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Review

The Basic Requirement of Tight Junction Proteins in Blood-Brain Barrier Function and Their Role in Pathologies

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Abstract: This review addresses the role of tight junction proteins at the blood-brain barrier (BBB). Their expression is described, and their role in physiological and pathological processes at the BBB is discussed. Based on this, new approaches are depicted for paracellular drug delivery and diagnostics in the treatment of cerebral diseases. Recent data provide convincing evidence that, in addition to its impairment in the course of diseases, the BBB could be involved in the aetiology of CNS disorders. Further progress will be expected based on new insights in tight junction protein structure and in their involvement in signalling pathways.

Keywords: tight junction proteins; blood-brain barrier; tight junction structure; cerebral disease; claudins; occludin

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1. Introduction

Endothelial and epithelial cells separate tissues from each other and from internal and external influences by regulating the fluxes of solutes and xenobiotics; their function is indispensable to life in higher organisms. The plasma membranes of neighbouring cells are linked, so restricting lateral diffusion results in polarised cells with polarised membranes, thus generating structural and functional differences in their apical and basolateral portions [1].

Cell-cell contacts consist of tight junctions (TJs), adherens junctions, gap junctions, and (in epithelia only) desmosomes [2]. The TJ function is essential for tissue borders, such as those in the liver, kidney, or brain. Adherens junctions (AJ) are formed by cadherins [3] and stabilise intercellular contacts by connecting adjacent cytoskeleton arrays [4]. AJ are involved in the development and maintenance of cell barriers [5] and support the formation of TJs [4]. TJs and AJs form mixed contacts in endothelia, are largely indistinct in the brain [6] (whilst appearing separate in most epithelia), and are termed the apical junctional complex [7]. In gap junctions, connexins establish intercellular channels that provide intercellular communication and coordination by exchanging ions, messengers, and small metabolites [8].

Tissue barrier function is also determined by paracellular channels, influx and efflux transport systems, transcellular pathways, metabolic regulation, and by the cellular neighbourhood. Individual barriers vary considerably in tightness and molecular selectivity depending on the expression of junctional proteins and permeability factors. The presence of TJs is the principal determinant of barrier and passage mechanisms. The exact mode of interactions of TJ proteins and their involvement in signalling pathways is far from being well understood. The processes that determine the balancing of tightness and selective permeation will be discussed to contribute to a better understanding of normal barrier function and barrier-related pathologies. This review focuses on transmembrane

TJ proteins, on their structure, expression, regulation, and interactions in tissue borders. The principles are exemplified by well-studied brain barriers, in particular the blood-brain barrier (BBB, Figure 1). In this context, we also address TJ protein modulators and their potential pharmacological use.

