4. The critical role of endothelial cells in the development of inflammatory diseases

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Abstract. Vascular endothelial barrier dysfunction occurs early and sustains persistently in the progression of many pathological process, playing a critical role in the development of trauma injury and inflammatory diseases. Various mechanisms have been implicated in the pathogenesis of edema, including the increases of filtration pressure, the enhancement of microvascular permeability, as well as the blockage of lymphatic drainage. This review focus on the pathogenesis of microvascular hyperpermeability by emphasizing the role of endothelial barrier dysfunction during the development of tissue edema. The basic structure and the functional regulation of endothelial barrier are introduced at first. Then, based on the development of recent research, the features, as well as the pathogenesis of vascular barrier dysfunction of endothelial cells are presented and discussed.

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Introduction

Endothelial cell is a type of squamous cell that lines the interior surface of heart, blood vessels and lymphatic vessels. These cells align and elongate in the direction of blood flow with a single layer, covering 400 m² ~ 500 m² of the vascular luminal surface. A human adult has about $10^{12}$ vascular endothelial cells, which weigh about 1.5 kg and are equivalent about the liver weight. Vascular endothelial cells are the lining tissue of blood vessel wall, and provide a smooth surface for the normal blood flow. Being a semipermeable membrane, endothelial cells serve as a barrier to regulate metabolite exchange of intra- and extra-vascular spaces. Endothelial cells are also an active metabolic and endocrine organ that play an important role in the modulation of circulatory functions. As an important turning point in the development of the modern vascular biology, endothelial cells were first successfully isolated from the umbilical vein by Jaffé et al in 1973 [1]. Endothelial biology entered a new stage since then and much has been accomplished in the study of physiological functions and pathological alterations of endothelial cells [2]. The discovery of endothelium-derived relaxing factor (EDRF), or nitric oxide (NO), by Furchgott et al in 1980 facilitated the research of vascular endothelial functional regulations in various related diseases [3]. In the initiation and development of vascular-related diseases, such as atherosclerosis, hypertension, diabetes, ischemic diseases, congenital heart disease, stroke, thrombosis and even tumor, endothelial cells bear the brunt of stimulation of oxidation products, inflammatory mediators and other large number of active factors in the blood, and thus the activation, injury and dysfunction of endothelial cells become the early and critical pathological events of those diseases.

1. The specificity of endothelial cell

Endothelial cells are different from other tissue cells in molecular expressions and cellular functions. And even endothelial cells themselves vary in different tissues and organs. The protein expression of endothelial cells is also various in different developmental stages of the cells and in different species.

1.1. The specificity of molecular expression in endothelial cell

Endothelial cells have specific molecular markers. Compared with other tissue cells, endothelial cells specifically express a class of molecules which determine the characteristics of endothelial cell function. These specifically expressed molecules are often used as tools to identify endothelial cells and
blood vessels in cytology and histology. The mature human vascular endothelial cells positively express endothelial nitric oxide synthase (eNOS), vascular endothelial-cadherin (VE-cadherin), vascular endothelial growth factor receptor 2 (VEGFR2), chemokine receptor (CXC, CXCR4), von Willebrand factor/Factor γ-related antigen (vWF/γ: Ag), CD31, CD34, and CD145. Among them, Weibel-Palade bodies (WP bodies), mainly composed of vWF/γ: Ag, are a special secretory rod-like organelles of vascular endothelial cell and contain a variety of bioactive molecules (Tab. 1). When endothelial cells are stimulated or injured, those molecules could be rapidly released from WP bodies and then participate in the regulation of hemostasis, inflammation, angiogenesis and other physiological functions [4]. The concentration of vWF in normal human plasma, is about 10 mg/L. And von Willebrand disease (vWD) occurs when the availability and quality of vWF are insufficient to achieve its normal hemostatic function in which vWF gene suffers substitution, deletion, insertion, or the early formation of transcription termination signal [5].

**Table 1.** The main bioactive molecules in WP bodies of endothelial cells.

<table>
<thead>
<tr>
<th>Name</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>von Willebrand factor/Factor γ-related antigen (vWF/γ:Ag)</td>
<td>hemostasis</td>
</tr>
<tr>
<td>P-selectin</td>
<td>cell adhesion</td>
</tr>
<tr>
<td>CD63/lamp3</td>
<td>P-selectin cofactor</td>
</tr>
<tr>
<td>interleukin-8 (IL-8)</td>
<td>chemotaxis</td>
</tr>
<tr>
<td>endothelin-1 (ET1)</td>
<td>vasoconstriction</td>
</tr>
<tr>
<td>angiopoietin-2 (Ang-2)</td>
<td>angiogenesis</td>
</tr>
<tr>
<td>α-1,3--fucosyltransferase VI (α-1, 6FucT VI)</td>
<td>immune adhesion</td>
</tr>
<tr>
<td>osteoprotegerin (OPG)</td>
<td>vascular calcification</td>
</tr>
<tr>
<td>eotaxin 3</td>
<td>chemotaxis</td>
</tr>
</tbody>
</table>

1.2. The specificity of endothelial function

Endothelial cells play essential role in many physiological functions and in the development of inflammatory diseases [6], which will be presented in the later section of this chapter. One of the special function for endothelial cell identification is the uptake of acetyl-low-density lipoprotein (Ac-LDL), which could only be uptaken by endothelial cells and macrophages. Using this characteristic, combined with the adherent cell growth and
morphological features of the cells, Endothelial cells could be identified in culture condition by observation of the uptake ability of fluorescently labeled Ac-LDL (Dil-Ac-LDL) by adherent cultured cells. Another functional specificity of endothelial cells is the tendency of tube formation in vitro multi-cell culture and they are even easier to fuse into tubes in three-dimensional media.

1.3. Heterogeneity of endothelial cells

The structure and functions of vascular endothelial cells show significant phenotypic varieties in different developmental stages of the body, in different organs and even in different location of one blood vessel. This manifestation is termed as heterogeneity. Endothelial cells also have significant heterogeneity in physiological and pathological condition.

1.3.1. Tissue heterogeneity of endothelial cells

Using DNA microarray, Chi et al elucidated the heterogeneity of endothelial cells by illustrating the gene expression profiles in endothelial cells from different types of blood vessels and different anatomical sites under the same culture conditions [7]. The results showed that endothelial cells from different tissues have corresponding specific gene expression profiles, which determine the further differentiation of endothelial cells, and affect their unique adaption to physiological and pathological environment. For example, cerebral vascular endothelial cells express abundant tight junction proteins, and endothelial cells without fenestra are important structural basis of the blood-brain barrier. Liver sinusoidal endothelial cells in metabolically active liver constitute a continuous but sieve-lined lining, conducive to transport and exchange of a variety of substances. Glomerular endothelial cells are highly differentiated, forming numerous pores of different sizes, which are responsible for special filtering functions.

