Protein Interactions at Endothelial Junctions and Signaling Mechanisms Regulating Endothelial Permeability

Yulia A. Komarova, Kevin Kruse, Dolly Mehta, Asrar B. Malik

Abstract: The monolayer of endothelial cells lining the vessel wall forms a semipermeable barrier (in all tissue except the relatively impermeable blood–brain and inner retinal barriers) that regulates tissue–fluid homeostasis, transport of nutrients, and migration of blood cells across the barrier. Permeability of the endothelial barrier is primarily regulated by a protein complex called adherens junctions. Adherens junctions are not static structures; they are continuously remodeled in response to mechanical and chemical cues in both physiological and pathological settings. Here, we discuss recent insights into the post-translational modifications of junctional proteins and signaling pathways regulating plasticity of adherens junctions and endothelial permeability. We also discuss in the context of what is already known and newly defined signaling pathways that mediate endothelial barrier leakiness (hyperpermeability) that are important in the pathogenesis of cardiovascular and lung diseases and vascular inflammation. (Circ Res. 2017;120:179-206. DOI: 10.1161/CIRCRESAHA.116.306534.)

Key Words: adherens junctions ■ blood cells ■ endothelial cells ■ lung diseases ■ signal transduction

Endothelial Barrier Integrity in Health and Disease

The endothelium lining the intima of all blood and lymphatic vessels forms a semipermeable barrier between circulating plasma and the interstitium. Interendothelial junctions connect endothelial cells into a contiguous monolayer to restrict the transport of proteins across the endothelial barrier in a size-selective manner.1–3 Molecules of ≤3-nm radii passively diffuse through junctions, whereas high molecular weight proteins such as albumin (67 kDa), constituting 75% of protein in the plasma, and blood cells are largely retained in the circulation.2,4

Interendothelial junctions are the main structures maintaining tissue–fluid homeostasis. The plasma oncotic pressure derived from the circulating albumin is the main factor contributing to passive reabsorption of fluid and solutes back into the circulation.5 Loss of interendothelial junctions as the result of an acute or chronic process leads to flux of proteinaceous fluid into the interstitium causing tissue edema. This is a common cause of a broad range of pathological conditions in humans including systemic capillary leak syndrome,6 angioedema,7 anaphylaxis,8 acute respiratory distress syndrome (ARDS),9,10 age-related and diabetic-associated eye diseases,11–13 and various disorders of central nervous system.14–17 Hence, elucidating signaling mechanisms responsible for control of junction integrity is of fundamental importance to developing novel therapeutic strategies for treating edema.

Role of Interendothelial Junctions in Regulating Endothelial Barrier Function

Interendothelial junctions are composed of protein complexes of adherens junctions (AJs), tight junctions (TJs), and gap junctions (GJs)18–21 (Figure 1A). Both AJs and TJs form pericellular zipper-like structures along endothelial cell borders through adhesion of distinct adhesive proteins.18–20 In contrast, GJs are intercellular channels enabling direct electrical and chemical communication between endothelial cells through the passage of ions and signaling molecules with a size of ≤1 kDa.22

Gap Junctions

A functional GJ is composed of 2 hemichannels aligned in the plasma membrane of adjacent endothelial cells.23 Each hemichannel consists of 6 connexin molecules, assembled within the endoplasmic reticulum or trans-Golgi.24–26 The hemichannel can be either homomeric or heteromeric, ie, assembled by the same or distinct connexin isoforms, respectively.27 Channels composed of different isoforms might exhibit altered activities in respect to ion selectivity and permeability when compared with homomeric channels.28–30

The 3 major connexin isoforms expressed in systemic arteriolar endothelial cells are Cx37, Cx40, and Cx43.31,32 These GJs are responsible for communication between endothelial and endothelial-smooth muscle cells.33,34 In animal models, deletion of Cx43 in endothelial cells causes hypotension,35 whereas deletion of Cx40 leads to hypertension associated...
Disruption of TJs is associated with BBB and iBRB leakage, a characteristic of multiple human diseases including diabetic and oxygen-induced retinopathy\(^\text{48}\) and disorders of the central nervous system such as stroke.\(^\text{59}-\text{51}\)

TJs are composed of several adhesive proteins including occludin, claudins, and junctional adhesion molecules\(^\text{52}-\text{57}\) (for reviews, see\(^\text{66}\)). Claudin-5 is ubiquitously expressed in all vascular beds, whereas claudin-1, claudin-3, and claudin-12 are specific to the brain microvasculature.\(^\text{59}-\text{61}\) Claudin-1, claudin-2, and claudin-5 are found in TJs of retinal vessels.\(^\text{48}\) Claudins and occludins, in association with cytosolic zonula occluden (ZO)-1, ZO-2, and ZO-3 proteins assemble zipper-like structures along the rim of endothelial cells.\(^\text{62,63}\) The role of junctional adhesion molecule-A in the organization of TJs is less understood.

The integrity of the BBB is crucial to the proper functioning of the central nervous system. Disruption of the BBB associated with trauma, hemorrhagic stroke, rupture of cerebral aneurysm, and inflammation leads to serious consequences ranging from progressive neuronal dysfunction, sclerosis, brain edema, paralysis, and death\(^\text{44}\) (for reviews, see\(^\text{65,66}\)). Studies in animal models demonstrate that deletion of the claudin-5 gene \textit{cldn5} causes death in newborn animals because of increased permeability of the BBB in a size-selective manner.\(^\text{50}\) Claudin-5 is targeted for degradation by matrix metalloproteinase after an ischemic insult, and loss of claudin-5 is responsible for disruption of the BBB in ischemic stroke.\(^\text{51}\) In contrast, loss of claudin-3 but not of claudin-5 or occludin accounted for breakdown of the BBB in experimental models of allergic encephalomyelitis and glioblastoma multiforme\(^\text{60}\) suggesting that claudin proteins might have distinct and indispensable roles in regulating the organization of TJs in brain circulation. Deletion of the occludin gene \textit{ocln} causes no apparent defects in the organization or strength of TJs,\(^\text{67}\) suggesting that its function is compensated by other adhesive proteins.

In contrast to the BBB, neither claudin nor occludin proteins are downregulated in the iBRB using experimental models of diabetic and oxygen-induced retinopathy.\(^\text{48,68}\) In fact, the expression of claudin-2 and claudin-5 is upregulated in oxygen-induced retinopathy,\(^\text{48}\) suggesting that breakdown of the iBRB is associated with post-translational modifications of adhesion proteins of TJs. PKC\(_\varepsilon\) is known to facilitate the formation of TJs through phosphorylation of occludin.\(^\text{69}\) Hyperactivation of PKC\(_\varepsilon\) observed in type 2 diabetes induced the mislocalization of occludin and disruption of TJs resulting in increased leakage of plasma proteins into the retina.\(^\text{70,71}\)

TJs are linked to the actin cytoskeleton through the ZO proteins ZO-1, ZO-2, and ZO-3 expressed in endothelial cells.\(^\text{52,63}\) They interact with adhesive proteins of TJs and anchor the actin cytoskeleton with TJs.\(^\text{62,63}\) ZO-1 plays a crucial role in the assembly of functional TJs and AJs.\(^\text{62,72,73}\) As discussed below, ZO-1 might regulate the cross-interaction between TJs and AJs through control of intracellular tension and assembly of the vascular endothelial (VE)-cadherin mechanosensory complex.\(^\text{73}\) Decreased expression of ZO-1 is associated with severe plasma leakage observed in multiple sclerosis\(^\text{74}\) and diabetic rats.\(^\text{75,76}\)

<table>
<thead>
<tr>
<th>Nonstandard Abbreviations and Acronyms</th>
</tr>
</thead>
<tbody>
<tr>
<td>AJ</td>
</tr>
<tr>
<td>ARDS</td>
</tr>
<tr>
<td>BBB</td>
</tr>
<tr>
<td>ECM</td>
</tr>
<tr>
<td>eNOS</td>
</tr>
<tr>
<td>FA</td>
</tr>
<tr>
<td>FAK</td>
</tr>
<tr>
<td>GAP</td>
</tr>
<tr>
<td>GEF</td>
</tr>
<tr>
<td>GJ</td>
</tr>
<tr>
<td>iBRB</td>
</tr>
<tr>
<td>MLCl</td>
</tr>
<tr>
<td>MLCK</td>
</tr>
<tr>
<td>MRCK</td>
</tr>
<tr>
<td>NO</td>
</tr>
<tr>
<td>N-WASP</td>
</tr>
<tr>
<td>PAF</td>
</tr>
<tr>
<td>PECAM-1</td>
</tr>
<tr>
<td>PI3K</td>
</tr>
<tr>
<td>PK</td>
</tr>
<tr>
<td>PTP</td>
</tr>
<tr>
<td>ROCK</td>
</tr>
<tr>
<td>S1P</td>
</tr>
<tr>
<td>TJ</td>
</tr>
<tr>
<td>TNF</td>
</tr>
<tr>
<td>TRP</td>
</tr>
<tr>
<td>VE</td>
</tr>
<tr>
<td>VEGF</td>
</tr>
<tr>
<td>VEGFR</td>
</tr>
<tr>
<td>ZO</td>
</tr>
</tbody>
</table>
In a study, in mice with inducible endothelial cell–restricted disruption of β-catenin, it is shown that endothelial β-catenin signaling was essential for maintaining BBB integrity through regulation of claudin-1 and claudin-3 in adult brain endothelial cells. These mice developed multiple brain petechial hemorrhages accompanied by neuronal injury and central nervous system inflammation. Thus, nuclear β-catenin is an essential mechanism in regulating BBB via the expression of claudin-1 and claudin-3. This conclusion is supported by the evidence that Wnt/β-catenin signaling regulates expression of claudin-3.

