indicated that MVD of endometrial cancer increases from well differentiated to poorly differentiated adenocarcinomas[4,21,22] but this relationship is not universally accepted.[23] Moreover, most of them have failed to consider the vessel numbers in normal endometrium and to compare it with those of adenocarcinoma. In the present study, we confirmed that the vessel number increased from normal endometrium to endometrial cancer, and that this increase was significantly augmented in poorly differentiated adenocarcinoma.

Recently, focus has been given to the importance of lymphangiogenesis for tumor metastasis.[24,25] Here, we investigated LVD in normal endometrium and endometrioid adenocarcinoamas. Our analysis revealed that LVD increased significantly in poorly differentiated adenocarcinoma, similar to MVD. Some studies have reported the presence of peritumoral lymphatic vessels in 40% of the cases in endometrial cancer and demonstrated that high LVD was strongly associated with the features of aggressive endometrial carcinomas, including high histological grade, presence of necrosis, and vascular invasion by tumor cells.[26–28] Although it was expected that frequent LVD correlated with lymph node metastasis, there was no significant correlation between LVD and lymph node metastasis. In the present study, only two cases out of 78 cases exhibited lymph node metastasis. Therefore, investigation of a greater number of cases with lymph node metastasis in endometrioid adenocarcinoma will be necessary to further elucidate this correlation. The

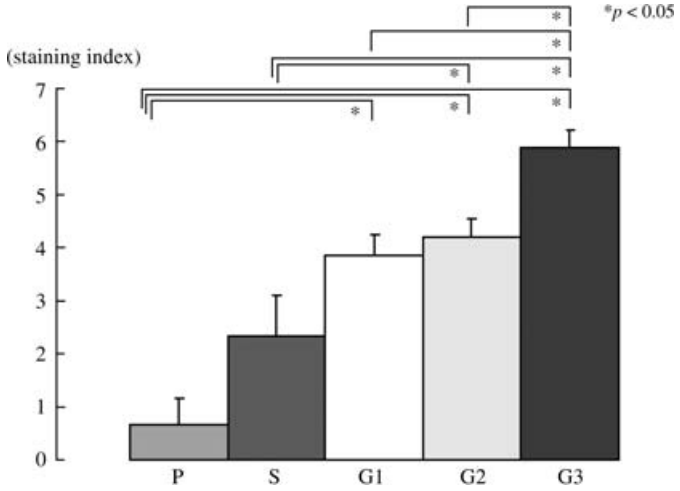


**Fig. 5.** Vascular endothelial growth factor-alpha (VEGF-A) staining indexes of the cytoplasm of the tumor cell and cyclic endometrial glands. *$P < 0.05$.

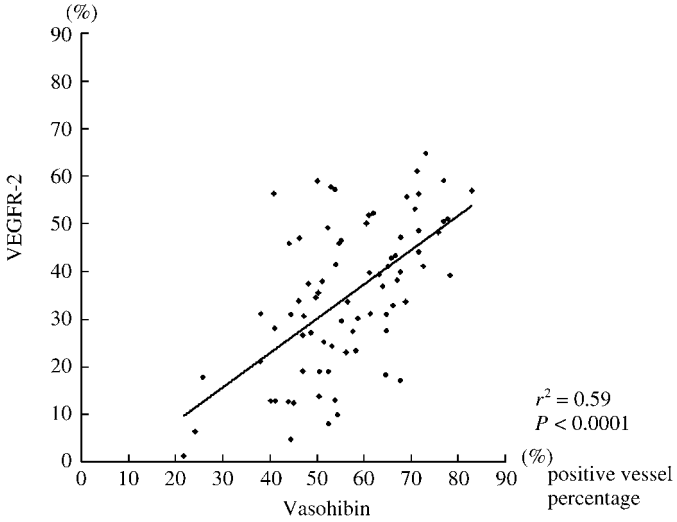


**Fig. 6.** Correlation between vasohibin and vascular endothelial growth factor receptor-2 (VEGFR-2). A strongly positive correlation was found between vasohibin- and VEGFR2-positive ratios in microvessels in endometrioid adenocarcinomas ($P < 0.0001$, $r^2 = 0.591$).

mechanism of the alteration of lymphangiogenesis from cyclic endometria to endometrioid adenocarcinoma remains unclear. Our analysis of MVD and LVD demonstrated that the secretory phase is similar to well differentiated and moderately differentiated endometrioid adenocarcinomas, which suggests that the function of cyclic endometria may be retained until adenocarcinomas become moderately differentiated.

