Endothelial dysfunction as a cellular mechanism for vascular failure

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Hirase T, Node K. Endothelial dysfunction as a cellular mechanism for vascular failure. Am J Physiol Heart Circ Physiol 302: H499–H505, 2012. First published November 11, 2011; doi:10.1152/ajpheart.00325.2011.—The regulation of vascular tone, vascular permeability, and thromboresistance is essential to maintain blood circulation and therefore tissue environments under physiological conditions. Atherogenic stimuli, including diabetes, dyslipidemia, and oxidative stress, induce vascular dysfunction, leading to atherosclerosis, which is a key pathological basis for cardiovascular diseases such as ischemic heart disease and stroke. We have proposed a novel concept termed “vascular failure” to comprehensively recognize the vascular dysfunction that contributes to the development of cardiovascular diseases. Vascular endothelial cells form the vascular endothelium as a monolayer that covers the vascular lumen and serves as an interface between circulating blood and immune cells. Endothelial cells regulate vascular function in collaboration with smooth muscle cells. Endothelial dysfunction under pathophysiological conditions contributes to the development of vascular dysfunction. Here, we address the barrier function and microtubule function of endothelial cells. Endothelial barrier function, mediated by cell-to-cell junctions between endothelial cells, is regulated by small GTPases and kinases. Microtubule function, regulated by the acetylation of tubulin, a component of the microtubules, is a target of atherogenic stimuli. The elucidation of the molecular mechanisms of endothelial dysfunction as a cellular mechanism for vascular failure could provide novel therapeutic targets of cardiovascular diseases.

endothelial cell; barrier; microtubule

The regulation of vascular tone, vascular permeability, and thromboresistance is essential to maintain blood circulation and therefore tissue environments under physiological conditions. Atherogenic stimuli, including diabetes, dyslipidemia, and oxidative stress, induce vascular dysfunction, leading to atherosclerosis, which is a key pathological basis for cardiovascular diseases such as ischemic heart disease and stroke. We have proposed a novel concept termed “vascular failure” to comprehensively recognize the vascular dysfunction that contributes to the development of cardiovascular diseases.

Vascular endothelial cells form the vascular endothelium as a monolayer that covers the vascular lumen. The optimally placed healthy endothelial cells are not merely constituents of the vessel wall but are able to respond to physiological stress. The variety of endothelial cell functions that play important roles in the maintenance of vascular integrity includes the regulation of vascular tone, vascular permeability, vessel wall inflammation, and thromboresistance (Fig. 1). Atherogenic stimuli activate cell signaling and therefore modulate cellular function in endothelial cells. The interaction between endothelial cells and immune cells is augmented in response to atherogenic stimuli by an upregulated expression of adhesion molecules. Vascular smooth muscle function is also modified through the altered production of vasoactive substances by endothelial cells. Accordingly, the endothelial dysfunction that is induced by atherogenic stimuli is considered to play crucial roles as a cellular mechanism in the development of vascular failure.

Regulation of Vascular Tone by Endothelial Cells

Endothelial cells not only respond to but also produce and release vasoactive substances that relax or constrict blood vessels. Nitric oxide (NO), generated from L-arginine by the action of endothelial NO synthase (eNOS) in the presence of cofactors such as tetrahydrobiopterin, diffuses to the vascular smooth muscle cells and activates guanylate cyclase, which results in cGMP-dependent vasodilation (12). The endothelium also mediates the hyperpolarization of vascular smooth muscle cells via a NO-independent pathway, which increases K+ conductance and subsequently propagates the depolarization of vascular smooth muscle cells, maintaining vascular tone through the production of EDHFs (7).

Dysregulation of vascular tone by endothelial cells is characterized by decreased NO bioavailability that not only up-regulates the endothelial expression of adhesion molecules, which induces immune cell recruitment to vascular wall, but also promotes smooth muscle cell activation, which leads to proliferation and migration (28). A number of clinical studies have demonstrated that impaired NO-dependent vasodilation is closely related to atherosclerosis (21). It is well recognized...
that EDHF is able to partially compensate for the loss of NO-mediated vasomotor tone in the microcirculation, particularly in case of reduced NO bioavailability, depending on the types of vascular beds.

