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Impact of Vessel Maturation on Anti-Angiogenic Therapy in Ovarian Cancer

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Abstract

Condensation—Pericytes contribute to endothelial survival in tumor vasculature and represent a potential therapeutic target.

Objective—To examine the functional and therapeutic significance of pericytes in ovarian cancer vasculature.

Study Design—Tumor vessel morphology and efficacy of endothelial and pericyte targeting were examined using *in vivo* ovarian cancer models. The expression of platelet derived growth factor (PDGF) ligands and receptors was examined in endothelial, pericyte-like, and ovarian cancer cells.

Results—Relative to normal vessels, tumor vasculature was characterized by loosely attached pericytes in reduced density. PDGF-BB was expressed predominantly by the endothelial and cancer cells whereas PDGFR β was present in pericyte-like cells. PDGF-BB significantly increased migration of and VEGF production by pericyte-like cells while PDGFR β blockade abrogated these effects. Dual VEGF (VEGF-Trap) and PDGF-B (PDGF-Trap) targeted therapy was more effective in inhibiting *in vivo* tumor growth than either agent alone.

Conclusions—Aberrations in the tumor microenvironment contribute to endothelial cell survival. Strategies targeting both endothelial cells and pericytes should be considered for clinical trials.

Keywords

endothelial cells; pericytes; ovarian cancer; VEGF; PDGF

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Introduction

The growth of primary tumors and metastases is dependent on the development of new vasculature. Vascular endothelial growth factor (VEGF), also known as vascular permeability factor (VPF), plays an essential role in physiological and pathological neovascularization.^{1–3,4–6} We and others have shown that elevated tumor VEGF expression and serum VEGF levels in ovarian cancer patients are associated with poor overall survival.^{7–11} Anti-VEGF therapies such as bevacizumab are efficacious in many human tumors, including ovarian cancer.^{11–13} However, despite improvement in overall survival of patients with many cancer types with VEGF-targeted therapies, most eventually develop progressive disease.¹⁴ One possible explanation for this observation may be related to the role of perivascular cells called pericytes, which can provide local survival signals for endothelial cells.¹⁵

Most blood vessels consist of at least two cell types, endothelial cells and pericytes.¹⁵ Pericytes wrap around endothelial cells and play an essential role in stabilization and hemodynamic functions of blood vessels.^{16,17} In contrast, tumor vessels have multiple morphological abnormalities and are often leaky and hemorrhagic, in part due to overproduction of VEGF.¹⁸ Tumor vessel walls are thin, probably due to abnormalities in pericytes and other supporting cells.¹⁹ The immature or poorly covered tumor vasculature appears to be particularly vulnerable to anti-VEGF therapies, but the mechanisms underlying the survival of remaining vasculature are not fully understood. Interestingly, the remaining vasculature appears to have greater pericyte coverage, suggesting that this cell type may limit the efficacy of some anti-angiogenic therapies.^{20,21}

Pericyte homeostasis is regulated to a large extent by the platelet-derived growth factor (PDGF) ligand/receptor system.^{22–24} PDGF is composed of four polypeptide chains that form homodimers PDGF-AA, BB, CC, and DD as well as the heterodimer PDGF-AB. The PDGF receptors (PDGFR) α and β mediate PDGF functions.^{25,26} Specifically, PDGFR α binds to PDGF AA, BB, AB, and CC, whereas PDGFR β interacts with -BB and -DD.²⁶ PDGFR β signaling is known to play a functionally significant role in pericyte development and recruitment by endothelial cells. The knockout of PDGF-B or PDGFR β in mice is known to cause perinatal death due to vascular abnormalities resulting from lack of pericytes.^{19,22,27} However, the role of pericytes in regulating endothelial function in ovarian tumor vasculature is not well understood. In the current study, we examined the morphological characteristics as well as functional roles of pericytes in ovarian cancer vasculature. We also tested the efficacy of dual endothelial and pericyte targeting using an established orthotopic mouse model of ovarian cancer metastasis.

