—Review—

Dynamic Regulation of Vascular Permeability by Vascular Endothelial Cadherin-Mediated Endothelial Cell-Cell Junctions

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Endothelial cells lining blood vessels regulate vascular barrier function, which controls the passage of plasma proteins and circulating cells across the endothelium. In most normal adult tissues, endothelial cells preserve basal vascular permeability at a low level, while they increase permeability in response to inflammation. Therefore, vascular permeability is tightly controlled by a number of extracellular stimuli and mediators to maintain tissue homeostasis. Accordingly, impaired regulation of endothelial permeability causes various diseases, including chronic inflammation, asthma, edema, sepsis, acute respiratory distress syndrome, anaphylaxis, tumor angiogenesis, and diabetic retinopathy. Vascular endothelial (VE)-cadherin, a member of the classical cadherin superfamily, is a component of cell-to-cell adherens junctions in endothelial cells and plays an important role in regulating vascular permeability. VE-cadherin mediates intercellular adhesion through trans-interactions formed by its extracellular domain, while its cytoplasmic domain is anchored to the actin cytoskeleton via α- and β-catenins, leading to stabilization of VE-cadherin at cell-cell junctions. VE-cadherin-mediated cell adhesions are dynamically, but tightly, controlled by mechanisms that involve protein phosphorylation and reorganization of the actomyosin cytoskeleton. Phosphorylation of VE-cadherin, and its associated-catenins, results in dissociation of the VE-cadherin/catenin complex and internalization of VE-cadherin, leading to increased vascular permeability. Furthermore, reorganization of the actomyosin cytoskeleton by Rap1, a small GTPase that belongs to the Ras subfamily, and Rho family small GTPases, regulates VE-cadherin-mediated cell adhesions to control vascular permeability. In this review, we describe recent progress in understanding the signaling mechanisms that enable dynamic regulation of VE-cadherin adhesions and vascular permeability. In addition, we discuss the possibility of novel therapeutic approaches targeting the signaling pathways controlling VE-cadherin-mediated cell adhesion in diseases associated with vascular hyper-permeability. (J Nippon Med Sch 2017; 84: 148–159)

Key words: vascular permeability, vascular endothelial-cadherin, Rap1, actin cytoskeleton, Rho

Introduction

Endothelial cells lining the interior surfaces of blood vessels function as a semipermeable barrier to regulate the vascular permeability that is implicated in tissue fluid homeostasis, extravasation of circulating cells, and supplying essential nutrients. Vascular permeability is dynamically, but tightly, controlled by a number of extracellular stimuli and mediators to maintain vascular homeostasis (**Fig. 1**). Thus, endothelial barrier function impairment causes vascular hyper-permeability, promoting the development and progression of various diseases, such as chronic inflammation, asthma, edema, sepsis, acute respiratory distress syndrome, anaphylaxis, tumor angiogenesis, diabetic retinopathy[1,2] (**Fig. 1**). When inflammation occurs, inflammatory mediators such as histamine, bradykinin, thrombin, platelet-activating factor (PAF) and

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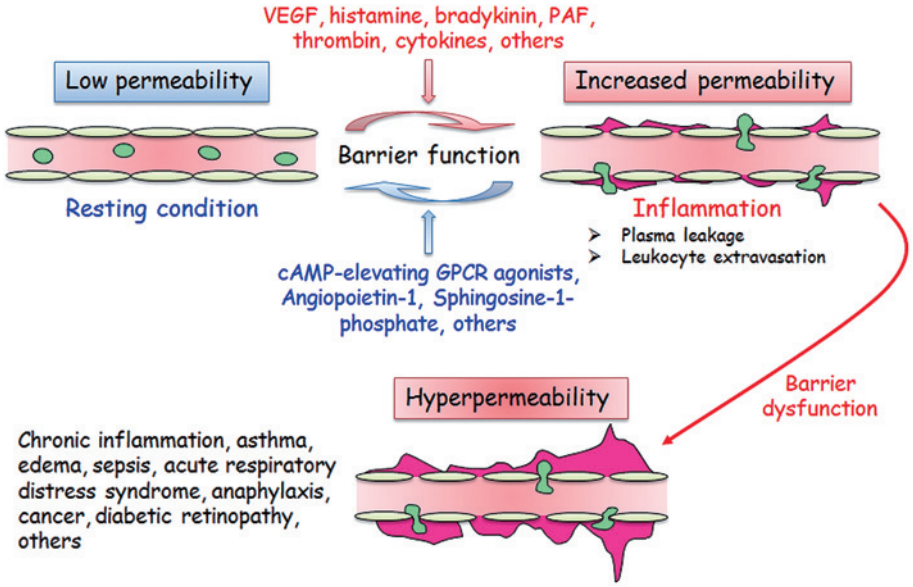

