ENDOTHELIAL CELL–CELL JUNCTIONS: HAPPY TOGETHER

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Junctional structures maintain the integrity of the endothelium. Recent studies have shown that, as well as promoting cell–cell adhesion, junctions might transfer intracellular signals that regulate contact-induced inhibition of cell growth, apoptosis, gene expression and new vessel formation. Moreover, modifications of the molecular organization and intracellular signalling of junctional proteins might have complex effects on vascular homeostasis.

THROMBOTIC REACTIONS
The reactions that lead to blood coagulation in the vascular lumen. Endothelial cell damage causes platelet deposition and aggregation, activation of the coagulation system and thrombin generation.

ANGIOGENESIS
The process of forming new vessels by sprouting from pre-existing ones.

DIAPEDESIS
The crossing of endothelial borders by leukocytes, which squeeze between adjacent endothelial cells.

ADHERENS JUNCTION
A cell–cell adhesion complex that contains cadherins and catenins that are attached to cytoplasmic actin filaments.

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Endothelial cells are one of the main cellular constituents of blood vessels, and one of their most important properties is to separate blood from underlying tissues. These cells function as gatekeepers, controlling the infiltration of blood proteins and cells into the vessel wall. This unique characteristic is achieved through specialized transcellular systems of transport vesicles and by the coordinated opening and closure of cell–cell junctions[1,2]. The specialized transcellular vesicle systems include endothelial cell organelles that are known as vesiculo-vacuolar organelles, which participate in the regulated transendothelial passage of soluble macromolecules. These systems must be tightly regulated to maintain endothelial integrity and to protect the vessels from any uncontrolled increase in permeability, inflammation or THROMBOTIC REACTIONS.

However, an important and new concept is that cell–cell junctions are not only sites of attachment between endothelial cells; they can also function as signalling structures that communicate cell position, limit growth and apoptosis, and regulate vascular homeostasis in general. Therefore, any change in junctional organization might have complex consequences, which could compromise endothelial reactions with blood elements or modify the normal architecture of the vessel wall.

Junctional complexes trigger intracellular signalling in different ways. They can do it directly, by engaging signalling proteins or growth-factor receptors, or indirectly, by tethering and retaining transcription factors at the cell membrane, thereby limiting their nuclear translocation[3–6].

In this review, I describe recent studies on the molecular organization of endothelial junctions and their signalling properties. Although structural and functional similarities of endothelial and epithelial junctions are discussed, the focus is on the role of these structures in endothelial-specific functions, such as ANGIOGENESIS, the control of permeability, leukocyte DIAPEDESIS and the response to blood flow.

The organization of endothelial junctions

In contrast to many types of epithelial cell, endothelial cells have less rigidly organized junctions. Electron-microscope images show that interendothelial cell–cell contacts are frequently complex and that there is a significant amount of overlap between the cells (FIG. 1).

Types of junction. Similar to epithelial cells, endothelial cells have specialized junctional regions that are comparable to ADHERENS JUNCTIONS (AJs) and TIGHT JUNCTIONS (TJs). However, whereas in most epithelia TJs are concentrated at the more apical side of the intercellular cleft, in the endothelium TJs are frequently intermingled with AJs all the way along the cleft[7]. Furthermore, in contrast to epithelial cells, endothelial cells lack DESMOSOMES[8]. However, certain types of endothelial cells — such as those of the lymphatic system or veins — have desmosomal-like structures that are called complexus adhaerentes, which contain some of the same components as epithelial desmosomes, such as plakoglobin and desmoplakin that are associated with vascular endothelial CADHERIN (VE-cadherin)[9]. The reason

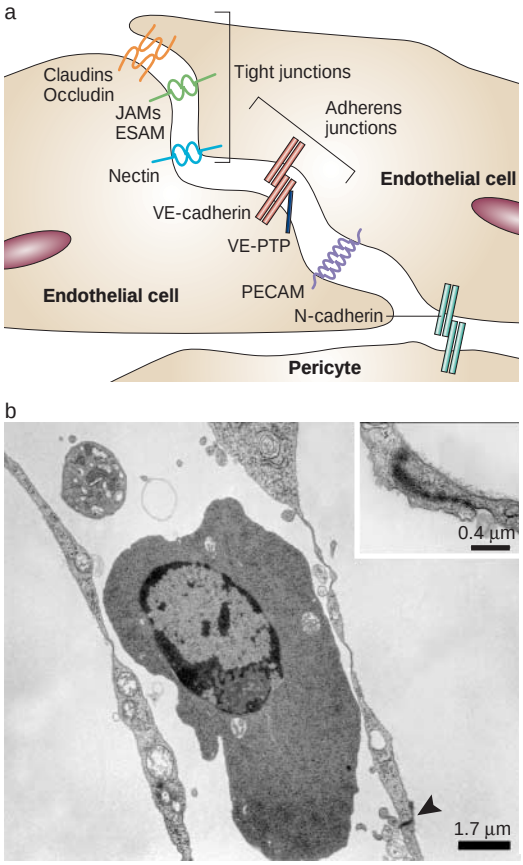


Figure 1 | **The organization of endothelial cell–cell junctions. a** | Transmembrane adhesive proteins at endothelial junctions. At tight junctions, adhesion is mediated by claudins, occludin, members of the junctional adhesion molecule (JAM) family and endothelial cell selective adhesion molecule (ESAM). At adherens junctions, adhesion is mostly promoted by vascular endothelial cadherin (VE-cadherin), which, through its extracellular domain, is associated with vascular endothelial protein tyrosine phosphatase (VE-PTP)[106]. Nectin participates in the organization of both tight junctions and adherens junctions. Outside these junctional structures, platelet endothelial cell adhesion molecule (PECAM) contributes to endothelial cell–cell adhesion. In endothelial cells, neuronal cadherin (N-cadherin) is not concentrated at adherens junctions, but instead probably induces the adhesion of endothelial cells to pericytes and smooth muscle cells. For more detail, see BOX 1. **b** | A transmission-electron-microscopy image of a blood vessel containing a monocyte. The arrow indicates an endothelial junction. The junctional zone frequently appears complex and cells partially overlap (inset). The image in part **b** was provided courtesy of S. Liebner, FIRC Institute of Molecular Oncology, Milan, Italy.

