Regulation of claudins in blood-tissue barriers under physiological and pathological states

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Claudins are pivotal building blocks of tight junctions that form the paracellular barrier in epithelia and endothelia. In mammals, claudins are a 27-gene family that encodes tetraspan membrane proteins, playing a crucial role in the formation and integrity of tight junctions and regulate the barrier function. Claudin isoforms are expressed in a tissue- and/or developmental stage-dependent manner. A growing body of evidence indicates that pathological states characterized by neuroinflammation, such as Alzheimer disease, multiple sclerosis, diabetic retinopathy and retinopathy of prematurity share a common feature: the barrier breakdown. This review aims integrating and summarizing the most relevant and recent work developed in the field of claudins, with particular attention to their role in blood-brain and blood-retinal barriers, as well as describing their regulation in the aforementioned human diseases.

Tight Junctions and Paracellular Transport in the Brain and Retina

The blood-brain barrier (BBB) concept was first derived from experimental observations made by Ehrlich in 1885[1] and later by Goldman in 1913,[2] after injecting colored dyes into the blood stream. The experiments conducted by Goldman showed that the trypan blue dye stained almost all tissues except the brain. When the dye was injected into the cerebrospinal fluid (CSF) that surrounds the brain, this organ was stained, but the rest of the tissues in the body were not, providing the first demonstration that there is a kind of compartmentalization between the bloodstream and the CSF. Subsequent studies performed by Stern confirmed the presence of a special filter at the blood-brain interface that protects the brain, which was called blood-brain barrier (until then called hematoencephalic barrier).

Several decades later, morphological studies using transmission electron microscopy, showed the presence of "zonula occludens" between retinal endothelial cells. Moreover, permeability measurements, after systemic or intravitreal injection of fluorescein, were the basis of the concept of a blood-retinal barrier (BRB).[3,4] Based on those and other findings, it was proposed that the BRB consists of two anatomical components, an inner BRB (iBRB) composed by tight junctions (TJ) between retinal capillary endothelial cells and an outer BRB (oBRB) formed by TJ between retinal pigment epithelial (RPE) cells.[5,6]

In the brain and retina, the presence of a barrier between the vascular lumen and neural layers and parenchyma, respectively, allows the maintenance of a regulated microenvironment and the proper neuronal function. BBB is formed by a continuous monolayer of endothelial cells, separating the nervous system from circulating blood.

The BBB is comprised of brain microvascular endothelial cells, astrocytes and smooth muscle cells, the pericytes. It has been claimed that astrocytes secrete soluble factors that are important to strengthen the barrier function in BBB.[7,8] Pericytes have also an important role in maintaining and strengthen the barrier function, as demonstrated by in vitro BBB models.[9]

Cerebral homeostasis also results from the ability of the blood-cerebrospinal fluid barrier (BCSFB) at the choroid plexus to control the composition of the CSF and cerebral extracellular fluid. Unlike the capillaries that form the BBB, choroid plexus capillaries do not have TJ and are fenestrated and therefore do not form a barrier to the passage of small molecules. Instead, the BCSFB at the choroid plexus is formed by TJ between the epithelial cells and TJ linked to the arachnoid membrane that envelops the brain.

The permeability barriers, as a result of the compartmentalization created by epithelia and endothelia with TJ, regulate the paracellular movement of ions and small molecules between adjacent cells. TJ strands are complex structures, composed of transmembrane and cytosolic proteins that function as a gate, which is sensitive to rapid changes on the microenvironment. TJ provide at least dual functions to the tissues, as barrier and fence, which are essential for the tissue development and homeostasis, as well as for the maintenance of cell polarity as a boundary between the apical and basolateral plasma membrane domains.[10-12] Recent advances have improved our understanding about the molecular components, regulation and function of the TJ permeability. TJ are dynamic complexes in which the extracellular domains of TJ proteins associate with extracellular domains of proteins on adjacent cells. The branching network of sealing strands of proteins found in endothelial and epithelial TJ includes a series of transmembrane proteins embedded in the plasma membrane, such as junctional adhesion molecules (JAMS), claudins, occludin and tricellulin,[13,14] which in turn are attached to several cytoskeleton and cytoplasmic scaffold proteins, including zonula occludens (ZO)-1/2/3, MAGI-1, MAGI-3, CASK/LIN-2, MUPP1, AF6, ASIP, PALS1, PATJ and cingulin.[15]

It is now clear that size-, charge-selectivity and permeability of TJ are tissue specific and depend on their essential components, the claudins.[16-20] In this review, we will summarize recent progress with respect to claudins, giving particular attention to their function and regulation in the brain and retinal barriers in health and pathological conditions.

