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Review

Endothelial adherens and tight junctions in vascular homeostasis, inflammation and angiogenesis

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Abstract

Endothelial cells lining the vessel wall are connected by adherens, tight and gap junctions. These junctional complexes are related to those found at epithelial junctions but with notable changes in terms of specific molecules and organization. Endothelial junctional proteins play important roles in tissue integrity but also in vascular permeability, leukocyte extravasation and angiogenesis. In this review, we will focus on specific mechanisms of endothelial tight and adherens junctions.

Keywords: Adherens junction; Tight junction; VE–cadherin; Claudin; Endothelium

Contents

1. Introduction

The endothelium is located at the inner side of all vessel types and is constituted by a monolayer of endothelial cells.

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Interendothelial junctions contain complex junctional structures, namely adherens junctions (AJ), tight junctions (TJ) and gap junctions (GJ), playing pivotal roles in tissue integrity, barrier function and cell–cell communication, respectively. The endothelium constitutes the vascular barrier with regulated permeability properties between the blood and the underlying tissues. Quiescent endothelium may be subjected to stimuli inducing leukocyte extravasation at inflammatory sites and sprouting angiogenesis. Both processes have a strong impact on endothelial cell–cell junctions. In this review, we will focus on endothelial AJ and TJ as well as interendothelial-specific molecules or mechanisms in resting and activated vessels.

1.1. Histology of endothelial junctions

The junctional structures located at the endothelial intercellular cleft are related to those found in epithelia; however, their organization is more variable and in most vascular beds their topology is less restricted than in epithelial cells. AJ, TJ and GJ are often intermingled and form a complex zonular system with variations in depth and thickness of the submembrane plaque associated with the junctional structure [1,2]. As opposed to epithelial cells, GJs are often observed close to the luminal surface. Therefore, the term "apical junction" used to collectively designate epithelial TJ and AJ may not be applied to the endothelium.

Another distinction comes from the difference in cell thickness. With some exceptions, cell body thickness of microvascular endothelium is less than 0.3 μm [2]. Overlapping strands of adjacent endothelial cells form contact domains of 0.5–0.9 μm. However, endothelial cell–cell contacts of some other vessels, including arteries and high endothelial venules, may reach 3–10 μm (Fig. 1) (deduced from [1,2]). Outside of the electron-dense junctional structures, the intercellular cleft is lined by parallel plasma membranes of neighbor cells separated by 10–20 nm.

Finally, endothelial intercellular domains differ from those of epithelial cells by the absence of desmosomes [2]. The intermediate filaments, constituted in the endothelium by vimentin molecules, are poorly linked to cell–cell contacts. However, as opposed to the situation in epithelia, the vimentin filaments may be linked to endothelial AJ in junctional structures similar to desmosomes, called complexus adherens [3–8]. These structures originally described in lymphatic endothelium may have a broader distribution in the vascular tree.

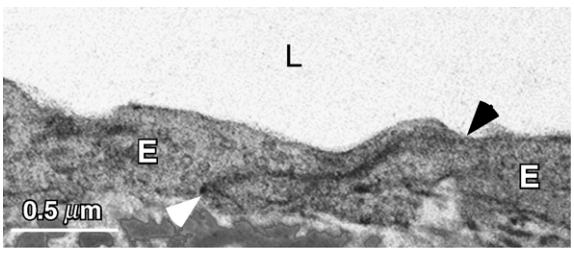

Fig. 1. Electron micrograph showing an endothelial junction in mouse aorta. The intercellular cleft (between arrowheads) is lined by two endothelial (E) strands and extands from basal lamina to lumen (L).

It must be stressed that interendothelial junctions are dynamic structures, subjected to multiple regulations. Furthermore, leukocytes extravasate at inflammatory sites (mostly in post-capillary venules) either through transcellular or paracellular routes. Extravasation through the intercellular junction is a rapid and regulated process, during which the leukocyte is squeezed in the cleft (diapedesis), followed by rapid junction reformation.

1.2. Adhesive proteins located at endothelial cell–cell contacts

A number of proteins exhibiting homophilic adhesive activities are located at interendothelial contacts (Fig. 2). Some of them are specific to endothelial cells (e.g., VE–cadherin [9,10], claudin-5 [11]) while others are common with epithelial cells (e.g., occludin [12], junctional adhesion molecule (JAM)-A [13], nectins [14,15], claudins (see references below) and connexins [16]), blood cells (e.g., PECAM/CD31 [17], endothelial cell-selective adhesion molecule (ESAM) [18,19], JAM-A, -C, CD99 (reviewed in [20])), smooth muscle cells (S-endo-1/CD146 [21]) or mesangial/trophoblast cells (protocadherin (Pcdh)12/VE–cadherin-2 [22]). These proteins may be part of organized junctional structures, such as VE–cadherin in AJ, claudins and occludin in TJ, or connexins in GJ, while others are independent, such as PECAM, CD99, S-endo-1 or Pcdh12. The JAMs are associated with TJ through intracellular components without being directly involved in TJ strand formation.

Interendothelial adhesive proteins are implicated at different levels in endothelial cell–cell interaction and tissue integrity. Some of them have a dual function as they also participate in leukocyte extravasation via homophilic (PECAM, CD99, JAM-A, -C) or heterophilic (JAM-A, -B, -C) interactions (reviewed in [20]).

2. The endothelial adherens junction

2.1. The VE–cadherin-based complex and its physiological role

VE–cadherin is the transmembrane component of endothelial AJ [23]. It is a type II cadherin harboring high adhesive activity [24]. Several biochemical evidences showed that VE–cadherin extracellular domains form hexamers in solution [25,26]. Electron microscopy data support this view and allowed to propose a model in which VE–cadherin dimers interact in trans through their extracellular domain 1 and VE–cadherin trimers interact in cis via their extracellular domain 4 (Fig. 3) [27].

VE–cadherin intracellular domain is similar to other classical cadherins. It contains a proximal binding site for p120 and p0071, and a distal binding site for β-catenin and plakoglobin (Fig. 4, left). Both β-catenin and plakoglobin are linked to α-catenin, which may further interact with α-actinin and vinculin (see [28] and references therein). The identity of the molecular link for the actin filament anchorage to the cadherin–catenin complex is still a question of debate [29]. As previously indicated, the VE–cadherin complex may associate with the vimentin cytoskeleton in some vascular locations [3–8]. This association is mediated by either plakoglobin or p0071, both interacting with desmoplakin, which in turn associates with

