Facio-Genital Dysplasia-5 Regulates Matrix Adhesion and Survival of Human Endothelial Cells

Maryam Nakhaei-Nejad,* George Haddad,* Qiu-Xia Zhang, Allan G. Murray

Objective—The function of the endothelial cell (EC)-enriched Rho family guanine nucleotide exchange factor, facio-genital dysplasia-5 (FGD5), is poorly understood. We sought to determine whether FGD5 regulates endothelial cytoskeletal reorganization and angiogenesis.

Methods and Results—We observed that FGD5 is expressed in primary human EC isolated from sites across the vasculature. Inhibition of FGD5 expression using RNA interference decreased the protein by ≈70%. In 3-dimensional vascular endothelial growth factor–stimulated angiogenesis in vitro, FGD5-deficient endothelial sprout protrusion was markedly blunted versus nonsilenced controls. FGD5 knockdown impaired adhesion to fibronectin and collagen IV and remodeling of matrix adhesion complexes. Similarly, monolayer electric impedance was decreased, and impedance increased at a slower rate after seeding FGD5-deficient cells versus controls, reflecting decreased EC spreading. Further, FGD5 plays a role in cell survival, because expression of cleaved caspase-3 was increased in FGD5-deficient EC after loss of cell–matrix contacts, and proapoptotic tumor necrosis factor-α stimulation elicited EC with subdiploid DNA content among FGD5-deficient EC. Mechanistically, the phosphatidylinositol 3-kinase/Akt pathway that regulates both adhesive and survival signal transduction pathways requires FGD5. Vascular endothelial growth factor–stimulated Akt phosphorylation and downstream forkhead box protein-O1 inactivation is inhibited by FGD5 loss.

Conclusion—FGD5 regulates endothelial adhesion, survival, and angiogenesis by modulating phosphatidylinositol 3-kinase signaling. (Arterioscler Thromb Vasc Biol. 2012;32:2694-2701.)

Key Words: vascular endothelium ■ neovascularization ■ cytoskeleton ■ phosphatidylinositol 3-kinase ■ vascular endothelial growth factor

Gene expression profiling studies have identified a series of genes characteristic to endothelial lineage. Although the function of many of these genes is known, several novel molecules have not been well studied. Among these is facio-genital dysplasia-5 (FGD5), a member of a subfamily of Rho GTP-GDP exchange factors.1–3 FGD5 expression is conserved from fish to mammals. In zebra fish, FGD5 is expressed throughout vascular development in the embryo and angiogenesis during tissue repair in adults.5 The effect on the endothelium is mediated through activation of 3 VEGF receptors and 2 coreceptors to elicit specific responses. However, signaling through vascular endothelial growth factor receptor-2 (VEGF-R2) is the dominant pathway to induce proliferation, survival, and angiogenesis. In the embryo, deficiency of VEGF or the VEGF-R2–mediated signals results in failure of the embryo to develop.7,8 New vessel growth in response to a developing tumor, ischemic tissue, or an inflammatory focus is dependent on VEGF signaling.9,10 Indeed, in the adult VEGF-R2 signal transduction is required even for maintenance of EC viability in the established vasculature.11 Thus, VEGF signaling is central to vascular homeostasis.

