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Review

THE MOLECULAR ORGANIZATION OF ENDOTHELIAL JUNCTIONS IN VASCULAR PERMEABILITY

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ABSTRACT

Vascular permeability is an innate function of the circulatory system that regulates the flux of fluid, proteins, and immune cells from blood to tissue. Vascular permeability is regulated by a molecular mechanism that involves the endothelial barrier. Endothelial barrier function and vascular permeability are regulated by intercellular junctions that control the extravasation of plasma and its macromolecular constituents. The number and arrangement of these junctions determine permeability differences in the vasculature in an organ- and tissue-specific manner. The barrier is mediated by endothelial cell-cell adhesions. Adjacent endothelial cells are connected by protein complexes that are part of the Gap junctions (GJs), adherens junctions (AJs), Tight junctions (TJs), and additional other adhesion receptors such as CD31/Platelet Endothelial Cell Adhesion Molecule-1 (PECAM-1) and nectins, which are connected to the actin cytoskeleton via different adaptor molecules. This review focuses on the molecular organization and regulation of endothelial junctions in vascular permeability in health and disease. Additional work should also be directed to the understanding of mechanisms that influence altered vascular permeability in specific diseases and to strategies for preventing or reversing vascular leakage, which can result in harmful consequences.

1. Introduction

Vascular permeability is an innate function of the circulatory system that regulates the flux of fluid, proteins, and immune cells from blood to tissue. In most non-inflamed tissues, vascular permeability is controlled by the "barrier" formed by the microvascular wall, which includes the endothelial glycocalyx, the endothelium, basement membrane, and any accessory cells (i.e., pericytes or smooth muscle cells) wrapped around the outer surface of the vessel (1).

Vascular permeability also contributes to the pathophysiology of many diseases. Increased permeability is a prominent feature of asthma and other inflammatory airway diseases, arthritis, chronic bowel disease, cancer, infections, trauma, ischemic stroke, and many other conditions where leakage can result in edema, impaired function, and morbidity.

This review focuses on the molecular organization and regulation of endothelial junctions in vascular permeability in health and disease (2).

2. Intercellular junctions

The control of vascular permeability is regulated by a molecular mechanism that involves the endothelial barrier. Endothelial barrier function and vascular permeability are regulated by intercellular junctions that control the extravasation of plasma and its macromolecular constituents (3). Intercellular junctions of endothelial cells are also involved in forming structures that develop into sprouts and primitive vascular tubes (4).

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The vascular endothelium lines the inner layer of the blood vessel and actively controls extravasation of fluids, ions, molecules, and leukocytes (3). The number and arrangement of these junctions determine permeability differences in the vasculature in an organ- and tissue-specific manner. The barrier is mediated by endothelial cell-cell adhesions CD31/Platelet Endothelial Cell Adhesion Molecule-1 (PECAM-1). Adjacent endothelial cells are connected by protein complexes that are part of the Gap junctions (GJs), adherens junctions (AJs), Tight junctions (TJs), and additional other adhesion receptors such as PECAM-1 and nectins, which are connected to the actin cytoskeleton via different adaptor molecules. GJs are formed by connexin-mediated transmembrane channels allowing direct communication between ECs via the passage of ions and small signaling molecules (5).

In contrast, AJs and TJs form adhesion structures, mediate and control cell contact integrity and molecular permeability across the endothelial barrier, and can be disrupted during EC activation.

2.1 Gap junctions

GJs are communication junctions (gap junctions, pannexins, ion channels, and chemical synapses). Gap junctions are clusters of intercellular channels that facilitate a direct connection between the cytoplasm of two neighboring cells to mediate intercellular communication (6). Gap junctions communicate changes in ion currents and signals mediated by small molecules that spread between endothelial cells. Gap junctions are made of connexins arranged in hexamers that form hemichannels joining their counterparts in neighboring endothelial cells. Connexins 37, 40, and 43 are preferentially expressed in endothelial cells and have been implicated in modulation of basal permeability (7). Specifically, after vascular leakage is generated by endotoxin in the lung, changes in connexin 43 expression show an inverse correlation with VE-cadherin expression (8). Gap junctions can also contribute to angiogenesis (9), being associated with tight junction strands in endothelial cells (10), and play a role in regulating the opening of the vascular barrier; under some conditions, Gap junctions between endothelial cells and smooth muscle cells have been implicated in vasomotor activity (7).

