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Tight junctions in neurological diseases

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Tight junction, one of the type of cell-cell junctions, controls the paracellular permeability across the lateral intercellular space and maintains the cell polarity. Tight junctions consist of transmembrane proteins: members of tight junction-associated MARVEL protein (TAMP) family, claudins and junctional adhesion molecules (JAMs), and various cytoplasmic proteins that are necessary for the correct organization of the integral membrane components of the junction. Alterations in expression or localization of proteins of tight junctions have been described in several neurological disorders including multiple sclerosis, stroke, Alzheimer's disease, Parkinson's disease and epilepsy. In this review, we summarize the most recent data on components of tight junctions and focus on the implication of tight junction dysfunction in neurological diseases.

Key words: blood-brain barrier, endothelial cells, neurological diseases, tight junctions

ABBREVIATIONS:

Aβ – amyloid-β peptide
CAR – coxsackie and adenorevirus receptor
ESAM – endothelial cell-selective adhesion molecule
GUK – guanylate kinase-like
JAMs – junctional adhesion molecules
MMPs – matrix metalloproteinases
MT-MMPs – membrane-type matrix metalloproteinases
MUPP1 – multi-PDZ domain protein 1
PALS1 – protein associated with Lin seven 1
Par – partitioning defective
PATJ – PALS1-associated tight junction protein
PDZ – postsynaptic density/discs large/zonula occludens
pro-MMP-2 – pro-matrix metalloproteinase-2
PTZ – pentylenetetrazole
SH3 – SRC homology 3
TAMP – tight junction-associated MARVEL protein
TER – transepithelial electrical resistance
VEGF – vascular endothelial growth factor
ZO – zonula occludens

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INTRODUCTION

Tight junction is an intercellular adhesion complex forming the close contact between adjacent cells. Tight junctions are typical of epithelial cells where they are found in the apical region of the cell (Spring 1998). Tight junctions are also formed by some endothelial cells, however their localization is more variable than in epithelium (Dejana et al. 2009).

Tight junctions are composed of transmembrane proteins: members of tight junction-associated MARVEL protein (TAMP) family, claudins and junctional adhesion molecules (JAMs), and the cytoplasmic plaque consisting of many different proteins that form large complexes inside the cell (Fig. 1A) (Furuse et al. 1998b, Martìn-Padura et al. 1998, Matter and Balda 2003).

Tight junctions hold cells together as well as act as the barrier and the fence (Balda and Matter 1998, Tsukita et al. 1999, Niessen 2007, Paris et al. 2008). The term barrier refers to the control of paracellular permeability across lateral intercellular spaces preventing solutes and water from passing freely through the paracellular pathway (Tsukita et al. 1999, Cereijido et al. 2004). The term fence describes a function as a boundary between the apical and basolateral plasma membrane domains by preventing dif-

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fusion of proteins between the membrane compartments (Balda and Matter 1998, Balda and Matter 2000, Tsukita et al. 2001, Hartsock and Nelson 2008). In addition, tight junction proteins are important components of numerous signaling pathways controlling gene expression, cell differentiation and proliferation (Matter and Balda 2003, Schneeberger and Lynch 2004, Steed et al. 2009).

Tight junctions are dynamic and their structure and function are regulated on the level of protein expression, post-translational modifications or protein–protein interactions (Steed et al. 2010). Tight junctions undergo dynamic changes in response to various signaling pathways including protein kinase A, monomeric and heteromeric G proteins and various protein kinase C isotypes (Matter and Balda 2003, Paris et al. 2008, Steed et al. 2010).

This review aims at presenting the most recent knowledge of tight junction components and their involvement in neurological disorders.

COMPONENTS OF TIGHT JUNCTIONS

Tight junction-associated MARVEL protein (TAMP) family

Indispensable components of tight junctions are tight junction-associated MARVEL proteins containing the tetra-spanning MARVEL (MAL and related proteins for vesicle trafficking and membrane link) domain that is present in proteins involved in membrane apposition and concentrated in cholesterol-rich microdomains (Sanchez-Pulido et al. 2002). Main and most studied tight junction MARVEL proteins are occludin and tricellulin. Recently, Steed and coauthors (2009) identified a novel tight junction protein – marvelD3. This protein, like occludin and tricellulin, contains a conserved four-transmembrane MARVEL domain (Fig. 1A) (Steed et al. 2009, Raleigh et al. 2010).