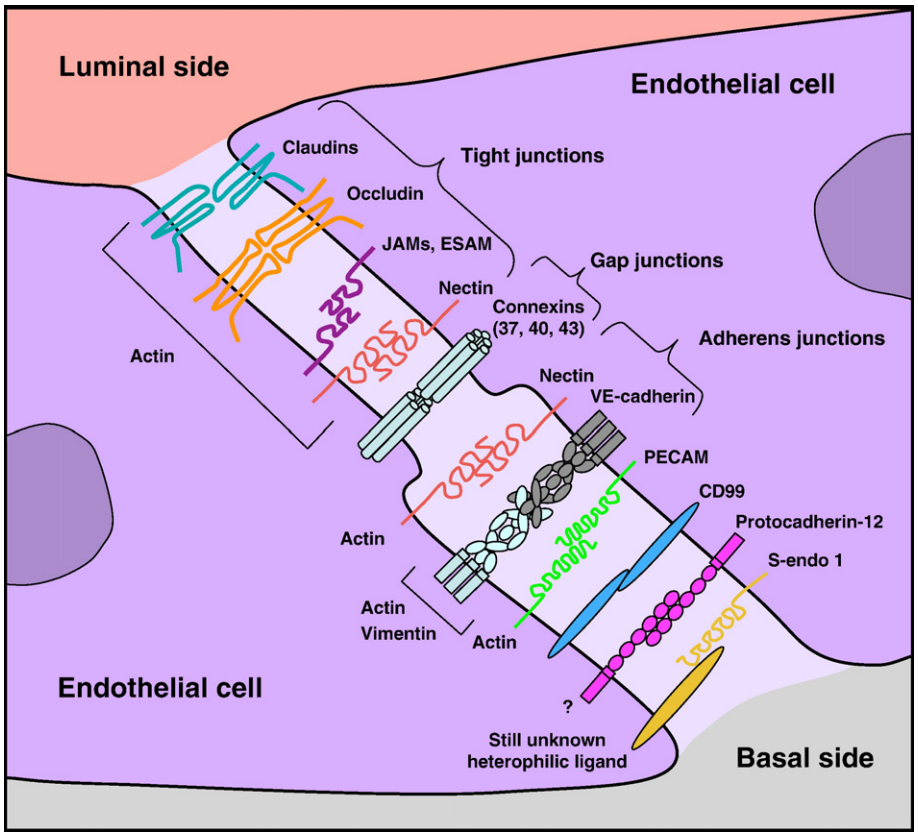

Fig. 2. Adhesive proteins within the interendothelial cleft. Claudins, occluding, JAMs and ESAM are located in TJs, VE–cadherin in Ajs and connexins in gap junctions. Nectin was detected in both Ajs and TJs, while PECAM, Pcdh12, CD99 and S-endo-1 are outside of these structures. TJs and Ajs are both linked to the actin cytoskeleton and VE–cadherin may also be associated with vimentin filaments in some vascular beds.

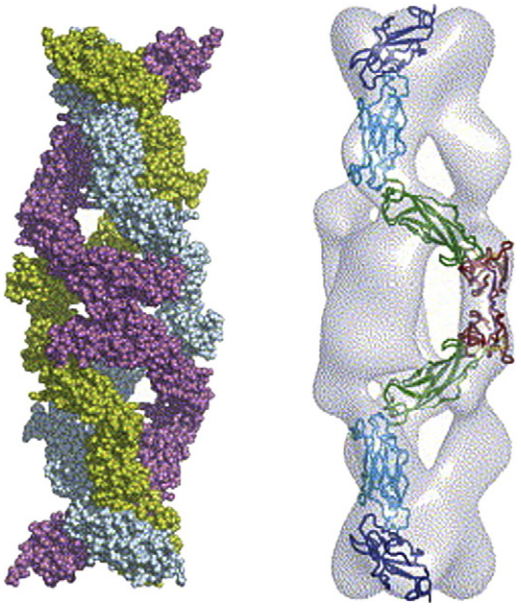

Fig. 3. Model of hexameric assembly of VE–cadherin extracellular domains deduced from biochemical analyses and electron-microscopy. The model includes VE–cadherin ectodomains (EC) 1–4. Left: model with the VE–cadherin dimers represented in violet, light-green and light-blue. Right: the fit of the VE–cadherin model in the reconstructed hexamer. EC1 (red), EC2 (green), EC3 (cyan) and EC4 (blue) are coloured as indicated. Reproduced from [27].

vimentin (Fig. 4, right). The VE–cadherin complex also transiently or permanently associates with signaling partners (see below), and with a specific apical–basal polarity complex, through direct interaction with PAR-3 and PAR-6 [30].

The precise mechanisms of VE–cadherin-based junction formation remain mostly unexplored. However, Delanoë-Ayari et al. [31] proposed a model in which VE–cadherin lateral clustering may be achieved by membrane tension and free VE–cadherin molecule diffusion rather than a cytoskeleton-driven mechanism. Consistent with this hypothesis, VE–cadherin clusters forming artificial AJ were observed by assembly of VE–cadherin extracellular domains at the surface of liposomes [32]. As described below, the first cell–cell contacts may be achieved by nectin interaction, which may facilitate cadherin binding.

VE–cadherin was shown to be specifically located at junctions of all endothelium subtypes [9]. VE–cadherin mRNA were observed at the onset of vasculogenesis in the mouse embryo [10]. Its extraendothelial expression has been observed in case of vascular mimicry (the extravillous cytotrophoblasts replacing the spiral artery endothelium [33] or melanoma cells lining tumor vessels [34]), in circulating endothelial progenitors [35] and in podocytes [36].

The VE–cadherin gene yields a single transcript and its promoter contains functional sites for transcription factors such

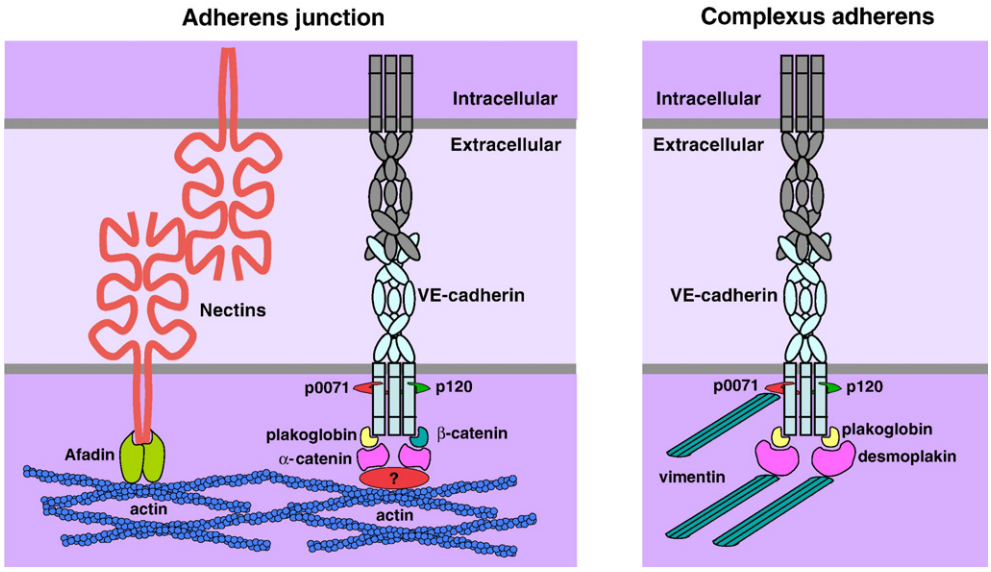

Fig. 4. Adherens junctions and complexus adherens in endothelial cells. In AJs, VE–cadherin directly interacts at a membrane-distal site with α- and β-catenins and at a juxtramembrane site with p120 and p0071. The exact molecular link between the VE–cadherin–catenin complex and the actin filaments is still obscure. The nectins are linked to the actin cytoskeleton via afadin. In complexus adherens, VE–cadherin associates with vimentin filaments via plakoglobin/desmoplakin or p0071.