1.3.2. Functional heterogeneity of endothelial cells

The heterogeneity of endothelial cells in histology leads to the diverse endothelial functions in cellular dynamics, morphology, antigen expression, secretion of active mediators (such as collagen and prostaglandin), as well as insulin response in different parts of vessels and organs. Moreover, different tissues, even different parts of microvascular blood vessels in the same tissue show specialized functions. For example, the endothelial cells at arterial end mainly produce and release NO through eNOS, which relaxes the vascular smooth muscle cells and regulates the blood flow. The continuous
endothelial cells in capillary can be manipulated, in particular, to prevent the protein leaking from the blood through the intercellular junctions. The small concave vesicle structure (Caveolae) in capillary endothelia also has the function of controlling the leakage of plasma protein and fluid, while the most typical inflammatory features of albumin leakage and leukocytes migration occur at venular end. Although leukocytes could be stagnant at the location of capillary, the expression of adhesion molecules, the rolling and adhesion of platelets and leukocytes, and the infiltration of inflammatory cells occur at the venular end [8, 9].

1.3.3. The development of endothelial cell heterogeneity

Aird et al’s studies have shown that microenvironmental changes and epigenetic modifications are important mechanisms leading to endothelial cell heterogeneity in the process of vascular differentiation and development. Endothelial cell experiences changes of tissue microenvironment such as pH, oxygen partial pressure, blood flow, shear stress, and reacts through signal transduction processes, resulting in diversities in molecular expression and cell function, which is confined to the related endothelial cells and could not be passed to endothelial cells lacking these stimulatory signals. The more they differentiated, the higher heterogeneity of endothelial cells caused by microenvironment. Epigenetic modifications caused by DNA methylation, histone methylation and histone acetylation lead to endothelial cell heterogeneity in gene expression. These differences can be passed to the offspring cells through the mitotic process. As the endothelial cells differentiate, epigenetic modification shows less importance to their heterogeneity, while microenvironment comes into prominence [10, 11].

2. The functions of endothelial cells

Vascular endothelial cells line the inner wall of the blood vessels, providing a smooth surface for normal blood flow in the vessels. Vascular endothelial cells are also very active in metabolic and endocrine functions, serving as blood-borne signal monitors, transducers, and regulators. Endothelial cells synthesize and release, as well as metabolize a variety of active substances for the regulation of vascular tone, coagulation, fibrinolysis, cell adhesion and angiogenesis through the perception and integration of alteration in physical and chemical environment signals in the blood. Endothelial cell functions are mainly summarized as the following aspects.
2.1. Lining function

Blood vessels are the container of circulating blood. The inner lining of vascular networks is formed by a single layer of endothelial cells, which provide smooth and antithrombotic surface for the blood flow by synthesizing and secreting related substances, such as thrombomodulin (TM), tissue factor pathway inhibitor (TFPI), tissue plasminogen activator (t-PA), and prostacycline, as well as heparinoid, to inhibit coagulation and platelet adhesion.

2.2. Material exchange

Endothelial cell is the structural basis for the exchange of blood and tissue substances, the distance between each tissue cell and its adjacent capillaries is no more than the length of 3 to 4 cells, only in this way can the cell achieve effective material exchange. As a selective permeability membrane, the endothelia serve as barrier by separating the blood inside the vessels and the tissue outside the vessels. The achievement of material exchange function of blood circulation depends on the regulation of vascular endothelial cells. In a certain range, the exchange of substances through the endothelial cells is passive, such as water and small molecules weigh solutes, including electrolytes, metabolic substrates and products, which filter through endothelial monolayer according to the concentration gradient. O₂, CO₂ and other lipophilic substances could easily pass through the blood vessels. Intercellular junctions of the vascular endothelial cells prevent the leakage of a variety of plasma components in the blood vessels effectively. Those high molecular weigh substances transport through active transportation of the vesicles of endothelial cells, or temporarily transendothelial channels formed by fusion of numerous vesicles. Endothelial cells of different tissues and organs, selectively transport the macromolecular substances or even blood cells through the endothelial cell barrier into the surrounding tissue, according to the needs of physiological functions.

2.3. Barrier function and vascular permeability regulation

As the understanding of endothelial cells and intercellular structures has reached to molecular level, the concept of endothelial permeability has changed from a sieve with pores to a wall with channels. These channels have the ability to discriminate specific chemicals, so as to realize their selective permeation function not only from the size but also from the
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chemical properties. The transportation of the substances can be achieved by transcellular pathways (intracellular vesicle) and paracelluar pathways (intercellular path) respectively. Transcellular pathways are those in which a transporter passes a single cell through an intracellular vesicle, with the molecule being transported in "solid state" (bound to vesicle membrane protein) or in "liquid state" (dissolved in vesicle fluid). Normally, macromolecular substances such as albumin, are transported mainly in the exchanging vessel, the capillaries, through the transcellular pathways. Paracellular pathways refer to the diffusion of transported substances through adjacent cells via the putative channels formed by intercellular junctions, which include the active diffusion of phospholipid complex, receptor-mediated shuttle movement and cell phagocytosis transport. During the development of inflammation, endogenous and exogenous stimulants activate endothelial cells through cellular signal transduction pathways, leading to the opening of the intercellular gap and the subsequent leakage of albumin and migration of leukocytes in the posterior venules and venules through paracellular pathways.

The maintenance of endothelial cell barrier function depends on the balance between the integrity of the cell-cell junction and the basement membrane connection with cell contractile force. Its structural and functional changes play an important role in the process of vascular permeability. The intercellular junctions between endothelial cells refer to the connection of cells from the luminal surface to the basement, mainly composed of tight junctions and adherens junctions. The basolateral side of endothelial cells form another cell-matrix adhesive junction with the basement membrane through integrins.

