Recent Advances in Research on Vascular Permeability to Establish Novel Therapeutic and Drug Delivery Strategies for Intractable Diseases

Review

Rap1 Small GTPase Regulates Vascular Endothelial-Cadherin-Mediated Endothelial Cell–Cell Junctions and Vascular Permeability

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The vascular permeability of the endothelium is finely controlled by vascular endothelial (VE)-cadherin-mediated endothelial cell–cell junctions. In the majority of normal adult tissues, endothelial cells in blood vessels maintain vascular permeability at a relatively low level, while in response to inflammation, they limit vascular barrier function to induce plasma leakage and extravasation of immune cells as a defense mechanism. Thus, the dynamic but also simultaneously tight regulation of vascular permeability by endothelial cells is responsible for maintaining homeostasis and, as such, impairments of its underlying mechanisms result in hyperpermeability, leading to the development and progression of various diseases including coronavirus disease 2019 (COVID-19), a newly emerging infectious disease. Recently, increasing numbers of studies have been unveiling the important role of Rap1, a small guanosine 5'-triphosphatase (GTPase) belonging to the Ras superfamily, in the regulation of vascular permeability. Rap1 enhances VE-cadherin-mediated endothelial cell–cell junctions to potentiate vascular barrier functions via dynamic reorganization of the actin cytoskeleton. Importantly, Rap1 signaling activation reportedly improves vascular barrier function in animal models of various diseases associated with vascular hyperpermeability, suggesting that Rap1 might be an ideal target for drugs intended to prevent vascular barrier dysfunction. Here, we describe recent progress in understanding the mechanisms by which Rap1 potentiates VE-cadherin-mediated endothelial cell–cell adhesions and vascular barrier function. We also discuss how alterations in Rap1 signaling are related to vascular barrier dysfunction in diseases such as acute pulmonary injury and malignancies. In addition, we examine the possibility of Rap1 signaling as a target of drugs for treating diseases associated with vascular hyperpermeability.

Key words vascular permeability; endothelial cell; Rap1; vascular endothelial-cadherin; actin cytoskeleton; acute lung injury

1. INTRODUCTION

Endothelial cells lining the inner surfaces of blood vessels regulate vascular permeability between the blood on the luminal side and the interstitium on the basolateral side by forming a semipermeable barrier. Vascular permeability is essential for tissue fluid homeostasis, extravasation of circulating cells and supplying essential nutrients. In most normal adult tissues, endothelial cells in blood vessels maintain basal vascular permeability at a relatively low level (Fig. 1). However, when inflammation occurs, inflammatory mediators such as histamine, bradykinin, thrombin, platelet-activating factor (PAF), and thromboxane A2 (TXA2), as well as cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), raise vascular permeability, thereby leading to plasma leakage and extravasation of immune cells as a defense mechanism (Fig. 1). Then, as the inflammatory response terminates, the barrier function of endothelial cells increases to restrict vascular permeability to the basal level. This dynamic but also tight regulation of vascular permeability by endothelial cells is responsible for maintaining homeostasis. Therefore, any impairment of proper regulation of endothelial barrier function will cause vascular hyperpermeability, leading to the development and progression of various diseases and syndromes such as sepsis, acute respiratory distress syndrome (ARDS), chronic inflammation, asthma, edema, anaphylaxis, cancer, diabetic retinopathy, and so on. (Fig. 1). For instance, many patients with severe coronavirus disease 2019 (COVID-19), a newly emerging infectious disease of major concern, have developed ARDS characterized by noncardiogenic pulmonary edema, which is the main cause of death.41

