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The Multiple Languages of Endothelial Cell-to-Cell Communication

Stefan Liebner, Ugo Cavallaro, Elisabetta Dejana

Abstract—Intercellular adhesion plays a key role during development and maintenance of tissue homeostasis. Within the vascular system, cell–cell adhesion is particularly important for the correct formation, networking, and remodeling of vessels. Although in vascular endothelial cells adhesive junctions account for the integrity of the vessel wall, they are not to be considered as static molecular structures that function as intercellular glue. This becomes evident during the remodeling of the endothelium in various physiological and pathological processes, requiring highly dynamic vascular adhesion complexes. Moreover, it has recently become evident that, besides their structural functions, adhesion molecules involved in endothelial cell–cell interaction play an important role in inducing and integrating intracellular signals that, in turn, impact on several aspects of vascular cell physiology. In this review, we describe these recent findings focusing on junctional proteins at adherens and tight junctions. The role of this adhesion molecule-mediated signaling is discussed in the context of developmental and pathological angiogenesis. (***Arterioscler Thromb Vasc Biol.*** **2006;26:1431-1438.)**

Key Words: adhesion ■ endothelial cells ■ junction proteins

Endothelial cells are maintained in contact to one another by a complex network of transmembrane adhesion proteins anchored to the actin cytoskeleton.[1] Growing evidence indicates that endothelial cell-to-cell adhesion is accompanied by intracellular signaling. It is well known that sparse and confluent cells present a different functional phenotype. Confluent cells have an epithelioid morphology, are contact-inhibited in growth, and are resistant to apoptotic stimuli. Furthermore, their motility and paracellular permeability to plasma solutes and/or inflammatory cells are reduced. Gene profiles of sparse and confluent cells also show that several genes are regulated by cell–cell contacts of which many are implicated in cell growth, apoptosis, matrix, and cytoskeletal remodeling. Thus, junction organization leads to rather dramatic changes in cell function.

The definition of junctional molecules and their cytoplasmic partners responsible for triggering intracellular signals is an issue not yet completely understood.[1–5] In general, signal

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From FIRC Institute of Molecular Oncology (U.C., E.D.) and Department of Biomolecular and Biotechnological Sciences (E.D.), School of Sciences, University of Milan, Milan, Italy; the Mario Negri Institute of Pharmacological Research (E.D.), Milan Italy; and the Institute of Neurology, Medical School (S.L.), University of Frankfurt/Main, Germany.

S.L. and U.C. contributed equally to this review.

Correspondence to Elisabetta Dejana, IFOM, Via Adamello 16, 20139 Milan, Italy. E-mail elisabetta.dejana@ifom-ieo-campus.it

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transduction by junctional components is sustained and directed to establish homeostasis of the cells. This differs from the quick and short-lasting activation induced by growth factors, suggesting that the type of signaling pathways triggered by cell–cell contacts are distinct.

In the past years, a large effort was made by several groups to decipher the molecular organization of intercellular junctions in endothelial cells. Several transmembrane adhesive proteins have been identified which include vascular endothelial (VE) and neural (N) cadherin at adherens junctions (AJs),[6,7] occludin,[8] and members of the claudin family,[9] as well as the junctional adhesion molecule (JAM) family at tight junctions (TJs).[10,11] PECAM-1[12,13] or S-endo-1/Muc 18/CD146[14] are proteins that are not components of the major junctional systems but localize along the intercellular cleft. Although AJs and TJs in the endothelium are comparable to those of epithelial cells, there are some cell-specific features. For example, VE-cadherin, claudin-5, or PECAM-1 have only been found in endothelial but not in epithelial cells. The morphology of the intercellular cleft in the endothelium differs from that of the typical epithelium, because TJs not only are located on the apical side but also are frequently intermingled with AJs.

The molecular organization of endothelial junctions has been described in detail.[1–5,10–13] Therefore, in this article we focus on the type of signals that may be transferred by AJ and TJ structures and on their biological significance.