Composed by integral membrane proteins and cytoplasmic proteins, tight junctions formed a primary barrier to the diffusion of solutes through intercellular space. The tight junction proteins, including transmembrane proteins occludin, claudin-1, claudin-2, claudin-5, and cytoplasmic banding proteins ZO-1, ZO-2, ZO-3, cingulin, rab13, create a boundary between the apical and the basolateral plasma membrane domains. These tight junction molecules are directly and indirectly linked to a variety of intracellular proteins, especially the scaffold proteins. The tight junction of endothelial cells is in a relatively closed state compared to adhesive junctions. One of the major functions of tight junction is to act as a selective intercellular barrier to regulate the filtration of various molecules and ions. The other function of tight junction is to serves as a fence for bilateral cytoplasmic
protein to guarantee that the proteins and lipids on top of the cell would not confound with those on basement membrane.

Adherent junction of endothelial cells centers on transmembrane cadherins, the calcium-dependent adhesion molecule, which connect with catenins in cytoplasm. Cadherin is a generic term for transmembrane family proteins and is a cell-cell adhesion molecule containing 720 to 750 amino acids. The major subfamilies include N-, P-, R-, B- and E-cadherins. The distribution of cadherin is tissue-specific. Vascular endothelial cadherin (VE-cadherin) specifically refers to calcium-dependent adhesion molecules on vascular endothelial cells. Cadherins form intercellular homodimers with adjacent cell, linking to each other through the extracellular amino terminus, and then connect to the cytoplasmic protein catenins through the carboxyl terminus of the cells. The cadherins further interact with cytoskeletal proteins such as actin. Cadherin bines with catenin β or γ subunit (also known as plakoglobin) at first, and then links with actin through the catenin α subunit. The function of cadherins depends on the presence of calcium, which protect cadherins from proteolytic enzymes. In the absence of extracellular calcium, cadherins will be degraded, leading to the loss of adherent attachment, and even destruction of multicellular tissue. Changes in intracellular or extracellular calcium concentration can affect the connection of cadherin with each other in adjacent cells, resulting in cell gap formation and barrier dysfunction. The disruption of the adhesive attachment structure can itself increase endothelial permeability, even without the contraction of endothelial cells.

The adhesion between endothelial cells and basement membrane is mainly composed of integrins and their related molecules. Integrin is a family of cell surface receptors that bind with extracellular matrix proteins such as fibronectin (FN) vitronectin, collagen, and laminin (also called plate protein). The binding site for the receptor and ligand is a short RGD (arginine-glycine-aspartic acid, Arg-Gly-Asp) sequence peptide. Integrin is a group of tetrameric glycoproteins, which has been found to have at least 16 α and 8 β subunits, constituting at least 20 different receptor subtypes. Integrin directly binds to cytosolic proteins such as talin and α-actinin through β-subunit of cytoplasmic region, and talin protein is in turn linked to paxillin and vinculin, and finally linked to actin. Alpha-actinin is directly attached to actin. Together, those components form a focal adhesion, also known as adhesive plaques, providing the "anchored” spots for the cell in the basement membrane (Fig. 1).
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Figure 1. The schematic image of simplified structure base of endothelial barrier. By interacting with cytoskeleton, tight junction (TJ) and adherens junction (AJ) are intercellular junctions crucial for endothelial barrier. Focal adhesions mediate the contact of endothelium and extracellular matrix.

In addition to compose the intercellular junctions, those macromolecular complexes of tight junctions, adhesive junctions, and cell-matrix connections also participate in cellular signal transduction, leading to changes in gene expression and cellular behavior, such as the alterations of morphology, motility, and even migration and proliferation of endothelial cells [12].

2.4. Vascular tone modulation

Endothelial cell modulates vascular tone not only through the passive responses to neurohumoral factors and mechanical stimulation, but also by the initiative synthesis and secretion of vasoactive substances, including diastolic as well as vasoconstrictive mediators. Working with neurotransmitters and active substances from the blood stream, endothelial cells are crucial in modulating smooth muscle cell relaxation or contraction to maintain a certain degree of vascular wall tension or change the diameter of blood vessels, and to
Table 2. The main active substance derived from endothelial cells.

<table>
<thead>
<tr>
<th>Category</th>
<th>Name</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vasodilation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nitric oxide (NO or EDRF)</td>
<td>Relaxing VSMC, inhibiting platelet aggregation, affecting vascular permeability</td>
</tr>
<tr>
<td></td>
<td>Prostacyclin (PGI$_2$)</td>
<td>Relaxing VSMC, inhibiting platelet aggregation</td>
</tr>
<tr>
<td></td>
<td>Endothelium-dependent hyperpolarizing factor (EDHF)</td>
<td>Relaxes VSMC</td>
</tr>
<tr>
<td></td>
<td>Calcitonin generelated peptide (CGRP)</td>
<td>Relaxing VSMC</td>
</tr>
<tr>
<td><strong>Vasoconstriction</strong></td>
<td>Endothelin/endothelin - converting enzyme system (ET/ECE)</td>
<td>Constricting VSMC, promoting VSMC proliferation, affecting vascular permeability</td>
</tr>
<tr>
<td></td>
<td>Cyclooxygenase endothelium - dependent contraction factor (EDCF)</td>
<td>Constricting VSMC</td>
</tr>
<tr>
<td></td>
<td>Angiotensins</td>
<td>Constricting VSMC</td>
</tr>
<tr>
<td></td>
<td>Platelet derived growth factor (PDGF)</td>
<td>VSMC, increasing vascular permeability, promoting VSMC proliferation</td>
</tr>
<tr>
<td><strong>Anticoagulation and fibrinolysis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tissue factor pathway inhibitor (TFPI)</td>
<td>Preventing thrombin generation, inhibiting coagulation and inflammatory response</td>
</tr>
<tr>
<td></td>
<td>Antithrombin-β (AT)</td>
<td>Form complex with thrombin, inhibiting coagulation, promoting the synthesis of prostacyclin</td>
</tr>
<tr>
<td></td>
<td>Tissue-type plasminogen activator (tPA)</td>
<td>Anticoagulant, promoting fibrinolysis</td>
</tr>
</tbody>
</table>
### Table 2. Continued