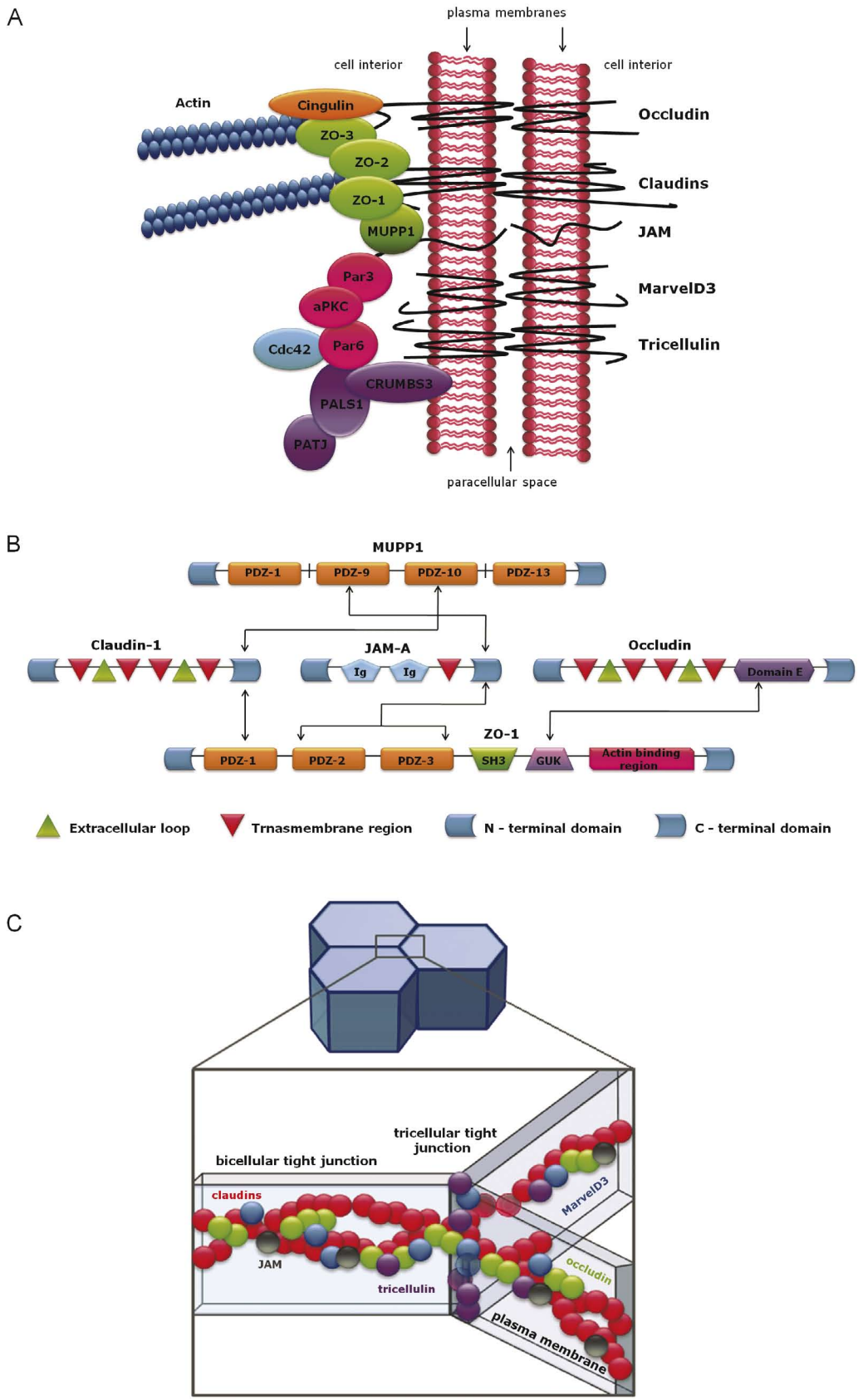
Occludin

Occludin, the first transmembrane protein to be associated with the tight junction (Furuse et al. 1993), is a ~60 kDa tetraspan membrane protein that forms two extracellular loops flanked by cytoplasmic N and C termini (Tsukita et al. 1999). It is expressed by essentially all epithelial and endothelial cells. In the brain, occludin is localized at the tight junctions of cerebral endothelial cells where it regulates paracellular permeability (Hirase et al. 1997). Expression of occludin by neurons and astrocytes has been also reported (Bauer et al. 1999).

Occludin localizes to the tight junction strands (Furuse et al. 1993), however is not crucial for the formation of the strands because occludin-deficient embryonic stem cells form morphologically normal tight junctions (Saitou et al. 1998). Although, barrier function of tight junctions is still present in intestinal epithelium of occludin knockout mice, histological abnormalities were found in several tissues, including chronic inflammation and hyperplasia of the gastric epithelium, calcification in the brain, loss of cytoplasmic granules in striated duct cells of the salivary gland and thinning of the compact bone (Saitou et al. 2000). It suggests that occludin functions are more complex than previously believed. It is possible that occludin plays an important role in the regulation of tight junction integrity rather than in the assembly of tight junctions through intracellular signaling.

Occludin interacts with the actin cytoskeleton and several intracellular proteins (Fig. 1A) (Furuse et al.

