Vascular Endothelial Growth Factor Upregulates Expression of ADAMTS1 in Endothelial Cells through Protein Kinase C Signaling

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PURPOSE. ADAMTS1 (a disintegrin and metalloproteinase with thrombospondin motifs) has been demonstrated to inhibit angiogenesis in vivo and to suppress endothelial cell proliferation in vitro. The purpose of this study was to investigate the expression of ADAMTS1 in endothelial cells and to study the regulation of ADAMTS1 expression in endothelial cells by vascular endothelial growth factor (VEGF). In addition, the potential function of endothelial cell–derived ADAMTS1 on cell proliferation was investigated.

METHODS. Expression of ADAMTS1 in human retinal endothelial cells (HRECs), human umbilical vein endothelial cells (HUVECs), and the mouse model of ischemia-induced retinal neovascularization was assayed by real-time PCR and Western blot analysis. The effect of ADAMTS1 on endothelial cell proliferation was evaluated using siRNA knockdown and [3H] thymidine incorporation.

RESULTS. ADAMTS1 mRNA and protein levels were increased in a mouse model of ischemia-induced retinal neovascularization, and VEGF induced time- and dose-dependent increases in ADAMTS1 mRNA and protein expression in endothelial cells. This upregulation was inhibited by the VEGF receptor (VEGFR)2 inhibitor SU1498, anti–VEGFR2 neutralizing antibody, and the phospholipase C (PLC)-γ inhibitor U73122. VEGF upregulation of ADAMTS1 expression was completely abolished by the inhibition of protein kinase C by calphostin C and largely blocked by the specific inhibition of PKCβ. Knockdown of endogenous ADAMTS1 resulted in increased proliferation of endothelial cells.

CONCLUSIONS. These results indicate that VEGF significantly induces ADAMTS1 expression in endothelial cells in a PKC-dependent fashion. ADAMTS1 expression is also increased, along with VEGF expression, in vivo in ischemia-induced retinal neovascularization. In addition, ADAMTS1 appears to be an endogenous regulator of endothelial cell proliferation. Therefore, VEGF upregulation of ADAMTS1, a potent angiogenesis inhibitor, may represent a mechanism for feedback inhibition of angiogenesis and retinal neovascularization. (Invest Ophthalmol Vis Sci. 2006;47:4059–4066) DOI:10.1167/iovs.05-1528

Angiogenesis, the formation of new blood vessels from preexisting ones, is a complex process that includes extracellular matrix breakdown, endothelial cell sprouting, proliferation, migration, differentiation, and recruitment of pericytes. Not only is it a natural physiologic process, it plays a critical role in a variety of pathologic disorders such as diabetic retinopathy, age-related macular degeneration, and tumor progression.1–5 It is postulated that angiogenesis results from an imbalance between angiogenic stimulators and inhibitors in a given tissue bed.6 With its ability to regulate many steps in the angiogenic process, including proliferation, migration, survival, and permeability, VEGF is one of the most important proangiogenic factors. The biologic effects of VEGF are mediated by its interaction with two high-affinity receptor tyrosine kinases, Flt-1 (VEGF receptor-1) and KDR/Flik-1 (VEGF receptor-2). VEGFR2 is considered the major mediator of several physiologic and pathologic effects of VEGF on endothelial cells, including mitogenesis and survival.7 On binding of VEGF to its receptors, several signaling cascades are activated, including the phosphorylation of phospholipase C (PLC)-γ, an increase in intracellular Ca2+ and diacylglycerol, and activation of protein kinase C (PKC). In addition, VEGF stimulates the activation of phosphatidylinositol-3 kinase (PI3K), p38 mitogen-activated protein kinase (p38MAPK), focal adhesion kinase (FAK), and Src.6

