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Perlecan Domain V Inhibits Amyloid- β Induced Brain Endothelial Cell Toxicity and Restores Angiogenic Function

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Abstract

In Alzheimer's disease (AD), amyloid- β (A β) deposits in the cerebrovasculature can result in neurovascular dysfunction and/or cerebral amyloid angiopathy. The accumulation of A β in blood vessels can cause endothelial cell damage, resulting in impaired A β clearance by the blood-brain barrier. Additionally, impaired endothelial cell function can result in decreased angiogenesis in the brains of AD patients, affecting cognitive function. VEGF is a crucial mediator of angiogenesis and is deficient in AD brains thus promoting angiogenesis could be an important component of successful AD treatment. The C-terminal portion of the extracellular matrix proteoglycan perlecan, Domain V (DV), promotes brain-derived endothelial cell proliferation and is proangiogenic in that it increases VEGFR2 expression and production of VEGF. In this study, we show that A β_{25-35} reduces proliferation of a mouse brain microvascular endothelial cell line (MBEC) *in vitro* and that DV and mouse LG3 (C-terminal fragment of DV) block these effects of A β_{25-35} . Additionally, we show that DV restores the ability of MBECs to form tube-like structures on Matrigel in the presence of A β_{25-35} and that this is $\alpha 5\beta 1$ dependent. Interestingly, the reduction in tube-like structure formation by A β_{25-35} was not due to endothelial cell death, suggesting that A β_{25-35} induces the downregulation of a cell surface molecule required for adhesion events critical to the angiogenic process. We propose a model suggesting that DV works through both the $\alpha 5\beta 1$ integrin receptor and VEGFR2 to increase VEGF production, causing competition with A β_{25-35} for VEGFR2 binding, thus ultimately increasing VEGF expression and restoring angiogenesis. This supports DV as a potential anti-amyloid therapy.

Keywords

Alzheimer's disease; amyloid- β_{25-35} ; blood-brain barrier; extracellular matrix; integrins; perlecan

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INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia among the elderly with symptoms including confusion, progressive loss of memory, and depression [1]. While the neuropathological hallmarks of AD include extracellular deposition of amyloid- β ($A\beta$) in the cortex and hippocampus and the intraneuronal accumulation of neurofibrillary tangles, the deposition of $A\beta$ on cerebral blood vessels is also a prominent component in AD [2], resulting in neurovascular dysfunction and/or cerebral amyloid angiopathy [3]. A link between AD and vascular disease has been suggested by many studies showing that hypertension, atherosclerosis, stroke, diabetes mellitus, smoking, and high cholesterol are common risk factors [4, 5]. Thus, neurovascular dysfunction is likely to be an important component of dementia and AD.

While increases in $A\beta$ production can contribute to AD pathogenesis, AD has been proposed to be a "retention" disease caused by inefficient clearance of $A\beta$ from the brain by the blood-brain barrier (BBB) [3]. In healthy individuals, $A\beta$ is cleared from the brain by transport across the BBB via receptor-mediated transcytosis. If $A\beta$ clearance pathways are disrupted, soluble $A\beta$ can accumulate [6] and promote the formation of neurotoxic $A\beta$ forms, resulting in neuronal and endothelial cell damage and death [2]. In traumatic brain injury, a known predisposing factor for AD [7], disruption of the BBB is thought to result in the higher risk of aberrant $A\beta$ accumulation [8]. Therefore, functional endothelial cells are critical to maintaining BBB integrity and regulating the BBB permeability for many solutes, preserving brain homeostasis. Disruption of endothelial integrity enhances $A\beta$ toxicity to endothelial cells, disrupts $A\beta$ clearance from the brain and contributes to the pathogenesis of AD [9]. Additionally, $A\beta$ can bind integrin receptors. Specifically, $A\beta_{1-40}$ binds to cells expressing the $\alpha 2\beta 1$ ($\alpha 2\beta 1$) integrin, resulting in both $A\beta$ deposition and initialization of a neurotoxic signaling cascade [10]. Furthermore, the $\alpha 5\beta 1$ ($\alpha 5\beta 1$) integrin can mediate cell adhesion to $A\beta_{1-40}$ and promote cellular internalization and degradation of $A\beta$, resulting in an increase in the clearance of $A\beta$, a reduction in the formation of an insoluble $A\beta$ matrix, and a decrease of $A\beta$ toxicity to cells [11].