Claudins Family: Structure, Function and Distribution

TJ constitute the most apical intercellular junctional complex between adjacent endothelial and epithelial cells. The TJ, a multiprotein complex consisting of transmembrane and cytosolic proteins, control and restrict the paracellular diffusion of macromolecules, ions and polar solutes.[21]

Based on transmission electron microscopy studies, TJ were first described as structures in which the outer leaflets of the membranes of two adjacent cells are merged into a single line or into a series of apparent fusions (kissing points).[22] Afterward, freeze-fracture electron microscopy showed that these fusions were composed by strands of intramembranous particles, corresponding to TJ.[23]

One of the major components of the TJ strands is the claudin family, which comprises 27 members in mice and humans.[16] Claudins were first purified and identified by Furuse and colleagues in 1998.[17] The name claudin derives from the latin *claudere* which means close. Based on sequence analysis of the mouse claudin proteins, Krause and colleagues proposed a subdivision of the claudin family into classic (claudin-1–10, 14, 15, 17, 19) and non-classic (claudin-11–13, 16, 18, 20–24) groups for mouse proteins.[24] Later on, it was proposed a similar division for the human proteins, with slight differences, considering that claudin-1–9, 14, 17, 19, 20 are classic claudins and claudin-10–12, 15, 16, 18, 21–24 are non-classic claudins.[25] Recently, it was proposed

another classification of the claudin family, based on their permeability attributes, dividing each member into different categories: sealing, channel forming (anion- or cation-selective and water-permeable), inconsistent functionality and limited functional characterization.[26]

Claudins are 20–34 kDa proteins, containing four transmembrane domais, N- and C-terminal cytoplasmic domains and two extracellular loops (ECL) (**Fig. 1**). The C-terminal tail of claudins is essential for their stability and intracellular transport to the TJ.[27-29] For some claudins, this domain can be phosphorylated to regulate barrier function, and its phosphorylation has been linked to either increases or decreases in TJ assembly and function. For example, it has been shown that cyclic AMP induces phosphorylation of Thr-207 in claudin-5 by protein kinase A (PKA), increasing the barrier function in brain endothelium.[30] However, when the same residue of claudin-5 is phosphorylated by PKA in lung endothelial cells, a size selective loosening of claudin-5-based barrier against small molecules was found.[31] In brain endothelial cells, Rho/ROCK signaling leads to the phosphorylation of claudin-5 at Ser and Tyr residues, increasing BBB permeability.[32] In lung endothelial cells, phosphorylation of claudin-1 at Thr-203 by MAPK promotes barrier functions.[33] In colon carcinoma cells, phosphorylation of claudin-4 at Tyr-208 by ephrin type-A receptor 2 (EphA2) attenuates its interaction with ZO-1 and reduces the integration of claudin-4 into TJ, enhancing paracellular permeability.[34] Similarly, PKA-dependent phosphorylation of claudin-3 at Thr-192 leads to its cytoplasmic localization and barrier dysfunction in ovarian cancer cells.[35] In summary, claudins can be phosphorylated by different kinases, which controls claudin localization and/or function. However, since the phosphorylation of claudins triggers different outcomes, it is not possible to make a general functional conclusion since the results differ enormously.

Other posttranslational modifications of claudins, including palmitoylation, have been described. Palmitoylation occurs at the conserved di-cysteine motifs, located right after the second and fourth transmembrane domains.[36] Palmitoylation of these motifs is thought to be required for incorporation of claudin-2, 4 and 14 into the TJ, enabling their translocation to detergent-resistant plasma membranes (lipid rafts).[36]

Additionally, almost every claudin has a PDZ binding motif at the C-terminus that allows binding to the PDZ domain of cytoplasmic scaffolding proteins: ZO-1/2/3,[37] PATJ[38] and MUPP1.[39] Association with the scaffold proteins indirectly links claudin strands to the actin cytoskeleton and regulates claudins function.

