Developmental Cell
Review

The Control of Vascular Integrity by Endothelial Cell Junctions: Molecular Basis and Pathological Implications

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DOI 10.1016/j.devcel.2009.01.004

Human pathologies such as vascular malformations, hemorrhagic stroke, and edema have been associated with defects in the organization of endothelial cell junctions. Understanding the molecular basis of these diseases requires different integrated approaches which include basic cell biology, clinical studies, and studies in animal models such as mice and zebrafish. In this review we discuss recent findings derived from these approaches and their possible integration in a common picture.

A variety of human vascular pathologies are due to or exacerbated by altered control of endothelial permeability. Defects in endothelial permeability can lead to edema and increase in interstitial pressure, which in turn induces compression and altered tissue perfusion. A typical example is ischemic stroke, where edema around the ischemic area extends brain damage. Inflammation is also associated with increases in vascular permeability, which favors leukocyte diapedesis through the vessel wall but may create pain and swelling. Edema is usually a reversible condition and the control of vascular permeability may be restored once the triggering cause is removed.

However, there are extreme conditions where the integrity of the endothelial monolayer is severely affected, cell-to-cell junctions are disrupted, and endothelial cells detach from the vessel wall, creating areas of vascular damage and possibly microthrombi. Altered permeability may also be accompanied by vascular fragility with the frank rupture of the vessels and formation of hemorrhages. This is a frequent condition in tumors where the newly forming vasculature is usually permeable and fragile (Carmeliet and Jain, 2000). In other more rare cases though, increased vascular fragility may be due to congenital alterations in vascular development (Brouillard and Vikkula, 2003, 2007). Molecular cloning of the defective genes from human disorders and gene inactivation approaches in model organisms such as mouse and fish have resulted in the identification of many genes involved in vascular remodeling and maintenance of vascular integrity. Deletion or reduced expression of these genes may result in early lethality due to diffuse hemorrhages in the embryo. However, in other cases the vascular defect may remain silent during development but manifest in the adult when the vessels are exposed to a triggering condition.

Defects in vascular permeability can have a number of different causes. Vascular permeability is mediated by at least two broad mechanisms, called the paracellular and transcellular pathways. The first is controlled by the dynamic opening and closing of endothelial junctions (Dejana et al., 2008), while the second includes vesicular transport systems, fenestrae, and biochemical transporters (Dvorak et al., 1996). Vascular fragility can be due to an altered organization of intercellular junctions and/or defective interaction of endothelial cells with pericytes or matrix proteins. The focus of this review is primarily on the role of intercellular junctions in the control of vascular permeability and integrity. We discuss current knowledge regarding the molecular and functional organization of adherens (AJ) and tight junctions (TJ), and attempt to show the correlations between experimental studies and related human pathologies.

The Molecular Organization of Endothelial Cell-to-Cell Junctions

The detailed architecture of endothelial cell-cell junctions has been described in detail in several other recent reviews (Bazzoni and Dejana, 2004; Gonzalez-Mariscal et al., 2008; Wallez and Huber, 2008) In Figure 1 we show a simplified version of some of the most important molecules involved in endothelial junction organization (Bazzoni and Dejana, 2004; Vestweber, 2008; Weber et al., 2007). Endothelial cells have at least two specialized adhesive junctional regions that are comparable to adherens junctions (AJs) and tight junctions (TJs) found in epithelial cells. In contrast to epithelial cells, however, endothelial cells lack typical desmosomes. Gap junctions are also present in the endothelium and play an important role in different endothelial functions but, as far as we know, are not involved in control of endothelial permeability and, for simplicity, will not be further considered in this review.

AJs and TJs have different functions. AJs initiate cell-to-cell contacts and promote their maturation and maintenance. TJs regulate the passage of ions and solutes through the paracellular route (Bazzoni and Dejana, 2004; Gonzalez-Mariscal et al., 2008). TJs may also act as a membrane "fence" to limit the free movement of lipids and proteins between the apical and the basolateral cell surfaces. Most importantly, both structures can transfer intracellular signals that control many endothelial

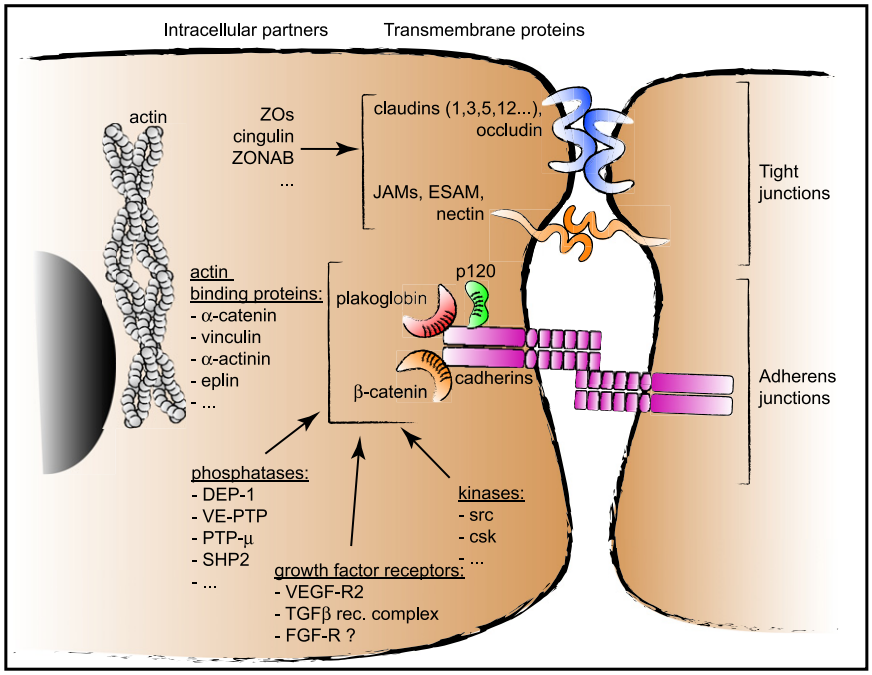

Figure 1. Schematic Representation of Tight Junctions and Adherens Junctions in Endothelial Cells

In endothelial cells, adhesion at tight junctions is mediated by claudins, occludin, members of the JAM family, and ESAM. The cytoplasmic components of tight junctions are ZO proteins, cingulin, ZONAB, and others. At adherens junctions, adhesion is promoted by cadherins (VE-cadherin and N-cadherin) which directly bind to p120, β-catenin, and plakoglobin. Nectins and their intracellular partner afadin/AF-6 participate in the organization of both tight and adherens junctions (Takai et al., 2008). A large set of actin binding proteins have been found to be associated to adherens junctions such as α-catenin, vinculin, α-actinin, eplin, and others. In addition, phosphatases (DEP-1, VE-PTP, PTPμ, SHP2, etc.) and kinases (src, csk, and others) are directly or indirectly associated to adherens junction components. Growth factor receptors: VEGF receptor 2 (also called flk-1 or KDR) and TGFβ receptor complex could bind to VE-cadherin complex. This interaction modulates their signaling properties. More details can be found in the text and, for review, in the following: Bazzoni and Dejana, 2004; Dejana, 2004; Engelhardt, 2003; Furuse and Tsukita, 2006; Gonzalez-Mariscal et al., 2008; Johnson-Leger and Imhof, 2003; Matter and Balda, 2003; Van Itallie and Anderson, 2006; Wallez and Huber, 2008; and Vestweber, 2008.

cell functions. The organization of intercellular junctions through the clustering of adhesion and signaling proteins is therefore an important process through which the cells sense their position, control growth and apoptosis, and form tubular structures (see below) (Matter and Balda, 2003; Bazzoni and Dejana, 2004; Dejana, 2004; Gonzalez-Mariscal et al., 2008).

Although the molecular components of TJs and AJs are different, they do have common features (Figure 1). In both types of junction, adhesion is mediated by transmembrane proteins that promote homophilic interactions and form a pericellular zipper-like structure along the cell border through their lateral aggregation in *trans* and *cis* (for review Bazzoni and Dejana, 2004; Gonzalez-Mariscal et al., 2008; Wallez and Huber, 2008) Endothelial cells express cell-type-specific transmembrane adhesion proteins such as VE-cadherin at AJs and claudin-5 at TJs. The restricted cell specificity of these components indicates that they might be needed for selective cell-cell recognition and/or specific functional properties of endothelial cells. Through their cytoplasmic tails, adhesion proteins of both types of junctions bind to cytoskeletal and signaling proteins that promote anchoring of junctions to actin microfilaments and transfer of intracellular signals to the inside of the cell. Cytoskeletal association is required for stabilization of the junctions, but also for the dynamic regulation of junction opening and closing. The interaction of junctional adhesion proteins with the actin cytoskeleton is also relevant in the maintenance of cell shape and polarity (Hartsock and Nelson, 2008). Many reports support the concept that AJs and TJs are interconnected and that AJs influence TJ organization (see below). AJs are formed at early stages of intercellular contacts and are followed by TJ organization. Some TJ components such as ZO-1 are found in AJs at early stages of junction formation and concentrate in TJs only subsequently when junctions are stabilized. Interestingly, however, AJs are required for TJ assembly but are dispensable for TJ maintenance in epithelial cells (Capaldo and Macara, 2007).

As described in the legend to Figure 1, the core components of TJs that promote cell-to-cell adhesion are members of the claudin family (Van Itallie and Anderson, 2006; Furuse and Tsukita, 2006). The claudin family has more than 20 members, only a few of which are expressed by endothelial cells. Claudin-5 is rather ubiquitous along the vascular tree. Other non-cell-specific claudins are also found in endothelial cells and their combination varies to respond to the different needs of the perfused organ. A variety of additional adhesion transmembrane proteins can also be found at TJs (JAMs, ESAM, occludin, etc.) and these contribute to intercellular adhesion in different ways (see below) (Wallez and Huber, 2008). Multiple intracellular partners of TJ adhesive proteins have been described. Among the best characterized are the members of the ZO family (ZO1 and 2 in the endothelium), a closely related subgroup of the membrane associated guanylate kinase (MAGUK) family that localize at TJs in most tissues including the endothelium. Other intracellular TJ proteins include signaling and actin-binding proteins. At AJs, adhesion is mediated by members of the cadherin family. VE-cadherin is expressed in essentially all types of vessels. N-cadherin is also present in the endothelium, but is frequently found localizing to non-AJ cellular structures both in vitro and in vivo. VE- and N-cadherins both bind catenins, in particular p120, β-catenin, and plakoglobin. β-catenin also binds α-catenin, which when released from junctions into the cytosol promotes actin bundling. As for TJs, many other actin-binding proteins and several kinases and phosphatases are also found at AJs (Wallez and Huber, 2008; Bazzoni and Dejana, 2004; Vestweber, 2008).

