CCM1 regulates vascular-lumen organization by inducing endothelial polarity

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Summary
Little is known about the molecular mechanisms that regulate the organization of vascular lumen. In this paper we show that lumen formation correlates with endothelial polarization. Adherens junctions (AJs) and VE-cadherin (VEC, encoded by CDH5) are required for endothelial apicobasal polarity in vitro and during embryonic development. Silencing of CDH5 gene expression leads to abrogation of endothelial polarity accompanied by strong alterations in luminal structure. VEC co-distributes with members of the Par polarity complex (Par3 and PKCζ) and is needed for activation of PKCζ. CCM1 is encoded by the CCM1 gene, which is mutated in 60% of patients affected by cerebral cavernous malformation (CCM). The protein interacts with VEC and directs AJ organization and AJ association with the polarity complex, both in cell-culture models and in human CCM1 lesions. Both VEC and CCM1 control Rap1 concentration at cell-cell junctions. We propose that VEC, CCM1 and Rap1 form a signaling complex. In the absence of any of these proteins, AJs are dismantled, cell polarity is lost and vascular luminal structure is severely altered.

Key words: Adherens junctions, Cell polarity, Endothelium, Vascular lumen

Introduction
The human disease cerebral cavernous malformation (CCM) is characterized by abnormal vascular structures mostly localized in the brain. Germline mutation in any of three independent genes, CCM1, CCM2 or CCM3, causes the inherited form of the disease (Plummer et al., 2005; Labauge et al., 2007), whereas local somatic mutation of these genes might induce the sporadic form (Limaye et al., 2009). The most dangerous clinical symptom in CCM is hemorrhagic stroke. Additionally, hemosiderin deposits in the tissue adjacent to lesions strongly suggest continuous blood leakage from the abnormal vessels, which could account for epilepsy and other neurological symptoms (Rigamonti et al., 1988).

Histological and ultrastructural analyses reveal that malformations consist of clusters of dilated vascular sacs (Wong et al., 2000), with endothelial cells (ECs) lacking both tight junctions (TJs) (Clatterbuck et al., 2001) and the support of astrocyte foot processes at the ablumenal aspect of vessels (Clatterbuck et al., 2001). Fibronectin, growth factors (VEGF, bFGF, TGFα), and smooth muscle actin are abundant in the ECs of lesions, whereas laminin and collagen IV are reduced (Hallmann et al., 2005), reminiscent of an immature or angiogenic situation (Kilic et al., 2000). Together, these observations strongly suggest that defects in lumen formation and vascular fragility are key features of CCM lesions.

Regulation of vascular permeability by CCM1 and CCM2 has been analyzed both in vitro and in vivo, and is associated with the organization of cell-cell junctions (Glading et al., 2007; Whitehead et al., 2009). In particular, CCM1 acts as an effector of the small GTPase Rap1, by enhancing the stability of endothelial adherens junctions (AJs).

The presence of vascular malformations might also contribute to vascular fragility. Abnormal blood flow generates turbulence and alterations in shear stress that can induce vascular rupture. The mechanism by which the absence of CCM proteins produces vascular malformation remains largely unexplored (Whitehead et al., 2004), possibly because of the gaps that still remain to be filled in our understanding of the pathways that direct vascular lumen formation (Iruela-Arispe and Davis, 2009). Kamei and co-workers (Kamei et al., 2006) showed, in cultured ECs and in zebrafish, that lumen formation requires the intercellular fusion of intracellular vesicles. However, other mechanisms, such as cavitation of endothelial cords, are also possible (Rubarsky and Krasnow, 2003; Iruela-Arispe and Davis, 2009; Blum et al., 2008; Kleaveland et al., 2009).