Materials and Methods

Cell Lines and Culture Conditions

The human ovarian cancer cell lines (HeyA8, SKOV3ip1, and A2780-Par) were cultured as previously described.^{25,28} The endothelial (HUVEC) and pericyte-like cells (hVSMCs & 10T1/2) were obtained from ATCC (Manassas, Va). Ovarian endothelial cells (EC-Ovary) of the immortomouse were derived by RRL and maintained in DMEM with 10% FBS, as previously described.²⁹

Animals

Female nude mice (NCr-*nu*; 8–12 weeks old) were purchased from the NCI (Frederick, MD). All mouse studies were approved by the M. D. Anderson Cancer Center Institutional Animal Care and Use Committee.

Drugs and Reagents

Recombinant human PDGF-BB and VEGF was purchased from R&D Systems, Inc. (Minneapolis, MN). Imatinib mesylate (Novartis Pharma, AG; Basel, Switzerland) and bevacizumab (Genentech Inc., South San Francisco, CA) were purchased from the institutional pharmacy. VEGF-Trap, PDGF-Trap and human F_C control were provided by Regeneron Pharmaceuticals (Tarrytown, NY).

Conditioned Media (CM) Collection

At 90% cell confluence, the medium was changed overnight to 5% FBS containing medium. Cells were washed with PBS and incubated in medium containing 5% FBS. After 48 h, the medium was collected, centrifuged, filtered, and stored at -20°C until use. To collect activated CM, hVSMCs were incubated with 1% FBS containing medium with PDGF-BB (10ng/ml) for 48 h before the CM was collected.

Quantitative Real-Time RT-PCR

RNA was extracted, and reverse transcription was performed using an oligo(dT) primer and Moloney murine leukemia virus (M-MLV) reverse transcriptase (Life Technologies, Gaithersburg, MD). After PCR amplification, quantitative values were obtained, as previously described.² The primers were obtained from Applied Biosystems.

Assessment of VEGF Levels

We quantified VEGF concentration in supernatants using an ELISA kit (R&D Systems) according to the manufacturer's instructions.²

Migration

For assessment of migration of the different cell types, the Membrane Invasion Culture System (MICS) assay was used, as previously described.^{30,31} Cells were treated with PDGF-BB at 10 ng/ml and/or imatinib mesylate at 5 μM for 6 h for these assays.

Flow Cytometry

For apoptosis assays, HUVECs were incubated for 24 h with MEM containing 15% FBS and 10 ng/ml bFGF. The medium was changed to 5% FBS containing MEM overnight. At 70–80% confluence, cells were incubated with either MEM containing 1% FBS (control), or the cytokine of interest. After 24 h, cells were collected, labeled according to manufacturer's instructions and analyzed on an EPICS XL flow cytometer (Beckman-Coulter, Miami, FL).

Orthotopic Implantation of Tumor Cells and Tissue Collection

For establishing the intra-ovarian orthotopic model, nude mice ($n = 10$) were injected with either HeyA8 (5×10^5) or SKOV3ip1 (1×10^6) cells into the left ovarian parenchyma in 30 μl total volume. For bioluminescence imaging, luciferase transfected HeyA8 (HeyA8-Luc) cells were used and the animals were imaged longitudinally, as described previously.²¹ For the intraperitoneal metastasis model, mice ($n = 10$ per group) were injected i.p. with HeyA8 cells (2.5×10^5) and 7 d later, randomized into 4 groups: 1) control (hFc 25 mg/kg, 2 times/week), 2) VEGF-Trap (12.5 mg/kg i.p., 2 times/week), 3) PDGF-Trap (12.5 mg/kg i.p., 2 times/week), 4) VEGF-Trap plus PDGF-Trap (same dosing as above). Mice were sacrificed after 3 wk of therapy when animals in any group began to appear moribund and a necropsy was performed. The investigators were blinded to the treatment groups during therapy and dissection.

Immunofluorescence Double Staining

Dual immunofluorescence studies were performed with CD31 (red) and desmin (green) antibodies, as described previously.¹⁵ The samples were counterstained with Hoechst. Vessels with at least 50% coverage of associated desmin-positive cells were considered positive for pericyte coverage. For assessment of endothelial apoptosis, frozen sections were stained for CD31 (red) followed by TUNEL (green; Promega, Madison, WI), as described previously.²⁸ An apoptotic endothelial cell was represented by yellow fluorescence. To quantify apoptotic endothelial cells, the number of CD31/TUNEL double-positive cells was calculated in 10 random fields at original magnification $\times 200$.