Adherens Junctions

AJs are composed of VE-cadherin and associated α-, β-, and p120-catenin adhesion complexes. In addition, there is also a variety of other recently described junctional proteins, ie, vinculin, N-WASP (neural Wiskott–Aldrich syndrome protein), and Arp2/3, which interact with catenins involved primarily in stabilizing VE-cadherin–mediated adhesion (discussed below). Multiple lines of evidence showed that VE-cadherin adhesion is the primary adhesion event during vascular development. VE-cadherin–mediated adhesion promotes activation of forkhead box transcriptional factor FoxO1, which is also required for claudin-5 expression. Knockout of β-catenin in endothelial cells leads to the disruption of TJs, indicating the importance of AJs in assembly and maintenance of TJs. Disassembly of AJs compromised by the integrity of the VE-cadherin adhesion complex is the leading cause of tissue edema associated with a broad range of pathological conditions.

Another major cadherin present in endothelial cells, Neural (N)-cadherin, has been shown to mediate the interaction between endothelial cells and the surrounding mural cells (smooth muscle cells and pericytes) and is critical for endothelial vessel
maturation and stabilization. N-cadherin adhesions are excluded from AJs both in vitro and in vivo. Deletion of N-cadherin gene cdh2 in endothelial cells causes embryonic lethality because of severe vascular defects. Multiple lines of evidence indicate a specific role of pericytes in the formation of the BBB and iBRB. The study demonstrated that interaction between pericytes and endothelial cells was required for the formation of TJs in iBRB and BBB. An attractive hypothesis is that N-cadherin adhesion, which is involved in the recruitment of pericytes, also contributes to the assembly of TJs (although the mechanisms of this unknown, this idea deserves scrutiny). Another study demonstrating that N-cadherin adhesion-induced signaling contributes to the resolution of lung vascular injury through an AMP kinase–dependent mechanism is consistent with this concept.

T-cadherin (cadherin 13) is also highly expressed in the vasculature. Unlike most cadherins, T-cadherin lacks a transmembrane and cytoplasmic region and is not involved in cell–cell adhesion or anchorage to the actin cytoskeleton. T-cadherin is anchored to lipid raft regions via a glycosylphosphatidylinositol anchor, where it acts as a signaling molecule. T-cadherin has been suggested to act as a receptor for low-density lipoprotein and may play a role in angiogenesis by a yet undefined mechanism. Furthermore, T-cadherin enhances endothelial barrier function in monolayers, but seems to negatively regulate the barrier when challenged with thrombin.

Retinal (R)-cadherin is critical for retinal vascular formation and relies on a similar network patterning as found in neurons. It has also been reported that R-cadherin forms functional, heterotypic interactions with N-cadherin, suggesting a possible role for R-cadherin in endothelial–mural cell interactions.

VE-cadherin 2 (protocadherin 12) is also localized to endothelial cell–cell junctions, and while sharing a common extracellular cadherin sequence, it has a cytosolic region with unknown homology to typical cadherins. VE-cadherin 2 does not bind catenins and is only weakly associated with the cytoskeleton. VE-cadherin 2 does not seem to affect endothelial permeability and seems to be only involved in cell–cell adhesion. Transgenic mice deficient in VE-cadherin 2 had no gross morphological defects. However, recent studies showed that arteries lacking VE-cadherin 2 had medial elastic lamellae, increased inner diameter, and circumferential midwall stress indicating that it is required for both the structure and function of arteries.

Mechanisms of VE-Cadherin cis and trans Interactions

VE-Cadherin Homophilic Dimerization

VE-cadherin is a member of the classical cadherin family that possess a modular structure of 5 ectodomains, a transmembrane domain, and a cytoplasmic tail. VE-cadherin displays characteristics of both type I and type II cadherins. Like type I cadherins, it lacks the hydrophobic nonswapped region that extends the hydrophobicity of the docking surface. Similar to type II cadherins, it contains 2 conserved tryptophans, Trp2 and Trp4, important for its adhesive property. Anchorage of these tryptophans to a hydrophobic pocket of the partner ectodomain 1 induces strand-swap binding mode, resulting in the so-called trans-dimerization of VE-cadherin (Figure 1B and 1C). Trans interaction reduces the flexibility of the extracellular domain, which enables a secondary adhesion event between ectodomains 1 and 2 of 2 cadherins on the same side of an endothelial cell (Figure 1D). This low-affinity cis interaction is proposed to be responsible for lateral clustering of VE-cadherin, which may increase the strength of adhesive bonds. Formation of both trans and cis interactions is an intrinsic property of the extracellular moiety of VE-cadherin that does not require the intracellular portion of the protein or assembly of the cadherin–catenin complex.

Tethering of VE-Cadherin Adhesion Complex to Actin Cytoskeleton

The strength of adhesive bonds, defined specifically as the ability of VE-cadherin adhesion to sustain mechanical stresses from blood flow and pressure, is regulated through attachment of the adhesion complex to the actin cytoskeleton. The actin cytoskeleton contributes to the strength of AJs by several fundamental mechanisms. It generates intracellular tension and clustering of VE-cadherin at AJs and facilitates assembly of the VE-cadherin mechanosensory complex.

α-catenin is the only member of the cadherin-associated catenin proteins that contain an actin-binding domain, enabling the direct association between VE-cadherin adhesion and the actin cytoskeleton. α-catenin can either tether pre-existing actin filaments to the VE-cadherin complex or alternatively induce de novo polymerization of actin filaments at sites of AJs. The latter mechanism involves recruitment of actin-binding proteins such as α-actinin, epithelial protein lost in neoplasm, and vinculin to VE-cadherin adhesion in the presence of intracellular tension (for review, see ).

α-catenin and vinculin are allosteric molecules that undergo a rapid and reversible switch between conformational states depending on the applied tension. α-catenin–mediated recruitment of vinculin, along with N-WASP, vasodilator-stimulated phosphoprotein, and myosin-II to AJs enhances the strength of VE-cadherin adhesion by promoting Arp2/3-dependent polymerization of de novo actin filaments. Recent work from our group has shown that p120-catenin forms a complex with Arp2/3 and N-WASP. Knockdown of Arp2 did not inhibit N-WASP interaction with p120-catenin, suggesting that N-WASP binds directly to p120-catenin and induces organization of cortical actin. Stabilization of F-actin filaments occurs through the binding of a variety of capping proteins including capping protein (aka β-actinin), CapZ, FSGS3/CD2-associated protein (FSGS3/CD2AP) to the barbed (plus) end of F-actin and is required for actin assembly. Hence, the strength of VE-cadherin adhesive bonds and therefore integrity of the endothelial barrier is regulated by a complex network involving the aforementioned regulators of actin polymerization.

Role of Intracellular Tension in Regulating VE-Cadherin Adhesion

Intracellular tension is a critical component regulating stable anchorage of VE-cadherin to the actin cytoskeleton.
Simultaneous binding of α-catenin to both β-catenin and F-actin filaments occurs only in the presence of tension. Tension of up to 10 pN induces stable bond formation between the β-catenin/α-catenin complex and F-actin in vitro. VE-cadherin adhesion at AJs is formed in a tension-dependent manner, indicating an important role of the actomyosin apparatus at AJs. Endothelial cell monolayers generate an intracellular tugging force of ~40 nN with an average tension on a VE-cadherin molecule from the actin cytoskeleton ranging from 1.8 to 2.4 nN per molecule. Proinflammatory mediators such as α-thrombin increase traction forces and the resultant mechanical stress at AJs (up to 8 nN/m²) that uncouples the VE-cadherin complex from the actin cytoskeleton.

Intracellular tension is generated by the actomyosin contractile apparatus (Figure 2A and 2B). The ubiquitously expressed nonmuscular actin motor myosin-IIA and B are central to control of intracellular tension at endothelial AJs. Myosin II binds to F-actin filaments and generates tension by sliding these filaments along each other. The ability of myosin-II to assemble antiparallel filaments consisting of 10 to 30 motors is the main determinant of the magnitude of intracellular tension (Figure 2A).

Phosphorylation of regulatory myosin light chain (MLC) at Thr18 and Ser19 is a prerequisite for motor activity. The canonical pathway involves phosphorylation of MLC by endothelial-specific myosin light chain kinase (MLCK), which is commonly activated by Ca²⁺/calmodulin binding or Src-dependent phosphorylation at Tyr464 and Tyr471. MLC phosphatase counteracts MLCK activity by dephosphorylating MLC. Therefore, a fine balance between MLCK and MLC phosphatase is essential for limiting myosin-II phosphorylation and thereby magnitude of contractile forces at endothelial AJs.

Activity of MLC phosphatase (PP1, type 1 protein phosphatase) is downregulated by RhoA signaling. RhoA activates Rho-associated coiled-coil forming protein kinase (ROCK), which in turn, elicits its effect through phosphorylation of PP1 at Thr-695, Ser-894, and Thr-850. The latter inhibits PP1 activity, allowing for myosin-II phosphorylation by MLCK and assembly of the actomyosin apparatus.