Fig. 1. Molecular structure of tight junctions formed by the tight junction-associated MARVEL protein family (occludin, tricellulin and marvelD3), claudins, junctional adhesion molecules (JAMs) and a cytoplasmic plaque consisting of many different proteins such as cingulin (orange), MUPP1 (dark green), ZO (light green) as well as proteins of polarity complexes: CRUMBS3–PALS1–PATJ (purple) and Par3-Par6-aPKC (pink) (A). Interactions between membrane components of tight junctions and adapters via defined domains (arrows). The association with ZO-1 requires domain E of occludin (dark purple) and C - terminal domains of claudin-1 and JAM-A. ZO-1 interacts with another proteins via PDZ (orange) and GUK (light purple) domains. Claudin-1 and JAM-A can also associate with different PDZ (orange) domains of MUPP1 (B). The organization of proteins within bicellular and tricellular regions of tight junction strands, which are composed of claudins (red spheres). MarvelD3 (blue spheres), tricellulin (purple spheres), occludin (green spheres) and JAM (black spheres) incorporate into claudin-based tight junction strands (C). (aPKC) atypical protein kinase C; (Cdc42) a Rho GTPase; (GUK) guanylate kinase-like; (MUPP1) multi-PDZ domain protein 1; (PALS1) protein associated with Lin seven 1; (Par) partitioning defective; (PATJ) PALS1-associated tight junction protein; (PDZ) postsynaptic density/discs large/zonula occludens; (SH3) SRC homology 3; (ZO) zonula occludens.


A
plasma membranes
cell interior
cell interior
Actin
Cingulin
ZO-3
ZO-2
ZO-1
MUPP1
Par3
aPKC
Cdc42
Par6
CRUMBS3
PALS1
PATJ
Occludin
Claudins
JAM
MarvelD3
Tricellulin
paracellular space
B
MUPP1
PDZ-1
PDZ-9
PDZ-10
PDZ-13
Claudin-1
JAM-A
Ig
Ig
Occludin
Domain E
ZO-1
PDZ-1
PDZ-2
PDZ-3
SH3
GUK
Actin binding region
Extracellular loop
Trnasmembrane region
N - terminal domain
C - terminal domain
C
tricellular tight junction
bicellular tight junction
claudins
JAM
tricellulin
MarvelD3
occludin
plasma membrane

1993). Even though C-terminus of occludin can bind actin directly, the interaction of occludin with the cytoskeleton occurs through adapters, such as zonula occludens (ZO) proteins and cingulin (Cordenonsi et al. 1999, Wittchen et al. 1999). In particular, occludin associates directly with ZO-1 (Furuse et al. 1994), ZO-2 (Itoh et al. 1999b) and ZO-3 (Haskins et al. 1998). The association with ZO-1 and ZO-2 requires the C-terminal portion of the domain E in the cytoplasmic tail of occludin (Fig. 2B) and this interaction is critical for the assembly of occludin into tight junctions (Furuse et al. 1994, Li et al. 2005). Interestingly, Shen and colleagues (2008) demonstrated that eighty percent of occludin is mobile and moves within the junctional membrane. This membrane traffic may be crucial to the maintenance of tight junction integrity. It is also possible that the constant movement of occludin and other junctional proteins within the tight junctions and the exchange of these proteins with extra-tight junction protein pools contribute to tight junction assembly (Shen et al. 2008).

Phosphorylation appears to be a key mechanism for regulating localization and biological function of occludin (Sakakibara et al. 1997, Wong and Gumbiner 1997, Farshori and Kachar 1999). Multiple kinases and phosphatases have been proposed to regulate occludin phosphorylation state.

Tyrosine phosphorylation of both occludin and ZO-1 induced by oxidative stress in Caco-2 cells mediates disruption and relocalization of the occludin-ZO-1 complex from tight junctions, contributing to an increase in paracellular permeability (Rao et al. 2002, Basuroy et al. 2003). Basuroy and coauthors (2003) suggested that c-Src plays a role in these processes. Another study has revealed that c-Src binds to C-terminal tail of GST-fused C-occludin and phosphorylates it on tyrosine residues causing reduction in affinity of occludin to ZO proteins (Kale et al. 2003).

It has been proposed that tyrosine phosphorylation of occludin induced by oxidative stress and dissociation of occludin from the actin cytoskeleton and ZO-1 resulting in disassembly of the tight junctions is mediated by phosphatidylinositol 3-kinase (Sheth et al. 2003). Similarly, tyrosine phosphorylation of occludin, but not ZO-1, in airway epithelium during mainstream cigarette smoke exposure decreased interaction between the two proteins contributing to increased permeability (Olivera et al. 2010).

There are also some evidences demonstrating that tyrosine phosphorylation of occludin is essential for assembly of tight junctions (Meyer et al. 2001, Chen et al. 2002). For example, phosphorylation of occludin, presumably by non-receptor kinase c-Yes, is crucial for tight junction formation and maintenance of its barrier function in epithelial cells (Chen et al. 2002).

Protein kinase C appears as a key enzyme, which directly phosphorylates occludin on serine/threonine or activates signaling pathways leading to increased serine/threonine phosphorylation of occludin. In epithelial cells, protein kinase C induces phosphorylation of occludin, causing its translocation from cytoplasm to the regions of cell-cell contact resulting in the tight junction formation (Andreeva et al. 2001). Whereas, in primary culture of retinal endothelial cells protein kinase C-dependent phosphorylation of occludin following vascular endothelial growth factor (VEGF) treatment contributes to increased endothelial permeability (Harhaj et al. 2006). The opposite effects of occludin phosphorylation by protein kinase C may be determined by the tissue-specific isoforms of protein kinase C involved (Andreeva et al. 2001). Dephosphorylation of occludin on serine/threonine, mediated by protein phosphatase 2A, leads to the increase in paracellular permeability (Nunbhakdi-Craig et al. 2002).