<table>
<thead>
<tr>
<th>Anticoagulation and fibrinolysis</th>
<th>Protein C system (PC)</th>
<th>Activated protein C hydrolyzes Va and VIIIa, anti-inflammatory and anti-apoptosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thrombomodulin (TM)</td>
<td>Binding and inactivating thrombin as a receptor, activating TFPI and protein C.</td>
</tr>
<tr>
<td></td>
<td>Heparan sulfate proteoglycan (HSPG)</td>
<td>Enhancing the activity of AT, Promoting leukocyte adhesion and migration, proinflammatory</td>
</tr>
<tr>
<td>Procoagulant anti-fibrinolysis</td>
<td>Plasminogen antivator inhibitor (PAI)</td>
<td>Binding with tPA, promoting thrombosis</td>
</tr>
<tr>
<td></td>
<td>Von Willebrand factor / factor VIII related antigen (vWF/FvAg)</td>
<td>Promoting platelet aggregation and thrombosis</td>
</tr>
<tr>
<td></td>
<td>Platelet-activating factor (PAF)</td>
<td>Promoting platelet aggregation, vasodilatation, increasing vascular permeability</td>
</tr>
<tr>
<td></td>
<td>Thrombospondin-1(TSP-1)</td>
<td>Promoting platelet activation and aggregation and vascular smooth muscle cell proliferation, inhibiting endothelial cell growth</td>
</tr>
<tr>
<td>Angiogenesis</td>
<td>Vascular endothelial growth factor receptor (VEGFR)</td>
<td>Promoting endothelial cell proliferation, increasing vascular permeability, proinflammatory</td>
</tr>
<tr>
<td></td>
<td>Angiopoietin 2 (Ang-2)</td>
<td>Antagonizing endothelial proliferation, increasing vascular permeability, proinflammatory</td>
</tr>
</tbody>
</table>
regulate the blood flow of tissue and organs. In particular, vasoactive substances derived from endothelial cells play more important roles in regulation of micro-vascular tone than in that of macro-vessel tension (Tab. 2).

2.5. Manipulating coagulation and fibrinolysis process

All vascular endothelial cells have anticoagulant function with anti-thrombotic properties under physiological conditions. Intact endothelial cell monolayer provides an anticoagulant interface and inhibit platelet activation and aggregation through the production and adsorption of a variety of anticoagulant substances. Under the pathological state, the damage of endothelial cell itself, the production of a variety of coagulation factors, the expression of adhesion molecules, and the reduction of anticoagulant synthesis together, can mediate platelet and leukocyte adhesion and aggregation, which promote hemostasis, thrombosis and the occurrence and development of inflammation. Endothelial cells are critical to the dynamic balance not only between clotting and anticoagulation, but also between fibrinolysis and antifibrinolysis by producing various factors for promoting or inhibiting fibrinolysis under physiological or pathological situation [13, 14] (Tab. 2).

2.6. Regulation of angiogenesis

Angiogenesis is the process of new capillary formation in tissues that accompanies the embryonic development and individual growth. The first specific marker expressed in primary endothelial cells is the receptor Flt-1 of vascular endothelial growth factor (VEGF). However, in the adult stage, the generation of new blood vessels is rare, except wound healing, female physiological cycles. Pathological angiogenesis happens in several diseases such as cancer, atherosclerosis, and diabetic proliferative retinopathy. Under the situation of hypoxia and the stimulation of other related metabolic factors, hypoxia inducible factor (HIF) activates the transcription and translation of VEGF and promotes the expression of VEGF receptors in endothelial cells, mediating the activation, migration and proliferation of endothelial cells. Endothelial cell-specific Tie2 is involved in the proliferation of endothelial cells and the formation of new blood vessels under the effect of Angiopoietin-1 and -2, (Ang-1 and Ang-2). The process of angiogenesis mainly includes the following steps: 1) the activation of endothelial cells and pericytes and the increased of vascular
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permeability; 2) the formation of endothelial tip cell and the degradation of vascular basement membrane; 3) the proliferation and differentiation of stalk cells immediately after the tip cells; 4) vascular tube formation and basement membrane reconstruction; 5) capillary network formation and angiogenesis process recession. In addition, endothelial cells secrete a variety of bioactive substances and specific receptors to inhibit or promote vascular smooth muscle cell proliferation respectively (Tab. 2), and finally affecting the vascular structure and maturation.

2.7. The metabolic function of bioactive substances

In addition to synthesis and release of multiple active substances, vascular endothelial cells also uptake and transform or inactivate active substances from blood circulation or locality. Endothelial cells can uptake arachidonic acid from blood and transform it to prostacyclin 2 (PGI2) by cyclooxygenase and prostaglandin synthetase. PGI2 has the functions of relaxing vascular smooth muscle cells and inhibiting platelet aggregation. Angiotensin I converting enzyme (ACE) in endothelial cells, particularly in pulmonary vascular endothelia, converts blood inactive precursor angiotensin I into active angiotensin II which is potent vasoconstrictor. Endothelial cells can also uptake and inactivate amine substances such as catecholamines, 5-hydroxytryptamine and histamine, as well as lipids such as prostaglandins and other substances. Vascular endothelial cells maintain a certain concentration of local active substances in a certain ratio through these metabolic functions, which play important role in regulating blood circulation, maintaining body homeostasis, and implementing further physiological functions.

2.8. Immunoregulatory function of endothelial cells

Different from the classical immunomodulatory cells, endothelial cells perform the immunoregulatory function in multiple ways [15]. Endothelial cells can either limit or promote the migration of immune cells, such as T cells and dendritic cells, from the blood vessels to the tissues for the induction or suppression of immune response. These regulatory functions can maintain the health of the body in the physiological circumstances, and might also lead to diseases in the pathological state. For example in the blood-brain barrier, endothelial cells form a barrier to suppress immune cells into the brain tissue in order to maintain the immune privilege of the
central nervous system. But the immunosuppressive effect on monocytes make them unable to enter the nerve tissue to remove the deposited amyloid protein, which is one of the important pathogenesis of Alzheimer's disease. Pathogens and toxins, such as LPS, can activate endothelial cells, resulting in the increased secretion of adhesion molecules, cytokines and chemokines, so as to recruit immune cells and to enhance natural immune response, which is the first defense line against bacteria, viruses and other invasions. However, the over activation of endothelial cells may also result in excessive immune inflammatory responses and diseases. As defined in The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) [16], sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. The pathogen and toxin-induced over-activation of endothelial cells play a crucial role in this “dysregulated host response”, leading to the tissue damage and organ dysfunction [17].

Theoretically, endothelial cells are the ideal place to recruit and activate tumor immune cells. Endothelial cells can also enhance the activation of immune cells during the migration process. But in fact, most of the endothelial cells in tumor tissue fail to execute this tumor defense function because endothelial cell immune function is adjusted under the tumor microenvironment. For example, the expression of adhesion molecules in endothelial cells is decreased, the tolerance to T cells is enhanced, and the suppressive deviation for immune cells is also induced by endothelial cells. These changes in endothelial functions result in not only the inhibition of the immune response to the tumor, but also the promotion of tumor proliferation.