In endothelial monolayers, myosin-II activity is finely tuned at VE-cadherin adhesions by a yet unknown mechanism. A basal level of ROCK activity seems to be essential for the maintenance of endothelial AJs. Recent studies utilizing the RhoA/B/C biosensors show that both RhoA and RhoB are constitutively activated at AJs. It remains unclear, however, how the basal level of Rho activity is maintained at AJs. To date, we have a better understanding of the regulation of RhoA signaling at epithelial AJs. In epithelial cells, the RhoA zone at E-cadherin adhesion represents the main molecular mechanism for generation of apical–lateral patterns of junctional contractility. Both p120-catenin and myosin-IIA recruit ROCKI to nascent adhesions and provide positive feedback regulation of RhoA activity at E-cadherin adhesions. ROCKI phosphorylates Rnd3 and prevents cortical recruitment of the GTPase-activating protein (GAP), p190RhoGAP to AJs, and hence preserves RhoA from inactivation.

It is unlikely that a similar mechanism operates in endothelial cells where p190RhoA accumulation and activity at AJs is required for maintenance of stable AJs. A possible mechanism of myosin-II might involve Cdc42 signaling as evident by the finding that Cdc42 also activates myosin-II (Figure 2B). Cdc42 mediates the assembly of myosin-II filaments through its effectors Pak2, Pak4, and myotonic dystrophy kinase-related Cdc42-binding kinase (MRCK). MRCK phosphorylates and thereby inhibits MLC phosphorylation although it is less potent than ROCK in activating myosin-II. This makes MRCK the best candidate for induction of low-grade tension at endothelial AJs. Therefore, it is possible that Cdc42 coordinates N-WASP–mediated polymerization of actin filaments with that of p120-catenin, such that the activation of myosin-II at VE-cadherin adhesion is able to strengthen AJs (Figure 2B).

Mechanosensing at the Level of AJs

Cells experience external mechanical forces from neighboring cells and the extracellular matrix (ECM), as well as the internal force generated by the actomyosin contractile machinery. In the vascular system, endothelial cells are also exposed to hemodynamic forces resulting from hydrostatic pressure in vessels and blood flow. VE-cadherin forms a mechanosensory complex with platelet endothelial cell adhesion molecule 1 (PECAM-1) and with vascular endothelial growth factor receptor (VEGFR) 2 and VEGFR3 enabling the endothelium to sense changes in hemodynamics and thus activate a variety of signaling pathways. These signaling pathways in turn orchestrate a coordinated cellular response resulting in reorganization of the actin cytoskeleton, redistribution of intracellular tension, and a shift in phosphorylation of VE-cadherin and associated catenins. Using a recently developed biosensor that measures actomyosin-mediated tension across VE-cadherin adhesion and PECAM-1, it was shown that shear stress applied to an endothelial monolayer reduces tension across VE-cadherin adhesion concomitant with a decrease in total cell–cell force. Thus, it seems that the distribution of intracellular tension is tightly regulated in response to external mechanical forces thus allowing AJs to align in the direction of flow.

The current concept of mechanosensing at AJs involves a series of distinct (and perhaps linear) sequences of signal transduction events (Figure 3). Signaling is initiated with conformational changes in PECAM-1 followed by activation of Src family kinase Fyn at AJs. Fyn in turn phosphorylates PECAM-1 and activates the receptor tyrosine kinase VEGFR2 in a ligand-independent manner. VEGFR2, in turn, induces phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K), Akt, and endothelial nitric oxide synthase (eNOS). PI3K subsequently activates Rac1, relieving the tension at AJs, whereas eNOS mediates vasorelaxation through the effect of nitric oxide on vascular smooth muscle cells.
adhesions (FAs) in the mechanosensory response. In cells adherent to collagen, mechanosensing at AJs resulted in Gαs-dependent protein kinase A (PKA) signaling and subsequent phosphorylation of RhoA. PKA phosphorylates RhoA at serine 188 and promotes the association of RhoA with Rho-guanine dissociation inhibitor, which sequesters RhoA in the cytosol. PKA-mediated inhibition of RhoA signaling is responsible for blunted stiffening of endothelial cells in response to hemodynamic shear stress. Hence, specifics of ECM composition might permit a differential response of Rho-guanine dissociation inhibitor, which sequesters RhoA in the cytosol.

Figure 2. Role of actomyosin apparatus in stabilizing adherens junctions (AJs). A, Domain structure of nonmuscle myosin-II (NM-II). The NM-II consists of a globular head domain containing both actin-binding and motor domains, essential light chains (ELCs), regulatory light chains (RLCs), and heavy chains. NM-II possesses a head-to-tail interaction in the absence of phosphorylation. Phosphorylation of regulatory light chain at Thr18/Ser19 by myosin light chain kinase (MLCK) unfolds the molecule, enabling assembly of antiparallel filaments through interactions between their rod domains. Activation of Rho-associated kinase (ROCK), which inhibits phosphatase activity of myosin light chain phosphatase (MLCP) in a phosphorylation-dependent manner, also favors RLC phosphorylation. NM-II filaments bind to actin filaments, which slide along each other, and cause a cell contraction. Modified from Vicente-Manzanares et al with permission of the publisher. Copyright ©2009, Nature Publishing Group. B, Proposed mechanism of regulation of NM-II activity at AJs in confluent endothelium. NM-II regulates attachment of the vascular endothelial (VE)-cadherin adhesion complex to the actin cytoskeleton, thereby generating mechanical tension required for binding of α-catenin to both β-catenin and f-actin. NM-II phosphorylation is controlled by MLCK and MLCP activities. In the model, we propose that Src and Cdc42 pathways cooperate in regulating NM-II activity at AJs. Cdc42 facilitates activation of NM-II through myotonic dystrophy kinase–related Cdc42-binding kinase (MRCK)–dependent phosphorylation of MLCP, whereas Src phosphorylates MLCK at sites of VE-cadherin adhesion. CaM indicates calmodulin; GAP, GTPase-activating protein; and GEF, guanine nucleotide exchange factor.

Rho-guanine dissociation inhibitor, which sequesters RhoA in the cytosol, PKA-mediated inhibition of RhoA signaling is responsible for blunted stiffening of endothelial cells in response to hemodynamic shear stress. Hence, specifics of ECM composition might permit a differential response of
endothelial cells to shear stress. In the fibronectin-rich aortic arch, which is prone to atherosclerosis, endothelial cells are stiffer and more permeable to protein-rich fluids and leukocytes. Failure to activate PKA and reduce stiffness of endothelial cells in these regions of the aorta might contribute to the development of atherosclerosis. In this context, remodeling of the ECM, itself a function of endothelial cell activation, is likely a key determinant of change in endothelial barrier integrity at the level of AJs.

**Figure 3. Mechanotransduction at adherens junctions (AJs).** The mechanosensory complex in endothelial cells is composed of vascular endothelial (VE)-cadherin, platelet endothelial cell adhesion molecule (PECAM)-1, and vascular endothelial growth factor receptor (VEGFR) 2. Mechanosensing of shear stress occurs through PECAM-1-dependent activation of Fyn, which in turn facilitates VEGFR2-mediated signaling in a ligand-independent manner and activates phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K). PI3K activates both Rac1 and endothelial nitric oxide synthase (eNOS) signaling pathways. Rac1 relieves tension at AJs, whereas NO concomitantly promotes vasorelaxation of smooth muscle cells. PECAM-1-dependent sensing of shear stress also promotes α2β1 integrin signaling and consequently activation of protein kinase A (PKA) in atheroresistant regions. PKA phosphorylates RhoA and decreases RhoA-dependent cellular stiffness allowing the endothelial cell to align in the direction of blood flow. GPCR indicates G-protein–coupled receptor; and sGC, soluble guanylyl cyclase.

### Endothelial Permeability

The steady-state dynamics of VE-cadherin at AJs is a critical determinant of AJ integrity. This includes several interdependent events concerning both biophysical properties of VE-cadherin adhesive bonds and the integration of intracellular proteins within VE-cadherin. VE-cadherin adhesive bonds undergo continuous assembling, disassembling, and remodeling at AJs; the kinetics of these events are defined by the affinity of trans-dimerization. This primary adhesion event requires neither energy nor attachment of the VE-cadherin complex to the actin cytoskeleton.

In contrast, turnover of VE-cadherin molecules at AJs, specifically the exchange between junctional and intracellular pools, is tightly regulated by the interaction of VE-cadherin with associated catenin proteins and the actin cytoskeleton. The steady-state kinetics of VE-cadherin at AJs is controlled through the stability of the cadherin–catenin complex, intracellular tension, and organization of the actin cytoskeleton. Disassembly of VE-cadherin adhesion in response to extracellular stimuli is triggered by phosphorylation of VE-cadherin and associated catenins and the redistribution of the actin cytoskeleton to the sites of FAs. Depending on the duration and magnitude of the intracellular response, changes in VE-cadherin dynamics at AJs can lead to weakening or disassembly of AJs, causing either transient or prolonged increase in junctional permeability. For example, tumor vessels represent a case of chronic vascular leakage that is associated with downregulation of VE-cadherin expression.