as Ets and Sp1 family members, as well as Tal [37–40]. Although constitutively expressed by endothelial cells, VE–cadherin expression is enhanced by basic FGF in angiogenic situations [41]. Furthermore, Tal, a bHLH transcription factor dramatically upregulated during angiogenesis, activates VE–cadherin transcription [40].

VE–cadherin adhesive properties are essential for optimal homotypic cell interaction; however, the observation that VE–cadherin-deficient embryos retained electron-dense endothelial junctions indicates that some redundancy exist between the several homophilic adhesive proteins located at the endothelial surface [42]. Furthermore, VE–cadherin-deficient embryos exhibited defective capacities in sprouting angiogenesis resulting in embryonic death at midgestation, suggesting that VE–cadherin has signaling properties required for vascular morphogenesis. The dramatic phenotype of VE–cadherin-deficient embryos was similar to that obtained with partial truncation of VE–cadherin cytoplasmic domain [43]. This feature further points to the implication of VE–cadherin in intracellular signaling.

The contribution of VE–cadherin in neovessel formation has been further demonstrated in adults by experiments using antibodies directed against specific VE–cadherin epitopes, unmasked during angiogenesis, that abrogated angiogenesis and tumor growth in mice [44–46].

Other cadherins, namely N-cadherin [47], T-cadherin [48] and Pcdh12 [22,49], have also been located in endothelial cells. Albeit abundant in endothelium, N-cadherin is not or poorly located at interendothelial junction [47]. Several converging studies indicate that this molecule promotes heterotypic interactions with perivascular cells such as pericytes [50,51]. T-cadherin has been identified in endothelium of human aorta [48]. In vitro studies indicate that T-cadherin may participate to various cellular processes, including migration, proliferation and survival [52–55]. Pcdh12 is located at interendothelial junctions, mostly in angiogenic endothelium, and its function is presently unknown [22,49].

2.2. β-catenin trafficking in endothelial cells

As described for epithelial cells, β-catenin may translocate to the nucleus and activate several genes including cyclin D1 and myc whose products induce entry in the cell cycle [56].

During human embryonic development, β-catenin was detected in endothelial cell nuclei and cytoplasms of capillaries (in particular in the brain), arteries and veins [57–59]. In quiescent endothelial cells of adults, β-catenin is detectable neither in the nucleus nor in the cytoplasm, but appears to be concentrated at cell–cell contacts. Conversely, β-catenin is often observed in the nucleus and cytoplasm of endothelial cells during pathological angiogenesis or vascular remodeling. Consistently, intracellular (nuclear or cytosolic, as opposed to junctional) accumulation of β-catenin correlates with BrDU incorporation, which therefore links the presence of intracellular β-catenin to a proliferative state.

The conditional inactivation of β-catenin in the endothelium leads to morphological alterations of vessels, increased vascular permeability and eventually results in embryonic lethality at midgestation [60,61]. In endothelial cells devoid of β-catenin, plakoglobin solely binds to VE–cadherin. As opposed to β-catenin, plakoglobin may bind to either α-catenin or desmoplakin and vimentin. Therefore, the VE–cadherin complex may interact more strongly with the vimentin cytoskeleton in this context.

Altogether, these features suggest that physiological regulation of endothelial junctions depends on (i) a subtle balance between β-catenin and plakoglobin binding to VE–cadherin and (ii) the proportion between intracellular and junctional β-catenin.

2.3. Regulation of VE–cadherin barrier function

Besides its role in maintaining endothelium integrity, VE–cadherin participates in the control of vascular permeability and leukocyte transmigration, as documented by injection of VE–cadherin function blocking antibodies in the mouse [62–64]. During leukocyte transmigration, VE–cadherin–catenin complexes are pushed aside to facilitate diapedesis [65–67]. An alternative mechanism has been described that implicates the degradation of VE–cadherin molecules by various proteases, as discussed below.

VEGF (vascular endothelial growth factor) is a pleiotropic cytokine relatively specific to endothelial cells or their progenitors inducing vascular permeability, cell survival or proliferation in a context-dependent manner [68]. In confluent endothelial cells, VEGF induces a rapid and transient tyrosine phosphorylation of VE–cadherin as well as β-catenin and plakoglobin [69]. These molecular events are accompanied by increased paracellular permeability and decreased association of the VE–cadherin complex with the cytoskeleton. Furthermore, VEGFR2, the receptor transducing VEGF signals in endothelial cells, interacts with the VE–cadherin complex, probably through β-catenin [43,70]. VE–cadherin tyrosine phosphorylation and association to VEGFR2 was confirmed in vivo in a model of physiological angiogenesis dependent on VEGF [71]. Src tyrosine kinase is constantly associated with VE–cadherin [71]. Chou et al. [72] showed that Src also associates with VEGFR2 and gets activated upon VEGF stimulation. Src inhibitors prevent VEGF-induced VE–cadherin phosphorylation, thereby indicating that Src is an obligatory mediator in this pathway [71]. Furthermore, Src directly phosphorylates VE–cadherin cytoplasmic domain on tyrosine 685 (Fig. 5A) [73]. The functional consequences of Y685 phosphorylation remain elusive, although a direct binding of C-terminal Src kinase (Csk) to phospho-Y685 has been reported (see below) [74]. Other VE–cadherin tyrosine phosphorylation sites (Y658 and Y731) have been described in correlation with increased permeability [75].

Recently, Gavard et al. [76] revealed an additional role of VEGF in VE–cadherin endocytosis and thus junction opening (Fig. 5B). VEGF induces the activation of the exchange factor Vav2 via Src. Vav2 activates Rac, which in turn activates PAK kinase, enabling phosphorylation of VE–cadherin cytoplasmic domain on serine 665. Phospho-S665 VE–cadherin becomes a docking site for β-arrestin-2, the binding of which triggers VE–cadherin endocytosis.

VEGF is not the sole endothelial effector inducing tyrosine phosphorylation of the VE–cadherin complex. Other permeability factors, including histamine, tumor necrosis factor (TNF)-α, platelet-activating factor (PAF) as well as activated neutrophils or integrin engagement phosphorylate VE–cadherin [77–83].