2.9. The function of endothelial glycocalyx

Endothelial glycocalyx is a 0.2 μm-0.5 μm thick gel-like layer mainly consisted of glycosaminoglycans (GAGs) such as proteoglycans and glycoproteins. The glycocalyx lines the luminal membrane of the endothelial cells and provides not only a natural physical barrier, but also a critical signalling platform to integrate the extracellular haemodynamic forces and chemical signalling for determining the fate of endothelial cells and vascular diseases. The glycocalyx mediates several key physiological processes such as the vascular barrier function, hemostasis, leukocyte and platelet adhesion, the transmission of shear stress to the endothelium and anti-inflammatory and antioxidant defenses [18].
3. The manifestations of EC functional alteration

The alterations of endothelial cell functions can be described in two ways, one is endothelial activation and the other is endothelial dysfunction. Endothelial activation is an adaptive change in cells. Responding to the changes of hemodynamics and metabolism, and the stimulation of cytokines, endothelial cell is activated through NF-κB-based signaling pathways, which induce gene transcription and protein expression, leading to the increase synthesis and secretion of various adhesion molecules in endothelial cells. Endothelial dysfunction is a non-adaptive changes, which is usually observed in situations of oxidative stress, hyperlipidemia and invasion of pathogens and toxins, resulting in a variety of endothelial cell dysfunctions, and even apoptosis and necrosis. The difference between activation and dysfunction of endothelial cells is mainly due to the difference of description angle. They all lead to vasoconstriction, platelet aggregation, leukocyte adhesion, low density lipoprotein oxidation and vascular smooth muscle cell proliferation. Endothelial activation and dysfunction are the important landmark in the development of pathological process of sepsis and other inflammatory diseases [19]. The acute activation and functional damage of endothelial cells play critical roles in microvascular dysfunction, leading to dysfunction of endothelium-dependent vasodilation, coagulation disorders, tissue edema, and even shock and organ failure.

3.1. Endothelium-dependent vasomotor dysfunction

In the early stages of hypoxia, ischemia and lower shear stress, the first change in endothelial cells is the functional impairment. The characteristic manifestations of endothelial cell dysfunction are the tamper of endothelium-dependent vasodilatation and the enhancement of vascular constriction due to the decrease of the constitutive vasodilators, such as NO, and the increase production of vasoconstrictors, such as endothelin (ET) in endothelial cells. In the later stages of ischemic hypoxia, severe trauma, sepsis, and other critical illness, the structure damage of endothelial cells results in the exposure of vascular smooth muscle cells to the blood stream and this is one of the major reasons for low vascular reactivity.

3.2. Coagulation dysfunction

Inflammatory mediators interact with endothelial cells and induce a significant coagulating status. The synthesis of anticoagulants thrombomodulin (TM) and heparinoids are decreased. The expression of
coagulants tissue factor and plasminogen activator inhibitor-1 (PAI-1) are increased. The activated endothelial cells also attract platelets, neutrophils and mononuclear macrophages and subsequently enlarge the coagulating effects. The activation of endothelial cell causes the disorganization of phospholipid layer on the cell surface, providing an interface for platelet adhesion. vWF released from endothelial cells could interact with platelet, promoting the coagulation cascade. Endothelial cell apoptosis itself is one of reason for its coagulating effects.

3.3. Increase of vascular permeability

The increase of vascular endothelial permeability is an early pathologic event of inflammation-related diseases and is a major feature of microcirculatory disturbances in conditions such as sepsis, burn and trauma. The loss of body fluids due to increased vascular permeability is an important reason for low blood volume and hypotension. Tissue edema is the main mechanisms for the development of organ dysfunction. The dilatation of blood vessels and the blood stasis during inflammation lead to the increase of hydrostatic pressure in venules and accelerate the leakage of fluid. The major reason for hyperpermeability in microvessels is the dysfunction of vascular endothelial barrier. The increase of endothelial permeability in the inflammatory state is mainly manifested in the opening of intercellular junctions due to the disarrangement of junctional proteins. The contraction of endothelial cell will further widen the intercellular gaps, resulting in endothelial hyperpermeability and the leakage of macromolecules through this paracellular pathway, and ultimately the formation of edema.

For example, burn-induced edema is a typical pathological event mainly due to the disruption of endothelial barrier and the increase of vascular permeability that happens not only in the location of burn insult but also in distal organs and tissues. The rapid release of pro-inflammatory mediators from injured tissue, as well as from activated endothelia and neutrophils exert this enhancement of vascular permeability by affecting the structure of intercellular junctions of endothelial cells. In our research of burn-induced edema, the crucial roles of junctional structure and endothelial barrier function were elucidated by challenging the culture human umbilical vein endothelial cells (HUVECs) or intact vessels with
Figure 2. The relationship of junctional structure and endothelial barrier function. Tight junction protein ZO-1 (a) and adherens junction protein VE-cadherin (b) were disassembled in HUVECs treated with burn plasma. ZO-1 was disorganized in isolated mesenteric venule of dorsal burn injured rats (c). The monolayer permeability of cultured HUVECs (d), as well as the permeability of isolated mesenteric venules (e) was increased concomitantly with the opening of inter-endothelial junctions.
burn serum for different time. The results demonstrated that the tight connection between adjacent cell membranes indicating by staining of ZO-1 was destroyed, and there was serration of cellular cortex, accompanying with deviation of ZO-1 in intercellular junctional area and internalization of ZO-1 into the cytoplasm (Fig. 2a) [20, 21]. The distribution of VE-cadherin was also disorganized by treatment of HUVECs with burn serum (Fig. 2b) [22]. In mesentery microvessels isolated from dorsal burn injured rats, similar manifestation of ZO-1-indicated intercellular junction was seen (Fig. 2c). The disruption of inter-endothelial junctions resulted in increasing in permeability not only in cultured HUVEC monolayer, but also in isolated mesentery microvessels (Fig. 2d, e).