Multiple lines of evidence suggest that the hyperpermeability response to proinflammatory mediators can be mitigated if the integrity of VE-cadherin internalization is preserved. Various strategies have been developed to stabilize VE-cadherin adhesion. They include overexpression of p120-catenin, which blocks clathrin-mediated VE-cadherin internalization; expression of a VE-cadherin-α-catenin chimera, which directly tethers adhesion to the actin cytoskeleton; and artificial bridging of opposing VE-cadherin molecules at AJs with a cyclic peptide. This evidence suggests that it is possible to manipulate the integrity of VE-cadherin adhesion, the main gatekeeper of the endothelial barrier.
Kinase-Mediated Regulation of VE-Cadherin Turnover at AJs

The spatiotemporal control of VE-cadherin turnover at AJs is an integral part of the intracellular response to environmental cues. Destabilization of VE-cadherin adhesion occurs during transendothelial migration of leukocytes and in response to extracellular stimuli associated with opening AJs and increased barrier permeability. Intracellular signaling such as phosphorylation of VE-cadherin and associated catenin proteins (Table 1) induce disassembly of the VE-cadherin–catenin complex. In particular, dissociation of p120-catenin from the juxtamembrane region of VE-cadherin masks the binding site for AP2, an adaptor protein complex of the endocytic machinery, and primes VE-cadherin for internalization (Figure 4). Vascular endothelial growth factor (VEGF) promotes VE-cadherin internalization via β-arrestin 2–mediated endocytosis. In this context, VEGF induces c-Src–dependent phosphorylation of the guanine nucleotide exchange factor Vav2, which in turn activates Rac1 and p21-activated kinase. p21-activated kinase phosphorylates VE-cadherin at S665 and targets VE-cadherin for β-arrestin 2–mediated internalization. Moreover, VEGF signaling decreases VE-cadherin/p120-catenin association, promoting clathrin-dependent VE-cadherin endocytosis.

Multiple proinflammatory mediators including thrombin, histamine, platelet-activating factor (PAF), VEGF, and tumor necrosis factor (TNF)-α facilitate disassembly of VE-cadherin adhesion although they do not seem to function by inducing a similar pathway. This is evident by the finding that they induce differential phosphorylation of VE-cadherin and p120-catenin. Some mediators, such as thrombin, function through phosphorylating VE-cadherin at Tyr658.200 In addition, activation of FAK down-stream of VEGF induces the phosphorylation of β-catenin at Y142 and its subsequent dissociation from the VE-cadherin complex. Deficiency of FAK in endothelial cells decreases extravasation of tumor cells and prevents spontaneous orthotopic melanoma metastasis. Together these studies suggest a critical role of FAK in regulating endothelial barrier integrity; however, this function of FAK may either be through direct interaction or upstream of another kinase such as Src.

It is also important to note that many of these kinases are themselves constituents of the VE-cadherin adhesion complex in the resting endothelium. Many of them are basally inactive because their activity is suppressed by phosphatases and other kinases at AJs.

Table 1. Role of Kinases at AJs

<table>
<thead>
<tr>
<th>Kinases</th>
<th>Activity Within VE-Cadherin Complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-Src (p60 Src, tyrosine kinase)</td>
<td>Phosphorylates VE-cadherin at Y658 and reduces binding to p120-cat; phosphorylates VE-cadherin Y685 and increases binding to CSK; phosphorylates β-catenin Y654 and reduces binding to VE-cadherin</td>
</tr>
<tr>
<td>CSK (cytosolic C-terminal Src kinase, tyrosine kinase)</td>
<td>Phosphorylates c-SRC Y530 and inhibits c-Src activity at AJs</td>
</tr>
<tr>
<td>Fer (tyrosine kinase)</td>
<td>Phosphorylates PTP1B Y152 and induces binding to VE-cadherin</td>
</tr>
<tr>
<td>Fyn (SFK, tyrosine kinase)</td>
<td>Phosphorylates β-catenin Y142 and reduces binding to VE-cadherin</td>
</tr>
<tr>
<td>Yes (SFK)</td>
<td>Phosphorylates β-catenin Y142 and reduces binding to VE-cadherin</td>
</tr>
<tr>
<td>Abelson (tyrosine kinase)</td>
<td>Phosphorylates β-catenin Y489 and reduces binding to VE-cadherin</td>
</tr>
<tr>
<td>PYK2 (proline-rich tyrosine kinase)</td>
<td>Phosphorylates VE-cadherin Y731 and reduces binding to β-catenin</td>
</tr>
<tr>
<td>PAK (Ser/Thr kinase)</td>
<td>Phosphorylates VE-cadherin S665 and targets VE-cadherin for β-arrestin–mediated internalization</td>
</tr>
</tbody>
</table>

AJ indicates adherens junction; PTP, protein tyrosine phosphatase; and VE, vascular endothelial.
Figure 4. Role of specialized kinases and phosphatases in stabilizing adherens junctions (AJs). A, Stable adherens junctions are characterized by low phosphorylation of vascular endothelial (VE)-cadherin and associated catenin proteins. Protein tyrosine phosphatases density-enhanced phosphatase-1 (DEP1), VE-protein tyrosine phosphatase (PTP), PTPμ, Src homology 2-domain containing tyrosine phosphatase (SHP2), and PTP1B at AJs counteract the effect of tyrosine kinases (Src, Fen, Fyn, and Ableson) to stabilize the VE-cadherin–catenin complex. Focal adhesion kinase (FAK) also stabilizes VE-cadherin adhesion by inhibiting RhoA signaling through phosphorylation-dependent activation of p190-Rho GTPase-activating protein (RhoGAP). B, Phosphorylation-dependent activation of kinases by vascular endothelial growth factor (VEGF), histamine, thrombin, platelet-activating factor (PAF), and tumor necrosis factor (TNF)-α leads to phosphorylation of VE-cadherin, β-catenin, and p120-catenin (residues are indicated) by distinct kinases. This results in destabilization of the VE-cadherin complex. Dissociation of p120-catenin because of phosphorylation of VE-cadherin at Y658 or p120-catenin at S879 exposes a VE-cadherin binding site for AP2 to facilitate VE-cadherin endocytosis via clathrin-coated pits. Phosphorylation of β-catenin induces the uncoupling of VE-cadherin adhesion from the actin cytoskeleton. Activation of RhoA leads to phosphorylation of myosin light chain (MLC), formation of stress fibers, and increased tension across VE-cadherin adhesion. Ang1 indicates angiopoietin-1; AP2, adaptor protein 2; PKCα, protein kinase C alpha; and RhoGEF, Rho-guanine nucleotide exchange factor.
oxidative stress, a common signal activated by multiple pro-inflammatory stimuli, is one such example. Whereas c-Src activity is basally suppressed by Csk at AJs, this suppression is relieved by Gt13 interaction with VE-cadherin downstream of oxidative redox signaling.210

**Role of AJ Localized Phosphatases in Regulating Junctional Integrity**

Protein tyrosine phosphatases (PTP) such as PTP1B, PTPμ, PTPβ (also known as vascular endothelial [VE]-PTP), Src homology 2-domain containing tyrosine phosphatase, and density-enhanced phosphatase-1 are also constituents of the VE-cadherin adhesion complex203,204,206,207 (Figure 4). They stabilize the cadherin–catenin complex by opposing the barrier-disruptive action of kinases (Table 2).204-207 PTP1B is required for continuous dephosphorylation of β-catenin at Tyr654, thus preventing the dissociation of β-catenin from AJs.206 Src homology 2-domain containing tyrosine phosphatase also induces dephosphorylation of β-catenin and promotes reassembly of AJs after inflammatory insult.207

VE-PTP, the most studied of the AJ-associated phosphatases in endothelial cells, interacts with VE-cadherin through the membrane proximal fibronectin-like extracellular domain.205 It stabilizes basai VE-cadherin adhesion by decreasing the rate of VE-cadherin internalization.165,169 PTP activity per se may not be required for this effect because inhibition of VE-PTP activity with a small-molecule inhibitor stabilizes AJs and restores tissue-fluid balance in eye and lung vascular inflammation models.177,208 The therapeutic effect of VE-PTP inhibitor has been explained by angiopoietin-1 signaling, which is suppressed by VE-PTP-dependent dephosphorylation of Tie-2177,208 (Figure 4). Angiopoietin-1 elicits a barrier protective effect in the endothelium by activating Tie-2 receptor signaling that uncouples Src kinase from VEGFR2 and inactivates VEGFR2 signaling.179 The angiopoietin-1/Tie-2 axis also triggers sequential activation of Rap1 and Rac1 in the endothelium.177,209 Rac1, in turn, causes dissolution of actin stress fibers and stabilizes VE-cadherin trans-interaction by preventing RhoA-mediated intracellular tension at AJs.182 As discussed below, this tug of war between RhoA and Rac1 at AJs is a major determinant of the stability and plasticity of VE-cadherin adhesion both basally and in response to permeability-increasing stimuli.

Table 2. **Role of Phosphatases at Adherens Junctions**

<table>
<thead>
<tr>
<th>Phosphatases</th>
<th>Activity Within VE-Cadherin Complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTP1B</td>
<td>Deyphosphorylates β-catenin Y654 and increases binding to VE-cadherin</td>
</tr>
<tr>
<td>SHP2 (tyrosine phosphatase)</td>
<td>Dephosphorylates β-, γ-, and p120-catenins</td>
</tr>
<tr>
<td>PTPμ (tyrosine phosphatase)</td>
<td>Dephosphorylates VE-cadherin</td>
</tr>
<tr>
<td>DEP1 (tyrosine phosphatase)</td>
<td>scaffold function</td>
</tr>
<tr>
<td>VE-PTP (tyrosine phosphatase)</td>
<td>Dephosphorylates VE-cadherin and γ-catenin</td>
</tr>
</tbody>
</table>

| DEP1 indicates density-enhanced phosphatase-1; PTP, protein tyrosine phosphatase; SHP2, Src homology 2-domain containing tyrosine phosphatase; and VE, vascular endothelial. |

**Role of Nitric Oxide Synthases**

eNOS is responsible for constitutive synthesis of nitric oxide (NO) in the resting endothelium.210-211 Basal production of NO controls vascular tone and vasorelaxation in response to increased blood flow, whereas hyperactivation of eNOS in response to VEGF or proinflammatory stimuli such as PAF triggers S-nitrosylation of VE-cadherin, β-catenin, and p120-catenin.213-215 S-nitrosylation, the covalent attachment of S-nitrosothiol to a cysteine thiol,216 represents another regulatory pathway of AJ stability. Similar to phosphorylation, S-nitrosylation reversibly modulates affinity of β- and p120-catenin proteins to VE-cadherin.213-215,217 S-nitrosylation of β-catenin on the Cys619 residue promotes dissociation of β-catenin from VE-cadherin causing destabilization of AJs and resultant hyperpermeability of the endothelial barrier.213-215,217 Deletion of eNOS causes a blunted VEGF-mediated permeability response,217 further supporting the role of NO redox signaling in regulating endothelial barrier function.