We then examined the expression of vasohibin. Vasohibin is an endogenous endothelium-derived angiogenesis inhibitor that we have previously isolated.[13] Here we confirmed that the expression of vasohibin was restricted to vascular endothelium, and further observed that the ratio of vasohibin-positive vessels increased from normal endometrium to poorly differentiated adenocarcinomas. This is the first study to profile the expression of vasohibin in human gynecologic malignancy.

Several clinicopathologic studies have demonstrated a direct association between VEGF expression and increased MVD in human solid tumors, including breast[29] lung,[30] and gastric[31] malignancies. A similar association has been reported for normal[32] and malignant endometrium.[33–35] Between the two VEGF signal transducing receptors, VEGFR-2 transduces most of the angiogenesis-related signals in ECs. The VEGF/VEGFR-2 signaling pathway is also important for the induction of vasohibin in ECs.[16] We previously revealed that the VEGF-A-mediated induction of vasohibin was preferentially mediated via the VEGFR-2 signaling pathway.[16]

Our present analysis revealed that the ratio of VEGFR-2-positive vessels, as well as the ratio of vasohibin-positive vessels, also increased from normal endometrium to poorly differentiated endometrial adenocarcinomas. In addition, a significantly positive correlation existed between the positive ratios of vasohibin and VEGFR-2 expression in endometrioid endometrial carcinomas. This is the first study to elucidate this correlation between expression of these factors in human cancer. This result suggested the value of vasohibin as a biomarker of angiogenesis at least in endometrial cancer.

Angiogenesis is determined by the local balance between angiogenic stimulators and inhibitors. Therefore, one may anticipate the application of angiogenesis inhibitors towards antiangiogenic therapy for the treatment of human malignancies including endometrial cancer. A number of angiogenesis inhibitors have been investigated and identified, including pigment epithelium-derived factor (PEDF), angiostatin, endostatin, and thrombospondin-1 (TSP-1). Vasohibin is a newly identified negative feedback regulator for angiogenesis. We previously reported that transfection of Lewis lung carcinoma (LCC) cells with the vasohibin gene did not affect the proliferation of cancer cell *in vitro*, but did inhibit tumor growth and tumor angiogenesis *in vivo*.[13] The growth of vasohibin-producing LLC cells in mice was significantly attenuated. In addition, tumors of mock-transfectants contained large luminal vessels, whereas those of vasohibin-producing LLC cells contained very small vessels, even when the size of tumors did not differ extremely.[14] These results suggest that vasohibin may play a very important role in regulating tumor angiogenesis.

Among the various angiogenesis inhibitors, thrombospondin-1 (TSP-1) has been extensively studied in cancers, although the role of TSP-1 in endometrial tumor angiogenesis and progression still remains controversial.[36] The expression of TSP-1 in epithelial cells and/or caner cells is up-regulated by the tumor suppressor gene *p53*, and down-regulated by oncogenes such as Myc and Ras. Thus, the mutation of *p53* or activation of *myc* and *ras* results in the down-regulation of TSP-1, which may alter tumor growth by modulating angiogenesis in a variety of tumor types. Herein, we demonstrated that the expression of vasohibin increased in ECs of endometrial cancer. As the expression of vasohibin was restricted to normal ECs, the alteration of tumor suppressor gene and/or oncogenes in tumor cells would not influence the expression of vasohibin. However, angiogenesis inhibitors may function in a concerted manner. Therefore, vasohibin alone may not be sufficient to control tumor angiogenesis, if other inhibitors become deregulated.

Nevertheless, since the expression of vasohibin increased in correlation with that of VEGFR-2, vasohibin could be an important biomarker of angiogenesis in both normal endometrium and endometrial cancer. However, further investigations are required to clarify the precise roles of vasohibin in regulating antiangiogenic activity in normal endometrium and its disorders.