The organization of TJs and AJs varies along the vascular tree depending on the functional needs of the vessels. For instance,

TJs are particularly abundant and complex in the brain microcirculation where there is a need to strictly control permeability, whereas the junctions are relatively poorly organized in postcapillary venules where exchange between blood and tissues is quite dynamic (Engelhardt, 2003; Dejana, 2004). An example of highly specialized junctions is found in peripheral lymphatic vessels, where intercellular junctions between lymphatic endothelial cells control entry of fluid and cells that drain from surrounding tissues. These lymphatic capillaries possess highly specialized junctions that, although formed by the same molecular components as blood vessels, have a strikingly different morphology. Endothelial borders have discontinuous button-like junctions with intermingled flaps resembling valve-like structures (Baluk et al., 2007). At the molecular level, AJ and TJ proteins are concentrated at the buttons, leaving the flaps free to open without disrupting the overall junctional organization. The larger, more proximal collecting lymphatic vessels have continuous zipper-like junctions resembling those in the endothelium of blood vessels.

The Dynamic Regulation of Vascular Permeability

An important emerging concept is that intercellular junctions are dynamic structures undergoing continuous remodeling not only during morphogenesis in the embryo or upon exposure of cells to agents that increase permeability, but also in confluent and resting cells. Continuous recycling of adhesive proteins and signaling partners may occur at AJs and also at TJs. Cadherins, and in particular VE-cadherin, show a flow-like movement in a basal to apical direction which is accompanied by actin reorganization (Kametani and Takeichi, 2007). Furthermore, recent data have shown that in *Drosophila* E-cadherin forms stable adhesion foci that undergo continuous, actin-controlled, mobility along intercellular contacts (Cavey et al., 2008). All of this suggests that even apparently stable AJs are dynamic structures able to continuously adapt to tissue requirements. The endothelium is continuously exposed to hemodynamic stimuli such as shear stress or the rhythmic changes in pressure due to heart beating, as well as vessel contraction and dilation. Junctions and the cell cytoskeleton need to continuously reshape to allow the endothelial monolayer to adapt to the dynamic conditions to which it is exposed. Junctional proteins such as VE-cadherin may also serve as flow sensors and transfer intracellular stimuli which help the cell to react to changes in flow conditions (Tzima et al., 2005).

TJs and Permeability Control

As discussed above, the role of junctions in endothelial and epithelial cell permeability is now well established, and these are clearly highly dynamic structures regulated in response to environmental conditions. A number of recent studies have focused on the importance of claudins in TJ formation and maintenance (Furuse and Tsukita, 2006; Van Itallie and Anderson, 2006). Although inactivation of claudin-5 gene in mice did not morphologically alter the vascular network or the ultrastructural appearance of TJs, claudin-5-deficient pups died within 10 hr of birth due to a size-selective loosening of the blood-brain barrier against molecules less than 800 Da. Other claudins may form the TJ strands in claudin-5 mutants and maintain the barrier against larger molecules (Furuse and Tsukita, 2006). Claudin-3 is likely responsible for the complex organization of TJ in brain vessels. Claudin-3 and -5 therefore appear to act in concert to form the tightly organized strand network at TJs of the brain microcirculation. Gene inactivation of claudin-1, which is also expressed in endothelial cells, did not result in a vascular phenotype during embryonic development, suggesting that it plays a lesser role in TJ in endothelium compared to claudin-5 and -3 (Gonzalez-Mariscal et al., 2008). Occludin is another transmembrane protein structurally similar to claudins, although not strongly homologous at the sequence level, which becomes incorporated into claudin-based junctional strands. Occludin is present in endothelial cells and in particular in the brain (Hirase et al., 1997). However, no effects on vascular morphology or blood-brain barrier permeability have been reported in mice lacking occludin.

Junction adhesion molecule-A (JAM-A) and its related family members JAM-B, JAM-C, endothelial cell-selective adhesion molecule (ESAM), and cocksackie- and adeno-virus receptor (CAR) are transmembrane glycoproteins that associate with TJ strands but are not part of the strands per se (Weber et al., 2007). All JAM family members and ESAM are expressed in endothelial cells, but inactivation of their respective genes in mice does not cause any defect in the development of the vascular system in the embryo (Weber et al., 2007; Wegmann et al., 2006). In adult mice, all these molecules play an important role in modulating leukocyte diapedesis through endothelial cells. Unlike other junctional proteins, JAM-C increases endothelial permeability when expressed at the endothelial cell surface, suggesting it may play a role in promoting and/or organizing junction formation (Orlova et al., 2006). This activity is mediated by changes in actin organization and VE-cadherin activity. Several kinases and phosphatases have been shown to modulate TJ protein phosphorylation and endothelial permeability in vitro and in some conditions also in vivo.

The Role of AJs and VE-Cadherin in Vascular Permeability

AJs, and the AJ component VE-cadherin in particular, play an important role in the control of vascular permeability and integrity. In vivo data using blocking antibodies to VE-cadherin show profound alterations of lung and heart vascular permeability accompanied by endothelial cell retraction and partial detachment with exposure of the subendothelial matrix (for review see Dejana et al., 2008). Stimuli such as high concentrations of histamine, thrombin, or growth factors may increase endothelial cell permeability through an effect on cell contractility mediated by phosphorylation of myosin light chain and activation of p21-activated kinase (PAK) (Stockton et al., 2004). However, increased permeability in vitro and in vivo could also be observed in the presence of more subtle changes in AJ organization. Histamine, tumor necrosis factor, platelet activating factor, and vascular endothelial growth factor (VEGF) induce tyrosine phosphorylation of VE-cadherin, β-catenin, and p120. This phosphorylation of AJ proteins parallels increases in permeability in cell culture systems (Dejana et al., 2008). Src is likely implicated in phosphorylation of AJs as it is directly associated with the VE-cadherin/catenin complex, and src gene inactivation or treatment with inhibitors blocks VEGF-induced VE-cadherin phosphorylation (Weis et al., 2004). VE-cadherin may also be phosphorylated through inhibition of associated phosphatases. The phosphatase VE-PTP is of particular interest as it is endothelial-specific and

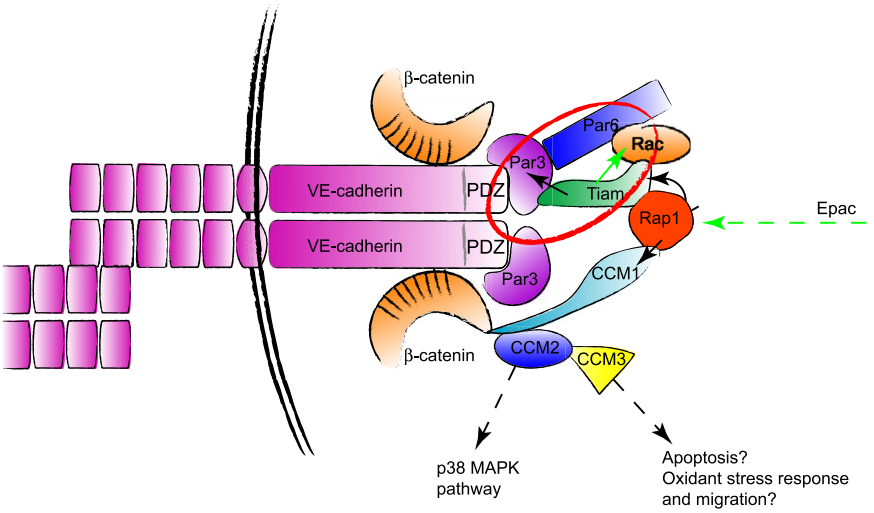

Figure 2. Protein Complexes that Associate with VE-Cadherin and Other Cadherins and Contribute to Adherens Junction Stability

VE-cadherin binds to PAR-3 through its PDZ domain and PAR3 can associate with PAR6 (Vestweber, 2008); the small GTPase Rap-1 stabilizes adherens junctions and is activated by increase in cAMP through Epac. Rap1 can bind the Rac-GEF Tiam (Michiels et al., 1995) which was found to codistribute with VE-cadherin complex (Lampugnani et al., 2002); CCM1,CCM2, and CCM3 form a complex which may associate to cadherins via β-catenin or Rap1 (Serebriiskii et al., 1997; Glading et al., 2007; Voss et al., 2007). For simplicity, other potential partners of the complex, such as ICAP-1 or afadin/AF6, are not illustrated in the figure. More details can be found in the text.

associates with VE-cadherin. Inactivation of the VE-PTP gene leads to a phenotype comparable to that of VE-cadherin null embryos. This suggests that vessels cannot form correctly if VE-cadherin is constantly phosphorylated (Baumer et al., 2006). Other phosphatases such as Dep-1, PTP-μ, and SHP2 may also associate with VE-cadherin and, directly or indirectly, decrease phosphorylation and increase barrier function (Dejana et al., 2008). There are also other kinases besides src that may be associated with the VE-cadherin/catenin complex and modulate permeability. This includes Csk, which binds to phosphorylated VE-cadherin and inhibits Src (Vestweber, 2008). Permeability may also be regulated by VE-cadherin internalization. VE-cadherin can be internalized in a clathrin-dependent manner. Binding of p120 to VE-cadherin prevents internalization, suggesting that p120 may act as a plasma membrane retention signal. Therefore, any condition that reduces VE-cadherin affinity for p120, such as tyrosine phosphorylation, may increase its internalization. A recent report found that VEGF disrupts endothelial barrier function by activating src, which in turn phosphorylates Vav2, a guanine exchange factor for Rac. Activated Rac induces VE-cadherin phosphorylation in Ser665. This process induces the recruitment of β-arrestin 2, which promotes clathrin dependent VE-cadherin internalization. In this scenario, phosphorylation of VE-cadherin in Ser665 together with tyrosine would be the crucial step for increase in permeability (Gavard and Gutkind, 2006). Importantly, the same authors found that angiopoietin 1, which in many conditions reduces vascular permeability, induces src trapping by mDia, reducing its activity at AJs (Gavard et al., 2008).

