Chapter

The Role of Occludin in Vascular Endothelial Protection

Yunhui Du, Yanru Duan and Shihan Zhang

Abstract

Endothelial tight junction proteins play an important role in maintaining the integrity of vascular endothelial structure and physiological function. In recent years, studies have found that alterations in the expression, distribution, and structure of endothelial tight junction proteins may lead to many related vascular diseases and pathologies (such as diabetes, atherosclerosis, neurodegenerative diseases, and hypertension). Therefore, related strategies to prevent and/or tight junction proteins dysfunction may be an important therapeutic target. Occludin, as the most representative one among tight junction proteins, is mainly responsible for sealing intercellular junctions, maintaining cell permeability and the integrity of vascular endothelium. Here, we review the published biological information of occludin. We highlight the relationship between occludin and vascular endothelial injury-related disease. At the same time, we show our current knowledge of how vascular endothelial occludin exerts the protective effect and possible clinical applications in the future.

Keywords: occludin, vascular endothelial cells, protective effect

1. Introduction

The normal vascular endothelium is taken as a gatekeeper of cardiovascular health, whereas abnormality of vascular endothelium is a major contributor to a plethora of cardiovascular ailments, such as atherosclerosis, hypertension, myocardial infarction, coronary artery disease [1]. Therefore, it is important to study the occurrence and development mechanism of vascular endothelial injury. Recent studies have shown that alterations in expression, distribution, and structure of endothelial tight junctions (TJ) may lead to atherosclerosis, neurodegenerative diseases, and pulmonary hypertension, suggesting that TJs play an important role in the vascular endothelium [2].

Occludin, the most representative tight junction proteins, can control the permeability of cells by regulating the connection between cells to play a barrier function. Occludin is involved in the formation of cell polarity *via* forming a fence to prevent cells from spreading to the top and base outer membranes [3]. Meanwhile, occludin can promote cell proliferation and migration [4]. In addition, the expression level of occludin in different vascular beds is positively correlated with the properties of the endothelial barrier of the vascular beds. For example, the permeability of the arterial

vascular endothelial barrier is lower than that of the venous vascular endothelial barrier, and the expression of occludin in arterial vascular endothelial is about 18 times higher than that in the venous blood vessels [3], suggesting that occludin is a critical factor of cell permeability and plays an important role in maintaining vascular homeostasis.

Alterations in occludin expression play an important role in vascular endothelial dysfunction. For example, the expression of occludin in retinal vascular endothelial cells of diabetics decreased, resulting in vascular dysfunction such as vascular permeability increased, new vascular formation disorders, and inflammatory response increased, suggesting that the decreased level of occludin may be one of the factors for vascular dysfunction in diabetes [5]. Liu et al. [6] isolated primary mouse retinal endothelial cells for *in vitro* culture and found that occludin S490 phosphorylation is one of the important conditions for retinal endothelial cell tube formation, cell proliferation, and migration. In addition, in the rat with cerebral ischemia at 24 h and 72 h, the expression of occludin in the blood-brain barrier first increased and then decreased [7]. In view of this, understanding the role and mechanism of occludin in vascular endothelial protection is significant for the prevention, diagnosis, and treatment of cardiovascular diseases. We will summarize recent advances in the relationship between occludin and vascular endothelial injury based on the biological information of occludin, the signaling pathway of occludin to protect the vascular endothelium, and the relationship between occludin and vascular endothelial injury-related diseases in this chapter.

2. Biological information of occludin

There are four main types of intercellular connections in vertebrates: tight junctions, adhesion junctions, gap junctions, and desmosome junctions. Intercellular tight junctions, which can seal intercellular spaces, control hydronium, water, and other molecular pathways, and maintain cell polarity, as discovered by Farquhar and Palade [8]. Discovery of tight junctions revealed the complexity of cellular internal structural, and cellular tight junction proteins (cingulin [9], Zos [10], Tricellulin, JAM [10], and occludin [11]) further clarify the structural complexity and functional diversity of cells.

2.1 Structure of occludin

Occludin has four transmembrane segments, two extracellular loops (the first extracellular loop rich in tyrosine and glycine and the second extracellular loop rich in tyrosine) and two extracellular loops internal domains (NH2-terminal cytoplasmic domain and COOH-terminal cytoplasmic domain) (**Figure 1**). The main function of the COOH-terminal cytoplasmic domain of occludin is to mediate the basolateral transport and endocytosis of proteins, while occludin lacking the C-terminus can localize at tight junctions, the tight junctions cannot be assembled correctly and function is lost [12]. In addition, Bamforth et al. [13] found that occludin lacking or truncating the N-terminus of the extracellular domain can still target tight junctions and co-localize with ZO-1, but the function of tight junction barrier disappears, suggesting that the C- and N-terminal domains of occludin are involved in tight junction assembly and play a barrier function. In addition, the two extracellular loop domains of occludin are critical for the localization of cellular tight

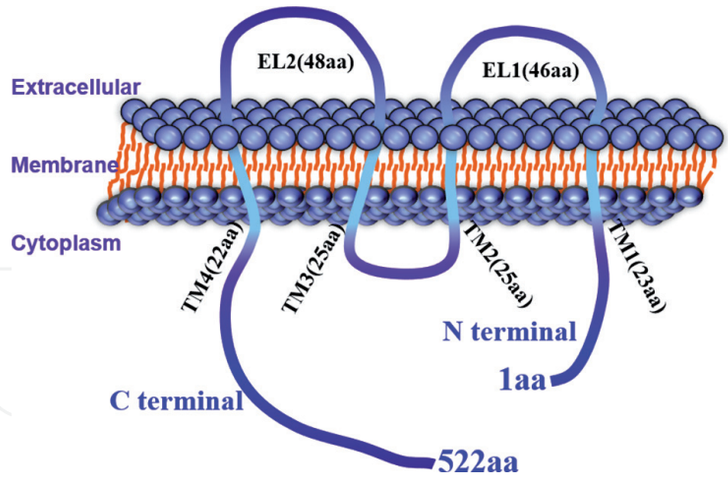

Figure 1.
Structural insight into occludin. Occludin shares with general architecture as tetraspan transmembrane proteins colored in a gradient ranging from yellow at the N-terminus [N] to yellow at the C-terminus [C]. aa: Amino acid; G: Glycine; T: Tyrosine; EL1/2, extracellular loops 1 and 2; TM1 to 4, transmembrane domains 1 to 4.

junctions. Occludin lacking the two extracellular loops is only present on the surface of basal cells but not cellular TJ [14].

2.2 Tissue distribution and expression regulation of occludin

Occludin is expressed in different cells and tissues with different expression level, and it is related to the function of tissues and organs. Occludin can be expressed in human, rat, mouse and other species, and it is mainly localized in arterial and venous vascular endothelial cells, blood-brain barrier, and blood-retinal barrier [14]. Although the expression of occludin could not be detected at the capillary-endothelial junction in mouse heart and skeletal muscle, occludin was highly expressed in brain capillaries [3], suggesting that occludin is essential for regulating the endothelial permeability of the blood-brain barrier. Morcos et al. [15] confirmed that under physiological conditions, occludin is highly expressed in retinal capillaries. However, in pathological conditions, the expression of occludin in the vascular endothelium will decrease significantly accompanied by different stress responses (inflammation, diabetes, cardiovascular diseases, neurodegenerative diseases, and atherosclerosis), and the permeability of vascular endothelium and the apoptosis of cell will increase [4], which suggests that occludin plays an important role in the blood-retinal barrier. In conclusion, under physiological and pathological conditions, the different expression levels of occludin in different tissues and cells are closely related to the tissue barrier properties.

3. The signaling pathway of occludin exerting the protective effect of vascular endothelium

In recent years, the study of cellular tight junction proteins has increased dramatically. Occludin, the most typical cell tight junction protein, has attracted much attention. A large number of studies have shown that many classical signaling pathways are involved in the regulation of occludin, affecting the distribution and expression of occludin.