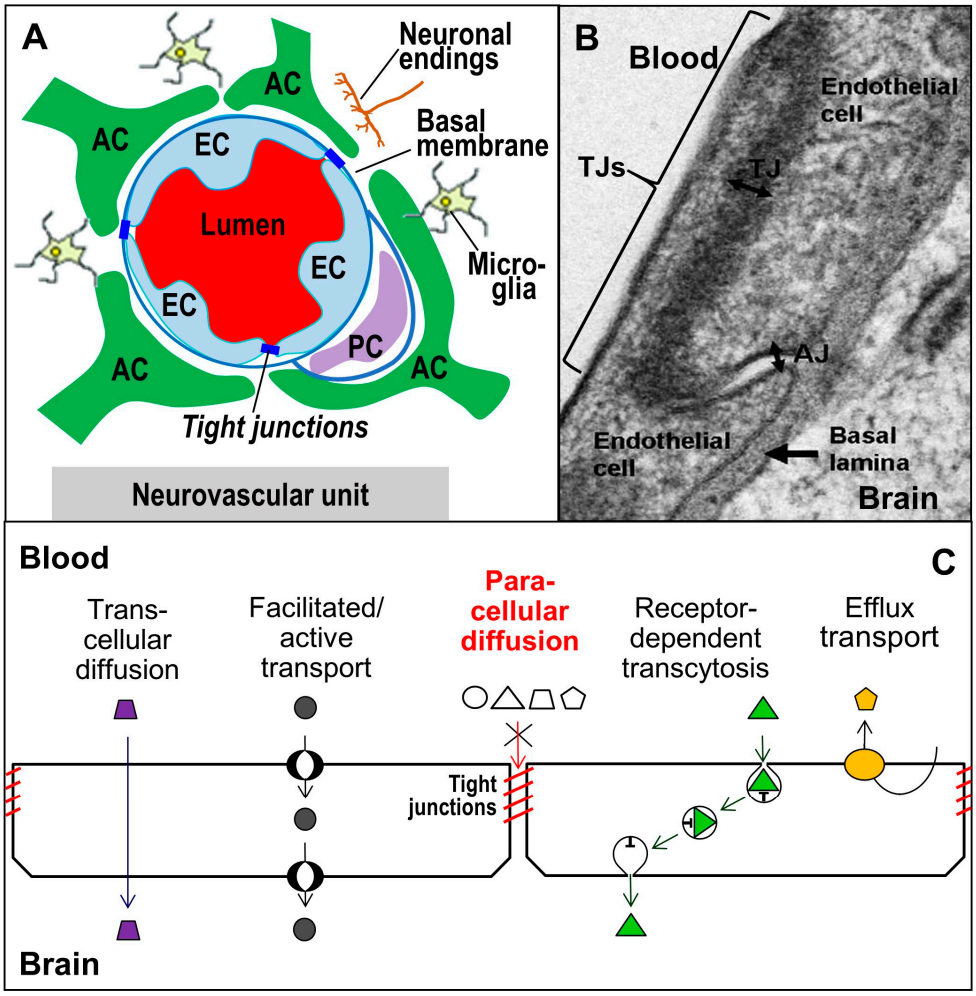

Figure 1. The blood-brain barrier (BBB). (**A**) The BBB is formed by capillary endothelial cells (EC) under the influence of other neurovascular components, such as astrocyte endfeet (AC) and pericytes (PC). (**B**) Tight junctions (TJs) seal the gap between ECs. AJ, adherens junction [9]. (**C**) Routes of passage at the BBB. TJs virtually prevent paracellular diffusion (red) and mandate transcellular transport (plasma membrane/cytosol, purple), active/facilitated transport (transporters/membrane channels, black), receptor-mediated transcytosis (green), or activation of efflux transporters (orange).

2. Tight Junctions: Proteins, Functions, and Structures

Tight junctions (TJs) are apical cell contacts (Figure 1B) formed from protein strand networks between neighbouring plasma membranes [2] (Figure 2B). These strands appear in freeze fracture electron micrographs as closely spaced particles (diameter ~10 nm, Figure 3) which, in some tissues, provide a seal against ions, proteins, immune cells, and toxic or pharmaceutical compounds [10–12]. In many epithelia and endothelia, the TJ configuration allows a size- and charge-dependent paracellular diffusion for molecules or ions [13,14]. For example, in the brain, the blood-brain barrier is much denser than the blood-cerebrospinal fluid barrier [15]. Similarly, the epithelium in the renal tubule system varies considerably from leaky proximal tubules to the tight loop of Henle and collecting ducts [16]. The TJ network is arranged similarly to a belt in adjacent cell membranes (Figure 2B). The transmembrane proteins of the TJs (Figure 2D) provide the basis for differences in apical and basolateral membrane composition [17] (membrane polarity), which limits the lateral

diffusion of lipids and proteins [18]. Moreover, in addition to their sealing function, TJs contribute to the regulation of cellular proliferation and differentiation [19].