Signaling Through AJ

AJs are ubiquitous and form rather early during the development of the vascular system. Inhibition of AJ organization causes major defects at early stages of development, as reported for VE-cadherin–deficient and N-cadherin–deficient mice.[7,15] It is accepted that AJs organize before TJs, and considerable evidence indicates that TJs cannot form in absence of AJs. However, the signals transferred by AJs that promote TJ assembly are still largely unknown.

Adhesion at AJs is induced by members of the cadherin family. VE-cadherin and N-cadherin are the major cadherins present in endothelial cells.[1] However, only VE-cadherin is found in the majority of interendothelial AJs, where it is linked through its cytoplasmic tail to a complex network of cytoskeletal and signaling proteins.

VE-Cadherin and PI3K Activation

VE-cadherin may transfer intracellular signals in different ways (Figure 1). We found that the p85 component of the phosphatidyl inositol 3-kinase (PI3K) can associate with the VE-cadherin/catenin complex.[15] PI3K and Akt phosphorylation is activated by VE-cadherin clustering. This may lead to inhibition of endothelial cell apoptosis. Interfering with VE-cadherin expression or function renders endothelial cells more susceptible to pro-apoptotic stimuli. Others found that E-cadherin expression and clustering could trigger PI3K activation in epithelial cells, suggesting that this might be a common feature of cadherins.[16] Because C-terminal truncated cadherins are unable to bind β-catenin and because they lose their ability to activate PI3K, it is suggestive that β-catenin-cadherin interaction is required for PI3K activity.[15]

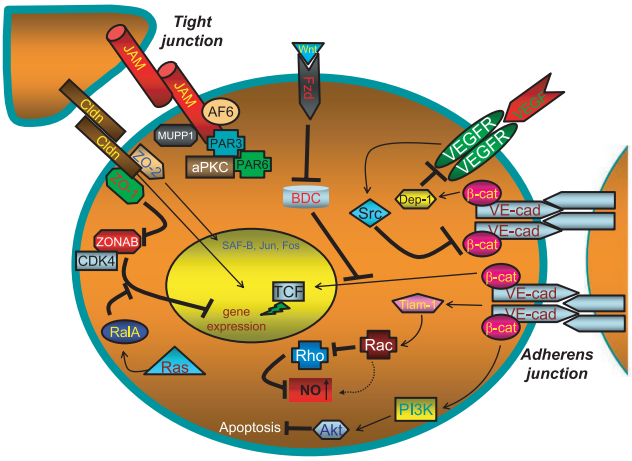

Figure 1. Schematic representation of signals emanating from endothelial cell junctions. Signaling pathways modulated by AJs have been described in endothelial cells, while those controlled by TJs are based on studies on different cell types, because little information is available on vascular cells. The anchorage of junctions to the cytoskeleton has been omitted for simplicity. β-cat, β-catenin; BDC, β-catenin degradation complex; Cldn, claudin; Fzd, frizzled; NO, nitric oxide; TCF, T cell factor; VE-cad, VE-cadherin.

We have also reported that, similar to E-cadherin,[17] VE-cadherin clustering activates the small GTPase Rac and inhibits Rho.[18] This effect is persistent, because Rac activity remains high in confluent cells expressing VE-cadherin, in contrast to VE-cadherin$^{-/-}$ cells. The Rac-specific guanosine exchange factor Tiam-1 is recruited to junctions by VE-cadherin, and the VE-cadherin/Tiam-1/Rac signaling axis facilitates the organization of the cytoskeleton and adhesion plaques in endothelial cells.[18] In contrast, Nelson et al reported that the engagement of VE-cadherin in bovine pulmonary artery endothelial cells induces a transient and sustained activation of RhoA and its effector ROCK, leading to a reduction and induction of focal adhesion (FA), respectively.[19] Additionally, more recent work provided evidence for signaling through VE-cadherin/Tiam-1/Rac, which may indirectly influence the bioavailability of nitric oxide (NO) via the downregulation of active Rho in porcine pulmonary artery endothelial cells. Rho signaling was demonstrated to exert its inhibitory function through the downstream effector ROCK by decreasing the mRNA stability of endothelial NO synthase (eNOS), as well as by decreasing eNOS phosphorylation.[20] Evidence that Rac1 and RhoA may have opposing effects in endothelial cells is further supported by Wojciak-Stothard et al, showing the differential regulation of these Rho GTPases in hypoxia/reoxygenation in pulmonary artery endothelial cells.[21] Interestingly, the authors reported that only a balanced amount of active Rac1 supports VE-cadherin-based junctional stability, whereas overexpression of a dominant active form of Rac1 leads to junction destabilization. Although the signaling of VE-cadherin via the small GTPases Rac and Rho has not been deciphered completely, it becomes evident that a balanced activation/inhibition is required for the quiescent and activated/angiogenic endothelium.