PAF induces S-nitrosylation of p120-catenin on multiple cysteine residues, Cys579, Cys429, Cys450, Cys618, and Cys692.214 S-nitrosylation of Cys579, located within the VE-cadherin–interacting domain, might represent a critical event associated with NO redox signaling in regulating endothelial hyperpermeability.214 PAF can also induce S-nitrosylation of VE-cadherin and consequent disruption of AJs.215 In this context, S-nitrosylation of VE-cadherin is required for tyrosine phosphorylation and internalization of VE-cadherin.216 These data indicate that S-nitrosylation of junctional proteins is an important mechanism for destabilization of AJs.

Another post-translational modification induced by reactive nitrogen species such as anion (ONOO−) is nitration of tyrosine residues.218 Nitration of junctional proteins, such as p190RhoGAP, which is associated with p120-catenin,157 and β-catenin itself,219 facilitates disassembly of the VE-cadherin adhesion complex. The proinflammatory mediator serine protease α-thrombin triggers nitration of p190RhoGAP on Tyr1105 downstream of eNOS-mediated NO redox signaling.157 This inhibits GAP activity, consequently activating RhoA signaling at AJs and corresponding actomyosin cell contraction.157 Hence, nitration of p190RhoGAP represents a crucial mechanism in the activation of RhoA signaling implicated in hyperpermeability of the endothelial barrier during inflammation.

Nitration of β-catenin occurs in the context of chronic or acute inflammation associated with the expression of inducible iNOS. Many inflammatory processes including diabetes mellitus, atherosclerosis, and systemic inflammation are associated with protein nitration because of activation of iNOS in the endothelium.220-225 Induction of iNOS in macrophages triggers nitration of β-catenin in endothelial cells and the resultant dissociation of VE-cadherin adhesion-mediated complex.219 Nitration of β-catenin also promotes its translocation to the nucleus where it is associated with T-cell factor/Lef transcription factors.219 This ultimately leads to vascular remodeling after injury.226 In this context, nitration of β-catenin induces vascular leakage, but at the same time limits endothelial injury by promoting prosurvival pathways.219,226
Role of Acetyltransferases

A growing body of evidence suggests that lysine acetylation of β-catenin might also provide an important mechanism for regulating endothelial barrier permeability. Acetylation is a reversible process controlled by acetyltransferases and deacetylases. Acetylation of β-catenin induces its association with the plasma membrane and modulates β-catenin activity toward specific genes. Acetylation of β-catenin at Lys49 is mediated by CREB-binding protein acetyltransferase and is known to modulate Wnt signaling in a promoter-specific fashion. In contrast, acetylation of β-catenin at Lys345 by the transcriptional coactivator p300 increases its affinity for T-cell factor 4, suggesting that acetylation might differentially modulate β-catenin transcriptional activity.

In contrast, deacetylation of β-catenin at Lys49 is controlled by a member of class II histone deacetylase HDAC6. Knockout of HDAC6 or treatment of animals with the specific HDAC6 inhibitor tubastatin A protects against endotoxin-induced pulmonary edema and acute lung injury and improves survival of mice in septic shock.

Role of Transient Receptor Potential Channels

Multiple pathological conditions are associated with calcium signaling, which represent a crucial pathway in mediating hyperpermeability of endothelial barrier. A superfamily of transient receptor potential (TRP) channels that are responsible for regulation of Ca2+ entry in endothelial cells are TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), and therefore, have been extensively studied for their role in mediating hyperpermeability response.

Transient Receptor Potential Canonical

Endothelial cells express 5 nonselectively permeable TRP cation channels (canonical): TRPC1, TRPC3, TRPC4, TRPC5, TRPC6, and TRPC7. These channels mediate store- and receptor-operated Ca2+ entry from extracellular spaces in response to edemagenic and proangiogenic mediators.

Store-operated calcium entry involves TRPC1 and TRPC4. These channels are activated by G-protein–coupled receptors, and receptor tyrosine kinases coupled to activation of phospholipase C. Multiple proinflammatory mediators such as serine protease α-thrombin, histamine, and PAF induce disruption of the endothelial barrier through binding to G-protein–coupled receptors on the surface of endothelial cells. This triggers activation of phospholipase C, which catalyzes phosphoinositol 4,5-bisphosphate (PIP2) to inositol 1,4,5-trisphosphate and diacylglycerol. Inositol 1,4,5-trisphosphate activates Ca2+ release from intracellular stores and consequent Ca2+ entry through store-operated calcium entry.

The critical role of store-operated calcium entry in the hyperpermeability response is evident from genetic deletion of murine trpc1 and trpc4 genes. These animals demonstrate markedly reduced hyperpermeability responses in lungs and reduced mortality induced by endotoxins. Intriguingly, full activation of TRPC1 is required for RhoA-mediated reorganization of the actin cytoskeleton, enabling the interaction of TRPC1 with inositol 1,4,5-trisphosphate receptors, calcium channels on the endoplasmic reticulum membrane responsible for Ca2+ release from stores, indicative of a positive amplification loop. Other studies demonstrate that TRPC1 downregulates both the expression and activity of sphingosine kinase 1, the kinase responsible for production of the barrier-enhancing mediator sphingosine-1-phosphate (S1P), thereby weakening AJs in both resting and inflammatory (activated) endothelium. This function of TRPC1 seems to be independent of Ca2+ entry because expression of a TRPC1 pore-defective mutant is sufficient to limit the expression of sphingosine kinase 1 and reduce the permeability response of the endothelial barrier.

Receptor-activated calcium entry is mediated through TRPC6 and TRPC7 in endothelial cells. These channels are activated by a diacylglycerol-dependent mechanism and are independent of intracellular store depletion. Multiple lines of evidence show that TRPC6 promotes both histamine- and lipopolysaccharide-mediated increases in endothelial permeability. Importantly, TRPC6 colocalizes with PECAM-1 at AJs during transendothelial migration of leukocytes. In this context, activation of TRPC6 is mediated through homophilic PECAM-1 adhesion between endothelial cells and leukocytes. Furthermore, TRPC6-mediated receptor-activated calcium entry is required for neutrophil transendothelial migration because expression of a pore-defective channel or knockdown of TRPC6 in endothelial cells arrests neutrophils within AJs.

Transient Receptor Potential Vanilloid

The subfamily members of TRPV induce Ca2+ entry in response to osmolar, thermal, mechanical, and chemical stimuli. In endothelial cells, TRPV4 is activated by heat and endogenous lipid mediators such as epoxyeicosatrienoic acids (14,15-EET) and phorbol ester 4a-phorbol 12,13-didecanoate (4αPDD). In mice, knockout of trpv4 gene inhibits permeability responses of lung microvasculature to both 4αPDD and 14,15-EET without affecting store-operated calcium entry. Other studies indicate that TRPV4 might also be involved in the mechanism of Ca2+ entry in response to shear stress.

Transient Receptor Potential Melastatin

The TRPM family is presented by TRPM2 and TRPM4 in endothelial cells. TRPM2 is activated by intracellular ADP-ribose, hydrogen peroxide, and nicotinamide adenine dinucleotide. TRPM2 has been shown to induce Ca2+ entry in response to H2O2 in a dose-dependent manner. It is plausible that TRPM2 may serve as a cellular redox sensor in endothelial cells.

Role of RhoGTPases in Regulating Integrity of AJs

The subfamily of Rho (Ras homologous) GTPases belongs to the Ras-sarcoma (Ras)–related superfamily of low molecular weight monomeric G proteins with highly conserved sequence homology. RhoA, Rac1, and Cdc42 are the best-studied members of the RhoGTPases subfamily because of their critical role in organization of the actin cytoskeleton and profound effect on the integrity of AJs.

A fine balance among RhoA, Rac1, and Cdc42 at AJs is regulated by VE-cadherin outside-in signaling. Formation of nascent VE-cadherin adhesions activates Rac1. Rac1, in turn, induces polymerization of actin filaments specifically
at sites of VE-cadherin adhesion and contributes to the stabilization of AJs. 270 Rac1 also stabilizes VE-cadherin trans-interaction by counteracting RhoA activity and suppressing actomyosin tension. 182 Hence, a subtle balance between RhoA and Rac1 activities is a critical control point of VE-cadherin turnover at AJs. 182

RhoGTPases are also involved in destabilization and realnnealing of AJs in response to mechanical and humoral stimuli. The net effect of RhoGTPases on barrier integrity depends on the nature of extracellular stimuli and activation of convergent signaling pathways that are able to rewire RhoGTPase signaling to specific intracellular locations and establish their interactions with particular downstream effectors. As described below, the complexity of these biological outcomes can be explained by the combinatorial effects of activation of multiple RhoGTPases.