TIGHT JUNCTION
A circumferential ring at the apex of epithelial cells that seals adjacent cells to one another. Tight junctions regulate solute and ion flux between adjacent epithelial cells.

DESMOSOME
A junctional structure that is formed by transmembrane proteins that are homologous to cadherins and are called desmocollins and desmogleins. These are linked to plakoglobin and desmoplakin and are anchored to intermediate filaments.

CADHERIN
A cell-type-specific calcium-dependent transmembrane adhesion protein. Cadherins promote homophilic binding and are preferentially located at adherens junctions.

CATENIN
A cytoplasmic protein that is directly or indirectly linked to the cytoplasmic tail of cadherins. In this complex, catenins promote the anchoring of cadherins to actin and junction stabilization.

PDZ DOMAIN
(Postsynaptic-density protein of 95 kDa, Discs large and Zona occludens-1). A region that is present in several scaffolding proteins and is named after the founding members of this protein family. PDZ domains bind to specific short amino-acid sequences that are found in several proteins at or outside junctions.

VASCULAR TREE
The complete vascular system, which includes the arteries, veins, capillaries and lymphatic system.

IMMUNOGLOBULIN FAMILY
A large family of proteins that includes antibodies and adhesive transmembrane proteins. Their structure is characterized by 'immunoglobulin loops' that are formed by disulphide bonds.

why these structures are present in the lymphatic system is not yet clear, but it is possible that they respond better than AJs and TJs to the need for the dynamic passage of fluids and cells.

TJs and AJs are formed by different molecules, but they have common features (BOX 1; FIG. 1). In both types of junction, regardless of the cell type, adhesion is mediated by transmembrane proteins that promote homophilic interactions and form a pericellular zipper-like structure along the cell border[10–17]. Endothelial cells express cell-type-specific transmembrane adhesion proteins, such as VE-cadherin at AJs[18] and claudin-5 at TJs[19]. The restricted cell specificity of these components indicates that they might be needed for selective cell–cell recognition and/or specific functional properties of endothelial cells.

Through their cytoplasmic tail, junctional adhesion proteins bind to cytoskeletal and signalling proteins, which allows the anchoring of the adhesion proteins to actin microfilaments and the transfer of intracellular signals inside the cell[3–6]. The association with actin is required not only for stabilization of the junctions, but also for the dynamic regulation of junction opening and closure. In addition, the interaction of junctional adhesion proteins with the actin cytoskeleton might be relevant in the maintenance of cell shape and polarity[2,20–22].

Besides acting as adaptors in mediating the binding of adhesion proteins to actin, some intracellular junctional proteins, when released from junctions, translocate to the nucleus and modulate transcription[3,23,24] (TABLE 1). Another characteristic of some junctional proteins is that they might function as scaffolds, binding several effector proteins and facilitating their reciprocal interaction. A typical example is the TJ component zona occludens-1 (ZO1), which can associate with many transmembrane proteins, such as claudins, occludin or junctional adhesion molecules (JAMs); with cytoskeletal binding proteins such as cortactin, cingulin, α-CATENIN and, albeit indirectly, vinculin and α-actinin; with other PDZ-DOMAIN-containing proteins such as ZO2; or with signalling mediators such as ZONAB (ZO1-associated nucleic-acid binding)[3,25,26] (BOX 1; TABLE 1).

Many reports in the literature support the idea that AJs and TJs are interconnected and that AJs might influence TJ organization. AJs are formed at early stages of intercellular contact and are eventually followed by the formation of TJs. Some TJ components such as ZO1 are found at AJs at early stages of junction formation and concentrate at TJs only subsequently, when junctions are stabilized[27]. In some, but not all[28], cellular systems, blocking AJs inhibits the correct organization of TJs[29].

The organization of the junctions varies in composition and morphological features along the length of the VASCULAR TREE, in a way that is related to different permeability requirements. AJs are ubiquitous in all types of vessels. By contrast, TJs are poorly organized where dynamic and rapid interchanges between blood and tissue are required, as occurs in post-capillary venules, but extremely complex where permeability is strictly controlled, as is required in the brain microvasculature[30].

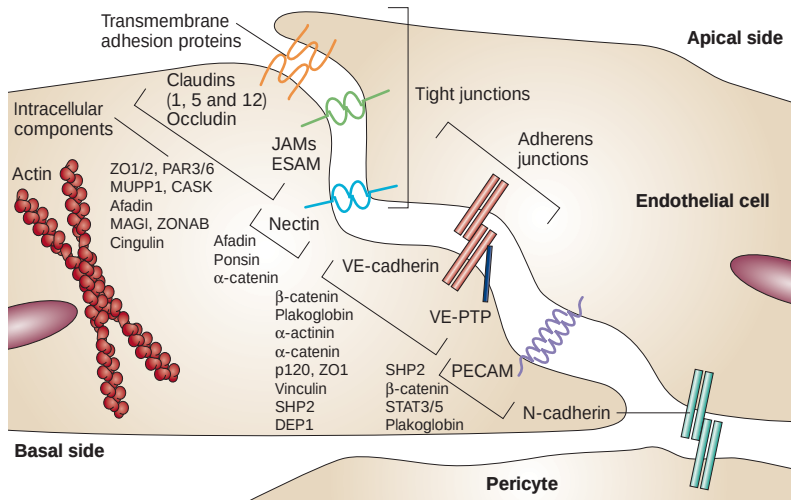
Nectin, afadin, PECAM and S-endo-1. An important role in both AJ and TJ organization is carried out by the nectin–afadin system, which has been described mostly in epithelial cells but also seems to be present in endothelial cells (BOX 1). Nectin is a member of the IMMUNOGLOBULIN FAMILY and is linked inside cells to afadin (also known as AF6), and through afadin to ponsin and actin[31]. This complex is required for AJ formation, but both afadin and nectin might also interact with TJ

MAGUKS
A family of proteins that contain membrane-associated guanylate kinase, PDZ and SRC-homology-3 (SH3) domains.

proteins such as ZO1 and JAMs, which indicates that they might also have a role in TJ formation[32].