Fig. 1 Dynamic regulation of vascular permeability
Endothelial permeability is dynamically, but tightly, controlled by a number of mediators that increase (blue) or decrease (red) endothelial barrier function. In normal adult tissues, endothelial cells preserve basal vascular permeability at a low level (left). When inflammation is induced, inflammatory mediators increase vascular permeability to induce plasma leakage and leukocyte extravasation. Dysregulation of endothelial permeability facilitates the development and progression of various diseases that are associated with vascular hyper-permeability.

cytokines increase vascular permeability by inducing intercellular gap formation[2–6]. Vascular endothelial growth factor (VEGF), which is one of the most potent angiogenic factors, also induces vascular permeability[7,8]. Indeed, VEGF was originally described as a vascular permeability factor[9]. On the other hand, various mediators such as angiopoietin-1 (Ang1) and sphingosine-1-phosphate (S1P) suppress vascular permeability, thereby improving endothelial barrier functions[10,11]. In addition, production of intracellular cyclic AMP (cAMP), a second messenger downstream from Gs-coupled G protein-coupled receptors (GPCRs), has been shown to reduce vascular permeability[12–14]. Consistently, cAMP-elevating GPCR agonists such as adrenomedullin, prostacyclin (PGI_2), prostaglandin E2 (PGE_2), and β-adrenergic agonists limit hyper-permeability induced by these inflammatory mediators[15–17].

Permeability across the endothelium is mediated by transcellular and paracellular pathways: the former involves caveolae-mediated transcytosis of soluble macromolecules, whereas the latter relies on the opening and closing of endothelial cell-cell junctions[18]. Similar to epithelial cells, endothelial cells form two specialized intercellular junctional domains: adherens junctions (AJs) and tight junctions (TJs)[19]. In endothelial cells, AJs are constituted by vascular endothelial (VE)-cadherin and nectin, while TJs are composed of members of the family of junctional adhesion molecules, such as claudins and occludins[19]. Endothelial cells in brain capillaries comprise the blood-brain barrier, in which claudin forms highly selective semipermeable TJs, thereby protecting the central nervous system by strictly separating the circulating blood from the brain's extracellular fluid[20]. However, endothelial cells in most peripheral tissues establish less rigidly organized intercellular junctions in which AJs and TJs are intermingled, thus allowing dynamic regulation of vascular permeability[19]. Such dynamic regulation of permeability is predominantly controlled by VE-cadherin-based AJs. Consistently, the intravenous administration of anti-VE-cadherin antibodies into mice leads to a dramatic increase in permeability, vascular fragility and hemorrhages[21], highlighting the importance of VE-cadherin in the maintenance of vascular barrier functions. Thus, VE-cadherin-mediated cell-cell junctions are dynamically controlled by the aforementioned factors that induce or suppress vascular permeability to maintain vascular homeostasis[6,19,22,23].

In this review, we will focus on the regulation of vas-

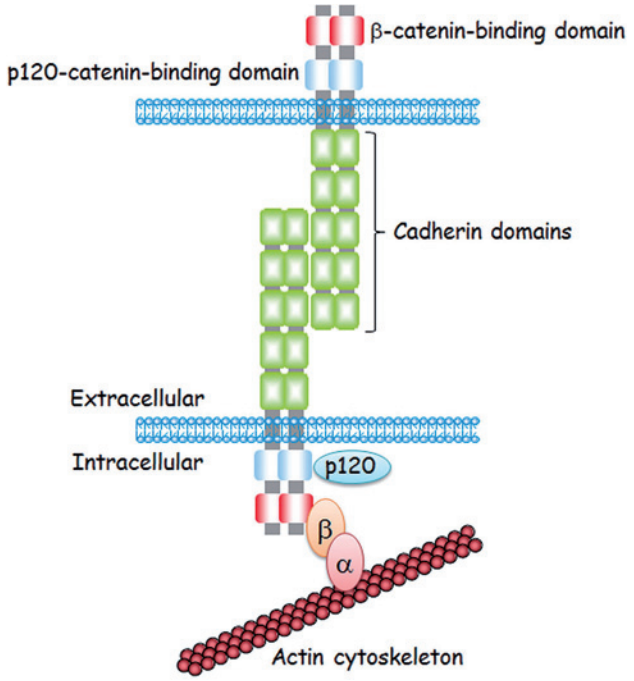

Fig. 2 Schematic representation of VE-cadherin-mediated endothelial cell junctions
VE-cadherin is composed of five extracellular cadherin domains, a transmembrane domain, and a highly conserved cytoplasmic tail, and mediates calcium-dependent intercellular adhesions of endothelial cells through cis- and trans-dimerization. The cytoplasmic tail of VE-cadherin binds to p120-catenin and β-catenin. β-catenin further interacts with α-catenin, which, directly or indirectly, links to the actin cytoskeleton. p120: p120-catenin, α: α-catenin, β: β-catenin.