In quiescent vessels, VE–cadherin is in a dephosphorylated state. Several protein tyrosine phosphatases are associated with VE–cadherin: Dep-1 [84], VE-PTP [85], PTP-μ [86,87] and

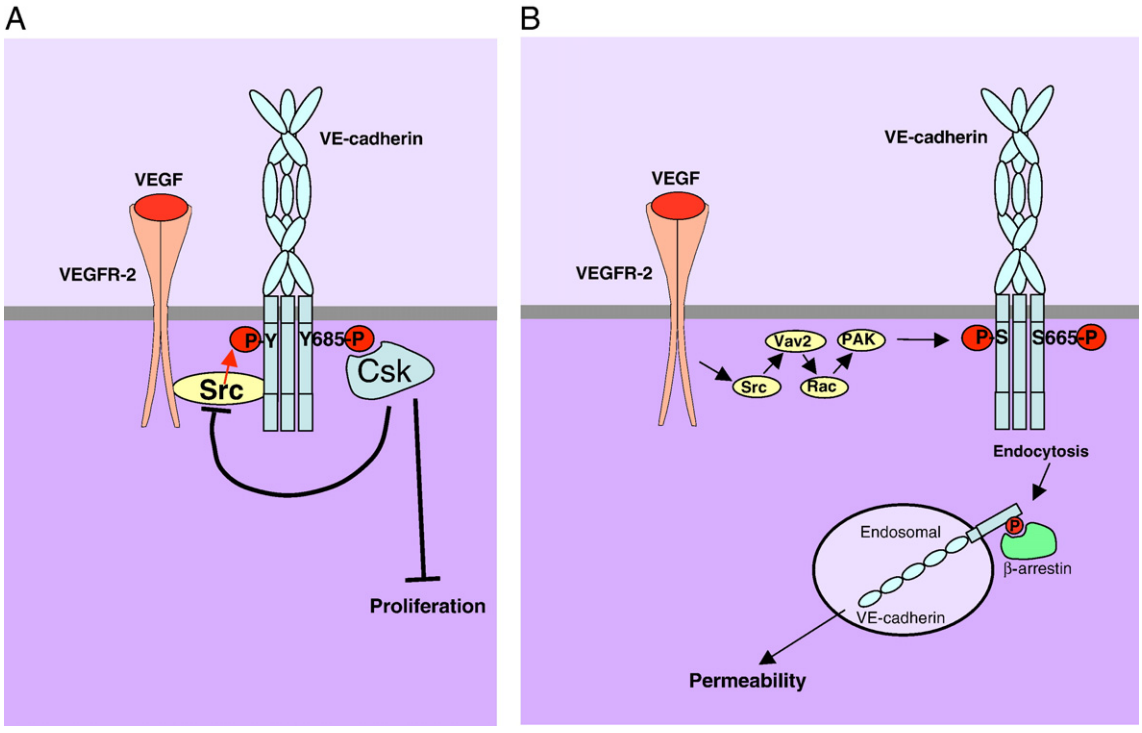

Fig. 5. VEGF-induced VE–cadherin phosphorylation and internalization via Src kinase. (A) VEGF activation induces VEGFR2 dimerization, enabling Src activation, which in turn phosphorylates VE–cadherin on Tyr-685 [71,73]. Csk may associate with VE–cadherin phospho-tyr-685. This interaction was shown to inhibit proliferation in high density cell cultures [74]. Csk-VE–cadherin interaction may potentially inhibit VE–cadherin-associated Src by phosphorylating an inhibitory tyrosine in Src C-terminus domain. (B) Independently, VEGF was shown to induce an activation cascade (Src → Vav2 → Rac → PAK kinase) leading to phosphorylation of VE–cadherin cytoplasmic domain on serine 665. Phospho-S665 VE–cadherin becomes a docking site for β-arrestin-2, the binding of which triggers VE–cadherin endocytosis and degradation, and thus, junction opening [76]. More work is necessary to examine the interplay between both pathways.

SHP-2 [88]. VE-PTP and PTP-μ, two integral membrane proteins, directly interact with VE–cadherin extracellular and intracellular domains, respectively. Both phosphatases were shown to dephosphorylate VE–cadherin after VEGF stimulation, leading to increased barrier function.

VE–cadherin extracellular domain is highly sensitive to proteolysis and a 90-kDa related protein corresponding to its extracellular domain is often detected in tissue extracts [71].

Whereas VE–cadherin cleavage by activated neutrophils has been a question of debate, some evidences indicate that proteases liberated by or at the surface of stimulated neutrophils [89], namely neutrophil elastase and cathepsin G [90] and probably others [91], cleave VE–cadherin, thereby facilitating neutrophil transmigration.

VE–cadherin degradation by matrix metalloproteinase, such as MMP-2, -7 and -9, has been documented in several settings, including apoptosis, diabetes and Dengue virus infection [92–96].

VE–cadherin is also degraded by clathrin-dependent endocytosis and lysosomal targeting by a mechanism involving p120 [97–100]. P120 downregulation in endothelial cells induces a dramatic decrease in barrier function associated with a loss of VE–cadherin protein levels. P120 may thus be an intracellular regulator of VE–cadherin degradation.

In conclusion, several pathways co-exist to reduce VE–cadherin-dependent barrier function: VE–cadherin/catenins complex dissociation from the cytoskeleton, VE–cadherin internalization, junctional movements and VE–cadherin degradation.

2.4. Contact inhibition of cell proliferation as a consequence of junction formation

The essential role of VE–cadherin in contact inhibition was revealed by two in vitro experiments. In a first study, Dejana's group showed that proliferation was inhibited when endothelial cells were seeded on dishes onto which the extracellular VE–cadherin domain had been adsorbed [101]. More recently, the same group reported that VE–cadherin-deficient endothelial cells had lost their contact inhibition and reached increased cell density [84].

Several signaling pathways were identified that may explain the growth inhibition controlled by VE–cadherin.

(1) VE–cadherin might sequester β-catenin at the membrane, thereby preventing transcriptional activation of proliferative genes. Interestingly, a study exploited this property by ectopically expressing the VE–cadherin cytoplasmic domain in tumor cells to successfully block β-catenin transcriptional activity and specifically kill these cells [102].

(2) VE–cadherin binding to VEGFR2 leads to a dramatic decrease in VEGFR2 tyrosine phosphorylation [84]. Consequently, both MAP kinase activation and cellular proliferation are reduced. The protein tyrosine phosphatase Dep1, associated to the VE–cadherin complex, might be responsible for VEGFR2 dephosphorylation (Fig. 6). More recently, the mechanism by which VE–cadherin attenuates VEGF proliferative signal has been further deciphered [103]. Junctional VE–cadherin reduces VEGFR2 internalization after VEGF activation and decreases receptor signaling from endosomal compartments by a Dep1-dependent mechanism. Conversely, in sparse cells, VEGFR2 is internalized faster and its proliferative activity lasts longer.