In response to VEGF, quiescent vascular ECs undergo a phenotypic change12 driven by VEGF-R2 signaling and modulated by VEGF-R1 and VEGF-R3 initiation of a specialized protrusive tip cell.13–15 Structural changes to the cytoskeleton include disassembly of interendothelial junctions to initiate capillary sprout formation.16 Polarization in response to matrix-bound VEGF17 involves remodeling of the cell cytoskeleton and endothelial matrix adhesive contacts as tip EC protrude into the surrounding matrix.18–20 Invasion of the extracellular matrix and subsequent cell remodeling to form tubes is regulated by Rho family GTP–binding proteins in the EC.21,22
VEGF-R2 is coupled to several key signal transduction pathways required for growth and survival. Regulated activity of phosphatidylinositol 3-kinase (PI3 kinase) converts PtdIns(4,5)P2 to PtdIns(3,4,5)P3-enriched membrane microdomains that support recruitment and assembly of downstream effector molecules, such as Akt, that mediate these events.20,21 ECs express 3 class I PI3 kinase isoforms, α, β, and γ, that associate with a regulatory subunit (eg, p85α). After VEGF stimulation, PI3 kinase-α activity accounts for most PI3P generation to in turn regulate Akt.22 Loss of endothelial PI3 kinase-α is associated with defective EC motility and early embryonic death from a malformed vasculature.26

We describe a critical role for FGD5 in the endothelial cell response to VEGF stimulation. FGD5 is broadly expressed among primary human EC lines in culture. Deficient expression of FGD5 markedly impairs angiogenic sprout formation in vitro. FGD5-deficient EC fail to migrate. Moreover, the cells are susceptible to proapoptotic stimuli. This is associated with impaired VEGF-stimulated activation of Akt and impaired inhibition of downstream forhead box protein O–dependent expression of proapoptotic molecules.

Materials and Methods

Reagents

M199, HBSS, FBS, and ECGS were from Invitrogen (Burlington, ON). Human tumor necrosis factor-α (TNF) was from Cedarlane Laboratories (Mississauga, ON). Cycloheximide and propidium iodide were purchased from Sigma-Aldrich (St. Louis, MO). VEGF-A was from R&D Systems (Minneapolis, MN). Anti-phospho-AKT (T454), anti-phospho-FOXO1 (S256), and anti-FOXO1 antibodies were from Cell Signaling Technology (Danvers, MA). Anti-tubulin-α was from Millipore Corporation (Temecula, CA). Anti-FGD5 and anti-Akt were from Protein Tech (Chicago, IL). Fluorophore-conjugated secondary antibodies were from Jackson Immunoresearch (West Grove, PA). Hiperfect, nonsilencing short interfering RNA (siRNA) and coding sequence FGD5 siRNA (TTGGATGACATGGACCATGAA; cat no.: S1386880) were from Qiagen Inc (Mississauga, ON, Canada). The siRNA against the 3 untranslated regions of FGD5 is from Thermo Scientific (cat no. L-028077-01-0005; Lafayette, CO).

Cell Culture

Human umbilical vein ECs were isolated and cultured, as described previously.22 Umbilical vein ECs under passage 6 were used for experiments. Human glomerular endothelial cell and aortic ECs were purchased from Angio-Proteomie (Boston, MA) and cultured as described previously.23 To detect VEGF-induced signals, transfected cells were starved overnight with M199 containing 1% FBS. Cells were washed with M199 and briefly incubated in M199 without FBS before stimulation. M199 containing vehicle control or 20 ng/mL TNF was added to cells as indicated.

Human endothelial progenitor cells (hEPCs) were isolated from peripheral blood as described.24,25 Briefly, monoclonal blood cells were isolated by density separation over a Ficoll gradient and CD34+ cells were isolated using magnetic beads coated with antibody (StemCell Technologies, Vancouver, BC). The late outgrowth endothelial colony-forming cell clones were monitored for expression of endothelial marker gene products using quantitative real-time polymerase chain reaction (RT-PCR), flow cytometry, or Western blot.

RNA Interference

To inhibit FGD5 expression, EC were seeded at 50% confluency and transfected twice, on consecutive days, with either 50 nmol/L nonsilencing (siNS) or 50 nmol/L FGD5-silencing (siFGD5) siRNAs using Hiperfect according to the manufacturer’s direction. FGD5 expression was optimally inhibited 72 hours after the first transfection. FGD5 inhibition was monitored by Western blotting and TaqMan RT-PCR.