Epoxyeicosatrienoic acids (EETs) are postulated EDHFs. Cytochrome P-450 (CYP)2J2 is an epoxygenase that is expressed in vascular endothelial and smooth muscle cells and cardiomyocytes and metabolizes arachidonic acid to biologically active EETs (42). 11,12-EET, produced by CYP2J2, has anti-inflammatory properties such as the prevention of leukocyte adhesion to endothelial cells by inhibiting NF-κB, which stimulates the expression of proinflammatory gene products in endothelial cells (42). 5,6-EET, 8,9-EET, 11,12-EET, and 14,15-EET have vasodilatory effects by the polarization and relaxation of vascular smooth muscle cells. EETs have been reported to have antithrombotic effects by upregulating tissue plasminogen activator and antimigratory effects against vascular smooth muscle cells. EETs have been demonstrated to have positive effects against vascular failure.

The Permeability Barrier Created by Endothelial Cells

Vascular endothelial cells covering the luminal surface of blood vessels serve as an interface between circulating blood and surrounding tissues. Ions and water-soluble solutes have been shown to move across endothelial cells via paracellular pathways through intercellular gaps in addition to transcellular pathways. On the other hand, immune cells have been demonstrated to migrate across endothelial cells most likely through paracellular pathways (58). Organelles defined as cell-to-cell junctions composed of tight junctions (TJs) and adherens junctions (AJs) that are formed at cell-to-cell contact sites create a barrier by regulating the permeability of solutes and immune cells (14). Cell-to-cell junctions are formed by protein complexes that are composed of both transmembrane proteins and associating cytoplasmic proteins that link transmembrane proteins to the actin-based cytoskeleton (Fig. 2).

Vascular-endothelial (VE)-cadherin, a single membrane-spanning protein localized at AJs, is an adhesion molecule that binds with VE-cadherin expressing on the cell surface of adjacent cells in a homophilic manner. It has been shown that VE-cadherin is indispensable for the formation of endothelial cell-to-cell adhesion, which plays key roles in vascular development as well as in the maintenance of vascular integrity (10, 62). For the formation of TJs, a claudin that has four transmembrane domains and is linked to the actin-based cytoskeleton via zonula occludens (ZO)-1, ZO-2, and ZO-3 is essential (22). The claudin protein family is composed of 24 members in humans; claudin-1, claudin-3, claudin-5, claudin-12, and claudin-15 are expressed in endothelial cells (3, 25, 39, 64). Claudin-5 knockout mice have demonstrated that claudin-5 is essential for the size-selective barrier function of TJs in the brain endothelial cells that form the blood-brain barrier (41). On the other hand, it has been demonstrated that claudin-1 deletion using small interfering RNA increases TJ permeability in cultured human endothelial cells, suggesting that claudin-1 plays a pivotal role in TJ permeability regulation in endothelial cells from non-neural tissue (1). Junctional adhesion molecule (JAM), which belongs to the Ig superfamily and has a single transmembrane domain with two Ig-like domains in the extracellular portion, is also localized to TJs in endothelial cells (32).