Outside specialized junctional structures, endothelial cells express other cell-specific homophilic adhesion proteins at intercellular contacts. The best studied are platelet endothelial cell adhesion molecule (PECAM; also known as CD31) (BOX 1; FIG. 1) and S-endo-1 (also known as Muc18 or CD146), both of which belong to the immunoglobulin family. PECAM is also present in leukocytes and platelets and S-endo-1 in smooth muscle cells[33–35], but both PECAM and S-endo-1 are absent from epithelia.

Box 1 | Molecular organization of endothelial junctions



Endothelial junctions are formed by transmembrane adhesion proteins and their intracellular partners. At tight junctions (TJs), adhesion is mediated by members of the claudin family (claudins 1, 5 and 12), occludin, junctional adhesion molecules (JAMs) A, B and C[86,104], and endothelial cell selective adhesion molecule (ESAM). At adherens junctions (AJs), the main adhesive protein is vascular endothelial cadherin (VE-cadherin). The nectin–afadin complex has been found at both junctions. In this complex, ponsin binds to afadin, vinculin and α-catenin and thereby helps to anchor the complex to actin. More-comprehensive lists of junctional components can be found in recent reviews[6,7,11,14–18,23,32,33,86,99,100,105].

Many intracellular components of TJs — such as zona occludens-1 (ZO1) and -2 (ZO2), calcium/calmodulin-dependent serine protein kinase (CASK), afadin (also known as AF6), partitioning defective-3 (PAR3) and multi-PDZ-domain protein-1 (MUPP1) — contain PDZ domains. CASK, ZO1, ZO2 and membrane-associated guanylate kinase inverted (MAGI) belong to the MAGUK (membrane-associated guanylate kinase) family. By binding to α-catenin, ZO1 can associate with the cadherin–catenin complex in non-epithelial cells[27]; in cells with well-organized TJs, however, it is mostly concentrated in these structures. ZONAB (ZO1-associated nucleic-acid binding) is a transcription factor that binds to ZO1 in confluent cells but can translocate to the nucleus in sparse cultures, which increases proliferation.

Some intracellular proteins at AJs are kinases and phosphatases, such as Src-homology-2 (SH2)-domain-containing protein tyrosine phosphatase-2 (SHP2) and density enhanced protein-1 (DEP1; also known as CD148). Through its extracellular region, VE-cadherin can also associate with VE-PTP (vascular endothelial protein tyrosine phosphatase), which modulates cadherin and catenin phosphorylation and vascular permeability[106]. Many components of AJs or TJs — for example, ZO1, ZO2, β-catenin, α-catenin, α-actinin, plakoglobin, vinculin, cingulin and CASK — interact directly or indirectly with actin filaments.

Endothelial cells also express neuronal cadherin (N-cadherin), which is not concentrated at AJs[52], but instead probably mediates binding to pericytes or other mesenchymal cells. TJs are not only on the apical side of endothelial cells but might also intermingle with AJs along the interendothelial cleft. Endothelial cells also express platelet endothelial cell adhesion molecule (PECAM), which promotes homophilic adhesion. PECAM can associate with STATs (signal transducers and activators of transcription) and modulate their tyrosine phosphorylation and nuclear localization[32].

Signals and endothelial homeostasis

In adults, the physiological state of endothelial cells is similar to their state in *in vitro* confluence. In this condition, the cells are contact inhibited in their growth, protected from apoptosis and in full control of permeability.

By contrast, when endothelial cells are growing — for example, during angiogenesis — their behaviour is comparable to that of *in vitro* sparse/subconfluent cells, which, in turn, behave similarly to fibroblasts or other mesenchymal cells. They are elongated, highly motile and sensitive to growth-factor stimulation. When they reach confluence and their junctions become organized, they lose the ability to respond to growth factors and switch to a resting condition[36,37]. These observations argue in favour of a role for junctional proteins in maintaining the cells in a resting state. In support of this, after vascular damage and disruption of intercellular contacts, endothelial cells regain the ability to respond to growth stimuli and to migrate into the wounded area (BOX 2).

Recently, it has become possible to define some of the signalling pathways that are activated by junction assembly. Within minutes of an initial cell–cell contact, junctional proteins trigger rapid and short-lived responses[38,39] that are important for the quick communication of cell position. Subsequently, once junctions are established, junctional proteins might transfer continuous and lasting signals that contribute to the stabilization of the cell monolayer[22,40].

Contact inhibition of cell growth. Cadherins are implicated in contact-induced inhibition of cell growth. Decreased cadherin expression has been associated with a negative prognosis in cancer patients, and with increased cell invasion[13]. The contact inhibition of growth is mediated, at least in part, by the induction of cell-cycle arrest at the G1 phase as a result of the dephosphorylation of retinoblastoma protein, an increase in the levels of the cyclin-D1-dependent kinase inhibitor p27KIP1, and a late reduction in cyclin D1 levels[41–44]. These effects might in turn be due to the ability of cadherins to interact with β-catenin at the cell membrane and thereby limit its nuclear translocation. In the nucleus, β-catenin upregulates the transcription of cyclin D1 and MYC, and so inhibition of this activity would indirectly limit growth[23,45–47] (TABLE 1).