RT-PCR

RNA was isolated by the RNAeasy kit (Qiagen). Five hundred nanograms of total RNA were reverse transcribed into cDNA using the qScript synthesis kit (Quanta). The FGD5 primers and probe for TaqMan qRT-PCR were from Applied Biosystems (cat no.: Hs00537299_m1; Foster City, CA).

RT-PCR was performed using a Fast 7500 thermocycler (Applied Biosystems). Negative controls included template without reverse transcriptase. Fold change in mRNA expression was calculated by the comparative Ct method.

Apoptosis

Apoptosis was measured in cells in resting conditions and in response to apoptotic stimuli. To induce apoptosis, EC monolayers were incubated with cycloheximide (3 μg/mL) and TNF (10 ng/mL) for 3 hours (for caspase-3 detection) or overnight (for subdiploid DNA detection) in reduced serum conditions (1% FBS). Resting or cycloheximide–TNF human umbilical vein ECs were incubated with the FITC-conjugated active caspase-3 reporter, DEVD-FMK, for 30 minutes at 37°C as directed by the manufacturer (Promokine, Heidelberg, Germany). Cells were trypsinized and combined with the floating cells in the medium. To measure apoptosis in suspended cells, cells were trypsinized first and then suspended in complete medium before activated caspase-3 detection. After brief washes, cells were analyzed by flow cytometry.

The fraction of subdiploid DNA content in cells was measured using propidium iodide. Briefly, trypsinized adherent cells and the floating cells were pooled and fixed in 70% ethanol. ECs were resuspended in 500 μL PBS and an equal volume of 0.2 mol/L NaHPO4 (pH 7.8)+0.005% Triton X-100, then stained with 40 μg/mL propidium iodide+1 mg ribonuclease, and analyzed by flow cytometry.

Flow Cytometry

Flow cytometry was performed, as described earlier.21 Briefly, ECs were harvested into suspension using nonenzymatic dissociation solution (Sigma), then treated with primary antibody for 60 minutes at 3°C, washed, and stained with FITC-conjugated 2′-acid antibody.

Western Blot

EC monolayers were washed once with ice cold PBS and then lysed immediately in hot 2x loading buffer (60 mmol/L Tris, pH 6.8, 25% glycerol, 2% SDS, 15 mmol/L 2-mercaptoethanol and 0.1% bromophenol blue) followed by boiling at 95°C for 7 minutes. Lysates were resolved on SDS-PAGE and then transferred onto nitrocellulose (Biorad) as described.25 The membranes were immunoblotted for phosphorylated proteins overnight at 4°C in 5% BSA/TBS-Tween20 blocking solution and then proteins were visualized using ECL (GE Life Sciences, Baie d’Urfe, Canada). The membranes were stripped using Restore buffer (Thermo Scientific, Rockford, IL) and reprobed for the total protein.

Angiogenesis

Vasculogenesis in vivo was evaluated as described.26 Briefly, EC derived from hEPCs transfected with siNS or FGD5-silencing (siFGD5) siRNAs were mixed with human aortic smooth muscle cells (American Type Culture Collection) in growth factor–depleted Matrigel, then injected subcutaneously into Rag2−/− mice (Taconic). The gels were harvested 1 week later, immersed in Zn-fixative, mounted in OCT, and then sectioned. Gel sections were then immunostained for hCD31 (mAb IC70; Dako) or mCD31 (mAb MEIC13.3; BD Biosciences) and photographed using a CCD camera (Nikon). Sections were examined qualitatively for hCD31-stained capillaries with patent lumen.
Angiogenesis was quantitated in vitro as described earlier with minor modifications. EC derived from hEPCs were transfected with siNS or FGDS-silencing (siFGD5) siRNAs, stained with CellTracker Green (Life Technologies), then were loaded onto Cytodex beads (~400 cells/bead) and cultured for 2 hours. The beads were resuspended in fibrinogen (2 mg/mL solution containing aprotonin (0.15 U/mL), and 0.625 U/mL thrombin was added and then cultured with 20% FBS to facilitate fibronectin incorporation into the gel. Images of the beads after 18 hours culture in EBM-2/EGM-2 Bulletkit (Lonza) without fibroblasts or smooth muscle cells were captured using a 20x objective and a CCD camera-equipped inverted microscope (Leica, Concord, ON, Canada). The number and length of sprouts was analyzed using image analysis software (OpenLab, Lexington, MA). Sprout length was grouped into tertiles established from mock-transfected hEPC (<75, 76–125, >126 μm).