As reported for other cadherins, VE-cadherin clustering also induces short-lasting mitogen-activated protein kinase

(MAPK) activation.[22] It is possible that MAPK activation is induced when cells first touch each other and then declines when confluence is reached and junctions are fully stabilized.

VE-Cadherin Association With VEGF Receptor-2

A novel and accepted paradigm is that adhesion molecules such as integrins and cadherins may associate to growth factor receptors and modulate their intracellular signaling properties.[23] The general consequence of this is that the cellular response to growth factors is dictated by the cell density or by the composition of the extracellular matrix.

In the case of VE-cadherin, it was found that when endothelial cells are activated by VEGF, VEGFR2 associates to the junctional clustered VE-cadherin/catenin complex (Figure 1). Although the molecular basis of this association remains to be characterized, data show that it occurs at the intracellular level and requires the association of β-catenin to VE-cadherin. This is further supported by the finding that in endothelial cells lacking β-catenin or expressing a truncated mutant of VE-cadherin unable to bind β-catenin, VEGFR2 cannot interact with the complex.[24] The association of VEGFR2 to the VE-cadherin/β-catenin complex has multiple consequences. First, in the presence of VE-cadherin tyrosine, phosphorylation of the receptor is markedly reduced and this is accompanied by inhibition of MAPK activation and induction of cell proliferation.[24] VEGFR2 seems to be dephosphorylated by the phosphatase Dep-1, which is present in the complex. However, other phosphatases may also take part in this response. Interestingly, PI3K activation by the same receptor is not affected by VE-cadherin expression. This suggests that dephosphorylation of VEGFR2 occurs in a specific way and likely on specific tyrosine residues. In contrast, when cells are sparse and junctions are disorganized, VEGF receptor effectively signals for proliferation, whereas the anti-apoptotic function of the receptor is strongly reduced.

Overall, this shows that the same receptor on the same cell may respond to the same growth factor in different ways depending on cell density and VE-cadherin clustering.

Second, activation of VEGF receptor may cause tyrosine phosphorylation of VE-cadherin, thus reducing the strength of its association to β-catenin and the actin cytoskeleton. This, in turn, may account for the increase in permeability observed on VEGF activation of endothelial cells.[25] Phosphorylation of VE-cadherin may be mediated by kinases of the src family, which are activated by VEGFR2.[26,27] Other kinases and phosphatases have been found to be constitutively associated to VE-cadherin. VE-PTP, for instance, has been implicated in maintaining vascular permeability, likely by dephosphorylating VE-cadherin.[28] Csk, which is an inhibitor of src, was found to be important for contact inhibition of cell growth. The downregulation of Csk in endothelial cells induces a significant increase in proliferation. Csk binding to VE-cadherin requires phosphorylation of tyrosine 685, and the mutation of this tyrosine inhibits Csk association to VE-cadherin, partially blocking its inhibitory activity on cell growth.[29]

Finally, it was found that VE-cadherin contributes to the endothelial cell response to shear stress. From a mechanosensory complex, formed by VE-cadherin, which functions as an adaptor, PECAM-1, which directly transmits mechanical forces and VEGFR2, which activates phosphatidylinositol-3-OH kinase, early responsiveness to flow is conferred.[30]