Another pathway that may induce vascular permeability is VE-cadherin cleavage. The VE-cadherin protein is particularly susceptible to enzymatic proteolysis. Exposure to elastase, Adam-10, and others induces digestion of VE-cadherin in cultured cells (for review see Dejana et al., 2008). Leukocytes and tumor cells can release high amounts of these enzymes, promoting VE-cadherin cleavage and thus increasing cell extravasation and vascular leakage. Permeability control may also be achieved through up or downregulation of VE-cadherin expression. Analysis of the VE-cadherin promoter showed different binding sites for several transcription factors known to act in endothelial cell differentiation. Among these TAL-1, Ets-1, ERG, or hypoxia inducible factors were found to effectively upregulate VE-cadherin (Birdsey et al., 2008; Deleuze et al., 2007). Although VE-cadherin is present in high molecular number on cultured endothelial cell membranes (more than 9×10^5 per cell), its concentration may change in different types of vessels in vivo and a modification in its expression may modify vascular barrier function.

cAMP and Rap-1

It is known that cAMP-elevating drugs reduce permeability and attenuate inflammatory edema. The intracellular mediators are PKA and Epac/Rap1. PKA promotes barrier function in brain endothelial cells through its activity on TJ and AJ proteins. In recent years the small GTPase Rap1 has received specific attention, and the emerging picture is complex and intriguing (Kooistra et al., 2007). Rap1 is a ubiquitous mediator that acts in a complex signaling network to control several actin-regulated processes in addition to the organization of cell-to-cell junctions. A cooperative multifaceted relationship exists between VE-cadherin and Rap1 in endothelial cells. Rap1 enhances the adhesive properties of VE-cadherin (Kooistra et al., 2007). An Epac/Rap1-specific c-AMP analog decreases monolayer permeability by increasing VE cadherin adhesion (Fukuhara et al., 2005). On the other hand, VE-cadherin is required for junctional recruitment of MAGI-1, a scaffold for Rap1-activator PDZ-GEF (Sakurai et al., 2006). Therefore Rap1 and VE-cadherin can reciprocally influence one another to modulate endothelial responses and barrier function. Several other small GTPases are also able to modulate AJ organization and endothelial permeability, as discussed in an excellent and detailed review by Wojciak-Stothard and Ridley (2002) on this subject. The picture that emerges from the many different results published over the past few years suggests that cadherin adhesive and signaling activities are controlled by a complex machinery of intracellular partners (Figures 2 and 3). Together, the components of this machinery form a dynamic structure that can be modified and adapted according to the functional circumstances of the cells. As discussed below, mutations in many of the genes encoding the interacting components of this complex can either directly lead to, or increase susceptibility to, defects in vascular permeability and/or vascular integrity.

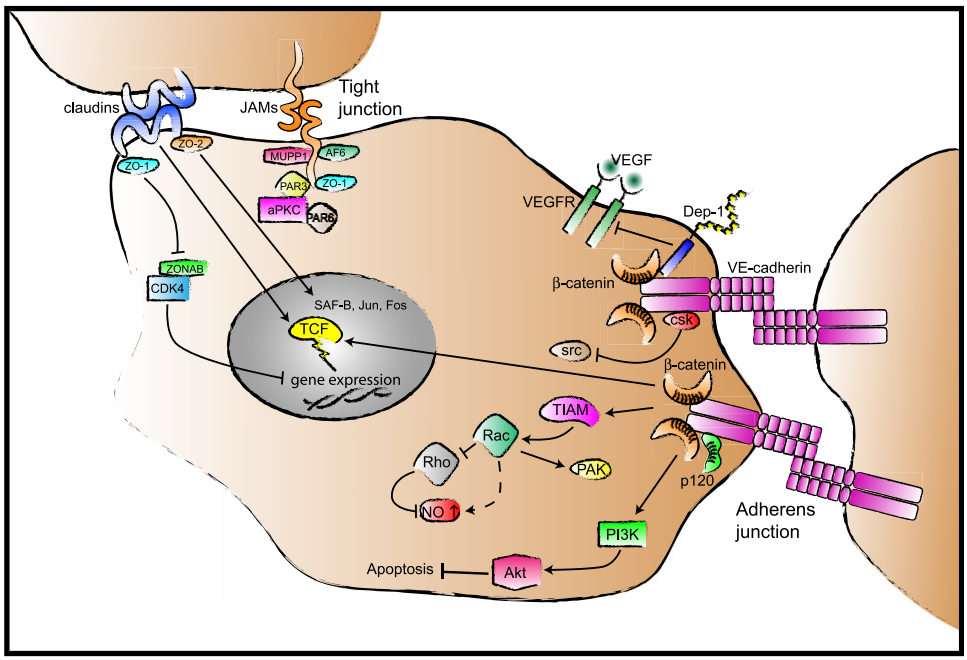

Figure 3. Signaling Pathways Downstream of AJs and TJs

At AJs, VE cadherin-β catenin complex binds different intracellular partners such as the kinase Csk which is a Src inhibitor, VEGFR-2 which may be dephosphorylated by the phosphatase Dep-1, TIAM which induces Rac and PAK activation, and PI3-kinase which in turn activates Akt and limit cell apoptosis. When it is stabilized in the cytosol, free β-catenin can translocate to the nucleus and modulate cell transcription. At TJs claudins can link to members of the ZO family which can also act as transcriptional factors. Members of the JAM family can associate to different signaling proteins which include the PAR3/PAR6 aPKC polarity complex, ZO1, AF6/afadin, or the phosphatase MUPP1. More details are found in the text and in Liebner et al., 2006, and Vestweber, 2008.

Endothelial Junctions and the Maintenance of Vascular Integrity

There is a close relationship between the control of permeability and vascular integrity. In many cases the opening and closure of endothelial junctions is transient and permeability control is re-established in a relatively short time. However, in some pathological conditions, junction dismantling may induce more dramatic and irreversible changes in vascular integrity. One possible reason for this is that endothelial junctions not only mediate cell-to-cell adhesion, but can also transfer intracellular signals that communicate cell position, limit growth and apoptosis, and regulate vascular stability. Therefore, modifications of the molecular architecture of junctions may have complex consequences for vascular homeostasis (Dejana, 2004). Junctional complexes trigger intracellular signals in endothelial cells in a variety of different ways (Figure 3). They can do so directly by engaging signaling proteins, or indirectly by limiting the nuclear translocation of proteins that modulate transcription. Examples of direct signaling through PI3-kinase activation, mitogen-activated protein kinase (MAPK), or small GTPases have been reported for different cadherins (for review see Dejana, 2004). Cadherins may also associate with growth factor receptors and modulate their signaling properties. For instance, VE-cadherin can form a multiprotein complex with VEGFR2 and limit its internalization and proliferative signals (Lampugnani et al., 2006). VE-cadherin association with the TGF-β receptor complex, however, induces coupling of TGFβ receptor II and receptor I (Alk 1 and Alk 5) and increases TGFβ signaling to inhibit cell growth and motility (Rudini et al., 2008). In other cell types, N-cadherin has been shown to bind to the FGF receptor and reduce its internalization, although direct evidence that this also occurs in endothelial cells is still lacking, and E-cadherin has been shown to interact with the EGF receptor. Together these results suggest that cadherin modulation of cellular responses to growth factors is a general feature of these proteins (for review see Liebner et al., 2006).

As mentioned above, junctional proteins can also shuttle from the membrane to the nucleus to influence transcription. β-catenin is a well-studied example of this paradigm. β-catenin is a crucial member of the canonical Wnt signaling pathway, where it modulates the transcriptional activity of lymphoid enhancer factor (Lef-1)/T cell factor (TCF) proteins. When β-catenin is free and stabilized in the cytoplasm it translocates to the nucleus, displaces the transcriptional repressors Groucho/TLE from their binding to Lef-1/TCF, and activates target gene transcription (Clevers, 2006). Similarly, the armadillo protein $p120^{ctn}$ also translocates to the nucleus under certain conditions to facilitate the release of the repressor *Kaiso* from Lef-1/TCF and increase target gene transcription (Park et al., 2006). In endothelial cells, β-catenin is mainly known as a structural component of AJs, and very little information is available on its function in signaling. Endothelial-specific deletion of the β-catenin gene in mice causes an embryonic lethal phenotype due to alterations in vascular and heart valve development (Liebner et al., 2004). Recent evidence indicates that Wnt/β-catenin signaling is responsible for endothelial cell expression of blood brain barrier characteristics (Liebner et al., 2008). By analogy to findings in other cell types, it seems likely that when cadherins are downregulated, or when the binding of β-catenin to the

cadherin tail is decreased by phosphorylation, free β-catenin protein will be able to translocate to the nucleus and modulate endothelial cell transcription. This is supported by recent work showing that in VE-cadherin null endothelial cells or in sparsely plated endothelial cells in culture, β-catenin signaling at the nucleus is increased and acts in concert with FOXO1 to modulate gene transcription (Taddei et al., 2008). ZO proteins associated with TJs also contain several nuclear localization and export signals. In other cell types, ZO-1 and ZO-2 become concentrated in the nucleus when cells are cultured in sparse conditions, i.e., they do not contact each other and junctions are not organized, or when they are exposed to stress. ZO proteins may associate with different transcription factors such as ZONAB, which is also found at TJs. These interactions promote cell proliferation which is conversely limited when ZO proteins are entrapped at the junctions (Matter and Balda, 2003).

Overall, most of the data suggest that the direct or indirect signals transferred by junctional structures mediate cell stability. In the endothelium this may be translated into maintenance of vascular integrity. These observations suggest that the resting state of endothelial cells is an active rather than constitutive/passive condition, and that it requires complex and sustained signaling processes for its maintenance. In support of this idea, recent work shows that continuous and physiological FGF signaling is required to maintain the integrity of endothelial AJs. When FGF signaling is blocked by a soluble receptor in vitro and in vivo, endothelial cells retract and eventually detach from the vessel wall leading to strong alterations in vascular integrity. This effect is mediated by phosphorylation and internalization of VE-cadherin, which cause dismantling of junctions and endothelial damage (Murakami et al., 2008).

Recent reports identified other mechanisms of inhibition of vascular permeability mediated by the junctional recruitment of the angiopoietin receptor Tie2 (Saharinen et al., 2008; Fukuhara et al., 2008). However, it is still unclear whether vascular stability mediated by these mechanisms is mediated by junctional proteins and/or VE-cadherin in particular.

Pathologies of Vessel Morphogenesis and Junctional Assembly

Altered vascular integrity and its major complication, bleeding, are hallmarks of vascular malformations in human patients. Vascular malformations are localized defects associated with abnormal angiogenesis. Most of these defects are sporadic, but Mendelian inheritance occurs in some families. The recent identification of genes involved in some of these Mendelian conditions has revealed new factors playing essential and often unsuspected roles in angiogenesis, vascular homeostasis, and vascular integrity. The challenge now is to determine the in vivo function of these new genes. In a step toward this goal, increased understanding of the mechanisms involved in vascular morphogenesis and vascular integrity has provided essential clues to help decipher the mechanisms of these conditions.