Endothelial cells utilize transcellular and paracellular pathways to regulate vascular permeability across the endothelium. This transcellular permeability is mediated by caveolae-mediated transcytosis of soluble macromolecules, while paracellular permeability depends on the opening and closing of the cell–cell junctions formed by endothelial cells. Two types of intercellular junctional domains, including adherens junctions (AJs) and tight junctions (TJs), regulate paracellular permeability across the endothelium. In endothelial cells, AJs are mainly comprised of vascular endothelial (VE)-cadherin, a member of the cadherin family of adhesion receptors, while TJs are organized by factors belonging to the family of junctional adhesion molecules such as claudins and...
occludins. Brain microvascular endothelial cells cooperate with pericytes and astrocytes to form a specialized barrier, termed the blood–brain barrier (BBB), which protects the central nervous system by strictly separating the circulating blood from the brain extracellular fluid. At the BBB, claudin-5 and claudin-3 organize highly selective semipermeable TJs in microvascular endothelial cells. On the other hand, in most peripheral tissues, microvascular endothelial cells in capillaries and post-capillary venules develop less rigidly organized intercellular junctions, in which AJs and TJs are intermingled, allowing dynamic regulation of vascular permeability. Particularly, VE-cadherin-based AJs play a crucial role in the regulation of microvascular permeability in peripheral tissues. Therefore, VE-cadherin-mediated endothelial cell–cell junctions are dynamically, but tightly, controlled by various extracellular stimuli and intracellular signaling pathways that induce or suppress vascular permeability to maintain vascular homeostasis. Herein, we review the molecular mechanisms underlying the dynamic regulation of vascular permeability, focusing especially on the small guanosine 5'-triphosphatase (GTPase) Rap1, a key regulator of VE-cadherin-mediated endothelial cell–cell junctions.

2. A RAP1 SMALL GTPASE

Rap1 (also known as Krev-1) is a small GTPase belonging to the Ras superfamily. Rap1 was originally identified as an antagonist of Ras-induced oncogenic transformation. In mammals, the two genes encoding Rap1 protein, Rap1A and Rap1B, share 95% identity and are well-conserved across species. Rap1 acts as a molecular switch to stimulate the downstream signaling pathways by cycling the guanosine 5'-diphosphate (GDP)-bound inactive state and the GTP-bound active state similarly to other GTPases (Fig. 2a). Rap1 becomes the GTP-bound active form in response to several guanine nucleotide exchange factors (GEFs), which catalyze the exchange of GDP for GTP (Fig. 2a). The GTP-bound active form of Rap1 is, conversely, inactivated by GTPase-activating proteins (GAPs) which stimulate the GTPase activity of Rap1 to catalyze GTP hydrolysis (Fig. 2a).

Rap1 controls diverse cellular processes including cell-extracellular matrix (ECM) adhesions, the formation of cell–cell junctions, cell migration, and cell polarity (Fig. 2b). Rap1 reportedly binds the Rap1-GTP-interacting adaptor molecule (RIAM) and talin to promote cell-ECM adhesions through inside-out activation of integrins (Fig. 2b). Rap1 is also involved in the formation and maturation of cadherin-based cell–cell adhesions in both endothelial and epithelial cells. Furthermore, it regulates the establishment of different types of cell polarity such as front-rear, apicobasal, and neuronal polarity. Therefore, Rap1 is implicated in a broad range of physiological and pathological processes including immune responses, hematopoiesis, neuronal development, and malignant progression. In addition, Rap1 plays multiple roles in vascular system development and functions. Analyses of endothelial cell-specific Rap1-deficient mice showed an essential role of Rap1 in vascular development and angiogenesis. Furthermore, Rap1 in endothelial cells is required for the maintenance of vascular tone. Mechanistically, Rap1 regulates the mechano-sensing property of shear stress in endothelial cells, thereby inducing the production of nitric oxide which decreases blood pressure. In addition, Rap1 plays a crucial role in regulating both VE-cadherin-mediated endothelial cell–cell junctions and vascular permeability.
3. ROLE OF RAP1 IN REGULATING VASCULAR PERMEABILITY VIA VE-CADHERIN