cular permeability by VE-cadherin-mediated endothelial cell-cell junctions and describe recent progress in understanding the signaling mechanisms that enable VE-cadherin adhesions and vascular permeability to be dynamically regulated. In the first part of this review, we will highlight the significance of VE-cadherin phosphorylation in the regulation of vascular permeability and leukocyte extravasation. In the latter part, we will introduce the regulatory mechanisms of VE-cadherin-mediated cell adhesions through reorganization of the actomyosin cytoskeleton, focusing especially on Rap1 and Rho-family small GTPases.

Molecular Organization of VE-cadherin-based AJs

VE-cadherin, also known as cadherin-5 and CD144, belongs to the classical cadherin superfamily[6,19]. It is composed of five extracellular cadherin domains, a transmembrane domain and a highly conserved cytoplasmic tail, and mediates calcium-dependent intercellular adhesions by initially forming cis-dimers at the cell surface via its extracellular cadherin domains followed by an antiparallel trans-interaction of dimers on opposing cells (**Fig. 2**). The cytoplasmic tail of VE-cadherin binds to armadillo family proteins, p120-catenin, β-catenin, and plakoglobin. β-catenin and plakoglobin further interact with α-catenin, which directly or indirectly links to the actin cytoskeleton[19,24–26]. Thus, α-catenin was believed to establish a stable linkage between the VE-cadherin-catenin complex and the actin cytoskeleton. Although this model was challenged by the observation that α-catenin does not bind simultaneously to both the cadherin-catenin complex and actin filaments[27,28], several recent reports have further supported the idea that α-catenin anchors the VE-cadherin-catenin complex to the actin cytoskeleton possibly through linker proteins such as vinculin, α-actinin, and the epithelial protein lost in neoplasm (EPLIN)[29–31].

Regulation of Vascular Permeability through Phosphorylation of VE-cadherin and Its Associated Catenins

Permeability-increasing agents such as VEGF, histamine, bradykinin, tumor necrosis factor, PAF, and thrombin induce phosphorylation of the VE-cadherin cytoplasmic tail and of its associated catenins, in turn leading to increased vascular permeability via VE-cadherin/catenin dissociation and VE-cadherin internalization[6,32,33]. The phosphorylation of VE-cadherin is also induced by leukocyte adhesion to endothelial cells through intercellular cell adhesion molecule-1 (ICAM-1) and is involved in diapedesis of leukocytes[3,33].

VEGF induces vascular permeability through activation of non-receptor tyrosine kinase Src[23,34,35] (**Fig. 3**). Stimulation with VEGF induces phosphorylation of several tyrosine residues of its receptor, VEGF receptor-2 (VEGFR2)[36]. T cell-specific adaptor (TSAd) binds to phospho-Y951 within VEGFR2 and subsequently recruits and activates Src, which directly phosphorylates VE-cadherin to increase vascular permeability[37,38]. In addition, Src reportedly induces activation of p21-activated kinase, which in turn phosphorylates the VE-cadherin on serine residues, leading to its internalization and weakening of cell-cell junctions[39]. In addition to Src, focal adhesion kinase (FAK) is activated by VEGF and promotes vascular permeability by inducing phosphorylation of β-catenin and its subsequent dissociation from VE-cadherin[40] (**Fig. 3**).

