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Minireview

Breaking the VE-cadherin bonds

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ABSTRACT

Exchanges between the blood compartment and the surrounding tissues require a tight regulation by the endothelial barrier. Recent reports inferred that VE-cadherin, an endothelial specific cell–cell adhesion molecule, plays a pivotal role in the formation, maturation and remodeling of the vascular wall. Indeed, a growing number of permeability inducing factors (PIFs) was shown to elicit signaling mechanisms culminating in VE-cadherin destabilization and global alteration of the junctional architecture. Conversely, anti-PIFs protect from VE-cadherin disruption and enhance cell cohesion. These findings provide evidence on how endothelial cell–cell junctions impact the vascular network, and change our perception about normal and aberrant angiogenesis.

Fluids, cells, and nutrients are normally exchanged between the blood compartment and the surrounding tissues, under physiologic conditions and depending on the vascular sites. In this dynamic network, endothelial cells that line the vascular wall act as gatekeepers to control the infiltration of blood proteins and circulating cells to the underlying tissues. This vascular permeability contributes to normal angiogenesis, blood pressure control, as well as immune responses [1]. Abnormal increase in vascular permeability is often observed in many pathological conditions and human diseases, such as tumor-induced angiogenesis, inflammation, macular degeneration, allergy, and brain stroke. Plasma molecules and cells can pass through (transcellular) or between (paracellular) endothelial cells to ensure endothelial permeability. Transcellular passage requires either cell fenestration or a complex system of transport vesicles, called vesiculo-vacuolar organelles. The paracellular pathway, by contrast, mostly relies on the coordinated opening and closure of endothelial cell–cell junctions. This latter function must therefore be tightly regulated to maintain the endothelial integrity.

1. Endothelial cell–cell junctions required functional VE-cadherin adhesion

The adhesive contacts between cells underlie many morphogenetic processes during embryonic development as well as growth control, turnover, and regeneration of post-natal and adult tissues. Formation, maintenance, and remodeling of the intercellular contacts are achieved through physical and functional cooperation between two main adhesive structures: tight junctions and adherens junctions (Fig. 1). Cadherins, which are the main constituent of the adherens junctions, belong to a conserved family of cell–cell adhesion molecules and link the plasma membrane with the intracellular actin cytoskeleton through catenin family proteins [2]. Tight junctions form a dense ultra-structure organization, observable by electron microscopy, which involves numerous adhesive molecules, including the claudin and occludin family of tetraspan transmembrane proteins, the junctional adhesion molecules (JAMs), and the intracellular adapters, namely zona occludens proteins, ZO-1 and ZO-2 [3]. Although tight junctions are often located apically with respect to adherens junctions in epithelial cells, both junctions are intermingled throughout cell–cell contact areas in endothelial cells [4].

The barrier properties require the adhesive activities of VE-cadherin and claudin-5, which are key components of the adherens and tight endothelial junctions, respectively (Fig. 1). Indeed, macromolecule flux is enhanced through claudin-5 and VE-cadherin-deficient endothelial monolayers in vitro [5,6]. In endothelial cells, VE-cadherin is highly expressed and located at adherens junctions. Its expression profile appears early and is restricted to the endothelial cell lineage, such that its promoter has been used to generate transgene expression specifically in the endothelial compartment [7]. In addition, N-cadherin is also found at high levels in endothelial cells but exhibits a weak clustering at the endothelial cell–cell junctions and occupied preferentially apical locations.

