The role of adherens junctions and VE-cadherin in the control of vascular permeability

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Summary

Endothelial cells control the passage of plasma constituents and circulating cells from blood to the underlying tissues. This specialized function is lost or impaired in several pathological conditions – including inflammation, sepsis, ischemia and diabetes – which leads to severe, and sometimes fatal, organ dysfunction. Endothelial permeability is regulated in part by the dynamic opening and closure of cell-cell adherens junctions (AJs). In endothelial cells, AJs are largely composed of vascular endothelial cadherin (VE-cadherin), an endothelium-specific member of the cadherin family of adhesion proteins that binds, via its cytoplasmic domain, to several protein partners, including p120, β-catenin and plakoglobin. Endogenous pathways that increase vascular permeability affect the function and organization of VE-cadherin and other proteins at AJs in diverse ways. For instance, several factors, including vascular endothelial growth factor (VEGF), induce the tyrosine phosphorylation of VE-cadherin, which accompanies an increase in vascular permeability and leukocyte diapedesis; in addition, the internalization and cleavage of VE-cadherin can cause AJs to be dismantled. From the knowledge of how AJ organization can be modulated, it is possible to formulate several pharmacological strategies to control the barrier function of the endothelium. We discuss the possible use of inhibitors of SRC and other kinases, of agents that increase cAMP levels, and of inhibitors of lytic enzymes as pharmacological tools for decreasing endothelial permeability.

Key words: Permeability, VE-cadherin, Adherens junctions, Endothelial cells

Introduction

Endothelial cells act as gatekeepers to control the infiltration of blood proteins and circulating cells into the vessel wall and the underlying tissues. Endothelial permeability is mediated by the so-called transcellular and paracellular pathways – that is, solutes and cells can pass through (transcellular) or between (paracellular) endothelial cells. Transcellular passage requires either cell fenestration [the appearance of specialized pore-like fenestrae that can control cellular permeability to water and solutes (Roberts and Palade, 2000)] or a complex system of transport vesicles, which includes organelles called vesiculo-vacuolar organelles. These organelles, or similar structures, can also fuse and appear as channels that traverse single cells and allow the passage of leukocytes and solutes through the endothelium (Carman et al., 2007; Engelhardt and Wolburg, 2004; Feng et al., 1999; Millan et al., 2006; Nieminen et al., 2006). The paracellular pathway, by contrast, is mediated by the coordinated opening and closure of endothelial cell-cell junctions. This function must be tightly regulated to maintain endothelial integrity and to prevent exposure of the subendothelial matrix of blood vessels, a highly thrombogenic event (Dejana, 2004; Muller, 2003; Vestweber, 2007).

In the case of vascular damage, when the endothelial layer is seriously compromised and endothelial cells are retracted, the resultant increase in permeability can be accompanied by obvious disruption of the vessels, along with hemorrhages, adhesion of leukocytes and the formation of small thrombi. This extreme condition can be irreversible, which causes major problems for vascular and tissue homeostasis. By contrast, many agents that increase permeability, such as histamine, thrombin and vascular endothelial growth factors (VEGFs) can do so in a reversible way that does not necessarily affect endothelial-cell viability or functional responses (Andriopoulos et al., 1999; Weis and Cheresh, 2005). In this case, the increase in permeability might also have beneficial effects, such as an increase in the access of nutrients and oxygen to tissues, an increase in leukocyte trafficking to inflammatory regions, and the induction of the accumulation of fibrinogen and fibrin outside the vessels, which improves tissue repair.