2.2 Adherens junctions

AJs are generally considered to provide stability to inter-endothelial-cell-contacts and regulation for large molecular weight plasma components. The main component of endothelial junctions, VE-cadherin, forms Ca2+-dependent homophilic interactions between adjacent endothelial cells that are essential for maintenance of the endothelial barrier (11-12). VE-cadherin is made of extracellular cadherin motifs, a transmembrane domain, and an intracellular domain that mediates interactions with β-catenin, p120-catenin, and γ-catenin, also known as plakoglobin (13-14). The intracellular complex of VE-cadherin with catenins is essential for junctional stability. In the heart and lung, inhibition of VE-cadherin causes the formation of endothelial gaps and leakage. VE-cadherin is also complexed with vascular endothelial growth factor receptor-2 (VEGFR2), Tie2, and other receptors that mediate the actions of permeability modulators. VE-cadherin also forms complexes with VE-protein tyrosine phosphatase (VE-PTP) and density-enhanced phosphatase 1 (DEP1) (14-19), which play a pivotal role on junctional stability.

VE-cadherin is essential for the development of the vascular system (20,21) and is probably the adhesion molecule with highest endothelial specificity. Mice with a genetically engineered VE-cadherin-α-catenin fusion construct, were resistant to VEGF and histamine-induced vascular leakage (22).

Although VE-cadherin was often described as exclusively expressed in blood vascular endothelial cells, it was also found in lymphatic endothelial cells, specifically a type of hematopoietic cells (23-25). Adhesion of VE-cadherin is controlled by phosphorylation and dephosphorylation on specific tyrosine residues (26). In response to vascular permeability stimulators, such as VEGF and bradykinin, some tyrosine residues of VE-cadherin become phosphorylated (Y685 and Y658), resulting in unstable AJs and an increased vascular permeability (27).

AJs are typically located more basally and join adjacent endothelial cells. Increases in endothelial permeability, resulting in focal changes of junctions, cause endothelial gaps and leakage. Gap formation requires detachment of complexes involving the AJs protein VE-cadherin, which changes its distribution from a continuous band along cell borders to a distinctive serrated or zig-zag pattern (28,29).

Nectins belong to an additional family of cell–cell adhesion molecules associated with AJs, which includes four types: Nectin-1, Nectin-2, Nectin-3, and Nectin-4. Unlike the cadherins, Nectins are independent of Ca2+ (30). Nectins contain three Ig-like domains in their extracellular regions (31). Ig-like domains reportedly form homo-and hetero-dimers with other Nectins, playing a pivotal role in cell-cell adhesion and migration (32). The physiologic functions of Nectins involve several downstream signaling events that are related to VEGFR-2 in human umbilical endothelial cells (33–35). VEGFR-2 is known to be regulated by direct interactions with several cell adhesion proteins such as Nectin-3, VE-Cadherin and Integrin in angiogenesis and cell proliferation (36).

In conclusion, monolayer integrity is controlled by endothelial cell–cell junctions (adherens junctions). This complex consists of the transmembrane protein VE–cadherin and intracellularly associated catenins and adaptor proteins that link VE–cadherin to the F-actin cytoskeleton. Integrins interact with a structural and functional link to the F-actin cytoskeleton.