The specific tyrosine residues of VE-cadherin that are

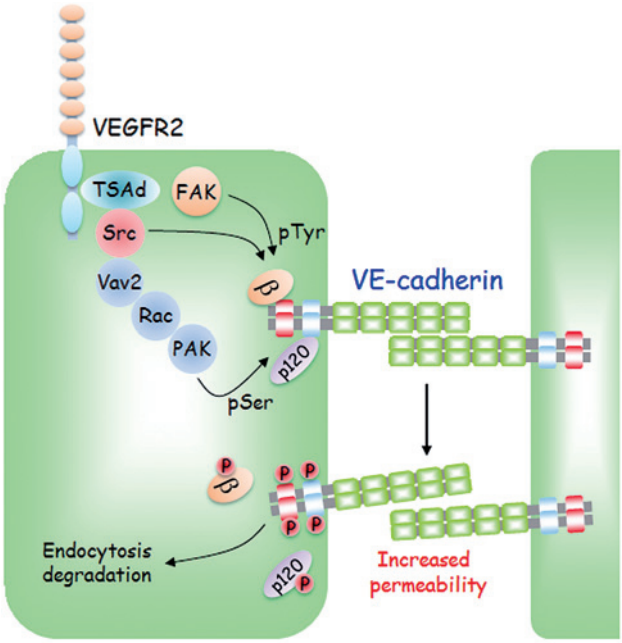

Fig. 3 Signaling pathways involved in VEGF-mediated phosphorylation of VE-cadherin leading to increased vascular permeability
VEGFR2 stimulated by VEGF induces activation of non-receptor tyrosine kinase Src by recruiting TSAd. Activated Src directly phosphorylates tyrosine residues of the cytoplasmic domain of VE-cadherin and indirectly phosphorylates its serine residues through a Vav2-Rac-PAK signaling pathway. In addition, VEGF/VEGFR2 signaling induces activation of FAK, which in turn phosphorylates β-catenin. Phosphorylation of VE-cadherin, and its associated catenins, results in dissociation of the VE-cadherin/catenin complex and internalization of VE-cadherin, leading to an increased vascular permeability. p120: p120-catenin, β: β-catenin.

phosphorylated by different stimuli and are responsible for the weakening of endothelial cell-cell junctions have been identified[6,41]. Orsenigo et al. reported that Y658 and Y685 in VE-cadherin are phosphorylated in veins, but not in arteries, through shear stress-induced junctional Src activation and suggested that phosphorylation of these tyrosine residues might be a prerequisite for inflammatory mediator-induced vascular permeability[42]. Interestingly, Wessel et al. showed, by generating knock-in mice expressing a Y685F or Y731F mutant of VE-cadherin, that leukocyte extravasation and vascular permeability are each controlled by different tyrosine residues of VE-cadherin[43]. Y731, but not Y685, in VE-cadherin is constitutively phosphorylated under basal conditions. Leukocyte adhesion induces dephosphorylation of Y731 in VE-cadherin through SH2-containing protein tyrosine phosphatase (SHP2) followed by AP2-mediated endocytosis of VE-cadherin, thereby facilitating leukocyte extravasation. In contrast, inflammatory mediators such as VEGF and histamine increase vascular permeability by inducing phosphorylation of Y685 in VE-cadherin. However, Sidibé et al. have also generated and analyzed VE-cadherin Y685F knock-in mice independently and shown that these mice exhibit spontaneously leaky microvessels and show increased sensitivity to VEGF-induced vascular permeability[44]. Since a previous *in vitro* study indicated that phospho-Y685 in VE-cadherin is a binding site for COOH-terminal Src kinase (Csk), a negative regulator of Src[45], VE-cadherin phosphorylated at Y685 might suppress endothelial permeability through Csk-mediated inhibition of Src. Therefore, the role of Y685 phosphorylation in the regulation of vascular permeability by VE-cadherin remains controversial.

The level of tyrosine phosphorylation of VE-cadherin is also regulated by several AJ-associated phosphatases such as VE-protein tyrosine phosphatase (VE-PTP)[46,47], density-enhanced phosphatase-1 (DEP-1)[48], protein tyrosine phosphatase receptor type M (PTPμ)[49], and SHP-2[50]. The VE-PTP is known to associate with VE-cadherin and maintain VE-cadherin in a dephosphorylated state, thereby enhancing the adhesive activity of VE-cadherin[46,47]. Conversely, VEGF stimulation and leukocyte adhesion lead to dissociation of VE-PTP from VE-cadherin to induce vascular permeability and leukocyte extravasation[47,51].

Dynamic Regulation of VE-cadherin Adhesions and Vascular Permeability through Actin Cytoskeleton Reorganization

Cadherin-based AJs are strengthened by the actin cytoskeleton to maintain tissue integrity. AJs mainly exist in two forms: stable linear AJs that provide a strong barrier and discontinuous focal AJs whose formation is a hallmark of reduced barrier function. Stable linear AJs are supported by circumferential actin bundles (CABs), which are defined as linear actin bundles that align along the cell-cell junctions, while perpendicularly oriented focal AJs are connected by radial actin bundles[52–56]. In addition, endothelial cells also form a distinct type of linear AJs that are not directly supported by the actin cytoskeleton, instead being aligned by the cortical actin bundles that run parallel to the cell-cell junctions[54,57]. Permeability-increasing agents such as histamine, bradykinin, PAF, and thrombin increase vascular permeability by inducing the formation of cytoplasmic stress fi-

bers and endothelial cell contraction, which exerts a pulling force on the VE-cadherin-based cell-cell contacts, thereby generating focal AJs[25,53,54,57]. In contrast, the factors that promote endothelial barrier function such as cAMP-elevating GPCR agonists, S1P, and Ang1 disrupt focal AJs and the formation of linear AJs supported by CABs[10,23,31,58–60]. Since VE-cadherin stabilizes at cell-cell junctions by anchoring to the CABs through α- and β-catenins, these factors promote endothelial barrier function by creating a stable linkage between the CAB-associated linear AJs and VE-cadherin through α- and β-catenins[29,31]. Consistently, it has been reported that forced linkage between VE-cadherin and the actin cytoskeleton blocks leukocyte extravasation and vascular permeability *in vivo*, possibly through stabilization of VE-cadherin-mediated cell-cell junctions[30]. Thus, transition between focal AJs and linear AJs through actin cytoskeleton reorganization is important for dynamic regulation of VE-cadherin-based cell adhesions and vascular permeability.