In general, the possibility of controlling vascular permeability has several therapeutic implications. An uncontrolled and lasting increase in permeability that is not balanced by the re-absorption of lymphatic fluid causes edema, which, in turn, increases ischemic tissue injury in conditions such as stroke or myocardial infarction. Furthermore, vascular leakage in tumors not only facilitates tumor-cell penetration into the vessels and metastatic dissemination, but also contributes to the accumulation of fluid in the stroma and the elevated interstitial pressure that are common to several solid tumors (Weis and Cheresh, 2005). Elevated interstitial pressure is probably the cause of altered tumor perfusion, the development of necrotic areas and impaired drug delivery. Conversely, increasing vascular permeability in a reversible manner might be beneficial because it might increase drug accessibility to different tissues in which fluid exchange between blood and tissues is limited, such as in the brain.

To develop targeted therapies for manipulating the permeability of the endothelial barrier we need to understand the molecular mechanisms that regulate paracellular and transcellular permeability. Transcellular permeability is probably governed by signaling pathways that are responsible for endocytosis and vesicular trafficking in cells (Mehta and Malik, 2006). By contrast,
paracellular permeability is governed by the opening and closing of cell junctions, which implies that a complex rearrangement of adhesion proteins and the related cytoskeleton must occur. It is likely that the two pathways are interconnected in some way, because many permeability-increasing agents increase vesicular transport and also disrupt the integrity of endothelial cell-cell junctions (Dejana, 2004; Feng et al., 1999; Weis and Cheresh, 2005), however, whether this occurs in the same vessels and at the same time is still a matter of debate. It is possible that, in some areas of the vasculature, such as the microvasculature of the glands, the transcellular pathway is better developed, whereas in others, such as the postcapillary venules, the paracellular pathway is favored. It will be of interest, once more knowledge is available on the vesicular-transport systems in endothelial cells, to try to integrate both systems into a more comprehensive picture.

This Commentary focuses on the role of cell-cell junctions – particularly adherens junctions (AJs) – in the barrier function of the endothelium. We pay particular attention to the function, molecular organization and regulation of vascular endothelial (VE)-cadherin, the endothelium-specific transmembrane component of AJs. Finally, we show that pharmacological strategies for modulating endothelial permeability can be obtained by using our knowledge of the structure and function of AJs.

Adherens junctions and vascular integrity

Previous work has described the molecular organization of the different types of endothelial cell-cell junctions, and has established a basis for understanding how these structures might crosstalk and reciprocally interact (Bazzoni and Dejana, 2004; Muller, 2003; Vestweber, 2007; Wallez and Huber, 2007). Endothelial-cell junctions present a particularly complex network of adhesion proteins that are linked to intracellular cytoskeletal and signaling partners. These proteins are organized into distinct structures called tight junctions (TJs) and AJs. In addition, several adhesion proteins (such as platelet endothelial cell adhesion molecule (PECAM1), MUC18, intercellular adhesion molecule 2 (ICAM2), CD34, endoglin and others) cluster at cell-cell contacts that are distinct from TJs and AJs. There are data to suggest that the assembly of AJs is required for the correct organization of TJs, but the molecular basis of this interaction is not fully understood (Bazzoni and Dejana, 2004). Changes in AJ composition or function might therefore have complex effects on overall vascular homeostasis.

AJs are formed by members of the cadherin family of adhesion proteins. Endothelial cells express relatively high levels of two cadherins: a cell-type-specific cadherin called VE-cadherin and neuronal cadherin (N-cadherin), which is also present in other cell types such as neural cells and smooth muscle cells (Bazzoni and Dejana, 2004). Other non-cell-type-specific cadherins, such as T-cadherin and P-cadherin, are variably expressed in different types of endothelial cells (Ivanov et al., 2001).