In addition to phosphorylation, occludin function is regulated by small GTPases (Barrios-Rodiles et al. 2005). Jou and coauthors (1998) generated MDCK cells that expressed RhoA and Rac1 GTPases mutants to investigate the role of these proteins in the spatial organization of tight junction proteins including occludin. In constitutively active RhoA and Rac1 mutants, occludin localization is disorganized. While in control cells it is restricted to the tight junctions, in RhoA and Rac1 mutants it is translocated to the cytoplasm and discontinuously distributed along the cell membrane (Jou et al. 1998). Additionally, the constitutive activation of RhoA or Cdc42 causes occludin redistribution from the membrane to cell interior (Bruewer et al. 2004).

Tricellulin

Tricellulin is a four transmembrane domain protein recently identified at tricellular tight junctions – which are formed where three cells meet (Fig. 1C) (Ikenouchi et al. 2005). Similar to occludin, tricellulin reveals extended N- and C-terminal cytoplasmic domains, with the C-terminal tail exhibiting homology to the

occludin C-terminus (Ikenouchi et al. 2005). Tricellulin mRNA was expressed in large amounts in epithelial tissues (Ikenouchi et al. 2005). Studies of subcellular localization revealed that tricellulin is predominantly concentrated at the vertically oriented tight junction strands of tricellular contacts. In addition, tricellulin participates in bicellular tight junctions formed between two adjacent cells (Fig. 1C) (Ikenouchi et al. 2005). When tricellulin expression was suppressed by RNA interference, tricellular contacts and bicellular tight junctions were disorganized resulting in disturbed continuity of occludin distribution at bicellular tight junction (Ikenouchi et al. 2005). Moreover, in occludin knockdown cells, the level of tricellulin in bicellular tight junctions was significantly increased suggesting that occludin deficiency resulted in a redistribution of tricellulin from tricellular tight junctions to bicellular tight junctions. These results enabled to postulate that tricellulin is localized to tricellular tight junctions, where is targeted to both edges of elongating bicellular tight junctions during the formation of tight junctions (Ikenouchi et al. 2008). It is not clear if tricellulin and occludin interact with each other. Although, coimmunoprecipitation studies demonstrated that tricellulin and occludin do not associate (Raleigh et al. 2010), other findings indicate that tricellulin forms homomeric tricellulin-tricellulin and heteromeric tricellulin-occludin complexes (Westphal et al. 2010). Ikenouchi and coauthors (2008) proposed that tricellulin and occludin are transported together to the edges of elongating bicellular junctions and get separated when tricellular contacts are formed.

MarvelD3

MarvelD3 was identified by Steed and coworkers (2009) as a novel integral membrane component of epithelial tight junctions. MarvelD3, a four-pass transmembrane protein (Fig. 1A) of about 40 kDa, is a member of the occludin family, a subgroup of the Marvel domain proteins. Bioinformatics analysis revealed the existence of two human MarvelD3 isoforms: isoform 1 contains 410 amino acids and isoform 2 with 401 amino acids (Steed et al. 2009). Both isoforms exhibit a broad tissue distribution and are expressed by different types of epithelial as well as endothelial cells (Steed et al. 2009). MarvelD3 co-localizes with the tight junction protein occludin but not with the adherens junction marker E-cadherin (Steed et al. 2009). Similar to occludin, expression of MarvelD3 is not essential for the formation of functional tight junctions (Steed et al. 2009). Nevertheless, MarvelD3 level can be a determinant of paracellular ion permeability. It has been shown that knockdown of MarvelD3 in Caco-2 cells causes an increase in transepithelial electrical resistance (TER) but does not affect permeability to fluorescent dextran tracers (Steed et al. 2009). In addition, Raleigh and coauthors (2010) demonstrated that MarvelD3, occludin and tricellulin make distinct contributions to tight junction structure and function. Although MarvelD3 is able to partially compensate for occludin or tricellulin loss, it cannot fully restore their function (Raleigh et al 2010).

Claudins

The claudin family consists of at least 24 members with molecular weight from 20 to 27 kDa (Furuse et al. 1998a, Tsukita et al. 2001, Schneeberger and Lynch 2004). Claudins are integral membrane proteins with four membrane-spanning regions, two extracellular and one intracellular loop, and N- and C-terminal cytoplasmic domains (Fig. 1A) (Furuse et al. 1998b). Individual claudins are generally expressed only in a restricted number of specific cell types, suggesting that they are associated with tissue-specific functions of tight junctions (Itoh et al. 1999a). For example, claudin-5 is a major component of tight junctions in the brain endothelial cells (Morita et al. 1999). Newborn claudin-5-deficient mice die within one day of birth without any morphological abnormalities in the brain. However, the function of blood-brain barrier of claudin-5-deficient mice is impaired. Tracer experiments and MRI revealed that in these mice, the tight junction endothelial barrier is loosened for small molecules – molecules smaller than ~800 Da can pass across tight junctions (Nitta et al. 2003).