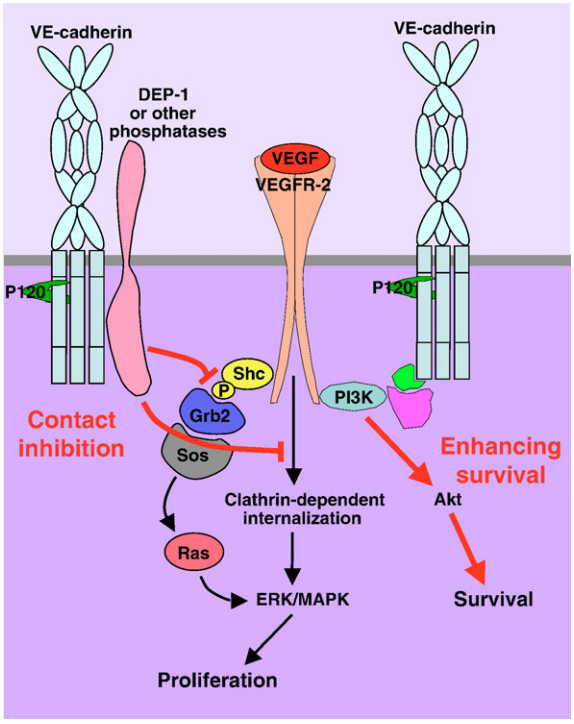

Fig. 6. Participation of VE–cadherin to VEGF signaling. It is clearly established that VE–cadherin has a pivotal role in contact inhibition of cell proliferation. VE–cadherin, through its interaction with Dep-1 or other tyrosine phosphatases inhibits VEGF proliferation signal via MAPK activation in three ways: (i) by reducing VEGFR2 phosphorylation, (ii) by inhibiting VEGFR2-associated Shc phosphorylation and (iii) by decreasing VEGFR2 internalization and signaling from endosomal compartments. VE–cadherin complex interaction with activated VEGFR2 also enhances VEGF survival signaling by PI3kinase/Akt pathway.

(3) VE–cadherin interacts with Shc, a protein known to activate the MAP kinase cascade, after stimulation with VEGF [104]. This binding leads to Shc dephosphorylation probably by junctional VE–cadherin-associated phosphatases. Phospho-Shc is able to couple Grb2-Sos to Ras, which eventually activates the MAP kinases (Fig. 6) [105]. Therefore, junctional docking of Shc and its subsequent dephosphorylation may inhibit VEGF proliferative activity.

(4) The protein tyrosine kinase Csk (C-terminal Src kinase) is a negative regulator of the Src family kinases that inactivates these enzymes by phosphorylation of their inhibitory tyrosine, thereby imposing a locked conformation [106]. Analysis of chimeric embryos containing Csk-deficient and wild type cells revealed defects in angiogenic sprouting and vascular remodeling, a phenotype reminiscent of VE–cadherin-deficient embryos [107]. Csk binds to VE–cadherin cytoplasmic domain when Y685 is phosphorylated (Fig. 5A) [74] and Csk siRNA inhibition in endothelial cells increases proliferation. It is thus likely that VE–cadherin participates in cell contact

inhibition by recruitment of Csk and inhibition of Src activity.

(5) Finally, by culturing endothelial cells on patterned substrates, Nelson and Chen [108] showed that VE–cadherin may inhibit proliferation by actively decreasing cell spreading. By preventing the VE–cadherin-induced change in morphology, the authors revealed that VE–cadherin also elicit a growth signal mediated by actin cytoskeleton tension.

2.5. VE–cadherin implication in VEGF survival signaling and the endothelial hemodynamic force response

Quiescent endothelial cells are resistant to harmful stimuli, such as serum deprivation. Similar to other cadherin members, VE–cadherin protects endothelial cells from serum deprivation-induced apoptosis. Yet, VEGF-induced phosphorylation of PI3 kinase and subsequent activation of the serine-threonine kinase Akt is enhanced by VE–cadherin and its binding to the VEGFR2 complex (Fig. 6) [43].

In addition, VE–cadherin induces Gas1 (growth arrest-specific 1) expression [109]. This protein efficiently protects cells from apoptosis. Interestingly, VE–cadherin-blocking antibodies prevent VEGF-induced stimulation of Gas1 expression, suggesting that junctional assembly of VE–cadherin is required for Gas1 induction.

Therefore, VE–cadherin acts both as a junctional sensor and a molecular switch, directing VEGF signaling towards proliferation or survival.

Endothelial cells are also exposed to and activated by the bloodstream. Hemodynamic forces are variable along the vascular tree and induce a complex response in the endothelium, including the induction or repression of several genes as well as modifications in cell morphology. Junction resistance to flow stress is dependent upon VE–cadherin, and more specifically upon its binding to plakoglobin [110]. Furthermore, loss of VE–cadherin precludes transcriptional activation of a shear stress-responsive promoter. In fact, VEGFR2, VE–cadherin together with PECAM/CD31 form a mecanosensor complex that is sufficient to activate a flow-response to heterologous cells [111,112].

2.6. VE–cadherin and cytoskeleton organization through G protein activation

Cadherins are good candidates as mediator of cytoskeletal organization modifications, such as those observed during epithelio-mesenchymal transition. An increasing number of studies indicate that cadherin engagement induces activation of Rho family GTPases.

Rac activity is associated with endothelial junction strengthening. Yet, its inhibition induces endothelial junction disruption [113]. Rac may act by promoting actin polymerization or by coupling the VE–cadherin complex to the cytoskeleton [114].

A recent study shows that acute hypoxia transiently inhibits Rac1, leading to RhoA activation, actin stress fiber formation, adherens junction opening and eventually increased endothelial permeability. Conversely, re-oxygenation strongly activates Rac1 and restores the cortical location of actin filament by inhibiting RhoA [115].

The endothelium is separated from interstitial collagens by a basal lamina constituted by several proteins, including laminins. Laminin I induces a sustained activation of Rac, correlated with endothelial quiescence, while collagen I, to which sprouting cells are exposed, triggers adherens junction disruption and capillary morphogenesis associated with and dependent of Rho activation [116].

Intriguingly, a recent study reports the activation of Rac1 in VEGF-treated endothelial cells, which transiently prevents VEGF-induced paracellular permeability [117]. A dominant-negative form of Rac1 abolishes the transient barrier effect, whereas the established and sustained effect of VEGF is unmodified.

Numerous reports indicate that Rac activity increases the endothelial barrier. However, this notion was challenged by van Wetering et al. [118], who showed that expression of constitutively active Rac in endothelial cells caused a rapid and ROS (reactive oxygen species)-dependent disruption of cell junctions. The same group further demonstrated that activation of Rac and subsequent production of ROS resulted in β-catenin phosphorylation by the tyrosine kinase Pyk2 [119]. As mentioned above, Rac also participates in VE–cadherin internalization after VEGF stimulation [76].