VE-cadherin, similar to many other members of the cadherin family, is linked through its cytoplasmic tail to the AJ proteins p120, β-catenin and plakoglobin. β-catenin and plakoglobin bind to α-catenin, which interacts with several actin-binding proteins, including α-actinin, ajuba, zonula occludens-1 (ZO-1) and others (Weis and Nelson, 2006). The cadherin complex (which comprises VE-cadherin and associated catenins) influences and is influenced by the actin cytoskeleton, but the molecular basis of this interaction is still an open issue. In the past, it was believed that the anchorage to actin was mediated by α-catenin, but recent work has shown that α-catenin cannot bind to both β-catenin and actin simultaneously (Weis and Nelson, 2006). It is possible to define a model in which monomeric α-catenin is recruited to the cell membrane by cadherin clustering when cells establish cell-cell contacts, reaching high local concentrations. Because α-catenin is bound to cadherin, via β-catenin or plakoglobin, in a dynamic way, molecules of α-catenin would detach from cadherins and associate to form homodimers that, in turn, would promote actin bundling. In this model, actin organization would be only indirectly influenced by cadherins. However, in conditions of increased permeability, endothelial cells retract and cause intercellular gaps to open. This process is probably mediated by the contraction of actomyosin that is anchored to AJs (Dudek and Garcia, 2001). Several candidate mediators of this interaction have been suggested, but further work is needed to define this process at a molecular level (Weis and Nelson, 2006) (Fig. 1 and Table 1).

Whatever the mechanism of action, the association of VE-cadherin with catenins is certainly required for full cellular control of endothelial permeability and junction stabilization. A mutant form of VE-cadherin that is truncated in the domain that is responsible for binding to β-catenin or to plakoglobin still forms intercellular zipper-like structures through the lateral clustering of the extracellular domain, but the strength of the junctions is strongly decreased (Navarro et al., 1995). Importantly, the induction of a similar VE-cadherin mutation in mouse leads to fetal lethality owing to major alterations in vascular remodeling; this effect is comparable to those caused by inactivation of the entire gene (Carmeliet et al., 1999). In the adult mouse, an indication of the importance of VE-cadherin in the maintenance of vascular integrity is the observation that the administration of anti-VE-cadherin antibodies leads to a dramatic increase in permeability, vascular fragility and hemorrhages (Corada et al., 1999). The effect of the antibodies is particularly strong, perhaps because they remain bound

![Fig. 1. Molecular organization of endothelial AJs.](image-url)

**Fig. 1.** Molecular organization of endothelial AJs. VE-cadherin is represented as a dinner, which is the minimal functional unit of cadherins; see Gumbiner (Gumbiner, 2005), in which various models of the intercellular association of cadherin dimers are discussed. EC1-EC5 are the five homologous extracellular domains of VE-cadherin. Clustering of VE-cadherin at cell-cell contacts promotes the formation of multimolecular complexes that comprise signaling, regulatory and scaffold proteins. Proteins that are well known to interact with VE-cadherin include the catenin proteins p120, β-catenin (βcat) and plakoglobin (plako). β-catenin and plakoglobin associate directly with VE-cadherin and α-catenin (αcat). Other AJ proteins are listed in Table 1. Some VE-cadherin-interacting proteins have enzymatic activity (tyrosine or serine kinases, tyrosine phosphatases and GTPases). Others have a scaffolding function, which might allow the organization of very complex molecular clusters. The interaction of such proteins with VE-cadherin can be either direct or indirect. The proteins shown in the figure and in Table 1 assemble into AJs – multimeric complexes that can modulate endothelial-barrier function by regulating the activity of VE-cadherin and transducing intracellular signals. There is likely to be local specificity of the molecular composition of such complexes, depending on the type and the state of activation of the vessels.
to VE-cadherin for several hours, thereby blocking junction reorganization (Fig. 2). Other agents such as histamine or low concentrations of VEGF (Weis and Cheresh, 2005), which only affect the adhesive strength of VE-cadherin, induce milder effects that are usually reversible (Fig. 2).