The first ECL (ECL1) of the claudin family is composed by ~50–60 amino acids being much longer than the second one, which is composed only by ~10–30 amino acids. ECL1 appears to be involved in the ionic properties of claudin strands. The role of ECL1 in determining the selectivity for ions was demonstrated using mutagenesis assays. For example, the replacement of acidic by basic amino acid residues in claudin-15 led to a reversal in paracellular charge selectivity, from a preference for cations to anions.[40] This has also been demonstrated in other claudin isoforms.[40-42] ECL1 also contains a GLWCC motif, which acts as a receptor for Hepatitis C virus (HCV) entry. This has been demonstrated for claudin-1, 6 and 9, which are widely expressed in the liver and
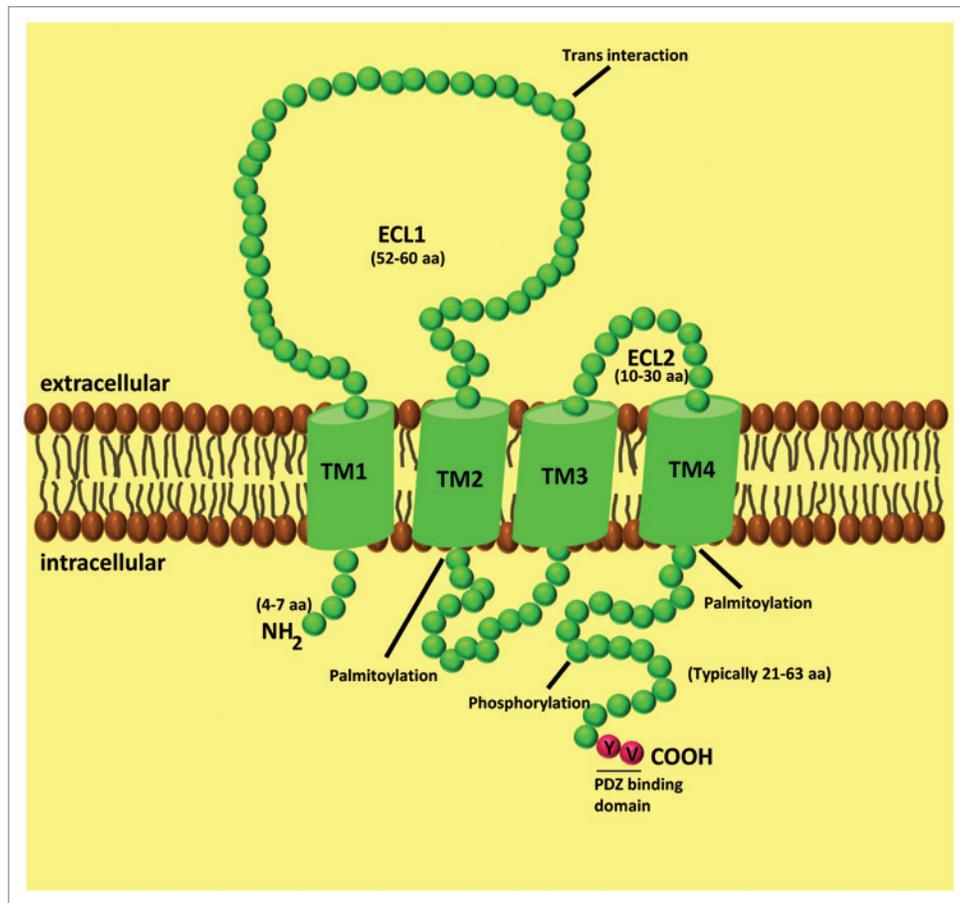
Figure 1. Schematic representation of claudin proteins. Claudins have four transmembrane spanning regions, two extracellular loops, one intracellular domain, with the amino and carboxyl terminus oriented toward the cytoplasm.

in peripheral blood mononuclear cells, precisely the major HCV replication sites.[43,44]

The second ECL (ECL2), due to its predicted helix-turn-helix motif, appears to be more important for the transcellular binding that narrows the paracellular space.[45] Moreover, it has been demonstrated that the ECL2 of claudin-3 and 4 is a receptor for *Clostridium perfringens* enterotoxin.[46,47]

The ECL from different claudins can interact with each other between opposing cells (trans-interaction) or in the same cell (cis-interaction).[24] The cis-interaction can involve the same claudin subtype (homomeric interaction) or different claudin subtypes (heteromeric interaction). The trans-interaction can be homotypic or heterotypic.[24,45,48]

Transfection of L-fibroblasts with different claudin isoforms, and their co-cultivation, show that both claudin-1 and claudin-2 can trans-interact with claudin-3 but not to each other.[49] More recently, Piontek and colleagues have demonstrated homotypic trans-interactions between claudin-1, 2, 3 and 5 (but not between claudin-12) and heterotypic trans-interactions between claudin-1 and 5, claudin-1 and 3 and claudin-3 and 5.[48] Despite nearly identical ECL domains and cis-type interaction, claudin-3 and 4 are heterotypically incompatible,[50] pointing out that the amino acid

sequence of the ECL is not the only determinant for the trans-interaction between claudins.