Subfamily of RhoGTPases
Monomeric RhoGTPases cycle between active (GTP-bound) and inactive (GDP-bound) states and thus act as binary molecular switches. 263,271,272 In the GTP-bound state, they interact with the downstream effectors to elicit a physiological response. 181,184,186 RhoGTPases interact with a wide spectrum of downstream effectors that are structurally different from each other. 196,205 and yet the RhoGTPase domain structure itself is highly conserved. All members of the RhoGTPase subfamily contain a G domain structure at the N terminal, which is composed of 5 sets of G-box binding motifs 273 (Figure 5A). The G domain consists of the nucleotide binding site (also called the p-loop), core effector domain, and switch regions (I and II) forming the interface for interaction with guanine nucleotide exchange factors (GEFs; Figure 5B). The p-loop motif inside the switch I and switch II regions represents the site of GDP to GTP exchange and the interface for interaction with downstream effectors on binding to GTP. 274–276 This ability to interact with effectors is lost when the switch region possesses a conformational change because of the release of the hydrolyzed phosphate. 273

Because of the high binding affinity of GTPases for both GDP and GTP and slow rate of intrinsic GTP hydrolysis, the GTPase cycle is controlled by upstream regulators, specifically GAPs (GTPase-activating proteins), GEFs, and guanine nucleotide dissociation inhibitors (Figure 5C). GAPs accelerate the rate of GTP hydrolysis and switch off RhoGTPase activity, whereas GEFs promote GDP to GTP exchange, thus turning RhoGTPases on. 277–281 The latter is a multistep process involving formation of a ternary complex between the GTPase, GEF, and nucleotide followed by nucleotide release (Figure 5B). Rebinding of GTP, predominantly because of higher concentration in the cell, restores GTPase activity. GEFs promote GTP exchange by increasing the rate of GDP release. 282,283 Another regulator, guanine nucleotide dissociation inhibitor, interacts with the GDP-bound form and prevents GTP exchange. 280,281 Guanine nucleotide dissociation inhibitors shield the hydrophobic tail by binding to a prenylated COOH terminus, and hence sequesters GTPase from the membrane compartment. 284,285

The interaction between RhoGTPases and downstream effectors requires translocation of GTPases from the cytosol to the plasma membrane. 284,285 This is controlled by post-translational modifications by the lipids farnesyl pyrophosphate and geranylgeranyl pyrophosphate. CAAX sequence at the C terminus serves as both membrane targeting signal and a recognition motif for farnesylation and geranylgeranylation. 266–288 Some members of the RhoGTPase family such as RhoA and RhoC are only geranylgeranylated and are localized in the cytoplasm, whereas others such as RhoB possess geranylgeranylated, farnesylated, or palmitoylated sites and can be localized at the plasma membrane or in the cytoplasm (localized to endosomes). 288–290

The recruitment of RhoGTPases to membranes, as demonstrated for Rac1, occurs preferentially at the boundaries between the cholesterol-rich, ordered domains (ie, lipid rafts) and the liquid disordered phase. 291 Rac1 then diffuses into both raft and nonraft domains, where it interacts with either downstream effectors inside of ordered domains or can be selectively inactivated by GAPs that prefer nonraft regions. 291 Hence, on the one hand, post-translational modifications target small RhoGTPases to distinct subcellular localizations, allowing them to interact with a specific set of downstream effectors and thus elicit distinct biological outputs through spatially regulated signaling networks. On the other hand, the organization of plasma membrane domains modulates RhoGTPase signaling by limiting their activities in the nonraft regions.

Rac1 and Cdc42 Signaling Pathways Regulate Stability of VE-Cadherin Adhesion
The role of Rac1 and Cdc42 on assembly and maturation of VE-cadherin adhesion is predominantly associated with their ability to induce nucleation, polymerization, and organization of the actin cytoskeleton through interactions with actin-binding proteins. 186,292,293 Whereas Rac1 promotes polymerization of branched actin networks within lamellipodia protrusions, 187,189,270,294 Cdc42 facilitates polymerization of linear F-actin filaments into filopodia. 295,296 On activation, Rac1 interacts with several downstream effectors including the WAVE (WASP family verprolin-homologous protein), IQGAP1 (Ras GAPS), PAR6 (partitioning-defective polarity protein), and members of p21-activated kinase (Pak) family 186 (Figure 6). Among the members of Pak family, Pak1 facilitates actin polymerization through activation of Lin1, Isl-1, and Mec-3 kinase. 297 The latter phosphorylates the actin-binding protein cofillin at Ser3 and consequently blocks actin monomer depolymerization. 298

The Cdc42 downstream effectors include WASP, neuronal (N)-WASP, diaphanous-related formin-1, IQGAP1, PAR6, and MRCK (Figure 6). 186 Cdc42 induces nucleation and polymerization of actin filaments through WASP and mDia pathways. 163 It can also bind to the insulin receptor substrate p53 that coordinates actin nucleation and polymerization through binding to both WASP and mDia at the plasma membrane. 299,300 The Cdc42-MRCK pathway activates myosin-II and strengthens AJs by generating low-magnitude intracellular tension. 160 Hence, in addition to nucleation, polymerization, and stabilization of the actin cytoskeleton at AJs, the Cdc42 signaling pathway is also capable of generating intracellular tension independent of RhoA signaling.
Cdc42 plays a crucial role in assembly and maintenance of AJs. Cdc42 deletion in endothelial cells results in loss of apical-basal polarity and disrupted AJs. Consistent with the proposed role of Cdc42 in activating both actin polymerization and stabilization, these defects are associated with formation of aberrant filopodia and impaired assembly of the actomyosin apparatus. The current model suggests a critical role of Cdc42 signaling in the assembly and maturation of AJs via effectors Pak2, Pak4, and N-WASP (Figure 6). Cdc42 signaling thus elicits an endothelial barrier protective effect in...
inflammatory lung injury and also promotes reannealing of the barrier in inflammatory endothelium through N-WASP–mediated actin polymerization. Moreover, Cdc42 can also act as a competitive inhibitor of Rac1 and thereby counteract the barrier-disruptive effect of p67phox signaling and reactive oxygen species production.

In contrast to Cdc42 that promotes AJ assembly, the outcome of Rac1 signaling on endothelial barrier integrity highly depends on intracellular context. In some cases, in response to shear stress or the bioactive lipid mediator S1P, the activation of Rac1 signaling enhanced endothelial barrier function. In other cases, such as stimulation of endothelial cells with TNF α, PAF, or VEGF, activation of Rac1 caused disruption of the endothelial barrier. Recent work utilizing a photoactivatable Rac1 probe sheds light on the biological outcome of Rac1 signaling at AJs independent of convergent signaling events. Recent work utilizing a photoactivatable Rac1 probe sheds light on the biological outcome of Rac1 signaling at AJs independent of convergent signaling events.

Rac1 signaling may also cause disassembly of VE-cadherin adhesion and disruption of the endothelial barrier. This is evident by the finding that the proinflammatory mediator TNFα leads to a transient and robust increase in Rac1 activity through phosphatidylinositol (3,4,5)-trisphosphate–dependent Rac exchanger 1 (P-Rex1). In this case, Rac1 signals through p67phox effector leading to production of reactive oxygen species and subsequent activation of Src and VE-cadherin phosphorylation. Another proinflammatory mediator PAF also induces Rac1 signaling through T-lymphoma invasion and metastasis-inducing protein 1 (Tiam1). PAF-induced activation of Rac1 is associated with profound reorganization of the actin cytoskeleton and vascular leakage. Furthermore, VEGF activates Rac1 through Src-dependent phosphorylation of Vav2 and causes Pak-mediated phosphorylation of VE-cadherin at Serine 665 and subsequent VE-cadherin internalization by β-arrestin. In conclusion, it seems that Rac1 signaling can have divergent effects on AJs ranging from stabilization to disassembly of VE-cadherin adhesions. These responses exemplify the central importance of intracellular environment, localized signaling, and interaction with specific partners in the net biological outcome of Rac1 signaling.

**RhoA Signaling Pathway**

In contrast to Rac1 and Cdc42 that mediate the assembly, stabilization, and maturation of AJs, RhoA signaling mainly contributes to destabilizing AJs and increasing endothelial permeability. RhoA promotes the formation of actin stress fibers and actomyosin contraction through activation

![RhoA, Rac1, and Cdc42 regulation of endothelial adherens junctions (AJs).](image-url)
of downstream effectors such as ROCK and mDia (Figure 6). The reorganization of the actin cytoskeleton via the mDia pathway and concurrent assembly of the contractile apparatus through activation of ROCK signaling leads to the generation of intracellular tension at junctions that disassemble AJs.

The mDia and ROCK pathways demonstrate a cooperative behavior downstream of RhoA activation. ROCKI and ROCKII are differentially regulated in endothelial cells. ROCKI-mediated activation of myosin-II. ROCKI and ROCKII are basally active and contribute to early responses of endothelial cells to proinflammatory mediators such as TNFα and lipopolysaccharide. In contrast, activation of ROCKI in response to proinflammatory stimuli is required for the long-term effects of lipopolysaccharide and TNFα in disrupting endothelial barrier integrity. Evidence also indicates that ROCKII maintains basal junctional tension and primes the endothelium for hyperpermeability responses such as during thrombin challenge, independent from subsequent ROCKI-mediated contractile stress fiber formation. Both ROCKs maintain MLC in a phosphorylated state through interaction with the PI3K/AKT pathway. ROCKs also block ROCK-mediated activation of myosin-II. ROCKI and ROCKII in response to proinflammatory stimuli is required for the long-term effects of lipopolysaccharide and TNFα in disrupting endothelial barrier integrity. ROCKII in response to proinflammatory stimuli is required for the long-term effects of lipopolysaccharide and TNFα in disrupting endothelial barrier integrity.