The endothelial paracellular barrier remains leaky to facilitate the movement of solutes between blood and the surrounding tissues in the development of blood vessels as well as in the regeneration of damaged tissues. Even after formation of the vascular network is completed, vascular permeability is dynamically controlled to maintain the tissue environment. Localization and interactions with associating proteins are important for junctional components to properly function. Adhesion molecules are recruited to the plasma membrane by transport proteins through internalization and recycling. EFA6, which is an activating factor for the small GTPase Arf6, has been implicated in AJ assembly by regulating E-cadherin internalization by early endosomes (52a). Colocalization of claudin-16 with an early endosome marker, early endosome antigen-1, and late endosome-mediated transport of claudin-3 have been demonstrated (33, 40). It has also been shown that the early endosome is involved in claudin-1 localization in endothelial cells (1). The Rab family of small GTPases has been implicated in the regulation of vesicular transport, Rab13 and Rab3b localize at TJs and contribute to TJ assembly (33, 63). It has been demonstrated that Rab13 specifically mediates the continuous endocytic recycling of a TJ protein, occludin, to the cell surface (38). In addition, Rab13 is involved in the transport of claudin-1 from the cytosol to cell-cell junctions and regulates TJ assembly via PKA signaling (26). In endothelial cells, Rab5a regulates claudin-1 localization, which is an important determinant of TJ permeability (1). Thus, Rab GTPase-mediated transport and localization of junctional proteins have been implicated in the regulation of endothelial barrier function.
Relocalization of the VE-cadherin-catenin complex occurs in endothelial cells exposed to hypoxia-reoxygenation, which is blocked by eNOS overexpression in endothelial cells (4, 45). In cultured endothelial cells, hydrogen peroxide causes the disappearance of cadherin and occludin from cell-to-cell contacts (23, 24). Signaling mediated by ROS may play a role in the disorganization of junctions caused by hypoxia-reoxygenation. The involvement of Rab GTPase-mediated transport and localization of junctional proteins in the disorganization of junctions caused by hypoxia-reoxygenation has not been elucidated.

Previous studies have revealed the importance of the posttranslational modification of junctional proteins in the regulation of endothelial barrier function. The adhesion properties of VE-cadherin are dynamically regulated through phosphorylation and association with cytoplasmic proteins such as catenins, which link VE-cadherin with the actin cytoskeleton. Accumulating data have demonstrated that various signaling molecules are concentrated at AJs, including Src family tyrosine kinases, receptor tyrosine kinases, and protein tyrosine phosphatases (34). VE-cadherin mediates the localization of VEGF receptor 2 to endothelial AJs, which is essential for VEGF signaling. VE-protein tyrosine phosphatase, which binds with the cadherin-catenin complex, has been shown to be involved in the disruption of VE-cadherin-based AJs during the transmigration of leukocytes across endothelial cells (44). Thus, roles of AJs as a communication center have been widely noticed, but future studies are required to identify the specific substrates in AJ components for such kinases or phosphatases. Recent studies have suggested that junctional components are targets of “permeability-modulating” signals and that the phosphorylation/dephosphorylation of junctional proteins plays crucial roles in the regulation of TJ permeability (54). Serine/threonine phosphorylation/dephosphorylation of junctional proteins have been reported. G protein-coupled receptor signaling triggered by lysophosphatidic acid, which is secreted from activated platelets, or histamine has been shown to induce occludin phosphorylation on serine/threonine residues and concomitantly increase TJ permeability via a RhoA/Rho kinase-dependent or -independent pathway, respectively (18). It has been demonstrated that the hypercholesterolemia-induced increase in endothelial permeability against LDL, which is an initial step of atherosclerosis, is mediated possibly by the activation of Rho and that a 3-hydroxy-3-methylglutaryl CoA reductase inhibitor (statin) has an antipermeability effect by Rho inactivation (60). These data implicate RhoA/Rho kinase as a key regulator of the endothelial barrier. In addition to Rho, Cdc42 and Rac1, members of the Rho small GTPase family, participate in the regulation of the endothelial barrier by controlling the assembly of the actin cytoskeleton and therefore the localization of junctional proteins (65). Rac1 activation by the insulin-dependent phosphatidylinositol 3-kinase/Akt pathway has been shown to stabilize the endothelial barrier through the assembly of the actin cytoskeleton (15). As Rho is activated in downstream of Cdc42 and Rac1, Rho-dependent as well as Rho-independent pathways may be involved in the regulation of the endothelial barrier.