Endothelial cell contractility change is considered to be the common pathway in the development of hyper-permeability. The changes of endothelial cell morphology and contractility are mainly affected by the contractile force which is generated by cytoskeleton proteins such as actin and myosin. The phosphorylation of myosin light chain (MLC) by its kinase MLCK and the consequent rearrangement of F-actin, or the formation of stress fiber are the major driving forces for the contraction of endothelial cells. Rho kinase (ROCK) is capable of maintaining the phosphorylated state of MLC by attenuating the phosphatase activity. In previous study, we have demonstrated that the exposure of HVUECs to burned plasma resulted in a rapid reassembly of F-actin and the formation of stress fibers, which could be partially inhibited by ROCK inhibitor Y-27632 (Fig. 3a) [23]. The application of Y-27632 dose-dependently induced the recovery of actin filament arrangement and attenuated the increase of permeability in venule wall after scalding (Fig. 3b) [23]. These results indicate that the activation of RhoA/ROCK signal transduction pathway is involved in burn-induced increase of venular permeability with endothelial cytoskeleton depolymerization and disruption. In our other studies of agonist-induced endothelial hyperpermeability responses, RhoA activity, as well as phosphorylation of ROCK was enhanced by agonists, such as advanced glycalation endproducts-modified human serum albumin (AGE-HSA) or high-dose sphingosine-1-phosphate (S1P), in time- and dose-dependent manners (Fig. 3d, e). Down-regulation of RhoA activity with RhoA N19 transfection abolished these agonist-induced changes, while transfection of activated RhoA L63 reproduced the agonist-evoked alterations (Fig. 3c). [21, 24].
Figure 3. The role of RhoA/ROCK pathway in burn-induced endothelial barrier dysfunction. Inhibition of ROCK with Y-27632 attenuated the formation of endothelial stress fiber induced by burned plasma (a), and inhibited the increase of vascular permeability of isolated mesenteric venule of dorsal burn injured rats (b). Down-regulation of RhoA activity with N19 and inhibition of ROCK activation with H-1152 altered AGE-BSA-induced endothelial monolayer hyperpermeability (c). RhoA activity (d) and ROCK phosphorylation (e) were enhanced with high dose S1P.

The heterogeneity of endothelial cells in venular end makes this part of microcirculation more vulnerable to the stimulation of inflammatory mediators. In most tissues and organs, venular endothelial cells are highly sensitive to inflammatory mediators due to the strong expressions of related receptors of mediators, such as the receptors of histamine, substance P receptor, etc., and adhesion molecules, such as P-selectin, and surface
glycoprotein [25, 26]. Activated leukocytes and platelet are more likely to adhere in venules than in arterials and capillaries.

The endothelial glycocalyx compromises some 20% of the intravascular volume and works as a dynamic natural barrier on endothelial cell surface. Its impairment also plays an important role in endothelial barrier disruption and its shedding occurs in the presence of oxidants, hyperglycemia, cytokines, and bacterial endotoxins, and is associated with many states of diseases.

3.4. Pro-inflammatory effects of endothelial cells

The effect of endothelial activation is also reflected in other common cellular inflammatory responses. The activation of endothelial cells can rapidly induce the synthesis and release of cytokines and chemokines, enhances the expression and activation of adhesion molecules, and mediates leukocyte-endothelial interaction. Endothelial activation is a critical factor for leukocyte rolling, adhesion, and infiltration. Xanthine oxidase is abundantly expressed in vascular endothelial cells, the activation of xanthine oxidase provides an important source of oxygen free radicals in ischemia-reperfusion injury. After the reperfusion, a large amount of superoxide anion and hydrogen peroxide is produced, the latter forms more active hydroxyl radicals in the presence of iron, causing the damages to interstitial structure, cell membrane, proteins, enzymes, nucleic acids, as well as chromosomes, and aggravating the inflammatory responses.

3.5. Angiogenesis abnormalities

Angiogenesis is one of the later events for wound healing during the development and resolution of inflammation. Since the activation, migration and proliferation of endothelial cells are the prerequisite of angiogenesis, all the factors that can regulate endothelial cell function, morphological changes are likely to affect the occurrence, development and outcome of angiogenesis. The abnormalities of angiogenesis play an important role in the development of various diseases, such as inflammation, cancer, proliferative diabetic retinopathy, rheumatoid arthritis and other diseases.

4. The markers of endothelial cell activation and dysfunction

Endothelial cell activation and dysfunction play important role in the occurrence and development of various acute and chronic diseases. The
evaluations of the features and extent of endothelial cell activation and injury become crucial in the management of various diseases in identifying the reason, predicting the progression, determining the prognosis and guiding the treatment.

4.1. The substance released from Weibel-Palade bodies

The activation of endothelial cells involves the rapid response conducting by those molecules that have been synthesized and stored in the cells, and the slow response of transcriptional translation and expression of new mediators. The endothelial cell-specific molecular reservoir is the Weibel-Palade bodies, which contain abundant bioactive molecules (Tab. 1). These substances are involved in the rapid activation of endothelial cells, the increases of above composition in serum have become important markers of endothelial cell activation.

4.1.1. Von Willebrand factor and thrombomodulin

The increase of von Willebrand factor (vWF) in plasma may reflect the severity of damage of endothelial cells and even organ damage in a certain extent. vWF is a glycoprotein synthesized by vascular endothelial cells and bone marrow megakaryocytes and is normally present in small amount in plasma and plenty in platelets. A large amount of vWF will be released into blood stream when vascular endothelial cells are damaged. The significant increase of vWF concentration in plasma can be used as a marker of vascular endothelial damage. vWF binds to platelet membrane glycoprotein GPI b/\eta/V, activates GP\alpha\beta/IIIa, thereby promoting platelet aggregation. vWF also mediates platelet adhesion to the exposed collagen surface after endothelial cell injury, thereby forming platelet thrombosis. In addition, thrombomodulin (TM) expressed on the surface of endothelial cells could bind with thrombin and activate protein C and play a role in anticoagulation and fibrinolysis. When the vascular endothelial cells are damaged, TM will be hydrolyzed to form soluble TM (soluble, sTM) and TM concentration in plasma will increase. Study has showed that the extent of severe acute brain injury can be determined by detecting the levels of serum TM and vWF. The results indicated that the concentration of TM and vWF in local brain injury was higher than that in diffuse brain injury. Patients with delayed traumatic intracranial hematoma had higher serum TM and vWF concentrations than those patients without. Older patients with brain injury had higher serum TM and vWF concentrations than younger patients did.
4.1.2. Angiopoietin-2