Altogether, these results show that Rac is involved in several signaling pathways leading to either endothelial barrier reinforcement or increased permeability.

Whether it is clearly demonstrated that Rho and Rac are potent effectors of VE–cadherin adhesive activity, the reverse is also true. Hence, VE–cadherin re-expression in VE–cadherin-deficient endothelial cells increases Rac activity by augmenting the expression of Rac-specific guanosine-exchange factor Tiam1 [120]. Furthermore, VE–cadherin re-expression increases the membrane-associated pools of Tiam1, Rac and its effector PAK (p-21 activated kinase). These properties are lost when VE–cadherin lacks domains interacting with p120 or β-catenin.

VE–cadherin also regulates RhoA, which in turn transmits signals via the actin cytoskeleton to adhesive proteins implicated in cell-matrix attachment [108,121]. As previously mentioned, following cell–cell contacts, this signaling may limit endothelial cell spreading.

Hence, VE–cadherin, by interfering with small G-protein activity, may act on cytoskeleton organization, cell spreading and matrix adhesion.

The GTPase Cdc42 also controls VE–cadherin activity. Indeed, a dominant-negative mutant of Cdc42 dramatically decreases endothelial junction reformation following an inflammatory stimulation like thrombin [122]. The same group further demonstrated that Cdc42 acts on adherens junctions by controlling the binding of α-catenin to the β-catenin/VE–cadherin complex [123]. Remarkably, the cytoplasmic domain of extrajunctional VE–cadherin is capable to induce formation of extended membrane protrusions by a Cdc42-dependent mechanism [124]. Hence, Cdc42 controls VE–cadherin protrusive

activity by an inside-out signaling pathway utilizing actin polymerization. This mechanism, together with the potent heterophilic adhesion of VE–cadherin with fibrin [125], may play a pivotal role in capillary morphogenesis.

Cyclic AMP (cAMP) is a second messenger acting downstream of G-protein-coupled receptors, whose production increases endothelial barrier function [126]. Receptor agonists, such as prostacyclins or prostaglandins, attenuate the hyperpermeability induced by inflammatory stimuli [127]. A recent study shed light on the role of Rap1, a Ras family GTPase, in this process [128]. Elevated levels of intracellular cAMP enable the activation of the exchange factor Epac, which is a specific activator of Rap1. Epac or Rap1 activation decreases permeability by promoting the extension of VE–cadherin-based junctions (Fig. 7) [129]. Another report indicates that the homophilic engagement of VE–cadherin is sufficient to activate Rap1, by a mechanism dependent on the adaptor protein MAGI-I [130]. This protein, probably recruited at the junction by β-catenin, is associated with the exchange factor PDZ-GEF1, which is a direct Rap1 activator (Fig. 7). Hence, first homophilic VE–cadherin contacts may autonomously enhance adherens junction assembly. Both the cAMP-Epac-Rap1 and the MAGI-I-PDZ-GEF1-Rap1 pathways may interfere with VE–cadherin-based junctions by controlling the cortical actin cytoskeleton.

Although the regulation mechanisms linking AJ and the cytoskeleton are probably not fully elucidated, it is possible to conclude that the VE–cadherin complex is an actor of vascular morphogenesis through actin remodeling, as well as an integrator of endothelial stimuli and physiological state.

3. The endothelial tight junction

TJs show considerable variability among different segments of the vascular tree [2]. This disparity constitutes a major evidence of vascular bed differentiation of endothelial cells and has a strong impact on vascular permeability and leukocyte extravasation. Variations concern the complexity degree of the occluding strands as well as TJ composition.

Large artery endothelial cells, which are exposed to high flow rates, display a well-developed system of TJ. Within the microvasculature, TJ are less complex in capillaries than in arterioles, and even less in venules. As previously mentioned, post-capillary venules are the primary site of leukocyte extravasation, and accordingly, they display a high content of permeability mediator receptors, such as those for histamine, serotonin and bradykinin. At the opposite, the blood–brain barrier (BBB) and the blood retinal barrier (BRB) are particularly rich in TJ and endothelial TJs have been mostly studied in these locations (as reviews, see [2,28,131–135]). It is currently admitted that the

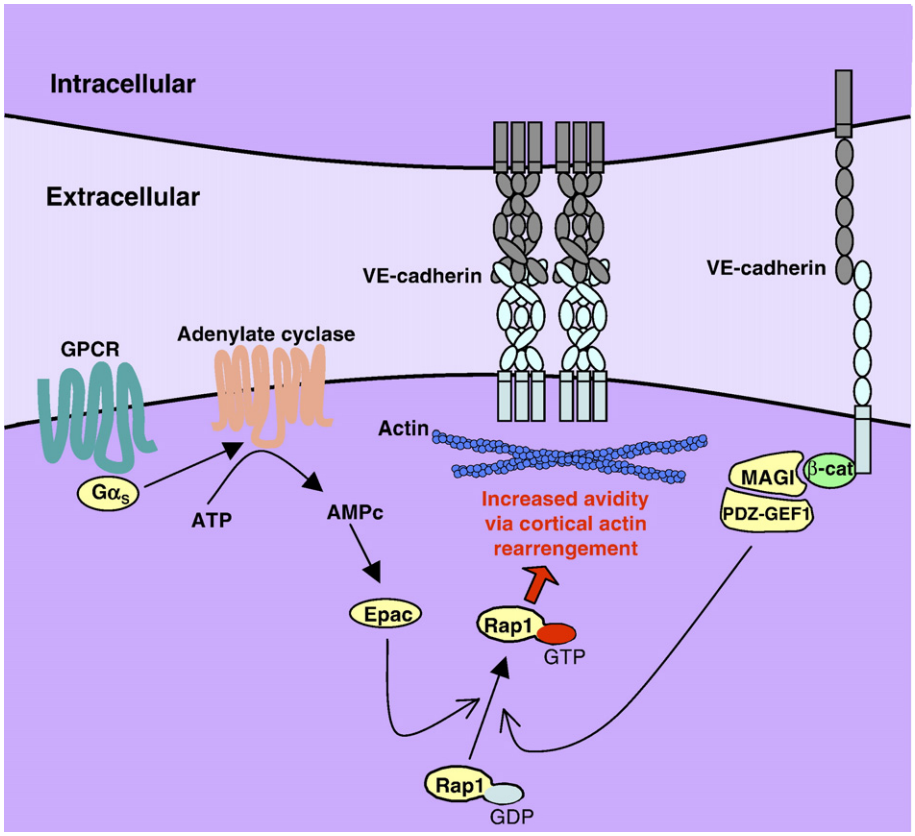

Fig. 7. Rap1 activation pathways and stabilization of the endothelial barrier. Increased levels of intracellular cAMP by G-protein-coupled receptors enable the activation of the exchange factor Epac, which is a specific activator of Rap1, a Ras family GTPase. Rap1 activation promotes AJ extension by increasing the avidity of cortical actin to the VE–cadherin complex. Rap1 may also be activated by the adaptor protein MAGI-I together with the exchange factor PDZ-GEF1, recruited at the VE–cadherin complex. These data are derived from [128–130].