Although different mechanisms might regulate lumen formation in different types of vessels, the establishment of endothelial apical-basal polarity is expected to be an absolute requirement for all of them. In epithelial cells, apical-basal polarity is a prerequisite of correct lumen organization (Martin-Belmonte and Mostov, 2008; Jaffe et al., 2008). Since the partitioning defective (Par) polarity complex is a key determinant of cell polarity (Goldstein and Macara, 2007), it is likely to be important in the organization of vascular lumen. The specific localization and activity of this complex is determined, at least in part, by the small GTPases Cdc42 (Martin-Belmonte et al., 2007) and Rac (Chen and Macara, 2005), the Rac-specific GEF Tiam (Mertens et al., 2005), the adaptor and regulatory molecules Par3 and Par6, and the effector PKCζ (Goldstein and Macara, 2007; Iden and Collard, 2008). Cdc42 and Par3 have been shown to regulate lumen formation of in three-dimensional (3D) cultures of ECs (Koh et al., 2008). In addition, the small GTPase
Rap1 controls lumen formation (Itoh et al., 2007) and anteroposterior polarity (Gerard et al., 2007; Schwamborn and Puschel, 2004). Excessive Rap1 activity leads to altered epithelial acinar polarity (Itoh et al., 2007). Furthermore, indirect data support the concept that AJs are involved in regulating EC polarity and vascular lumen formation. VE-cadherin (VEC) promotes endothelial cell-cell adhesion at AJs. In the early murine embryo, a homozygous null mutation of the gene encoding VEC (Cd5h) results in a reduction in lumen size of the aorta and of cardinal veins, although the lumens of other vessels, such as cephalic vessels, become enlarged (Carmeliet et al., 1999; Gory-Faure et al., 1999). Consistent with this data, impaired lumen formation has been reported in zebrafish upon knockdown of expression of the gene encoding VEC (Monteiro-Balagué et al., 2009). VEC can directly bind Par3, which is an element of the polarity complex (Iden et al., 2006), and it is required for junctional organization of Tiam (Lampugnani et al., 2002); however, the actual pathway through which VEC regulates endothelial polarity remains unclear.

Here, we present data indicating that VEC is needed for the correct localization and to promote activation of the polarity complex, which, in turn, establishes endothelial apical-basal polarity. CCM1 stabilizes VEC at AJs and thus promotes the concentration of the polarity complex at intercellular junctions. In the absence of CCM1, apical-basal polarity of ECs is altered, and the size and organization of vascular lumens is severely affected. These data support the concept that CCM1 acts in concert with VEC and the members of the polarity complex to modulate the vascular lumen and offer a mechanistic explanation for the vascular malformations observed in CCM disease.

**Results**

VEC regulates the formation of a polarized vascular lumen

HUVECs cultured in collagen gel rapidly organize into a network of hollow structures (supplementary material Fig. S1) (Bayless and Davis, 2002). Using podocalyxin (Podxl) and collagen IV (Coll IV) as markers of the apical and basal surfaces, respectively (Horvat et al., 1986; Hallmann et al., 2005), we observed that cells forming these vascular-like structures were correctly polarized (Fig. 1A). Knockdown of VEC protein expression using siRNA against Cd5h resulted in the formation of several small lumens lined with ECs (supplementary material Fig. S1A) that were not regularly polarized (Fig. 1A). This effect was not due to reduced expression of polarity markers (supplementary material Fig. S2C). Consistently, in wild-
type embryos at the 5-somite stage, ECs in the dorsal aorta (Fig. 1B) were polarized and expressed Podxl and Coll IV at the apical and basal sides, respectively. Conversely, in Cdh5-null embryos, the vascular lumen was strongly altered and ECs were abnormally polarized (Fig. 1B). In addition, the absence of VEC protein (in VEC-null cells) or the lack of its engagement in cell-cell contacts (in sparse VEC-positive cells, which were genetically modified to express human CDH5) impaired the apical distribution of Podxl in 2D culture (Fig. 1C).

We then tested whether VEC could determine the localization and activate the Par polarity complex that has a crucial role in the establishment and maintenance of apical-basal polarity (Goldstein and Macara, 2007; Iden and Collard, 2008). We first observed that Par3, phosphorylated Thr410 PKCζ and Tiam (all of which are components of the Par polarity complex) required the presence of VEC for their correct localization at cell-cell junctions (supplementary material Fig. S3A). In addition, phosphorylated PKCζ expression levels were higher in ECs expressing VEC than in VEC-null cells (Fig. 4C). Higher levels of GTP-bound Rac and Cdc42 were also observed in control cells than in VEC-null cells (supplementary material Fig. S3B). Finally, the knockdown (KD) of components of the polarity complex, Tiam and Par3, inhibited lumen formation in HUVECs (supplementary material Fig. S1A), but did not affect the expression and distribution of VEC (supplementary material Fig. S2B and M.G.L., unpublished results), suggesting a role for these molecules as effectors, rather than activators, of the VEC complex.