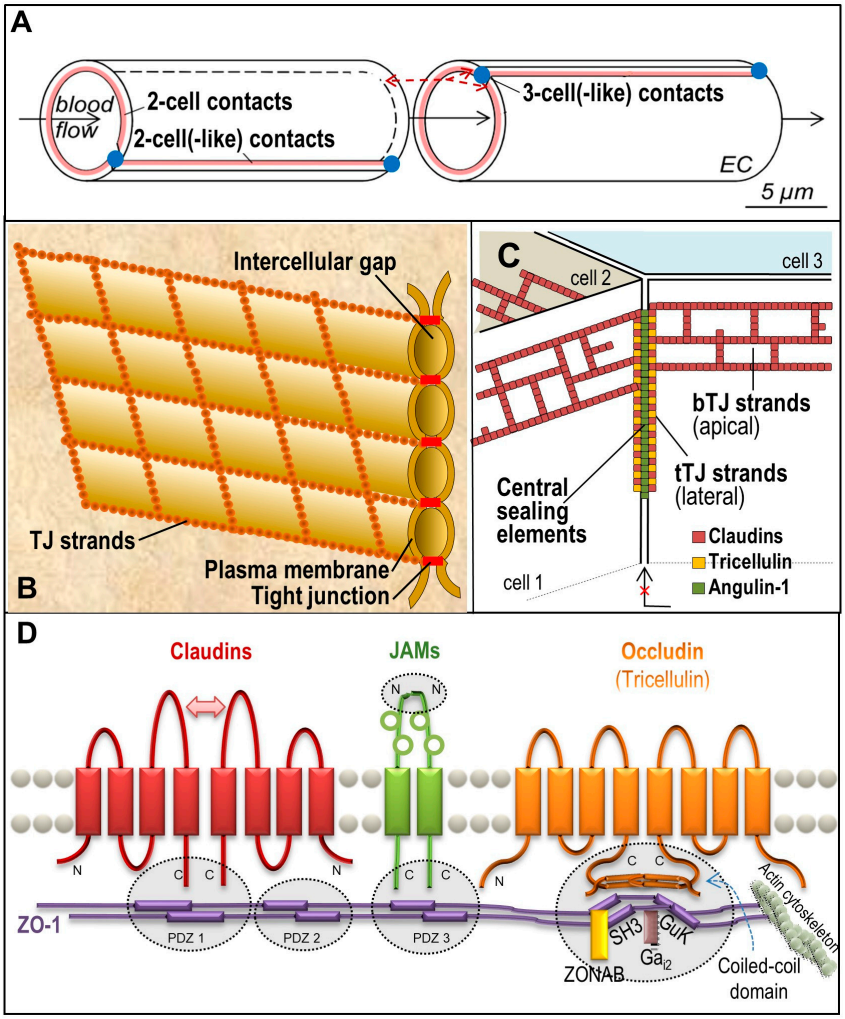

Figure 2. Scheme of tight junctions (TJs). (**A**) Longitudinal and circular TJ areas at the most luminal part of the lateral plasma membrane of brain capillary endothelial cells (EC). (**B**) Paracellular sealing by transmembrane TJ proteins forming a belt-like strand network of neighbouring plasma membranes. (**C**) Tricellular TJs as formed by non-endothelial cells (bicellular TJ, bTJ; tricellular TJ, tTJ). (**D**) Protein interactions at the TJs: claudins, occludin, JAMs, and tricellulin oligomerise along the plasma membrane and between two and/or three cell (-like) membranes. These proteins are recruited by the scaffolding protein ZO-1. Self-association occurs extracellularly (claudins), N-terminally (JAMs), C-terminally (occludin), or via PDZ2 domain (ZO-1). C-termini bind to PDZ1 (claudins), PDZ3 (JAMs), or to the SH3-Hinge-GuK unit (occludin) of ZO-1. The SH3-Hinge-GuK unit of ZO-1 also interacts with regulatory molecules, such as guanine nucleotide-binding protein G(i) subunit alpha-2 ($G\alpha_{i2}$) or Y-box transcription factor ZONAB (ZO-1-associated nucleic acid binding protein).

There are more than 30 transmembrane proteins that are involved with TJ composition, including members the claudin family [20,21], TJ-associated MARVEL proteins (TAMPs) [22], and junctional adhesion molecules (JAMs) [23]. They are connected to intracellular structures via a number of cytosolic adaptor proteins, such as the *zonula occludens* (ZO) proteins [24], which provide a link to the cytoskeleton (Figure 2D). JAMs and ZO proteins alone do not form TJs. The transmembrane TJ proteins are subject of continuous turnover, in most cases via clathrin-mediated endocytosis [25,26], caveolin [27], or macropinocytosis [28] with subsequent lysosomal decomposition or recycling to the membrane [27,29,30].

Claudins, a multigene family of 27 members with a molecular mass of 20–29 kDa [31] (first described in 1998 [20]), form the key component of the TJs and are essential for paracellular sealing of tissue barriers [13,32] (Figure 2). Based on sequence comparison, claudins can be divided into (homologous) classic and (less homologous) non-classic claudins. A functional distinction can be made between sealers (e.g., claudin-5) and sealers that simultaneously form paracellular channels (e.g., claudin-15). The function of certain claudins is unclear (e.g., claudin-25–27) or described controversially (e.g., claudin-4, Table 1).

Crystal structural data (obtained for claudin-15 [33], -19 [34], -4 [35], and -9 in complex with a toxin [36]) show a bundle of the four transmembrane helices and a joint extracellular domain consisting of a β sheet (five β-strands) and usually two short helices (Figure 4A, Supplementary Figure S1). Two cysteines in the first extracellular loop (ECL1) and the disulfide bridge included therein are part of a consensus sequence (G-L-W-x-x-C-[7–9 polar/charged amino acids]-C) [13,37,38]. In their C-terminal part, many claudins (e.g., Claudin-1–8) contain a conserved binding motif (Y/L/F/P)-x-K/R/L/V-K/R/T/S-x-Y-VCOOH [39], enabling association with the PDZ1 domain of ZO proteins [40,41] (Figure 2D). There are several sites of post-translational modification, e.g., at the conserved Tyr of the PDZ-binding motif (Table 2), that regulate claudin functions, including oligomerisation, transport processes, interactions, subcellular localisation, and homoeostasis [42]. Frequently, tyrosine is phosphorylated at the conserved PDZ binding motif [43]. Cys-palmitoylation at the intercellular loop or at the cytosolic C-terminal portion is essential for building the TJs [44].