2.3 Tight junctions

In contrast to AJs, TJs control permeability for ions and small molecules (<800 Dalton). Therefore, TJs are not evenly distributed throughout the vasculature. Endothelial cell TJs are made of multiple transmembrane proteins that interact with membrane lipids and cytoplasmic proteins, as in epithelial cell barriers. Endothelial cell TJs proteins include members of the claudin (Cldn) family and junction-associated molecule (JAM) families, occludin, endothelial cell-selective adhesion molecule (ESAM), and other adhesion molecules, sharing many features with TJs in epithelial cells (37).

Endothelial barrier tightness is influenced by the composition of TJs complexes and the abundance of TJs strands (38,39). TJs strands are more abundant in endothelial cells of arterioles than venules (38,39).

Endothelial gap formation depends also on TJs changes corresponding to changes in AJs (39). Central components of TJs are claudins tetra-spanning membrane proteins. A Cldn member, claudin-5 (Cldn5), is particularly important among tight junction proteins because it is relatively specific to endothelial cells, but Cldn5 expression is not uniform throughout the vasculature. VE-cadherin can influence TJs and stabilize junctions by promoting Cldn5 expression (39). On the other hand, TJs can influence AJs stability through the junctional adaptor protein ZO-1, which regulates functional coupling of VE-cadherin to the cytoskeleton (40). In cultured endothelial cells, evidence that VEGFA induces Cldn5 down-regulation, through a protein kinase C-β (PKCβ)-dependent pathway, confirms that tight junction changes in VEGFA induced leakage (39).

ESAM is widely expressed in the vasculature, but its contribution to barrier function depends on whether Cldn5 or other tight junction proteins are also expressed. Mice with constitutive deletion of ESAM showed a modest leakage in lungs, but not in brain, heart, or skin. When ESAM deletion was combined with VE-cadherin ablation, mice rapidly died from lung leakage and thrombosis (40). These findings support the organ-specific importance of ESAM in tight junctions.

Junctional adhesion molecule (JAM) family proteins include three classical members, JAM-A, JAM-B, and JAM-C, and three distantly related members, JAM4, JAM-like (JAM-L) protein, and Coxsackie and adenovirus receptor (CAR) (41-43). JAMs are characterized by the molecular structure featuring two extracellular immunoglobulin domains, a single transmembrane domain and a cytoplasmic domain with a PDZ-binding motif (44). The crystal structure of the extracellular domain in JAM-A has been resolved (45). Although all members of the JAM family proteins have a PDZ-binding motif at the carboxyl terminus, they show variable affinities towards the ZO proteins. Only the classic JAMs, JAM-A, JAM-B, and JAM-C, have been confirmed to bind to the ZO proteins (45,46). The JAM family proteins also mediate cell adhesion, either directly, via the extracellular domains, or indirectly, via AJs interaction. In contrast to claudins, JAMs do not reconstitute the TJ strands in transfected mouse fibroblasts.

3. Modulators of endothelial barrier function

Changes in vascular permeability are associated with many modulators of the endothelial barrier function. Different factors that contribute to plasma leakage and edema formation in disease were identified, and molecular mechanisms causing changes in vascular permeability have been studied.

VEGFA, angiopoietins (ANGPT1, ANGPT2), and the inflammatory cytokines histamine and bradykinin, have direct actions on the endothelial barrier function. Some mediators can induce the formation of smaller or larger gaps between endothelial cells leading to extravasation. Many factors that affect the barrier cause changes in endothelial TJs and AJs.

3.1 VEGF and VEGF Receptor

VEGF subtypes include VEGFA (47**)** VEGFB (48), VEGFC (49**)**, VEGFD (50**)**, and placenta growth factor (PlGF) (51**)**. The VEGF family acts through three structurally related transmembrane tyrosine kinase receptors, VEGFR1, VEGFR2, and VEGFR3. VEGFR2 is the most abundant receptor of this family on endothelial cells and is essential for VEGFA-induced increase in vascular permeability (52-54**).**

All three receptors can bind VEGFs, leading to vascular permeability increase, angiogenesis, and other effects of VEGFs.