The AD affected brain has an increase in proangiogenic factors such as vascular endothelial growth factor (VEGF) [12]. This suggests that possible compensatory mechanisms exist to counteract damaged vasculature in the brain. However, the low density of blood vessels seen in AD suggests that this reparative angiogenic mechanism fails [13]. $A\beta$ has been shown to be anti-angiogenic *in vitro* and *in vivo* and thus its presence in the AD brain likely contributes to the lack of new blood vessel formation [13]. $A\beta$ also binds growth factor receptors such as VEGFR2 thus blocking the angiogenic activity of VEGF on endothelial cells [14]. Additionally, VEGF has been shown to co-aggregate with $A\beta$ in plaques seen in the AD brain [15], decreasing available VEGF to promote angiogenesis in the brain. Therefore, $A\beta$ appears to act as an antagonist for the proangiogenic factors in the brain, opposing their effects on angiogenesis and maintenance of the cerebrovasculature [13]. Therefore, finding a way to re-establish the angiogenic system and promote blood vessel formation could be an important component of successful AD treatment.

The C-terminal portion of the extracellular matrix proteoglycan perlecan, termed Domain V (DV), promotes brain-derived endothelial cell proliferation and is proangiogenic in the brain in rodent stroke models [16]. It increases the production and release of VEGF in brain endothelial cells via the $\alpha 5\beta 1$ integrin and is neuroprotective [16]. DV consists of three laminin-globular-like repeats (LG domains) separated by two type-1 epidermal growth factor-like repeats. The C-terminal LG domain, LG3, has been shown to have many of the functional activities of full-length DV [17]. LG3 is physically smaller than DV (26 kDa versus 85 kDa, respectively) and thus, may be more tractable as a therapeutic molecule as it is easier to produce and less susceptible to degradation.

$\alpha 5\beta 1$ integrin is expressed in many cell types, including brain microvascular endothelial cells, the latter during brain development and in circumstances such as brain injury, where it helps promote angio-genesis and revascularization [18]. DV promotes angiogenesis through the $\alpha 5\beta 1$ integrin in brain microvascular endothelial cells and generally increases endothelial cell health and function via expression and secretion of VEGF [19]. We hypothesized that DV and LG3 could decrease A β -induced endothelial cell death and prevent its antiangiogenic properties. In this study, we show that human DV and LG3 rescue mouse brain endothelial cells from A β -induced cytotoxicity *in vitro* and that DV restores A β -disrupted angiogenesis in an $\alpha 5\beta 1$ integrin dependent manner. Additionally, we demonstrate that both human and mouse LG3 are active in mouse brain endothelial cells.

MATERIALS AND METHODS

Cell culture

In vitro experiments were performed using the mouse cerebrovascular endothelial cell line, MBEC. The MBEC cells were kindly provided by Dr. Jane Welsh (Texas A&M University) and were isolated from the C57BL/6 strain of mice [16, 20]. The cells (passage 11–17) were grown in Iscove's Modified Dulbecco's Medium (IMDM, Invitrogen, CA), 10% FBS, 1% antibiotic/antimycotic (Cellgro), and 1% L-glutamine (Cellgro) on flasks coated with porcine-derived gelatin (Sigma Aldrich).