The ultimate function of a TJ barrier depends on a number of parameters, such as cell type, tissue, organ, or even species, in addition to the role of any individual claudin and the presence and stoichiometry of other family members. For example, rat cholangiocytes or human-colon-derived Caco-2 cells express a large but considerably different subset of claudins [45]. Claudin-11 is expressed in oligodendrocytes to form the electrical seal of the myelin sheet around nerve fibres and forms the blood-testis barrier in Sertoli cells [46,47]. Claudin-13 has been found in mouse but not, so far, in human tissues (Supplementary Figure S1). The claudin profile is dynamically regulated during development and by environmental conditions (reviewed by refs. [48,49] and others). It should be noted that expression data collected in vitro often differ from those collected in vivo [50], similar to mRNA and protein expression levels. Interestingly, claudins (1, 7) have also been found at the basolateral membrane (but with unknown local function) [51,52].

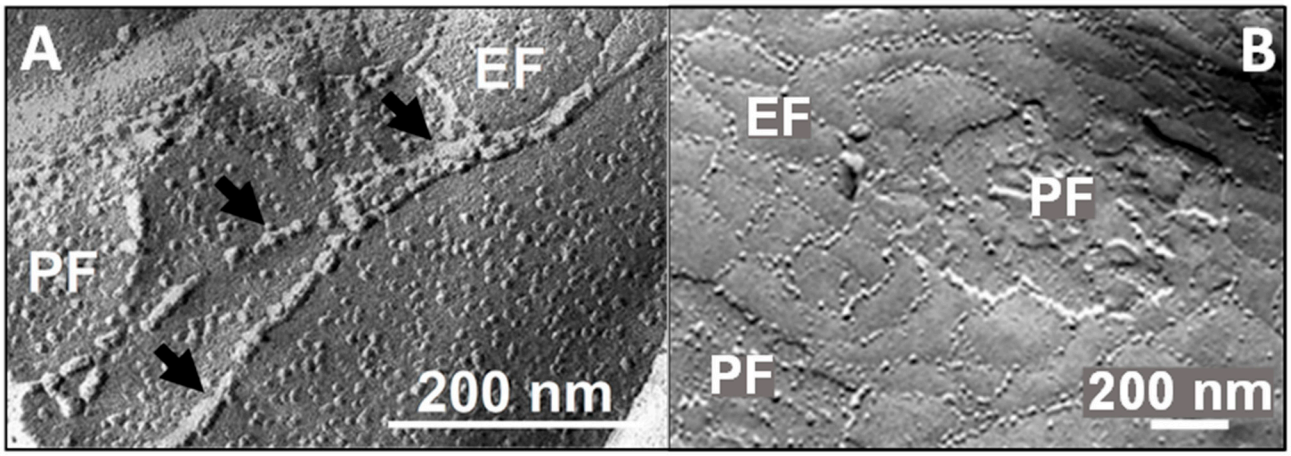

Figure 3. Freeze fracture electron microscopy of tight junction (TJ) strands. Strand networks between mouse brain capillary endothelial cells. TJ strands on exoplasmic (E-face, EF) and protoplasmic (P-face, PF) fracture of the plasma membrane indicated by arrows. (**A**) Cell culture model [49]. (**B**) Isolated brain capillaries [53].

TJ structures are mainly formed via oligomerisation of the extracellular claudin domains of adjacent cell membranes [54]. These *trans* interactions create—in conjunction with *cis* associations along the cell membrane (Figure 4B)—TJ strands (Figure 3), which are responsible for paracellular sealing and channel function (Table 1). Certain combinations of heterophilic interactions in *cis* and *trans* are preferred, while others do not occur. Claudin-1, -3, and -5 can interact in *cis* and *trans* with each other, claudin-2 and -11 only interact with themselves (Figure 4C). Thus, levels of the different claudin subtypes localised at the TJs determine the permeability and specificity of the paracellular pathway [49].