Vascular malformations can affect any part of the vascular tree, including arteries, veins, capillaries, or a combination of these segments (for review see Brouillard and Vikkula, 2007). They may affect any organ, but the most common and pathologically significant locations are the skin, the gastrointestinal tract, and the brain. In addition to pain and major esthetic problems, they may threaten life due to bleeding. In the context of this review, we will focus on vascular malformations of the brain (VMB) which cause serious neurological disability or death in a significant proportion of patients due to cerebral hemorrhage.

The two main classes of VMB are *b*rain *a*rterio-*v*enous *m*alformations (BAVM) and *c*erebral *c*avernous *m*alformations (CCM). BAVM are high flow malformations characterized by a complex conglomeration of dilated arteries and veins that lacks a capillary bed and results in arteriovenous shunting (Friedlander, 2007). Both conditions are thought to arise during embryonic, fetal, and/or postnatal stages. Most occurrences are sporadic, but they can also occur in families segregating as autosomal dominant conditions such as *c*apillary *m*alformation-*a*rteriovenous *m*alformations (CM-AVM) and *h*ereditary *h*emorrhagic *t*elangiectasia (HHT). The gene associated with CM-AVM encodes RASA1/p120RasGAP, a Ras GTPase activating protein which negatively regulates the Ras MAP kinase pathway (Revencu et al., 2008). CM-AVM patients are heterozygous for loss-of-function mutations in this gene. However, the localized and multifocal nature of CM-AVM lesions suggests that a somatic second hit of this gene may be necessary for their manifestation, as shown in other vascular hereditary malformations (Limaye et al., 2008). Recent data on the activation of p190RhoGAP by RASA1 and the role of the p190RhoGAP and p120-catenin in AJ assembly has provided some clues on the possible role of RASA1 in cell-cell junction homeostasis (Wildenberg et al., 2006). RASA1 has also been shown to bind to and activate Akt. However, the exact mechanisms leading to CM-AVM malformations are still currently unknown. BAVM also occur in HHT. The two HHT genes identified so far, endoglin and ACVRL1/ALK1, encode TGFβ superfamily receptors mainly expressed in endothelial cells and are associated to VE-cadherin (see above) (Rudini et al., 2008). The important role played by TGFβ in vascular remodeling and vessel wall integrity through its functions in endothelial cells and differentiation of vascular smooth muscle cells has been well established (for review see ten Dijke et al., 2008).

CCM are slow flow malformations characterized by densely packed vascular sinusoids embedded in a collagen matrix without intervening neural tissue (Figures 4A and 4B). These clusters are lined by a thin endothelium and rare subendothelial cells. Ultrastructural analysis shows structural defects, a paucity of endothelial cell TJs, and an absence of astrocyte end feet within the lesions. These data suggest that the leakiness of these lesions and the heavy hemosiderin deposits underlying the vessels might be caused by a blood brain barrier dysfunction (Clatterbuck et al., 2001). CCM bleeding has been involved in 10% of young patients showing intracerebral hemorrhage and recurrent bleeding is the major risk endured by patients affected with a familial form of the disease (FCCM), which is characterized by lesion multiplicity (Labauge et al., 2007). The three CCM genes identified so far (KRIT1/CCM1, MGC4607/CCM2, and PDCD10/CCM3) encode nonhomologous proteins whose role in angiogenesis and vascular homeostasis was completely unsuspected. All mutations of FCCM patients lead to a loss of function. Biallelic loss-of-function mutations are most likely required for CCM lesions to arise (Gault et al., 2005). Recent data strongly suggest that CCM proteins are members of a large complex involved in cell-cell junction homeostasis and

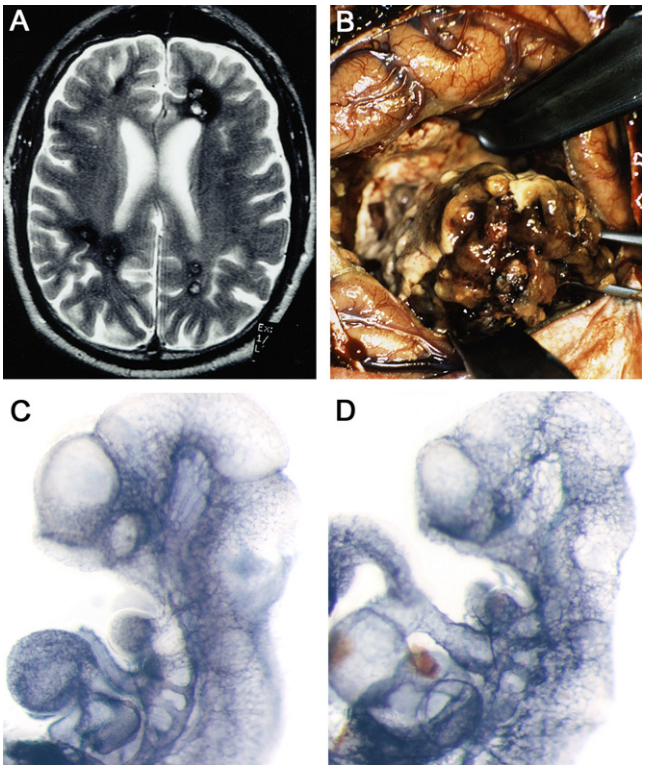

Figure 4. CCM Lesions from a Human Patient and Abnormal Vessel Remodeling in a Transgenic Mouse Embryo in which CCM2 Has Been Ablated from Endothelial Cells
(A) Cerebral MRI showing multiple CCM lesions.
(B) Surgical view of a CCM lesion.
(C and D) Whole mount PECAM staining of control (C) and transgenic embryos (D) at E10.5 showing abnormal vascular remodeling in the aorta, outflow tract, and head vessels.

cytoskeleton remodeling (Hilder et al., 2007). CCM1 and CCM3 interact with CCM2 (Figure 2). CCM2 is a scaffold protein that binds to actin, the GTPase Rac, and the upstream kinases MEKK3 and MKK3, and is involved in p38 MAP kinase-mediated control of osmolarity stress (Uhlik et al., 2003). CCM1 interacts with ICAP1, a modulator of integrin β1, and CCM3 binds to various STK kinases and phosphatases (Zawistowski et al., 2002; Goudreault et al., 2008). Recent data strongly suggest that CCM1 is a specific Rap1 effector regulating endothelial cell-cell junctions (Glading et al., 2007). The CCM1 FERM domain is unmasked by the activated Rap1 and controls the junctional localization of CCM1, which has been shown to interact with β-catenin and AF6/afadin. Interestingly, ICAP1 and CCM2 are involved in Rho GTPase pathways, which in turn are known to regulate the integrity of tight junctions. Altogether these data strongly suggest that CCM proteins may be involved in cell-cell junction integrity.

Animal Models of Vascular Malformation Disorders

Despite the identification of the affected genes in several inherited vascular disorders, our understanding of the mechanisms underlying vascular lesion formation in these diseases is still very incomplete. Most of the human disorders are highly variable in penetrance and onset, and the location and timing of lesion formation are unpredictable, making it impossible to observe and study the earliest, proximal steps in disease manifestation. Animal models, in particular mice and zebrafish (Figure 5A), have been critical for in vivo experimental analysis of the molecular and cellular mechanisms underlying vascular diseases, and have begun to yield new insights into their etiology. We will briefly discuss, as examples, HHT and CCM and some of the novel insights that have come from exploring the in vivo roles of disease genes using animal models.

Animal Models of Hereditary Hemorrhagic Telangiectasia

As discussed above, defects in the TGFβ receptor superfamily members Endoglin and ALK1 have been shown to be the causative lesions in HHT type 1 (HHT1) and HHT type 2 (HHT2), respectively (Lebrin and Mummery, 2008). Murine models have been developed for both HHT1 (Arthur et al., 2000; Bourdeau et al., 1999) and HHT2 (Bourdeau et al., 1999; Seki et al., 2003; Urness et al., 2000). Endoglin null embryos die at approximately E10.5 with vascular and cardiac defects (Arthur et al., 2000). Endothelial cells are specified and the yolk sac vasculature develops into a primitive vascular plexus, but it fails to remodel and mature beyond this stage. Vascular channels throughout the animals appear dilated, and the animals also develop extensive hemorrhaging indicative of vascular fragility. Heart defects are also noted, with the atrioventricular canal endocardium failing to undergo mesenchymal transformation and cushion-tissue formation. Mice homozygous null for ALK1 also die by mid-gestation, with severe vascular malformations including fusion of major arteries and veins (Seki et al., 2003; Urness et al., 2000) and defects in placental vascular development (Hong et al., 2007). As the human disorders are autosomal dominant conditions, mice heterozygous for Endoglin or ALK1 null mutants have been carefully examined for progressive signs of HHT-like lesions. Although initial work suggested a very low penetrance for lesion formation in heterozygous mice, it was found that the incidence of lesion formation is highly dependent on genetic background and/or the presence of additional genetic defects (Arthur et al., 2000; Bourdeau et al., 1999; Marchuk et al., 2003). Expression of ACVRL1 proteins harboring different mutations found in human patients in either cell culture or in zebrafish embryos confirmed that most of these mutants do not act in a dominant-negative fashion, supporting the view that HHT is a haploinsufficiency disorder that requires additional environmental and/or genetic "triggers" to initiate lesion formation (Baumer et al., 2006).