The ubiquitin-proteasome system plays important roles in the regulation of protein modification and degradation. Target proteins are ubiquitinated by ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and E3 ubiquitin ligase. Ubiquitination of plasma membrane proteins is a mechanism to control their endocytic trafficking by promoting their interactions with cytosolic proteins that contain ubiquitin-binding domains (17). Traweger et al. (56) demonstrated that itch, an E3 ubiquitin ligase, interacts with the TJ protein occludin and is involved in the ubiquitination of occludin. In addition, cAMP induces itch expression, which triggers occludin ubiquitination, resulting in TJ disruption (30). On the other hand, the ubiquitination of claudins has not yet been reported. In epithelial cells, hepatocyte growth factor and the activation of
Src induced cell scattering through junctional dissociation and increased the solubility of protein components of TJs and AJs, which was prevented by proteasome inhibitors (57). Hypoxia or hypoxia mimetic ATP depletion caused a dissociation of cell-to-cell contacts and decreased E-cadherin turnover in a proteasome-dependent manner (6). Thus, stability of the junctional proteins mediated by the proteasome may be involved in the regulation of junctional permeability enhanced by hypoxia or angiogenic signaling.

Inflammatory mediators, such as thrombin, impair the endothelial barrier by proteolysis of VE-cadherin (49). Thrombin-induced shedding of the VE-cadherin ectodomain by a disintegrin and metalloproteases (ADAM)-10 followed by cleavage by \( \gamma \)-secretase disrupts the endothelial barrier against solutes and migrating T cells (51). The accumulation and activation of proteases such as matrix metalloproteinases have been documented. Shedding of junctional transmembrane proteins by proteases seems to contribute to the increased vascular permeability induced by inflammatory stimuli in the development of atherosclerosis.

Direct evidence linking TJ protein and vascular failure has been provided for JAM. It has been reported that the gene and protein expression of JAM-A are upregulated in atherosclerotic lesions in atherosclerosis-prone apolipoprotein E (apoE)\(^{-/-}\) mice and humans (2). JAM-A knockout in atherosclerosis-prone apoE\(^{-/-}\) mice decreased neointimal lesion formation accompanied with monocyte infiltration (67). Also, JAM-C blockade with antibody prevented neointimal hyperplasia and monocyte infiltration induced by wire injury of the endothelium in apoE\(^{-/-}\) mice (52). Impaired endothelial barrier function caused by atherogenic stimuli facilitates the infiltration of immune cells as well as plasma contents into the vascular wall. Infiltrated plasma contents, including modified LDL, and substances produced by infiltrated immune cells, such as cytokines and chemokines, alter smooth muscle function, which is able to contribute to the development of atherosclerosis.

The Endothelial Microtubule as a Target of Vascular Failure

Vasoactive substances as well as mechanical stress triggered by hemodynamic forces, such as mechanical stretch and shear stress, stimulate endothelial cell signaling. Accordingly, chronic changes in vasoactive substances and hemodynamic...
forces caused by hypertension and metabolic disorders such as diabetes and dyslipidemia induce endothelial dysfunction, which leads to vascular failure. Microtubules, one of the key cytoskeletal fibers in endothelial cells, regulate numerous cellular functions that contribute to the maintenance of vascular integrity (27).

The endothelial cell functions controlled by microtubules include cell shape, mitosis, intracellular transport, adhesion, and migration. Endothelial migration is required for angiogenesis and reendothelialization in wound repair and after angioplasty or coronary artery bypass procedures (35). Microtubules polymerized with heterodimers formed by an α-tubulin and a β-tubulin are one of the major components of the cellular cytoskeletal system. Microtubules show dynamic instability and an interconversion of assembly and disassembly (20, 36, 37). Posttranslational modifications of tubulin, such as deacetylation, detyrosination, phosphorylation, and polyglutamation, are involved in the regulation of microtubule dynamics and microtubule-based cellular functions. Acetylation of α-tubulin on Lys^{40} plays important roles in the regulation of microtubule stability and structure (19, 61). Microtubules contain acetylated α-tubulin in quiescent cells. In contrast, tubulin is deacetylated in the leading edge of migrating cells (29, 47). Hyperacetylated microtubules decrease microtubule dynamics, increase microtubule stability, and decrease cell migration (55). Thus, the tubulin acetylation level, which is a key determinant of microtubule function, changes in response to extracellular stimuli, and the regulatory mechanism of tubulin acetylation/deacetylation by acetylase/deacetylase has attracted much attention.