Claudins form the backbone of the tight junction strands (Fig. 1C) (Furuse et al. 1998a) which show dynamic behavior, breaking and associating with each other. This is required for network formation and its constant remodeling without losing overall integrity (Sasaki et al. 2003).

Claudin proteins directly regulate the gate/barrier function as paracellular tight junction channels (Furuse et al. 2002, Tang and Goodenough 2003, Umeda et al. 2006, Krause et al. 2008). Changes in the type of clau-

din expressed, or single amino acid substitutions in claudin protein affect claudin ion selectivity. For example, a single substitution of amino acid Lys^{65} from a negative to positive charge within the first extracellular loop of claudin-4 causes an increase in Na^+ permeability (Colegio et al. 2002). In addition to their function in paracellular permeability, claudins modulate subcellular signaling and posses non-junctional functions in the regulation of cell proliferation (Tsukita et al. 2008).

Like occludin, also claudins interact with the ZO proteins as well as multi-PDZ domain protein 1 (MUPP1) and PATJ, which is a regulator of cell polarity (Fig. 1B). Claudins contain a PDZ-binding motif at their C terminus, which binds to PDZ domain-containing peripheral membrane proteins (Itoh et al. 1999a). Umeda and colleagues (2006) have shown that ZO-1 and ZO-2 can independently determine whether and where claudins are polymerized during the formation of tight junction strands. Furthermore, claudins recruit all membrane-type matrix metalloproteinases (MT-MMPs) and pro-matrix metalloproteinase-2 (pro-MMP-2) on the cell surface and participate in the MT-MMP-mediated activation of pro-MMP-2 (Miyamori et al. 2001). This mechanism may be important for vascular functions of matrix metalloproteinases (MMPs). There is little data supporting role of MMPs in regulation of tight junctions besides reports suggesting the participation of MMPs in cleavage of claudins after stroke by unknown mechanism (Yang et al. 2007, Zhao et al. 2011).

Junctional adhesion molecules

JAMs (junctional adhesion molecules) with a molecular mass of ~40 kDa have a single transmembrane domain (Fig. 1A) and belong to the immunoglobulin superfamily (Martìn-Padura et al. 1998, Balda and Matter 2000). They consist of two extracellular Ig-like domains, a single transmembrane region and a C-terminal cytoplasmic domain (Fig. 1B). The JAM family proteins are divided into two groups based on their sequence similarities: the closely related molecules JAM-A, -B and -C, and the more distantly related coxsackie and adenorevirus receptor (CAR), endothelial cell-selective adhesion molecule (ESAM) and JAM-4 (Bazzoni 2003, Ebnet et al. 2004).

These proteins are the components of tight junctions and may be involved in the barrier function of tight junctions in both epithelial and endothelial cells (JAM-A) (Mandell et al. 2004) or only in epithelial cells (CAR) (Walters et al. 2002). JAM-A interacts with MUPP1 (Hamazaki et al. 2002) and ZO-1 (Ebnet et al. 2000). It has been also demonstrated that JAM-A affects the formation of cell polarity in epithelial cells (Rehder et al. 2006).

JAMs are not only found in cells that form tight junctions but also are localized to circulating leukocytes regulating the leukocyte-endothelial cell interactions (Ebnet et al. 2004). In particular, junctional adhesion molecules participate in leukocyte adhesion and transmigration across endothelial and epithelial cells (Martìn-Padura et al. 1998, Chavakis et al. 2004, Khandoga et al. 2005). This process is mediated by integrins α4β1 or LFA-1 present on leukocytes, which bind endothelial JAM-A or JAM-B respectively (Cunningham et al. 2002, Ostermann et al. 2002). The JAM-A-LFA-1 interaction supports the adhesion of T cells to endothelial cells as well as T-cell and neutrophil transendothelial migration (Ostermann et al. 2002). It has been shown that JAM-C present on leukocytes binds to endothelial JAM-B (Arrate et al. 2001, Liang et al. 2002).

Cytoplasmic plaque

The cytoplasmic plaque of tight junctions is formed by different components that include adapters, such as zonula occludens (ZO), multi-PDZ domain protein 1 as well as cingulin and other proteins like signaling molecules, kinases and polarity complexes (Par3-Par6-aPKC and PATJ-PALS-CRUMBS3) (Fig. 1A).