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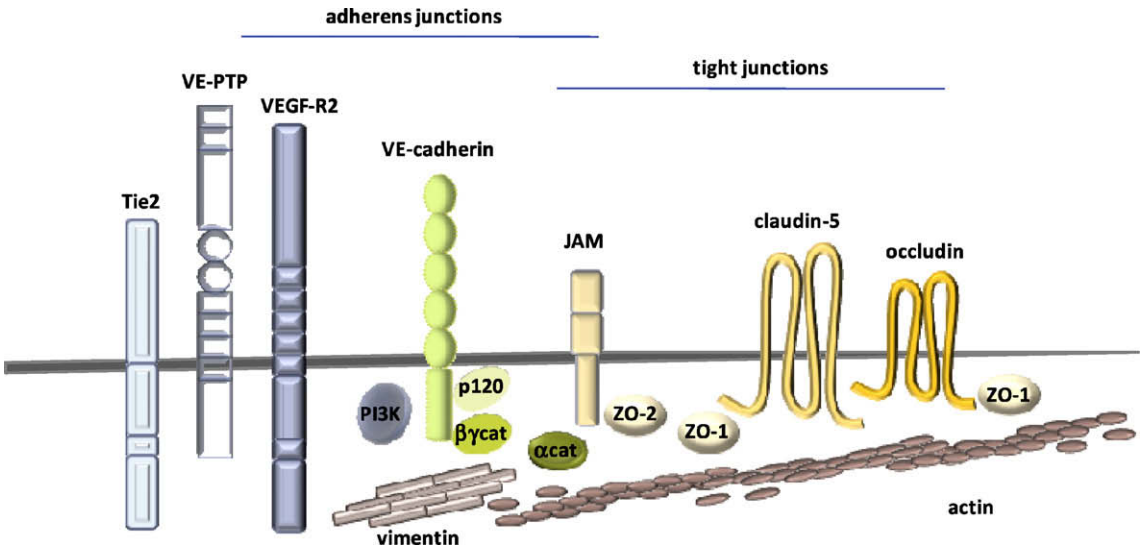

Fig. 1. Endothelial cell–cell junctions. Adherens and tight junctions sealed the endothelial cells through specific cell–cell adhesion molecules: VE-cadherin, junctional adhesion molecules (JAM), claudin-5, occludin. These adhesion complexes are linked to the actin filament cytoskeleton and the vimentin intermediate filament network through intracellular mediators: p120, α, β and γ catenins (cat) and zona occludens (ZO) proteins. Endothelial cell–cell junctions can also concentrate signaling components such as phospho-inositide 3 kinase (PI3K), vascular endothelial growth factor receptor type 2 (VEGF-R2), vascular endothelial cell-specific phospho-tyrosine phosphatase (VE-PTP) and the angiopoietin-1 receptor, Tie2.

Indeed, N-cadherin was suggested to play a more prominent role in vascular stabilization through heterotypic adhesion between endothelial cells and pericytes [8]. Interestingly, the phenotype of knockout mice for claudin-5 and VE-cadherin drastically differs. While claudin-5 knockout mice develop normally but have a defective blood–brain barrier function and die shortly after birth [9], VE-cadherin knockout mice are embryonic lethal and exhibit multiple severe defects during developmental angiogenesis [10]. The severity of the phenotype due to VE-cadherin deficiency as compared to the one observed in the absence of claudin-5 suggests a functional hierarchy in the organization of the endothelial cell–cell junctions. In agreement, VE-cadherin is required to prevent the disassembly of the blood vessel walls [11], and to coordinate the passage of macromolecules through the endothelium in vitro [5,12]. Finally, VE-cadherin has been recently shown to directly enhance the expression level of claudin-5 by tethering repressive transcription factors away from the claudin-5 promoter [6]. Conversely, the absence of functional VE-cadherin is associated with loss of claudin-5 expression, suggesting that VE-cadherin adhesion might act upstream in the formation and maintenance of the endothelial barrier integrity. Hence, VE-cadherin function in endothelial cells is intimately linked to vascular integrity and endothelial barrier plasticity.

2. Molecular basis for VE-cadherin adhesive function

VE-cadherin belongs to the cadherin super-family of cell–cell adhesion molecules, which are encoded by more than 200 genes in the human genome [13]. Among them, classical cadherins are Ca^{2+}-dependent, homophilic, cell to cell adhesion molecules expressed in nearly all cells within solid tissues. These molecules also participate in cell–cell recognition, a property that confers the ability of cells to aggregate with and ultimately sort their most physiologically relevant cell partners. It is generally admitted that cadherins form a core adhesion complex that consists of a cadherin dimer, binding through its extracellular region to another dimer of cadherins expressed in adjacent cells, while its intracellular region is anchored to the plasma membrane and linked to the cytoskeleton [14].