Endothelial cells are exposed to mechanical forces, such as cyclic stretch and shear stress. Excessive strain is involved in the pathogenesis of atherosclerosis. Previous reports (8, 9, 46, 66) have shown that mechanical stretch increases ROS generation, induces the reorganization of integrins, and regulates cytoskeletal reorganization in endothelial cells (Fig. 3). On the other hand, mechanical forces, such as cyclic stretch and shear stress, modulate the vascular renin-angiotensin system. It has been reported that cyclic stretch induces the release of ANG II in human endothelial cells (11). A number of studies have revealed that ANG II plays important roles in the development of cardiovascular diseases (50). The beneficial effects of ANG II type 1 receptor antagonists for the prevention and treatment of cardiovascular diseases have been well established. Recently, it has been demonstrated that ANG II as well as cyclic stretch trigger microtubule reorganization associated with tubulin deacetylation in endothelial cells (16). Therefore, cyclic stretch is considered to synergistically augment the effects of ANG II. As an ANG II type 1 receptor antagonist inhibited ANG II-induced microtubule reorganization in endothelial cells, vascular failure induced by ANG II could be partly attributable to endothelial dysfunction mediated by microtubule reorganization (16). The molecular mechanisms responsible for tubulin acetylation/deacetylation in endothelial cells are not yet fully understood. Histone deacetylase 6 (HDAC6) and sirtuin 2 (SIRT2) are candidate tubulin deacetylases that regulate microtubule function in endothelial cells (16, 59). Further studies are necessary to reveal the involvement of endothelial HDAC6 and SIRT2 in vascular failure. Gene silencing selectively in endothelial cells and conditional knock-out of these genes in mouse models of vascular failure such as atherosclerosis need to be studied. Also, selective inhibitors for these deacetylases could be potential therapeutic agents for vascular failure.

Concluding Remarks and Future Directions

A number of studies have revealed a variety of endothelial cell functions. Dysfunction of endothelial cells promotes the dysfunction of smooth muscle cells as well as immune cells, which leads to vascular failure. Dysfunction of endothelial cell-to-cell junctions, which disrupts the endothelial barrier and increases vascular permeability, appears to be involved in the pathogenesis of vascular failure, including atherosclerosis. Recent progress has contributed to the understanding of the molecular architecture of endothelial cell-to-cell junctions that create paracellular barriers to restrict movements of solutes and immune cells across endothelial cells. Biochemical changes, including the phosphorylation/dephosphorylation of junctional proteins that determine localization and interactions with associating proteins, may play a central role in the control of junctional permeability. The kinases/phosphatases specifically responsible for the phosphorylation/dephosphorylation of junctional proteins would be a key regulator of junctional permeability. Intervention to the cell signaling that controls the vascular permeability by pharmacological (kinase/phosphatase inhibitors) and genetic (mutant kinases/phosphatases) methods are proposed as novel therapeutic strategies for dysregulated vascular permeability in vascular failure. Previous studies have shown that the cytoskeleton (such as the microtubules), which changes biochemical properties in response to chronic mechanical and metabolic stress, is a target of atherogenic stimuli. Modifications in microtubule properties contribute to the endothelial cell dysfunction that leads to vascular failure. Accordingly, analysis of the molecular basis for endothelial dysfunction would lead us to identify novel therapeutic targets for vascular failure in cardiovascular diseases.

DISCLOSURES

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