3.1 Occludin and mTOR pathway

mTOR, consisting of two distinct complexes (mTORC1 and mTORC2), is a sensor of ATP. mTOR1, a classical metabolic pathway in mammals, is involved in the proliferation and migration of vascular endothelial cells [16] and the occurrence and development of various cardiovascular diseases. Currently, a large number of studies have focused on the role of the mTOR pathway in the regulation of occludin expression [1] In diabetic rat model, as the phosphorylation level of mTOR increases, the downstream 4EBP1 and S6K1 proteins are activated, and the expression level of ROS is increased, which leads to the reduction of NO production in vascular endothelial cells and the decrease of the expression of occludin protein, and the vascular endothelium is damaged. However, the expression of occludin increased after adding the mTOR inhibitor rapamycin [2, 17] inhibiting the production of occludin *via* the inhibition of the PI3K/Akt/mTOR signaling pathway in the cerebral vascular endothelium may lead to the age-related leakage of the blood-brain barrier [3, 18]. Hepatocyte growth factor [HGF] secreted by mesenchymal stromal cells can activate endothelial cell mTOR/STAT3 signaling pathway to promote endothelial occludin expression, maintaining vascular endothelial permeability homeostasis and reducing endothelial cell apoptosis [19]. In conclusion, mTOR signaling pathway is involved in the regulation of occludin expression.

3.2 Occludin and VEGF pathway

The VEGF family consists of five vascular growth factors: VEGFA, VEGFB, VEGFC, VEGFD, and placental growth factor (PIGF). VEGF binds to tyrosine kinase cell receptors (VEGFR1/fms-like tyrosine kinase 1(FLT1), VEGFR2/human kinase insertion domain receptor (KDR)/mouse fetal liver kinase 1 (FLK1), and VEGFR3/fms-like tyrosine kinase 4 (FLT4)) to exert biological effects. Under physiological conditions, VEGF may cause neovascularization, and aggravate vascular inflammation, vascular endothelial cell proliferation, migration and invasion, and endothelial cell survival [20]. A variety of studies revealed the relationship between the VEGF pathway and occludin: (1) Phosphorylation of occludin S490 could induce endothelial cell VEGF expression and promote endothelial cell proliferation and angiogenesis both *in vivo* and *in vitro* [6]; (2) in rat model of cerebral artery occlusion, the lack of VEGF expression in microvascular endothelial cells can prevent the expression of occluding *via* inhibiting the VEGFR2/eNOS signaling pathway to further affect the permeability of the blood-brain barrier [21]; and (3) in mouse mammary cancer model, VEGF secreted by cancer cells can inhibit the expression of occludin in pulmonary vascular endothelium, increase pulmonary vascular permeability, and induce cancer cell metastasis, while overexpression of occludin can alleviate vascular endothelial disorder [22]. In conclusion, the interaction between VEGF and occluding could affect the occurrence and development of the disease.

3.3 Occludin and PKC pathway

PKC, a second messenger-regulated serine/threonine kinase, belongs to the AGA kinase family. Studies have shown that PKC can participate in the regulation of vascular endothelial integrity by interacting with the vascular endothelial marker tight junction protein [23]. Presently, a variety of *in vivo* and *in vitro* disease models have been studied to explore the role of the PKC pathway in regulating the expression and

distribution of occludin: (1) In diabetes, metformin improves TJ barrier function by promoting the abundance and assembly of full length occludin at the TJ and that this process involves phosphorylation of the protein *via* an AMPK-PKCζ pathway [24]; (2) high glucose/ethanol induction increases the activity of NAD(P)H and promotes the phosphorylation level of subunit p47phox *via* inhibiting the activity of PKCα and PKCβ to increase the activity of matrix metalloproteinase 2 and reduce the expression of occluding, ultimately increasing vascular endothelial permeability, leading to the loss of blood-brain barrier integrity [25]; (3) in rat model of hypoxia and pulmonary ischemia-reperfusion injury, PKCα inhibits the expression of occludin in cerebral blood vessels and affects cerebral angiogenesis [26]; and (4) endothelial monocyte-activating polypeptide-II (endothelial monocyte-activating polypeptide II, EMAP-II) induced-redistribution of occludin by activating the PKCζ/PP2A signaling pathway is another mechanism in the impairment of the blood-tumor barrier [27].

3.4 Occludin and PKA pathway

PKA, a cAMP-dependent kinase involved in the regulation of vascular endothelium, belongs to AGA kinase. Recently, it was reported in the literature that a novel β-adrenergic receptor agonist [complex 49b]-treated diabetic retinal endothelial cells could activate the PKA signaling pathway, promote the expression of occludin in retinal vascular endothelium, increase vascular tight junctions, and reduce endothelial cell apoptosis [27]. cAMP/PKA signal transduction is involved in the increase of blood-tumor barrier permeability mediated by bradykinin and promotes the up-regulation of occludin expression [28]. Glucagon-like peptide-1 (GLP-1) activates the cAMP/PKA signaling pathway to promote occludin expression and maintain the integrity of the blood-brain barrier in rat primary brain capillary endothelial cells [29].

3.5 Occludin and AMP-activated protein kinase [AMPK] pathway

AMPK is a serine/threonine protein kinase involved in the regulation of cellular and body metabolism. AMPK activation counteracts oxidative stress by inhibiting the production of reactive oxygen derived by NAD[P]H oxidase in endothelial cells [30]. Stimulation of lipopolysaccharide in aging mice can significantly inhibit the activation of AMPK pathway in cerebral vascular endothelial cells, up-regulate the production of NAD[P]H oxidase, and reduce the expression of occludin protein, leading to blood-brain barrier disorders [31]. AMPK kinase inhibits the activation of inflammasome NLRP3 through the mTOR/ULK1 pathway-mediated autophagy, promotes the expression of occludin, and protects the blood-brain barrier in human brain capillary endothelial cells cultured *in vitro* [18]. Studies reported that occludin can negatively regulate AMPK activity to affect blood glucose uptake and energy production [32]. In conclusion, there is a strong connection between the energy metabolism pathway AMPK and occludin.

3.6 Occludin and MAPKs pathway

MAPKs including extracellular signal-related kinases (ERK1/2), p38, and c-Jun N-terminal kinase [JNK] are a family of serine/threonine protein kinases [33]. Under the stimulation of various extracellular factors (such as inflammatory signals), MAPK kinase promotes the activation of nuclear proteins and transcription factors, and

regulates gene expression, differentiation, apoptosis and other processes. MAPK kinase is an important intracellular signal transduction that regulates various intracellular functions. Many studies have found that MAPK pathway activation can affect endothelial cell occludin expression and modification in physiological and pathological conditions: (1) Exposure of cerebral microvascular endothelial cells to lipopolysaccharide can affect the p38MAPK/JNK signaling pathway and MMP2 expression, thereby regulating the level of occludin protein in endothelial cells and leading to central nervous system inflammation and brain edema [34]; (2) ERK1/2 inhibits the activation of the NF-κB signaling pathway resulting in the increase of occludin and decrease of endothelial barrier permeability to protect the TJ barrier in human lung microvascular endothelial cells [35]; (3) after lipopolysaccharide stimulates human umbilical vein endothelial cells, it can promote the mRNA and protein expression of CXCL4 and its receptor CXCR3 activates the downstream p38 signaling pathway, thereby inhibiting the expression of occludin in endothelial cells, promoting endothelial cell apoptosis, and increasing endothelial cell permeability [36]; (4) exposure of human umbilical vein endothelial cells to γ-rays can promote the expression of MAPK pathway molecules p38, p53, p21, and p27, induce the activation of NF-κB signaling pathway, and inhibit the expression of occludin in endothelial cells, resulting in the increase of cell permeability, oxidative stress, nitrification, and inflammatory [37]; (5) in human brain microvascular endothelial cells, reduction of occludin can upregulate PI3K/AKT and ERK signaling pathways, and promote cytokine secretion, inflammatory factor activation, and apoptosis protein expression. However, overexpression of occludin can inhibit endothelial cell apoptosis and inflammation [25]. In conclusion, the MAPK signaling pathway is closely related to the regulation of occludin.