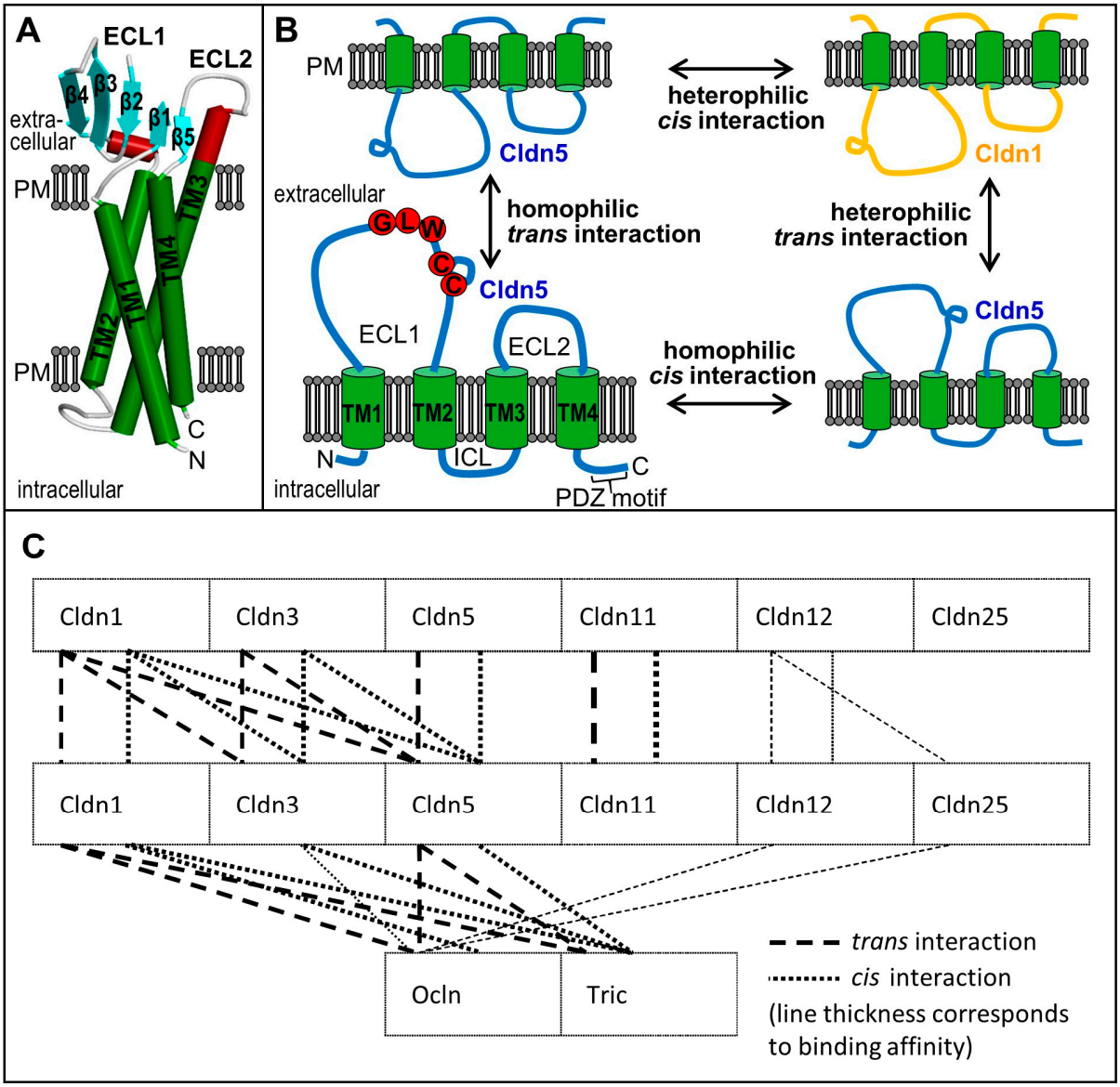

Figure 4. Structural and interaction models of claudins. (**A**) General model based on crystal structure of claudin (Cldn)-15 ([33], C-terminus truncated by 33 amino acids). ECL1 and -2 form an extracellular domain consisting of a β-sheet (five antiparallel β-strands, blue) and two α-helices (red) and unstructured areas (grey). TM, transmembrane domain; ECL/ICL, extra-/intracellular loop; PM, plasma membrane. (**B**) *Trans* interactions between claudins of neighbouring cell membranes and *cis* interactions along a plasma membrane. Red, conserved residues including two cysteines form an intramolecular disulfide bridge. PDZ, binding motif for PDZ1 domain in *zonula occludens* proteins. (**C**) Scheme of different interactions of blood-brain barrier-expressed claudins, occludin, and tricellulin [50,55].

Table 1. Claudin (Cldn) functions in tight junctions.