ADAMTS1 (also known as METH1) is a member of the ADAM (a disintegrin and metalloproteinase) family of proteases.7 The ADAMTS1 gene encodes a multidomain protein containing a metalloproteinase domain, a disintegrin domain, and thrombospondin (TSP) type 1 motifs.8 ADAMTS1 is secreted as a 110-kDa zymogen form and may undergo processing by furin to its 87-kDa active form. Both forms can anchor to the extracellular matrix through their C-terminal TSP motifs.9 ADAMTS1 knockout mice exhibit a number of morphologic defects in the kidney, adrenal gland, and adipose tissue in addition to growth retardation and impaired female infertility, demonstrating its important role for normal growth, fertility, and organogenesis.10–13 ADAMTS1 is a potent inhibitor of angiogenesis: it inhibits endothelial cell proliferation in vitro, suppresses fibroblast growth factor-2–induced vascularization in the cornea pocket assay, and inhibits VEGF-induced angiogenesis in the chorioallantoic membrane assay.14 In addition, ADAMTS1 directly binds VEGF and blocks VEGFR2 phosphorylation, with consequent suppression of endothelial cell proliferation.15

The possible role of ADAMTS1 in retinal neovascularization and the regulation of ADAMTS1 in endothelial cells have not been studied extensively. Here we investigated the expression of ADAMTS1 in the murine oxygen-induced retinopathy (OIR) model and its upregulation by VEGF through several signaling pathways in retinal microvascular and umbilical vein endothelial cells. In addition, we investigated the role of endogenous...
ADAMTS1 on endothelial cell proliferation. We found that ADAMTS1 expression is increased in the retina in the OIR model and that ADAMTS1 expression in endothelial cells is significantly upregulated by VEGF. Furthermore, the suppression of endogenous ADAMTS1 expression enhances endothelial cell proliferation, consistent with an angio-inhibitory role for this molecule.

**MATERIALS AND METHODS**

**Materials**

The following materials were used: human recombinant VEGF, placental growth factor (PIGF), FGF-2, TNF-α, IL-1β, and monoclonal anti-human VEGFR2 neutralizing antibody (R&D Systems, Minneapolis, MN); calphostin C, G6109205X, LY335551, rottlerin, SU1498, U73343, U0126, PD98059, and actinomycin D (CalBiochem, San Diego, CA); U73122 (BIOMOL International, Plymouth Meeting, PA); and complete mini-protease inhibitor cocktail tablet (Roche Diagnostic). Other chemicals and reagents were obtained from Sigma Chemical Co. (St. Louis, MO) unless otherwise indicated.

**Cell Culture**

Human retinal endothelial cells (HRECs; Cell Systems, Kirkland, WA) and human umbilical vein endothelial cells (HUVECs; Clonetics, San Diego, CA) were cultured in EGM2-MV medium (Clonetics) and used between passages 4 and 10. HRECs were cultured in fibronectin-coated dishes. Cells were grown in 5% CO2 at 37°C, and media were changed every 2 to 3 days.

**Animal Studies**

Oxygen-induced retinopathy was elicited in mice as previously described. In brief, postnatal day (P) 7 C57BL/6 mice were exposed to 75% O2, along with their nursing mothers for 5 days. On P12, the mice were returned to room air and killed at the indicated times. Retinas were extracted, snap-frozen in liquid nitrogen, and stored at −80°C until further processing. The study protocol adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Real-time PCR Analysis**

Cells were harvested in reagent (TRizol; Invitrogen, Carlsbad, CA), and total RNA was isolated according to manufacturer’s instructions. Single-stranded cDNA was synthesized from 1 μg total RNA using an oligo (dT) 18-mer as primer, and reverse transcription (MMLV Reverse Transcriptase; Invitrogen) in a final reaction volume of 25 μL real-time PCR was performed (QuantiTect SYBR Green PCR Kit; Qiagen, Valencia, CA) with a light-cycling system (LightCycler; Roche Diagnostics GmbH, Mannheim, Germany). Primers were hADAMTS1 sense (5'-ATCAAGGCAAGTTGTTAAT-3') and antisense (5'-CTCTTGGAGCGCGTGGTTC-3'); hADAMTS1 sense (5'-AGTTGGTTGTCACTCGAGGA-3') and antisense (5'-TCTCTGGGGGTGTTGTC-3'); and mVEGF sense (5'-TACTGGCCTACTACACC-3') and antisense (5'-ACAGGACCCTTAAATG-3'). GAPDH was used as the reference for normalization with hGAPDH sense (5'-GAGTCAAGGATTGTGTTGT-3') and antisense (5'-GACAAGCTCCGTTCTACG-3') and mGAPDH sense (5'-AAGCACCCTTCTATTGAC-3') and antisense (5'-TCAAGAGGATATCCTGAC-3') primers. Values of ADAMTS1 or VEGF mRNA expression were normalized to GAPDH gene expression.