CCM1 acts in concert with VEC to establish and maintain correct EC polarity and vascular lumen

When the expression of CCM1 was downregulated in HUVECs by siRNA (which knocked down CCM1 protein levels by some 70%, independently of the oligos used, supplementary material Fig. S2A), the lumen of vascular-like structures in 3D collagen gel was highly abnormal, both at early and late stages of culture (Fig. 2A; supplementary material Fig. S1A). CCM1-knockdown cells lining the lumen were poorly polarized. In several regions, Coll IV presented apical distribution (Fig. 2A) and, although the expression levels of Podxl were not reduced by CCM1 gene silencing (supplementary material Fig. S2C), it did not show the normal distinct apical polarization (Fig. 2A).

Podxl was also depolarized in the endothelium of CCM lesions from a patient with mutated CCM1 (Fig. 2B). In the areas of tortuous, lace-like vessels, Podxl was often lacking from the luminal surface and was instead concentrated at the basal aspect of ECs. Podxl appeared normally polarized in peri-lesion vessels (Fig. 2B).

CCM1 has been shown to associate indirectly with AJs (Glading et al., 2007). As reported above, VEC is important in endothelial polarization and lumen formation. Furthermore, the 3D phenotype observed in CDH5-knockdown cells resembles that of CCM1-knockdown cells. CCM1 might, therefore, act in concert with VEC to promote cell polarity and regular lumen formation. As reported in Fig. 3A, we found that CCM1 associated with VEC. This was further supported by the observation that CCM1 was lost from junctions when CDH5 gene

**Fig. 2. Endothelial lumen and polarity are abnormal in CCM1-silenced HUVECs and in human CCM1 vascular lesions.** (A) HUVEC vascular structures in 3D culture. At early stages (12 hours) a single central lumen (L) is formed with control siRNA, whereas two eccentric lumens (L and L') are typically observed with CCM1 siRNA. At later stages (24 hours), CCM1-silenced ECs form an irregular lumen with cell aggregates at the wall. Podxl (arrowheads) is cytoplasmic or ablumenal whereas collagen IV appears to be luminal (arrows) (see boxed magnification). Dashed lines outline the lumen. Scale bars: 50 μm (left), 50 μm (central) and 10 μm (magnified areas). (B) Vascular lesion in a patient with heterozygous mutation of CCM1. In vessels at the periphery of the lesion (Peri-lesion) the lumen (L) is correctly polarized, with apical Podxl (arrowheads). By contrast, within the lesion, tortuous and multiple lumens (L) often lack apical Podxl, which appears to be basal or displays a diffuse pattern of expression (arrowheads). Dashed lines outline the lumens. Scale bars: 115 μm (top), 60 μm (bottom left) and 10 μm (bottom right).
expression was silenced (Fig. 3B), indicating that VEC is needed for CCM1 organization at AJs.

We then examined the effects of CCM1 on junction organization and localization of components of the polarity complex. Silencing of CCM1 expression strongly affected junctional clustering of VEC and β-catenin. Both proteins relocated to lateral junctions towards large areas flanking the junctions (Fig. 3D). VEC and β-catenin were often distributed toward the apical membrane (Fig. 3C, top panels, z-axis) and toward the basal aspect of HUVECs in 3D culture (Fig. 3C, bottom panels). Similarly to VEC, junctional staining of components of the Par polarity complex (Par3 and phosphorylated PKCζ and Tiam) was strongly altered after knockdown of CCM1 (Fig. 4A). Comparable effects were observed using RNA-silencing oligos that targeted independent sequences of CCM1 mRNA (our unpublished results), that were as effective at silencing the expression of CCM1 as the oligos used for experiments described in Figs 3 and 4 (see also supplementary material Fig. S2A). Most importantly, ablation of the Ccm1 gene in ECs isolated from Ccm1 fl/fl mice produced effects comparable with those described after siRNA treatment (Fig. 3E, Fig. 4B, and supplementary material Fig. S2A).