Class	Function			
	Paracellular Sealing	Paracellular Sealing/Channel-Forming	Other	Not Clear
classic *	Cldn1 [56]	Cldn2 [57]/(Na^+, K^+) [58]		
	Cldn3 [59]			
	Cldn4 [60]	Cldn4 [61]/Na^+ [62]		
	Cldn5 [63]	Cldn7 [64]/Na^+ [65]		
	Cldn6 [66]	Cldn10 sealing/-10a an^-, -10b cat^+ [67]	Cldn6 [68]	
	Cldn8 [69]	Cldn15 sealing/Na^+, K^+ [62]		
	Cldn9 [66]	Cldn17 sealing/an^- [70]	Cldn9 [68]	
	Cldn14 [71]			
	Cldn19 [72]			
non-classic	Cldn11 [73]	Cldn16 sealing/cat^{++} [72]	Cldn13 [68]	Cldn12, -13, -20 [50]
	Cldn18 [74]	Cldn21 sealing/Na^+, K^+, solutes $\leq$ 0.56 nm [75]		Cldn22, -23, -24 [50]
	Cldn25 indirectly [76] via structure of TJ [50]			Cldn25, -26, -27 [50]

*, high sequence homology [13]; an, anion; cat, cation.

Table 2. Characteristics of selected claudins (Cldns) of the blood-brain barrier.

Expression	Function	Structure/Interactions	Regulation/Signalling
Claudin-1 (*Senescence-associated epithelial membrane protein*) - gene *CLDN1*, chromosome 3 (human), -16 (mouse) - protein: human [77,78], mouse [78,79] - cell membranal at TJs [80] and cytoso- lic [50] localisation [53] - KO mouse: postnatal dehydration, lethal [81]	- causes tightness (TER) [56,82], sealing [56,82,83] - receptor for hepatitis C- virus [84]	- 211 aa; M.W., 22.7 kDa; pI, 8.41; N-/C-terminal tail, 7/27 aa; ECL1/ECL2, 53/27 aa (human) - homophilic *cis*/*trans* interactions [85,86], dissociation constant ECL1 to Cldn1 47 ± 0.6 nM [86] - heterophilic *cis* Cldn3, -5 [87], Ocln, Tric, MD3 [55], PDZ1 of ZO-1 [88]; *trans* Cldn3,-5 [87], Ocln, Tric, [55] - continuous P-face TJ-strands [55] - low membrane mobility [55]	- GPR30 via ERK and/or Akt-domain [89] - dehydroepiandrosteron/Gnα11 [90] - hypoxia inducible factor-complex [91] - cAMP/PKA, down-regulation and cytosolic localisation [92] - down-regulated by hypoxia, focal cerebral ischemia [50], glioblastoma [93] - down-regulated by TGFβ [93], Cu [94], miR212/132 [95] - differentiated regulation upon virus infection [96–100]
Claudin-3 (*Clostridium perfringens enterotoxin receptor* 2) - gene *CLDN3*, chromosome 7 (human), 5 (mouse) - protein: human [77,78], mouse [78,79], rat [101] - KO mouse: amount of Cldn5 and Ocln, paracellular permeability reduced [53]; no changes found by other authors [102]	- enhances BBB integrity in vivo [78], increases complex-ity of TJ-strand network [53] - controls paracellular tightness [59,103,104] (particularly small molecules/ions) - limits endocytosis; pro-motes infarction/oedema [53] - supports embryogenesis/ postnatal development, stabilises BBB/TJ [105]	- 220 aa; M.W., 23.3 kDa; pI, 8.37; N/C-terminal tail, 8/40 aa; ECL1/ECL2, 51/23 aa (human) - homophilic interaction *cis*/*trans* [87] - heterophilic *cis* Cldn1, -5 [87], Tric, MD3 [55], associates ZO-1-PDZ1 [40]; *trans* Cldn1, -5 [87], Tric, MD3 [55] - continuous P-face strands [87,106] - high membrane mobility >Cldn5 [87] - strengthens TJ strand network/-branching [53]	- Wnt/β-catenin controlled barrier development [105] - expression modulated by Na/K-ATPase [107] - down-regulated by hypoxia/middle cerebral artery occlusion [50] - down (haemorrhage) (PI3K, sphingosine 1-phosphate receptor 1) [104] - loss in EAE, glioblastoma [78] - down-regulated at low Cu [94]