$\alpha 5$ integrin knockdown

Cells were prepared as described in Lee et al. [16]. Briefly, MBECs were treated with $\alpha 5$ siRNA (Mission siRNA, Sigma-Aldrich) containing media with Lipofectamine 2000 (Invitrogen) and selected for resistance to puromycin to develop a stable cell line. $\alpha 5$ knockdown (75%) was previously confirmed by $\alpha 5$ quantitative PCR and by $\alpha 5$ western blot analysis [16]. Cells were grown on flasks coated with porcine-derived gelatin (Sigma Aldrich) in the media described for parental MBEC supplemented with 2 μ g/ml puromycin.

DV and LG3 protein production

Human DV (hDV) and human LG3 (hLG3) were produced and purified from 293-EBNA cells as previously published [16, 21]. In some experiments, hDV was heat inactivated for 15 min at 90°C. Mouse LG3 (mLG3) was cloned with a 3' Histidine tag into the vector pCep-pu (provided by Maurizio Mongiat, Center for Cancer Research, Aviano, Italy) using the following primers: Mouse LG3 5' Nhe I #2 5' -

AGGCTAGCGTATGGGGTAGTGGAGTCAGACTG-GCAC-3' and Mouse LG3 3' Xho I #2 5'-AGCTCGAGCATGATGATGATGATGATGTGAGG-GGCAGGGCCGTGTGTTGG-3'. Mouse LG3 was amplified from full-length mouse DV using Pfx50 (Invitrogen, Grand Island, NY). 293-EBNA cells were transfected via Lipofectamine 2000 (Invitrogen) and stable cells were selected by growing the transfected cells in basal medium supplemented with 0.05 μ g puromycin. After transfection, conditioned media from the transfected 293 EBNA cells was collected and mLG3 was purified using Ni-NTA agarose beads (Qiagen, Valencia, CA). Eluted fractions containing mLG3 were dialyzed against PBS and the purity was confirmed via SDS-PAGE stained with Brilliant Blue G Colloidal (Sigma, St. Louis, MO) and western blot using an anti-6X His antibody (Genetex, Irvine, CA). Human DV, hLG3, and mLG3 were used at 300 nM unless otherwise indicated.

A β ₂₅₋₃₅ preparation

Human A β ₂₅₋₃₅ and the reverse peptide A β ₃₅₋₂₅ were purchased from California Peptide Research, Inc. (Napa, CA) and were resuspended in sterile distilled H₂O (Invitrogen, Carlsbad, CA) to a final concentration of 1 mM. They were incubated for 3–4 days at 37°C to allow the peptide to aggregate to its toxic form. Aggregated A β was stored at –80°C.

Capillary tube-like structure formation on a Matrigel matrix (in vitro angiogenesis assay)

MBECs in IMDM/1% FBS (1×10^5 per well) were treated in suspension for 20 min at 37°C/5% CO₂ with hDV (300 nM), A β ₃₅₋₂₅ (25 μ M) alone (reverse peptide), A β ₂₅₋₃₅ (25 μ M) alone, A β ₂₅₋₃₅ plus hDV or IMDM/1% medium alone (control) and then added to Matrigel (Becton Dickinson, Franklin Lakes, NJ) coated wells for 3–6 h also at 37°C/5% CO₂. The assay was stopped with 4% paraformaldehyde. Adobe Photoshop CS 5.0 for Apple Macintosh was used to quantify pictures (three randomly selected microscopic fields per well) for tube formation and calculating the average tube pixels per high power field as previously published [16]. Results were expressed as a percentage of the control (arbitrarily set to 100%) with error bars representing standard deviation. After the assay was stopped, non-adherent cells in the media were harvested via centrifugation and quantified (percent difference from control) with a hemocytometer using trypan blue stain to distinguish live from dead cells.