It has long been recognized that cAMP-elevating G protein-coupled receptor (GPCR) agonists reduce the vascular permeability triggered by inflammatory stimuli. cAMP-dependent protein kinase A (PKA) is the most well-known cAMP effector. Furthermore, since PKA inhibits RhoA-induced elevations in actomyosin contractile activity, PKA was previously considered to mediate cAMP-induced enhancement of vascular barrier function. However, we and other groups additionally identified exchange protein directly activated by cAMP (Epac) as a cAMP effector responsible for vascular barrier enhancement (Fig. 3). Concomitantly, the importance of Epac as a cAMP effector responsible for vascular barrier function became clear, since Epac is a GEF for Rap1. Thus, cAMP potentiates VE-cadherin-mediated cell–cell contacts to restrict endothelial permeability via an Epac-Rap1 signaling pathway (Fig. 3). Subsequent studies further clarified that Rap1 is activated not only by cAMP-elevating GPCR agonists but also by other upstream signals to regulate VE-cadherin-mediated endothelial cell–cell junctions. For instance, VE-cadherin engagement leads to Rap1 activation at nascent cell–cell contacts through the PDZ domain containing GEF1 (PDZ-GEF1, also known as RAPGEF2), another GEF for Rap1, which in turn promotes maturation of VE-cadherin-based AJs. Other group has also shown that angiopoietin-1, a unique angiogenic signaling molecule involved in both angiogenesis and vascular stabilization, activates Rap1 to decrease vascular permeability. Furthermore, low-magnitude cyclic stretch was shown to promote Rap1-induced enhancement of VE-cadherin-mediated cell–cell adhesions in pulmonary endothelial cells. Along with VE-cadherin, Rap1 is also involved in the formation and stabilization of E-cadherin-based cell–cell adhesions in epithelial cells. Thus, Rap1 was recently recognized as a key mediator of not only integrin-mediated cell-ECM adhesions but also cadherin-based cell–cell adhesions.

4. MOLECULAR MECHANISMS BY WHICH RAP1 POTENTIATES VE-CADHERIN-MEDIATED ENDOTHELIAL CELL–CELL JUNCTIONS TO ENHANCE VASCULAR BARRIER FUNCTION

Organization of the actin cytoskeleton determines the adhesive function of VE-cadherin and vascular barrier integrity. VE-cadherin-based AJs exist mainly in two forms: stable linear AJs supported by circumferential actin bundles (CAB), defined as linear actin bundles that align along the cell–cell junctions, and discontinuous focal AJs connected by radial stress fibers that have a perpendicular orientation to the cell–cell junctions. In linear AJs, VE-cadherin stabilizes at cell–cell junctions by anchoring to the CABs through α- and β-catenins, thereby providing a strong barrier. In contrast, formation of focal AJs is a hallmark of reduced barrier function. In focal AJs, radial stress fibers are connected to and generate a pulling force on VE-cadherin-based cell–cell junctions, which together result in the formation of discontinuous focal AJs and thereby increased vascular permeability. Therefore, controlling the balance between linear and focal AJs allows dynamic regulation of vascular barrier function.

Rap1 potentiates VE-cadherin-mediated cell–cell adhesions by inducing marked reorganization of the actin cytoskeleton (Fig. 4). Inflammatory mediators such as histamine, bradykinin, thrombin, PAF, and TXA2 trigger the formation of focal AJs through Rho signal activation, thereby increasing vascular permeability. Rho and its downstream effector, Rho-associated coiled-coil containing protein kinase (ROCK), induce actomyosin contractility and the formation of radial stress fibers through cytoplasmic activation of non-muscle myosin II (NM-II), leading to the formation of focal AJs. Activation of Rap1, on the other hand, disrupts focal AJs and induces the formation of linear AJs, which in turn promote the function of VE-cadherin-mediated cell–cell junctions and thereby enhance the vascular barrier. Disruption of focal AJs by Rap1 involves inhibition of the Rho-ROCK-NM-II signaling pathway (Fig. 4). Rap1 suppresses Rho activity via two highly related Rap1 effectors, Ras-association and dilute domain-containing protein (Radil) and Ras-interacting protein 1 (Rasip1). In response to Rap1 activation, Radil and Rasip1 are translocated to the plasma membrane, resulting in the recruitment of RhoGAP ArhGAP29 to suppress Rho activity. Furthermore, the cerebral cavernous malformation protein CCM1, which is also known as Krev interacting trapped protein 1 (Krit-1), reportedly acts downstream from Rap1 to inhibit the Rho-ROCK pathway. In addition to inhibiting Rho-mediated formation of focal AJs, Rap1 induces the formation of CABs through activation of Cdc42, another member of the Rho family of small GTPases. In pulmonary endothelial cells, along with VE-cadherin, Rap1 is also involved in the formation and stabilization of E-cadherin-based cell–cell adhesions in epithelial cells. Thus, Rap1 was recently recognized as a key mediator of not only integrin-mediated cell-ECM adhesions but also cadherin-based cell–cell adhesions.