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## References


1 Jemal A, Tiwari RC, Murray T *et al*. Cancer statistics, 2004. *CA Cancer J Clin* 2004; **54**: 8–29.
2 Abulafia O, Triest WE, Sherer DM, Hansen CC, Ghezzi F. Angiogenesis in endometrial hyperplasia and stage I endometrial carcinoma. *Obstet Gynecol* 1995; **86** (4): 479–85.
3 Kirschner CV, Alanis-Amezcua JM, Martin VG *et al*. Angiogenesis factor in endometrial carcinoma. a new prognostic indicator? *Am J Obstet Gynecol* 1996; **174**: 1879–82; discussion 1882–4.
4 Kaku T, Kamura T, Kinukawa N *et al*. Angiogenesis in endometrial carcinoma. *Cancer* 1997; **80**: 741–7.
5 Salvesen HB, Iversen OE, Akslen LA. Independent prognostic importance of microvessel density in endometrial carcinoma. *Br J Cancer* 1998; **77**: 1140–4.
6 de Gois Speck NM, Focchi J, Alves AC, Ribalta JC, Osorio CA. Relationship between angiogenesis and grade of histologic differentiation in endometrial adenocarcinoma. *Eur J Gynaecol Oncol* 2005; **26**: 599–601.
7 Stefansson IM, Salvesen HB, Akslen LA. Vascular proliferation is important for clinical progress of endometrial cancer. *Cancer Res* 2006; **66**: 3303–9.
8 Hirai M, Nakagawara A, Oosaki T, Hayashi Y, Hirono M, Yoshihara T. Expression of vascular endothelial growth factors (VEGF-A/VEGF-1 and VEGF-C/VEGF-2) in postmenopausal uterine endometrial carcinoma. *Gynecol Oncol* 2001; **80**: 181–8.
9 Fujisawa T, Watanabe J, Akaboshi M, Ohno E, Kuramoto H. Immunohistochemical study on VEGF expression in endometrial carcinoma – comparison with p53 expression, angiogenesis, and tumor histologic grade. *J Cancer Res Clin Oncol* 2001; **127**: 668–74.
10 Holland CM, Day K, Evans A, Smith SK. Expression of the VEGF and angiopoietin genes in endometrial atypical hyperplasia and endometrial cancer. *Br J Cancer* 2003; **89**: 891–8.
11 Saito M, Watanabe J, Fujisawa T *et al*. Angiopoietin-1, 2 and Tie2 expression in endometrial adenocarcinoma – the Ang2 dominant balance up–regulates tumor angiogenesis in the presence of VEGF. *Eur J Gynaecol Oncol* 2006; **27**: 129–34.
12 Mazurek A, Kuc P, Terlikowski S, Laudanski T. Evaluation of tumor angiogenesis and thymidine phosphorylase tissue expression in patients with endometrial cancer. *Neoplasma* 2006; **53**: 242–6.
13 Watanabe K, Hasegawa Y, Yamashita H *et al*. Vasohibin as an endothelium-derived negative feedback regulator of angiogenesis,. *J Clin Invest* 2004; **114**: 898–907.
14 Sonoda H, Ohta H, Watanabe K, Yamashita H, Sato Y. Multiple processing forms and their biological activities of a novel angiogenesis inhibitor Vasohibin. *Biochem Biophys Res Comms* 2006; **342**: 640–6.
15 Shibuya M, Claesson-Welsh L. Signal transduction by VEGF receptors in regulation of angiogenesis and lymphangiogenesis. *Exp Cell Res* 2006; **312**: 549–60.
16 Shimizu K, Watanabe K, Yamashita H *et al*. Gene regulation of a novel angiogenesis inhibitor, Vasohibin, in endothelial cells. *Biochem Biophys Res Commun* 2005; **327**: 700–6.
17 World Health Organization classification of tumours. In: Tavassoli FA, Devilee P, eds. *Pathology and Genetics of Tumours of the Breast and Female Genital Organs*. Lyon: IARC Press, 2003; 217–32.
18 Creasman WT. Announcement FIGO stages: 1988 revisions. *Gynecol Oncol* 1989; **35**: 125–7.