However, this model might not explain all of the published observations. For instance, in sparse cells, β-catenin remains associated with cadherins, but cells are not inhibited in their growth. This could be explained by the fact that even small — and, in some cases, undetectable — increases in the levels of nuclear β-catenin might be enough to achieve transcriptional activation[43]. Alternatively, there is evidence for the presence of functionally distinct pools of β-catenin that are involved in adhesion and in signalling, and which might be regulated

Table 1 | Junctional proteins with transcriptional/signalling activity

Junctional protein	Target	Effects	Associated factors	References
Adherens junction				
β-Catenin	Cyclin D1, MYC, Twin, Conductin, Siamois, TCF, UBX	Induction of cell growth, inhibition of apoptosis, modulation of cell differentiation	TCF/LEF, groucho, SMAD4, CBP, SWI/SNF, pygopus	23,24,112
p120	Matrilysin?	Developmental events, cadherin expression and function	Kaiso	113
Plakoglobin		Cell proliferation, transformation	TCF/LEF	114
Tight junction				
ASH1		Homeotic gene expression		115
ZONAB	ERBB2	Cell proliferation		3

The indicated adherens-junction and tight-junction proteins are linked to specific transmembrane adhesive proteins and, under particular conditions, can detach from junctions, translocate to the nucleus and modulate cell transcription. The table shows some of the known targets of these junctional proteins, their effects and, when known, other associated transcription factors. ASH1, absent, small or homeotic disc 1; CBP, cyclic AMP response element (CREB)-binding protein, a histone acetyl transferase; LEF, lymphoid enhancer factor; SMAD4, similar to mothers against decapentaplegic-4; SWI/SNF, a chromatin-remodelling complex; TCF, T-cell factor; UBX, ultrabithorax; ZONAB, zona occludens-1 (ZO1)-associated nucleic-acid binding.

independently[46]. We recently found that endothelial cells that are null for the *CDH5* gene, which encodes VE-cadherin, lose the contact inhibition of cell growth and reach higher densities than *CDH5*-positive cells[40]. VE-cadherin expression and clustering strongly reduces the cellular response to vascular endothelial growth factor (VEGF). This action seems to be due to the association of VE-cadherin with VEGF receptor-2 (VEGFR2; also known as FLK1 (fetal liver kinase-1) or KDR (kinase insert domain containing receptor)) and with density enhanced phosphatase-1 (DEP1; also known as CD148), which causes receptor dephosphorylation on activation of the receptor by its ligand (FIG. 2).

Other growth-factor receptors, such as the fibroblast growth factor receptor-1 (FGFR1) and the epidermal growth factor receptor (EGFR), can interact with neuronal cadherin (N-cadherin) and epithelial cadherin (E-cadherin), respectively[38,48,49], which indicates that this phenomenon might not be exclusive to VE-cadherin and VEGFR2, and could be considered to be a general model. Therefore, cadherins can, rather like integrins[50], form multiprotein complexes with growth-factor receptors and modulate their activation and/or stability at the cell membrane. However, the general model seems to be that, whereas integrins usually function synergistically with growth-factor receptors and promote proliferation and motility signals, VE- or E-cadherins instead limit growth. It is possible that when cells are sparse and their junctions are disorganized, the association of growth-factor receptors with integrins prevails. By contrast, after cells reach confluence — when the junctions are fully stabilized — growth-factor receptors might preferentially associate with cadherins, which, in turn, would attenuate proliferation signals.

However, an exception to this rule is N-cadherin. In tumour cells, N-cadherin has been associated with increased cell invasion[51]. Interestingly, its association with FGFR1 maintains the receptor on the membrane, which thereby inhibits its internalization and induces a state of continuous cell activation[48]. It is tempting to speculate that, as N-cadherin is recruited not at endothelial cell–cell junctions but instead at sites where endothelial cells meet PERICYTES (BOX 1; FIG. 1), N-cadherin-mediated engagement of FGFR1 would promote endothelial motility and vessel elongation[52].

In addition to associating with growth-factor receptors, cadherins have also been found to co-precipitate with signalling mediators such as: Src-family kinases; phosphatases such as protein tyrosine phosphatases μ and B (PTPμ and PTPB) and Src-homology-2 (SH2)-domain-containing protein tyrosine phosphatase-1 (SHP1), SHP2 and so on[40,53–56]; and the adaptor protein SHC (SH2-domain-containing protein), which participates in RAS activation. SHC can directly bind to the cytoplasmic tail of VE-cadherin, but only after several minutes of activation by VEGF. Binding of SHC to VE-cadherin is associated with SHC dephosphorylation, indicating that cadherins might function by sequestering SHC, thereby favouring its dephosphorylation and reducing the activation of RAS protein[57]. As RAS signalling to extracellular-signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) is known to be an important growth-promoting pathway, this would effectively attenuate proliferation.

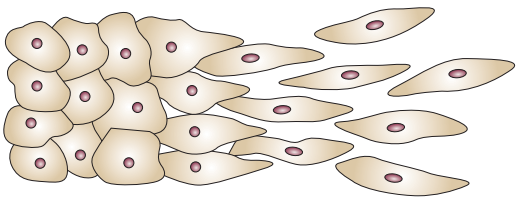
PERICYTE
A cell that is found around capillaries and is related to smooth muscle cells. Pericytes surround the endothelium as single cells. Association with pericytes reduces endothelial apoptosis and stabilizes the vasculature.

Box 2 | Phenotypes of confluent and sparse cells

Endothelial cells behave differently in confluent or sparse conditions (see figure; from left to right, respectively).

Confluent cells
- Epithelioid phenotype
- Contact inhibition of growth and motility
- Rearrangement of actin microfilaments
- Protection from apoptosis
- Apical–basal polarity

Sparse cells
- Fibroblastoid morphology
- Active growth
- Motility



Junctional structures contribute to the 'resting' confluent phenotype by transducing signals within the cells and changing gene expression. Sparse cells, which lack cell–cell junctions, are unable to transduce such signals.