Statistics
Data are shown as mean±SEM. Statistical analysis was performed using 1-way or 2-way ANOVA as appropriate. Pairwise comparisons were done by paired Student t test using Prism 5 (Graphpad, San Diego, CA). P values <0.05 were considered significant.

Results
We analyzed FGD5 expression in human primary EC originating from 4 different sites: arterial, microvascular, umbilical vein (human umbilical vein ECs), and circulating late outgrowth hEPC. As shown in Figure 1, we found robust expression of FGD5 mRNA and protein in the primary human ECs isolated from each site in the vasculature. Similarly, we identified FGD5 expression in late outgrowth ECs derived from CD34+ circulating progenitor cells. Treatment of EC with either an siRNA directed to the coding sequence or a pool of siRNAs against the 3′ untranslated region resulted in marked loss of FGD5 protein. Quantitative RT-PCR had FGD5 mRNA reduced by 69±3% or 80%±3% (mean±SEM; P<0.05; n=4 experiments) versus nonsilencing control siRNA using the coding or 3′ untranslated region siRNAs, respectively.

To examine the role of FGD5 in angiogenesis, we tested the effect of transfection of hEPC with nonsilencing or FGDS-specific siRNA on microvascular assembly in vivo. EC derived from hEPC formed hCD31-positive tube structures in Matrigel in the presence of human aortic smooth muscle cells. Similarly, nonsilencing siRNA-treated hEPC formed human CD31-positive capillary structures (Figure 2A) but only rare tubes were identified among FGD5-deficient hEPC preparations. Overall, fewer human CD31-positive cells were noted in the FGD5-deficient gels, but little difference was observed in mouse CD31-positive microvessel ingrowth into the Matrigel plugs.
To quantitate the effect of FGD5 deficiency on VEGF-stimulated angiogenesis, we examined sprout outgrowth into a fibrin/fibronectin gel in vitro. As shown in Figure 2B and 2C, FGD5 deficiency impaired the number of sprouts extending from hEPC-coated beads at 18 hours and decreased the length of the sprouts (Figure 2D). Longer culture of the FGD5-deficient EC in the absence of fibroblasts or human smooth muscle cell resulted in the loss of the nascent sprouts and apparent death of the FGD5-deficient EC.

Angiogenic sprout extension is generated by dynamic protrusive contacts into the matrix. We hypothesized that FGD5 deficiency disrupted endothelial tip cell reorganization of the cytoskeleton and matrix adhesion complexes. To assess cell–matrix interaction, we evaluated the effect of FGD5 deficiency on EC adhesion to matrix components. EC deficient in FGD5 adhered poorly to matrix when replated, with 64±4% of FGD5-silenced cells versus 90±4% nonsilenced control cells adherent at 2 hours to the αvβ3 and α5β1 integrin ligand, fibronectin. Similarly, FGD5 loss impaired adhesion by endothelial αvβ1 and α2β1 integrins to collagen IV (Figure 3A). As shown in Figure 3B, EC spreading on fibronectin was similarly impaired, reflected by a decreased rate of increase in electric impedance among FGD5-deficient versus control EC monolayers. Adherent ECs were fixed, and immunostained for vinculin after culture for 24 hours to evaluate the formation of focal adhesions (Figure 3C). These appeared grossly normal, suggesting a defect in dynamic remodeling of matrix adhesion complexes. These observations indicate that FGD5 is involved in the regulation of EC cytoskeletal remodeling. However, in long-term culture the most striking abnormality was a decrease in cell number and an increase in nonadherent cells.