Prominent components of tight junction plaque are the zonula occludens proteins ZO-1, -2 and -3 (Mitic and Anderson 1998). They belong to the MAGUK (membrane-associated guanylate kinase-like homologs) family and contain three PDZ domains, one SH3 domain and one guanylate kinase-like (GUK) domain (Fig. 1B) (Anderson 1995, Schneeberger and Lynch 2004). Due to the presence of multiple domains, zonula occludens proteins can function as adapters that connect transmembrane tight junction proteins to actin cytoskeleton and to various signaling molecules (Guillemot et al. 2008). MUPP1 can also directly interact with transmembrane components of tight junctions, claudins as well as JAM-A (Hamazaki et al. 2002). Another adapter, cingulin links tight junction proteins to the actin and myosin (Cordenonsi et al. 1999). Many

additional proteins of cytoplasmic plaque, such as conserved multiprotein polarity complexes Par3-Par6-aPKC and PATJ-PALS-CRUMBS3 are essential for tight junction functions as well as correct recruitment and localization of proteins during tight junction formation (Rajasekaran et al. 1996, Fogg et al. 2005, Shin et al. 2005). The polarity complexes determine the location of the tight junction in the process of cell polarization (Shin et al. 2006). For example, in MDCK cells, RNAi-mediated reduction of PATJ expression results not only in polarity defects but also in a severe delay of tight junction formation, including mislocalization of occludin and ZO-3 to the lateral membrane (Michel et al. 2005, Shin et al. 2005). Several cytoplasmic signaling molecules have been reported to be recruited by adapters to tight junctions where they participate in the regulation of tight junction assembly and disassembly (Matter and Balda 2003). These include protein kinase A, monomeric and heterotrimeric G proteins and various protein kinase C isotypes (Balda et al. 1991, Matter and Balda 2003). For example, Meyer and coauthors (2002) revealed that ZO-1 directly interacts with $G\alpha_{12}$ and the expression of $G\alpha_{12}$ increases paracellular permeability of epithelial cells. The existing data on the remaining signaling molecules are sparse and contradictory, so their role has not been elucidated.

TIGHT JUNCTIONS AND THE BLOOD-BRAIN BARRIER

The blood-brain barrier is a dynamic and complex cellular system strictly controlling the exchanges between the blood and brain compartments that is essential for the maintenance and regulation of the neuronal microenvironment. Therefore, blood-brain barrier contributes to brain homeostasis and provides protection against many toxic compounds and pathogens (Huber et al. 2001, Ballabh et al. 2004, Bernacki et al. 2008, Cardoso et al. 2010). The blood-brain barrier is formed by the endothelial cells of cerebral blood vessels which display a unique phenotype characterized by the presence of tight junctions and transport systems (Petty and Lo 2002, Weiss et al. 2009). Tight junctions constitute a crucial component of the blood-brain barrier which controls the intracellular diffusion and maintains the structural and functional polarity of the blood-brain barrier of endothelial cells (Cereijido et al. 1998, Bazzoni et al. 1999). Any abnormality in the structure or function of tight junctions can lead to the blood-brain barrier dysfunction that consequently may contribute to the development of neurological diseases. Blood-brain barrier disruption that is associated with alterations of tight junctions has been implicated in the pathogenesis of a number of acute and chronic neurodegenerative disorders including multiple sclerosis (Minagar and Alexander 2003), stroke (Mark and Davis 2002), Alzheimer's disease (Zipser et al. 2007) and Parkinson's disease (Kortekaas et al. 2005).

TIGHT JUNCTIONS IN NEUROLOGICAL DISEASES

Multiple sclerosis

Multiple sclerosis is a neuro-inflammatory disease in which the fatty myelin sheaths around the axons of the brain and spinal cord are damaged leading to the occurrence of a broad spectrum of symptoms. This disease affects mostly young adults, and it is more common in women (Noseworthy et al. 2000). A key factor in multiple sclerosis progression appears to be loss of blood-brain barrier integrity and transendothelial migration of activated leukocytes into the brain (Minagar and Alexander 2003, Minagar et al. 2006). The decrease or inhibition of expression of endothelial junctional proteins may play a significant role in this pathology. Minagar and coworkers (2003) demonstrated that serum from patients with multiple sclerosis containing elevated levels of pro-inflammatory cytokines down-regulated occludin expression in cultured endothelial cells and hypothesized that this mechanism contributes to the disruption of the blood-brain barrier in multiple sclerosis. It has been shown that translocation from the membrane, or lack of ZO-1 and occludin in the brain of multiple sclerosis patients promotes the blood-brain barrier impairment (Plumb et al. 2002, Kirk et al. 2003, Leech et al. 2007). Interestingly, occludin phosphorylation is involved in forming more competent blood-brain barrier. In the rat model of multiple sclerosis, occludin dephosphorylation is associated in time with the onset of clinical signs of multiple sclerosis and appears just prior to apparent changes in blood-brain barrier permeability (Morgan et al. 2007). Furthermore, Wosik and coauthors (2007) revealed that in brain endothelial cells occludin phosphorylation on threonine, within lipid raft membrane microdomains, leads to the decreased permeability of the

blood-brain barrier. All these results indicate the correlation between blood-brain barrier permeability and alterations of brain endothelial junctions in multiple sclerosis.

Stroke

Stroke is the rapidly developing dysfunction of the brain tissue caused by the disturbance in the blood supply to the brain. This can be due to ischemia (lack of blood flow) caused by blockage of arteries (thrombosis, arterial embolism), or a hemorrhage. As a result, the affected area of the brain is damaged and unable to function (Hossmann 2006). Approximetely 90% of stroke cases are caused by ischemia, therefore the great majority of reports focus on alterations of brain endothelium after cerebral ischemia (hypoxia) and reperfusion (post-hypoxic reoxygenation).