**Fig. 3. Molecular Mechanism(s) by Which cAMP-Elevating GPCR Agonists Potentiate VE-Cadherin-Mediated Cell–Cell Junctions to Restrict Vascular Permeability**

GPCR, G protein-coupled receptor; AC, adenylyl cyclase; PKA, protein kinase A; Epac, exchange protein directly activated by cAMP. (Color figure can be accessed in the online version.)
thought to catalyze the exchange of GDP for GTP on Cdc42, triggering junctional accumulation of active Cdc42. Active Cdc42 at cell–cell contacts subsequently recruits and stimulates myotonic dystrophy kinase-related CDC42-binding kinase (MRCK), which in turn triggers junctional activation of NM-II via phosphorylation of its regulatory light chain subunit, thereby promoting vascular permeability. Therefore, Rap1 inhibits inflammatory mediator-induced increases in vascular permeability by suppressing Rho activity. In addition, Rap1 promotes the formation of circumferential actin bundles (CABs) and linear AJs through the Cdc42-MRCK pathway-mediated junctional activation of NM-II as well as Cdc42/Rac-mediated actin polymerization which stabilizes VE-cadherin-mediated cell–cell junctions, thereby decreasing vascular permeability. In endothelial cells developing linear AJs, VE-cadherin stabilizes at cell–cell junctions by anchoring to the CABs through α- and β-catenins. Fluorescence images show VE-cadherin (green) and F-actin (red) in human pulmonary artery endothelial cells (HPAECs) stimulated without (left image) or with (right image) 007, an Epac activator. Note that the unstimulated HPAECs exhibit formation of radial stress fibers and focal AJs, while 007-mediated activation of Rap1 in these cells induces the disruption of radial stress fibers and focal AJs as well as the formation of CABs and linear AJs. (Color figure can be accessed in the online version.)

5. **IN VIVO ROLE OF RAP1 IN THE REGULATION OF VASCULAR PERMEABILITY**

Whether Rap1 is essential to maintaining vascular barrier function in vivo remains unknown, although several in vitro studies have clearly shown that it potentiates VE-cadherin-mediated endothelial cell–cell junctions, as discussed in detail above. Endothelial specific Rap1A and Rap1B double knockout (Rap1 EC-KO) mice were reported to exhibit embryonic lethality with severe hemorrhage, suggesting Rap1 to play a role in vascular barrier formation and/or function during development. However, unexpectedly, Chrzanowska-Wodnicka and colleagues found that endothelial cell-specific deletion of both...
Rap1A and Rap1B after birth did not exert a marked effect on vascular barrier function, although the affected mice showed a slight increase in pulmonary vascular permeability as compared with controls. However, Rap1 EC-KO mice, when injected with lipopolysaccharide (LPS), exhibited increased vascular leakage in the lungs as compared with controls. These results raised the possibility that endothelial Rap1 may not make a major contribution to the maintenance of basal vascular barrier function in normal adult tissues, while playing a barrier-protecting role against inflammation-induced vascular leakage and/or promoting remodeling of the vascular barrier after inflammation-induced disruption of endothelial cell–cell junctions.

However, research has supported the role of Rap1 in maintaining vascular barrier function in normal tissues. The transcription factor lymphoblastic leukemia-derived sequence 1 (Lyl1), a member of the basic helix-loop-helix family, is known to specifically be expressed in hematopoietic and endothelial cells during development as well as in adulthood. Lyl1-deficient mice showed increased pulmonary vascular permeability as compared to wild type mice. It is noteworthy that endothelial cells derived from Lyl1-deficient mice exhibited reduced expressions of C3G and Dock4 (an atypical GEF for Rap1) mRNAs and increased Rho activity, raising the possibility that downregulated Rap1 activity might account for GEFS for Rap1, thereby stimulating Rap1 activation of vascular barrier dysfunction in a variety of disorders associated with hyperpermeability (right). Hence, Rap1 signaling might be maintained by physiological cyclic stretch-induced normalization of Rap1 activity via the formation of focal AJs (Fig. 5). In normal tissues, Rap1 shifts the balance from focal to linear AJ formation, thereby maintaining vascular barrier function (Fig. 5). However, when inflammation is induced, permeability-increasing factors raise vascular permeability via induction of Rho-dependent formation of focal AJs (Fig. 5). In addition, excessive formation of focal AJs by Rho results in hyperpermeability which promotes both the development and the progression of various diseases and syndromes including sepsis, ARDS, chronic inflammation, asthma, edema, anaphylaxis, cancer, diabetic retinopathy, and other disorders (Fig. 5). Thus, Rap1 signaling activation may provide protection against vascular barrier dysfunction which would alleviate the pathology underlying these disorders, based on restricting hyperpermeability by normalizing the balance between