Proliferation assay

MBECs were plated in 96-well tissue culture treated plates (1500 cells per well, triplicate wells) in IMDM/1% FBS immediately after being exposed to hDV, hLG3, or mLG3 with or without A β ₂₅₋₃₅ (25 μ M) or media alone (control). Additionally, the cells were tested for their response to an A β ₂₅₋₃₅ titration at 10, 25, or 50 μ M. On day 3, the cells were treated with alamar blue (AbD Serotec, Raleigh, NC) for 2–6 h followed by measurement with a fluorescent plate reader, excitation at 560 nm and emission at 590 nm. The results were expressed as percent change from the absorbance of the control (arbitrarily set at 100%).

Statistical analysis

All experiments were repeated three independent times. The proliferation assay was performed in triplicate per condition and the Matrigel experiments were performed in duplicate per repeat. Individual experimental values (from repeated measures) were averaged and used in subsequent statistical analyses. Statistical significance ($p < 0.05$) was determined by Student's *t* test. Error bars represent standard deviation unless otherwise stated.

RESULTS

hDV and LG3 promote proliferation and overcome A β toxicity in mouse brain endothelial cells

Cell proliferation is a required step during angiogenesis and DV has previously been reported to increase proliferation in MBECs [16]. However, LG3 has not been evaluated for this ability in these cells. Therefore, we tested the ability of hDV, hLG3, and mLG3 to promote proliferation of MBECs (Fig. 1A). Cells were plated immediately after being exposed to the test conditions and incubated in the wells for 72 h prior to alamar blue analysis. hDV, but not heat inactivated hDV, promoted proliferation of MBECs. hLG3 significantly increased MBEC proliferation at 600 nM and 900 nM but not at the lower concentration of 300 nM (Fig. 1A). Likewise, mLG3 was also unable to significantly increase proliferation in the MBECs when at the lower concentration of 300 nM.

We next tested the capability of hDV, hLG3, and mLG3 to rescue A β -mediated endothelial toxicity. MBECs were plated in the presence of A $\beta_{25-35} \pm$ DV, hLG3, or mLG3, and the ability of the cells to proliferate was measured as described above. When the cells were treated with A β_{25-35} only, MBECs showed decreased viability in a dose-dependent fashion (Fig. 1B). The reverse peptide A β_{35-25} did not have any measurable effect in this assay. Interestingly, hDV and mLG3 significantly rescued the MBECs from A β_{25-35} toxicity but hLG3 did not.

DV rescues A β_{25-35} -induced inhibition of capillary tube-like formation by MBECs on a Matrigel matrix

We next performed *in vitro* capillary “tubulogenesis” assays to determine if DV was able to rescue MBEC from A β_{25-35} -induced inhibition of the formation of capillary tube-like structure formation on Matrigel (a growth factor enriched substance that promotes and supports such tube-like structure) (Fig. 2). MBECs were seeded on Matrigel and treated with media only, DV alone, A β_{25-35} , DV plus A β_{25-35} , or the reverse peptide A β_{35-25} . We first verified that DV increases the formation of tube-like structures in the MBECs as previously published [16]. A β_{25-35} significantly inhibited tube formation while DV overcame A β_{25-35} inhibitive effects by promoting tube-like formation. The reverse peptide A β_{35-25} had no toxic effects on the cells.

Reduction of capillary tube-like formation by A β_{25-35} is not due to cell death

During progression of the Matrigel assay, we noted that MBEC that were not part of the tube-like structures were floating in the A β treated wells. To investigate if this was due to

cell death, media containing the non-adhered cells was collected after assay progression was stopped and cells were counted in the presence of trypan blue. Interestingly, upon examination of the collected non-adhered cells, less than 1% were non-viable from all of the treatment conditions, including the A β ₂₅₋₃₅ wells. MBEC treated with media only or DV did not have many non-adhered cells and quantitation revealed that more than 90% of the cells seeded were able to form tube-like structures. However, at 25 μ M A β ₂₅₋₃₅, 49% of the original MBECs seeded were alive yet unable to adhere to form tubes (Fig. 3). Moreover when DV was added along with A β ₂₅₋₃₅ at 25 μ M, it overcame A β ₂₅₋₃₅'s inhibition of cell adhesion to the Matrigel matrix, showing a 35% decrease when compared to the 25 μ M A β ₂₅₋₃₅ alone wells.