BBB/BRB TJ phenotype is induced by 2 perivascular cell types: the astrocytes through their cellular processes (end foot) and the closely associated pericytes. Both cells produce factors, including angiopoietin-1, TGFβ and probably other uncharacterized molecules, that are required to induce the barrier phenotype [136–138].

As discussed below, some of TJ components are specific to endothelial cells. In addition, the same molecules might be differentially assembled and regulated in endothelial versus epithelial TJs. As previously mentioned, we will concentrate herein on TJ molecules and mechanisms specific to endothelial cells. The general organization of TJs is described in chapters 2–3 and in previous reviews [28,139]. It should be noted that information regarding TJ component properties mostly derives from studies on epithelial cells because of lack of convenient endothelial cell models retaining TJ.

3.1. Claudins, occludin and the submembrane TJ complex

Claudin family members are the major TJ transmembrane constituents, exhibiting homophilic and heterophilic (with other claudin subtypes) adhesive activities through their extracellular domains and forming the TJ strands [135]. As previously mentioned, endothelial TJs specifically and highly express claudin-5, with a few exceptions [140]. Claudin-5-deficient mice have selective impairment in BBB function for molecules smaller than 800 Da [141]. This phenotype suggests a partial redundancy between claudin subtypes. Yet, claudin-3 and -11 have also been characterized in endothelial cells [142–145]. Initial identification of claudin-1 and -12 [141,146] in the endothelium is now largely questioned and was not confirmed. Besides their barrier function, claudins also behave as specific channels and their diversity reflects their differential function in paracellular transport [147]. A number of pathological settings associated with decreased BBB function were correlated with lower claudin expression, thereby indicating that claudins may be therapeutic targets in brain edema lesions [144,146,148–152].

Another transmembrane component of TJ strands is occludin. Although not necessary for TJ strand formation, occludin is associated with increased TJ barrier function. In the endothelium, this molecule is specifically expressed in the BBB and the BRB [12] and its expression is increased with pericyte-derived angiopoietin-1 [136]. Occludin downregulation has been observed in various disease state associated with BBB or BRB disruption, including stroke, diabetes as well as hypoxia/aglycemia [153–158]. Nevertheless, the fact that genetic ablation of occludin does not affect endothelial TJ organization or permeability is intriguing and may indicate differential functions in man and mouse [159].

Decreased occludin contents together with increased paracellular permeability were observed in VEGF-treated retinal endothelial cells. This involves a proteolytic mechanism depending on urokinase plasminogen activator (Fig. 8) [153,160]. Occludin proteolysis by metalloproteinases was also observed after protein tyrosine phosphatase inhibition or monocyte diapedesis [161,162]. These features suggest that occludin degradation may be one of the mechanisms increasing vascular permeability. VEGF activation also leads to occludin phosphorylation on serine/threonine residues through PKC activation in correlation with increased permeability (Fig. 8) [163,164]. Upon VEGF treatment, occludin is phosphorylated on multiple serine and threonine residues, however the identification of the precise phosphorylation sites and their molecular function remain elusive [154]. Other factors, namely lysophosphatidic acid, histamine, oxidized phospholipids, monocyte chemoattractant protein-1 (MCP-1/CCL-2) or shear-stress, induce both occludin phosphorylation on serine/threonine residues and increased permeability (Fig. 8) [165–168]. But paradoxically, angiotensin-2 was shown to increase blood–brain barrier function in correlation with increased threonine phosphorylation of occludin together with its mobilization to lipid rafts (Fig. 8) [169]. Thus, the functional consequences of occludin phosphorylation may be phospho-site-dependent. Stamatovic et al. [168] demonstrated that MCP-1, in addition to occludin, also targets ZO-1, ZO-2 (see below) and claudin-5 phosphorylation on serine/threonine residues by a signaling pathway involving Rho and PKCα. Occludin may also be phosphorylated on tyrosine residues following cerebral ischemia and angiotensin-2 has a negative impact on occludin tyrosine phosphorylation level (Fig. 8) [168,170]. In conclusion, inflammatory or angiogenic mediators alter BBB function in part by acting on occludin integrity, localization or phosphorylation level. Conversely, hydrocortisone treatment, through its anti-inflammatory properties, increased occludin levels and barrier properties of retinal endothelial cells [171].

Claudins and occludin are linked to numerous intracellular partners, including ZO-1, -2 and -3, AF-6/afadin, PAR-3, cingulin and 7H6 antigen, also present in epithelial TJs [28]. These proteins form a molecular complex at the submembrane side of TJs. Detailed properties of these proteins and their molecular interactions are reviewed in other chapters and reviews [149]. Interestingly, two versions of ZO-1 resulting from alternative splicing may be found. An 80 amino-acid domain, called α, may be inserted in the central part of the protein. Whereas the ZO-1α^+ variant is found in most epithelial cells, the ZO-1α^- isoform is only observed in endothelial cells, Sertoli cells and podocytes of kidney glomeruli [172–174]. In general, the ZO-1α^-isoform characterizes more dynamic junctions. Symplekin, another protein located in the TJ complex of some epithelia, is absent in endothelial cells [175].

3.2. The JAMs

JAM-A, -B, and -C constitute a family of transmembrane adhesive proteins belonging to Ig superfamily that colocalize with TJ, although not included in TJ strands [20]. Of note, JAM-B localization at TJ has been challenged [176]. JAM-A is located in epithelial and endothelial intercellular junctions and at the surface of platelets and leukocytes [13,177], while JAM-C expression is restricted to endothelial cells of lymphatic sinuses and high endothelial venules of lymphatic organs, as well as smooth muscle cells, fibroblasts and some blood cells; however its tissue distribution varies between mouse and human