Together, these data show that VEC and CCM1 interact at AJs, and that both are required for junctional localization of the polarity complex. However, VEC was needed for phosphorylation of PKCζ at Thr410 (Fig. 4C), whereas CCM1 controlled the junctional localization of this molecule. Analysis of lesions from a patient with mutated CCM1 confirmed that both VEC (Fig. 3F) and phosphorylated PKCζ (Fig. 4D) are strongly delocalized from endothelial junctions in these areas: their focal junctional distribution is lost, but is maintained in the endothelium of peri-lesion vessels.

Finally, we observed that Rap1 was also important in EC polarity. Indeed, localization of the components of the Par polarity complex phosphorylated PKCζ (Fig. 5A), as well as Par3 and Tiam (supplementary material Fig. S3C) were strongly reduced at junctions upon treatment of ECs with RAP1B siRNA. Consistent with this observation, RAP1B siRNA altered endothelial cell polarity and lumen formation (Fig. 5C). Dismantling AJs by silencing either CCM1 (Fig. 5D) or CDH5 (Gore et al., 2008) led to loss of Rap1b.
CCM proteins and vascular lumen

from intercellular contacts and to its relocalization to focal contacts, as previously reported in other cell types (Balzac et al., 2005). The active form of Rap, Rap1 G12V, was observed at junctions in a CCM1-dependent manner (Fig. 5E), although knockdown of VEC abrogated Rap1b activation (Fig. 5B).

Discussion
In the present paper, we have shown that VEC and CCM1 form a complex and act in concert to determine the correct junctional localization of the Par polarity complex that is required for endothelial polarization and formation of vascular lumen. This conclusion is supported by the observation that cadherin proteins might act as scaffolds for the construction of polarized structures (Capaldo and Macara, 2007). We did not perform detailed studies on the molecular basis of the interaction of CCM1 with VEC, but Glading and colleagues (Glading et al., 2007) reported that CCM1 could bind β-catenin directly. It is therefore likely that CCM1 associates indirectly with VEC, via binding to β-catenin. These authors also found that CCM1 is an effector of Rap1. The active state of this small GTPase induces a conformational change in CCM1 that is required for CCM1 binding to β-catenin. Rap1, which also concentrates at intercellular contacts, is known to stabilize AJs, thus reducing vascular fragility (Kooistra et al., 2005). CCM1 has therefore been proposed to act downstream of Rap1 in the stabilization of AJs. We show here that Rap1b, which is recruited to the complex formed by VEC and CCM1, contributes to polarization and lumen formation by stabilizing AJs. The need for VEC homophilic interactions to permit Rap1b activity has been previously reported. Clustering of VEC would allow junctional localization of guanosine-exchange factors such as C3G or PDZ-GEF to promote local activation of Rap1 (Kooistra et al., 2007; Sakurai et al., 2006). VEC and CCM1 exert a different type of control on PKCζ, the effector component of the polarity complex. Both VEC and CCM1 direct junctional localization of PKCζ. However, only VEC is required for the phosphorylation of PKCζ at Thr410, which is needed for expression of the catalytic activity of the enzyme (Standaert et al., 1999). Nevertheless, segregation of PKCζ is fundamental to allow phosphorylation of the correct molecular targets in the appropriate subcellular compartment (Wirtz-Peitz et al., 2008). Loss of segregation of the Par polarity complex and PKCζ can have a strong impact on their signaling and functional outcome (Goldstein and Macara, 2007; Martin-Belmonte et al., 2007). This has recently been shown in vivo during the organization of tubular structures in the morphogenesis of the mammary gland (McCaffrey and Macara, 2009).