Recent studies suggest that the increase of Angiopoietin-2 in circulating blood is a relatively specific indicator of endothelial cell activation and dysfunction. Receptor Tie2 expresses on the vascular endothelium, which is the only common receptor for all known angiopoietin (Ang). The endothelium-specific Ang/Tie2 ligand-receptor system (Ang/Tie2) is an important medium for endothelial cell activation. The combination of angiopoietin 1 (Ang-1), which is synthesized by microvascular pericytes, with receptor Tie2 protects the endothelial functions, especially barrier function, from inflammation damage by maintaining the construction and function of adhesion protein VE-cadherin, attenuating the activation of NF-κB, reducing the expression of adhesion molecules, and inhibiting endothelial cell apoptosis. Whilst the binding of inducible angiopoietin 2 (Ang-2) with Tie2 antagonizes the signal of Ang-1/Tie2, leading to damage of endothelial barrier function. Therefore, the constitutive Ang-1/Tie2 signal is an important stabilizing factor for vascular endothelial cell, while the inducible expression of Ang-2 is an antagonist of Ang-1/Tie2 functional axis. Ang-2 is stored in the Weibel-Palade body of endothelial cells in quiescent state, and is released into the blood stream when endothelium is damaged and activated. The expression of Ang-2 mRNA is almost undetectable in resting blood vessels. It is significantly increased after endothelial cell activation. Studies have shown that the levels of Ang-2 in blood circulation in normal, non-septic patients, sepsis patients, and septic shock patients were successively increased, and the levels of Ang-2 in the blood were significantly correlated with the degree of tissue hypoxia, the ratio of arterial oxygen partial pressure, and the inhaled oxygen concentration (PaO$_2$/FiO$_2$), as well as organ damage and patient survival. Thus, the increased expression of Ang-2, which is specifically expressed by endothelial cell, is an important independent indicator for prognosis in critically ill patients.

4.2. Adhesion molecule

Another important marker of endothelial cell activation is the increase of expression and enhancement of function of adhesion molecules on cell surface. Under inflammation situation, the synthesis of varieties of adhesion molecules, such as E-selectin, (also known as endothelial
leukocyte adhesion molecule-1 (ELAM-1), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) is increased, which plays an important role in the process of leukocyte-endothelial cell interaction. ELAM-1 is endothelial cell-specific, and ICAM-1 and VCAM-1 are also expressed in leukocytes and other cell types. The increase of soluble (s) component of adhesion molecule in the plasma after membrane shedding can be used as a marker for endothelial cell activation during inflammation and sepsis. Studies reported that the levels of sELAM-1, sVCAM-1 and sICAM-1 in the plasma of patients with sepsis were significantly increased, which was associated with systemic inflammatory response, organ damage and disease outcome. Especially, the sustained increase in sICAM-1 concentration indicated that patient were not optimistic about the outcome. Those finally non-survivors had the most significant increase in plasma sICAM-1 concentration.

4.3. Other markers

The activation of endothelial cells leads to the activation and consumption of proteins, as well as the increase synthesis and secretion of cytokines. For example, endothelial cell activation can lead to alterations in activities of coagulation system-related factors. Activated Protein C (APC) functions as anticoagulant anti-inflammatory facilitator and is also capable in maintenance of endothelial cell stability. APC content was significantly reduced in sepsis and inflammation. This consumptive shortage of protein C can be used as a clinical indicators for the evaluation of endothelial cell functions and diseases. Recombinant human APC (rh-APC) can also be used for supplement in the treatment of related diseases. Antithrombin β is a plasma glycoprotein synthesized by hepatocytes, its content may be significantly reduced because of over consumption in pathological situations accompanied with disseminated intravascular coagulation. The reduction of antithrombin β may reflect the activation and dysfunction of endothelial cells. Endothelial cells can synthesize different cytokines, so the increase of cytokine contents, such as IL-6 level, could also partially reflects the degree of inflammatory response in endothelial cells.

4.4. Endothelial microparticles

Microparticles are tiny particles with cell markers formed during cell activation and apoptosis. The endothelial microparticles (EMPs) have a
diameter of 100 nm to 1 μm, which shed from the vessel wall and retains most molecular expression characteristics of the endothelial cell. They are rich in endothelial cell active components and can reach the distant tissue and organ through circulation. EMPs are involved in the processes of coagulation and inflammation, affecting endothelial cell function and angiogenesis. The presence of EMPs in plasma can be used as a marker of endothelial cell activation and apoptosis. Study has indicated that the increase of EMPs in blood is closely related to endothelial dysfunction and the occurrence of atherosclerosis caused by hypertension and smoking [27, 28]. Syndecan-1, a cell surface proteoglycan, will be released along with the shedding of endothelial glycocalyx, and its increase level in serum has also been implicated in many diseases.

5. Strategies for improving endothelial cell function

Since endothelial activation and dysfunction are so important in the pathogenesis of various diseases, it is critical and also potential to improve endothelial cell functions for the prevention and treatment of different diseases. While endothelial barrier dysfunction is always an early and sustaining pathological event in the development of inflammatory diseases, the barrier function protection becomes our focus. Several permeability anti-increasing factors have been proved to be effective in antagonizing and attenuating the barrier disruption effects of those permeability-increasing mediators. A batch of endogenous bioactive factors was also found of capable in protecting and improving the integrity of endothelial cell monolayer in vascular intima and reduce vascular permeability. These factors include cAMP, ATP, adenosine, adrenomedullin, sphingosine-1-phosphate, as well as the previously mentioned Ang-1/Tie2 system, activated protein C, etc. They are called the protective agents of endothelial barrier function or the stabilizing mediators of vascular permeability.

5.1. Antioxidants in endothelial function protection

There has been a long-standing interest in the efficiency of anti-oxidative reagents in modulation of endothelial barrier function. It is showed that vitamin C can protect vascular endothelial cells with antioxidant properties [29]. As early as in 1992, Matsuda T et al have showed that vitamin C application was able to attenuate the increase in capillary permeability in postburn animals. With adjuvant high-dose vitamin C administration, 24-h resuscitation fluid volume could be reduced significantly, while cardiac output was still maintained adequate [30]. Some
Roles of endothelial cells in inflammation

Other experimental studies in different animal models have shown that vitamin C is beneficial to burns by reducing endothelial damage, preventing capillary leakage, lymph flow and resuscitation fluid requirements [31, 32]. Further, a human study showed a reduction in resuscitation volume with vitamin C treatment after severe burn [33]. But the efficiency of vitamin C in enhancement of endothelial barrier function is still contradictory, while Aliabadi-Wahle et al found that vitamin C did not attenuate the increase of microvascular permeability and the formation of tissue edema when vitamin C was given after burn in dogs [34].

5.2. Activated protein C

As mentioned above, activated protein C (APC) has anticoagulant and fibrinolytic functions in normal human body. APC has a unique receptor in endothelial cells, endothelial cell protein C receptor (EPCR). By binding with EPCR on endothelial cells, APC is capable in stabilizing intercellular junctions and strengthening the barrier function. By degrading thrombin receptor protease-activated receptor-1 (PAR-1) and inhibiting the expression of pro-apoptotic genes, the formation of APC-EPCR complex also exhibit anti-apoptotic effect on vascular endothelial cells, helping in maintaining endothelial barrier integrity.