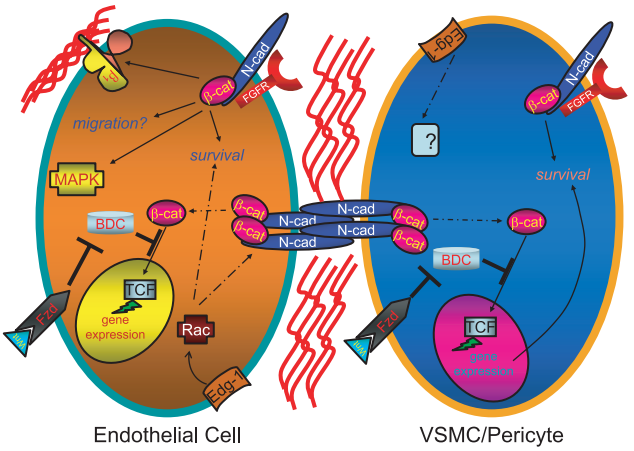

Figure 2. Schematic representation of signals modulated by junctional molecules in endothelial cells and pericytes. The signaling properties of N-cadherin and Edg-1/S1P$_1$, emerging from recent studies on ECs and VSMC/pericytes, are summarized, emphasizing the cross-talk with growth factor receptors and integrins. The anchorage of adhesion molecules to the cytoskeleton has been omitted for simplicity. β1, β1 integrin; Edg-1, endothelial differentiation gene 1; FGFR, FGF receptor; N-cad, N-cadherin.

N-Cadherin and Growth Factor Receptor Interaction

N-cadherin is expressed by endothelial cells at rather high levels, often comparable to those of VE-cadherin. Nevertheless, the function of endothelial N-cadherin long remained elusive. Unlike VE-cadherin, N-cadherin does not appear to be implicated in endothelial cell–cell junctions, at least in mature vessels.[2] However, recent data show that endothelial specific deletion of N-cadherin in mice leads to a decrease in VE-cadherin expression and a severe vascular phenotype, which resemble that of VE-cadherin$^{-/-}$ embryos.[7] The mechanism through which N-cadherin regulates VE-cadherin expression is unknown. Work on N-cadherin–deficient ECs in vitro indicates that N-cadherin mediates the heterotypic adhesion between endothelial cells and pericytes, a crucial event during vessel maturation and stabilization.[7,31] These heterotypic junctions are organized in so-called peg-socket contacts where the basement membrane is missing. N-cadherin becomes targeted to heterotypic junction in ECs through the activation of the G-protein–coupled receptor Edg-1/S1P$_1$ (endothelial differentiation gene), which is a receptor for platelet-derived sphingosine-1-phosphate (S1P), inducing Rac activation and microtubule polymerization.[32] Although Edg-1/S1P1 is present also on vascular smooth muscle cells (VSMCs) and pericytes, the phenotype of endothelial specific gene deletion suggests that Edg-1/S1P1 exerts its major function in ECs (Figure 2).[33]

The signaling cascades modulated by N-cadherin at the endothelial–pericyte interface, however, remain largely obscure. It is noteworthy that during chick development, N-cadherin associates exclusively with β-catenin and not with γ-catenin/plakoglobin, at the endothelial-pericytic junction, speaking in favor of a specific involvement of β-catenin

in the downstream signaling events.[34] At least in VSMCs it was demonstrated that β-catenin plays a role for cell proliferation and survival through the canonical Wnt pathway.[35]

Furthermore, the cross-talk between N-cadherin and the fibroblast growth factor receptor (FGFR) has been documented in various cell types, and the effect of this interaction seems to depend on the cellular context. For example, in pancreatic beta tumor cells, a ternary complex, which includes NCAM, N-cadherin, and FGFR, modulates the activity of β1 integrin and, hence, cell–matrix adhesion.[36] Based on these observations, it seems plausible that N-cadherin may interact with growth factor receptors also in vascular cells. Together with the recent observation that N-cadherin controls the level of β1 integrin in endothelial cells,[7] this raises the intriguing hypothesis that the integrated N-cadherin/FGFR signaling is implicated in β1-mediated endothelial cell adhesion. Interestingly, N-cadherin supports the motility and the metastatic potential of breast cancer cells by binding to the FGFR, preventing its internalization. This results in a sustained cell response to FGF, leading to the activation of the MAPK pathway and the induction of matrix metalloproteinase (MMP)-9 expression.[37] Moreover, a functional association between N-cadherin and FGFR has long been proposed in neurons, where it would contribute to neurite outgrowth,[38] a process that share several molecular and cellular features with the endothelial cell sprouting in the early phases of angiogenesis. Thus, future studies should be devoted to the understanding whether N-cadherin/FGFR cross-talk is implicated in endothelial cell motility and in neovascularization (Figure 2). Recent observations support the hypothesis that N-cadherin–mediated modulation of FGFR activity is involved in endothelial cell survival (Figure 2). A peptide interfering with N-cadherin's adhesive function in endothelial cells inhibits FGFR signaling, thus resulting in apoptosis.[39] This anti-apoptotic effect of the N-cadherin/FGFR complex would not be restricted to endothelial cells, as it has been reported in other cell types.[23] It remains to be clarified whether the stimulation of FGFR downstream of the N-cadherin–mediated adhesion between pericytes and endothelial cells supports vascular cell survival.