Similarly to other typical cadherins, the VE-cadherin extracellular domain consists of five cadherin-type repeats, called EC (extracellular cadherin) domains that are bound together by calcium ions in a rod-like structure [14]. Interestingly, VE-cadherin lacks the HAV tripeptide, localized in the EC1 repeat, which has been discussed to participate in cell–cell adhesion recognition in the case of E- and N-cadherin-mediated contacts. By the use of purified recombinant proteins containing either fragments or total ECs, it was shown that multiple EC interactions promote the formation of a completely inter-digitated hexameric configuration. Of note, this hexameric structure might be specific for VE-cadherin, and has been proposed to favor its membrane stability [15]. Once engaged in homophilic interactions, the extracellular domain develops additional forces, strengthening the adhesive contacts while retaining the ability to associate and to dissociate rapidly in response to dynamic changes in the cellular environment.

Classical cadherins are single-pass transmembrane proteins that interact with a number of different cytoplasmic partners to carry out their functions, which include cell–cell adhesion, cytoskeletal anchoring, immediate intracellular signaling and control of gene expression. The cadherin-associated proteins, catenins, are universally present in classical cadherin complexes [13]. In particular, β-catenin interacts with the distal part of the cadherin cytoplasmic domain and p120 catenin with a more proximal region. By contrast, α-catenin does not bind directly to cadherin, but instead associates with β-catenin, and provides a physical links to the actin cytoskeleton, either by directly binding to actin filaments or indirectly through other actin-binding proteins such as vinculin, α-actinin, and formins thus regulating actin polymerization, cross-linking and dynamics at the cell–cell contact zones [16]. Under some conditions, β-catenin could be substituted to γ-catenin, which has been suggested to associate preferentially with the vimentin network, and therefore may provide a link between VE-cadherin-mediated junctions and the intermediate filament cytoskeleton [4]. On the other hand, p120 catenin seems to influence cadherin function by a variety of mechanisms, which includes plasma membrane stability, recycling, and cell surface targeting [17].

Therefore, disrupting the endothelial cell–cell junctions implies to disconnect VE-cadherin intracellular domain from essential

cohesive mediators, as well as weakening its extracellular homophilic interactions.

3. How do permeability inducing factors (PIFs) disrupt VE-cadherin bonds?

There is a growing number of angiogenic and inflammatory agents that have been shown to modulate vascular integrity in vivo and barrier properties of endothelial monolayers in vitro. These permeability inducing factors (PIFs) may vary in their mode of actions, and dissecting their mediated signaling pathways has been an intense field of investigation (Table 1). Indeed, several studies have primarily focused on PIF effects on the endothelial cell–cell junction organization and have therefore permitted to unveil key molecular mechanisms involved in the endothelial barrier plasticity. One can distinguish four interconnected biochemical routes, elicited by PIFs leading to the loss of VE-cadherin function, and ultimately culminating at the increase of vascular permeability, through: (1) phosphorylation-driven VE-cadherin/catenin complex destabilization, (2) reduction of cell surface expression and exposure, (3) crosstalk with tight junctions, and (4) tension and mechanical forces (Fig. 2).

Table 1
Permeability inducing factors (PIFs) and their proposed modes of action.

PIFs	Effects on VE-cadherin	Proposed signaling pathways
VEGF	Phosphorylation	Src
	Internalization	Rac/PAK
	Catenin dissociation	Ca^{++}/PKC
Thrombin		Ca^{++}/PKC
	Catenin dissociation	Rho
	Phosphorylation	Rac/PAK
		MAPK
Histamine	Phosphorylation	Ca^{++}/PKC
	Catenin dissociation	Rho
	Internalization	Rac/PAK
TNFα		Ca^{++}/PKC
	Phosphorylation	Rho
		Rac/PAK
LPS		Rho
	Phosphorylation	Indirect
		Src
ROS	Phosphorylation	Rac/PAK
	Catenin dissociation	Src
		Ca^{++}/PKC

LPS: lipopolysaccharide; PAK: p21-activated kinase; PKC: protein kinase C; ROS: reactive oxygen species; TNF: tumor necrosis factor; VEGF: vascular endothelial growth factor. Some PIFs are not listed here, such as bradykinine, as their effects have been suggested to be specific of the endothelial cell models used.