Claudins form ladders of stable homomultimers in native gel electrophoresis with increased molecular mass, corresponding to hexamers, which suggests a similar strand conformation to connexins in gap junctions.[51,52] The formation of claudin-4 monomers and claudin-2 homodimers occurs via the second transmembrane domain (helix–helix interaction).[53] Cis homo-dimerization was detected by fluorescence resonance energy transfer (FRET) analysis in claudin-5.[54] Cis-homomeric interactions have also been described for claudin-1, 2 and 3, along with heteromeric interactions between claudin-5 and 1, claudin-3 and 1 and claudin-3 and 5.[45,48] Interactions between claudin-4 and 8 and claudin-16 and 19, have also been studied. Claudin-4 and claudin-8 are co-transported from the Golgi apparatus to the TJ, cis-interacting with each other.[55] Claudin-16 and claudin-19, like claudin-3 and 4 share high similarity in their ECL. They show cis, but not trans-interaction.[56] The claudin-claudin interactions, in the same cell and between two adjacent cells, are responsible for the establishment of a continuous barrier within the intercellular clefts of the monolayer. This should mean that the network of strands formed by various claudin isoforms in each tissue leads to a selective

permeability of the solutes or compounds with different molecular weights or differently charged ions.

The diverse claudin subtypes are expressed in a tissue- and cell type-specific manner varying with the stage of development and differing in their barrier properties.[24] They can functionally be divided in barrier-forming or sealing claudins (for example claudin-1, 3 and 5) that are found in several epithelia and endothelia with a moderate to high transepithelial or endothelial resistance (TER), respectively.[57] Additionally, they can be pore-forming claudins; claudin-2 and a complex between claudin-16 and claudin-19 form pores for cations,[56,58] while claudin-17 forms an anion-selective pore.[59] Claudin-2 also forms a water channel, mediating paracellular water transport in leaky epithelia.[60] Interestingly, alternative splicing of claudin-10 gene originates claudin-10a and b, a cation and anion-selective claudin pore-forming, respectively.[61]

The function of several claudins, like for example claudin-4, 7 or 8, are not yet fully understood, mainly because very different and sometimes contradictory results have been obtained, namely in studies where claudins are overexpressed or knockdown. However, this strongly depends on the cell type used, and one cannot discard the possibility of interactions, or lack of them, with other claudins or other TJ proteins endogenously expressed in a specific cell type.

Some physiological roles of claudins have been clarified from studies with transgenic and knockout mice or human diseases. Claudin-1 knockout mice die shortly after birth due to dermal water loss indicating the essential role of claudin-1 in contributing to the tightness of skin epithelia.[20] Mutations in claudin-1 gene cause neonatal ichthyosis and sclerosing cholangitis in humans.[62] Deficiency in claudin-5 also causes neonatal death, due to size-selective loosening of the BBB.[63] Mice deficient in claudin-11 show myelin defects and the male animals are sterile due to the breakdown of the blood-testis barrier.[64] Mutations in claudin-14 gene lead to autosomal recessive deafness in mice and humans.[65] Several mutations in claudin-16 gene are seen in patients of familial hypomagnesemia with hypercalciuria and nephrocalcinosis, an autosomal recessive disorder that leads to renal calcification processes and renal failure.[66,67] Mutations in claudin-19 cause renal hypomagnesemia with ocular involvement.[68]

Claudins in Blood-Brain and Blood-Retinal Barriers

The BBB is a selective interface between the blood and the brain that maintains ionic homeostasis within the brain microenvironment.[69] The lack of fenestrations, decreased pinocytotic activity and presence of TJ, contribute to a high TER (1500–2000 Ω/cm^2) and to the restrictive nature of the BBB. On the contrary, systemic capillaries present a TER of only 5–10 Ω/cm^2.[70,71]

At the BBB, claudins-1, 3, 5 and 12 participate in the formation of TJ between brain microvascular endothelial cells (see **Table 1**).[18,63,72,73] Claudin-5 is the most abundant claudin at the BBB and is a critical regulator of brain endothelial cells permeability. In claudin-5 knockout mice, Nitta and colleagues demonstrated that the size-selectivity of the BBB was affected, allowing the diffusion of molecules smaller than 800 Da, but not of larger molecules.

These mice present morphologically normal TJ but died within 10 h of birth. It remains unclear whether this was due to BBB defects. Furthermore, overexpression of claudin-5 in cultured brain microvascular endothelial cells increases barrier properties.[74] Moreover, the expression of the TJ protein claudin-1 is lost in brain tumor microvessels, while claudin-5 is only downregulated, suggesting a relationship between claudin-1 suppression and the alteration of TJ morphology, which is likely to be correlated with the increase in endothelial cell permeability.[72] Similarly, the selective loss of claudin-3 from the TJ in pathological conditions demonstrates that it may be important for determining permeability and BBB integrity.[73] Claudin-12 at the cell-cell boundaries of brain capillary endothelial cells was detected in the brain of mice embryo.[63] In adult tissue, the levels of claudin-12 mRNA were relatively lower than those of claudin-5 at rat brain capillaries, and when the whole brain was analyzed, claudin-12 was shown not to be restricted only to capillaries.[57] Possibly, claudin-12 expression in BBB changes during development.