Another open issue is how N-cadherin homophilic interactions affect the N-cadherin/FGFR signaling axis. Although the protein motif involved in N-cadherin homophilic binding is distinct from the one mediating FGFR activation, experimental evidence indicates that N-cadherin–dependent cell–cell adhesion influences FGFR signaling.[39–42] However, whether this interplay between the 2 systems is dictated by spatial organization (ie, the recruitment of FGFR to N-cadherin-containing junctions) or relies on specific signal transduction remains to be elucidated.

Signaling Through β-Catenin

In AJs, cadherins are constitutively bound to β-catenin, γ-catenin/plakoglobin, and p120. β-catenin (and potentially also γ-catenin/plakoglobin) is a crucial member of the canonical Wnt signaling pathway, in which it acts by modulating the transcriptional activity of lymphoid enhancer factor-1 (Lef-1)/T-cell factor (TCF) in the nucleus (Figure 1). The canonical Wnt pathway is induced by Wnt growth factor-mediated activation of the designated frizzled receptors (Fzd), which trigger a cascade of events through the activation of dishevelled (Dvl), leading to the inhibition of the β-catenin degradation complex (BDC) formed by casein kinase 1 (CK1), glycogen synthase kinase 3β (GSK-3β), axin (Axn), and adenomatous poliposis coli (activated protein C [APC]). Stabilized β-catenin translocates to the nucleus, directly displacing the transcriptional repressors Groucho/TLE from their binding to Lef-1/TCF, resulting in activation of target gene transcription.[43] Concomitantly, under certain conditions, the armadillo protein p120^{ctn} also translocates to the nucleus and accomplishes the release of the repressor *Kaiso* from Lef-1/TCF, which also leads to an increase in target gene transcription.[44]

In endothelial cells, β-catenin is mainly known as a structural component of AJs, and only little information is available on its function in signaling. However, the lethal phenotype of the endothelial-specific deletion of the β-catenin gene in mice, and the phenotype of APC mutants in zebrafish provided evidence that β-catenin–dependent signaling participates in the transformation of endothelial into mesenchymal cells during heart valve development.[45–47] In cultured endothelial cells, stabilization of β-catenin induces capillary formation, likely caused by increased phosphorylation of VEGFR-2 and activation of Akt.[48] Canonical Wnt signaling in human umbilical vein endothelial cells (HUVECs) induces proliferation, survival, and the expression of interleukin (IL)-8.[49] Interestingly, this effect was enhanced by FGF-2. Previous reports on mammary tumor cells argue in the same direction, namely that FGF and Wnt/β-catenin signaling may cooperate.[50] The interaction and cooperation may further be relevant for the signaling downstream of the N-cadherin/FGFR complex at the heterologous junction between ECs and pericytes as discussed in the previous section.

The importance of the Wnt signaling pathway through β-catenin in the vasculature is supported by the analysis of several Wnt-signaling relevant genes mutated in humans and/or knocked out in mouse models, such as FZD4, FZD5, Norrin, LRP5, Wnt-2, -4, and -7.[51] The underlying mechanisms and specific action of one or the other Wnt pathway component at the specific site of the vascular tree are not yet completely understood.

Tight Junction Proteins in Signal Transduction

Signaling of TJ-related proteins was mainly described in nonendothelial cells, because of the lack of suitable in vitro systems of TJ-bearing ECs. However, the available results open new concepts for signaling events downstream of TJs in ECs (Figure 1).