α 5 β 1 mediates rescue of capillary tube-like formation by DV

As stated earlier, DV induces VEGF secretion via the α 5 β 1 integrin receptor [16]. If DV is mediating rescue of the A β -induced reduction of capillary tube-like formation via the α 5 β 1 integrin in the MBEC, we reasoned that DV should not rescue when the α 5 integrin was knocked down. α 5 integrin- knockdown MBECs (MBEC α 5 KD) that express 25% of normal α 5 β 1 integrin levels [16] were tested to determine if DV could restore their ability to form tube-like structures in the presence of A β ₂₅₋₃₅ in the Matrigel assay (Fig. 4). Surprisingly, DV was able to increase tube-like formation in the MBEC α 5 KD cells by 72% suggesting that less than complete knockdown of α 5 β 1 integrin was insufficient to block DV tube promoting effects but also raises the possibility that other factors additionally contribute in these MBECs. However, in contrast to wild-type MBECs, DV was unable to rescue A β -induced reduction in tube formation.

DISCUSSION

In this study, we demonstrate that A β ₂₅₋₃₅ reduces proliferation of a mouse brain microvascular endothelial cell line *in vitro*. Furthermore, we demonstrate that perlecan DV, the 85 kDa C-terminal protein fragment of perlecan (itself a prominent heparan sulfate proteoglycan component of the vascular basement membrane in the brain and throughout the body), and mLG3 (the C-terminal LG domain of DV) blocks these A β ₂₅₋₃₅ effects on proliferation. Interestingly, hLG3 required a 2-fold higher concentration than DV to increase proliferation of MBECs. Mouse LG3 was more effective than hLG3 in blocking A β ₂₅₋₃₅ proliferation effects on MBECs but not more so in increasing MBEC proliferation by itself without A β ₂₅₋₃₅. Mouse LG3 possesses a single glycosaminoglycan attachment site [22, 23] not present in hLG3, potentially explaining the species specificity of the interaction. Importantly, to the best of our knowledge, this is the first study that has examined the function of mouse recombinant LG3.

In this study, we performed Matrigel assays to determine the ability of DV to overcome the deleterious effects of A β ₂₅₋₃₅ on capillary tube-like formation. The formation of these structures is thought to mimic the process by which endothelial cells form capillaries *in vivo* [24]. We show that A β ₂₅₋₃₅ inhibits mouse brain microvascular endothelial cells from forming a capillary network on Matrigel. It has been reported that A β ₁₋₄₀ has similar effects on human brain-derived endothelial cells [13]. Furthermore, DV is able to restore the ability

of MBECs to form tube-like structures in the presence of A β ₂₅₋₃₅. To our surprise, A β ₂₅₋₃₅ did not reduce these biological functions by inducing cell death as would be expected from a cytotoxic agent. Angiogenesis is a tightly regulated process involving endothelial cell proliferation, migration, attachment, recruitment of supporting cells, and the formation of tubes [25]. Growth factors such as VEGF initiate angiogenesis and growth factor receptors and integrins are crucial for attachment. In our study, there is a clear correlation between the extent of tube formation by MBECs on Matrigel and cell adhesion to the matrix. Additionally, the fact that cells treated with A β ₂₅₋₃₅ were alive but unable to form tubes suggests that A β ₂₅₋₃₅ may cause the downregulation of a cell surface molecule critical for adhesion or modify other events required for cell adhesion. We hypothesize that A β ₂₅₋₃₅, like A β ₁₋₄₀, may bind to the integrins required for the cells to bind to Matrigel such as α 5 β 1 and/or α 2 β 1 and thereby inhibit their adhesion to Matrigel.