4. Protein post-translational modifications (PTMs)

In general, various protein post-translational modifications (PTMs) increase the functional diversity of the proteome through adding covalent functional groups, proteolytically cleaving regulatory subunits, or degrading the entire protein. These covalent modifications of proteins involving in phosphorylation, glycosylation, ubiquitination, nitrosylation, methylation, acetylation, lipidation, and proteolysis have affected all the details of cellular physiology and pathology. The post-translational modifications of proteins further contribute to the biological complexity from genome to proteome. PTMs play an important role in regulating activity, localization, and interaction with cellular molecules (such as proteins, nucleic acids, lipids, and cofactors) [38, 39].

Therefore, better understanding and analysis of protein post-translational modifications may be crucial for the study of cell biology, disease treatment and disease prevention including cardiovascular diseases, several forms of cancers, neurodegenerative diseases and diabetes, etc. [40].

4.1 Post-translational modifications of occludin

In recent years, post-translational modifications of occludin, as representative tight junction proteins, have become a research hotspot. The reported post-translational modifications of occludin include proteolysis, phosphorylation, and ubiquitination, which have all been shown to play vital roles in the course of disease occurrence, development, and convalescence [41, 42].

4.2 Proteolytic degradation of occludin

Studies have found that degradation of tight junction proteins play an important regulatory feature in pathological and physiological tissue remodeling [43]. Basic studies demonstrated that the two fragments of cleaved Occludin released into circulation and the levels of blood occludin correlate well with the extent of blood brain barrier in cerebral ischemic model of rats [44]. In addition, occludin serve as a potential biomarker to predict the severity of acute ischemic stroke, hemorrhagic transformation, and patient prognosis [45]. These results suggested that the degradation of Occludin may be involved in the occurrence and development of many diseases.

4.3 MMPs-dependent degradation of occludin

Matrix metalloproteinases (MMPs) are secreted by astrocytes, endothelial cells, pericytes and peripheral circulating cells and are capable to degrade extracellular matrix (ECM) proteins as well as non-ECM proteins, including cytokines, chemokines, membrane receptors, and antimicrobial peptides [46, 47]. Studied showed that MMPs are related to the development of cancer infiltration and metastasis, inflammatory response, and angiogenesis. Within the endothelial layer, MMPs can degrade intercellular junction molecules (such as cadherin, occludin, and claudins) and intracellular structural proteins (e.g., actins), enhancing the permeability of endothelial barrier [48].

Currently, a number of data have showed that occludin was mainly proteolytically cleaved *via* MMPs to inactive fragments, leading to endothelial barrier disruption. (1) Feng Chen et al. demonstrated MMP9 induced the degradation of occludin and suppressed the synthesis and expression of Occludin in brain endothelial cells and in brains of mice with experimental acute liver failure (ALF), which can cause severe vasogenic brain edema [49]. (2) Related studies showed that LPS/hypoxia induced brain blood barrier (BBB) leakage by MMP2/MMP9 contributed to the degradation of occludin in brain microvascular endothelial cells [34]. (3) TGF-β can promote the production of MMP9 in brain microvascular endothelial cells and retinal endothelial cells, accelerate the degradation of Occludin, and lead to increased vascular endothelial permeability [50]. (4) Several studies demonstrated that MMP2/9 leads to occludin fragmentation in brain microvessels from rat model of cerebral ischemic injury, with resultant brain leakage and brain edema [51–53]. (5) At the same time, Yang et al. firstly described the temporal dynamics of occludin degradation by MMPs in rodent models of cerebral ischemic injury, suggesting that MMP-2 cleaved occludin during the early phase of the ischemia (3 h), while MMP-9 caused further occludin degradation and more long-term (24-h) alterations to BBB integrity. In addition, MMP9 can promote the degradation of occludin through HIF-1α and AQP-4, ultimately triggering BBB disruption and brain edema [54]. (6) Simultaneous data show that MMP2/9-mediated occludin hydrolysis can be used as a marker of blood-brain barrier and blood-retinal barrier in type 2 diabetes and diabetic retinopathy [55, 56]. (7) Caron et al. have suggested that elevated ProMMP-2/9 and MMP9 correlate with increased levels of occludin degradation in rodent kidney endothelium in ischemic injury [57]. (8) The degradation of tight junction proteins (occludin, claudins) through MMP9 secreted by glioma cells is an important mechanism in the BBB breakdown mediated by TGF-β [58]. (9) In acute leukemia, MMP9 secreted by leukemic cells degraded occludin, which constituted an extreme mechanism of the BBB breakdown that contributes to the invasion of the central nervous system [59]. Overall, occludin contains

extracellular MMP cleavage sites and are a substrate of MMPs. In endothelial cells, the degradation of Occludin mediated by MMPs leads to vascular leakage.

4.4 MMP-independent proteolysis of occludin

At present, a large number of data focus on MMPs-dependent occludin degradation; however, there are also some studies showing the existence of MMPs-independent occludin degradation. (1) Qian et al. found that tryptase can act on mouse brain microvascular endothelial cells to promote the production of MMP9/2, degrade the tight junction proteins occludin and Claudin5, and lead to the destruction of the blood-brain barrier [60]. (2) Wan et al. and Runs et al. verified both serine and cysteine peptidases cleavage the occludin with elevation of epithelial permeability, which reveals a pathological mechanism for allergen delivery across lung and nasal epithelial barriers in asthma and allergic rhinitis sufferers [35]. (3) Caspase-mediated cleavage of the occludin C-terminal promotes apoptosis in MDCKs [61]. The studies about the MMP-independent proteolysis of occludin occurred in the epithelial cells; therefore, more research is needed to further define the MMP-independent proteolysis of occludin in endothelial cells.

4.5 Occludin phosphorylation

Protein phosphorylation is a ubiquitous type of post-translational modification, whereby protein kinase catalyzes the phosphorylation reactions by transferring the phosphate group of ATP to the substrate protein amino acid residues, typically serine, threonine, and tyrosine, or bind GTP under the action of signal transduction. It was widely demonstrated that protein phosphorylation is the most basic and the most common key mechanism for regulating and controlling protein biological activity and function [62]. Notably, the phosphorylation status of occludin regulating endothelial barrier protection has been received extensive attention.

More than 40 phosphorylation residues are in human occludin; however, only nine sites are confirmed in cell levels by different kinases on certain stimuli, including Y398, T400, Y402, T403, T404, S408, T424, T438, and S490[46]. All confirmed phosphorylation residues lie in the occludin C terminal. As early as 1997, Sakakibara et al. firstly observed increased phospho-serine [pSer] and phospho-threonine [pThr] occludin selectively localized to intact epithelial TJs as a detergent-insoluble form [63]. Subsequently, Kale and Elias et al. confirmed occludin phosphorylation on key serine, threonine, and tyrosine residues plays a crucial role in the assembly and maintenance of TJs in Caco-2 and MDCK cells [64, 65]. Dörfel and colleagues in 2013 studied that CK2-mediated phosphorylation [T400A/T404A/S408A] of occludin in MDCK-C11 cells bind with ZO1/2 interaction and protect the epithelial barrier [66]. The regulation of occludin phosphorylation in endothelium has also received extensive attention, with many studies focusing on how the phosphorylation status of occludin regulates the vessel barrier.

Different phosphorylation sites of occludin exerts specific functions in endothelial cells: (1) Antonetti and his colleagues investigate the role of tight junction protein occludin phosphorylation at S490 in modulating barrier properties and its impact on visual function. They found that endothelial-specific expression of the S490A form of occludin completely prevented diabetes-induced permeability to label dextran and inhibit leukostasis. Importantly, vascular-specific expression of the occludin mutant completely blocked the diabetes-induced decrease in visual acuity

and contrast sensitivity in the retinas of streptozotocin-induced diabetic mice [67]. (2) Treatment with glutamate increased tyrosine phosphorylation and decreased threonine phosphorylation of occluding in brain microvascular endothelial cells. It affects the redistribution of occludin. These may lead to opening of the blood-brain barrier (BBB) and induce further brain damage [68]. (3) The phosphorylation of occludin and claudin-5 by RhoK at specific sites disrupted the integrity of BBB. Antibodies against specific phosphorylation sites of occludin could be useful reagents for monitoring BBB dysfunction *in vivo* [69]. (4) Other recent studies confirm the importance of threonine phosphorylation with occludin C-terminal for mediating its ability to localize to the tight junction [70–72]. According to the above studies, it is demonstrated that the specific phosphorylation sites of occludin regulate the different function of endothelial cells. Other studies need to further focus on other phosphorylation residues of occludin in correlating with endothelial function.