The Small GTPase Rap1 is a Key Regulator of VE-cadherin-mediated Cell-cell Adhesions

cAMP is a well-known intracellular signaling molecule that improves endothelial barrier functions[12–14]. Previously, we, and others, investigated the molecular mechanism underlying cAMP-induced endothelial barrier enhancement and identified Rap1 as a key downstream molecule of cAMP for potentiating VE-cadherin-mediated cell adhesions[58,61,62]. Rap1 is a small GTPase that belongs to the Ras subfamily and was originally identified as an antagonist of Ras-induced transformation[63,64]. In addition, Rap1 is known to promote cell-extracellular matrix (ECM) adhesions by stimulating integrin activity[63,65]. Rap1 becomes the GTP-bound active form via several guanine nucleotide exchange factors (GEFs), among which, exchange protein directly activated by cAMP (Epac) is a cAMP-responsive GEF for Rap1[66]. Thus, cAMP potentiates VE-cadherin-mediated cell-cell contacts to enhance endothelial barrier function through an Epac-Rap1 signaling pathway[58,61,62]. In addition, it has been shown that VE-cadherin engagement leads to Rap1 activation at nascent cell-cell contacts through PDZ-GEF, another GEF for Rap1, which in turn facilitates maturation of VE-cadherin-based AJs[67,68]. Similarly, Rap1 is involved in the formation and stabilization of E-cadherin-based cell-cell adhesions in epithelial cells[69–71]. Thus, Rap1 has recently been recognized as a key mediator not only for integrin-mediated cell-ECM adhesions but also for cadherin-based cell-cell adhesions.

Molecular Mechanism Underlying Rap1-induced Endothelial Barrier Enhancement

Rap1 potentiates VE-cadherin-based cell-cell adhesions by disrupting focal AJs connected by radial actin bundles and the formation of linear AJs supported by CABs[57,59,72] (**Fig. 4**). Rap1 inhibits Rho, a member of the Rho family small GTPases, to disrupt focal AJs[59,72–75]. Rho and its downstream effector, Rho-associated coiled-coil containing protein kinase (ROCK), induce actomyosin contractility and the formation of stress fibers through cytoplasmic activation of non-muscle myosin II (NM-II), which leads to the formation of focal AJs[25,54,57] (**Fig. 4**). Consistently, most permeability-increasing agents are known to activate the Rho-ROCK-NM II pathway to induce vascular permeability[1,76,77]. Therefore, Rap1 blunts the formation of focal AJs to reduce vascular permeability through suppression of the Rho-ROCK-NM-II pathway not only under resting conditions but also in the presence of permeability-increasing agents[59,72] (**Fig. 4**). Signaling mechanisms underlying the Rap1-mediated inhibition of Rho have also been investigated. The cerebral cavernous malformation protein CCM1, also known as Krev interacting trapped protein 1 (Krit-1), is identified as a Rap1 effector that downregulates Rho activity, although the underlying molecular mechanism remains unknown[78,79]. Recently, Ras-interacting protein 1 (Rasip1) has also been found to regulate Rap1-mediated inhibition of Rho. Activated Rap 1 binds to Rasip1 and activates its binding partner, ArhGAP29, a GTPase-activating protein for Rho, thereby suppressing Rho activity[74,75].

In addition to suppressing Rho activity, Rap1 induces accumulation of active Cdc42, another member of the family of Rho GTPases, at cell-cell junctions, thereby inducing formation of CABs supporting the linear AJs[57,59,72]. Rap1 increases the active Cdc42 at cell-cell contacts by inducing junctional localization of Cdc42 and its GEF, FYVE, RhoGEF, and PH domain-containing protein-5, also known as facio-genital dysplasia-5 (FGD5)[59]. Subsequently, active Cdc42 at cell-cell contacts triggers the activation of junctional NM-II and subsequently develops the CAB-associated linear AJs by recruiting myotonic dystrophy kinase-related CDC42-binding kinase (MRCK) that phosphorylates the regulatory light chain subunit of NM-II[59]. Thus, Rap1 promotes CAB formation by inducing the Cdc42-MRCK pathway-dependent activation of junctional NM-II, thereby enhancing endothelial barrier function (**Fig. 4**).