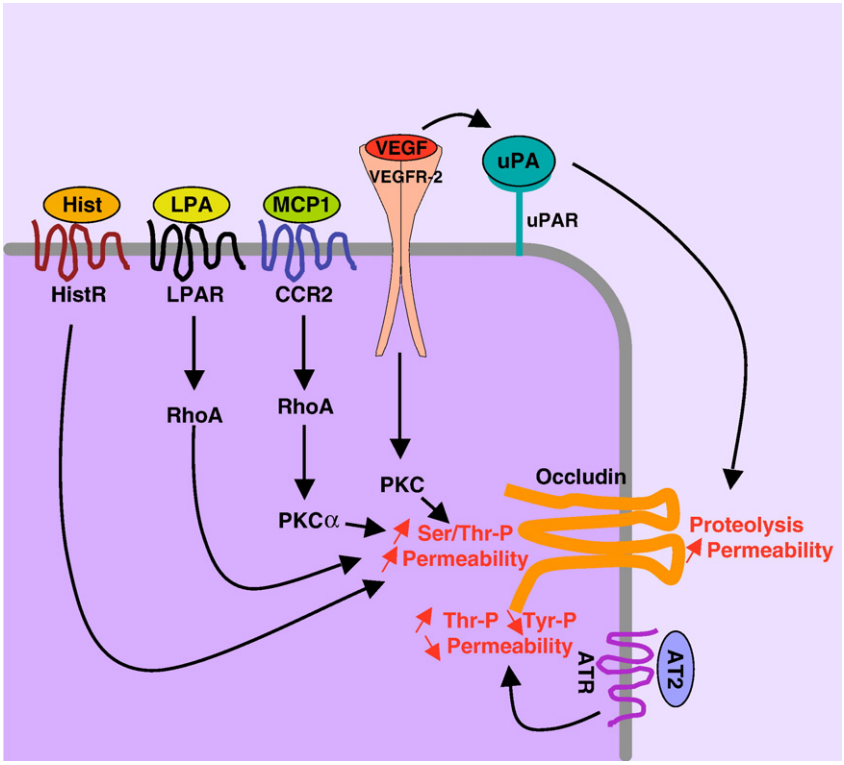

Fig. 8. Cytokines activating signaling pathways leading to occludin functional alterations (phosphorylation or proteolysis). VEGF, MCP-1, lysophosphatidic acid (LPA) and histamine (Hist), by interaction with their cognate receptors, VEGFR2, CCR2, LPAR and HistR, respectively, induce increased occludin phosphorylation on serine/threonine residues, in correlation with increased permeability. Signaling pathways involve PKC activity, PKCα and/or RhoA, as indicated [163,164,167,168]. VEGF also increases permeability by inducing occludin proteolysis through activation of the urokinase (uPA)/uPAR system [153,160]. Conversely, angiotensin-2 (AT2) binding to type 1 angiotensin receptor (ATR) decreases permeability by inducing threonine phosphorylation and tyrosine dephosphorylation of occludin [169].

(see [178] and references therein and [179]). JAM-B is expressed at interendothelial junctions [180] but its tissue distribution is less documented than the two other members.

JAM-A develops homophilic adhesive activity, suggesting that it may mediate endothelial cell–cell interaction. Its barrier function is further demonstrated by use of blocking antibodies or peptides [181,182]. Furthermore, the presence of JAM-A at intercellular junctions reduces paracellular permeability [13]. Intracellularly, it interacts with several members of TJ complex (see [183], for more details), as well as the PAR-3/aPKC/PAR-6 polarity complex, suggesting that JAM-A may be involved in the establishment of apical–basal polarity. Most recent studies on JAM-A are focused on its role in leukocyte transmigration. In inflammatory conditions, JAM-A is redistributed at the apical surface [184,185]. JAM-A, together with PECAM, CD99 [20] and other JAMs (see below), facilitates leukocyte transmigration through the intercellular cleft. During diapedesis, JAM-A binds heterophilically to $\alpha_L\beta_2$ integrin located on leukocytes [186]. The phenotype of JAM-A-deficient mice confirmed its activity in neutrophil transmigration and unveiled other functions including the restriction of dendritic cell trafficking in lymph nodes or its participation in bFGF-induced angiogenesis [187–190].

JAM-C develops homophilic binding at interendothelial junctions, where it may also interact, even with higher affinity, with JAM-B [191,192]. As opposed to JAM-A, junctional localization of JAM-C increases paracellular permeability, possibly by modulating VE–cadherin cell–cell contacts [176,193]. JAM-C promotes neutrophil transmigration via its binding to the leukocyte integrin $\alpha_M\beta_2$ [192], but this activity is lost under shear-stress conditions, suggesting a weak interaction [194]. Two reports indicate that tumor cells also express JAM-C at their surface [195,196]; this feature may promote the metastatic potential of these cells by facilitating their adhesion to endothelial cells via homophilic binding and their subsequent transmigration. JAM-C also regulates tumor cell migration by regulating integrin activity [197]. JAM-C-deficient mice exhibit growth retardation and pneumonia causing poor survival of the mice [198]. Additionally, the number of circulating granulocytes is increased, which seems to be caused by loss of endothelial JAM-C as its rescue in endothelial cells is sufficient to restore homeostasis and better survival.

ESAM is a transmembrane Ig protein related to JAMs [18]. Its expression is restricted to endothelial cell TJs and to the surface of activated platelets. ESAM mediates homophilic binding and its only known cytoplasmic binding partner is MAGI-I [199]. ESAM-deficient mice showed defective tumor angiogenesis, whereas physiological angiogenesis was normal [200]. Endothelial ESAM also participates in neutrophil extravasation by a mechanism involving Rho activation and TJ destabilization [201].

Collectively, these data indicate that JAM/ESAM family members have pivotal and selective functions in leukocyte trafficking and may also influence TJ dynamics.

3.3. Nectins

Nectins form another group of cell–cell adhesion molecules belonging to the Ig superfamily [15,202,203]. Four members have been described, nectin 1–4, as well as five nectin-like molecules, Necl 1–5. Nectin C-terminus interacts with the PDZ domain of afadin/AF-6, an actin filament-binding protein, which connects them to the actin cytoskeleton (Fig. 4). Nectin/afadin were found associated with TJ and AJ proteins. They participate in the initial step of junction formation and play a fundamental role in the establishment of polarity. The nectin-like molecules do not exhibit a PDZ-binding motif and do not interact with afadin.

So far, only nectin-2 and Necl-5 were identified in endothelial cells [14,204]. Nectin-2 is an α-herpes receptor and Necl-5 is the poliovirus receptor [202] and thus both receptors may be involved in viral dissemination. Nectin-2 develops homophilic as well as heterophilic interactions with nectin-3, while Necl-5 does not develop homophilic binding but interacts with nectin-3 [15]. Necl-5 is a potential receptor for DNAM-1/CD226, a transmembrane glycoprotein expressed on a subset of B cells and all T cells, natural killer cells, monocytes and platelets. Necl-5 facilitates monocyte transmigration through endothelial monolayers and may thus participate in inflammatory processes in vivo. Nectin-2, which is also a DNAM-1 ligand in other cell types, does not seem to play a similar role in endothelial cells [14].

4. Future directions

In the recent years, the number of adhesive proteins at interendothelial junctions has stabilized and their contribution to barrier function has been relatively well established. Nevertheless, a comprehensive view of the proteins located on the cytoplasmic side of AJ and TJ is still missing. For example, after years of investigation, the molecular link between the actin cytoskeleton and the AJ remains elusive. The molecular network in both junction types is probably more complex and variable according to location and pathophysiological state than hitherto thought. A number of endothelial adhesive proteins are implicated in leukocyte adhesion and transmigration; their specific and sequential actions in these processes remain to be clarified. Finally, some of these proteins have demonstrated function in angiogenesis or cell migration through mechanisms that need to be fully investigated.

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