**Spatial Control of RhoGTPases at AJs**

**Spatial Control of RhoGTPases at AJs**

VE-cadherin adhesion modulates the organization of the actin cytoskeleton at AJs through the recruitment of signaling and scaffolding proteins such as upstream regulators and downstream effectors of RhoGTPases. Engagement of VE-cadherin at cell–cell contacts initiates spatial activation of Rac1 and Cdc42 signaling. Rac1 signaling is induced through the activation of PI3K and recruitment of the RhoGEFs Tiam1, Vav2, and TRIO (triple functional domain protein) to AJs. Tiam1 serves as the scaffold for Rac1 at AJs, whereas Vav2, a common GEF for RhoA, Rac1, and Cdc42, promotes Rac1 GTP loading and hence facilitates the activation of Rac1 signaling.

Some evidence suggests that TRIO, a GEF for both RhoA and Rac1, is also recruited to nascent VE-cadherin adhesion where it activates Rac1 signaling and promotes the formation of AJs. IQGAP1, which stabilizes both Cdc42 and Rac1 in the GTP-bound state and protracts the activity of these GTPases, is also recruited to AJs through binding to β-catenin. Recent data suggest that IQGAP1 is responsible for Rac1 activity at the sites of AJs and hence is an important regulator of AJ integrity and vascular leakage in acute lung injury.

In contrast to Rac1 and Cdc42, RhoA activity is suppressed at endothelial AJs by multiple convergent pathways. RhoA activity is finely counterbalanced by Rac1 signaling. Rac1-mediated activation of p190RhoGAP, a RhoA-specific GAP, and phosphorylation of p190RhoGAP by Src and FAK play a central role in inhibiting RhoA signaling at endothelial AJs. Whether Cdc42 can also counteract RhoA signaling remains unclear. One tenable mechanism involves Cdc42/MLCK-dependent assembly of myosin-II filaments, which can then bind to and suppress activities of Db1 family GEFs containing a DH-PH module at AJs.

It is an attractive possibility that the interaction between myosin-II and the RhoGEFs expressed in endothelial cells (TRIO, GEF-H1, Db1, leukemia-associated Rho GEF, Tiam1, and Vav2) might provide a mechanism for switching small RhoGTPases on and off at AJs.

**Spatial Control of RhoGTPases at TJs**

In contrast to endothelial AJs, which are characterized by low RhoA activity, TJs are shown to be sites of RhoA activation; however, there are important differences. RhoA activity is induced by p114GEF at endothelial TJs, an adaptor protein of TJs, folds a scaffold consisting of junction-associated coiled-coil protein and p114GEF to provide spatial activation of RhoA. Conversely, both Rac1 and Cdc42 activities are suppressed at TJs. Rich1, a GAP for both Cdc42 and Rac1, is associated with angiomotin, a scaffolding protein of TJs, where it controls cell polarity and endothelial junction integrity through inhibition of Rac1 and Cdc42. High activity of RhoA and low activities of Rac1 and Cdc42 are required for generation of intracellular forces at the level of TJs that are transmitted to VE-cadherin adhesion allowing formation of stable AJs. This finely compartmentalized regulation of RhoGTase signaling in endothelial cells might be critical for the stability of VE-cadherin adhesion. Activation of RhoA at the level of TJs rather than AJs might be beneficial for achievement of a proper balance, magnitude, and directionality of mechanical forces across VE-cadherin adhesion, the main gatekeeper of junctional permeability in endothelial cells.

**Role of RhoGTPases in Response of Endothelium to Mechanical and Humoral Stimuli**

Endothelial cells express at least 17 different RhoGAPs and 20 RhoGEFs at high levels. This broad spectrum of upstream regulators of RhoGTPases might be important for spatiotemporal control of intracellular tension at endothelial AJs exposed to pulsatile blood pressure and blood flow. Both RhoA and Rac1 contribute to cell responses induced by mechanical forces. At least 11 different GEFs including Abr, alsin, ARHGEF10, Bcr, GEF-H1, leukemia-associated Rho GEF, p190RhoGEF, PLEKHG1, P-REX2, and a-PIX mediate endothelial cell adaptation to the cyclic stretch response to pulsatile blood pressure and flow. In particular, a GEF for RhoA, Solo, transduces mechanical force at cell–cell adhesion sites, whereas leukemia-associated Rho GEF and GEF-H1 are involved in integrin-dependent mechanotransduction. Both responses contribute to cell alignment and stress fiber reorientation in the endothelium exposed to cyclic stretch.

Conversely, mechanosensing of laminar shear stress and resultant stabilization of VE-cadherin adhesion occurs through activation of Rac1 at AJs. Mechanotransduction emanates at the level of the sensory complex composed of...
VE-cadherin/PECAM-1/VEGFR2. PECAM-1 induces activation of Src, which in turn promotes phosphorylation of Vav2 and hence Vav2-mediated activation of Rac1 signaling at AJs.174 Rac1 functions as a reversible modulator of intracellular tension at mature AJs and induces stabilization of VE-cadherin adhesion without notable reorganization of the actin cytoskeleton.162

It is important to note that the vast majority of responses to humoral stimuli involve a broad spectrum of RhoGTPases expressed in endothelial cells. Activation of RhoA signaling in response to proinflammatory stimuli is induced by p115RhoGEF,197 GEF-H1,359 and TRIO.360,361 In contrast, secondary messengers such as cAMP and cGMP elicit a barrier protective effect by inhibiting RhoA signaling. cGMP blocks RhoA signaling through PKG-mediated phosphorylation of RhoA,362,363 whereas cAMP inhibits RhoA activity by both activating p190RhoGAP358 and preventing RhoA dissociation from Rho-guanine nucleotide dissociation inhibitors.180 Hence, modulation of RhoA activity might represent an attractive strategy for preventing or treating vascular leakage in disease states.

RhoGTPases as Therapeutic Targets in Vascular Inflammation

As discussed above, RhoGTPases are fundamental to the biology of endothelial AJs. They serve as a control point for many signaling pathways, making them ideal targets for ameliorating inflammatory disease. Thus, a possible therapeutic approach for treating vascular inflammation may depend on rewiring of signaling pathways to restore AJs quickly by shifting the balance from RhoA toward Rac1 and Cdc42 activities. In the following sections, we provide an overview of agents and describe newer targets preventing or resolving inflammation due to leaky AJs.

Small-Molecule Inhibitors

Rho Kinase Inhibitors

Although many targets downstream of RhoA have been identified, Rho kinase, a serine/threonine PK of ≈160 kDa (also referred to as ROCK) is the major RhoA downstream effector.176 Two isoforms of ROCK, namely, ROCK1 (also known as ROCKβ) and ROCKII (ROCKα), are encoded by 2 independent genes.365 Kinase activities of both ROCKs are auto-regulated by the COOH-terminal domain, which folds into the active site and inhibits kinase activity. Despite their structural similarity, the 2 proteins have distinct functions in endothelial cells. ROCKII but not ROCK1 regulates basal tension across AJs and shifts the endothelium toward hyperpermeability.126

The deletion of either ROCK or ROCKII genes in mice has no apparent phenotype except for a defective placenta–embryo interaction.366,367 The above results suggest that ROCK potentially can be used as a drug target.

The small-molecule inhibitors targeting ROCK kinase activity can be grouped into 4 classes: isoquinolines, 4-amino-pyridines, indazoles, and amide and urea derivatives. Fasudil, dimethyl-fasudil (H-1152), and compound 4 belong to the isoquinoline series of Rho kinase inhibitors. Fasudil shows higher potency toward ROCK1 (Ki of 330 nmol/L),368 whereas H-1152 and compound 4 inhibited both ROCKs with Ki values of 1.6 to 23 nmol/L, respectively.369,370 Fasudil has demonstrated high efficacy in preclinical models of pulmonary hypertension, pulmonary fibrosis, and vascular leakage.371-373 Fasudil is approved in Japan for the treatment of cerebral vasospasm after aneurysm rupture174 and has been tested in US clinical trials of angina and pulmonary hypertension.372,373 Y-27632, a member of the 4-aminopyridine series, binds the ATP binding pocket of ROCK and has a Ki of 220 to 300 nmol/L.176 Like fasudil, Y-27632 has been widely used in animal models of hypoxia-induced pulmonary hypertension, lung injury, and cerebral vasospasm.197,377 However, Y-27632 has been much less investigated clinically.