Rap1-induced activation of junctional NM-II, through the Cdc42-MRCK pathway, generates tension on the

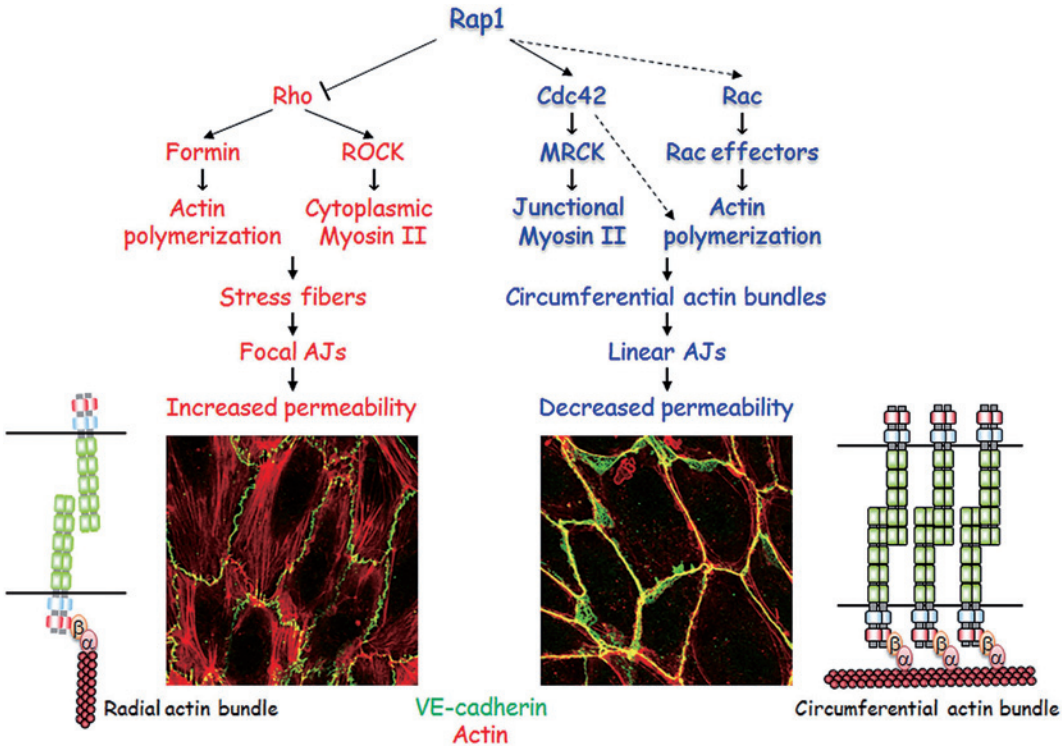

Fig. 4 Dynamic regulation of VE-cadherin-mediated cell adhesion and vascular permeability through actomyosin cytoskeleton reorganization.
VE-cadherin-mediated cell adhesions and vascular permeability are controlled by the balance between two types of AJs; focal and linear. Rho signaling induces the formation of focal AJs through ROCK-mediated activation of cytoplasmic NM-II and formin-induced actin polymerization, which weakens VE-cadherin-mediated cell adhesions to increase vascular permeability. On the other hand, Cdc42/Rac signaling induces the formation of CAB-associated linear AJs through the Cdc42-MRCK pathway-mediated activation of junctional NM-II and possibly via Rac-mediated actin polymerization. In endothelial cells developing linear AJs, VE-cadherin stabilizes at cell-cell junctions by anchoring to the CABs through α- and β-catenins, thereby potentiating barrier function to reduce vascular permeability. Rap1 acts as an upstream regulator and potentiates vascular barrier function through inhibition of Rho signaling and activation of Cdc42/Rac signaling. Images (bottom): human umbilical vein endothelial cells stimulated without (left image) and with (right image) forskolin, an activator of adenylyl cyclase, were stained with anti-VE-cadherin antibody and rhodamine-phalloidin to visualize VE-cadherin (green) and actin filaments (red), respectively. Note that stimulation with forskolin results in Rap1 activation through a cAMP-Epac pathway.