Zonula occluden-1 (ZO-1), together with the homologous proteins ZO-2/-3, are members of the membrane-associated guanylate kinase homologues (MAGUKs), which exhibit a PDZ-binding domain in the C-terminus and a src homology region 3 (SH3). PDZ domains are known to mediate the anchorage of transmembrane proteins to the cortical actin cytoskeleton. ZO-1 exists in 2 splicing variants, characterized

by the presence of the 80-amino acid α domain. The α^+ isoform is present in epithelial cells, whereas the α^- isoform is restricted to ECs and Sertoli cells.[52] Interestingly, ZO-1 localizes to the nucleus in sparse or migrating cells when TJs are not or only poorly developed.[53] The loss-of-function mutation of the *Drosophila* MAGUK family member discs-large-1 (dlg) leads to an overgrowth phenotype, suggesting that these proteins are involved in the downstream signaling from cell-to-cell junctions and may regulate contact inhibition of growth.[54] Interestingly, ZO-1 was recently demonstrated to interact with N-cadherin and to participate in the regulation of invasiveness in human melanoma cells.[55] Previously, in cell lines devoid of TJs and during the assembly of TJs, the interaction of ZO-1 with the adherens junction system could be demonstrated.[56] At AJs, ZO-1 functions as a cross-linker between α-catenin and the actin cytoskeleton during the establishment of epithelial cell polarity and interacts at this site with β- and γ-catenin.[57] In this context, it should be mentioned that also the product of the Armadillo-repeat gene deleted in Velo-cardio-facial syndrome (ARVCF) was demonstrated to be a binding partner of ZO-1 and ZO-2, which in turn differentially regulated ARCF localization to the membrane and the nucleus, respectively.[58] Furthermore, ZO-2 was shown to translocate to the nucleus, and to interact with scaffold attachment factor-B (SAF-B),[59] Jun, Fos, and C/EBP.[60] These data support the hypothesis that ZO-2, in cooperation with other transcription factors, is involved in signaling events that confer junctional plasticity.

Also, the ZO-1-associated nucleic acid binding protein (ZONAB), which belongs to the Y-box transcription factors, associates with ZO-1 and with the cell division kinase 4 (cdk4), participating in the regulation of cell proliferation.[61] Recently, ZONAB was shown to directly interact with the Ras family member RalA when cells become confluent, leading to de-repression of target gene promoters normally repressed by ZONAB in nonconfluent cells.[62] It was suggested that also the interaction of ZONAB with ZO-1 decreases the transcriptional repression by, and the nuclear localization of, ZONAB, which might be analogous to the membrane recruitment of ARCF by ZO-1. Interestingly, oncogenic Ras alleviates transcriptional repression by ZONAB,[62] underlining the concept of multilateral crosstalk of TJ proteins with other signaling pathways.

Beside the direct participation of MAGUK proteins in nuclear signaling, at least ZO-1 could be demonstrated to interact with the $G\alpha_O$ subunit of heterotrimeric G-proteins.[63] Expression of constitutively activated Ga_O leads to an increased transepithelial resistance (TER) of the cell monolayer and accelerates TJ biogenesis.[64] Additionally, TJ formation in epithelial cells is regulated by the small GTPases Rho and Rac, because Rho inhibition results in TER disruption.[65] In contrast, in brain ECs the activation rather than the inhibition of Rho by lysophosphatidic acid leads to decreased TER,[66,67] supporting a different mode of TJ regulation in epithelial and endothelial cells.

In the vascular system, the TJ transmembrane proteins occludin, claudin-1/-3/-12, and the EC-specific claudin-5 were shown to be expressed.[9] The formation of TJs does not seem to be an intrinsic program of ECs as it is for epithelia, given that ECs of the brain rapidly lose TJs in vitro, whereas epithelial cells retain the morphological and physiological properties of a barrier tissue.[68] Claudin-1 (Cldn1) was found to frequently localize to the nucleus of metastatic colon carcinoma cells and to promote the metastatic phenotype,[69] most likely through the interaction and the positive coregulation of the β-catenin/Lef/TCF pathway, which is often constitutively activated in colon carcinoma.[70]