Table 2. *Cont.*

Expression	Function	Structure/Interactions	Regulation/Signalling
Claudin-5 (*Transmembrane protein deleted in velocardiofacial syndrome*) - gene *CLDN5*, chromosome 22 (human), 16 (mouse) - very high expression [50,78,108], embryonically starting with cerebral angiogenesis [109] - KO mouse: abnormal TJs, brain capillaries permeable for molecules < 800 Da, lethal 10 h postnatally [63] - KD: BBB breakdown in tissue culture, human BEC [110] - $t_{1/2}$ 70 min [111] - protein amount: Cldn5 > −25, Ocln, Cldn1 > −11, −12 (isolated brain capillaries, TX-100 extract) [50]	- causes paracellular tightness for molecules < 800 Da [63] - induces/maintains TJ tightness [21,112] mediated via ECL1 [113] and ECL2 [54]	- 218 aa; M.W., 23.1 Da; pI, 8.25; N-/C-terminal tail, 7/38 aa; ECL1/ECL2, 53/16 aa (human) - homophilic *cis*/*trans* interaction [54,87] - heterophilic *cis* Cldn1, -3 [87], Tric, MD3; *trans* Cldn1, -3, Ocln, Tric [55] - discontinuous E-face-associated TJ-strands (in TJ-free cells) [21,54] - low membrane mobility [87] - mixed E-/P-face strands by Cldn3 [87] - associates ZO-1-PDZ1 [40] - no effect on ZO-1 clustering [114] - C54S, C64S (mouse ECL1, aa exchange) weaken barrier [113] - ECL1-G60R, human channelopathy: Cl^-/small molecule flux [115] - transferred from BEC to leukocytes in EAE, possibly supporting transmigration into CNS [116]	- Thr(207)-phosphorylation opens porcine BBB, protein kinase A [117] - TGF-β/activin signalling increases Cldn5 [118] - VE-cadherin via Akt-activation: phosphorylation of FoxO1 induces Cldn5 [119] - adrenomedullin: enhanced expression and TER, decreases permeability [112] - increase (mRNA, protein, promoter): gluco-corticoids TER up [120,121], estrogen [122] - ROCK via EphA2: down-regulation [123] - ROCK up in dementia: Cldn5 down [124] - C/EBP-α (stimulated by JAM-A) up-regulation, reduced permeability [125] - serum Cldn5 up: autistic children [126], severe stroke [127] -down-regulated by EphA4/Tie2/Akap12 signalling mediating microvascular dysfunction and trauma [128] - down-regulated at low Cu [94] - oxidative stress inhibitor improves Cldn5, ZO-1, TER via Nrf2/HO-1 [129]
Claudin-11 (*Oligodendrocyte-specific protein*) - gene *CLDN11*, chromosome 3 (human), 3 (mouse) - mRNA/protein: very high expression in BEC (human, mouse, rat) in vivo equal to Cldn5, in vitro strongly down-regulated [50,130] - less expressed in human brain oligodendrocytes [50] - KO mouse: mild neurological deficits [131], deafness (low endocochlear potential) [47] - KD: enhanced dextran permeability through BEC layers [130]	- contributes to tightness of BEC layers [50,130] and BBB [132]	- 207 aa; M.W., 22.0 Da; pI, 8.22; N-/C-terminal tail, 1/29 aa; ECL1/ECL2, 50/14 aa (human) - very strong homophilic *cis*/*trans* interaction (Cldn11 >> other Cldns, Ocln, Tric [55,133]) - no heterophilic binding [55]; Cldn5 colocalisation in junctions [50,130] - continuous P-face oriented TJ-strands, modulated by Ocln [50] - very low membrane mobility <other Cldns [50], Ocln, Tric, MD3 [55]	- reduced in multiple sclerosis [130] - decreased in EAE by activated annexin A2 signalling (brain capillaries) [134] - decrease in BEC by podocalyxin KD [135] - increased in blood of human autism spectrum disorder [126] - ischemia reduces Cldn11; KO of leucine-rich alpha-2 glycoprotein 1 improves Cldn11 and BBB in ischemia [132]
Claudin-12 - gene *CLDN12*, chromosome 7 (human), 5 (mouse) - in BEC [63,94,136]; mRNA in vivo > in vitro [50] - expressed at TJs [50,63] - lack of Cldn12: intact BBB; neurological/behavioral changes [137] - knock-in mouse: mRNA in BEC, pericytes, oligodendrocytes, smooth muscle cells, astrocytes [137]	- not crucial in establishing or maintaining BBB TJ integrity [137]	- 244 aa; M.W., 27,1 kDa; pI, 8.80; N-/C-terminal tail, 10/49 aa; ECL1/ECL2, 56/18 aa (human) - homophilic: no *cis*- [87], but weak *trans* interaction [55] - heterophilic: weak *trans* interactions with Cldn22, -24, -25, Ocln [50] - no C-terminal PDZ-binding motif [87] - no strand formation [87], very high paracellu-lar flux in TJ-free cells [138]	- ouabain-activated Na/K-ATPase reduces expression [107] - high-energy diet decreases mRNA-, increases hippocampal permeability [139] - hyperammonia reduces mRNA in vitro [140] - down-regulated in hypoxia/ischemia [50,53] and in diet-induced diabetes (in latter case attenuated by carbonic anhydrase inhibitor [141]) - regulated by Cu exposure [94]