CABs supporting the linear AJs for their stabilization. Therefore, in addition to activation of junctional NM-II, Rap1 promotes actin polymerization to form the CABs through additional pathways. Neuronal Wiskott-Aldrich Syndrome protein (N-WASP) and Formin-like 3 are actin regulatory proteins acting downstream from Cdc42 and are known to regulate endothelial cell-cell junctions[80–83]. Thus, these Cdc42 effectors might regulate Rap1-induced CAB formation. Another candidate is Rac. Rac is activated by barrier protective molecules such as S1P, PGE2, PGI2, and hepatocyte growth factor and is known to enhance endothelial barrier function by inducing reorganization of the actin cytoskeleton[1,10,84,85]. Rap1 reportedly promotes cell spreading by associating and localizing with a subset of Rac GEFs such as Vav2 and Tiam1 to active lamellipodia extension[86]. In addition, it has been shown that Rap1 activation by cAMP promotes endothelial barrier function through Rac-dependent cytoskeletal reorganization[84,87]. Therefore, Rap1 might regulate the formation of the CAB-associated linear AJs not only through the Cdc42-MRCK pathway-mediated activation of junctional NM-II but also by inducing Rac-dependent actin polymerization (**Fig. 4**). However, since Rac also negatively regulates endothelial barrier functions[39,88,89], the role

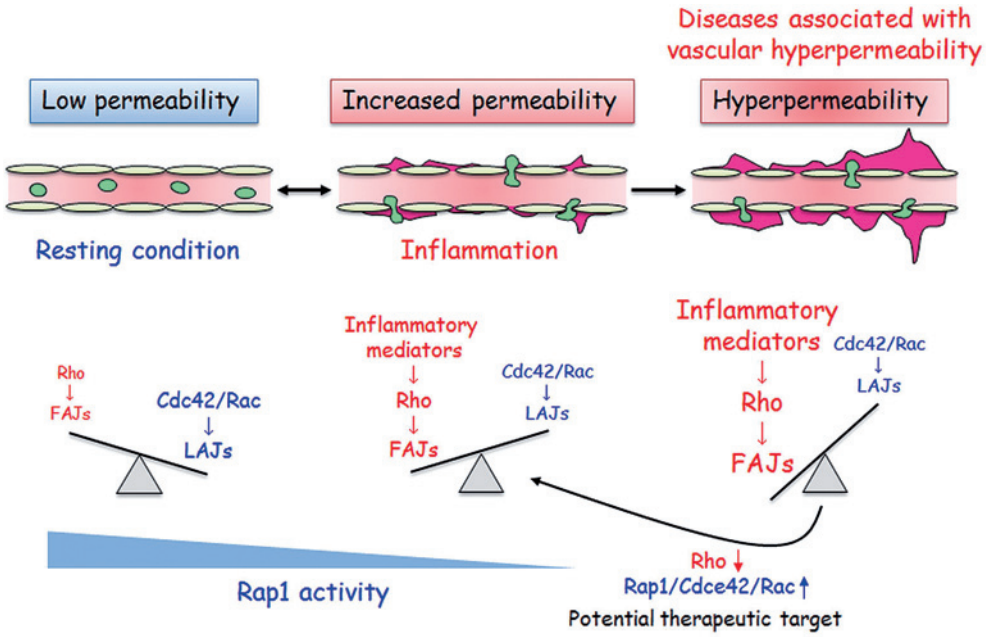

Fig. 5 A proposed model for how endothelial permeability is regulated under resting and inflammatory conditions and how dysregulation of endothelial permeability causes various diseases.
Under resting conditions, high basal Rap1 activity results in the formation of linear AJs through the Cdc42/Rac pathway, thereby restricting vascular permeability (left). When inflammation occurs, inflammatory mediators increase vascular permeability by inducing Rho-mediated formation of focal AJs (middle). Hyper-activation of the Rho pathway, possibly through overproduction of the inflammatory mediators, induces vascular hyper-permeability, which promotes the development and progression of various diseases (right). Thus, the signaling molecules that control VE-cadherin-mediated cell adhesions, such as Rho, Rap1, Cdc42, and Rac, might be potential therapeutic targets for diseases associated with vascular hyper-permeability. FAJ: focal AJs, LAJ: linear AJ.

of Rac in regulating endothelial cell-cell junctions is more complex than was expected. Other Rap1 effectors that are involved in regulation of the actin cytoskeleton such as AF-6, also known as afadin, and Rap1-interacting adaptor molecule (RIAM) might be involved in Rap1-induced formation of CAB-associated linear AJs[57,90,91].

In summary, Rap1 potentiates VE-cadherin-mediated cell adhesions by disrupting focal AJs and by developing linear AJs (**Fig. 4**). Rap1 forms linear AJs by inducing the Cdc42-MRCK pathway-mediated activation of junctional NM-II and possibly via Rac-mediated actin polymerization. Simultaneously, Rap1 disrupts focal AJs through suppression of the Rho-ROCK-NM-II pathway.