The barriers that surround the central nervous system are critical for its protection and homeostasis. While the BBB has been investigated intensively, only recently the choroid plexus-blood barrier, also known as the BCSFB, has received attention. BCSFB is characterized by having lower TER values (150 Ω/cm^2) and being less restrictive than the BBB. The molecular organization underlying that difference is probably related to the expression of different claudins, since those proteins play an important role in barrier size-selectivity and in the control of paracellular movement of ions. In fact, claudins-1, 2, 3 and 11 are expressed in the choroid plexus epithelium (see **Table 1**).[75,76] The expression of claudin-2 greatly increases the permeability of this barrier to both cations and water, and one cannot forget that interactions between different claudins can also influence paracelullar tightening. Despite low evidences showing the contribution of claudin-11 in the BCSFB, an important role of this isoform was pointed out in other cell types, namely in oligodendrocytes and Sertoli cells.[64] Immunohistochemical analysis of human/rat fetal and postnatal brains for claudin-1, 2 and 3 demonstrated their early presence and localization at the apico-lateral border of the choroid plexus epithelial cells.[76] Increased mRNA expression of claudin-6, 9, 19 and 22 also displayed a previously undescribed choroidal selectivity, although the authors could not confirm the presence of claudin-6 and 22 at the TJ due to lack of appropriate antibodies.[76] It was also detected a developmental upregulation of claudin-2, 9 and 22 and downregulation of claudin-6, reflecting changes in selective blood to CSF transport functions during development, which may be crucial for brain protection.

In the eye, the blood-tissue barrier is divided into two regions. The iBRB is formed by two beds of capillary endothelia. The inner capillary bed lies in the ganglion cell layer and its barrier function is modulated by astrocytes. The outer capillary bed lies in the inner and outer plexiform layers, where the function of astrocytes is replaced by Muller cells. The oBRB is formed by the RPE and lies on the outer surface of the photoreceptor layer.

At the iBRB, claudin-1, 2 and 5 are the most abundant claudins (see **Table 1**).[77-79] In comparison with BBB, the iBRB might be more permeable to ions in general, due to the lack of claudin-3

Table 1. Claudin expression changes in blood-brain and blood-retinal barriers in several neuroinflammatory diseases

Barrier	Claudin	Expression	Alterations
Brain			
BBB	Claudin-1	mRNA and protein detected in mouse[57] and human[72]	Expression suppressed in brain tumor vessels[72]
	Claudin-3	mRNA and protein detected in mouse[57] and human[73]	Loss of the TJ strands in a MS animal model and in brain tumor vessels[73]
	Claudin-5	mRNA and protein detected in mouse[18,57,63] and human[72]	Knockout mice have selective blood–brain barrier dysfunction for molecules < 800 Da;[63] Decreased expression[124] and subcellular redistribution[114] in a MS animal model
	Claudin-12	High mRNA levels in mice embryos, relative low expression in adult tissue[63]	No known alteration
BCSFB	Claudin-1	mRNA and protein detected in mouse,[75] rat[152] and human[76]	No known alteration
	Claudin-2	mRNA and protein detected in mouse,[75] rat[152] and human[76]	No known alteration
	Claudin-3	mRNA and protein detected in rat and human[76]	No known alteration
	Claudin-11	Protein detected in mouse[75]	No known alteration
Retina			
iBRB	Claudin-1	mRNA and protein detected in mouse,[77] rat[79] and rabbit[78]	No known alteration
	Claudin-2	mRNA and protein detected in mouse[77]	Overexpression in OIR animal model[77]
	Claudin-5	mRNA and protein detected in mouse[77] and rat[79,130]	Overexpression in OIR animal model;[77] Reduced expression and subcellular redistribution in diabetes animal models[79,132,136,137]
oBRB	Claudin-1	mRNA and protein detected in chick embryo[80,81]	Increased expression after ER stress induction;[141] Subcellular redistribution after high glucose and IL-1β exposure[95]
	Claudin-2	mRNA and protein detected in chick embryo[80,82]	No known alteration
	Claudin-3	mRNA and protein detected in human[83,84]	No known alteration
	Claudin-4L2	mRNA detected in chick embryo[81]	No known alteration
	Claudin-5	mRNA and protein detected in chick embryo[80,81]	No known alteration
	Claudin-10b	mRNA and protein detected in human[84]	No known alteration
	Claudin-11	mRNA detected in chick embryo[81]	No known alteration
	Claudin-12	mRNA and protein detected in chick embryo[80]	No known alteration
	Claudin-19	mRNA and protein detected in human[83,84]	siRNA against claudin-19 eliminates TER in vitro;[83] Mutations in claudin-19 gene cause renal hypomagnesemia with severe visual impairment[68]
	Claudin-20	mRNA detected in chick embryo[81,82]	No known alteration

and more permeable still to the cation sodium, due to the presence of claudin-2.