Historically, phosphorylation has been early described as a prominent mechanism by which cadherin and catenin interaction can be modulated, either positively or negatively, and therefore might decipher for overall adhesion forces [4]. To this regard, VE-cadherin bears nine putative phospho-tyrosine sites, among which Y658, Y685, and Y731 have been individually found to be implicated in the barrier integrity [18,19]. Recently, serine phosphorylation on the conserved S665 residue emerged as a pivotal regulator of VE-cadherin exposure at the plasma membrane [5]. Of note, vascular endothelial growth factor (VEGF) was demonstrated to trigger VE-cadherin tyrosine phosphorylation and remodeling in endothelial cells [20]. Similarly, most of the PIFs identified so far promote an increase in VE-cadherin tyrosine phosphorylation and diminution of VE-cadherin/catenin binding. Although several kinases were proposed to contribute to VE-cadherin phosphorylation, how exactly tyrosine phosphorylation promotes vascular leakage remains elusive [4]. However, the non-receptor tyrosine kinases of the Src family might be involved in this biological process. Indeed, Src-deficient mice show decreased vascular leakage and VE-cadherin tyrosine phosphorylation in response to VEGF

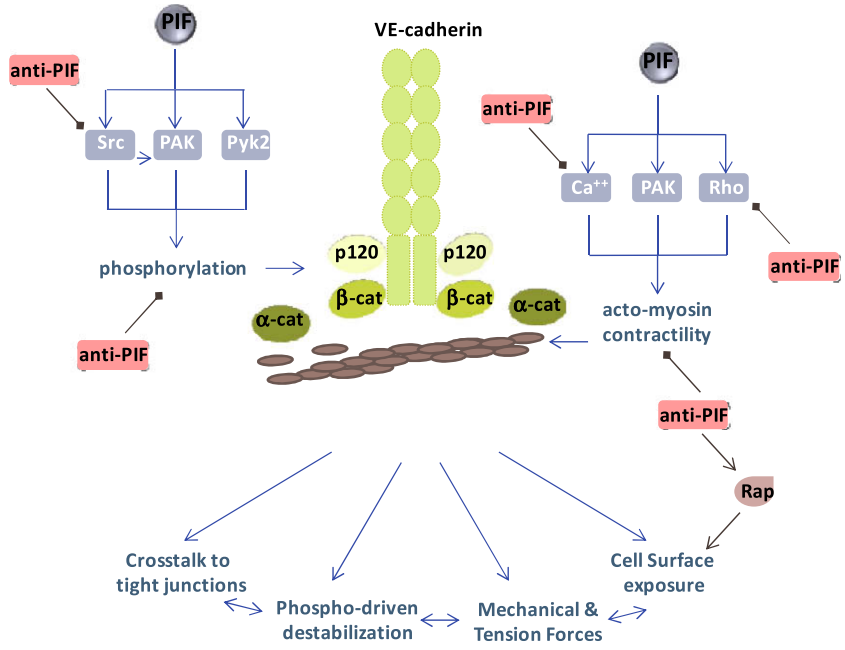

Fig. 2. VE-cadherin adhesion controls the endothelial barrier properties. Four interconnected pathways can be elicited by permeability inducing factors (PIFs) to increase endothelial permeability, leading ultimately to the loss of VE-cadherin function, through: (1) crosstalk to tight junctions, (2) phosphorylation-driven VE-cadherin/catenin complex destabilization, (3) mechanical and tension forces, and (4) reduction of cell surface expression and exposure. Essential mediators such as the kinases: Pyk2, PAK, and Src and the biochemical routes involving the RhoA and calcium signaling axis have been shown to act downstream angiogenic and inflammatory PIFs. At the opposite, anti-PIFs elicit signaling mechanisms hampering on Src and Rho activation, calcium signaling, VE-cadherin phosphorylation, acto-myosin contractility and actin polymerization, or can enhance VE-cadherin cell surface exposure through Rap signaling axis. Ultimately, anti-PIFs can reinforce VE-cadherin adhesion and therefore the integrity of the endothelial barrier.

but not inflammatory agents [21]. Moreover, blocking Src activity can efficiently restore the barrier integrity in a pathological context such as brain stroke, tumor cell extrasavation, and retinal hyperpermeability [22–24].