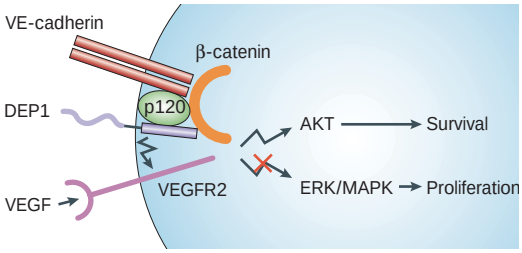


Figure 2 | **Modulation of VEGFR2 signalling by VE-cadherin.** In confluent endothelial cells, vascular endothelial cadherin (VE-cadherin) is clustered at junctions and forms a complex with the vascular endothelial growth factor (VEGF) receptor-2 (VEGFR2). The phosphatase density enhanced protein-1 (DEP1; also known as CD148) associates with the complex, probably through p120 and β-catenin, and dephosphorylates VEGFR2 (jagged arrow pointing towards VEGFR2). This phosphatase specifically targets tyrosine residues that, when phosphorylated, would recruit phospholipase Cγ (PLCγ; not shown) and signal proliferation through extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK). Tyrosine residues in VEGFR2 that are required for activation of phosphatidylinositol 3-kinase (PI3K) and AKT/protein kinase B (PKB) are not targeted. The net effect is to inhibit cell proliferation while promoting survival.

Circumstantial evidence indicates that TJ proteins might also contribute to the inhibition of the growth of confluent cultures[3], but the mechanism of action and the molecules that are involved remain to be fully defined. The transcription factor ZONAB accumulates either at cell junctions or in the nucleus, depending on cell confluence. ZONAB promotes cell proliferation at least in part by interacting with cyclin-dependent kinase-4 (CDK4). Similar to the situation with β-catenin, ZONAB binding to ZO1 at TJs in confluent cultures would restrain its ability to access the nucleus and so would indirectly inhibit cell proliferation[58]. Other possible proliferation-suppressive signalling pathways that are triggered by TJs have also been partially delineated. ZO1 can indirectly associate with β-catenin[59], presumably sequestering it away from the nucleus. In epithelial cells, the expression of deletion mutants of ZO1 causes a transition to a mesenchymal and tumorigenic phenotype[60], probably through modulation of β-catenin signalling. TJ components might also interact with members of the RAS family and with RAS effectors that are involved in the regulation of cell growth[3].

PECAM has also been implicated in the control of cell growth. Interestingly, this protein can bind β-catenin and limit its transcriptional activity[32,33]. Therefore, although PECAM is localized outside AJs, it might have activities that are similar to those of VE-cadherin.

Protection from apoptosis. In the normal vasculature, resting endothelial cells are protected from pro-apoptotic stimuli. Cadherin engagement induces the activation of phosphatidylinositol 3-kinase (PI3K), probably by recruiting the enzyme to the membrane[39]. Activation of this pathway in endothelial cells leads to the phosphorylation of AKT/protein kinase B (PKB) and the inhibition of apoptosis[61,62]. In these cells, PI3K activation by VEGFR2 is increased by VE-cadherin[61]. The association of VEGFR2 with VE-cadherin is therefore expected to decrease its ability to induce proliferation but increase its anti-apoptotic activity. This indicates that the effect of VE-cadherin is complex, and that this protein can direct VEGFR2 signalling to specific pathways while inhibiting others. A possible explanation is that the phosphatases that are associated with VE-cadherin, such as DEP1, might dephosphorylate some specific tyrosine residues on the receptor tail, but not others[40]. This would inhibit receptor interaction with some effectors without affecting other pathways. For instance, specific tyrosines are required for the binding and activation of phospholipase Cγ (PLCγ), which would then trigger proliferation[63]. However, these tyrosines would be irrelevant for PI3K binding and activation. Therefore, in confluent endothelial cells, when VE-cadherin is clustered at junctions, VEGFR2 would preferentially signal through PI3K for survival. By contrast, in sparse cells or in cells lacking VE-cadherin, VEGFR2 would mostly promote cell growth (FIG. 2; FIG. 3), probably through the recruitment of PLCγ.

Other junctional proteins protect endothelial cells from apoptosis. PECAM, which belongs to the immunoreceptor tyrosine-based inhibitory motif (ITIM) family, can activate AKT/PKB[32] and also suppress mitochondrion-dependent apoptosis[64], by suppressing BAX-induced cytochrome *c* release, caspase activation and nuclear fragmentation — all hallmarks of apoptosis. This activity requires PECAM to form homophilic contacts, to cluster at intercellular contacts and to recruit the phosphatase SHP2 (REF. 64). The hypothesis is that a PECAM–SHP2 signalling complex might modulate either the location or the activation state of pre-existing pro-apoptotic components of the cell-death pathway.

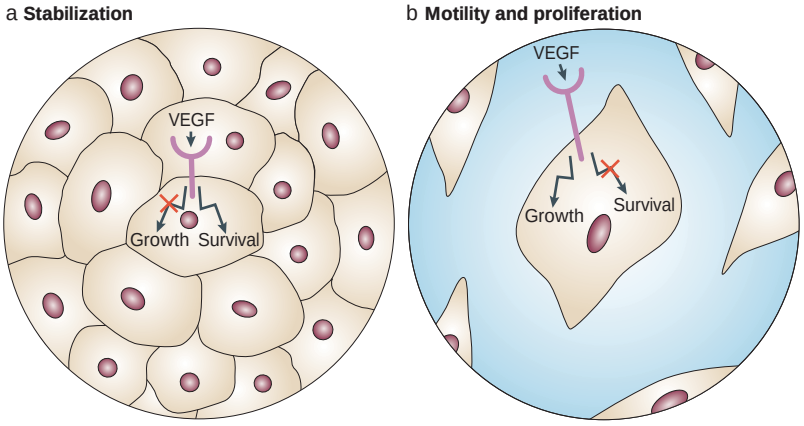


Figure 3 | **VEGF signalling in confluent and sparse endothelial cells. a** | In confluent cells, vascular endothelial cadherin (VE-cadherin) is clustered at junctions, and vascular endothelial growth factor (VEGF) preferentially induces survival and not cell proliferation, as outlined in FIG. 2. This stabilizes the endothelial sheet. **b** | In sparse cells, where junctions are dismantled and VE-cadherin is diffuse on the cell membrane, VEGF preferentially mediates proliferation and not survival. This also allows the cells to move. So, the same receptor in the same cell type responds differently to the same ligand, depending on cell confluency and VE-cadherin clustering.