It has been shown in rats that ischemia following microsphere-induced cerebral embolism evokes an increase in paracellular permeability of the blood-brain barrier (Betz 1996) and this increase is due to Src-mediated tyrosine phosphorylation of occludin (Fukumoto et al. 2010). Reperfusion injury leads to a biphasic increase in permeability of the blood-brain barrier with the early opening occurring several hours after the onset of reperfusion and the later opening – 24 to 48 hours after the initial one (Kuroiwa et al. 1985, Rosenberg et al. 1998, Rosenberg and Yang 2007). Rosenberg and coworkers (1998) indicated that blood-brain barrier opening after reperfusion is associated with activity of MMPs in animal model of stroke. Interestingly, an increase in blood-brain barrier permeability after reperfusion is caused by MMP-mediated disruption of tight junction proteins such as occludin and claudin-5 and MMPs inhibitor prevents degradation of tight junction proteins by MMPs (Rosenberg and Yang 2007, Yang et al. 2007). The most recent studies revealed that PI3-Kγ is responsible for ischemia/reperfusion-induced blood-brain barrier disruption which is correlated in time with the degradation of claudin-5 in acute experimental stroke (Jin et al. 2011).

In vitro, it has been observed that the increase in paracellular permeability coincides with alterations in localization or expression pattern of tight junction proteins during hypoxia (Mark and Davis 2002, Fischer et al. 2004). The exposure to a hypoxic environment triggers relocation of some tight junction proteins such as claudin-1, ZO-1 and ZO-2 from the plasma membrane to the cytoplasm (Mark and Davis 2002, Fischer et al. 2004). It is not clear if occludin localization after hypoxia is changed. According to study of Mark and Davis (2002) occludin translocates to cytoplasm, whereas another study indicates that occludin remains at the cell membrane but the immunofluorescent staining pattern appears more discontinuous with gap formation (Fischer et al. 2004). This rearrangement of endothelial tight junction proteins involves activation of phosphatidylinositol 3-kinase/Akt and phospholipase C pathway leading to the activation of protein kinase G resulting in hypoxia-induced permeability changes in endothelial cells (Fischer et al. 2004). *In vivo*, it has been shown that hypoxia-induced alterations in expression pattern of occludin as well as ZO-1 and vascular leakage in the brain are mediated by gelatinolytic activity of matrix metalloproteinase-9 which is elevated following hypoxia (Bauer et al. 2010).

Post-hypoxic reoxygenation results in the increase in expression level of claudin-1, ZO-1, ZO-2 and changes in their localization reinforcing cell-cell contact which contributes to a reduction in paracellular permeability in endothelial cells (Mark and Davis 2002). This suggests that O_2 reintroduced to the system stimulates cellular mechanisms which reverse alterations of tight junction proteins induced during hypoxic insult. It has been demonstrated that tyrosine phosphorylation in aortic endothelial cells exposed to 4-h hypoxia with 30-s post-hypoxic reoxygenation are increased (Crawford et al. 1996), but it is not clear which proteins are phosphorylated. Therefore, further work is required to determine which of intracellular signaling systems are involved in the molecular and functional changes during post-hypoxic reoxygenation.

Alzheimer's disease

Alzheimer's disease is a progressive neurodegenerative disorder characterized by a gradual loss of memory, orientation, judgment and reasoning (Roses 1996, Zlokovic 2008). Pathological hallmarks of Alzheimer's disease include loss of neurons and synapses in the cerebral cortex and certain subcortical regions, the development of neurofibrillary tangles consisting of hyper-phosphorylated Tau protein in neuronal cell bodies and the extracellular deposition of the amyloid-β

peptide (Aβ) in senile plaques (Trojanowski et al. 1995, Yankner 1996, Wenk 2003, Tanzi 2005). It has been proposed that inflammation and blood–brain barrier dysfunction play an important role in Alzheimer's pathogenesis in the adult brain (Stewart et al. 1992, Ujiie et al. 2003, Stolp and Dziegielewska 2009).

Stewart and coworkers (1992) observed subtle abnormalities in endothelial tight junctions in brain biopsies from patients with Alzheimer's disease and suggested that they may be responsible for the damage of blood-brain barrier. It has been revealed that treatment of endothelial cells isolated from rat cerebral cortex microvessels with $A\beta_{1-42}$ for 3 days caused relocation of claudin-5 and ZO-2 to the cytoplasm, whereas, under control conditions the transmembrane tight junction proteins occludin, claudin-1 and claudin-5, as well as the cytoplasmic accessory proteins ZO-1 and ZO-2 displayed a continuous distribution along the plasma membrane at cell-cell contacts (Marco and Skaper 2006). $A\beta_{1-42}$ treatment affected also protein expression: occludin expression decreased at 1st day, claudin-1 increased during the treatment, and ZO-2 expression increased after 1 day and then decreased (Marco and Skaper 2006). Consistent with the above findings authors suggest that changes in distribution and expression of tight junction proteins caused by $A\beta_{1-42}$ may alter blood–brain barrier integrity and contribute to the pathogenesis of Alzheimer's disease.