We hypothesized that FGD5 loss sensitized EC to apoptosis. ECs were transfected with FGD5 or control siRNA, cultured in complete media with growth factors, and then the EC cultures were assayed for the frequency of cells with subdiploid DNA content using flow cytometry. We found no difference between the control and FGD5-silenced EC under these optimal growth conditions. Next, we deprived the cultures of growth factors overnight and found a small increase in the frequency of subdiploid cells among EC deficient in FGD5 (Figure 4A). Strikingly, we observed a marked increase in subdiploid EC after treatment with the extrinsic pathway proapoptotic stimulus, TNF and cycloheximide, after FGD5 knockdown (Figure 4A). The ECs were similarly sensitive to apoptosis if the αvβ3 integrin complex was engaged by the fibronectin matrix or endothelial α1β1 or α2β1 integrins bound to collagen IV.

As an additional measure of apoptosis, we evaluated activated, cleaved caspase-3 expression in FGD5-deficient EC. Under suboptimal growth conditions, we found an increase in the fraction of adherent FGD5-deficient EC that express activated caspase-3 versus control monolayers (Figure 4B). Consistent with the analysis of DNA content, FGD5-deficient

![Figure 3.](image)

**Figure 3.** Facio-genital dysplasia-5 (FGD5) deficiency impairs endothelial–matrix interactions. Control short interfering RNA (siRNA)-transfected human umbilical vein endothelial cells (HUVEC; filled bar) or FGD5-silencing (siFGD5) siRNAs-HUVEC (open bar) were suspended using nonenzymatic cell dissociation solution and then replated on a fibronectin or collagen IV matrix. A. Attached EC were counted at 2 hours and expressed as a fraction of the total cells plated. B, Control or FGD5-deficient HUVEC were plated on fibronectin-coated electrodes, and the rate of change of impedance was measured over 2 hours. n=4 independent experiments; *P<0.05 by ANOVA. C, Control (left) or FGD5-deficient EC (right) were plated on a fibronectin matrix and stained for the formation of vinculin-rich focal adhesions.

![Figure 4.](image)

**Figure 4.** Facio-genital dysplasia-5 (FGD5) deficiency sensitizes EC to apoptosis. A, Human umbilical vein endothelial cells (HUVEC) monolayers transfected with control short interfering RNA (siRNA) or FGD5-silencing (siFGD5) siRNAs were cultured on fibronectin (Fn) or collagen IV (Col IV) matrices in low serum medium without growth factors for 18 hours, then the fraction of EC with subdiploid DNA content was quantitated by flow cytometry as described in the Materials and Methods section. Where indicated, EC were challenged with cycloheximide+ tumor necrosis factor-α (TNF). n=3 to 8 independent experiments; *P<0.05 by ANOVA. B, Control siRNA- or siFGD5-transfected HUVEC untreated monolayers, monolayers treated with cycloheximide+TNF, or HUVEC in suspension for 3 hours were stained for caspase-3 activity and quantitated by flow cytometry. n=4 independent experiments; *P<0.05 by ANOVA. CHX indicates cycloheximide.
EC monolayers were more susceptible to activated caspase-3 expression induced by TNF/cycloheximide treatment compared with the control monolayers.

Next, we tested the effect of loss of matrix contacts on EC apoptosis in FGD5-deficient versus control EC. ECs were placed in suspension for 30 minutes in the absence of growth factor and then evaluated for caspase-3 activity. As shown in Figure 4B, a higher fraction of cells with activated caspase-3 was found in FGD5 knockdown EC versus control EC. Taken together these observations suggest that FGD5 regulates signaling events opposing apoptosis in the endothelium.