Statistics

Continuous variables were compared with the Student's t-test (between two groups) or ANOVA (for all groups) if normally distributed, and the Mann-Whitney rank sum test or Kruskal Wallis test (for all groups) if nonparametric. All statistical tests were performed with SPSS (SPSS Inc., Chicago, IL). A $p < 0.05$ on two-tailed testing was considered significant.

Results

Alterations in maturation of tumor vasculature

Prior to examining the functional relationships between the cell types in tumor vasculature, we first examined morphological characteristics of the vasculature during establishment of ovarian carcinoma. For these studies, we established and characterized a fully orthotopic model of ovarian carcinoma. Nude mice ($n = 10$) were injected with either HeyA8 or SKOV3ip1 cells into the left ovarian parenchyma (Figure 1A). Tumor development occurred in 90–100% of injected ovaries, followed eventually by peritoneal dissemination and ascites formation (Figure 1B–C). In the SKOV3ip1 and HeyA8 models, mice became moribund at 75 and 29 d, respectively. Interestingly, para-aortic nodal metastasis occurred in 50% of animals in both models, which is not commonly observed in the i.p. injection model. To examine whether the metastases observed in this model could be related to tumor cell spillage at the time of injection, we used HeyA8-Luc cells followed by longitudinal bioluminescence imaging. Following tumor cell injection, a tumor arose in the injected ovary and dissemination occurred around day 21 (Figure 1D), suggesting that the metastases were not related to tumor spillage during injection.

Next, we examined pericyte coverage using dual immunofluorescence staining for CD31 and desmin. In the normal ovary, small vessels (venules and capillaries) were extensively covered by irregularly shaped pericytes that were tightly associated with the endothelial cells (Figure 1E). However, tumor vessels were morphologically abnormal and tortuous in shape and most lacked pericyte coverage early in development (Figure 1E–F). In the larger tumors (day 21; Figure 1F), while most tumor vessels had pericyte coverage, the pericytes were loosely attached and had extensions projecting both toward the endothelial cells and into the tumor stroma.

Expression of PDGF receptors and ligands in ovarian cancer, endothelial, and pericyte-like cells

Since the PDGF ligand/receptor system plays a critical role in pericyte homeostasis,^{22–24} we first examined the mRNA expression levels of PDGF ligands and PDGF receptors in ovarian cancer (HeyA8, A2780-PAR, SKOV3ip1), endothelial (HUVEC and EC-ovary), and pericyte-like (10T1/2, hVSMC) cell lines. The endothelial cells expressed the mRNA for all of the PDGF ligands (Figure 2), but had minimal expression of PDGFR α and PDGFR β . All 3 cancer cell lines expressed PDGFR α , but had low expression of PDGFR β . The cancer cell lines had strong expression of PDGF-B and varying levels of expression of the genes for other ligands.

The pericyte-like cells had strong expression of PDGFR β and PDGFR α , but minimal to no expression of PDGF ligands.

PDGF-BB promotes migration of pericyte-like cell

Based on the known role of the PDGFR β /PDGF-B axis in pericyte function during development,^{19,22,27} we next asked whether PDGF-BB played a role in pericyte recruitment and migration.³² Either cancer (HeyA8, SKOV3ip1), endothelial (HUVEC), or pericyte-like (hVSMC) cell lines were exposed to PDGF-BB 10 ng/ml in the presence or absence of a PDGFR β inhibitor imatinib mesylate. PDGF-BB treatment had no significant effect on HeyA8 or SKOV3ip1 cells, but there was a 57% increase in migration of HUVEC ($p < 0.05$), and 3-fold increase in hVSMC ($p < 0.001$; Figure 3). These results suggest that the greatest effect of PDGF-BB occurs on the migration of pericyte-like cells, providing a possible mechanism for pericyte recruitment in the maturing tumor vasculature. The effects of PDGF-BB on migration of endothelial or pericyte-like cells were blocked by Imatinib mesylate.

Effect of hVSMC-derived CM on HUVEC survival

We have previously demonstrated that even though the total vessel density decreases with VEGF-targeted therapies, the proportion of vessels with pericyte coverage increases.²¹ To examine potential mechanisms underlying this observation, HUVECs were exposed to conditioned media from pericyte-like cells (basal PCM), PDGF-BB or both. No protective effects against endothelial cell apoptosis were noted (Figure 4A). We next collected CM from hVSMCs following stimulation with PDGF-BB for 48 h ("activated" PCM). Exposure to this media, as well as VEGF, significantly protected endothelial cells from apoptosis. Relative to this reduction, bevacizumab partially abrogated this effect, suggesting that local production of VEGF by pericytes promotes endothelial survival likely through a paracrine mechanism (Figure 4A).