Although N-cadherin is expressed at levels that are comparable to VE-cadherin, it presents a diffuse distribution on the endothelial-cell membrane and is poorly clustered at intercellular junctions. Previous work has shown that when VE-cadherin is present at junctions, it excludes N-cadherin from those sites (Navarro et al., 1998). It is therefore possible that, in stabilized endothelial monolayers, N-cadherin does not play a role at endothelial cell-cell junctions but instead acts at heterotypic cell-cell contacts between endothelial cells and pericytes (mesenchymal cells that associate with the walls of small blood vessels) (Gerhardt et al., 2000; Paik et al., 2004). Interestingly, endothelial-cell-specific gene inactivation of N-cadherin induces a phenotype that is similar to that of VE-cadherin-null embryos. In cultured cells, it was found that the reduction of N-cadherin expression causes post-transcriptional inhibition of VE-cadherin expression; this effect might be the indirect cause of the observed vascular defects in the embryo (Luo and Radice, 2005).

The role of β-catenin in vascular permeability and integrity is suggested by the effect of its endothelial-cell-specific gene inactivation in mouse embryos (Cattelino et al., 2003). Vessels from β-catenin-null embryos have an abnormal lumen and are frequently hemorrhagic. Endothelial cells in these embryos present a different organization of AJs, in which plakoglobin substitutes for β-catenin and recruits desmoplakin and vimentin instead of α-catenin. This type of junction can be more fragile and leads to hemorrhage when vessels are exposed to elevated blood pressure (Cattelino et al., 2003).

**Permeability-increasing agents and adherens-junction organization**

Several studies focus on the effect of agents that increase vascular permeability on the organization of endothelial cell-cell junctions (Dudek and Garcia, 2001; Esser et al., 1998; Mehta and Malik, 2006; Schnittler, 1998; van Hinsbergh and van Nieuw Amerongen, 2002; Weis and Cheresh, 2005). Some agents, such as histamine or thrombin, act very rapidly, and the effect is quickly reversible once they are removed. By contrast, inflammatory cytokines require several hours to increase vascular permeability but the effect is sustained, even up to 24 and 48 hours. This suggests that the mechanism of action, and the functional implications of the increase in permeability, might vary. Most of the conditions that increase permeability, however, affect the organization of AJs. This can be accompanied by an obvious endothelial-cell retraction and by the opening of intercellular gaps. However, in many cases, the subsequent weakness of the junctions is not reflected by morphological changes in the endothelial monolayer – for instance, the internalization of VE-cadherin or the phosphorylation of AJ proteins reduces junctional strength without necessarily opening intercellular gaps (Fig. 2) (Andriopoulou et al., 1999; Esser et al., 1998).

### Endogenous pathways that modulate endothelial permeability

Endothelial cells possess several molecular mechanisms by which vascular permeability can be modulated. Such mechanisms focus on AJ organization and, in several cases, target VE-cadherin specifically – for instance, the phosphorylation, cleavage and internalization of VE-cadherin are all thought to affect endothelial permeability. These pathways, and others, are described below.

### Tyrosine phosphorylation of AJ components

It is generally accepted that the tyrosine phosphorylation of VE-cadherin and other components of AJs is associated with weak junctions and impaired barrier function. It has been reported that permeability-increasing agents such as histamine (Andriopoulou et al., 1999; Shasby et al., 2002), tumor necrosis factor-α (TNFα) (Angelini et al., 2006), platelet-activating factor (PAF)
SRC-deficient mice or in wild-type mice treated with SRC inhibitors and VEGF-induced phosphorylation of VE-cadherin is inhibited in the mechanism, because it associates directly with VE-cadherin, fully clarified. The tyrosine kinase SRC is probably implicated in pathological conditions only.

Whether the phosphorylation of VE-cadherin reflects a constitutive ischemic conditions in mouse tissues. However, we still do not know VE-cadherin can be phosphorylated in vivo in angiogenic and extracts (Lambeng et al., 2005; Weis et al., 2004) have shown that two papers that use organ extracts. Although most of the data that provide evidence of this relationship were obtained using cultured cells. Two papers that use organ extracts (Lambeng et al., 2005; Weis et al., 2004) have shown that VE-cadherin can be phosphorylated in vivo in angiogenic and ischemic conditions in mouse tissues. However, we still do not know whether the phosphorylation of VE-cadherin reflects a constitutive and dynamic state of AJs in vessels in vivo, or whether it is induced in pathological conditions only.