**Protein Extraction from Mouse Retinas**

Mouse retinas were sonicated in lysis buffer (50 mM Tris-HCl, pH 7.4, 1% Triton X-100, 1 mM EDTA, 150 mM NaCl, 1 mM PMSF, 10 μL complete mini-protease inhibitor cocktail) with an ultrasonic cleaner (model 2510; Branson Ultrasoundics, Torrance, CA) for 15 minutes. Retina samples were then pipetted 10 times and incubated on ice for 30 minutes. After centrifugation at 15,000g for 10 minutes, the precipitates were washed with ice-cold acetone 2 times, and 20 μL Laemmli sample buffer (Bio-Rad Laboratories, Hercules, CA). Cell culture supernatants were precipitated with 4 vol acetone at −20°C for 1 hour. After centrifugation at 15,000g for 10 minutes, the precipitates were washed with ice-cold acetone 2 times, and 20 μL Laemmli sample buffer was added to the dried precipitates.

**Western Blot Analysis**

Protein extracts prepared from mouse retinas or cultured endothelial cell lines were subjected to SDS-PAGE on 7.5% gels and were transferred to nitrocellulose membrane (Hybond ECL; Amersham Biosciences, Piscataway, NJ). Membranes were probed with rabbit polyclonal C-terminal of human anti-ADAMTS1 antibodies (diluted 1:2000 for the detection of human ADAMTS1 protein and 1:500 for the detection of mouse ADAMTS1 protein; Sigma Chemical Co.) and monoclonal anti-GAPDH antibodies. Blots were detected with the substrates (Supersignal West Pico Chemiluminescent Substrate; Pierce Biotechnology, Rockford, IL).

**Proliferation Assay**

Endothelial cell proliferation activity was assessed by measuring the incorporation of [3H] thymidine (Amersham Biosciences) into DNA. HUVECs were used for this assay because of the high efficiency of small interfering (si) RNA knockdown achievable in these cells. Briefly, subconfluent HUVECs in a 24-well plate were transfected with 100 nM ADAMTS1 or negative control siRNA (Ambion, Austin, TX) using a reagent (siPORT Amine; Ambion). After 24 hours, media were replaced by 10% calf serum in DMEM, treated with 25 ng/mL VEGF, and incubated for another 24 hours. The cells were pulsed with 1 μCi/mL [3H] thymidine during the last 6 hours of culture. Cells were washed twice with PBS and twice with cold 5% trichloroacetic acid (TCA) to remove the unincorporated [3H] thymidine. Cells were then washed twice with ethanol/ether (3:1 vol) and solubilized in 200 μL of 0.1 N NaOH, followed by neutralization in 0.1 N HCl. Aliquots of samples were added to 8 mL scintillation fluid, and radioactivity was determined with a liquid scintillation counter.

**Statistical Analysis**

All experiments were performed at least twice. Results were reported as mean ± SD. An unpaired Student’s t test was used to determine statistical significance. P < 0.05 was considered significant.