To characterize the effects of PDGF-BB signaling on VEGF production, 10T1/2 cells were treated with PDGF-BB or conditioned media (CM) from SKOV3ip1 or HUVEC cells. The VEGF mRNA levels were significantly increased after treatment with recombinant PDGF-BB or incubation with CM from SKOV3ip1 or HUVEC cells (Figure 4B). Imatinib mesylate blocked the VEGF induction mediated by SKOV3ip1 CM. Imatinib mesylate also blocked VEGF induction mediated by PDGF-BB or HUVEC CM (data not shown). Similarly, PDGF-BB treatment resulted in an almost 3-fold increase in VEGF protein production (949 pg/ml versus 225 pg/ml, $p < 0.01$) in hVSMC-derived CM.

Effect of dual endothelial and pericyte targeting in ovarian carcinoma

Since pericytes regulate endothelial survival, we hypothesized that dual targeting of endothelial cells (using VEGF-Trap) and pericytes (using PDGF-Trap) would be more efficacious than targeting either cell type alone. For these studies, we used an orthotopic model of metastatic ovarian cancer. Nude mice were injected i.p. with HeyA8 cells and 7 d later, randomized as follows: 1) control (hFc), 2) VEGF-Trap, 3) PDGF-Trap, and 4) VEGF-Trap plus PDGF-Trap. Compared to controls, PDGF-Trap alone had no effect on tumor growth (Figure 5). VEGF-Trap significantly inhibited tumor growth by 64% ($p < 0.01$), and was even more effective in combination with PDGF-Trap (75% growth inhibition; $p < 0.01$ compared to controls).

Effect of VEGF-Trap and PDGF-Trap on tumor vasculature

Based on our *in vitro* data suggesting the protective effects of pericytes on endothelial cells, we determined the extent of tumor-associated endothelial cell apoptosis *in vivo* using dual (CD31/TUNEL) immunofluorescence. Minimal endothelial cell apoptosis was apparent in either the control or single-agent PDGF-Trap treatment groups (Figure 6A). VEGF-Trap

significantly increased endothelial cell apoptosis, but the greatest increase was noted in the combination therapy group ($p < 0.05$ compared to VEGF-Trap treatment).

To further examine potential mechanisms, pericyte coverage of tumor blood vessels was determined using double immunofluorescence (desmin/CD31). Treatment with VEGF-Trap alone resulted in an overall decrease in vessel density (Figure 6C), but there was an increase in the proportion of vessels with pericyte coverage ($p < 0.05$; Figure 6B). In contrast, treatment with PDGF-Trap alone had no significant effect on vessel density, but there was a decrease in the proportion of vessels covered with pericytes (35%; $p < 0.05$). The combination of VEGF-Trap and PDGF-Trap resulted in lower vessel density and decreased pericyte coverage (Figure 6B–C).

Comment

The main findings from this study are that pericytes play key roles in maintenance of ovarian cancer vasculature and may provide a protective mechanism against anti-angiogenic therapies. These effects reflect complex paracrine interactions between the tumor endothelium, pericytes, and tumor cells (Figure 7). However, our findings suggest opportunities for novel anti-vascular therapeutic strategies based on dual-targeting of tumor endothelial cells and pericytes.

Despite many advances in surgical and medical management, ovarian cancer remains the most deadly gynecologic malignancy.^{33,34,35} Ongoing studies in the Gynecologic Oncology Group (GOG) suggest that addition of more chemotherapy drugs may not result in substantial improvements in the outcome of ovarian cancer patients. It is likely that combination of biologically-targeted agents will be required for additional gains in patient survival. Targeting the tumor vasculature is attractive because angiogenesis is required for tumor growth and endothelial cells are thought to be more genetically stable than the highly aneuploid cancer cells. Indeed, VEGF-targeted therapies have improved the outcome of patients with colorectal and other cancers.^{13,14} Remarkably, bevacizumab monotherapy resulted in an 18% response rate in recurrent ovarian cancer.¹² While these therapies are improving survival in many cancer patients, the cure rates do not appear substantially higher and new strategies are needed to make additional gains.