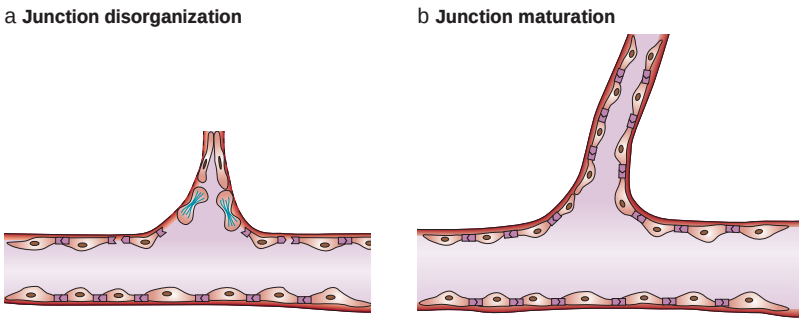


Figure 4 | **Modulation of junctions in angiogenesis. a** | During vascular sprouting, junctions are partially disorganized. This allows endothelial cells to migrate and proliferate, but increases vascular permeability. **b** | When the vessels are stabilized, as occurs after interaction with pericytes, junctional integrity is re-established and permeability is tightly controlled. Cell proliferation and apoptosis are inhibited.

Actin reorganization and epithelial–mesenchymal transition. Normal endothelial cells have a typical 'cobblestone' morphology at confluence, with an epithelioid phenotype. By contrast, when cells are sparse or intercellular junctions disrupted, a fibroblastoid/mesenchymal morphology predominates (BOX 2). The establishment of intercellular contacts probably transfers intracellular signals that can mediate changes in cytoskeletal organization, cell shape and polarity. Cadherins, and VE-cadherin in particular, are important determinants of the transition from a spread, epithelioid morphology to a fibroblastoid one — the so-called epithelial–mesenchymal transition (EMT)[22]. These changes are accompanied by actin-filament reshaping and an increased number of vinculin-positive FOCAL CONTACTS.

Important effectors of the EMT are RHO-FAMILY GTPASES[65,66]. In endothelial and epithelial cells, cadherin clustering induces sustained RAC activation and RHO inhibition[4]. The mechanism of RAC activation is still being debated and varies in different cell types. In Madin–Darby canine kidney (MDCK) cells, it requires PI3K activation by cadherins; in keratinocytes, EGFR signalling[4]. In the endothelium, VE-cadherin induces membrane localization of TIAM[22], a RAC-specific GUANINE NUCLEOTIDE-EXCHANGE FACTOR, which mediates RAC activation[67]. In epithelial cells, RHO inhibition is mediated by the GTPase-activating protein (GAP) p190RHO GAP, and it is likely that a similar mechanism also works in endothelial cells[68].

The response to blood flow. A specific characteristic of endothelial cells is that they are continuously exposed to blood flow. Haemodynamic forces cause a complex response in endothelial cells, with upregulation or downregulation of genes and a chronic restructuring of blood vessels. Changes in cytoskeletal organization and cell shape are among the most rapid and marked modifications that are induced by blood flow in the endothelium. A crucial question is how endothelial cells transduce mechanical forces into biological responses. Several membrane proteins have been implicated as mechanosensors, but, more recently, VE-cadherin and PECAM have been found to show specific activities in response to cell exposure to shear stress[69,70]. PECAM can be rapidly phosphorylated in response to shear stress, bind SHP2 and mediate ERK/MAPK activation[70]. Shear stress induces the association of VE-cadherin with VEGFR2 — in the absence of VEGF — and is required for shear-dependent gene expression[69]. These data strongly indicate that endothelial cell junctions are probably a potential site for mechanosensing and transducing shear-stress signals.

Junctional proteins and new vessels

If we accept the idea that junctions mediate 'stabilization' signals and maintain the resting state of endothelial cells, it is conceivable that these signals are attenuated and that the junctions become partially disorganized when endothelial cells migrate and proliferate, as occurs during the formation of new vessels (FIG. 4). However, this process must be tightly controlled. If junctions were fully dismantled, adverse effects — such as an increase in apoptosis and uncontrolled cell proliferation — would prevail, leading to the regression of newly formed vessels[71].

In support of an inverse relationship between junction strength and new vessel formation, angiogenesis is frequently accompanied by an increase in vessel permeability[72,73]. VEGF induces tyrosine phosphorylation of VE-cadherin and β-catenin[74], an effect that is probably mediated by SRC-family kinases[75]. Tyrosine phosphorylation of cadherin–catenin complexes is accompanied by decreased junctional strength and increased permeability. It is possible that, in microvascular endothelium — which has poorly organized junctions — VEGF could be responsible for this disorganization and the release of contact inhibition of cell growth.

Studies of transgenic mice have been very informative for understanding the role of junctions in angiogenesis and vasculogenesis. Inactivation of the genes coding for certain AJ proteins, such as VE-cadherin[61], β-catenin[76] or DEP1 (REF. 77), markedly inhibits normal vascular development in the embryo. By contrast, in the absence of certain TJ proteins, such as occludin[78] or claudin-5 (REF. 19), the vascular system can form as usual, but there are problems with the control of vascular permeability to fluids or circulating cells in the adult (BOX 3).