**RESULTS**

**Increased ADAMTS1 Expression in the Retina of the Mouse Model of Oxygen-Induced Retinopathy**

We investigated ADAMTS1 mRNA expression in the mouse model of oxygen-induced retinopathy. As shown in Figure 1A, VEGF mRNA levels were increased significantly in P12.5 mice compared with P12 mice (P < 0.01), consistent with previous studies.17,18 Similarly, ADAMTS1 mRNA levels were also significantly increased: mRNA levels in P12.5 mice were approximately three times the levels in P12 mice. Western blot analysis of this mouse model also demonstrated that ADAMTS1 protein levels were elevated in retinas from P13 to P17 compared with P12 (Fig. 1B). These results indicated that ADAMTS1 expression was upregulated during retinal neovascularization in this model and could potentially have a regulatory role in this process.
FIGURE 1. ADAMTS1 expression levels were increased in a mouse model of oxygen-induced retinopathy. (A) Levels of ADAMTS1 and VEGF mRNA were measured by quantitative real-time PCR in mouse retinas immediately after oxygen treatment (0) and 12 hours after return to room air (12h). **P < 0.01 compared with controls. Three mice were used for each time point. (B) Western blot analysis for ADAMTS1 protein in retinas from mouse model of OIR (ROP P12-P17).

Effect of Different Growth Factors on ADAMTS1 mRNA Expression in Human Endothelial Cells

Retinal neovascularization is associated with increases in extracellular proteinases such as the matrix metalloproteinases (MMPs) in humans and in animal models, and these proteinases are thought to play an active role in promoting and regulating the neovascular process. Endothelial cell production and activation of MMPs is thought to be critical for the angiogenic process and endothelial cell production of MMPs is regulated by extracellular cues including VEGF, angiopoietin-2, and TNF-α. Interestingly, endothelial cell production of ADAMTS1 is increased by lipopolysaccharide (LPS) and TNF-α. Therefore, we investigated whether other growth factors could regulate endothelial cell expression of ADAMTS1. Several growth factors associated with angiogenesis were used to treat serum-starved endothelial cells. ADAMTS1 mRNA expression was dramatically upregulated by VEGF and modestly upregulated by TNF-α and IL-1β (1.7- and 2.2-fold, respectively; Fig. 2). PlGF and FGF2 did not significantly induce the expression of ADAMTS1 mRNA.

Effect of VEGF on ADAMTS1 Gene Expression in Human Endothelial Cells

Because VEGF displayed a dramatic upregulation of ADAMTS1 expression, we further characterized its effect on ADAMTS1 expression. We performed real-time PCR and Western blot analysis in HRECs and HUVECs. As shown in Figure 3A, 25 ng/mL VEGF stimulated ADAMTS1 expression as early as 30 minutes after treatment, and ADAMTS1 mRNA expression peaked at 2 hours. VEGF upregulation of ADAMTS1 mRNA persisted at 24 hours after treatment. Western blot analysis showed similar results (Fig. 3B). We detected both thezymogen form (110 kDa) and the mature form (87 kDa) of ADAMTS1 in the cell layer, whereas only the zymogen form was detected in the culture medium (Fig. 3B). ADAMTS1 protein expression in the cell layer and culture medium was significantly increased after VEGF treatment with maximal expression at 12 hours and 24 hours, respectively. VEGF upregulated ADAMTS1 expression in a dose-dependent manner, with a peak effect at 25 ng/mL (Fig. 3C).

Effect of VEGF on Half-Life of ADAMTS1 mRNA

To investigate whether VEGF affects the stability of ADAMTS1 mRNA, the half-life of ADAMTS1 mRNA was measured in the presence of actinomycin D, which inhibits de novo gene transcription. As shown in Figure 4, the half-life of ADAMTS1 mRNA was 1.8 hours in unstimulated controls and 1.9 hours when treated with VEGF. There was no significant difference between control and VEGF treatment, suggesting that the increase in ADAMTS1 mRNA levels in response to VEGF treatment was caused by transcriptional activation.