The mechanism of VE-cadherin phosphorylation has not yet been fully clarified. The tyrosine kinase SRC is probably implicated in the mechanism, because it associates directly with VE-cadherin, and VEGF-induced phosphorylation of VE-cadherin is inhibited in SRC-deficient mice or in wild-type mice treated with SRC inhibitors (Weis and Cheresh, 2005). In addition to SRC, other kinases are thought to associate with the VE-cadherin–β-catenin complex and to modulate endothelial permeability (Table 1). These include c-SRC tyrosine kinase (CSK), which binds to phosphorylated VE-cadherin and inhibits SRC by phosphorylating it at Tyr527 (Baumeister et al., 2005). Proline-rich tyrosine kinase 2 (PYK2) might also play a role in the phosphorylation of the VE-cadherin–β-catenin complex. PYK2 can phosphorylate β-catenin directly, although it is still unclear whether it can directly target VE-cadherin as a substrate (Allingham et al., 2007). The extent of phosphorylation of VE-cadherin might also be increased by the inhibition of AJ-associated phosphatases. For example, vascular endothelial protein tyrosine phosphatase (VE-PTP) is an endothelium-specific phosphatase that associates with VE-cadherin and might inhibit its tyrosine phosphorylation (Nawroth et al., 2002). Intriguingly, inactivation of the gene that encodes VE-PTP leads to an embryonic-lethal phenotype in which the maintenance and remodeling of blood vessels is strongly affected. This phenotype, which is comparable to that of VE-cadherin-null embryos, suggests that vessels cannot form correctly if VE-cadherin is constantly phosphorylated (Baumer et al., 2006). Other phosphatases, such as density-enhanced phosphatase-1 (DEP1) (Lampugnani et al., 2003), protein tyrosine phosphatase receptor type M (PTPµ) (Sui et al., 2005) and SH2-containing phosphotyrosine phosphatase (SHP2) (Ukropec et al., 2000), might associate indirectly or directly with

![Fig. 2. Suggested functional modifications of endothelial AJs under in vitro conditions that increase endothelial permeability.](image-url)
VE-cadherin, decreasing the extent of its phosphorylation (Fig. 1) and enhancing barrier function.

It is important to define the specific tyrosine residues of VE-cadherin that are phosphorylated in response to different stimuli (Fig. 3). In Chinese hamster ovary (CHO) cells, single tyrosine-to-glutamic acid (Y-to-E) point mutants of tyrosines 658 and 731 of VE-cadherin cause an impaired barrier function and lose the capacity to bind to p120 and to β-catenin, respectively (Potter et al., 2005). In endothelial cells, it has been reported that SRC, when activated by VEGF, phosphorylates only tyrosine 685 of VE-cadherin (Wallez et al., 2006). In one recent report, the adhesion of neutrophils to endothelial cells via ICAM1 induced the phosphorylation of tyrosines 658 and 731 of VE-cadherin by SRC and PYK2 (Allingham et al., 2007), whereas in another study, the engagement of ICAM1 by antigen-activated lymphocytes induced the phosphorylation of tyrosines 645, 731 and 733, which is mediated by Rho GTPase, Ca²⁺ and dynamic actin but not by SRC (Turowski et al., 2008). VE-cadherin-null endothelial cells that express a mutant form of VE-cadherin in which a single tyrosine is substituted for phenylalanine at position 645, 731 or 733 are less permissive of lymphocyte diapedesis (Turowski et al., 2008). Some of the discrepancies reported in the literature might be the result of different experimental conditions (the types of stimuli and cells that are used, the incubation time, and so on). Further work is needed to understand these conflicting data more fully; this might involve extending the study of VE-cadherin phosphorylation to in vivo systems.