4.6 Occludin ubiquitination

Ubiquitination, also known as ubiquitylation, refers to the process in which the ubiquitin (a small 76-residue regulatory protein widely expressed in eukaryotes) molecules classify the proteins in cells under the action of a series of special enzymes, choose the target protein molecules from them, and specifically modify the target proteins. These special enzymes include ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), ubiquitin-protein ligase (E3), and degrading enzymes [73]. Ubiquitination plays an important role in protein localization, metabolism, function, regulation, and degradation [73, 74]. At the same time, ubiquitination takes part within the regulation of nearly all life activities, including cell cycle, proliferation, apoptosis, differentiation, metastasis, gene expression, transcriptional regulation, signal transmission, damage repair, inflammation, and immunity [73, 74]. In recent years, studies about the functional role of occludin ubiquitination in diseases have begun to emerge in a burst. And the modification way has become an important regulatory mechanism in epithelial and endothelial function.

Hannelore et al. identified a novel interaction between occludin N-terminal and the E3 ubiquitin-protein ligase Itch, a member of the HECT domain-containing ubiquitin-protein ligases by co-immunoprecipitation *in vivo* and *in vitro* [75]. In addition, the team provides evidence that Itch is specifically involved in the ubiquitination of occludin *in vivo*, and that the degradation of occludin is sensitive to proteasome inhibition. The team firstly confirmed that occludin can be ubiquitinated. Liu and Lee et al. reported that occludin degradation was associated with Itch and UBC-4 (an ubiquitin-conjugating enzyme), resulting in occludin ubiquitination to disrupt tight junctions in blood and testosterone barrier [76]. A slightly later study reported that a conserved C-terminal PY motif of occludin association with Nedd4-2 was involved in the paracellular permeability of mpk-CCD[c14] cells (a collecting duct epithelial cell line) by coimmunoprecipitation. These authors also showed that small interfering RNA [siRNA]-mediated knockdown of Nedd4-2 increased occludin expression and reduced the epithelial permeability, with Nedd4-2 overexpression having the opposite effects [77]. In conclusion, ubiquitinated occludin is taken part in the maintenance of cell barrier.

Currently, the study about the regulation of occludin ubiquitination in vascular endothelial function focuses on the following aspects. (1) Murakami et al. demonstrated that Ser-490 phosphorylation of occludin is an essential prerequisite for its ubiquitination in BRECs. The team showed that a C-terminal occludin-ubiquitin

chimera was internalized, bypassing the requirement for phosphorylation. Thus, VEGF, through PKCβ-mediated phosphorylation, promotes Itch-mediated ubiquitylation of occludin, which is required for its internalization and degradation, thereby enhancing retinal endothelial permeability [78]. (2) It is well known that blood-spinal cord barrier (BSCB) breakdown is a hallmark of amyotrophic lateral sclerosis (ALS). Results found that mutant SOD1 induced occludin phosphorylation, which promoted the subsequent occludin ubiquitination mediated by the E3 ligase ITCH. Moreover, ubiquitinated occludin interacted with Eps15 to initiate its internalization, then trafficked to Rab5-positive vesicles, and be degraded by proteasomes, resulting in a reduction in cell surface localization and total abundance [79]. (3) Feng et al. showed that the γ-secretase blocker DAPT reduced the permeability of the BBB by decreasing the ubiquitination and degradation of occludin during permanent brain ischemia [80]. Notwithstanding the information already generated about the role of occludin ubiquitination in endothelial cells, several avenues for future investigation still remain. The identification of new ubiquitin enzymes, characterization of tissue and cell-specific occludin ubiquitination, and deciphering the functional rapport between different modification events (e.g., phosphorylation, ubiquitination, proteolysis), will likely typify future studies in this field. This will ultimately yield a fuller understanding of how ubiquitination modifications to occludin affect TJ characteristics and will help to unlock the therapeutic potential of the TJ by identifying new cellular targets for intervention in diseases characterized by barrier dysregulation.

5. The relationship between occludin and vascular endothelial injury-related diseases

Vascular endothelial injury includes vasodilation dysfunction characterized by decreased endothelial NO and structural damage characterized by increased endothelial inflammatory response, endothelial cell apoptosis, and endothelial cell permeability. A large number of studies have found that the abnormal expression and modification of occludin is accompanied by damage to the endothelial structure of blood vessels in tissues and organs, resulting in increased vascular permeability, inflammatory cell infiltration, and apoptosis. Therefore, we will detail the relationship between occludin and diseases related to damage to the arterial vascular endothelial structure in this section.

5.1 Occludin and arterial vascular disease

5.1.1 Occludin and cerebrovascular injury-related diseases

Brain has received extensive attention because it is the most vulnerable to endothelial barrier dysfunction. Under normal circumstances, the blood-brain barrier is a semi-permeable interface, which is important for providing a neuronal microenvironment and exchanging water, ions, gases, and metabolites, but it is not suitable for exogenous harmful substances such as bacteria and viruses [4]. However, many triggers (e.g., inflammation, traumatic brain injury, ischemia) can lead to leakage of the blood-brain barrier, increasing the risk of cerebral edema, nerve damage, cerebral hemorrhage, and further increasing the risk of cerebral ischemia. The brain endothelial cell tight junction protein occludin plays an important role in maintaining the integrity of the blood-brain barrier. For example: (1) Both clinical and basic researchers have found

that under cerebral ischemia, and cysteinase can hydrolyze the blood-brain barrier tight junction protein occludin to promote its release into the blood, resulting in an increase of occludin levels in serum. Therefore, serum occludin levels can be used as an indicator for predicting the severity of acute ischemic stroke, hemorrhagic transformation, and prognosis of patients [45]; (2) studies have shown that the increased permeability of the blood-brain barrier in type 1 diabetic mice may be due to the increased level of serum extracellular vesicle occludin, which affects the distribution of occludin in the cerebral vascular endothelial cell membrane [81]; (3) in rat model of traumatic shock, the expression of occludin in cerebral vascular endothelial cells is reduced, which affects the integrity of the blood-brain barrier, the leakage of inflammatory cells, and deterioration of vascular inflammatory response [82]; (4) both *in vitro* and *in vivo* studies found that the occludin degradation caused by autophagy is an important factor of the blood-brain barrier disorder when the brain is exposed to an ischemic environment [83]; (5) the increased expression of occludin in cerebral microvascular endothelial cells can reduce the apoptosis of endothelial cells by inhibiting the expression of apoptosis-related proteins, and the degradation of occludin makes cerebral blood vessels more prone to reperfusion injury [25, 84]; (6) in diabetic animal model, the expression of occludin in the cerebral vascular endothelium is reduced, which is manifested as diabetes complicated with cerebrovascular disease, and nerve damage, etc. [85]. In conclusion, abnormal expression, modification, and degradation of occludin may induce vascular endothelial dysfunction, resulting in injury of blood-brain barrier function, and ultimately aggravating the occurrence and development of brain diseases.

5.1.2 Occludin and coronary vascular injury diseases

Coronary endothelial barrier dysfunction is closely related to ischemic heart disease. Endothelial barrier integrity and function are regulated by a variety of transmembrane proteins, including claudin family proteins, occludin, VE-cadherin, etc. In recent years, basic research on occludin and coronary artery injury-related diseases has found that in mouse model of coronary artery sclerosis, the expression of occludin in arterial endothelial cells decreased, and the atherosclerotic plaque was expanded. Conversely, up-regulation of occludin expression in arterial endothelial cells can alleviate the occurrence and development of plaque [86]. In conclusion, abnormal expression of occludin in coronary endothelial cells is directly related to the occurrence and development of heart disease.