The PI3 kinase–Akt serine/threonine kinase pathway has been implicated to regulate both cytoskeletal dynamics and cell survival and hence may be modulated by FGD5 knockdown in EC. To investigate this hypothesis, we examined the PI3 kinase–regulated Akt kinase activity downstream of VEGF stimulation in FGD5-deficient and control EC. We observed that FGD5 deficiency markedly attenuated VEGF-stimulated Akt phosphorylation but did not change total Akt in EC (Figure 5A). Similarly, forhead box protein–O1, a downstream substrate of Akt that regulates expression of several apoptosis-related genes, was poorly phosphorylated after VEGF stimulation of FGD5-deficient EC (Figure 5B). We detect no decrease in surface expression of the principal VEGF receptor, VEGF-R2, among FGD5-deficient EC (Figure 5C). Further, in contrast to the blunted Akt phosphorylation after VEGF stimulation, we observed robust mitogen-activated protein kinase activity reflected by extracellular signal–regulated kinase-1/2 phosphorylation in VEGF-stimulated FGD5-deficient EC (Figure 5D). These results suggest VEGF-R2 is expressed on the EC surface and is competent to respond to VEGF stimulation but fails to elicit Akt activation in the absence of FGD5.

To determine whether FGD5 is involved in the regulation of Akt after stimulation from other growth factors, we evaluated the effect of FGD5 deficiency on insulin receptor signaling. As shown in Figure 6, we observe inhibition of Akt phosphorylation after insulin stimulation of EC monolayers treated with either the coding- or 3′ untranslated region–targeted siRNAs. This implicates FGD5 in the regulation of diverse receptor tyrosine kinase–mediated recruitment of this survival pathway in EC.

Discussion

This article describes the function of the endothelial-specific gene, FGD5. We identified expression of FGD5 in EC isolated from diverse sites in the vasculature. In contrast, we did not identify FGD5 expression in lines derived from epithelial cells or fibroblasts. We demonstrated that loss of FGD5 function in human primary ECs impairs VEGF-stimulated angiogenic sprout formation in the robust in vitro sprout outgrowth model, which correlates with altered cell–matrix interaction and increased sensitivity to proapoptotic conditions. Mechanistically, these events are
associated with impaired PI3 kinase–mediated Akt activation among FGD5-deficient EC, coupled to signaling from endothelial receptor tyrosine kinases, such as VEGF and insulin receptors.

Angiogenesis and vascular integrity in the embryo and the adult are critically dependent on VEGF stimulation of VEGF-R2 on the EC. Loss of VEGF signaling in the mouse embryo results in failure to develop new vessels, and regression of established vessels. In the adult, isolated loss of autocrine VEGF signaling in the mature vasculature results in EC apoptosis and thrombotic microangiopathy. Interruption of VEGF signaling is also associated with catastrophic microvascular thrombosis in humans and has been exploited as adjuvant therapy in cancer treatment regimes.

Three endothelial receptors for VEGF have been characterized and all appear to participate in angiogenesis and recruit signal transduction pathways. For example, genetic ablation of VEGF-R2, mutation of cytoplasmic residues in VEGF-R2, and VEGF-R2 small molecule inhibitors have all been used to explore a variety of signal transduction pathways coupled to this receptor. These include p38SAPK, Src, phospholipase C-γ1, and PI3 kinase–dependent signals. The current results describe a distinct defect in VEGF receptor signal transduction, involving the PI3 kinase to Akt pathway, but relatively sparing mitogen-activated protein kinase activation that occurs after loss of FGD5. We link this defect in VEGF-stimulated signaling to increased susceptibility to proapoptotic stress, defective regulation of cytoskeleton-associated structures, and ultimately to impaired angiogenic sprout formation.