In a rabbit model of sporadic Alzheimer's disease, a cholesterol-enriched diet down-regulated the expression of the endothelial cell tight junction proteins occludin and ZO-1 which correlated with increased the leakage of the blood–brain barrier (Chen et al. 2008a). Furthermore, it has been demonstrated that chronic ingestion of caffeine protects against blood–brain barrier breakdown preventing decrease in expression of tight junction proteins in this model (Chen et al. 2008a).

Interestingly, expression of occludin is increased in neurons from Alzheimer's disease brains as compared to age-matched controls, in both frontal cortex and basal ganglia regions (Romanitan et al. 2007). This up-regulation of occludin can be driven by the elevated level of VEGF found in Alzheimer's disease patients (Tarkowski et al. 2002). Previously, it has been reported that VEGF increases the endothelial permeability by protein kinase C-dependent phosphorylation of occludin (Harhaj et al. 2006) leading to its dysfunction. Consequently, increased synthesis of occludin can occur to compensate the tight junctions function.

Parkinson's disease

Parkinson's disease is a chronic and progressive neurodegenerative disorder characterized by abnormal motor symptoms such as shaking, rigidity, slowness of movement as well as difficulty with walking and gait (Obeso et al. 2000, Dauer and Przedborski 2003). The pathological features of Parkinson's disease are loss of dopaminergic neurons mainly in the substantia nigra pars compacta, and depletion of dopamine in the striatum (Kortekaas et al. 2005, Savitt et al. 2006). The role in neuropathology of dopaminergic neurons has been suggested for reactive astrocytes (Forno et al. 1992), microglial activation (Kohutnicka et al. 1998), dysfunction in the blood–brain barrier transporter system (Kortekaas et al. 2005, Rite et al. 2007) and infiltration of peripheral immune cells (Hunot and Hirsch 2003, Choi et al. 2005).

Chen and colleagues (2008b) revealed decreased expression of the tight junction proteins ZO-1 and occludin in the striatum that was associated with disruption of the blood–brain barrier in MPTP-induced mouse model of Parkinson's disease. Moreover, they found that these changes in levels of tight junction proteins and blood-brain barrier leakage were blocked by caffeine.

Epilepsy

Epilepsy, that affects more than 1% of the population worldwide, is a chronic neurological disorder manifested by recurrent seizures. These seizures are transient signs and/or symptoms of abnormal, excessive or hypersynchronous neuronal activity in the brain (Fisher et al. 2005).

A well-known consequence occurring during epileptic seizures is an increase of blood–brain barrier permeability (Nitsch and Hubauer 1986, Arican et al. 2006, Marchi et al. 2007) that is associated with disruption of tight junctions and/or enhanced pinocytotic activity of endothelial cells in both humans and animal models of epilepsy (Westergaard et al. 1978, Grange-Messent et al. 1999, Lamas et al. 2002, Kaya et al. 2008, Ahishali et al. 2010). For example, Arican and coauthors (2006) proposed that stimulation of transcellular pathway rather than tight junction disruption is responsible for the pathogenesis of increased blood–brain barrier permeability because immunoreactivity for tight junction proteins, ZO-1 and occludin, was not changed in brain vessels of rats by pentylenetetrazole (PTZ)-induced

seizures. Subsequent findings confirmed that increased number of pinocytotic vesicles correlates with reduced integrity of blood-brain barrier whereas the immunoreactivity or the protein expression of occludin and the ultrastructure of endothelial tight junctions are not altered in PTZ-kindled rats with cortical dysplasia (Kaya et al. 2008). However, the most recent studies revealed a decrease of occludin immunoreactivity and protein expression and occasional opening of tight junctions as well as increased number of pinocytotic vesicles in capillary endothelial cells in rats with cortical dysplasia exposed to hyperthermia-induced seizures. These changes can be reversed by antiepileptic drug levetiracetam (Ahishali et al. 2010). In humans and in rat model of temporal lobe epilepsy, alterations in protein expression pattern of ZO-1 from uniform to irregular and discontinuous immunofluorescent staining have been observed (Rigau et al. 2007). Interestingly, decrease in expression of ZO-1 protein as a result of seizure-like events *in vitro* occurs via VEGF induced Src activation (Morin-Brureau et al. 2011). Additionally, a selective downregulation of expression of claudin-8 mRNA has been detected in kindling model of epilepsy, while decrease in claudin-5 protein was observed following epileptiform activity *in vitro* (Lamas et al. 2002, Morin-Brureau et al. 2011). All this suggests that seizures induced changes in local tight junction composition may lead to blood–brain barrier breakdown.

CONCLUSION

Proteins forming tight junctions are expressed in endothelial cell of brain vasculature and are crucial component of blood-brain barrier. Changes in their expression or localization accompany blood–brain barrier dysfunction in several neurological disorders. However, data are still surprisingly sparse. Additionally, few reports indicate other than blood–brain barrier localization of some of these proteins, possibly not even restricted to tight junctions. If it is of any relevance to brain pathology remains to be studied.

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