The roles of cadherins, catenins and their associated partners in angiogenesis are complex and are probably linked to their signalling properties. In addition to their role in proliferation and apoptosis, junctional proteins might also be important in vascular tubulogenesis. During the development of the vascular system, endothelial cells form tubes by switching between a fibroblastoid/migratory state, in which they lack in large part apical–basal polarity, and an epithelioid state, in which they form intercellular junctions and establish an apical–lumenal surface[79–84]. Junctions have a role in regulating cell polarity through the rearrangement of the cytoskeleton and the establishment of apical and basal surfaces that are required for lumen determination. RAC activation by cadherin clustering might be a key event in this system, as blocking RAC activity prevents

FOCAL CONTACTS
Regions of cell attachment to the extracellular matrix. Adhesion receptors and specific cytoskeletal proteins are clustered in these regions.

RHO-FAMILY GTPASES
RAS-related GTPases that are involved in controlling the polymerization of actin.

GUANINE NUCLEOTIDE-EXCHANGE FACTOR
A protein that facilitates the exchange of GDP for GTP in the nucleotide-binding pocket of a GTP-binding protein.

Box 3 | Mice vascular phenotypes produced by null mutations in endothelial junctional components

VE-cadherin
Embryos that are null for *Cdh5*, which encodes vascular endothelial cadherin (VE-cadherin), present significant defects in vascular remodelling and die *in utero* within 9.5 days after fertilization[61]. Although early phases of vascular development can occur, later stages are severely affected: vessels collapse, regress and large haemorrhages occur. The ENDOCARDIUM is markedly altered and cells detach from the matrix and form aggregates in the endocardial cavity.

β-catenin
Embryos that are null for the β-catenin gene, *Ctnnb1*, die within 11.5 days of fertilization, but early phases of VASCULOGENESIS and angiogenesis are not affected[76]. However, the vascular patterning is altered and, in many regions, the vessel lumen is irregular, with lacunae and haemorrhages occurring at bifurcations. In cultured *Ctnnb1*-null endothelial cells, junctions are weaker, and desmoplakin substitutes, in part, for α-catenin in binding plakoglobin, which leads to a different molecular composition of junctions. These structures are weaker than adherens junctions and might therefore result in vascular deformation and fragility when they are exposed to sustained blood flow.

N-cadherin
Embryos that are null for *Cdh2*, which encodes N-cadherin, die 10.5 days after fertilization[107]. This mutation mostly affects the development of the heart tube, but blood vessels in the yolk sac are also altered.

Desmoplakin
Dsp-null embryos die *in utero* with significant heart, neuroepithelium and skin epithelium defects[108]. However, they also have a reduced number of capillaries, which has been attributed to the weakening of endothelial cell–cell adhesion.

Density enhanced protein-1
Embryos that express an inactive mutant of *Ptprj*, which encodes density enhanced protein-1 (DEP1; also known as CD148), die *in utero*[77]. The overall vascular network is profoundly altered; vascular lumina are markedly enlarged and endothelial cells have a higher proliferation rate. These defects are consistent with a role for DEP1 in contact-induced inhibition of endothelial cell growth.

Claudin-5
No morphological defects are seen in the vasculature of embryos that are null for *Cldn5*, but there is a selective postnatal loss of the properties of the blood–brain barrier, which leads to death a few hours after birth[19].

Occludin
There is no apparent vascular phenotype in *Ocln*-null embryos[109].

Platelet endothelial cell adhesion molecule
There are no detectable developmental vascular defects in *Pecam*-null embryos. Inflammatory angiogenesis is reduced in the adult[110,111].

ENDOCARDIUM
The endothelial lining of the cardiac lumen.

VASCULOGENESIS
The formation of vascular structures through the differentiation of endothelial cells from specific progenitors and their subsequent organization into a tubular network.

ANASTOMOSIS
A cross-connection between adjacent channels, tubes, fibres or other parts of a network.

INNATE IMMUNE RESPONSE
This response is crucial during the early phase of host defence against infection by pathogens, before the antigen-specific adaptive immune response is induced.

ADAPTIVE IMMUNE RESPONSE
The antigen-specific response of T and B cells. It includes antibody production and the killing of pathogen-infected cells, and is regulated by cytokines such as interferon-α.

lumen formation, capillary assembly and vascular morphogenesis[79]. In epithelial cells, TJs have been implicated in establishing apical membrane biogenesis, possibly through the recruitment of the polarity components CRUMBS, PALS1 and the PAR3–PAR6–aPKC (atypical protein kinase C) complex[3]. The PAR3–PAR6–aPKC complex can bind to JAM-A in epithelial and endothelial cells[80,81]. Several examples from other models of tubulogenesis, such the *Drosophila melanogaster* tracheal system, confirm the hypothesis of an important role for TJs and other junctional structures in tube fusion (ANASTOMOSIS), as well as in forming and controlling the size of the lumen[82–84].

Endothelial junctions and leukocyte diapedesis

INNATE and ADAPTIVE IMMUNE RESPONSES are accompanied by leukocyte adhesion to the blood-vessel wall and their subsequent infiltration into the underlying tissues. This last process is regulated by the so-called transcellular and paracellular pathways. The transcellular pathway defines the passage of leukocytes through the endothelial cytoplasm, probably through vesicular/canalicular systems. This pathway has been described in detail at a morphological level[85], but little is known about the molecular structures that are involved.

When they follow the paracellular pathway, leukocytes cross the endothelium by squeezing through the border between apposed endothelial cells, a process that is known as diapedesis. This process is usually very rapid and is followed by an equally rapid reassembly of junctions, which prevents increased permeability[86].

Although we only have a partial picture of how leukocytes can open endothelial junctions, it is likely that, on adhesion to the inflamed endothelium, they transfer signals that direct junction rearrangement. Leukocyte adhesion to endothelial cells causes cell retraction by increasing levels of intracellular calcium and inducing the subsequent activation of myosin light chain kinase (MLCK)[86,87]. MLCK-mediated phosphorylation of MLC results in an enhanced interaction of MLC with actin, increased myosin ATPase activity and consequent contractility. Increases in intracellular calcium levels might also be triggered by the release of soluble cationic proteins during neutrophil activation[86,87].