Fig. 4. CCM1 is required for junctional localization of the components of the polarity complex Tiam, Par3 and phosphorylated PKCζ in cultured ECs and in human CCM1 vascular lesion. (A,B) Junctional staining of Tiam, Par3 and phosphorylated PKCζ, in both Ccm1 silenced VEC-positive cells and in Ccm1 fl/fl+Cre cells, appears convoluted and fragmented (arrowheads). This pattern is reminiscent of VEC and β-catenin distribution in Ccm1 silenced ECs (Fig. 3D,E). Scale bar: 20 μm. (C) Phosphorylation of PKCζ at Thr410 (p-PKCζ) is downregulated by 40% and 80%, respectively, in CDH5 silenced HUVECs and in VEC-null cells. Phosphorylation of PKCζ is not modified after CCM1 or Ccm1 siRNA treatment. Data shown are representative of three independent experiments. Vertical bars indicate that the bands, from the same blot, do not come from contiguous lanes. (D) Brain vascular lesion of a patient with heterozygous mutation of CCM1 gene. Phosphorylated PKCζ expression is diffuse over large areas in the endothelium of the vascular mulberry lesion (arrowheads, magnification of boxed area). It is instead concentrated to interendothelial junctions in peri-lesion vessels (arrowheads, magnification of boxed area). Scale bars: 50 μm and 10 μm (in magnifications). Asterisks indicate the nuclei of ECs lining the lumen (dashed line).
The mechanism through which VEC influences endothelial polarity has not been investigated, as yet. In epithelial cells, cadherins promote the organization of TJs. In turn, TJs determine cell polarity, and act as a barrier to the redistribution of proteins and lipids between the apical and basolateral surfaces (Martin-Belmonte et al., 2007; Nejsum and Nelson, 2007; Mertens et al., 2006). However, early phases of epithelial polarization occur also in the absence of TJs (Umeda et al., 2006; Qin et al., 2005). TJs are either absent or very poorly organized in cultured ECs or in vascular endothelium at early phases of embryo development (Liebner et al., 2008; Carmeliet et al., 1999), which are the experimental models used in the present study. VEC and AJs could therefore serve to control early phases of polarity and lumen formation in the presence of TJs (Yu et al., 2005). As both phosphorylation at Thr410 (Standaert et al., 1999), and GTP-Cdc42 (Martin-Belmonte et al., 2007) or GTP-Rac (Chen and Macara, 2005; Mertens et al., 2005) are required for full activation and correct positioning of PKCζ, our data strongly suggest that VEC induces the early steps of cell polarization by permitting the correct localization and activation of the polarity complex. However, since junctional clustering of VEC precedes and triggers TJ organization (Taddei et al., 2008), it is possible that when junctions are fully stabilized, the polarity complex re-localizes from AJs to TJs (Mertens et al., 2006) to maintain cell polarity (Capaldo and Macara, 2007).

CCM1 can form a complex with CCM2 and CCM3 (Zawistowski et al., 2005; Voss et al., 2007). Although CCM proteins are structurally distinct, gene inactivation of any one of them leads to a comparable clinical outcome (Plummer et al., 2005), suggesting that these proteins act in concert to promote similar cell functions. CCM2 limits Rho activation and, as a consequence, reduces endothelial cell contractility and vascular permeability (Whitehead et al., 2009). The organization of the actin cytoskeleton might also be implicated in cell polarity (Iden and Collard, 2008). It is therefore possible that CCM2 also contributes indirectly to the establishment of cell polarity through a reshaping of the cell cytoskeleton (Whitehead et al., 2009).

In conclusion, our data show that the presence of VEC and CCM1 is needed at AJs to establish cell polarity and lumen formation. These effects are mediated by recruitment and activation of the polarity complex and Rap1b. In the absence of any of these proteins, AJs are not correctly organized, cell polarity is lost and vascular lumen formation is severely altered. These observations suggest that the various proteins implicated in inducing cell polarity do not function independently, but interact with one another in a signaling network.