5.1.3 Occludin and pulmonary vascular injury diseases

Pulmonary vascular endothelial cells form a complete cell barrier, participate in the regulation of vascular homeostasis, and maintain the normal operation of the body. Under pathological conditions (diabetes, hypertension, and hyperlipidemia), the pulmonary vascular endothelial barrier is damaged, resulting in vascular endothelial dysfunction and chronic structural damage. Tight junction proteins play an important role in maintaining the integrity of the pulmonary vascular endothelial barrier. As an important component of TJ, occludin has been shown to be down-regulated in a variety of pulmonary vascular injury-related diseases. (1) Pulmonary arterial hypertension (PAH) is a progressive disease characterized by pulmonary endothelial cell dysfunction and vascular remodeling. Histological evaluation of mouse model of pulmonary arterial hypertension shows downregulation of occludin expression in pulmonary vessels [87]; (2) the expression of occludin in pulmonary artery endothelial

cells of diabetic and hypertensive model mice was reduced, and nitric oxide (NO), superoxide dismutase, and inducible NO synthase were severely imbalanced, suggesting that occludin may be involved in the production of vascular endothelial NO [88]; (3) studies have found that the occludin protein in the pulmonary artery endothelial cells of the rat model of acute lung injury is lost, the endothelial permeability is increased, the vascular inflammatory response is increased, and oxidative stress and other pathological states occur [89]. In conclusion, abnormal expression and distribution of occludin are closely related to pulmonary vascular lesions.

5.1.4 Occludin and renal vascular injury diseases

Kidney is one of the organs with the most abundant distribution of endothelial cells. Under physiological conditions, renal endothelium can mediate signal communication between various parts of the kidney, stabilize renal osmotic pressure, and regulate vascular permeability. Under pathological conditions such as ischemia, inflammation, and sepsis, renal vascular endothelial permeability is increased, renal metabolism is impaired, and the basal layer of endothelial cells is thickened, which induces endothelial damage and leads to plasma leakage. Occludin is involved in maintaining the barrier function of renal endothelial cells, and a large number of basic studies on occludin and renal vascular injury have found that (1) The abnormal expression and distribution of occludin in renal endothelial cells, the imbalance of electrolytes such as sodium, potassium, and chloride, and the deterioration of renal injury exist in the rat model of renal ischemia-reperfusion, suggesting that the abnormal expression and distribution of occludin in renal vascular endothelial cells affect renal function homeostasis [90]; (2) High glucose and high fat stimulate human glomerular endothelial cells, decrease the expression of occludin, and damage renal endothelial barrier function, which leads to development of diabetic nephropathy [91]; (3) renal dysfunction caused by hyperoxia is closely related to renal endothelial tight junction protein occludin [92]. In conclusion, the decreased expression of occludin in renal endothelial cells under pathological conditions may be a new marker of renal vascular injury.

5.1.5 Occludin and other arterial diseases

The blood retinal endothelial barrier maintains the integrity of retinal tissue. The level of occludin in endothelial cells can dynamically regulate the intracellular signal transduction system, promote the transport of nutrients, and limit the transport of harmful substances, which is extremely important for maintaining the blood retinal endothelial barrier. Studies have found that: (1) The phosphorylation of occludin S490 in retinal endothelial cells regulates the proliferation and angiogenesis of retinal endothelial cells [6]; (2) the decreased expression of occludin in endothelial cells of diabetic retinopathy can induce inflammatory cell infiltration, suggesting that the loss of occludin at the blood-retinal barrier leads to increased endothelial cell permeability, which is an important factor for mediating the aggravation of vascular inflammatory responses [93]; (3) when neonatal rats exposed to hypoxia, the expression of occludin in retinal endothelial cells decreased, vacuoles appeared in endothelial cytoplasm, and mitochondrial vacuoles and multivesicles accumulated in capillary lumen, suggesting that occludin was involved in the occurrence of hypoxic stress response [94]; (4) relevant studies have shown that exogenous stimuli (high sugar, long-term high-fat diet, long-term smoking) can inhibit the expression of occludin in the vascular endothelium, resulting in an increase in vascular permeability, which

in turn causes the occurrence of oxidative stress in vascular endothelial cells [89]. According to the above research results, it is suggested that the maintenance of blood retinal endothelial barrier integrity is closely related to occludin.

5.2 Occludin and venous vascular diseases

Venous vessels maintain venous barrier function by expressing abundant occludin. Recent studies on occludin in venous endothelial cells have shown that: (1) Serum occludin levels are higher in patients with jugular vein stenosis [95]; (2) Nitta et al. found that in mouse model of retinal vein occlusion, venous vascular inflammation increased, occludin expression decreased, and retinal edema occurred; conversely, inhibiting vascular inflammation could alleviate the decrease in occludin expression and maintain retinal homeostasis [96]; (3) studies on mice with ischemic stroke found that early cerebral venous filling and dilation were associated with occludin displacement and abnormal expression and distribution [97]. In conclusion, the maintenance of the venous homeostasis is inseparable from the regulation of occludin.

6. Conclusions

Occludin, as a cellular tight junction protein, mediates molecular communication between cells and maintains the integrity of various tissues and cells. More and more studies have confirmed that under pathological conditions, various signaling pathways can disrupt the integrity of cell barrier by regulating the expression and distribution of occludin, and participate in apoptosis, inflammation, cardiovascular and neurodegenerative diseases. Occludin plays an important role in cardiovascular disease, but current research also faces great challenges. A variety of classical signaling pathways can regulate the expression and distribution of occludin, but only some studies suggest that occludin can act as an upstream regulatory molecule to affect downstream signaling pathways, whether it affects multiple molecules and signaling pathways is an urgent problem to be solved. At present, most researches focus on occludin participating in cell barrier and maintaining cell integrity. Whether its overexpression plays a positive role in all systems is unknown. Loss of occludin can affect vascular endothelial permeability, leading to pathology such as inflammatory cell infiltration, apoptosis, and oxidative stress. However, whether inflammatory stimulation, apoptosis, and oxidative stress can directly affect the expression, modification, and redistribution of occludin is the current vacancy in current research field and needs further exploration and discovery. Relatively speaking, the research on occludin and vascular endothelial injury-related diseases is still very limited, but there is already relevant evidence that it is a close relationship between them. There is still a large gap in the relationship between occludin and vascular metabolic diseases needs to be filled. With further research in the future, the connection between occludin and many diseases related to vascular endothelial injury will become increasingly clear. In a word, whether it is possible to inhibit or use occludin to develop related drugs and apply them to the treatment of clinical diseases requires further research and discovery.

Conflict of interest

The authors declare no conflict of interest.

Author details

Yunhui Du[1*], Yanru Duan[2] and Shihan Zhang[3]

1 Beijing Institute of Heart, Lung and Blood Vessel Diseases, Beijing Anzhen Hospital, Capital Medical University, Beijing, P.R. China

2 Department of Physiology and Pathophysiology, School of Basic Medical Sciences, Capital Medical University, Beijing, P.R. China

3 Medical Oncology Department, Pediatric Oncology Center, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing Key Laboratory of Pediatric Hematology Oncology, Key Laboratory of Major Diseases in Children, Ministry of Education, Beijing, P.R. China

*Address all correspondence to: annahhdu820@mail.ccmu.edu.cn

References

[1] Sun HJ, Wu ZY, Nie XW, Bian JS. Role of endothelial dysfunction in cardiovascular diseases: The link between inflammation and hydrogen sulfide. Frontiers in Pharmacology. 2019;**10**:1568

[2] Li X, Wu TT, Chen J, Qiu W. Elevated expression levels of serum insulin-like growth factor-1, tumor necrosis factor-α and vascular endothelial growth factor 165 might exacerbate type 2 diabetic nephropathy. Journal of Diabetes Investigation. 2017;**8**(1):108-114

[3] Cong X, Kong W. Endothelial tight junctions and their regulatory signaling pathways in vascular homeostasis and disease. Cellular Signalling. 2020;**66**:109485