**6. RAP1 AS A POTENTIAL DRUG TARGET FOR VASCULAR HYPERPERMEABILITY**

The degree of vascular permeability might be determined by the balance between focal AJ formation by Rho and linear AJ formation by Cdc42 and Rac in endothelial cells (Fig. 5). In normal tissues, Rap1 shifts the balance from focal to linear AJ formation, thereby maintaining vascular barrier function (Fig. 5). However, when inflammation is induced, permeability-increasing factors raise vascular permeability via induction of Rho-dependent formation of focal AJs (Fig. 5). In addition, excessive formation of focal AJs by Rho results in hyperpermeability which promotes both the development and the progression of various diseases and syndromes including sepsis, ARDS, chronic inflammation, asthma, edema, anaphylaxis, cancer, diabetic retinopathy, and other disorders (Fig. 5). Thus, Rap1 signaling activation may provide protection against vascular barrier dysfunction which would alleviate the pathology underlying these disorders, based on restricting hyperpermeability by normalizing the balance between

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**Fig. 5. Proposed Model for How Vascular Permeability Is Regulated under Normal and Inflammatory Conditions and How Dysregulation of Vascular Barrier Function Causes the Diseases Associated with Hyperpermeability**

In normal tissues, Rap1 activity is increased and thereby disrupts focal AJs and induces the formation of linear AJs which restrict vascular permeability (left). When inflammation occurs, inflammatory mediators increase vascular permeability via the formation of focal AJs (middle). Inflammatory mediators may suppress Rap1 activity and thus disrupt linear AJs. Overproduction of these inflammatory mediators induces excessive formation of focal AJs, resulting in the induction of vascular hyperpermeability, which promotes the development and progression of various diseases (right). Hence, Rap1 signaling might be an ideal drug target for effectively preventing vascular barrier dysfunction in a variety of disorders associated with hyperpermeability. (Color figure can be accessed in the online version.)
focal and linear AJ formation through the respective inhibition and activation of Rho and Cdc42 (Fig. 5). Several lines of evidence supporting this possibility have revealed the protective effect of 007, a cAMP analog specific for Epac, against hyperpermeability under various pathological conditions. We previously reported administration of 007 to suppress vascular endothelial growth factor (VEGF)-induced vascular hyperpermeability in murine skin. Similarly, 007-initiated activation of the Epac/Rap1 pathway was shown to inhibit microvascular hyperpermeability caused by PAF in the rat mesentery, disruption of the endothelial barrier caused by toxins derived from *Pseudomonas aeruginosa*, a major nosocomial infection agent, ischemia-reperfusion-induced microvascular hyperpermeability in skeletal muscle, and cytokine-induced retinal vascular permeability. Furthermore, cAMP-elevating GPCR agonists such as prostaglandin I2 and its stable analogs like iloprost and beraprost, prostaglandin A2, and adrenomedullin are known to exert Rap1-dependent protective effects against pulmonary endothelial barrier dysfunction during acute pulmonary injury induced by LPS administration and mechanical ventilation. Thus, Rap1 signaling might be an ideal drug target for preventing vascular barrier dysfunction in diseases associated with vascular hyperpermeability (Fig. 5).