There is growing evidence that pericytes may play a role in limiting the efficacy of VEGF-targeted therapies.²⁰ The pathophysiological interaction of tumor cells, tumor-associated endothelial cells, and pericytes in the tumor microenvironment is complex and poorly understood. Nonetheless, understanding this relationship is critical to effective angiogenic targeting. We documented that pericytes are normally tightly associated with endothelial cells and extensively cover normal vasculature, but in ovarian cancer, pericytes are loosely associated with endothelial cells and irregularly oriented relative to the stroma. The interplay between these cells likely reflects the shifts in PDGF-BB gradients in the tumor microenvironment. Nevertheless, pericytes recruited and stimulated by PDGF-BB appear to provide survival signals for the tumor endothelial cells. Using highly specific decoy receptors for ligands (PDGF-Trap and VEGF-Trap), we demonstrated that while blockade of PDGF had little effect on tumor growth, VEGF blockade and dual targeting significantly reduced tumor growth. Mechanistically, this effect appears to be due, in part, to endothelial cell apoptosis following VEGF blockade, which is enhanced in the presence of PDGF blockade. We also observed that pericyte coverage of endothelial cells in ovarian cancer is reduced when PDGF-BB signaling is blocked, which enhances the effects of anti-VEGF therapy in ovarian cancer.

We previously examined the potential of endothelial and pericyte targeting using non-specific inhibitors of PDGFR (imatinib mesylate) and VEGFR (AEE788, Novartis, Basel, Switzerland) signaling in ovarian cancer models.²¹ In combination with paclitaxel, the triple-drug

combination was highly effective in chemotherapy-sensitive and resistant tumors. As was observed in the current study, interruption of the PDGF receptor/ligand axis reduced pericyte coverage and blockade of VEGF receptor phosphorylation induced endothelial cell apoptosis. However, we recognized that a confounding factor in our previous study was the broad end-target spectrum of the agents, particularly the co-blockade of epidermal growth factor receptor (EGFR) phosphorylation with AEE788. In the current study, we extend and confirm these results using highly specific decoy receptors for PDGF and VEGF ligand.

The consistency of these findings combined with those of others^{20,36,37} support the hypothesis that pericytes modulate endothelial cell function partially by producing survival signaling. Since PDGF-BB promotes VEGF production by pericytes and VEGF is a known survival factor for endothelial cells, pericytes appear to promote endothelial cell survival in a paracrine fashion. It is possible then that pericytes may serve as a local source of VEGF and other factors for the adjacent endothelial cells. Based on our data and published literature, we have developed a model (Figure 7) to describe the interactions between tumor, perivascular, and endothelial cells. In this model, the tumor and endothelial cells produce PDGF-BB, which promotes VEGF production by pericytes. Among other functions, VEGF protects endothelial cells from apoptosis.

We recognize that there are limitations to this work, which should be discussed. We have focused primarily on PDGF-BB due to its known significance for pericyte biology. However, other PDGF ligands may be important for production of survival factors from pericytes. In addition, other pathways such as Ang1/Tie2, TGF- β 1/Alk5, and MMPs may be involved in pericyte recruitment during angiogenesis,¹⁵ and could offer additional opportunities for therapeutic targeting. While the presence of pericytes is altered in cancer vasculature relative to normal, there are no standard ways to quantify this effect. We used the percent of vessels with 50% coverage of pericytes; however, a more precise measure of functional deficiency may be required for using this feature as a potential biomarker. Finally, we co-administered PDGF- and VEGF-Trap to demonstrate the differential improvement in VEGF sensitivity. However, it is possible that specific temporal sequencing of agents such as these, particularly in combination with cytotoxic chemotherapy, may be required for optimal anti-tumor effects.

In summary, our data show that paracrine signaling from pericytes provides survival cues for tumor vasculature. Blocking PDGF signaling enhances the effect of anti-VEGF therapy in ovarian cancer treatment, in part, by decreasing pericyte coverage and increasing apoptosis of tumor-associated endothelial cells. Therefore, dual targeting of endothelial cells and pericytes is more efficacious than targeting either cell type alone. This study provides the preclinical rationale for the development of more effective anti-vascular therapeutic strategies for human ovarian cancer.

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Figure 1.