[4] Keep RF, Andjelkovic AV, Xiang J, Stamatovic SM, Antonetti DA, Hua Y, et al. Brain endothelial cell junctions after cerebral hemorrhage: Changes, mechanisms and therapeutic targets. Journal of Cerebral Blood Flow and Metabolism. 2018;**38**(8):1255-1275

[5] Chen YH, Xu X, Sheng MJ, Zheng Z, Gu Q. Effects of asymmetric dimethylarginine on bovine retinal capillary endothelial cell proliferation, reactive oxygen species production, permeability, intercellular adhesion molecule-1, and occludin expression. Molecular Vision. 2011;**17**:332-340

[6] Liu X, Dreffs A, Díaz-Coránguez M, Runkle EA, Gardner TW, Chiodo VA, et al. Occludin S490 phosphorylation regulates vascular endothelial growth factor-induced retinal neovascularization. The American Journal of Pathology. 2016;**186**(9):2486-2499

[7] Zinnanti WJ, Lazovic J, Housman C, Antonetti DA, Koeller DM, Connor JR, et al. Mechanism of metabolic stroke and spontaneous cerebral hemorrhage in glutaric aciduria type I. Acta Neuropathologica Communications. 2014;**2**:13

[8] Farquhar MG, Palade GE. Junctional complexes in various epithelia. The Journal of Cell Biology. 1963;**17**(2):375-412

[9] Citi S, Sabanay H, Jakes R, Geiger B, Kendrick-Jones J. Cingulin, a new peripheral component of tight junctions. Nature. 1988;**333**(6170):272-276

[10] Itoh M, Nagafuchi A, Yonemura S, Kitani-Yasuda T, Tsukita S, Tsukita S. The 220-kD protein colocalizing with cadherins in non-epithelial cells is identical to ZO-1, a tight junction-associated protein in epithelial cells: cDNA cloning and immunoelectron microscopy. The Journal of Cell Biology. 1993;**121**(3):491-502

[11] Furuse M, Hirase T, Itoh M, Nagafuchi A, Yonemura S, Tsukita S, et al. Occludin: A novel integral membrane protein localizing at tight junctions. The Journal of Cell Biology. 1993;**123**(6 Pt 2):1777-1788

[12] Balda MS, Flores-Maldonado C, Cereijido M, Matter K. Multiple domains of occludin are involved in the regulation of paracellular permeability. Journal of Cellular Biochemistry. 2000;**78**(1):85-96

[13] Bamforth SD, Kniesel U, Wolburg H, Engelhardt B, Risau W. A dominant mutant of occludin disrupts tight junction structure and function. Journal of Cell Science. 1999;**112**(Pt 12):1879-1888

[14] Heinemann U, Schuetz A. Structural features of tight-junction proteins.

International Journal of Molecular Sciences. 2019;**20**(**23**):6020

[15] Morcos Y, Hosie MJ, Bauer HC, Chan-Ling T. Immunolocalization of occludin and claudin-1 to tight junctions in intact CNS vessels of mammalian retina. Journal of Neurocytology. 2001;**30**(2):107-123

[16] Li Y, Xu Q, Shi M, Gan P, Huang Q, Wang A, et al. Low-level laser therapy induces human umbilical vascular endothelial cell proliferation, migration and tube formation through activating the PI3K/Akt signaling pathway. Microvascular Research. 2020;**129**:103959

[17] Wang GH, Ma KL, Zhang Y, Hu ZB, Liu L, Lu J, et al. Platelet microparticles contribute to aortic vascular endothelial injury in diabetes via the mTORC1 pathway. Acta Pharmacologica Sinica. 2019;**40**(4):468-476

[18] Manthari RK, Tikka C, Ommati MM, Niu R, Sun Z, Wang J, et al. Arsenic induces autophagy in developmental mouse cerebral cortex and hippocampus by inhibiting PI3K/Akt/mTOR signaling pathway: Involvement of blood-brain barrier's tight junction proteins. Archives of Toxicology. 2018;**92**(11):3255-3275

[19] Meng SS, Guo FM, Zhang XW, Chang W, Peng F, Qiu HB, et al. mTOR/STAT-3 pathway mediates mesenchymal stem cell-secreted hepatocyte growth factor protective effects against lipopolysaccharide-induced vascular endothelial barrier dysfunction and apoptosis. Journal of Cellular Biochemistry. 2019;**120**(3):3637-3650

[20] Ashina K, Tsubosaka Y, Kobayashi K, Omori K, Murata T. VEGF-induced blood flow increase causes vascular hyper-permeability in vivo. Biochemical and Biophysical Research Communications. 2015;**464**(2):590-595

[21] Wang Q, Deng Y, Huang L, Zeng W, Chen S, Lv B, et al. Hypertonic saline downregulates endothelial cell-derived VEGF expression and reduces blood-brain barrier permeability induced by cerebral ischaemia via the VEGFR2/eNOS pathway. International Journal of Molecular Medicine. 2019;**44**(3):1078-1090

[22] Li R, Qi Y, Jiang M, Zhang T, Wang H, Wang L, et al. Primary tumor-secreted VEGF induces vascular hyperpermeability in premetastatic lung via the occludin phosphorylation/ubiquitination pathway. Molecular Carcinogenesis. 2019;**58**(12):2316-2326

[23] Jo H, Hwang D, Kim JK, Lim YH. Oxyresveratrol improves tight junction integrity through the PKC and MAPK signaling pathways in Caco-2 cells. Food and Chemical Toxicology. 2017;**108**(Pt A):203-213

[24] Kalsi KK, Garnett JP, Patkee W, Weekes A, Dockrell ME, Baker EH, et al. Metformin attenuates the effect of Staphylococcus aureus on airway tight junctions by increasing PKCζ-mediated phosphorylation of occludin. Journal of Cellular and Molecular Medicine. 2019;**23**(1):317-327

[25] Yu H, Wang C, Wang X, Wang H, Zhang C, You J, et al. Long-term exposure to ethanol downregulates tight junction proteins through the protein kinase Cα signaling pathway in human cerebral microvascular endothelial cells. Experimental and Therapeutic Medicine. 2017;**14**(5):4789-4796

[26] Xia W, Pan Z, Zhang H, Zhou Q, Liu Y. Inhibition of ERRα aggravates sepsis-induced acute lung injury in rats via provoking inflammation and

oxidative stress. Oxidative Medicine and Cellular Longevity. 2020;**2020**:2048632

[27] Li Z, Liu YH, Liu XB, Xue YX, Wang P, Liu LB. Low-dose endothelial monocyte-activating polypeptide-II increases permeability of blood-tumor barrier via a PKC-ζ/PP2A-dependent signaling mechanism. Experimental Cell Research. 2015;**331**(2):257-266

[28] Boardman R, Pang V, Malhi N, Lynch AP, Leach L, Benest AV, et al. Activation of notch signaling by soluble Dll4 decreases vascular permeability via a cAMP/PKA-dependent pathway. American Journal of Physiology. Heart and Circulatory Physiology. 2019;**316**(5):H1065-H1075

[29] Yue W, Li Y, Ou D, Yang Q. The GLP-1 receptor agonist liraglutide protects against oxidized LDL-induced endothelial inflammation and dysfunction via KLF2. IUBMB Life. 2019;**71**(9):1347-1354

[30] Xiong R, Zhou XG, Tang Y, Wu JM, Sun YS, Teng JF, et al. Lychee seed polyphenol protects the blood-brain barrier through inhibiting Aβ[25-35]-induced NLRP3 inflammasome activation via the AMPK/mTOR/ULK1-mediated autophagy in bEnd.3 cells and APP/PS1 mice. Phytotherapy Research. 2021;**35**(2):954-973

[31] Wang X, Xue GX, Liu WC, Shu H, Wang M, Sun Y, et al. Melatonin alleviates lipopolysaccharide-compromised integrity of blood-brain barrier through activating AMP-activated protein kinase in old mice. Aging Cell. 2017;**16**(2):414-421