When α 5 β 1 integrin expression was reduced by 75% in MBECs, DV was no longer able to restore the reduction in tube-formation caused by A β ₂₅₋₃₅, demonstrating the importance of the α 5 β 1 integrin in mediating this activity of DV. Our lab has previously shown that α 5 integrin knockdown in MBECs impaired their migratory capabilities when DV was the migratory stimulus [19] and that the CRRETAWAC peptide (a peptide specific for the binding pocket of the α 5 β 1 integrin [26]) inhibited DV-induced capillary morphogenesis in brain endothelial cells [16]. However, while blockade of α 5 β 1 integrin could block DV-induced capillary morphogenesis, the present study suggests that incomplete (75%)- α 5 knockdown could not. While this is likely due to the residual α 5 β 1 integrin still present on the endothelial cells in the context of a strong angiogenesis promoting environment (Matrigel is enriched in angiogenesis promoting growth factors), the possibility that other factors could contribute to DV's proangiogenic effect in this assay cannot be excluded and merits further study. Nevertheless, α 5 β 1 clearly plays an important, if not exclusive, role in mediating DV's activity on brain microvascular endothelial cells schematized in Fig. 5.

It is known that A β directly interacts with VEGFR2, blocking the ability of VEGF to bind to VEGFR2 [14], the primary receptor for transducing angiogenic signals, preventing brain microvascular endothelial cells from forming tube-like structures on Matrigel. A β disrupts VEGF/VEGFR2 signaling by antagonizing VEGF-induced Akt phosphorylation and Tyr996 phosphorylation of VEGFR2 [14] (Fig. 5A). Previous studies have shown that exogenous VEGF application can dose-dependently reverse the anti-angiogenic effect of A β [14], VEGF levels in the brain are closely related to cognitive function [27–30] and intraperitoneal injection of VEGF improved the cognitive impairment of AD mice [31]. Therefore, to reverse the deleterious effects of A β on angiogenesis, it is critical to disrupt A β interaction with VEGFR2. Importantly, DV has been shown to directly bind VEGFR2 [32] and increase tube-like structure formation in brain endothelial cells by increasing their VEGFR2 expression and VEGF secretion via the α 5 β 1 integrin [16]. Additionally, increasing VEGFR2 receptor and VEGF expression creates a proangiogenic positive feedback loop. DV also binds α 5 β 1 and when the α 5 β 1 integrin is reduced, DV is unable to induce VEGF secretion and thus is unable to rescue the reduction of A β in the formation of tube-like structures. DV signals through α 5 β 1 in MBECs, activating the Akt/ERK mitogen activated pathway [19] and similarly, VEGF/VEGFR2 can signal through the same pathway, inducing the expression of VEGF [33]. The blockade of ERK-dependent pathways upon

decreased $\alpha 5$ integrin expression blunts DV-induced proliferation in brain endothelial cells [19]. These proliferative signals induced by DV in endothelial cells might either overcome the A β toxicity or interfere with A β -induced endothelial cell death. Furthermore, DV might prevent A β from binding to VEGFR2 by directly binding to VEGFR2 itself and/or increase the VEGF amount to a level where it can outcompete with A β for receptor binding and subsequently induce an angiogenic signal.