Establishment and characterization of a fully orthotopic model of ovarian carcinoma. (A) Following establishment of anesthesia, a small incision was made on the left lateral abdomen and the left ovary was isolated. Using a 30 gauge needle, the tumor cells were injected into the left ovary. (B) Intraovarian tumor (left) and peritoneal metastasis (right) were noted 4 weeks after tumor cell injection into the ovary. (C) H & E stained sections of the uterus and ovaries 1 week after intraovarian tumor cell injection reveal a high-grade tumor within the ovarian parenchyma. (D) Longitudinal assessment of ovarian tumor (HeyA8-Luc) growth and metastasis by bioluminescence imaging. (E) Dual immunofluorescence staining for CD31 (red) and desmin (green) was performed to visualize endothelial cells and pericytes. Images were taken at original magnification x400. (F) Pericyte coverage of small vessels in normal ovary and ovarian carcinoma. The percentage of vessels with at least 50% coverage of associated desmin-positive cells were counted.

Figure 2. Expression of PDGF ligands and receptors in ovarian cancer (HeyA8, SKOV3ip1, A2780-Par), endothelial (HUVEC, EC-ovary), and pericyte-like cells (hVSMC, 10T1/2) was assessed by RT-PCR.

Figure 3.

Effect of PDGF-BB the *in vitro* migration ability of ovarian cancer (HeyA8, SKOV3ip1), endothelial (HUVECs), and pericyte-like (hVSMC) cell lines. Error bars represent SE. *p < 0.05, **p < 0.001.

Figure 4A **Figure 4B** **Figure 4.**

(A) Effect of pericyte-like cells on endothelial cell apoptosis. HUVEC were treated with 1% serum containing medium, VEGF (10 ng/ml), conditioned medium from hVSMC (basal PCM), basal PCM + PDGF-BB (20 ng/ml), activated PCM (conditioned medium from hVSMC treated with PDGF-BB), PDGF-BB alone, bevacizumab (2 μ g/ml), or activated PCM + bevacizumab. Apoptosis was assessed with flow cytometry. Error bars represent SE. * $p < 0.01$. (B) PDGF-BB promotes VEGF production by pericyte-like cells. The 10T1/2 cells were treated with PDGF-BB, imatinib mesylate (PDGFR β blocker) or conditioned medium from SKOV3ip1 or HUVEC cells. RNA was extracted and mRNA levels were determined by real-time RT-PCR. Error bars represent SE. * $p < 0.01$.

Figure 5.

Effect of dual endothelial and pericyte targeting on ovarian cancer growth in a mouse model. Mice were injected with HeyA8 human ovarian cancer cells. Seven days later, mice were randomized (n = 10) to receive the following regimens: (1) vehicle control (Fc 25 mg/kg i.p., 2 times/week); (2) VEGF-Trap (12.5 mg/kg i.p. 2 times/week); (3) PDGF-Trap (12.5 mg/kg i.p., 2 times/week); (4) VEGF-Trap plus PDGF-Trap (same doses as above for both). Mice were sacrificed when controls became moribund. Mean tumor weights with standard errors are shown. *p < 0.01 compared to vehicle controls; †p < 0.05 compared to the VEGF-Trap group.

Figure 6.

Effect of dual endothelial and pericyte targeting on tumor vasculature. (A) Immunofluorescence double staining for CD31 (endothelial marker, red) and TUNEL (apoptotic marker, green) were performed to determine apoptotic endothelial cells. Co-localization of endothelial cells undergoing apoptosis shows yellow fluorescence. * $p < 0.01$ compared to vehicle controls; † $p < 0.05$ compared to the VEGF-Trap group. (B) Dual immunofluorescence staining for CD31 (red) and desmin (pericyte marker, green) was used to determine the extent of pericyte coverage. The percentage of vessels with at least 50% coverage of associated desmin-positive cells were counted. * $p < 0.05$ compared to vehicle controls. Images were taken at original magnification x400. (C) Tumor sections were stained

with CD31 using IHC staining to reveal microvessel density (images taken at original magnification x200). Microvessels were counted and graphed. * $p < 0.01$ compared to vehicle control; † $p < 0.05$ compared to the VEGF-Trap group.

Figure 7.

Hypothetical model of interactions between tumor, endothelial cells, and pericytes in ovarian cancer. The tumor and endothelial cells produce PDGF-BB, which promotes local VEGF production by pericytes, providing local survival signals for the mature tumor vasculature.