A reduction in VE-cadherin stability at the plasma membrane might also contribute to compromise the endothelial barrier integrity. Indeed, VE-cadherin cell surface expression is frequently altered in hyper-permeability of the vascular network in pathological conditions such as inflammation, diabetes, and virus exposure [29–31]. Interestingly, internalization of VE-cadherin has been observed in response to monocyte extrasavation, inhibition of FGF signaling and activation of VEGF-R2 by VEGF [5,25,26]. At the molecular level, VEGF triggers the hierarchical activation of the Src tyrosine kinase, Vav2, a guanine-nucleotide exchange factor for the small Rho GTPase, Rac and its downstream effector the p21-activated kinase, PAK [5]. This signaling axis culminates at the PAK-dependent phosphorylation of VE-cadherin, on a highly conserved serine residue, which directs VE-cadherin to endocytosis [5]. Consistent with these results, active forms of PAK were observed to be targeted and localized at cell–cell junctions upon PIF exposure [27]. In addition, decreasing the association between VE-cadherin and p120 catenin leads to clathrin-dependent VE-cadherin endocytosis [28]. To this regard, blocking FGF signaling in endothelial cells triggers the dissociation between VE-cadherin and p120, leading to the reduction of VE-cadherin exposure at the cell surface, and the subsequent disassembly of endothelial cell–cell junctions [26]. Ultimately, PIF dose, kinetics and mode of action might control the fate of internalized VE-cadherin and drive it to temporary storage compartment, recycling pathway, or degradation.

As discussed above, crosstalk between VE-cadherin and tight junctions is now admitted [6]. Indeed, an increase in endothelial monolayer permeability is generally accompanied by the reorganization of junctional proteins, leading to a transient opening of the endothelial junctions and a subsequent increase in paracellular permeability. It has been demonstrated that not only VE-cadherin is relocated and phosphorylated upon stimulation by diverse PIFs, but similar observations have also been made for tight junction components [32]. Interestingly, the diminution of VE-cadherin plasma membrane exposure corroborates with compromised organization of the endothelial tight junctions [5,26]. As extrapolate from epithelial cells, adherens junctions are functionally linked tight junctions and vice versa [3]. This hypothesis has been further demonstrated since claudin-5 mRNA is down regulated in endothelial cells lacking VE-cadherin expression or in which VE-cadherin activity is lost [6]. Conversely, rescuing VE-cadherin function triggers claudin-5 transcription. Hence, VE-cadherin can directly control claudin-5 expression, and therefore the organization and maturation of endothelial tight junctions [2,6]. At the molecular level, VE-cadherin adhesion prevents β-catenin and FoxO binding to claudin-5 promoter, where they could repress its transcription. These data offer a fine molecular framework on how VE-cadherin encrypts global endothelial cell–cell junction architecture. On the other hand, the loss of expression of the junctional adhesion molecule, JAM-C, results in the stabilization of VE-cadherin-mediated adhesion, and the subsequent decreased in basal permeability and VEGF- and histamine-induced increases in vascular leakage [33], suggesting then a more complex bidirectional interplay between adherens and tight endothelial junctions at the cell–cell contact interface.