Effects of Inhibition of VEGFR2 and PLC-γ on VEGF-Stimulated ADAMTS1 Upregulation

It is well known that VEGF exerts its function through binding to two different VEGF-receptor tyrosine kinases, with VEGFR2 considered the major mediator of the mitogenic and angiogenic effects of VEGF. To investigate whether VEGFR2-mediated VEGF upregulation of ADAMTS1 in endothelial cells, we treated endothelial cells with SU1498, a potent inhibitor of VEGFR2. Although SU1498 had no effect on basal ADAMTS1 expression, it abrogated VEGF-induced ADAMTS1 upregulation (Fig. 5A). Pretreatment of HUVECs with 2 μg/mL neutralizing antibody against human VEGFR2 for 1 hour suppressed VEGF-induced ADAMTS1 expression by 70% (Fig. 5B). Western blot analysis using anti-phospho-KDR antibody confirmed that the VEGFR2-neutralizing antibody blocked VEGF-induced KDR phosphorylation in these cells (data not shown). Together, these results indicate that VEGFR2 plays a major role in VEGF-induced ADAMTS1 expression, though we cannot exclude a role for VEGFR1. Because PLC-γ activation is an important downstream mediator of VEGFR2 signaling, endothelial cells were pretreated with the PLC-γ inhibitor U73122. As shown in Figure 5C, treatment of HUVECs with 2 μM U73122 inhibited VEGF-induced ADAMTS1 expression by 79%. In contrast, U73343, an inactive analogue of U73122, had no effect on ADAMTS1 level, suggesting that VEGF-increased ADAMTS1 expression is dependent on PLC activity.

FIGURE 2. Effects of different growth factors on ADAMTS1 mRNA expression in human endothelial cells. HUVECs were treated with VEGF, PlGF, FGF2, TNF-α, or IL1-β at 25 ng/mL for 2 hours. Total RNA was isolated, and quantitative real-time PCR was used to detect the expression of ADAMTS1 mRNA. Results are representative of three independent experiments, each performed with duplicate samples. *P < 0.05 compared with untreated control.
Different Roles of PKC and MEK Pathways on VEGF-Induced Upregulation of ADAMTS1

We next investigated the role of the protein kinase C (PKC) pathway in VEGF-induced ADAMTS1 upregulation because PKC is a well-documented downstream target of PLC-γ. HUVECs and HRECs were pretreated with PKC inhibitors. As shown in Figures 6A and 6B, 1 μM calphostin C, a PKC inhibitor, completely inhibited VEGF-induced ADAMTS1 upregulation. GF109203X (3 μM), another PKC inhibitor, also abrogated VEGF-induced ADAMTS1 expression. Then we investigated the effect of two specific PKC inhibitors. Inhibition of PKCα with LY333531 significantly reduced ADAMTS1 protein expression by 60%, whereas inhibition of PKCβ with rotterlin resulted in slight inhibition, indicating that PKCβ is the primary isoform involved in VEGF-induced ADAMTS1 upregulation. We also investigated the role of the MAP/ERK kinase (MEK) pathway. Two different inhibitors of MEK (10 μM U0126 and 50 μM PD98059) failed to abrogate VEGF-induced ADAMTS1 upregulation, though the same dosage of these inhibitors completely inhibited the phosphorylation of extracellular response kinase (ERK), the downstream kinase in MEK pathway (data not shown). Taken together, these results demonstrate that VEGF-induced ADAMTS1 expression is dependent on PKC signaling but independent of MEK.

Effect of ADAMTS1 Small Interference RNA on Endothelial Cells Proliferation

Although several reports have demonstrated that exogenous administration of ADAMTS1 has potent antiangiogenic effects in vitro and in vivo, comparatively less investigation has been performed into the role of endogenous ADAMTS1 on endothelial cell activities, such as proliferation. We investigated the
Effect of siRNA-mediated knockdown of ADAMTS1. Transfection of ADAMTS1 siRNA into endothelial cells successfully knocked down ADAMTS1 mRNA expression by 90% (Fig. 7A). Concomitantly, the endothelial cell proliferation rate was significantly increased by twofold (Fig. 7B), suggesting that ADAMTS1 plays an inhibitory role in endothelial cell proliferation.