Early work identified the PI3 kinase/Akt pathway as a critical mediator of VEGF-mediated signals to promote angiogenesis in the aortic ring model. Indeed, modulation of this pathway by manipulation of the activity of the specific phosphatase, phosphatase and tensin homolog, confirmed that the pathway is recruited in formation of new vascular sprouts. In the developing embryo both the PLCγ1 and the PI3 kinase pathways are critical for vasculogenesis and viability of the embryo. Moreover, the regulation of vascular development appears to involve complex cross talk among the PI3 kinase and other signal transduction pathways recruited after VEGF stimulation.

Interestingly, ablation of the principal Akt isoform in the endothelium is not lethal, and the vasculature develops more or less normally but has a maladaptive stress response. However, the Akt kinase acting to phosphorylate and silence forkhead box protein–O1 transcriptional activity is known to be critical to vascular development and repair. Thus, FGD5 is a newly identified component of this key signal transduction pathway linked to defective blood vessel growth, early embryonic death, and impaired repair responses. The restricted expression of this molecule suggests it may support a unique function of the endothelium. However, FGD5 appears to be coupled to both endothelial-specific and ubiquitous growth factor receptors because endothelial Akt activity is impaired after either VEGF or insulin stimulation. This highlights a central role this molecule may play in endothelial cell biology.

The mechanism through which FGD5 contributes to coupling the PI3 kinase–Akt pathway to receptor tyrosine kinases in the EC is unclear. FGD5 has a consensus Rho guanine nucleotide exchange factor domain and similar to other subfamily members has been shown to mediate GDP to GTP exchange on Cdc42. The Rho GTP-binding protein, RhoB has been identified as a regulator of the Akt signal transduction pathway. However, in contrast to FGD5, loss of RhoB function, by either inhibition of farnesyl transferase or knockdown of the protein, caused loss of total Akt that was reversed by proteosome inhibition. FGD5 deficiency abrogated activating Akt phosphorylation but did not cause loss of total Akt. The Rho family proteins Rac1 and Cdc42 have both been implicated in regulating docking between PI3 kinase subunits and receptor tyrosine kinases. However, Rho GTPases may also regulate association of the phosphatidylinositol-3-phosphatase, phosphatase and tensin homolog, to the p85 complex. Further, defective phosphorylation of Akt S473 after FGD5 loss does not exclude interactions with undefined components of the mammalian target of rapamycin complex 2 pathway that ultimately phosphorylates Akt S473.

Recently, Kurogane et al published findings indicating little apoptosis among EC rendered deficient in FGD5 expression. Our findings agree that under optimal growth conditions, there is no increase in EC apoptosis. However, as we illustrate in Figure 4, FGD5-deficient ECs are more sensitive to proapoptotic stresses. Although, in part, this sensitivity is attributable to immediate effects of inhibition of Akt-dependent phosphorylation of propapoptotic Bcl-2 family members, an additional effect of FGD5 deficiency on the apoptosis machinery is mediated by disordered forkhead box protein O–regulated gene transcription, downstream of Akt. This additional time-dependent effect may contribute to our observations evident 2 days after FGD5 siRNA transfection versus more immediate studies reported by Kurogane. Conversely, Kurogane emphasized a decrease in extracellular signal–regulated kinase–driven proliferation in FGD5-deficient cells. Although we detect a trend to decreased extracellular signal–regulated kinase-1/2 phosphorylation at 5 minutes after stimulation, the difference in 2- to 3-fold induction above unstimulated ECs versus irrelevant siRNA controls was not sustained later in the time course and was not statistically significant.

In summary, the endothelial-restricted molecule, FGD5, is required for optimal angiogenesis and defense against proapoptotic stresses in the endothelium. We identify a critical permissive role of the molecule in recruitment of Akt signaling after receptor tyrosine kinase stimulation in EC. Hence, FGD5 may be a suitable molecule to target in antiangiogenesis treatments and deficiency may underlie some cases of atypical thrombotic microangiopathy associated with endothelial cell injury.

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Disclosures
None.

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Figure I

EPC NT

siRNA C

FGD5
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