Many reports also link the disruption of endothelial junctions with RHO or RAC activation[88]. Inflammatory cytokines, thrombin and histamine increase endothelial permeability and dismantle AJs and TJs through RHO/RHO-kinase activation[89,90]. RHO inhibits myosin light chain phosphatase (MLCP) and increases the

ICAM1
(Intercellular adhesion molecule-1). A member of the immunoglobulin family that is highly expressed on endothelial cell membranes on activation by inflammatory cytokines. It is one of the major adhesive proteins for leukocytes.

EXTRAVASATION
The process by which something is let or forced out from a vessel that naturally contains it.

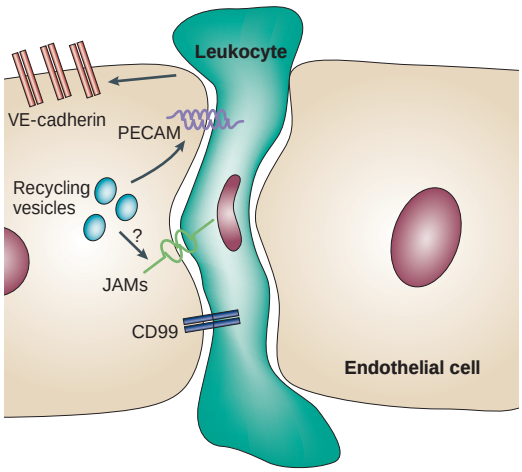


Figure 5 | **Leukocyte diapedesis through endothelial junctions.** During their passage through the interendothelial cleft, leukocytes encounter different junctional proteins. During this process, vascular endothelial cadherin (VE-cadherin) tends to redistribute to the endothelial surface, whereas platelet endothelial cell adhesion molecule (PECAM) and junctional adhesion molecules (JAMs) are concentrated along the endothelial cell borders, probably as a result of targeted recycling of specific vesicles. CD99, a membrane protein that is present in endothelial cells and leukocytes, functions independently in directing leukocyte diapedesis through the cleft. Blocking both PECAM and CD99 leads to an additive inhibitory effect on diapedesis.

phosphorylation and activity of MLCK, which, in turn, increases cell contractility, as described above[91]. Leukocyte adhesion to intercellular adhesion molecule-1 (ICAM1) on endothelial cell surfaces might similarly disrupt endothelial junctions by triggering a series of responses, including the activation of RHO and JUN-amino-terminal kinase (JNK)[92,93].

However, leukocyte diapedesis through the endothelium might also follow specific rules and exclusive molecular mechanisms (FIG. 5). During leukocyte migration, VE-cadherin is transiently removed from endothelial junctions[94]. By contrast, PECAM is constitutively contained in vesicles, located just beneath the plasma membrane, which tend to recycle continuously from this compartment along the endothelial border. When leukocytes transmigrate, PECAM in the endothelium recycles and concentrates around the migrating leukocyte, thereby establishing a homophilic interaction with PECAM that is expressed on the leukocyte membrane[95,96]. Recent data indicate that JAM-A might also contribute to leukocyte diapedesis, by forming a transient ring through which leukocytes can tunnel[97]. In addition, CD99, which is also expressed at the membrane of leukocytes and at interendothelial contacts, is required for this process and blocking it *in vitro* leads to the arrest of migrating monocytes as they cross intercellular junctions[98]. A general model seems to be that proteins at endothelial junctions establish homophilic interactions with identical proteins that are present on leukocytes. These interactions might then direct the passage of leukocytes through the endothelial border.

Experiments that were carried out *in vivo* using blocking antibodies or null mutations of the genes that encode junctional proteins indicate, however, that the picture is more complex. For instance, null mutation of *PECAM* has only limited effects on leukocyte EXTRAVASATION, whereas blocking antibodies were active in several models of inflammation[86,99,100]. Antibodies directed against JAM-A inhibited leukocyte infiltration in some models of inflammation, but not in others[101–103]. There could be different reasons for these discrepancies. First, when endothelial cell junctions are disrupted, as they are in some inflammatory conditions[103], leukocytes can easily infiltrate through the exposed subendothelial matrix, and so inhibition of junctional proteins would be ineffective. Second, several junctional proteins could act in parallel and compensate for the loss-of-function of the other proteins. Third, as mentioned previously, the molecular organization and composition of endothelial junctions varies along the vascular tree. When TJs are well developed, as they are in the brain microvasculature or large arteries, leukocyte infiltration is reduced. In this last case, leukocytes come into contact mostly with TJ adhesive proteins, such as claudins or occludin, and their diapedesis is probably regulated by different adhesive receptors.

Future directions

Endothelial cells have complex junctional structures that are formed by transmembrane adhesive proteins, which promote homophilic adhesion among the cells and create zipper-like structures along the cell borders. Inside the cells, junctional adhesive proteins are linked to the actin cytoskeleton and this interaction stabilizes adhesion. Several endothelial functions are regulated by junctions, including growth and apoptosis, and recent results indicate that these structures have a central role in stabilizing the endothelium in the resting condition that corresponds to its physiological state. The switch of these cells from a confluent and resting condition to a migrating and proliferative state, for example during the formation of new vessels, is modulated by junction organization and signalling.

Exciting new developments have shown that endothelial cell–cell junctional structures transfer intracellular signals, and some of these signalling pathways have been delineated. However, we are far from deciphering this complex system and from defining all of the molecular players and their reciprocal interactions. It is likely that more junctional proteins will be identified in the future and that different types of vessel (for instance, in the lymphatic system or the brain microvasculature) have different structures and specific molecular components.

Defining the architecture of junctions is instrumental not only for understanding the role of these structures in the control of vascular permeability, but also for understanding the mechanisms that regulate new vessel formation. Furthermore, these type of studies are also likely to be informative with regard to the pathologies that are related to uncontrolled vascular fragility and permeability, such as hereditary haemorrhagic telangiectasia, haemangiomas, or endothelial tumours such as angiosarcomas and Kaposi's sarcoma, in which the vascular network is disrupted.

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Competing interests statement

The author declares that she has no competing financial interests.

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