[32] Victor C, Marta S, Jorge L, Jane H, Neil S, Martina V. Occludin regulates glucose uptake and ATP production in pericytes by influencing AMP-activated protein kinase activity. Journal of Cerebral Blood Flow and Metabolism. 2018;**38**(2):317-332

[33] Liu Y, Tang J, Yuan J, Yao C, Hosoi K, Han Y, et al. Arsenite-induced downregulation of occludin in mouse lungs and BEAS-2B cells via the ROS/ERK/ELK1/MLCK and ROS/p38 MAPK signaling pathways. Toxicology Letters. 2020;**332**:146-154

[34] Qin LH, Huang W, Mo XA, Chen YL, Wu XH. LPS induces occludin dysregulation in cerebral microvascular endothelial cells via MAPK signaling and augmenting MMP-2 levels. Oxidative Medicine and Cellular Longevity. 2015;**2015**:120641

[35] Zhou Q, Wang YW, Ni PF, Chen YN, Dong HQ, Qian YN. Effect of tryptase on mouse brain microvascular endothelial cells via protease-activated receptor 2. Journal of Neuroinflammation. 2018;**15**(**1**):248

[36] Wang X, Zhao Z, Zhu K, Bao R, Meng Y, Bian J, et al. Effects of CXCL4/CXCR3 on the lipopolysaccharide-induced injury in human umbilical vein endothelial cells. Journal of Cellular Physiology. 2019;**234**(12):22378-22385

[37] Wang H, Segaran RC, Chan LY, Aladresi AAM, Chinnathambi A, Alharbi SA, et al. Gamma radiation-induced disruption of cellular junctions in HUVECs is mediated through affecting mapk/nf-κb inflammatory pathways. Oxidative Medicine and Cellular Longevity. 2019;**2019**:1486232

[38] Patwardhan A, Cheng N, Trejo J. Post-translational modifications of G protein-coupled receptors control cellular Signaling dynamics in space and time. Pharmacological Reviews. 2021;**73**:120-151

[39] Banks CJ, Andersen JL. Mechanisms of SOD1 regulation by post-translational

modifications. Redox Biology. 2019;**26**:101270

[40] Song L, Luo ZQ. Post-translational regulation of ubiquitin signaling. The Journal of Cell Biology. 2019;**218**:1776-1786

[41] Majolée J, Kovačević I, Hordijk PL. Ubiquitin-based modifications in endothelial cell-cell contact and inflammation. Journal of Cell Science. 2019;**132**:jcs227728

[42] Cummins PM. Occludin: One protein, many forms. Molecular and Cellular Biology. 2012;**32**:242-250

[43] Reiche J, Huber O. Post-translational modifications of tight junction transmembrane proteins and their direct effect on barrier function. Biochimica et biophysica acta. Biomembranes. 2020;**1862**:183330

[44] Pan R, Yu K, Weatherwax T, et al. Blood Occludin level as a potential biomarker for early blood brain barrier damage following ischemic stroke. Scientific Reports. 2017;**7**:40331

[45] Li W, Qi Z, Kang H, et al. Serum occludin as a biomarker to predict the severity of acute ischemic stroke, hemorrhagic transformation, and patient prognosis. Aging and Disease. 2020;**11**:1395-1406

[46] Beroun A, Mitra S, Michaluk P, et al. MMPs in learning and memory and neuropsychiatric disorders. Cellular and Molecular Life Sciences : CMLS. 2019;**76**:3207-3228

[47] Van Doren SR. Matrix metalloproteinase interactions with collagen and elastin. Matrix Biology : Journal of the International Society for Matrix Biology. 2015;**44-46**:224-231

[48] Yuan J, Zhang Y, Zhang Y, et al. Effects of metal nanoparticles on tight junction-associated proteins via HIF-1α/miR-29b/MMPs pathway in human epidermal keratinocytes. Particle and Fibre Toxicology. 2021;**18**:13

[49] Chen F, Hori T, Ohashi N, et al. Occludin is regulated by epidermal growth factor receptor activation in brain endothelial cells and brains of mice with acute liver failure. Hepatology [Baltimore, Md.]. 2011;**53**:1294-1305

[50] Nelson PT, Dickson DW, Trojanowski JQ, et al. Limbic-predominant age-related TDP-43 encephalopathy [LATE]: Consensus working group report. Brain : A Journal of Neurology. 2019;**142**:1503-1527

[51] Behzadian MA, Wang XL, Windsor LJ, et al. TGF-beta increases retinal endothelial cell permeability by increasing MMP-9: Possible role of glial cells in endothelial barrier function. Investigative Ophthalmology & Visual Science. 2001;**42**:853-859

[52] Bauer AT, Bürgers HF, Rabie T, et al. Matrix metalloproteinase-9 mediates hypoxia-induced vascular leakage in the brain via tight junction rearrangement. Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism. 2010;**30**:837-848

[53] Chen W, Hartman R, Ayer R, et al. Matrix metalloproteinases inhibition provides neuroprotection against hypoxia-ischemia in the developing brain. Journal of Neurochemistry. 2009;**111**:726-736

[54] Lischper M, Beuck S, Thanabalasundaram G, et al. Metalloproteinase mediated occludin cleavage in the cerebral microcapillary endothelium under pathological

conditions. Brain Research. 2010;**1326**:114-127

[55] Tada Y, Yagi K, Kitazato KT, et al. Reduction of endothelial tight junction proteins is related to cerebral aneurysm formation in rats. Journal of Hypertension. 2010;**28**:1883-1891

[56] Giebel SJ, Menicucci G, McGuire PG, et al. Matrix metalloproteinases in early diabetic retinopathy and their role in alteration of the blood-retinal barrier. Laboratory Investigation; A Journal of Technical Methods and Pathology. 2005;**85**:597-607

[57] Hawkins BT, Lundeen TF, Norwood KM, et al. Increased blood-brain barrier permeability and altered tight junctions in experimental diabetes in the rat: Contribution of hyperglycaemia and matrix metalloproteinases. Diabetologia. 2007;**50**:202-211

[58] Caron A, Desrosiers RR, Langlois S, et al. Ischemia-reperfusion injury stimulates gelatinase expression and activity in kidney glomeruli. Canadian Journal of Physiology and Pharmacology. 2005;**83**:287-300

[59] Ishihara H, Kubota H, Lindberg RL, et al. Endothelial cell barrier impairment induced by glioblastomas and transforming growth factor beta2 involves matrix metalloproteinases and tight junction proteins. Journal of Neuropathology and Experimental Neurology. 2008;**67**:435-448

[60] Lohmann C, Krischke M, Wegener J, et al. Tyrosine phosphatase inhibition induces loss of blood-brain barrier integrity by matrix metalloproteinase-dependent and -independent pathways. Brain Research. 2004;**995**:184-196

[61] Wan H, Winton HL, Soeller C, et al. Quantitative structural and biochemical analyses of tight junction dynamics following exposure of epithelial cells to house dust mite allergen Der p 1. Clinical and Experimental Allergy: Journal of the British Society for Allergy and Clinical Immunology. 2000;**30**:685-698

[62] Bojarski C, Weiske J, Schöneberg T, et al. The specific fates of tight junction proteins in apoptotic epithelial cells. Journal of Cell Science. 2004;**117**:2097-2107

[63] Bilbrough T, Piemontese E, Seitz O. Dissecting the role of protein phosphorylation: A chemical biology toolbox. Chemical Society Reviews. 2022;**51**:5691-5730

[64] Sakakibara A, Furuse M, Saitou M, et al. Possible involvement of phosphorylation of occludin in tight junction formation. The Journal of Cell Biology. 1997;**137**:1393-1401

[65] Farshori P, Kachar B. Redistribution and phosphorylation of occludin during opening and resealing of tight junctions in cultured epithelial cells. The Journal of Membrane Biology. 1999;**170**:147-156

[66] Tsukamoto T, Nigam SK. Role of tyrosine phosphorylation in the reassembly of occludin and other tight junction proteins. The American Journal of Physiology. 1999;**276**:F737-F750