We can reasonably speculate that cAMP-Epac-Rap1 signaling is affected by inflammatory cytokines, a process which disrupts vascular barrier function. Koga et al. reported that stimulation with TNF-α reduces the intracellular cAMP concentration to increase the permeability of the endothelial cell monolayer. It has also been shown that LPS-induced endothelial barrier breakdown depends on reducing the intracellular cAMP level. Mechanistically, TNF-α induces the expression and/or activation of phosphodiesterases (PDEs), enzymes that catalyze cAMP hydrolysis, thereby decreasing the intracellular cAMP concentration. Koga et al. demonstrated that TNF-α stimulates PDE-IV activity, without affecting either its protein or its mRNA level, to decrease the intracellular cAMP level. Seybold et al. also reported that TNF-α reduces the cAMP level by inducing the expression of PDE2 via activation of p38 mitogen-activated protein kinase, which increases endothelial permeability. Moreover, inflammatory cytokine-induced vascular barrier dysfunction has been shown to be alleviated by inhibition of PDEs. PDE inhibition suppressed microvascular leakage and reduced acute lung injury in a murine pneumococcal pneumonia model. Furthermore, PDE4 inhibition reportedly increased the cAMP level, thereby suppressing capillary leakage in states of sepsis and systemic inflammation. Since cAMP stimulates the Epac-Rap1 pathway, TNF-α and LPS may induce vascular hyperpermeability by promoting PDE-dependent cAMP degradation to suppress cAMP-Epac-Rap1 signal-mediated vascular barrier function. These observations raise the possibility that basal vascular barrier function in normal tissues is maintained by the cAMP-Epac-Rap1 pathway, which is inhibited by inflammatory cytokines that disrupt vascular barrier function. Therefore, drugs that activate Rap1 signaling can be expected to effectively prevent vascular barrier dysfunction during inflammatory processes.

Disorganized, tortuous, and hyperpermeable blood vessels characteristic of tumors hamper the anti-tumor efficacy of immune cells and prevent efficient diffusion of chemotherapeutic agents. Thus, normalizing the tumor vasculature by decreasing hyperpermeability has been speculated to improve the efficacies of chemotherapy, radiotherapy, and immunotherapy. VEGF signaling is known to induce vascular hyperpermeability in tumor tissues. Interestingly, Yamazaki et al. found that VEGF increases tumor vascular permeability by reducing endothelial PKA activity. Based on intravital imaging of tumors and normal blood vessels in mice, they found that endothelial PKA activity is lower in tumors than in normal tissues and also demonstrated that VEGF lowers the PKA activity in tumor blood vessels. Since PKA is a downstream effector of cAMP, it can reasonably be assumed that VEGF decreases the cAMP level in tumor endothelial cells. Indeed, a previous report described VEGF as decreasing the intracellular cAMP level in endothelial cells by reducing the functions of PDE2 and PDE4. Therefore, VEGF might increase tumor vascular permeability by inhibiting the cAMP-Epac-Rap1 pathway. If this is the case, pharmaceutical agents which can activate Rap1 signaling would presumably normalize the tumor vasculature and thus improve anti-tumor therapy.

7. CONCLUSION

In this review, we have elaborated on the roles of Rap1 in regulating vascular endothelial permeability and discussed how Rap1 potentiates VE-cadherin-mediated endothelial cell junctions to maintain vascular barrier function. We have also highlighted the potential of Rap1 signaling as a drug target for a range of diseases associated with hyperpermeability. Currently, there are no pharmaceutical agents that can decrease vascular permeability. ARDS is a severe medical condition characterized by noncardiogenic pulmonary edema. The mortality rate associated with ARDS remains very high. ARDS is, in fact, one of the main causes of death in severely ill COVID-19 patients. Since defective pulmonary vascular barrier integrity is regarded as one of the pathological hallmarks of ARDS, particularly in the very early stage of its development, it is widely assumed that medications enhancing vascular barrier function would ameliorate the pathological conditions underlying ARDS. Therefore, drugs capable of activating Rap1 signaling in endothelial cells might be used to treat ARDS. As noted above, cAMP-elevating GPCR agonists are known to exert Rap1-dependent protective effects against pulmonary endothelial barrier dysfunction during acute pulmonary injury. However, cAMP-elevating GPCR agonists such as adrenomedullin not only enhance vascular barrier function but also induce vasodilation and hypotension, and have thus been described as a “double-edged sword” in patients with sepsis. Indeed, strict relationships of high levels of the bioactive form of adrenomedullin upon intense care unit admission with both organ dysfunction and mortality have been reported. Hence, pharmaceutical agents that specifically activate Rap1 signaling are preferable to cAMP-elevating GPCR agonists. Epac might thus be a good drug target for improving vascular barrier function, since it is a GEF for Rap1 and is relatively highly expressed in endothelial cells. In fact, non-cyclic nucleotide and low-molecular weight compounds that activate Epac have recently been developed, though their efficacies for improving vascular barrier function in animal disease models have not yet been adequately tested. Therefore, low-molecular weight compounds with the capacity to activate Rap1 signaling, e.g., Epac activators, might be ideal agents for treating the
various diseases associated with vascular hyperpermeability.

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