Other TJ components in endothelial cells are the members of the JAM family. The family is formed by at least 5 members: JAM-A (also called F11R or JAM-1), JAM-B, JAM-C, JAM-4, and JAM-L. Another protein highly related was named ESAM and presents many similarities with the JAM group.[2,10,71] JAM-A, JAM-B, and ESAM were found to be concentrated at endothelial TJs, although they may also be found along the intercellular cleft. JAM-A (but also JAM-B and JAM-C) presents at its C-terminus a consensus binding sequence for type II PDZ domains. It was demonstrated that JAM-A is rather promiscuous and interacts with several PDZ containing partners such as ZO-1, AF6/afadin, partitioning defective (PAR) 3/6/atypical PKC complex, CASK/lin 2, and MUPP-1.[2,10,71] Some of these interactions are likely to be important for the anchorage of the protein to the actin cytoskeleton. Additionally, AF6 is a target for the small GTPases Ras and Rap-1. Rap-1 may modulate endothelial permeability by regulating cadherin adhesive strength.[72] This suggests that JAM-A may participate in the organization and function of AJs.

Several observations on different cell types suggest that one of the major functions of JAMs is the establishment and maintenance of cell polarity. The orthologue of the JAM-A partner MUPP-1 in *D. melanogaster* binds to the integral membrane proteins CRB1 and CRB3 and regulates cell polarization.[73] JAM-A (but also of JAM-C and likely JAM-B) binds the PAR3/PAR6 complex, which can associate to the lambda and theta isoforms of atypical protein kinase C (aPKC) and the small GTPase cdc42. This complex plays a central role in establishing cell polarity in *C elegans* and TJ organization in mammalian cells.[74,75] Angiogenesis also involves modulation of cell polarity. It was shown that JAM-C is required for tumor angiogenesis and hypoxia-induced retinal neovascularization.[76] The effect of JAMs on cell polarization can be observed also at the single cell level. JAM-$C^{-/-}$ mice exhibit a dramatic defect in spermatid polarization during egg fertilization.[77] Neutrophils lacking JAM-A are defective in their polarized movement and tissue infiltration under inflammatory conditions.[78] Abnormal polarized motility was also reported in JAM-A–deficient dendritic or endothelial cells, although in this case the final result was an increased random motility.[79,80]

Another important function of endothelial JAMs is the control of leukocyte migration through endothelial junctions.[10,71,81] Gene inactivation or the use of blocking antibodies inhibited infiltration of leukocytes in several models of inflammation, and ischemia reperfusion injury.[78,82] It is conceivable that the passage of leukocytes and their interaction

with junctional proteins, in particular JAMs, may transfer intracellular signals, which may influence endothelial cell responses to inflammation such as permeability or expression of chemokines or leukocyte adhesion molecules.

In conclusion, the complex molecular organization and function of endothelial TJs have been partially characterized. This is of particular interest for the vessels in the central nervous system (CNS), which develop extensive TJs and exhibit blood–brain barrier (BBB) characteristics. The BBB is of crucial importance for neuronal function, indicated by its disturbance in most brain pathologies, such as tumors, inflammatory responses in the CNS, stroke, and Alzheimer disease.[83]

Conclusion

As discussed, several proteins participate in endothelial cell-to-cell adhesion and transfer a complex array of intracellular signals. Signaling through intercellular contacts controls cell growth and apoptosis. In addition, intercellular junction proteins dynamically regulate vascular permeability to solutes and circulating cells. Some signals may be important for a sustained control of endothelial cell stability, whereas others, more rapid and short-lasting, may control permeability or leukocyte diapedesis. Another emerging concept is that, besides direct signaling, junction proteins may modulate cell function by interacting with growth factor receptors. In some cases this interaction requires cell confluence and results in impairment of downstream proliferative signaling of the receptors. In this way, once the cells get in contact with each other and junctional proteins cluster at the designated junctions, the response to growth factors declines. The increasing interest and effort in deciphering endothelial junction organization would not only help to understand how the endothelium "senses" its environment but also may open new possibilities for modulating angiogenesis and inflammatory responses.

Acknowledgments

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