**DISCUSSION**

During angiogenesis, vascular endothelial cells respond to various molecular cues in their environments, including growth factors such as VEGF, and they elaborate an array of molecules that promote and regulate the angiogenic process. Among these molecules are extracellular proteases, including urokinase and MMPs, which play an important role in matrix degradation and growth factor signaling modulation. ADAMTS1 is a member of a relatively novel group of MMPs, characterized by the presence of disintegrin-like and thrombospondin-like domains, that is beginning to receive attention for its ability to regulate endothelial cells. Little attention has been focused on the expression and regulation of ADAMTS1 in endothelial cells. In this study, we found that VEGF markedly stimulated microvascular and macrovascular endothelial cell production of ADAMTS1 at the mRNA and the protein levels. VEGF up-regulation of ADAMTS1 mRNA occurred very quickly after treatment, peaking at 2 hours, though the upregulation persisted for at least 24 hours. By comparison, TNF-α and IL-1β exhibited comparatively modest stimulation, while another strong proangiogenic factor, FGF-2, had no significant effect on ADAMTS1 expression. Upregulation of ADAMTS1 mRNA and protein also occurred in vivo in the murine OIR model, along with the upregulation of VEGF, indicating that ADAMTS1 might play a role in retinal neovascularization. Other extracellular proteases, such as uPA, MMP-2, and MMP-9, are also increased in the OIR model. Indeed, pharmacologic inhibition of MMPs and genetic disruption of MMP-2 have been reported to inhibit retinal neovascularization in this model.

VEGF is known to promote the activation of several signaling molecules, including PI3K, p38MAPK, PKC, and ERK. The mitogenic effects of VEGF are thought to be transduced
VEGFR2 mediates PLC-primarily through a PKC-dependent pathway; specifically, HRECs and HUVECs were pretreated with 1 μM calphostin C, 5 μM GF109203X, 2 μM LY335531, and 5 μM rottlerin for 30 minutes and were treated with 25 ng/mL VEGF for 6 hours. (*P = 0.01 compared with VEGF-treated samples. (C) Serum-starved HUVECs were pretreated with 5 μM and 10 μM U0126 and 50 μM PD98059 for 30 minutes and then were treated with 25 ng/mL VEGF for 6 hours. Total cell lysates were subjected to Western blot analysis for ADAMTS1. The location of zymogen form (110 kDa) and the mature form (87 kDa) of ADAMTS1 were indicated (arrows). The same membrane was blotted with anti-GAPDH antibody for normalization.

![Figure 6](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933235/)  
**FIGURE 6.** VEGF-induced ADAMTS1 expression was PKC-dependent and MEK-independent in human endothelial cells. (A) Serum-starved HRECs were pretreated with 1 μM calphostin C, 5 μM GF109203X, 2 μM LY335531, and 5 μM rottlerin for 30 minutes and were treated with 25 ng/mL VEGF for 6 hours. (B) After overnight serum starvation, HRECs and HUVECs were pretreated with 1 μM calphostin C and 2 μM LY335531 for 30 minutes and were treated with 25 ng/mL VEGF for 2 hours. Total RNA was isolated, and quantitative real-time PCR was used to detect the expression of ADAMTS1 mRNA. (**P < 0.01 compared with untreated control samples. (C) Serum-starved HUVECs were pretreated with 5 μM and 10 μM U0126 and 50 μM PD98059 for 30 minutes and then were treated with 25 ng/mL VEGF for 6 hours. Total cell lysates were subjected to Western blot analysis for ADAMTS1. The location of zymogen form (110 kDa) and the mature form (87 kDa) of ADAMTS1 were indicated (arrows). The same membrane was blotted with anti-GAPDH antibody for normalization.

primarily through a PKC-dependent pathway; specifically, VEGFR2 mediates PLC-γ phosphorylation, which in turn leads to PKC activation and endothelial cell proliferation through ERK. Our results indicate that the PKC signaling pathway plays a critical role in mediating VEGF stimulation of ADAMTS1 because this stimulation was dramatically inhibited by pharmacologic blockade of PKC and by VEGFR2 and PLC-γ. Our results also indicate a particularly important role in ADAMTS1 stimulation for the PKCβ isoform, which has previously been shown to play a major role in VEGF-induced endothelial cell proliferation.32 In contrast, we found that the upregulation of ADAMTS1 expression by VEGF was independent of the MEK/ERK pathway. VEGF stimulation of ADAMTS1 was mediated primarily through PKC, which is interesting given the importance of PKC in the mitogenic effects of VEGF and the anti-proliferative properties of ADAMTS1.