**Materials and Methods**

**Cells and 2D culture**

Human umbilical vein endothelial (HUVEC) cells and murine endothelial cells genetically ablated for Cdh5 (VEC-null) or genetically modified to express human
CDH5 (VEG-positive) were obtained and cultured as described (Lambagna et al., 2002). Lung endothelial cells from a mouse (57/BL/6N) in which exons 4-5 of Cdh5 gene were flanked by loxP sites (Cdh5/fl/fl) (produced by Artemis Taconis, Cologne, Germany) were isolated and immortalized as described (Balconi et al., 2000). Deletion of exons 4-5 by Cre-mediated recombination produces loss of function by removing the exons encoding part of the p53-like DNA-binding domain and generating a frame-shift in downstream exons. Cdh5/fl/fl was inactivated treating cells in culture with TAT-Cre recombinase (Lieberer et al., 2008). For Tat-Cre treatment: Cdh5/fl/fl ECs were treated with 100 μg/ml Tat-Cre for 60 minutes in HyClone ADFC-Mab medium (Thermo Scientific) and with 100 μM chloroquine for further 60 minutes (Wadia et al., 2004). Cells (Cdh5/fl/fl + Cre) were used for experiments after at least two in vitro passages from Tat-Cre treatment.

For two-dimensional (2D) cultures, cells were seeded in 35 mm Petri dishes or in 0.2 cm² wells created with flexiPERM frames (Greiner Bio-One, Germany) on polylysine cell culture slides (NUNC, Thermo Fisher, Denmark). In both cases, the coating was with gelatin (Difco Gelatin, BD, 0.1% for murine cells and 1.5% for HUVECs). Cells were cultured for 72-96 hours. The splitting ratio was such that confluence was reached overnight after seeding. HA-Rap1 G12V (a gift from Giorgio Scita, IFOM, Milano, Italy) and HA-Rap GAP (a gift from Johannes Bos, University Medical Center Utrecht, Utrecht, The Netherlands) were introduced in lentiviral plasmids to produce lentiviral vectors, as described (Taddei et al., 2008).

RNA interference
For small RNA interference (siRNA) the following oligos were used: anti-CCM1, human-specific (pool, L-003825-00) (ON-TARGET plus SMART pool, Dharmacon); murine-specific oligo 2 (KRIT1MS234457) and oligo 4 (KRIT1MS234436); human- and murine-specific oligo 3 (KRIT1MS234455) were all stealth oligo (Invitrogen). Anti-humanVE-cadherin: (L-03641-01); Anti-Rap1b: human-specific (L-010364-00) and murine-specific (L-062638-01); anti-human Tiam (L-00392-01); anti-human Par 3 (L-015620-02) were all ON-TARGET plus SMART pool, Dharmacon. Control oligos were ON-TARGET plus siControl (D-001810-10, Dharmacon) and Medium G-C control oligo (12935-300, Invitrogen) when Dharmacon SMART pool or Invitrogen oligos were used, respectively. For oligo transfection, cells were seeded at a density of 10⁴ cm². 20 hours later, they were transfected with 40 nM of small interfering oligo for 5 hours in Optimem (Invitrogen). The effects of silencing were tested 72-96 hours after transfection.

Antibodies
For immunofluorescence (IF), western blot (WB) and immunoprecipitation, the following antibodies were used: VE-cadherin (C-19, sc-6458, goat, Santa Cruz), Podxl (murine-specific, sc-106220, rabbit, Cell Signaling), total a-PKC (rabbit, Cell Signaling), total a-PKC (rabbit, Cell Signaling), Tiam 1 (C-16, sc-872, rabbit, Santa Cruz), Podxl (murine-specific (pool, L-003825-00) (ON-TARGET plus SMART pool, Dharmacon); for in vivo treatment and three-dimensional (3D) cultures, a murine anti-human VEC (clone BV9, mouse), which inactivates VEC (Corada et al., 2001) was used as specified in supplementary material Fig. S1. Secondary antibodies for IF were donkey antibodies to the appropriate species conjugated with Alexa Fluor 488, 555 or 647 (Molecular Probes). Western blot and immunoprecipitation
Total extracts were obtained using 2× Laemmki sample buffer. After 10 minutes of boiling, protein content was quantified (BCA, Pierce). Samples were then reduced by adding DTT (50 mM). Extraction for immunoprecipitation was as described (Rudini et al., 2008). Bands on films revealed through ECL were quantified through ImageJ (freely available at the NIH website). Several exposure times were used for each blot and values in the linear range of detection were used for quantification.

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References


Rap1 integrates tissue polarity, lumen formation, and tumorigenic potential in human breast epithelial cells. J. Biol. Chem. 283, 9146-9154.