Some of the evidence obtained from murine studies has suggested a role for ALK1/Endoglin signaling in arterial-venous differentiation. In an initial study it was reported that ALK1 null embryos develop large arterial-venous shunts, downregulate the arterial marker ephrinB2, and display intravascular hematopoiesis in both veins and arteries instead of only embryonic arteries (Urness et al., 2000). Vascular remodeling defects have been noted in mice with defects in arterial-venous (A-V) differentiation, including ephrinB2 or EphB4 (Adams et al., 1999; Gerety and Anderson, 2002; Gerety et al., 1999; Wang et al., 1998), Notch 1/4 (Krebs et al., 2000), Notch ligand Dll4 (Duarte et al., 2004; Gale et al., 2004; Krebs et al., 2004), Notch effector Rbpj and Mib, and Hey1/Hey2 double mutants (Fischer et al., 2004; Kokubo et al., 2004; Koo et al., 2005; Krebs et al., 2004). This

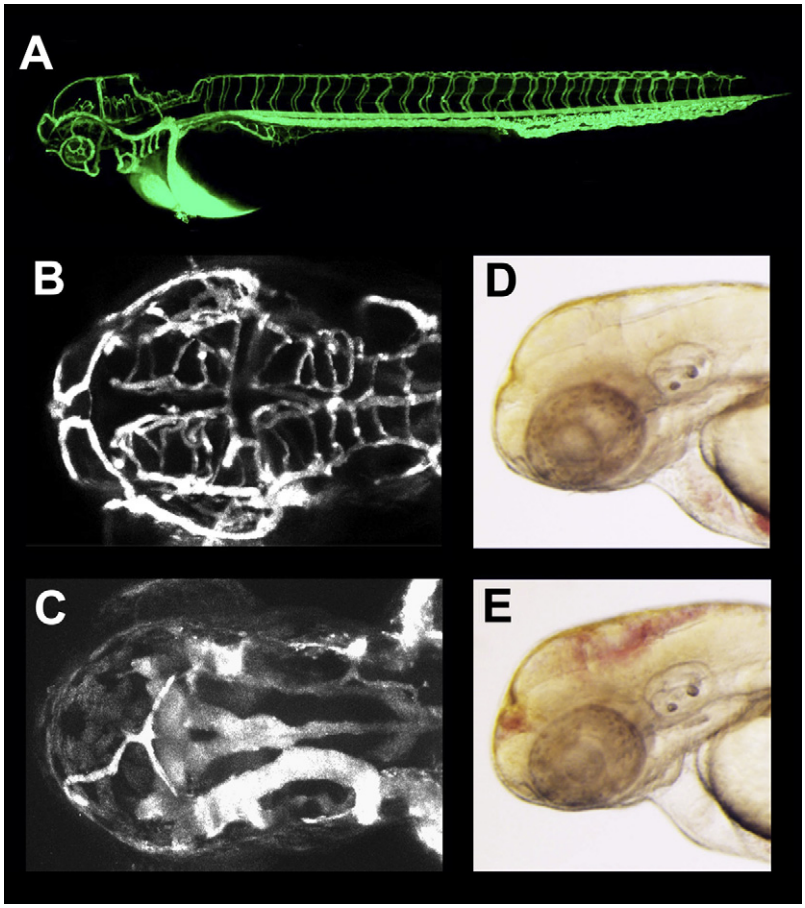

Figure 5. Zebrafish Models of Hereditary Hemorrhagic Telangiectasia, HHT, and Cerebral Cavernous Malfomations, CCM

(A) Blood vessels are readily visualized in living zebrafish embryos and larvae, as shown in this confocal microangiographic image of the vasculature of a 3 day old zebrafish larva.

(B and C) Confocal microangiographic images of the cranial vasculature of 3 day old wild-type (B) and *violet beauregarde (alk1/acvrl1)* mutant animals. Mutants display highly enlarged cranial vessels with increased numbers of endothelial cells.

(D and E) Transmitted light images of 2 day old wild-type (D) and CCM pathway gene knockdown (E) zebrafish.

Images are: (A) Lateral view, anterior to the left, from Isogai et al., 2001; (B and C) Dorsal view of the head, anterior to the left; (D and E) Lateral view of the head, anterior to the left, adapted from Gore et al., 2008.

has led to the suggestion that ALK1 is required for A-V identity and for developing distinct arterial and venous vascular beds. However, vascular remodeling defects and a failure to properly differentiate arteries and veins have been noted in a number of different knockout models that do not directly affect arterial-venous differentiation, and it is not always clear that A-V morphology defects represent proximal effects of loss of the targeted gene as opposed to secondary consequences. Brain arterial venous malformations most similar to those found in HHT have not been reported so far in human patients carrying Notch receptor/ligand mutants. Furthermore, analysis of Endoglin null mice showed that, unlike ALK1 mutants, they do not show profound vessel dilation or downregulate arterial ephrinB2 (Sorensen et al., 2003). Analysis of an ALK1 LacZ knockin mouse also showed that this gene is expressed primarily in larger arteries (Seki et al., 2003), suggesting it probably does not have a direct functional role in venous vessels.

A variety of recent evidence has suggested that rather than affecting A-V identity, the proximal role of ALK1 signaling might be to regulate proliferation and the "quiescence state" of the endothelium. In a zebrafish model for HHT2, the "*Violet beauregarde*" mutant (Figures 5B and 5C), affected vessels did not display any apparent defect in differentiation but did contain many more endothelial cells than normal, indicating that the mutant caused an endothelial over proliferation (Park et al., 2008; Roman et al., 2002). Other work, including a recent study identifying a probable in vivo ligand for ALK1 (see below), has supported the idea that signaling through this receptor regulates the "activation state" of the endothelium (Park et al., 2008). It may be that the defects in arterial-venous differentiation noted in some studies could be a secondary consequence of a general failure of ALK1-deficient endothelial cells to cease proliferating and take on a quiescent differentiated state.

As noted above, ALK1 and Endoglin are TGFβ superfamily receptors and a great deal of interest has been focused on potential signaling upstream and downstream from these genes. Based on a variety of evidence it has been proposed that TGFβ can activate downstream Smad signaling through both ALK1 and ALK5 TGFβ type1 receptors in endothelial cells, with opposing effects. TGFβ/ALK5 signaling inhibits endothelial cell migration and proliferation, while TGFβ/ALK1 signaling promotes endothelial cell migration and proliferation, with the balance between ALK1 and ALK5 signaling regulating the cellular response (Goumans et al., 2002). However, more recent studies have cast doubt on both the role of opposing signaling between ALK1 and ALK5 in vivo and whether TGFβ is the physiologically relevant ligand for ALK1. Examination of mice with lacZ knocked into either the ALK1 or ALK5 genes revealed that they are not expressed in the same cells; ALK1 is expressed in endothelial cells, while ALK5 is expressed in vascular smooth muscle cells (Seki et al., 2006). It was assumed that TGFβ activated downstream signaling by binding to either ALK1 or ALK5 Type I receptors in combination with the TGFβ type 2 receptor (TFGβR2). However, when Alk1-cre mice were used to perform cre-mediated restricted excision of the ALK1, ALK5, or TGFβR2 genes, only deletion of ALK1 resulted in a vascular malformation defect (Park et al., 2008), suggesting that ALK5 and TGFβR2 are not required for ALK1 signaling or involved in the pathology of HHT. Evidence from a recent study also indicates that the likely physiological ligand for ALK1 is BMP9, not TGFβ, and that this ligand is a potent anti-angiogenic factor (David et al., 2008). Interestingly, the results of this paper also suggest that high levels of circulating BMP9 play a role in maintaining adult blood vessel quiescence.

Animal Models of Cerebral Cavernous Malformation

As described above, human familial CCM syndromes have been linked to mutations at three loci: CCM1/Krit1, CCM2/Malcavernin, and CCM3/PDCD10. A number of studies (such as Hilder et al., 2007; Plummer et al., 2005; Zawistowski et al., 2005) have demonstrated that the three CCM proteins physically interact with one another and with a variety of additional protein partners including the small ras-family GTPase Rap1 (CCM1/Krit1 was originally cloned by virtue of its interaction with Rap1/Krev1, hence its name "Krev interaction trapped 1," or Krit1; Serebriiskii et al., 1997). Expression studies performed using in situ hybridization, immunohistochemistry, or analysis of knockin alleles have shown that all three CCM genes are similarly expressed in both neurons and blood vessels in the mouse brain (Petit et al., 2006; Plummer et al., 2006; Tanriover et al., 2008). CCM1 and CCM2 are also expressed in blood vessels and selected neural tissues in the zebrafish (Gore et al., 2008; Hogan et al., 2008; Mably et al., 2006). The strong expression of the CCM genes in neural tissue and the neural involvement in lesion formation in CCM patients has led to suggestions that cavernous malformations may be the result of a primary defect in surrounding neural cells rather than blood vessel endothelial cells (Plummer et al., 2006). As discussed below, however, recent animal studies suggest that despite their expression in neurons it is the endothelial function of these genes that is critical for maintaining vascular integrity.

CCM1/Krit1 knockout mice demonstrated that this gene is required for proper vascular development (Whitehead et al., 2004). Homozygous mutant embryos die in mid-gestation with dilated, thin-walled cranial vessels and enlarged caudal dorsal aorta with an increased number of endothelial cells. Downregulation of arterial-specific gene expression is also observed. The vascular defects emerge in the absence of obvious alterations in neural morphology or neural marker expression, leading the authors of the initial knockout study to suggest that the primary defect is in endothelial rather than neural cells. Like HHT, CCM is an autosomal dominant disorder, and virtually all mutants discovered to date in human CCM patients have been clear loss-of-function alleles. An initial screening of heterozygous mice failed to detect cavernous malformations. To test the hypothesis that the occurrence of CCM lesions might require a second somatic hit, CCM1 heterozygous mice were bred with $p53^{-/-}$ mice that show an increased rate of somatic mutations. Vascular lesions were observed in the brains of 55% of the double mutant animals (Plummer et al., 2004). However, no somatic hit was detected within the CCM1 wild-type allele in the double transgenic mutants, suggesting that the appearance of vascular lesions might reflect a direct role of p53 deficiency in promoting the genesis of vascular malformations. However, a second hit in any of the two other CCM genes or interacting genes cannot be excluded. The idea that otherwise phenotypically silent second site "hits" in interacting genes could be predisposing to or initiating lesion formation in CCM has been explored directly in more detail using the zebrafish (see below).

Fortuitously, a gene trap insertion was discovered in the CCM2 gene (Plummer et al., 2006). Heterozygous gene trap insertion mice develop cerebral vascular malformations, although the penetrance is low, as in many murine models of vascular malformation disorders. In heterozygotes, β-galactosidase from the gene-trap allele is strongly expressed in neurons and choroid plexus as well as larger vessels, but is not clearly visualized in vascular endothelium of small vessels in the brain (like CCM1 and CCM3; see above), leading the authors of this study to propose that "cerebral cavernous malformations arise as a result of defects in the neural parenchyma surrounding the vascular endothelial cells in the brain." However, in both zebrafish and mice, it appears that endothelial and not neural cell alterations are responsible for CCM lesions.