5.3. Sphingosine 1-phosphate

Sphingosine-1-phosphate, derived from the metabolism of sphingomyelin, is an important component in plasma. Intracellular S1P works as a second messenger to interact with regulatory molecules, such as different enzymes, channel proteins and transcription factors, etc. However, the primary role of S1P is working as an extracellular ligand that binds to specific S1P receptors (S1PRs). The binding of S1P with different S1PRs and the activation of downstream signal transduction pathways affect the motility, morphology, differentiation and survival of cells, exerting a wide range of biological effects. The specific receptors for S1P are originally called differentiation genes of endothelial cells, and are now generally known as S1P receptors (S1PR), which include five subtypes (S1PR1-5). Human vascular endothelial cells mainly express S1PR1 and S1PR3, whereas S1PR2 is expressed at lower levels. In the physiological concentration, S1P mainly binds to S1PR1 and interacts with Gi of G protein-coupled receptor, leading to the enhancement of Rac1 activity, and consequently initiating the protective process of endothelial barrier function. This function is considered to have important clinical therapeutic
significance, and may provide a treatment pathway for attenuation of vascular hyperpermeability and tissue edema in inflammatory process. Normally, platelet is an important source of plasma S1P, endothelial cell is another contributor to plasma S1P by secreting S1P in a constitutive manner. Based on the hypothesis that consumption of platelet at the early stage of burn injury exhausts the storage of S1P, we found that the supplement of physiological dose of S1P help to restore the proper

Figure 4. Effects of S1P and its receptors on modulation of endothelial junctional morphology. Proper amount of S1P helped to stabilize and restore the cortical assembly of F-actin and the continuous lining of VE-cadherin in endothelial membrane area upon the challenge of burned plasma (a). The activation of S1PR1 preserved the lining distribution of tight junction protein ZO-1, while the activation of S1PR2 enhance the disorganization of ZO-1 in HUVECs (b).
organization of F-actin and the integrity of intercellular junctions in burn serum-treated endothelial cells (Fig. 4a, b). The inhibition of S1PR1 with specific inhibitor VPC abolished this restoration. Using SEW, a specific agonist of S1PR1, we found that the lining distribution of tight junction protein ZO-1 was preserved in burn serum-treated endothelial monolayer (Fig. 4b) [23].

However, when the concentration of S1P is pathologically increased, whilst more than 10 mol/L in plasma, S1P will mainly bind to S1PR2/3 and interact with Gq and G12/13, leading to cell contraction and the disruption of endothelial cell barrier. Furthermore, the expression of S1PR2 can be induced and activated under inflammatory situation, which may be one of the mechanisms of S1P-induced vascular hyperpermeability. Using JTE, the specific inhibitor of S1PR2, we found that high dose S1P-induced disorganization of F-actin and ZO-1 was attenuated in burn serum-treated endothelial cells (Fig. 4b) [22].

### 5.4. Glucocorticoid and insulin

Both glucocorticoids and insulin have the efficiency of suppressing the activation of endothelial cells and protecting endothelial cells function. By inhibiting the activation of inflammatory cells and reducing the release of inflammatory mediators, glucocorticoids can suppress the activation of endothelial cells and exert protective effect on endothelial barrier function. As early as in 1966, Zhao K et al had reported that vascular permeability was increased in skin and muscle, as well as in distant organs, such as liver, spleen and kidney. This increase of vascular permeability could be prevented by pretreatment with cortisone [35]. But clinically, the usage of GC is limited because of their immunological suppression effects. Insulin protects endothelial function mainly by reducing the oxidative stress in endothelial cells through inhibition of activity of inducible nitric oxide synthase (iNOS) and enhancement of the phosphorylation of constitutive endothelial nitric oxide synthase (eNOS).

### 5.5. Other permeability anti-increasing factors

By applying unspecific serotoninergic receptor blocking agent methysergide, specific 5-HT2 antagonism Cinanserin, or specific 5-HT2a antagonism Ketanserin, respectively, Hernekamp JF and his coworkers demonstrated that the inhibition of 5-HT receptor could reduce FITC-albumin extravasation in burn-plasma transfer rat mesenteries. Zhao J et al reported that granulocyte/macrophage colony-stimulating
factor (GM-CSF) can be used as an anti-leakage agent for burn treatment. They showed that GM-CSF protects endothelial cells from thermal injury and attenuates endothelial hyperpermeability after thermal injury by maintaining the endothelial membrane expression of VE-cadherin through inhibiting the activation of RhoA [36]. It is also revealed that the activation of cholinergic anti-inflammatory pathway with cdp-choline administration could inhibit leukocyte activation and macromolecular efflux [37]. While matrix metalloproteinases (MMPs) have been shown to induce the proteolysis of adherens junction and extracellular matrix, tissue inhibitor of matrix metalloproteinases (TIMPs) have been demonstrated to be effective in inhibiting burn-induced disarrangements in microvascular endothelial cells. Doxycycline, best known as an antibiotic, has also been revealed with the inhibitory effect on MMPs. Childs EW et al revealed that MMP-9 in associated with the disruption of endothelial adherens junction and played a role in burn-induced microvascular hyperpermeability. This was attenuated by application of doxycycline [38]. Our study demonstrated that doxycycline could also alleviated LPS-evoked endothelial hyper-permeability and F-actin redistribution by inhibiting the phosphorylation of p38 and its downstream target, HSP27 [39].

**Summary**

We know that vascular system is widely distributed in almost all tissues of the body except lamina dura, so vascular endothelial cell is involved in the functional regulation of all tissues, organs, and even systems. Endothelial cells are also the mediating cells of water and electrolyte balance, inflammation, coagulation, hemodynamics, and cell migration. The alterations in endothelial functions and phenotypes are the acute and sub-acute reactions to the original reason of various diseases. Endothelial dysfunction is even the key mechanism for a variety of chronic cardiovascular diseases. The malfunction of endothelial cell is also the main mechanism for the acute onset of SIRS, ARDS and other local or systemic ischemic-reperfusion injury, including trauma, hemorrhagic shock and resuscitation, etc. In different pathological processes, the nature and extent of endothelial cell response are different, which need to deeper research and discussion. Clinically the treatment strategy to restore the homeostasis and functions of endothelial cell will contribute to the improvement of microcirculation and the attenuation of coagulation and inflammatory response.
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