Endothelial cells from the brains of AD transgenic mice are impaired in forming tube-like structures on Matrigel, suggesting altered angiogenic properties in these mice [34]. Additionally, multiple *in vitro* and *in vivo* assays have shown that angiogenesis is inhibited by soluble A β peptides. Finally, A β can inhibit the vascularization of xenografted human cancer cells and oppose VEGF-induced angiogenesis *in vivo* [14]. Thus, the anti-angiogenic properties of A β are well characterized and present a problem in the AD brain. Understanding the molecular mechanisms behind the anti-angiogenic activity of A β and discovering novel ways to overcome it will help us better evaluate the importance of angiogenesis to the AD process and in particular the contribution of the cerebrovasculature to the pathogenesis of AD. To this end, DV, a proteolytically cleaved C-terminal protein fragment of the major vascular basement membrane component perlecan, and thereby intimately associated with cerebrovasculature, may serve as part of the brain's natural A β defense mechanisms, inhibiting its deleterious effects on endothelial cell viability and angiogenesis when present. This concept is supported by the observation that the normally ubiquitously expressed (around brain blood vessels) perlecan is conspicuously absent in amyloid-laden brain blood vessels in human AD and cerebral amyloid angiopathy patients, but present in amyloid-free "normal" brain blood vessels, suggesting that a lack of perlecan (and its DV portion) could contribute to A β cerebrovascular pathology [35]. Our study suggests that DV is beneficial to brain endothelial cells exposed to A β_{25-35} *in vitro* in an integrin-mediated fashion. Future studies with other forms of A β , specifically A β_{1-40} and A β_{1-42} , as well as those involving *in vivo* models of AD and cerebral amyloid angiopathy, may further support DV as a potential novel anti-amyloid therapy.

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Fig. 1.

DV and LG3 enhance brain endothelial cell proliferation and block Aβ₂₅₋₃₅ toxicity. A) Quantification (% of control arbitrarily set at 100%) of alamar blue measured proliferation of MBECs after 72 h *in vitro* treated as labeled. **p* < 0.05 as compared to control. H.i. hDV = heat-inactivated hDV. B) Quantification (as in A) with added Aβ₂₅₋₃₅ ± DV or LG3 treatments as labeled demonstrating Aβ₂₅₋₃₅ dose dependent toxicity that was rescued by human DV and mLG3, but not hLG3 (all at 300 nM). **p* < 0.05 as compared to 25 uM Aβ₂₅₋₃₅.

Fig. 2.

DV restores angiogenic capabilities to Aβ₂₅₋₃₅-treated MBECs- No treatment control (A), DV treatment (B), Aβ₃₅₋₂₅ reverse peptide control (C), Aβ₂₅₋₃₅ only (D), DV plus Aβ₂₅₋₃₅ (E), and quantitation of three independent experiments (F). Calculations were based on the average tube pixels per high power field (as described in the methods section) from three randomly selected microscopic fields per well. Values are expressed as percent of control (no treatment) arbitrarily set to 100%. **p* < 0.05 as compared to indicated treatments.

Fig. 3.

Reduction of capillary tube-like formation by Aβ₂₅₋₃₅ is not due to cell death. Quantification of non-adhered cells from Matrigel tube assay, treated as labeled. * $p < 0.05$ as compared to control.

Fig. 4.

$\alpha 5\beta 1$ mediates rescue of capillary tube-like formation by DV. Quantification (% of control, arbitrarily set at 100%) of tube formation of MBEC $\alpha 5$ knockdown cells (75% knockdown) on Matrigel treated as indicated. * $p < 0.05$ as compared to control.

Fig. 5.

Schematic of proposed model. A) During AD pathogenesis, Aβ directly interacts with VEGFR2, blocking the ability of VEGF to bind to VEGFR2, disrupting signaling. The complete Aβ/VEGFR2 pathway is currently unknown. B) DV directly binds both VEGFR2 and the α5β1 integrin, increasing tube-like structure formation in brain endothelial cells by increasing VEGF secretion and VEGFR2 expression, creating a proangiogenic positive feedback loop. When α5 expression is reduced, DV is unable to induce VEGF secretion and thus is unable to rescue Aβ's reduction in the formation of tube-like structures. DV signals through α5β1 in MBECs to activate the Akt/ERK mitogen activated pathway. VEGF/VEGFR2 can signal through the same pathway, inducing the expression of VEGF. We propose that DV prevents Aβ from binding to VEGFR2 by directly binding to VEGFR2 itself and/or by increasing the VEGF amount to a level where it can outcompete with Aβ for receptor binding and subsequently induce an angiogenic signal.