Mutants in both CCM1 ("*santa*" mutants) and CCM2 ("*valentine*" mutants) have been isolated in the zebrafish (Mably et al., 2006). Both of these mutants were discovered based on their enlarged heart cardiac phenotypes (enlarged, poorly functional hearts are also noted in homozygous CCM1-deficient mice). Like their mammalian counterparts, the zebrafish CCM genes are also expressed in the vasculature, and several recent studies have examined the functional requirement for CCM genes in that context. Loss of CCM1 or CCM2 in fish leads to defects in the vasculature in addition to the cardiac defects, with formation of enlarged, thin-walled vessels (Gore et al., 2008; Hogan et al., 2008). In one report, blastomere transplantation methods were used to show that wild-type endothelial cells could adopt a "wild-type" morphology when they integrated into vessels in CCM1 mutant animals, suggesting that CCM1 is required cell autonomously for regulation of endothelial cell shape (Hogan et al., 2008). An endothelial cell-autonomous requirement for CCM1 function has also been demonstrated in zebrafish by using transgenic endothelial-specific expression of CCM1 to "rescue" cranial vascular hemorrhage caused by antisense morpholino-mediated knockdown of endogenous CCM1 (Gore et al., 2008). However, recent work in the mouse has provided what is probably the most conclusive evidence to date that the primary defects in CCM are endothelial-specific. Ubiquitous, endothelial-, or neural-specific CCM2 knockout mice were generated using cre-lox technology and examined for homozygous phenotypes. Animals homozygous for a ubiquitous deletion of CCM2 die during early embryogenesis, like CCM1 knockouts. However, despite the high level of expression of this gene in the neuroepithelium, targeting of CCM2 in neuroglial precursor cells does not lead to cerebrovascular defects. By contrast, endothelial-specific knockout of CCM2 severely affects angiogenesis and leads to major heart, arterial, and venous morphogenesis defects and embryonic lethality at mid-gestation (Figures 4C and 4D). Thus, while further analysis will be required to verify a specific functional requirement for the other CCM genes in endothelium versus neuroepithelium, it appears that the proximal defect in CCM is probably initiated within the endothelium (Boulday et al., 2009).

The results of a recent study in the zebrafish have also provided new insights into the etiology of CCM (Gore et al., 2008). As noted above, the incidence of lesion formation is highly variable within affected CCM families, and the factors that trigger intracranial hemorrhage (ICH) in either inherited or sporadic forms of the disease are not understood. Not all individuals harboring defective CCM genes develop ICH, reflecting incomplete penetrance of these mutations and/or involvement of additional genetic modifiers predisposing to lesion formation (Lucas et al., 2003). As also noted above, there is ample evidence that CCM genes act together in common intracellular complexes

and/or signaling pathways (Dupre et al., 2003; Hilder et al., 2007; Plummer et al., 2005; Voss et al., 2007; Zawistowski et al., 2005), suggesting that minor functional perturbations of different genes in these complexes or pathways might act together to precipitate ICH. However, demonstrating this sort of multigene association is not possible in the available small human CCM pedigrees. By injecting specialized morpholino antisense oligonucleotides into zebrafish embryos, Gore et al. (2008) simultaneously inhibited the expression of multiple CCM pathway genes in various combinations. They showed that a subtle decrease in each of these genes alone caused little or no effect independently, but when combined resulted in very high frequencies of ICH (Figures 5D and 5E). Thus, small, individually silent defects in the CCM pathway can strongly synergize to increase susceptibility to ICH. These findings have important implications. Single heterozygous mutations in CCM proteins may not be enough to induce stroke, but may require accompanying subtle secondary mutations to "trigger" lesion formation. Mutations in CCM pathway effectors may contribute to the highly variable penetrance of familial CCM disorders. Subtle genetic second hits in individuals that are outwardly normal but "sensitized" by minor deficits in CCM pathway genes could also lead to sporadic forms of hemorrhagic stroke. Together, the mouse and zebrafish studies discussed above illustrate the power of these animal models for dissecting the in vivo functional roles of identified human vascular malformation disease genes.

Concluding Remarks

Understanding the molecular basis for vascular malformation disorders and the defects in vascular integrity that lead to hemorrhage and stroke requires a three-pronged approach. In vitro studies are needed to study the basic cell biology of endothelial junction formation, and probe the functional roles of genes implicated in vascular pathobiology through in vivo studies in human patients and animal models. Clinical studies are needed to identify the genetic causes of vascular disease in humans and examine how vascular malformation lesions and endothelial integrity defects develop in human patients. And finally, animal models such as mice and zebrafish are a vital tool for testing the in vivo functional roles of identified junction/vascular disease genes, and to uncover additional interacting genes. It is important to fully integrate together each of these approaches to achieve a holistic understanding of the etiology of vascular malformation/junctional integrity disorders and provide the greatest opportunity for developing effective therapies to ameliorate or prevent these conditions. In this review we have attempted to illustrate how recent findings from each of these three approaches can be integrated to provide progress toward this goal.

ACKNOWLEDGMENTS

This work was supported by the Fondation Leducq Transatlantic Network of Excellence, Associazione Italiana per la Ricerca sul Cancro, Association for International Cancer Research UK, the European Community (Integrated Project Contract No LSHG-CT-2004-503573; NoE MAIN 502935; NoE EVGN 503254; EUSTROKE and OPTISTEM Networks), and Istituto Superiore di Sanita', Italian Ministry of Health, MIUR (COFIN prot: 2006058482_002). B.M.W. was supported by the Intramural Research Program of the NIH (NICHD).

REFERENCES

Adams, R.H., Wilkinson, G.A., Weiss, C., Diella, F., Gale, N.W., Deutsch, U., Risau, W., and Klein, R. (1999). Roles of ephrinB ligands and EphB receptors in cardiovascular development: demarcation of arterial/venous domains, vascular morphogenesis, and sprouting angiogenesis. Genes Dev. *13*, 295–306.

Arthur, H.M., Ure, J., Smith, A.J., Renforth, G., Wilson, D.I., Torsney, E., Charlton, R., Parums, D.V., Jowett, T., Marchuk, D.A., et al. (2000). Endoglin, an ancillary TGFβ receptor, is required for extraembryonic angiogenesis and plays a key role in heart development. Dev. Biol. *217*, 42–53.

Baluk, P., Fuxe, J., Hashizume, H., Romano, T., Lashnits, E., Butz, S., Vestweber, D., Corada, M., Molendini, C., Dejana, E., et al. (2007). Functionally specialized junctions between endothelial cells of lymphatic vessels. J. Exp. Med. *204*, 2349–2362.

Baumer, S., Keller, L., Holtmann, A., Funke, R., August, B., Gamp, A., Wolburg, H., Wolburg-Buchholz, K., Deutsch, U., and Vestweber, D. (2006). Vascular endothelial cell-specific phosphotyrosine phosphatase (VE-PTP) activity is required for blood vessel development. Blood *107*, 4754–4762.

Bazzoni, G., and Dejana, E. (2004). Endothelial cell-to-cell junctions: molecular organization and role in vascular homeostasis. Physiol. Rev. *84*, 869–901.

Birdsey, G.M., Dryden, N.H., Amsellem, V., Gebhardt, F., Sahnan, K., Haskard, D.O., Dejana, E., Mason, J.C., and Randi, A.M. (2008). Transcription factor Erg regulates angiogenesis and endothelial apoptosis through VE-cadherin. Blood *111*, 3498–3506.

Boulday, G., Blécon, A., Petit, N., Chareyre, F., Garcia, L.A, Niwa-Kawakita, M., Giovannini, M., and Tournier-Lasserve, E. (2009). Tissue-specific conditional CCM2 knock out mice establish the essential role of endothelial CCM2 for angiogenesis: implications for human Cerebral Cavernous Malformations. Dis. Model Mech., in press. Published online February 2. doi: 10.1242/dmm.001263.

Bourdeau, A., Dumont, D.J., and Letarte, M. (1999). A murine model of hereditary hemorrhagic telangiectasia. J. Clin. Invest. *104*, 1343–1351.

Brouillard, P., and Vikkula, M. (2003). Vascular malformations: localized defects in vascular morphogenesis. Clin. Genet. *63*, 340–351.

Brouillard, P., and Vikkula, M. (2007). Genetic causes of vascular malformations. Hum. Mol. Genet. *16*, R140–R149.

Capaldo, C.T., and Macara, I.G. (2007). Depletion of E-cadherin disrupts establishment but not maintenance of cell junctions in Madin-Darby canine kidney epithelial cells. Mol. Biol. Cell *18*, 189–200.

Carmeliet, P., and Jain, R.K. (2000). Angiogenesis in cancer and other diseases. Nature *407*, 249–257.

Cavey, M., Rauzi, M., Lenne, P.F., and Lecuit, T. (2008). A two-tiered mechanism for stabilization and immobilization of E-cadherin. Nature *453*, 751–756.

Clatterbuck, R.E., Eberhart, C.G., Crain, B.J., and Rigamonti, D. (2001). Ultrastructural and immunocytochemical evidence that an incompetent blood-brain barrier is related to the pathophysiology of cavernous malformations. J. Neurol. Neurosurg. Psychiatry *71*, 188–192.

Clevers, H. (2006). Wnt/β-catenin signaling in development and disease. Cell *127*, 469–480.

David, L., Mallet, C., Keramidas, M., Lamande, N., Gasc, J.M., Dupuis-Girod, S., Plauchu, H., Feige, J.J., and Bailly, S. (2008). Bone morphogenetic protein-9 is a circulating vascular quiescence factor. Circ. Res. *102*, 914–922.

Dejana, E. (2004). Endothelial cell-cell junctions: happy together. Nat. Rev. Mol. Cell Biol. *5*, 261–270.

Dejana, E., Orsenigo, F., and Lampugnani, M.G. (2008). The role of adherens junctions and VE-cadherin in the control of vascular permeability. J. Cell Sci. *121*, 2115–2122.

Deleuze, V., Chalhoub, E., El-Hajj, R., Dohet, C., Le Clech, M., Couraud, P.O., Huber, P., and Mathieu, D. (2007). TAL-1/SCL and its partners E47 and LMO2 up-regulate VE-cadherin expression in endothelial cells. Mol. Cell. Biol. *27*, 2687–2697.

Duarte, A., Hirashima, M., Benedito, R., Trindade, A., Diniz, P., Bekman, E., Costa, L., Henrique, D., and Rossant, J. (2004). Dosage-sensitive requirement for mouse Dll4 in artery development. Genes Dev. *18*, 2474–2478.

Dupre, N., Verlaan, D.J., Hand, C.K., Laurent, S.B., Turecki, G., Davenport, W.J., Acciarri, N., Dichgans, J., Ohkuma, A., Siegel, A.M., et al. (2003). Linkage to the CCM2 locus and genetic heterogeneity in familial cerebral cavernous malformation. Can. J. Neurol. Sci. *30*, 122–128.

Dvorak, A.M., Kohn, S., Morgan, E.S., Fox, P., Nagy, J.A., and Dvorak, H.F. (1996). The vesiculo-vacuolar organelle (VVO): a distinct endothelial cell structure that provides a transcellular pathway for macromolecular extravasation. J. Leukoc. Biol. *59*, 100–115.