Catenin proteins such as β-catenin and plakoglobin can also be tyrosine phosphorylated by the action of the same agents that stimulate VE-cadherin phosphorylation (Lampugnani et al., 1997; Esser et al., 1998). However, the specific consequences of catenin phosphorylation for vascular permeability are still unknown. In several systems, the tyrosine phosphorylation of β-catenin reduces its affinity for the cadherin cytoplasmic tail and increases its turnover at junctions (Huber and Weis, 2001; Lilien and Balsamo, 2005). It is possible that this compromises cytoskeletal organization and impairs the barrier function of the endothelium.

**VE-cadherin internalization**

Another mechanism by which endothelial permeability might be regulated is the internalization of VE-cadherin, a process that can occur in a clathrin-dependent manner (Xiao et al., 2005). Interestingly, the binding of p120 to VE-cadherin prevents its internalization, introducing the concept that p120 might act as a plasma-membrane-retention signal. In a recent report, it was found that VEGF disrupts endothelial-barrier function by activating SRC, which, in turn, phosphorylates VAV2, a guanine-nucleotide-exchange factor (GEF) for the GTPase Rac (Gavard and Gutkind, 2006). Activated (GTP-bound) Rac induces the phosphorylation of VE-cadherin at SerR665. This process induces the recruitment to VE-cadherin of β-arrestin-2, which promotes clathrin-dependent internalization of VE-cadherin. In this scenario, phosphorylation of VE-cadherin at Ser665, and not at a tyrosine residue, would be the crucial requirement for an increase in permeability (Fig. 3).

**Cleavage of VE-cadherin**

VE-cadherin is particularly susceptible to enzymatic lysis, suggesting that this might be another pathway that can induce an increase in vascular permeability or in leukocyte diapedesis. Exposure to metalloproteases (Herren et al., 1998; Luplertlop et al., 2006), elastase, cathepsin G or minimal concentrations of trypsin induces the digestion of the VE-cadherin extracellular domain in cultured cells (Lampugnani et al., 1992; Xiao et al., 2003). Leukocytes and tumor cells can release large amounts of these enzymes, which could promote VE-cadherin cleavage and thereby increase cell extravasation and vascular leakage.
cAMP production and RAP1 activity
It has long been known that cAMP-elevating drugs enhance vascular-barrier function both in vivo and in vitro (for details, see Waschke et al., 2004) and attenuate inflammatory edema in several tissues (Moy et al., 1998). Protein kinase A (PKA) and the ubiquitous Ras-related GTPase RAP1, along with its GEF EPAC1 (exchange protein activated by cAMP), are thought to transduce such responses (Waschke et al., 2004; Cullere et al., 2005; Kooistra et al., 2005). In recent years, RAP1 has received particular attention, and the emerging picture of its function is complex and intriguing (Kooistra et al., 2007).

RAP1 acts in a complex signaling network to control the organization of cell–cell junctions, in addition to its role in several actin-regulated processes (Bos, 2005). A cooperative and multi-faceted relationship exists between VE-cadherin and RAP1 in endothelial cells. RAP1 enhances the adhesive properties of VE-cadherin (Fukuhara et al., 2005). The EPAC1-specific cAMP analog 8-pCPT-2′-O-Me-cAMP (also known as 007) decreases monolayer permeability only in those endothelial cells that express VE-cadherin (Kooistra et al., 2005), indicating that RAP1 requires VE-cadherin for its activity. By contrast, VE-cadherin is required for the recruitment of the membrane-associated guanylate kinase MAGI1, a scaffold for the RAP1 activator PDZ-GEF1 (Sakurai et al., 2006). Therefore, RAP1 and VE-cadherin can influence each other reciprocally to finely modulate endothelial responses and barrier function.