The expression of claudins during the formation of the iBRB TJ also seems to vary. The mRNA levels of claudin-1, 2, 3, 4, 5, 12, 22 and 23 were shown to be developmentally altered in the retinas of mice pups, from postnatal day 8 (P8) until P21. Claudin-22 mRNA increased throughout this period, but the others exhibited transient peaks. The protein levels of claudin-1 and 5 remained high, even though the amount of their mRNA decreased at P21. On the contrary, the protein levels of claudin-2 paralleled the decrease in mRNA expression. Among all the claudins expressed in neural retina, only claudin-1, 2 and 5 were found in the blood vessels, which are present in the inner, outer and ganglion cell vascular layers. All three claudins co-localized with occludin in the lateral membranes of endothelial cells.[77] By contrast, claudin-3, 4, 12 and 23 were localized in extravascular cells.

The oBRB regulates the movement of solutes between the fenestrated capillaries of the choroid and the photoreceptor layer of the retina. The formation of TJ and TJ protein expression in RPE seem to vary considerably between species and developmental stage. The analysis of RPE from chick embryos demonstrates that claudins continue to be tightly regulated even after the barrier is fully functional. Claudin-1, 2, 4L2, 5, 11, 12 and 20 mRNA have been detected (see **Table 1**). For example, claudin-5 is transiently expressed, while claudin-1 appears in an intermediate phase and others, like claudin-20, appear later.[80-82] These results suggest that from the time that functional TJ form to the time they mature, the selectivity and permeability of the oBRB are likely to change. Human RPE expresses predominantly claudin-19 mRNA and protein, also with significant amounts of claudin-3 (see **Table 1**).[83-85] In a monolayer of a human fetal RPE, claudin isoforms have a diverse localization. For instance, although claudin-19 and claudin-3 were uniformly expressed across the monolayer, claudin-10 and claudin-1 were only detected in a subset of cells.[83,84] Moreover, knockdown of claudin-19 by siRNA in the same in vitro model eliminated the TER, while siRNAs for other claudins had minimal effects,[83] supporting the assumption that this claudin has an important role in ocular complications.[68]

Role of Claudins in Human Diseases

Changes in the integrity of the brain and retinal barriers may affect the neurovascular unit, a functional association of neurons, astrocytes and microvasculature. Findings have shown that blood stream derived-factors and signals from astrocytes and pericytes are involved in the regulation of claudins expression in endothelial cells.[86-88] In several barrier dysfunction-related diseases, the levels of claudins and occludin present in microvessels are altered, contributing to the barrier breakdown. In several pathologies of the nervous system characterized by a prominent neuroinflammatory component, such as Alzheimer disease, multiple sclerosis, diabetic retinopathy and retinopathy of prematurity, it has been claimed that brain and retinal barriers dysfunction contributes to the pathogenesis of those diseases, even in the early stages.[77,89-95] The increase in passive diffusion of blood-borne substances through TJ detected in several pathological conditions will be discussed below. The redistribution, protein levels and mRNA expression changes of claudin isoforms observed in those pathologies are outlined in **Table 1**.

Alzheimer Disease

Alzheimer disease (AD), the most common dementia in elderly, is characterized by learning and memory impairments.[96] AD patients present cerebral amyloid angiopathy and profound changes in cerebral microvessels.[97] It has been shown that β-amyloid peptide (1–42) might alter BBB integrity by affecting the TJ complexes.[98,99] The disruption of BBB is well documented in AD and may contribute to the progression of disease. Indeed, several reports have shown a positive correlation between increased BBB leakage in aged brains and the degree of AD,[100-102] suggesting that BBB disruption may be an early event in AD progression or even an independent factor involved in brain aging. Increased levels of oxidative stress markers associated with downregulation of TJ proteins and increased BBB permeability[103] have been found in the early stages of AD.[104-107]