All receptors act by homo-and hetero-dimerization with the activation of an intracellular tyrosine kinase, promoting a series of downstream reactions through multiple common signaling pathways. The effects of VEGFs on endothelial cells are large and composite. VEGFs are potent and powerful vascular permeabilizing agents (55) and potent vasodilators (56).

They increase migration of endothelial cells, new vessel formation (57, 58) and have effects on pericytes that surround endothelial cells (59). *In vivo* and *in vitro* activation of endothelial cells by VEGFs results in marked ultrastructural changes due to permeability responses.

VEGFs activation induce a reduction in staining of junctional proteins such as ZO1, (60) occludin, (61) and VE-cadherin (62**)** at the endothelial junctions, concomitant with the onset of increased vascular permeability. In particular**,** VEGFs activation of endothelial cells results in phosphorylation and disassembly of VE-cadherin, one of the main signaling and structural proteins associated with the AJs.

3.2 Angiopoietins and Tie Receptor

The angiopoietin (ANGPT) family is an important group of factors, specific for the vascular endothelium, which functions are mediated through two tyrosine kinase receptors, TIE1 and TIE2.

In humans, ANGPT1 and ANGPT2 are the two primary angiopoietins. ANGPT1 and ANGPT2 have complex positive and negative effects on vascular permeability through actions on Tie1 and Tie2 receptor tyrosine kinases. ANGPT1 is a vascular stabilizer acting on the TIE2 receptor (63). By contrast, ANGPT2 is an inhibitory ligand of the Tie2 receptor that disrupts the integrity of the blood vessel wall, thus counteracting vascular normalization (64-65).

ANGPTs also regulate inflammation in several disorders, including cardiovascular diseases (66-68).

VEGFs and ANGPT1 have similar effects on endothelial cell survival and proliferation but opposite effects on barrier function. ANGPT1 plays an important role in increasing junctional stability. At the endothelial junctions, VEGFs and bradykinin enhance endothelial permeability by promoting endocytosis of VE-cadherin (69). This effect is provoked by the phosphorylation of the VE-cadherin intracellular domain, which is protected by ANGPT1. Bradykinin and VEGFs also induce cytoskeletal rearrangement and endothelial cell contractile response leading to the disruption of intercellular contacts and the increase of permeability (70). Recently, the detection of missense *ANGPT1* variants in a family with Hereditary Angioedema (HAE) was reported (70). Bradykinin has shown to be the predominant mediator for the enhanced vascular permeability in HAE attacks. The mutant protein was not able to form multimers, leading to a reduction in the ability to bind to the TIE2 receptor and affecting the stabilization of the endothelial barrier function. *In vitro* experiments showed that the expression of VE-cadherin on the endothelial cell surface is strongly reduced in the presence of the mutant ANGPT1. ANGPT1 contributes to the formation of a strong adhesion through the linkage between VE-cadherin, β-catenin and the actin of the cytoskeleton.

Mutant ANGPT1 caused a change of β-catenin cellular distribution that appeared weak and dotted along the cell-cell borders of endothelial cells, suggesting that the ANGPT1 variant is not sufficient to restore endothelial cell-cell contact. In addition, stress fiber formation induced by both VEGFs and bradykinin was reduced and failed to restore the arrangement of the F-actin cytoskeletal elements (70).

4. Conclusions

For Vascular permeability contributes to the pathophysiology of many diseases. Altered endothelial barrier functions also play important roles in cancer, age-related macular degeneration, diabetic macular edema, angioedema, chronic inflammatory and neurodegenerative diseases, and many other pathological conditions.

Because of the importance of plasma leakage in the pathophysiology of many of these conditions, further studies are needed to increase our understanding of endothelial gap formation and the mechanisms that influence the regulation of the endothelial barrier function. Additional work should also be directed to the understanding of mechanisms that cause altered vascular permeability in specific diseases and to strategies for preventing or reversing vascular leakage, which can result in harmful consequences.

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