Finally, endothelial permeability largely depends on actomyosin-based cell contractility, as intracellular actin fibers exert centripetal tension on intercellular junctions [34]. Inhibition of actomyosin contractility by pharmacological agents, as well as examination of myosin light chain (MLC) phosphorylation status had placed mechanical forces in the pathway by which PIFs, such as thrombin and histamine exert their effects [35]. MLC phosphorylation mainly relies on the activation of the small GTPase, RhoA, which in turn controls the two serine/threonine kinases, ROCK, and PRK [35–37]. Interestingly, elevation of intracellular calcium signaling has been shown upstream RhoA activation in such biochemical routes [37,38]. In addition, blocking PAK-dependent acto-myosin contractility prevents from endothelial permeability increased by several PIFs, such as VEGF, histamine, tumor necrosis factor (TNFα), lipopolysaccharide from the bacterial wall (LPS), as well as atherosclerotic risk factors [27,39].

In conclusion, coordinated disruption of VE-cadherin intracellular interactions by phosphorylation, internalization, and mechanical forces most likely contributes collectively to the destabilization and the disengagement of VE-cadherin adhesion, culminating at the restructuration of both adherens and tight junctions and the subsequent opening of endothelial cell–cell junctions.

4. Looking for anti-permeability inducing factor (anti-PIFs) mechanisms

Few angiogenic mediators exert an anti-permeability action, since plasma leakage supplies essential growth factors, matrix proteins, and angiogenic factor-producing cells to the perivascular zones. Thus, blocking vascular leakage may have direct implications in modulating angiogenesis and inflammation, as well as therapeutic potentials in the treatment of many human diseases characterized by loss of vascular integrity. In this paragraph, the proposed mechanisms by which anti-permeability inducing factors (anti-PIFs) exert their effects on the endothelial barrier function will be discussed (Fig. 2). The most relevant anti-PIFs are reported in Table 2, among them: angiopoietin-1 and its cognate receptor Tie2, fibroblast growth factor (FGF), Robo-4, cAMP-elevating G protein-coupled receptor (GPCR) agonists, and sphingosine-1-phosphate (S1P).

Although angiopoietin-1 and VEGF are both potent pro-angiogenic factors, angiopoietin-1 stabilizes blood vessels and protects from VEGF-induced vascular permeability. Indeed, angiopoietin-1 administration or overexpression in the dermal compartment can protect from the potentially lethal actions of VEGF as a consequence of uncontrolled plasma leakage [40]. In this regard, angiopoietin-1 can potently block VEGF-induced endothelial permeability in vitro [41], suggesting that their opposing effects on vascular leakage may be exerted through direct stimulation of endothelial cells. In addition, angiopoietin-1 might exert a general anti-vascular permeability effect, protecting blood vessels from the plasma leakage caused by thrombin and bacterial wall components, such as LPS [41,42]. Thus, the angiopoietin-1/Tie2 endothelial signaling axis might play a key anti-inflammatory role in

Table 2
Anti-permeability inducing factors (anti-PIFs) and their effects on the endothelial barrier.

Anti-PIFs	Signaling system	Effects on the endothelial barrier
Angiopoietin-1	Tie-2 Rho-GAP mDia	VE-cadherin membrane stabilization Src–Ca^{++} pathway inhibition Acto-myosin contractility
Robo-4	Slit-2	VE-cadherin membrane stabilization Src inhibition
S1P	S1P-R1	Rac/PAK
cAMP-GPCR	Rap	VE-cadherin membrane stabilization
FGF	FGF-R1	VE-cadherin membrane stabilization

cAMP-GPCR: cyclic adenosine monophosphate elevating G protein-coupled receptor; FGF: fibroblast growth factor; GAP: GTPase activating protein; mDia: mammalian diaphanous; PAK: p21-activated kinase; R: receptor; S1P: sphingosine 1 phosphate.