Moreover, a detailed immunohistochemistry analysis for claudins in AD brains revealed higher levels of claudin-2, 5 and 11 (in neurons), claudin-2 and 11 (in astrocytes) and claudin-11 (in oligodendrocytes), as compared with aged controls.[108] The upregulation of these claudin isoforms in AD might be an endogenous protective response of the brain tissue. Neurons expressing claudins were identified as being mainly of pyramidal type, which are thought to support cognition, and are known to be affected in the early stage of AD,[109] bearing neurofibrillary tangles. In addition, changes in BBB may result from a deregulation of the interplay between different claudin isoforms, such as claudin-2, which induces a leaky strand type,[110] while claudin-5 and 11 are known to be responsible for increasing the TER.[111] Although the role of claudins in AD is currently poorly understood, the regulation of claudin expression in different cell types, others than endothelial cells, also suggests a role of claudins in cellular responses to neurodegeneration.

Multiple Sclerosis

Multiple sclerosis (MS) is characterized by microvascular inflammation and demyelination of the nerves of the central nervous system (brain and spinal cord),[112] with a relapsing-remitting profile. In MS, increased leukocyte migration leads to a reorganization of the actin cytoskeleton and loss or subcellular redistribution of the brain endothelial TJ proteins claudin-5, occludin and ZO-1,[113-115] with a consequent disruption of both BBB and BCSFB. The BBB breakdown allows the infiltration of activated immune effector cells, including T lymphocytes, which in turn activate a complex cascade leading to tissue damage.[116,117] Several pro-inflammatory cytokines (tumor necrosis factor (TNF)-α, interferon (IFN)-γ, interleukin (IL)-6 and IL-12)[118] and activated matrix metalloproteinases (MMPs) target TJ proteins in brain endothelial cells, compromising the BBB integrity.[115,119] In experimental autoimmune encephalomyelitis (EAE), a mouse model used for brain inflammation and MS, it has been suggested that the inflammatory cytokines TNF-α and IL-1 are key mediators that induce alterations in BBB permeability.[120] Moreover, it has been consistently shown that TNF-α is detected upon post-mortem examination of MS brain lesions, being abnormally elevated in the CSF of patients. Moreover, the levels of this cytokine have been correlated with the progression and severity of this disease.[121-123] Although the molecular mechanisms underlying the regulation of BBB and BCSFB in MS remain poorly understood, recent evidences have shown that Irgm-1, an immune-related GTPase, is involved in the regulation of those barriers. During the initiation and progression of EAE, Irgm-1 is upregulated in epithelial cells of the choroid plexus, ependymal layers and ventricular system, as well as in reactive astrocytes, promoting the disruption of BBB and BCSFB via downregulation of claudin-5 expression on brain microvascular endothelial cells and upregulation of CCL-20 expression in choroid plexus and ependymal cells, respectively.[124] It has been also described a compromised endothelial barrier function (decreased TER) that is associated with a protein and mRNA downregulation of claudin-5 and occludin and an upregulation of the matrix metalloproteinase MMP-9 when brain endothelial cells are incubated with sera from patients in the exacerbation or remission phase of MS.[125] The downregulation of claudin-5 and occludin, accompanied with an upregulation of vascular endothelial growth factor (VEGF)-A, correlated with the BBB breakdown in an animal model of EAE.[88] In the same animal model, a specific loss of claudin-3 immunostaining from the brain microvessels that were surrounded by inflammatory infiltrates was observed,[73] suggesting a direct role for inflammatory cells in disrupting BBB TJ.

Diabetic Retinopathy and Retinopathy of Prematurity

The breakdown of the outer and inner BRB in patients with diabetic retinopathy, due to disorganization of TJ proteins, is one of the main factors accounting for macular edema and major vision complications that frequently lead to severe vision loss in patients with diabetes.[5,126] Therefore, the BRB is a relevant target for the treatment of retinal diseases, as we have previously discussed.[127]

The increase in BRB permeability has been described to be associated with changes in the expression, protein levels, phosphorylation, ubiquitination and subcellular distribution of TJ proteins in retinal endothelial cells.[128-131] Several studies have shown that the levels of pro-inflammatory cytokines, namely IL-1β and TNF-α, and adhesion molecules, are increased in the retina, vitreous and serum of diabetic patients and rats, being key mediators of TJ proteins disorganization and consequently BRB breakdown.[94,132-135] Moreover, alterations in claudin-5 in retinal vessels have been associated with increased vessel leakage in the early stages of diabetes.[79,132,136,137] In addition to pro-inflammatory cytokines, growth factors like VEGF, also mediate the increase in BRB permeability and contribute to the pathophysiology of diabetic retinopathy. In vitro studies demonstrate that VEGF treatment disrupts cell border staining of claudin-1[138] and 5 and contributes to clathrin-mediated endocytosis of claudin-5.[139] Recent reports have also shown that claudin-5 may be downregulated in retinal endothelial cells due to endoplasmic reticulum (ER) stress, which has been involved in vascular impairment in diabetic retinopathy.[140] In contrast, in a RPE cell line, ER stress promotes the increase in both protein and mRNA claudin-1 expression, which is accompanied by an increase in TER.[141]