[67] Dörfel MJ, Westphal JK, Bellmann C, et al. CK2-dependent phosphorylation of occludin regulates the interaction with ZO-proteins and tight junction integrity. Cell Communication and Signaling : CCS. 2013;**11**:40

[68] Goncalves A, Dreffs A, Lin CM, et al. Vascular expression of permeability-resistant occludin mutant preserves visual function in diabetes. Diabetes. 2021;**70**:1549-1560

[69] András IE, Deli MA, Veszelka S, et al. The NMDA and AMPA/KA receptors are involved in glutamate-induced alterations of occludin expression and phosphorylation in brain endothelial cells. Journal of Cerebral Blood Flow and Metabolism : Official Journal of the International Society of Cerebral Blood Flow and Metabolism. 2007;**27**:1431-1443

[70] Yamamoto M, Ramirez SH, Sato S, et al. Phosphorylation of claudin-5 and occludin by rho kinase in brain endothelial cells. The American Journal of Pathology. 2008;**172**:521-533

[71] Seth A, Sheth P, Elias BC, et al. Protein phosphatases 2A and 1 interact with occludin and negatively regulate the assembly of tight junctions in the CACO-2 cell monolayer. The Journal of Biological Chemistry. 2007;**282**:11487-11498

[72] Suzuki T, Elias BC, Seth A, et al. PKC eta regulates occludin phosphorylation and epithelial tight junction integrity. Proceedings of the National Academy of Sciences of the United States of America. 2009;**106**:61-66

[73] Popovic D, Vucic D, Dikic I. Ubiquitination in disease pathogenesis and treatment. Nature Medicine. 2014;**20**:1242-1253

[74] Shaid S, Brandts CH, Serve H, et al. Ubiquitination and selective autophagy. Cell Death and Differentiation. 2013;**20**:21-30

[75] Traweger A, Fang D, Liu YC, et al. The tight junction-specific protein occludin is a functional target of the E3 ubiquitin-protein ligase itch. The Journal of Biological Chemistry. 2002;**277**:10201-10208

[76] Lui WY, Lee WM. cAMP perturbs inter-Sertoli tight junction permeability barrier in vitro via its effect on proteasome-sensitive ubiquitination of occludin. Journal of Cellular Physiology. 2005;**203**:564-572

[77] Raikwar NS, Vandewalle A, Thomas CP. Nedd4-2 interacts with occludin to inhibit tight junction formation and enhance paracellular conductance in collecting duct epithelia. American Journal of Physiology. Renal Physiology. 2010;**299**:F436-F444

[78] Murakami T, Felinski EA, Antonetti DA. Occludin phosphorylation and ubiquitination regulate tight junction trafficking and vascular endothelial growth factor-induced permeability. The Journal of Biological Chemistry. 2009;**284**:21036-21046

[79] Tang J, Kang Y, Zhou Y, et al. ALS-causing SOD1 mutants regulate occludin phosphorylation/ubiquitination and endocytic trafficking via the ITCH/Eps15/Rab5 axis. Neurobiology of Disease. 2021;**153**:105315

[80] Zhang GS, Tian Y, Huang JY, et al. The γ-secretase blocker DAPT reduces the permeability of the blood-brain barrier by decreasing the ubiquitination and degradation of occludin during permanent brain ischemia. CNS Neuroscience & Therapeutics. 2013;**19**:53-60

[81] Rom S, Heldt NA, Gajghate S, Seliga A, Reichenbach NL, Persidsky Y. Hyperglycemia and advanced glycation end products disrupt BBB and promote occludin and claudin-5 protein secretion on extracellular microvesicles. Scientific Reports. 2020;**10**(1):7274

[82] Nikolian VC, Dekker SE, Bambakidis T, Higgins GA, Dennahy IS, Georgoff PE, et al. Improvement of blood-brain barrier integrity in traumatic brain injury and hemorrhagic shock

following treatment with valproic acid and fresh frozen plasma. Critical Care Medicine. 2018;**46**(1):e59-e66

[83] Kim KA, Kim D, Kim JH, Shin YJ, Kim ES, Akram M, et al. Autophagy-mediated occludin degradation contributes to blood-brain barrier disruption during ischemia in bEnd.3 brain endothelial cells and rat ischemic stroke models. Fluids Barriers CNS. 2020;**17**:21

[84] Zhang Y, Li X, Qiao S, Yang D, Li Z, Xu J, et al. Occludin degradation makes brain microvascular endothelial cells more vulnerable to reperfusion injury *in vitro*. Journal of Neurochemistry. 2021;**156**(3):352-366

[85] Ogata S, Ito S, Masuda T, Ohtsuki S. Changes of blood-brain barrier and brain parenchymal protein expression levels of mice under different insulin-resistance conditions induced by high-fat diet. Pharmaceutical Research. 2019;**36**(10):141

[86] Higashi Y, Sukhanov S, Shai SY, Danchuk S, Snarski P, Li Z, et al. Endothelial deficiency of insulin-like growth factor-1 receptor reduces endothelial barrier function and promotes atherosclerosis in Apoe-deficient mice. American Journal of Physiology. Heart and Circulatory Physiology. 2020;**319**(4):H730-H743

[87] Good RB, Gilbane AJ, Trinder SL, Denton CP, Coghlan G, Abraham DJ, et al. Endothelial to mesenchymal transition contributes to endothelial dysfunction in pulmonary arterial hypertension. The American Journal of Pathology. 2015;**185**(7):1850-1858

[88] Lee JH, Chinnathambi A, Alharbi SA, Shair OHM, Sethi G, Ahn KS. Farnesol abrogates epithelial to mesenchymal transition process through regulating Akt/mTOR pathway. Pharmacological Research. 2019;**150**:104504

[89] Liu H, Gu C, Liu M, Liu G, Wang D, Liu X, et al. Ventilator-induced lung injury is alleviated by inhibiting NLRP3 inflammasome activation. Molecular Immunology. 2019;**111**:1-10

[90] Lee SY, Shin JA, Kwon HM, Weiner ID, Han KH. Renal ischemia-reperfusion injury causes intercalated cell-specific disruption of occludin in the collecting duct. Histochemistry and Cell Biology. 2011;**136**(6):637-647

[91] Yin Q, Xia Y, Wang G. Sinomenine alleviates high glucose-induced renal glomerular endothelial hyperpermeability by inhibiting the activation of RhoA/ROCK signaling pathway. Biochemical and Biophysical Research Communications. 2016;**477**(4):881-886

[92] Gonzalez A, Krieg R, Massey HD, Carl D, Ghosh S, Gehr TWB, et al. Sodium butyrate ameliorates insulin resistance and renal failure in CKD rats by modulating intestinal permeability and mucin expression. Nephrology, Dialysis, Transplantation. 2019;**34**(5):783-794

[93] Zhang T, Ouyang H, Mei X, Lu B, Yu Z, Chen K, et al. Erianin alleviates diabetic retinopathy by reducing retinal inflammation initiated by microglial cells via inhibiting hyperglycemia-mediated ERK1/2-NF-κB signaling pathway. The FASEB Journal. 2019;**33**(11):11776-11790

[94] Rathnasamy G, Sivakumar V, Foulds WS, Ling EA, Kaur C. Vascular changes in the developing rat retina in response to hypoxia. Experimental Eye Research. 2015;**130**:73-86

[95] Wu Y, Meng R, Rajah GB, Ding Y, Xu Y, Ji X. Blood-brain barrier disruption

may contribute to white matter lesions in the setting of internal jugular venous stenosis. Current Neurovascular Research. 2019;**16**(4):328-334

[96] Nitta K, Nishinaka A, Hida Y, Nakamura S, Shimazawa M, Hara H. Oral and ocular administration of crocetin prevents retinal edema in a murine retinal vein occlusion model. Molecular Vision. 2019;**25**:859-868

[97] Chai J, Long B, Liu X, Li Y, Han N, Zhao P, et al. Effects of sevoflurane on tight junction protein expression and PKC-α translocation after pulmonary ischemia-reperfusion injury. Experimental & Molecular Medicine. 2015;**47**(6):e167