VE-cadherin-based AJs Are a Potential Therapeutic Target for Vascular Hyper-permeability

Permeability-increasing factors elevate vascular permeability by inducing the Rho-dependent formation of focal AJs. In contrast, Rap1 potentiates vascular barrier functions by inducing the Cdc42/Rac-mediated formation of linear AJs and by suppressing the Rho-mediated formation of focal AJs. These findings imply that the degree of vascular permeability is determined by the balance between focal AJ formation via Rho signaling and linear AJ formation mediated by Cdc42/Rac signaling (**Fig. 5**). Therefore, impaired regulation of this balance might promote the development and progression of various disease states that are associated with hyper-permeability, such as chronic inflammation, asthma, edema, sepsis, acute respiratory distress syndrome, anaphylaxis, tumor angiogenesis, diabetic retinopathy (**Fig. 5**). Thus, normalizing the balance between the Rho signaling involved in focal AJ formation and the Cdc42/Rac signaling involved in linear AJ formation could represent an effective therapeutic intervention for these diseases (**Fig. 5**). Indeed, administration of 007, a cAMP analog specific for Epac, has been shown to suppress hyper-permeability induced by VEGF and PAF in mice and to improve lipopolysaccha-

ride (LPS)-induced acute lung injury and vascular barrier dysfunction through activation of Rap1, which leads to suppression of Rho signaling and activation of Cdc42 signaling, thereby correcting their balance[58,92,93]. Consistently, ventilator-induced lung injury and pulmonary endothelial barrier dysfunction in mice can be attenuated by a PGI2 analog, iloprost, that normalizes the balance between the Rho signaling involved in focal AJ formation and the Cdc42/Rac signaling involved in linear AJ formation through a cAMP-Epac-Rap1 pathway[94]. In addition, inhibition of the Rho-ROCK pathway by ROCK inhibitors such as Fasudil and Y27632 might represent an effective therapeutic intervention for diseases associated with hyper-permeability. It has been reported that inhibition of the Rho-ROCK pathway by Fasudil suppresses anaphylaxis-induced vascular leakage and thereby provides protection from lethal systemic anaphylaxis in mice[77]. Similarly, Y27632 has also been shown to alleviate endotoxin-induced lung edema in mice and acute lung injury in rats[95,96]. Furthermore, S1P reportedly inhibits vascular leakage in a murine model of acute lung injury through Rac-dependent reorganization of the actin cystoskeleton[97,98]. Moreover, Ang1 promotes vascular integrity and barrier function through its receptor Tie2, while angiopoietin-2 (Ang2) has been shown to exert the opposite effects by acting as an antagonist for Tie2[99]. Consistently, COMP-Ang1, a potent and stable Tie2 activator, has been shown to suppress LPS-induced acute lung injury and vascular leakage associated with choroidal neovascularization in mice[100,101]. Recently, Koh's group developed an unusual anti-Ang2 antibody, ABTAA (Ang2-binding and Tie2-activating antibody), and showed that, upon binding to Ang2, ABTAA triggers clustering of Ang2, assembling an ABTAA/Ang2 complex that can bind and activate Tie2[102]. Importantly, they further revealed that ABTAA is highly effective in augmenting survival from sepsis, at least in part, by reducing vascular leakage.

Conclusions and Future Perspectives

In this review, we have summarized recent progress in unraveling the molecular mechanisms underlying the dynamic regulation of VE-cadherin-mediated cell adhesions and vascular permeability. VE-cadherin-mediated cell adhesions are dynamically, but tightly, controlled by mechanisms that involve protein phosphorylation and actomyosin cytoskeleton reorganization. Phosphorylation of VE-cadherin, and its associated catenins, results in dissociation of the VE-cadherin/catenin complex and internalization of VE-cadherin, leading to increased vascular permeability. In addition, VE-cadherin-mediated cell adhesions are controlled by the balance between two types of AJs; focal and linear AJs. Rho signaling weakens VE-cadherin adhesion to increase vascular permeability by promoting the development of focal AJs. In contrast, Cdc42/Rac signaling potentiates VE-cadherin adhesions to restrict vascular permeability by favoring the development of linear AJs. Furthermore, Rap1 acts as an upstream regulator and potentiates vascular barrier function by suppressing Rho signaling and by stimulating Cdc42/Rac signaling. However, whether these mechanisms regulate VE-cadherin-mediated cell adhesions to control vascular permeability in an *in vivo* setting remains to be determined, since these findings mainly come from studies performed on *in vitro* cultured endothelial cells. Thus, it is anticipated that future studies using advanced intravital imaging techniques will contribute to answering this question. It is also important to clarify the molecular mechanisms controlling vascular permeability in different organs and in different types of vessels. Answers to these questions will facilitate the development of effective therapies for diseases associated with vascular hyperpermeability.

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