Previous studies have demonstrated that exogenous administration of ADAMTS1 can inhibit angiogenesis in vivo and endothelial cell proliferation in vitro. Indeed, its ability in suppressing vessel growth was even greater than that mediated by thrombospondin-1 or endostatin on a molar basis.34 We sought to determine whether the intrinsic production of ADAMTS1 by endothelial cells could have a functional effect on their proliferative activity. Our studies indicated that siRNA knockdown of endogenous ADAMTS1 stimulated the proliferation of endothelial cells, supporting a role for this molecule as a negative regulator of cell proliferation. These results, in conjunction with the rapid and marked stimulation of endothelial cell ADAMTS1 expression by VEGF, suggest that ADAMTS1 could represent a mechanism by which endothelial cells intrinsically regulate their proliferative capacity.

Angiogenesis, particularly in a physiologic context, is a tightly regulated process. As such, this process involves a complex interplay of stimulatory and inhibitory factors to prevent excessive angiogenesis and unchecked endothelial cell activation. It is becoming increasingly clear that endothelial cells play an active role in their self-regulation. Specifically, attention is being devoted to the involvement of endothelial cell “intrinsic” molecules.35 For instance, vascular endothelial cell growth inhibitor (VEGIF) TNFSF15 is expressed predominantly in endothelial cells and has been demonstrated to block angiogenesis and to inhibit endothelial cell growth.36 VEGF expression in endothelial cells is stimulated by TNF-α.37 Treatment of endothelial cells with VEGF, FGF-2, and IL-8 increases endothelial cell surface levels of Fas/CD95 receptor, thereby sensitizing these cells to FasL-mediated apoptosis.37 Some endothelial cell intrinsic molecules appear to function in a manner reminiscent of feedback inhibition. For instance, VEGF rapidly upregulates endothelial cell expression of DSCR1 by activating the calcineurin-NFAT pathway.38–41 DSCR1, in turn, acts to inhibit calcineurin and its stimulation of NFAT. Recently, VEGF was found to induce endothelial cell expression of vasohibin, which selectively inhibits proliferation, migration, and tube formation in endothelial cells and angiogenesis in vivo.42 VEGF upregulation of vasohibin is primarily mediated by PKC, particularly the delta isoform.43

In our case, we found that VEGF rapidly and strongly stimulates endothelial cell production of ADAMTS1 in a PKC-dependent fashion. Exogenous administration of ADAMTS1 has previously been demonstrated to inhibit endothelial cell proliferation and angiogenesis. In our study, we found that the knockdown of endogenous endothelial cell production of ADAMTS1 resulted in increased endothelial cell proliferation, further supporting a role for this molecule in regulating endothelial cells. Therefore, ADAMTS1 may represent another molecule used by endothelial cells for self-regulation to help prevent uncontrolled endothelial cell activation, thereby stabilizing and controlling the process of angiogenesis. It will be of great interest in future studies to determine the effect of ADAMTS1 on retinal neovascularization with the use of either ADAMTS1 null mice or siRNA-mediated knockdown of ADAMTS1.

In summary, we have demonstrated the upregulation of ADAMTS1 in the murine oxygen-induced retinopathy model and the induction of ADAMTS1 by VEGF in endothelial cells and have revealed an important role of the PKC signaling pathway in VEGF-induced ADAMTS1 expression. As a negative
feedback regulator of endothelial cells and angiogenesis, ADAMTS1 could represent a molecular target for therapeutic manipulation of vasculopathic disease states.

References