Retinopathy of prematurity (ROP), the major cause of vision loss in children, is associated with younger gestational age and lower birth weight as risk factors.[142] The key pathological change, namely retinal neovascularization, is associated with local ischemia followed by subsequent neovascularization. In more severe forms of the disease, the abnormal vascular changes may progress to retinal detachment, and once retinal detachment occurs the prognosis for recovery of good visual acuity is very low.[143] The oxygen-induced retinopathy (OIR) model is widely used for studies of retinal neovascular diseases such as ROP and proliferative diabetic retinopathy.[144] Normally, in the OIR model, there is neovascularization in the retina and increased vascular permeability,[145] being also detected an overexpression of claudin-2 and 5 (mRNA and protein), while the levels of occludin and claudin-1 were unaffected.[77] Moreover, each claudin was also mislocalized to the cytosolic compartment or distributed to nonjunctional regions of the plasma membrane, suggesting a break in tight junctional strands of each cell thus contributing to the formation of new leaky vessels.[77]

While the breakdown of the iBRB has been investigated extensively, the involvement of the breakdown of the oBRB (RPE barrier) in the progression of certain retinal diseases has not been widely addressed. The leakage through the RPE barrier causes excessive water influx to the retina, and so the breakdown of this barrier is likely to play a causative role in the development of some forms of diabetic macular edema, a major cause of vision loss in diabetic retinopathy, being also involved in the development of age-related macular degeneration.

As mentioned above, inflammation underlies many alterations detected in these retinal pathologies. In ARPE-19 cells, a spontaneously transformed cell line of human adult RPE, the pro-inflammatory cytokine IL-1β promotes a decrease in the TER, while stimulating the expression of claudin-1, although the expression of claudin-11 and 12 remains constant.[146] In the same cell line, exposure to high glucose and IL-1β leads to the disruption of claudin-1 staining, despite an increase in its protein levels, which is associated with an increase in the monolayer permeability.[95] Upregulation of claudin-1 can induce changes in TJ function by different arrangements, either by altering side-to-side oligomerization that is essential for the formation of TJ within a cell or head-to-head interactions (homophilic or heterophilic) between opposing cells.

Although ARPE-19 cells are widely used and studied, one should be careful when interpreting data from studies regarding the RPE tight junctions using this cell line. Cultured RPE can manifest a greater heterogeneity than the one observed in vivo. In fact, Luo and colleagues reported that the heterogeneity of the tight junctions in ARPE-19 cells was manifested by a nonuniform distribution of claudin-1 and 2 and that the expression of the claudins was very dependent on culture conditions.[147] Also, transcriptome analysis revealed that this cell line does not express claudin-19,[85] which is, as mentioned before, one of the most important claudin isoform in maintaining the monolayer resistance.

One of the most suitable culture models appears to be derived from a primary human fetal RPE cell culture, as it mimics the normal physiology, function and structure of native fetal and adult RPE, preserving the function of tight junctions and retaining barrier function.[148] In human fetal RPE, a mixture of inflammatory cytokines including IL-1β, TNF-α and IFN-γ, or IFN-γ alone, decreased TER after 24 h with an increase in net epithelial fluid absorption.[149,150] With longer incubation periods (two days) only TNF-α alone significantly decreased TER, although this decrease was not correlated with changes in claudin-2, claudin-3 or claudin-19 expression.[151] Moreover, the authors did not detect any alterations in TER when exposing the human fetal RPE cells to IL-1β,[151] contrarily to what was observed in the ARPE-19 cells.[146]

Conclusion and Perspectives

Claudins are key components of TJ that regulate the paracellular permeability. Although it is well established that claudins can polymerize into TJ strands in heteromeric and heterotypic claudin-claudin interactions, the mechanisms underlying claudin assembly are not yet well understood, as well as whether and how this heterogeneity contributes to barrier properties and tissue homeostasis. Disturbances in the content, distribution and postranslational modifications of claudins have been detected in several brain and retinal diseases characterized by barrier breakdown. Although the elucidation of the molecular mechanisms involved in TJ deregulation are pivotal to find a potential common denominator in many disease states, a better understanding of claudin biology may facilitate the development of novel claudin-targeted therapies.

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