Engelhardt, B. (2003). Development of the blood-brain barrier. Cell Tissue Res. *314*, 119–129.

Fischer, A., Schumacher, N., Maier, M., Sendtner, M., and Gessler, M. (2004). The Notch target genes Hey1 and Hey2 are required for embryonic vascular development. Genes Dev. *18*, 901–911.

Friedlander, R.M. (2007). Clinical practice. Arteriovenous malformations of the brain. N. Engl. J. Med. *356*, 2704–2712.

Fukuhara, S., Sakurai, A., Sano, H., Yamagishi, A., Somekawa, S., Takakura, N., Saito, Y., Kangawa, K., and Mochizuki, N. (2005). Cyclic AMP potentiates vascular endothelial cadherin-mediated cell-cell contact to enhance endothelial barrier function through an Epac-Rap1 signaling pathway. Mol. Cell. Biol. *25*, 136–146.

Fukuhara, S., Sako, K., Minami, T., Noda, K., Kim, H.Z., Kodama, T., Shibuya, M., Takakura, N., Koh, G.Y., and Mochizuki, N. (2008). Differential function of Tie2 at cell-cell contacts and cell-substratum contacts regulated by angiopoietin-1. Nat. Cell Biol. *10*, 513–526.

Furuse, M., and Tsukita, S. (2006). Claudins in occluding junctions of humans and flies. Trends Cell Biol. *16*, 181–188.

Gale, N.W., Dominguez, M.G., Noguera, I., Pan, L., Hughes, V., Valenzuela, D.M., Murphy, A.J., Adams, N.C., Lin, H.C., Holash, J., et al. (2004). Haploinsufficiency of delta-like 4 ligand results in embryonic lethality due to major defects in arterial and vascular development. Proc. Natl. Acad. Sci. USA *101*, 15949–15954.

Gault, J., Shenkar, R., Recksiek, P., and Awad, I.A. (2005). Biallelic somatic and germ line CCM1 truncating mutations in a cerebral cavernous malformation lesion. Stroke *36*, 872–874.

Gavard, J., and Gutkind, J.S. (2006). VEGF controls endothelial-cell permeability by promoting the β-arrestin-dependent endocytosis of VE-cadherin. Nat. Cell Biol. *8*, 1223–1234.

Gavard, J., Patel, V., and Gutkind, J.S. (2008). Angiopoietin-1 prevents VEGF-induced endothelial permeability by sequestering Src through mDia. Dev. Cell *14*, 25–36.

Gerety, S.S., and Anderson, D.J. (2002). Cardiovascular ephrinB2 function is essential for embryonic angiogenesis. Development *129*, 1397–1410.

Gerety, S.S., Wang, H.U., Chen, Z.F., and Anderson, D.J. (1999). Symmetrical mutant phenotypes of the receptor EphB4 and its specific transmembrane ligand ephrin-B2 in cardiovascular development. Mol. Cell *4*, 403–414.

Glading, A., Han, J., Stockton, R.A., and Ginsberg, M.H. (2007). KRIT-1/CCM1 is a Rap1 effector that regulates endothelial cell cell junctions. J. Cell Biol. *179*, 247–254.

Gonzalez-Mariscal, L., Tapia, R., and Chamorro, D. (2008). Crosstalk of tight junction components with signaling pathways. Biochim. Biophys. Acta *1778*, 729–756.

Gore, A.V., Lampugnani, M.G., Dye, L., Dejana, E., and Weinstein, B.M. (2008). Combinatorial interaction between CCM pathway genes precipitates hemorrhagic stroke. Dis. Model Mech. *1*, 275–281.

Goudreault, M., D'Ambrosio, L.M., Kean, M.J., Mullin, M., Larsen, B.G., Sanchez, A., Chaudhry, S., Chen, G.I., Sicheri, F., Nesvizhskii, A.I., et al. (2008). PP2A phosphatase high-density interaction network identifies a novel striatin-interacting phosphatase and kinase complex linked to the cerebral cavernous malformation 3 (CCM3) protein. Mol. Cell. Proteomics; Epub ahead of print.

Goumans, M.J., Valdimarsdottir, G., Itoh, S., Rosendahl, A., Sideras, P., and ten Dijke, P. (2002). Balancing the activation state of the endothelium via two distinct TGF-β type I receptors. EMBO J. *21*, 1743–1753.

Hartsock, A., and Nelson, W.J. (2008). Adherens and tight junctions: structure, function and connections to the actin cytoskeleton. Biochim. Biophys. Acta *1778*, 660–669.

Hilder, T.L., Malone, M.H., Bencharit, S., Colicelli, J., Haystead, T.A., Johnson, G.L., and Wu, C.C. (2007). Proteomic identification of the cerebral cavernous malformation signaling complex. J. Proteome Res. *6*, 4343–4355.

Hirase, T., Staddon, J.M., Saitou, M., Ando-Akatsuka, Y., Itoh, M., Furuse, M., Fujimoto, K., Tsukita, S., and Rubin, L.L. (1997). Occludin as a possible determinant of tight junction permeability in endothelial cells. J. Cell Sci. *110*, 1603–1613.

Hogan, B.M., Bussmann, J., Wolburg, H., and Schulte-Merker, S. (2008). ccm1 cell autonomously regulates endothelial cellular morphogenesis and vascular tubulogenesis in zebrafish. Hum. Mol. Genet. *17*, 2424–2432.

Hong, K.H., Seki, T., and Oh, S.P. (2007). Activin receptor-like kinase 1 is essential for placental vascular development in mice. Lab. Invest. *87*, 670–679.

Isogai, S., Horiguchi, M., and Weinstein, B.M. (2001). The vascular anatomy of the developing zebrafish: an atlas of embryonic and early larval development. Dev. Biol. *230*, 278–301.

Johnson-Leger, C., and Imhof, B.A. (2003). Forging the endothelium during inflammation: pushing at a half-open door? Cell Tissue Res. *314*, 93–105.

Kametani, Y., and Takeichi, M. (2007). Basal-to-apical cadherin flow at cell junctions. Nat. Cell Biol. *9*, 92–98.

Kokubo, H., Miyagawa-Tomita, S., Tomimatsu, H., Nakashima, Y., Nakazawa, M., Saga, Y., and Johnson, R.L. (2004). Targeted disruption of hesr2 results in atrioventricular valve anomalies that lead to heart dysfunction. Circ. Res. *95*, 540–547.

Koo, B.K., Lim, H.S., Song, R., Yoon, M.J., Yoon, K.J., Moon, J.S., Kim, Y.W., Kwon, M.C., Yoo, K.W., Kong, M.P., et al. (2005). Mind bomb 1 is essential for generating functional Notch ligands to activate Notch. Development *132*, 3459–3470.

Kooistra, M.R., Dube, N., and Bos, J.L. (2007). Rap1: a key regulator in cell-cell junction formation. J. Cell Sci. *120*, 17–22.

Krebs, L.T., Xue, Y., Norton, C.R., Shutter, J.R., Maguire, M., Sundberg, J.P., Gallahan, D., Closson, V., Kitajewski, J., Callahan, R., et al. (2000). Notch signaling is essential for vascular morphogenesis in mice. Genes Dev. *14*, 1343–1352.

Krebs, L.T., Shutter, J.R., Tanigaki, K., Honjo, T., Stark, K.L., and Gridley, T. (2004). Haploinsufficient lethality and formation of arteriovenous malformations in Notch pathway mutants. Genes Dev. *18*, 2469–2473.

Labauge, P., Denier, C., Bergametti, F., and Tournier-Lasserve, E. (2007). Genetics of cavernous angiomas. Lancet Neurol. *6*, 237–244.

Lampugnani, M.G., Zanetti, A., Breviario, F., Balconi, G., Orsenigo, F., Corada, M., Spagnuolo, R., Betson, M., Braga, V., and Dejana, E. (2002). VE-cadherin regulates endothelial actin activating Rac and increasing membrane association of Tiam. Mol. Biol. Cell *13*, 1175–1189.

Lampugnani, M.G., Orsenigo, F., Gagliani, M.C., Tacchetti, C., and Dejana, E. (2006). Vascular endothelial cadherin controls VEGFR-2 internalization and signalling from intracellular compartments. J. Cell Biol. *174*, 593–604.

Lebrin, F., and Mummery, C.L. (2008). Endoglin-mediated vascular remodeling: mechanisms underlying hereditary hemorrhagic telangiectasia. Trends Cardiovasc. Med. *18*, 25–32.

Liebner, S., Cattelino, A., Gallini, R., Rudini, N., Iurlaro, M., Piccolo, S., and Dejana, E. (2004). β-catenin is required for endothelial-mesenchymal transformation during heart cushion development in the mouse. J. Cell Biol. *166*, 359–367.

Liebner, S., Cavallaro, U., and Dejana, E. (2006). The multiple languages of endothelial cell-to-cell communication. Arterioscler. Thromb. Vasc. Biol. *26*, 1431–1438.

Liebner, S., Corada, M., Bangsow, T., Babbage, J., Taddei, A., Czupalla, C.J., Reis, M., Felici, A., Wolburg, H., Fruttiger, M., et al. (2008). Wnt/β-catenin

signaling controls development of the blood-brain barrier. J. Cell Biol. *183*, 409–417.

Limaye, N., Wouters, V., Uebelhoer, M., Tuominen, M., Wirkkala, R., Mulliken, J.B., Eklund, L., Boon, L.M., and Vikkula, M. (2008). Somatic mutations in angiopoietin receptor gene TEK cause solitary and multiple sporadic venous malformations. Nat. Genet. *41*, 118–124.

Lucas, M., Costa, A.F., Garcia-Moreno, J.M., Solano, F., Gamero, M.A., and Izquierdo, G. (2003). Variable expression of cerebral cavernous malformations in carriers of a premature termination codon in exon 17 of the Krit1 gene. BMC Neurol. *3*, 5.

Mably, J.D., Chuang, L.P., Serluca, F.C., Mohideen, M.A., Chen, J.N., and Fishman, M.C. (2006). santa and valentine pattern concentric growth of cardiac myocardium in the zebrafish. Development *133*, 3139–3146.

Marchuk, D.A., Srinivasan, S., Squire, T.L., and Zawistowski, J.S. (2003). Vascular morphogenesis: tales of two syndromes. Hum. Mol. Genet. *12*, R97–R112.

Matter, K., and Balda, M.S. (2003). Signalling to and from tight junctions. Nat. Rev. Mol. Cell Biol. *4*, 225–236.

Michiels, F., Habets, G.G., Stam, J.C., van der Kammen, R.A., and Collard, J.G. (1995). A role for Rac in Tiam1-induced membrane ruffling and invasion. Nature *375*, 338–340.