Statins

Statins can influence endothelial function by virtue of their direct effect on HMG-CoA reductase, a rate-limiting enzyme that generates cholesterol by converting HMG-CoA to mevalonic acid. However, statins can block the synthesis of isoprenoid intermediates such as farnesyl pyrophosphate and geranylgeranyl pyrophosphate, which are important lipid attachments for post-translational modification of a variety of proteins including attachment of small GTP-binding proteins at the plasma membrane. Statins are shown to prevent thrombin- and lipopolysaccharide -induced loss of endothelial barrier function using in vitro and in vivo models of vascular injury through their pleiotropic effects on RhoA- and Rac1-mediated cytoskeletal arrangements and reactive oxygen species generation.378 Interestingly, simvastatin reduces pulmonary and systemic inflammatory responses in healthy human patients after lipopolysaccharide inhalation179 and improves organ function in a small, single-center, randomized study on patients with acute lung injury.380 However, a recent large clinical trial failed to demonstrate such a protective effect of statins in patients with ARDS.381

SIP receptor agonists and antagonists. SIP acts as a ligand in an autocrine or paracrine manner for the G-protein–coupled receptor SIP receptors (SIP1R-5; formerly termed the endothelial differentiation gene [Edg] receptors). In several studies, SIP enhances endothelial barrier function through ligating its high-affinity receptor, SIP1R.385 SIP1R functions in endothelial cells through interaction with heterotrimeric Gi proteins and downstream activation of Rac1.382-384 FTY720 has a barrier-enhancing effect both in vitro and in vivo.386-388 However, FTY720P induces vascular leak in a mouse model through promoting phosphorylation of SIP1R at several serine residues, which trigger receptor ubiquitination and degradation.385,389 In contrast, newly developed and modified FTY720, (R)-methoxy-FTY720 ((R)-OMe-FTY), (R)/(S)-fluoro-FTY720 (FTY-F), and β-glucuronide-FTY720 (FTY-G) compounds were shown to display in vitro barrier-enhancing properties.380 Tyrosine phosphorylation of SIP1R at Y143 in endothelial cells also regulates receptor expression at the cell surface and hence the responsiveness to SIP.384 Thus, newer SIP analogs may be efficacious in repairing endothelial AJ integrity.

In contrast to SIP1R, SIP3R has a barrier-disruptive role.385 Lung endothelial cells shed SIP3R in microparticles after activation with lipopolysaccharide or low molecular
weight hyaluronan. Exposure of normal endothelial cells to S1PR3-containing microparticles significantly reduces AJ integrity, consistent with increased permeability response. These changes are attenuated by RNAi-mediated depletions of S1PR3. Intriguingly, elevated S1PR3 plasma concentration has been linked to sepsis and acute lung injury mortality, indicating S1PR3 antagonists as novel therapeutics targeting AJ integrity.

Growth Factors
Angiopoietins (Ang1 and 2) stabilize developing vessels by ligating the endothelial Tie-2 receptor. However, both have different effects on mature endothelium. Although Ang1 promotes endothelial survival, migration, and barrier formation, Ang2 induces vascular leak by disrupting AJs through activation of actin-myosin–induced stress fiber formation. Ang2 has emerged as a predictor of patient mortality from ARDS and sepsis, as circulating levels of Ang2 are consistently greater in patients who died of ARDS or sepsis as compared with control groups. Ang1 infusion has been thought to be a therapeutic approach to counteract Ang2 disruption of the endothelial barrier, but its use has been limited because of its side effect of inducing pulmonary hypertension.

Micro-RNAs
miRNAs are small (19–23 nucleotides) noncoding RNAs that can suppress or augment cellular signaling in several cell types, including endothelial cells, based on their ability to target mRNA. miR-27a targets VE-cadherin and induces vascular leak during ischemia and reperfusion injury, indicating inhibition of miR-27a may be a useful approach for preventing vascular barrier disruption. The expression of mature miR-150-5p but not of miR-150-3p is induced during recovery from endothelial injury post-lipopolysaccharide challenge. Loss of miR-150 does not alter AJ organization or barrier function under basal conditions but markedly impairs AJ annealing after lipopolysaccharide challenge leading to persistent vascular injury. miR-150 restores endothelial barrier function post-injury by suppressing Ang2 generation through targeting the transcription factor EGR2. Conversely, depletion of Ang2 in miR-150 null endothelial cells rescues AJ annealing and barrier function, demonstrating that miR-150 functions by suppressing Ang2 generation.

Cellular Therapy
Recent evidence suggests that trafficking and differentiation of nonresident and resident stem cells facilitate the repair of injured vessels. Stem cells can be mobilized from the bone marrow to the damaged tissue where they proliferate and function in the same manner as the original cell type. Hematopoietic and nonhematopoietic stem cells are located in the bone marrow and are critical in tissue repair. Mesenchymal stem cells have been shown to prevent lung vascular injury by secreting growth factors such as keratinocyte growth factor or S1P that may alter Rho-Rac1 signaling. Studies also demonstrated that transplanted bone marrow–derived hematopoietic stem cells are detected in several organs including lungs. Many studies have shown the so-called endothelial progenitor cells to be proangiogenic. Wary et al further showed that VE-cadherin+/Flk1+ endothelial progenitor cells promote endothelial barrier function and that loss of the barrier is prevented by activation of c4 and c5 integrins in a model of lipopolysaccharide-induced lung vascular injury. In addition, Zhao et al have demonstrated that bone marrow–derived mesenchymal stem cells can be directed to AJs where they restore normal endothelial permeability through the generation of S1P and thereby prevent lipopolysaccharide-induced lung injury.

Concluding Remarks and Future Directions
Here, we have discussed the current view on the organization and dynamics of VE-cadherin adhesion, the main gatekeeper of AJs, in resting and activated (or inflamed) endothelium. Although the list of constituents of the VE-cadherin complex is incomplete, it is apparent that it forms an extraordinary well-organized network of signaling molecules at AJs. VE-cadherin assembles a mechanosensory complex with another adhesion molecule PECAM-1 and receptor tyrosine kinases VEGFR2 and VEGFR3 at AJs enabling sensing and adaptation of endothelial cells to rapid shifts in local perfusion and pressure. The signals sensed by this complex are tuned and then transmitted to integrins at the sites of FAs to elicit coordinated responses to modify barrier properties of the junctions.

Small RhoGTPases at the level of AJs are key molecular switches that play a fundamental role in regulating the plasticity of VE-cadherin adhesion, and hence endothelial permeability. They are essential for signaling endothelial responses to both humoral and mechanical stimuli. They are therefore potential drug targets in a variety of inflammatory disorders. The endothelium expresses numerous upstream regulators of RhoGTPases that regulate GTPase activation in space and time. These are also drug targets that might be exploited.

Several fundamental questions remain unanswered, such as how PECAM-1, VE-cadherin, and VEGFR2 organize themselves to serve as a unified mechanosensor, and whether the mechanosensing function is different in the quiescent endothelium as opposed to the activated endothelium. Perturbation of the mechanosensory complex has consequences in disease pathogenesis. Another question is what dictates the balance between RhoA, Rac1, and Cdc42 activities. It is clear that Rac1 and Cdc42 activities (as opposed to RhoA activity) need to be exquisitely balanced, but how this is achieved remains unknown. Another question is how inflammatory mediators in disease states disrupt the sensing function of AJs and how this leads to short- or long-term disruption of AJs. Hence, better understanding of the formation of the mechanosensory complex in the endothelium and the function of RhoGTPases at AJs will be critical for development of novel therapeutic targets for treating inflammatory diseases.

VE-cadherin also assembles a complex with signaling molecules comprising kinases (eg, Src kinases), phosphatases (eg, V-PTP), and RhoGTPases, which in turn, provide spatial control of VE-cadherin adhesion under physiological and pathological conditions. An increasing body of evidence suggests that VE-cadherin adhesion undergoes continuous reorganization resulting in on-demand remodeling at AJs. Exchange of VE-cadherin molecules between junctional and cytosolic pools is a constitutive process accounting from the permeable nature of the endothelial barrier. VE-cadherin adhesion is finely regulated by specific
intracellular signaling pathways that assemble and disassemble AJs. Depending on the stimuli, these signals can also enhance or weaken the endothelial barrier. Disruption of VE-cadherin adhesion triggers increased endothelial permeability and tissue edema, a central feature of human diseases ranging from cancer to acute inflammatory disorders such as ARDS. Thus, an important concept evolving from these studies is whether a cross-talk mechanism exists between phosphatases such as VE-PTP and myosin phosphatase (PP1), which stabilize AJs.

It is also apparent that the tyrosine kinases Fyn, Src, and FAK differentially regulate AJs depending on the context and their mode of activation. For example, as described above, FAK is involved in both formation and disruption of AJs. FAK also influences RhoGTPases. What determines whether FAK functions one way or the other? It will be important in future studies to understand how the interplay between the underlying ECM and AJs dictates the function of FAK, Fyn, or Src and how the function of these kinases influences the endothelial barrier. It is likely that further clarification of how mechanical stimuli sensed by the AJ mechanosensor influence endothelial permeability in response to inflammatory mediators will advance our understanding of mechanisms regulating endothelial permeability.

Sources of Funding
This study was supported by National Institutes of Health grants R01 HL103922 to Dr Komarova, R01 HL84153 to Dr Mehta, AHA to acute inflammatory disorders such as ARDS. Thus, an important concept evolving from these studies is whether a cross-talk mechanism exists between phosphatases such as VE-PTP and myosin phosphatase (PP1), which stabilize AJs.

Disclosures
None.

References


α-catenin reveals rapid, reversible guidance cadherin cluster assembly, stability, and movement.  


Regulation of Endothelial Permeability

201


294. Axlrad TW, Deo DD, Ottino P, Van Kirk J, Bazan NG, Bazan HE, Hunt JD. Platelet-activating factor (PAF) induces activation of matrix...


Regulation of Endothelial Permeability

Kumar et al


371. Komarova et al

205

Downloaded from http://circres.ahajournals.org/ by guest on August 13, 2017


Protein Interactions at Endothelial Junctions and Signaling Mechanisms Regulating Endothelial Permeability
Yulia A. Komarova, Kevin Kruse, Dolly Mehta and Asrar B. Malik

Circ Res. 2017;120:179-206
doi: 10.1161/CIRCRESAHA.116.306534

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2017 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/120/1/179

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at: http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at: http://circres.ahajournals.org//subscriptions/