various diseases such as asthma, rheumatoid conditions and septic shock. Several signaling mediators, including calcium signaling or GTPase Activating Proteins (GAPs) for RhoA, have been proposed to impede on the endothelial barrier disruption [42,43], but the exact molecular mechanisms were still unknown. We therefore revisited our findings on phospho-serine-dependent internalization of VE-cadherin and investigated the effects of angiopoietin-1 on the control of endothelial monolayer permeability [5]. Our data indicated that angiopoietin-1 elicits a signaling pathway through Tie2, which can compete for Src activation by VEGF-R2, therefore halting the VEGF signaling to VE-cadherin internalization [44]. Similar Src inhibition has been recently demonstrated to oppose to VEGF-induced vascular permeability in response to Robo-4, a ligand for the endothelial Slit-2 receptor [45]. Interestingly, Tie2 is localized at cell–cell junctions in an intact endothelial monolayer, where it contributes to stabilize VE-cadherin adhesion, in association with the vascular endothelial phospho-tyrosine phosphatase (VE-PTP) [46,47]. Both Tie2 localization and downstream signaling are modified in the absence of endothelial cell–cell contacts, suggesting a regulatory feedback between VE-cadherin adhesion and Tie2 function. This "super" clustering of adhesion and signaling receptors at the contacting zones might then preserve controlled paracellular permeability in quiescent conditions and in response to acute exposure to angiogenic factors.

In addition to angiopoietin-1 action, membrane stability of VE-cadherin is also involved in the protective mechanism exerted by FGF signaling in the vascular maintenance [26]. FGF is a pleiotropic factor playing a protective effect on existing vasculature, primarily thought to be mediated by anti-apoptotic signaling. However, in contrast to VEGF that can activate pro-survival signals as well, FGF is unable to induce vascular leakage and endothelial fenestrations. Indeed, it has been recently shown that inhibition of FGF signaling reduces the interaction between p120 and VE-cadherin, while VE-cadherin internalization is enhanced [26]. Conversely, the VE-cadherin/p120 interaction is critical to prevent from VE-cadherin endocytosis [28], suggesting therefore that FGF signaling might prevent from the lost of p120 binding to VE-cadherin, culminating at VE-cadherin stabilization at the plasma membrane. Finally, cyclic adenosine monophosphate (cAMP)/Epac/Rap1 signaling axis promotes a decreased paracellular permeability in response to cAMP-elevating G protein-coupled receptor (GPCR) agonists, such as prostaglandin E2 and atrial natriuretic peptide (ANP) [12,48,49]. Indeed, Rap1 has been demonstrated to enhance VE-cadherin-mediated adhesion by stabilizing the adhesion complexes at the plasma membrane [12,33].

The sphingosine 1 phosphate (S1P), a bioactive lipid that binds the endothelial S1P receptors, type 1 and 2, alternatively known as Edg1 and Edg2, had emerged as an effective barrier-protective agonist in addition to its pro-angiogenic abilities. Cytoskeletal rearrangement and barrier enhancement through Rac activation have been on the other hand proposed to militate for vascular integrity by S1P signaling [50], while reinforcement of endothelial cell/pericyte interaction through N-cadherin adhesion cannot be excluded in its anti-PIF effects [8].

Thus, protection of VE-cadherin adhesion likely contributes to anti-PIF molecular mechanisms, such as through angiopoietin-1, FGF, and intracellular Rap signaling. Actin rearrangement and cell adhesion collectively cooperate to the control of the endothelial barrier properties. Screening for novel anti-PIFs has recently intensified as this research field has direct implications in the quest for therapeutic drugs designed to target aberrant vascular leakiness, inflammation, and edema.

Despite fantastic progresses in our understanding of the molecular mechanisms regulating VE-cadherin function in the endothelial barrier, the dynamics of VE-cadherin trafficking, including endocytosis and recycling are not fully elucidated yet. Ultimately, elucidating the biochemical route by which VEGF, angiogenic factors and oncogenes modulate VE-cadherin, cell–cell junctions and vascular integrity will help identify new therapeutic targets for the treatment of many human diseases that exhibit aberrant vascular leakage. Although anti-VEGF/VEGF-R drugs have been approved in colon cancer treatment and ocular diseases, they are however not suitable for all patients, and can affect normal vasculature and exhibit tumor recurrence upon therapy withdraw. Hence, it will be crucial in the future to ascertain the molecular basis for the development of novel therapeutic targets designed to promote normalization of the vascular wall and its microenvironment.

Competing interest statement

The author declares no competing financial interests.

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