Murakami, M., Nguyen, L.T., Zhang, Z.W., Moodie, K.L., Carmeliet, P., Stan, R.V., and Simons, M. (2008). The FGF system has a key role in regulating vascular integrity. J. Clin. Invest. *118*, 3355–3366.

Orlova, V.V., Economopoulou, M., Lupu, F., Santoso, S., and Chavakis, T. (2006). Junctional adhesion molecule-C regulates vascular endothelial permeability by modulating VE-cadherin-mediated cell-cell contacts. J. Exp. Med. *203*, 2703–2714.

Park, J.I., Ji, H., Jun, S., Gu, D., Hikasa, H., Li, L., Sokol, S.Y., and McCrea, P.D. (2006). Frodo links Dishevelled to the p120-catenin/Kaiso pathway: distinct catenin subfamilies promote Wnt signals. Dev. Cell *11*, 683–695.

Park, S.O., Lee, Y.J., Seki, T., Hong, K.H., Fliess, N., Jiang, Z., Park, A., Wu, X., Kaartinen, V., Roman, B.L., et al. (2008). ALK5- and TGFBR2-independent role of ALK1 in the pathogenesis of hereditary hemorrhagic telangiectasia type 2. Blood *111*, 633–642.

Petit, N., Blecon, A., Denier, C., and Tournier-Lasserve, E. (2006). Patterns of expression of the three cerebral cavernous malformation (CCM) genes during embryonic and postnatal brain development. Gene Expr. Patterns *6*, 495–503.

Plummer, N.W., Gallione, C.J., Srinivasan, S., Zawistowski, J.S., Louis, D.N., and Marchuk, D.A. (2004). Loss of p53 sensitizes mice with a mutation in Ccm1 (KRIT1) to development of cerebral vascular malformations. Am. J. Pathol. *165*, 1509–1518.

Plummer, N.W., Zawistowski, J.S., and Marchuk, D.A. (2005). Genetics of cerebral cavernous malformations. Curr. Neurol. Neurosci. Rep. *5*, 391–396.

Plummer, N.W., Squire, T.L., Srinivasan, S., Huang, E., Zawistowski, J.S., Matsunami, H., Hale, L.P., and Marchuk, D.A. (2006). Neuronal expression of the Ccm2 gene in a new mouse model of cerebral cavernous malformations. Mamm. Genome *17*, 119–128.

Revencu, N., Boon, L.M., Mulliken, J.B., Enjolras, O., Cordisco, M.R., Burrows, P.E., Clapuyt, P., Hammer, F., Dubois, J., Baselga, E., et al. (2008). Parkes Weber syndrome, vein of Galen aneurysmal malformation, and other fast-flow vascular anomalies are caused by RASA1 mutations. Hum. Mutat. *29*, 959–965.

Roman, B.L., Pham, V.N., Lawson, N.D., Kulik, M., Childs, S., Lekven, A.C., Garrity, D.M., Moon, R.T., Fishman, M.C., Lechleider, R.J., et al. (2002). Disruption of acvrl1 increases endothelial cell number in zebrafish cranial vessels. Development *129*, 3009–3019.

Rudini, N., Felici, A., Giampietro, C., Lampugnani, M., Corada, M., Swirsding, K., Garre, M., Liebner, S., Letarte, M., ten Dijke, P., et al. (2008). VE-cadherin is a critical endothelial regulator of TGF-β signalling. EMBO J. *27*, 993–1004.

Saharinen, P., Eklund, L., Miettinen, J., Wirkkala, R., Anisimov, A., Winderlich, M., Nottebaum, A., Vestweber, D., Deutsch, U., Koh, G.Y., et al. (2008). Angiopoietins assemble distinct Tie2 signalling complexes in endothelial cell-cell and cell-matrix contacts. Nat. Cell Biol. *10*, 527–537.

Sakurai, A., Fukuhara, S., Yamagishi, A., Sako, K., Kamioka, Y., Masuda, M., Nakaoka, Y., and Mochizuki, N. (2006). MAGI-1 is required for Rap1 activation upon cell-cell contact and for enhancement of vascular endothelial cadherin-mediated cell adhesion. Mol. Biol. Cell *17*, 966–976.

Seki, T., Yun, J., and Oh, S.P. (2003). Arterial endothelium-specific activin receptor-like kinase 1 expression suggests its role in arterialization and vascular remodeling. Circ. Res. *93*, 682–689.

Seki, T., Hong, K.H., and Oh, S.P. (2006). Nonoverlapping expression patterns of ALK1 and ALK5 reveal distinct roles of each receptor in vascular development. Lab. Invest. *86*, 116–129.

Serebriiskii, I., Estojak, J., Sonoda, G., Testa, J.R., and Golemis, E.A. (1997). Association of Krev-1/rap1a with Krit1, a novel ankyrin repeat-containing protein encoded by a gene mapping to 7q21-22. Oncogene *15*, 1043–1049.

Sorensen, L.K., Brooke, B.S., Li, D.Y., and Urness, L.D. (2003). Loss of distinct arterial and venous boundaries in mice lacking endoglin, a vascular-specific TGFβ coreceptor. Dev. Biol. *261*, 235–250.

Stockton, R.A., Schaefer, E., and Schwartz, M.A. (2004). p21-activated kinase regulates endothelial permeability through modulation of contractility. J. Biol. Chem. *279*, 46621–46630.

Taddei, A., Giampietro, C., Conti, A., Orsenigo, F., Breviario, F., Pirazzoli, V., Potente, M., Daly, C., Dimmeler, S., and Dejana, E. (2008). Endothelial adherens junctions control tight junctions by VE-cadherin-mediated upregulation of claudin-5. Nat. Cell Biol. *10*, 923–934.

Takai, Y., Miyoshi, J., Ikeda, W., and Ogita, H. (2008). Nectins and nectin-like molecules:roles in contact inhibition of cell movement and proliferation. Nat. Rev. Mol. Cell Biol. *9*, 603–615.

Tanriover, G., Boylan, A.J., Diluna, M.L., Pricola, K.L., Louvi, A., and Gunel, M. (2008). PDCD10, the gene mutated in cerebral cavernous malformation 3, is expressed in the neurovascular unit. Neurosurgery *62*, 930–938.

ten Dijke, P., Goumans, M.J., and Pardali, E. (2008). Endoglin in angiogenesis and vascular diseases. Angiogenesis *11*, 79–89.

Tzima, E., Irani-Tehrani, M., Kiosses, W.B., Dejana, E., Schultz, D.A., Engelhardt, B., Cao, G., DeLisser, H., and Schwartz, M.A. (2005). A mechanosensory complex that mediates the endothelial cell response to fluid shear stress. Nature *437*, 426–431.

Uhlik, M.T., Abell, A.N., Johnson, N.L., Sun, W., Cuevas, B.D., Lobel-Rice, K.E., Horne, E.A., Dell'Acqua, M.L., and Johnson, G.L. (2003). Rac-MEKK3-MKK3 scaffolding for p38 MAPK activation during hyperosmotic shock. Nat. Cell Biol. *5*, 1104–1110.

Urness, L.D., Sorensen, L.K., and Li, D.Y. (2000). Arteriovenous malformations in mice lacking activin receptor-like kinase-1. Nat. Genet. *26*, 328–331.

Van Itallie, C.M., and Anderson, J.M. (2006). Claudins and epithelial paracellular transport. Annu. Rev. Physiol. *68*, 403–429.

Vestweber, D. (2008). VE-cadherin: the major endothelial adhesion molecule controlling cellular junctions and blood vessel formation. Arterioscler. Thromb. Vasc. Biol. *28*, 223–232.

Voss, K., Stahl, S., Schleider, E., Ullrich, S., Nickel, J., Mueller, T.D., and Felbor, U. (2007). CCM3 interacts with CCM2 indicating common pathogenesis for cerebral cavernous malformations. Neurogenetics *8*, 249–256.

Wallez, Y., and Huber, P. (2008). Endothelial adherens and tight junctions in vascular homeostasis, inflammation and angiogenesis. Biochim. Biophys. Acta *1778*, 794–809.

Wang, H.U., Chen, Z.F., and Anderson, D.J. (1998). Molecular distinction and angiogenic interaction between embryonic arteries and veins revealed by ephrin-B2 and its receptor Eph-B4. Cell *93*, 741–753.

Weber, C., Fraemohs, L., and Dejana, E. (2007). The role of junctional adhesion molecules in vascular inflammation. Nat. Rev. Immunol. *7*, 467–477.

Wegmann, F., Petri, B., Khandoga, A.G., Moser, C., Khandoga, A., Volkery, S., Li, H., Nasdala, I., Brandau, O., Fassler, R., et al. (2006). ESAM supports neutrophil extravasation, activation of Rho, and VEGF-induced vascular permeability. J. Exp. Med. *203*, 1671–1677.

Weis, S., Shintani, S., Weber, A., Kirchmair, R., Wood, M., Cravens, A., McSharry, H., Iwakura, A., Yoon, Y.S., Himes, N., et al. (2004). Src blockade

stabilizes a Flk/cadherin complex, reducing edema and tissue injury following myocardial infarction. J. Clin. Invest. *113*, 885–894.

Whitehead, K.J., Plummer, N.W., Adams, J.A., Marchuk, D.A., and Li, D.Y. (2004). Ccm1 is required for arterial morphogenesis: implications for the etiology of human cavernous malformations. Development *131*, 1437–1448.

Wildenberg, G.A., Dohn, M.R., Carnahan, R.H., Davis, M.A., Lobdell, N.A., Settleman, J., and Reynolds, A.B. (2006). p120-catenin and p190RhoGAP regulate cell-cell adhesion by coordinating antagonism between Rac and Rho. Cell *127*, 1027–1039.

Wojciak-Stothard, B., and Ridley, A.J. (2002). Rho GTPases and the regulation of endothelial permeability. Vascul. Pharmacol. *39*, 187–199.

Zawistowski, J.S., Serebriiskii, I.G., Lee, M.F., Golemis, E.A., and Marchuk, D.A. (2002). KRIT1 association with the integrin-binding protein ICAP-1: a new direction in the elucidation of cerebral cavernous malformations (CCM1) pathogenesis. Hum. Mol. Genet. *11*, 389–396.

Zawistowski, J.S., Stalheim, L., Uhlik, M.T., Abell, A.N., Ancrile, B.B., Johnson, G.L., and Marchuk, D.A. (2005). CCM1 and CCM2 protein interactions in cell signaling: implications for cerebral cavernous malformations pathogenesis. Hum. Mol. Genet. *14*, 2521–2531.