It seems clear that RAP1 helps to control endothelial permeability; however, its role in the regulation of leukocyte transmigration is still controversial (Cullere et al., 2005; Wittchen et al., 2005). It is also important to emphasize that, although RAP1 has attracted particular attention in the recent literature, several other small GTPases are also able to modulate AJ organization and endothelial permeability. There are several excellent reviews on this subject in the literature (van Hinsbergh and van Nieuw Amerongen, 2002; Wojciak-Stothard and Ridley, 2002).

Association of VE-cadherin with the VEGF receptor
VE-cadherin can associate with VEGF receptor 2 (VEGFR2; also known as FLK1 or KDR) to reduce the proliferative signaling that is initiated by the receptor. The induction of phospholipase Cγ (PLCγ) and activation of MAP kinases by VEGF are both markedly reduced by the binding of VEGFR2 to VE-cadherin. VE-cadherin acts by inhibiting both the phosphorylation of the receptor and its internalization to intracellular signaling compartments (Miauczynska et al., 2004). This pathway, which requires the phosphatase DEP1, contributes to VE-cadherin-induced contact inhibition of cell growth (Lampugnani et al., 2003; Lampugnani et al., 2006). In addition, the association of VEGFR2 with VE-cadherin might facilitate the phosphorylation of AJ components by SRC (Weis and Cheresh, 2005), and might thereby impair endothelial-barrier function. Interestingly, the activation of SRC by VEGFR2 is not required for angiogenesis in vivo, which enables a distinction to be drawn between endothelial proliferation and increased endothelial permeability (Elcheiri et al., 1999).

The molecular basis of the association of VEGFR2 with VE-cadherin has been only partially characterized. VE-cadherin mutants that can bind to p120 but not to β-catenin lose the capacity to associate with VEGFR2 (Lampugnani et al., 2003). Kinase inhibitors that inhibit the tyrosine phosphorylation of VE-cadherin also reduce its association with the receptor (M.G.L., unpublished observations).

Pharmacological strategies for controlling endothelial permeability
The extent of our understanding of the structure and function of AJs makes it possible to formulate some working hypotheses regarding pharmacological strategies for the treatment of diseases in which vascular permeability is affected. The following list sets out five classes of potential therapy and the expected drawbacks to their pharmacological exploitation, the major drawback being our presently limited knowledge of the molecular mechanisms by which they act. (1) SRC inhibitors might be beneficial in the clinic, as they could limit the VEGF-induced increase in vascular permeability. Unfortunately, SRC modulates several fundamental cell functions, such as proliferation, survival, motility and invasion, and these agents are likely to present side-effects. (2) Other inhibitors might act by blocking the association between VEGFR2 and VE-cadherin, but our knowledge of the structural characteristics of the VE-cadherin–VEGFR2 complex and peptides or antibodies that might compete for binding to either component is still very limited. (3) The inhibition of VE-cadherin internalization might decrease vascular permeability. Blocking the association of p120 or β-arrestin-2 with VE-cadherin would promote the retention of the protein at the cell surface and thereby contribute to the stabilization of endothelial-barrier function. Because phosphorylated residues of VE-cadherin or the catenins can act as docking sites for p120 and β-arrestin-2, one drug-discovery approach would be to screen specific tyrosine- and serine-kinase inhibitors. (4) The cleavage of the extracellular domain of VE-cadherin might also influence the integrity of junctions in lasting inflammatory conditions or during cell extravasation. Inhibitors of metalloproteases and other lytic enzymes might therefore be beneficial in this context. (5) Last but not least, it might be possible to influence vascular permeability by modulating the activity of RAP1 or other small GTPases. Chemicals that are able to activate EPAC1, and in turn RAP1, have been described and could be further optimized to enhance their endothelial specificity.

Conclusion
VE-cadherin is an important determinant of the barrier function of the vascular endothelium. From the knowledge of how the expression and function of this protein are regulated, it should be possible to design specific agents that can increase or decrease vascular permeability. Further work is required, however, to address important issues such as the relationship between the transcellular and paracellular permeability pathways and their specific biological roles in different regions of the vascular tree.

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