19 Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis – correlation in invasive breast carcinoma. *N Engl J Med* 1991; **324**: 1–8.
20 Aas T, Borresen AL, Geisler S *et al*. Specific P53 mutations are associated with de novo resistance to doxorubicin in breast cancer patients. *Nat Med* 1996; **2**: 811–4.
21 Wagatsuma S, Konno R, Sato S, Yajima A. Tumor angiogenesis, hepatocyte growth factor, and c-Met expression in endometrial carcinoma. *Cancer* 1998; **82**: 520–30.
22 Salvesen HB, Iversen OE, Akslen LA. Prognostic significance of angiogenesis and Ki-67, 53, and p21 expression: a population-based endometrial carcinoma study. *J Clin Oncol* 1999; **17**: 1382–90.
23 Morgan KG, Wilkinson N, Buckley CH. Angiogenesis in normal, hyperplastic, and neoplastic endometrium. *J Pathol* 1996; **179**: 317–20.
24 Alitalo K, Mohla S, Ruoslahti E. Lymphangiogenesis and cancer: meeting report. *Cancer Res* 2004; **64**: 9225–9.
25 Sipos B, Klapper W, Kruse ML *et al*. Expression of lymphangiogenic factors and evidence of intratumoral lymphangiogenesis in pancreatic endocrine tumors. *Am J Pathol* 2004; **165**: 1187–97.
26 Ingunn M, Stefansson Helga Salvesen B &, Lars Akslenl A. Vascular proliferation is important for clinical progress of endometrial cancer. *Cancer Res* 2006; **66**: 3303–7.
27 Bono P, Wasenius VM, Heikkila P *et al*. High LYVE-1-positive lymphatic vessel numbers are associatedwith poor outcome in breast cancer. *Clin Cancer Res* 2004; **10**: 7144–9.
28 Shields JD, Borsetti M, Rigby H *et al*. Lymphatic density and metastatic spread in human malignant melanoma. *Br J Cancer* 2004; **90**: 693–700.
29 Toi M, Inada K, Suzuki H, Tominaga T. Tumor angiogenesis in breast cancer: its importance as a prognostic indicator and the association with vascular endothelial growth factor expression. *Breast Cancer Res Treat* 1995; **36**: 193–204.
30 Giatromanolaki A, Koukourakis MI, Kakolyris S *et al*. Vascular endothelial growth factor, wild-type p53, and angiogenesis in early operable nonsmall cell lung cancer. *Clin Cancer Res* 1998; **4**: 3017–24.
31 Giatromanolaki A, Koukourakis MI, Stathopoulos GP *et al*. Angiogenic interactions of vascular endothelial growth factor, of thymidine phosphorylase, and of p53 protein expression in locally advanced gastric cancer. *Oncol Res* 2000; **12**: 33–41.
32 Charnock-Jones DS, Sharkey AM, Rajput-Williams J *et al*. Identification and localization of alternately spliced mRNAs for vascular endothelial growth factor in human uterus and estrogen regulation in endometrial carcinoma cell lines. *Biol Reprod* 1993; **48**: 1120–8.
33 Guidi AJ, Abu-Jawdeh G, Tognazzi K, Dvorak HF, Brown LF. Expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in endometrial carcinoma. *Cancer* 1996; **78**: 454–60.
34 Fine BA, Valente PT, Feinstein GI, Dey T. VEGF, flt-1, and KDR/flk-1 as prognostic indicators in endometrial carcinoma. *Gynecol Oncol* 2000; **76**: 33–9.
35 Fujimoto J, Ichigo S, Hirose R, Sakaguchi H, Tamaya T. Expressions of vascular endothelial growth factor (VEGF) and its mRNA in uterine endometrial cancers. *Cancer Lett* 1998; **134**: 15–22.
36 Ren B, Yee KO, Lawler J, Khosravi-Far R. Regulation of tumor angiogenesis by thrombospondin-1. *Biochim Biophys Acta* 2006; **1765**: 178–88.