Table 2. *Cont.*

Expression	Function	Structure/Interactions	Regulation/Signalling
Claudin-25 (*Claudin domain-containing protein* 1) - gene *CLDND1*, chromosom 3 (human), 16 (mouse), - very high mRNA expression in vivo in BEC [50] - in human BEC localised at TJs [76] - KD: reduces mRNA/protein without cytotoxicity, paracellular permeability raises for small molecules [76]; P-face strands less structured, reduced mesh number, i.e., less particles, larger meshes] [50].	- contribution to cell adhe-sion and tightness for small molecules [76]	- 229 aa; M.W., 25.4 Da; pI, 5.37; N-/C-terminal tail, 10/44 aa; ECL1/ECL2, 50/19 aa (human) - no homophilic *trans* interaction in BEC [50] - weak heterophilic *trans* interaction (Cldn12, -22, -24, Ocln) [50] - no TJ strand formation, but strands supported indirectly (via Ocln) [50]	- xenobiotics-activated arylhydrocarbon-receptor [142], retinoic acid receptor-related orphan receptor α [143], and myeloid zinc finger 1 [144] increase mRNA expression - transcription inhibition by miR-124 [145] - cerebellar haemorrhage decreases expression in mouse BEC by [76]

aa, amino acids; Akt, protein kinase B; BEC, brain endothelial cells; BBB, blood-brain barrier; Cldn, claudin; C/EBP, CCAAT/enhancer-binding protein; *cis* interaction, cf. Figure 4B; EAE, experimental autoimmune encephalitis; E-face, exoplasmic-face; EphA2/4, ephrin type-A receptor 2/4; ERK, extracellular-signal regulated kinase; ECL, extracellular loop; FoxO1, forkhead box O1; G, G-protein; GPR, G-protein-coupled receptor; JAM, junctional adhesion molecule; KO/KD, knockout/-down; MD3, MarvelD3; M.W., molecular weight; Nrf2/HO-1, erythroid 2 like 2 nuclear translocation/haem oxygenase 1 signalling; Ocln, occludin; PI3K, phosphoinositide 3 kinase; P-face, protoplasmic-face; pI, (calculated) isoelectric point; PK, protein kinase: ROCK; Rho-associated protein kinase; $t_{1/2}$, half-life; TER, transendothelial electrical resistance; TGF, transforming growth factor; TJ, tight junction; *trans* interaction, cf. Figure 4B; Tric, tricellulin; ZO-1, *zonula occludens* protein 1.

3. Tight Junctions and Their Proteins at the Blood-Brain Barrier

The BBB ensures the brain function by maintaining a constant cerebral milieu and is thus a decisive factor for the homoeostasis of the CNS. It provides a highly efficient exchange of nutrients and metabolites and prevents the permeation of xenobiotics, peripheral metabolites, pathogens, and blood cells [146]. The BBB is formed by capillary endothelial cells (Figures 1B and 2A) influenced by the basal membrane, pericytes, (located within the basal membrane), neurons, microglia, and especially astrocytes. The whole ensemble is referred to as the neurovascular unit (Figure 1A). Pericytes surround a third of the endothelium [147] and support the formation and maintenance of the BBB. They contribute to angiogenesis and various brain functions. Microglia provide the immune defence of the brain by being activated under pathological conditions [148,149]. The endfeet of astrocytes almost completely envelop the capillaries [147], which is highly important for a functional BBB [150]. The capillaries exhibit an inner diameter of 3–5 µm. In humans, the capillary system has a total length of ~650 km and a surface of 10–20 m^2 [151–153]. The cleft between the endothelial cells is closed by an intercellular network of TJ strands, preventing direct diffusion of solutes including Na^+, K^+, and water [154–156] (Figures 2B and 3). Water transport is probably mainly diffusive since the measure of hydraulic conductance of 2×10^{-9} cm $(cmH_2O\ s)^{-1}$ is comparable to that of endothelial cell membranes [157].

The very high TJ density of the BBB results in a considerable transendothelial electrical resistance (~5 $k\Omega cm^2$) as it lacks paracellular channels, as seen for other barriers [158] such as the blood-cerebrospinal fluid barrier (where claudin-2 creates ion channels [15]). Flux rates through the BBB are very low for hydrophilic molecules for which transporters are lacking [159]. The barrier function is supported by low pinocytosis, little vesicular transport, and no fenestration in the cells [146,160]. The supply of the brain with substrates and regulatory molecules is enabled by selective transport systems, which are essential because the paracellular route is blocked by TJs. The TJs are thus the key element of the BBB [161–163]. Additionally, a metabolic barrier is formed by the high activity of endothelial enzymes, such as γ-glutamyl transferase, alkaline phosphatase, glucose-6-phosphatase, catechol-O-methyl transferase, monoaminoxidase, or cytochrome P450 [164]. They collectively prevent their substrates passing into the CNS owing to their being metabolised during entry into or within the cell.

In general, TJs largely prevent passive paracellular diffusion of polar compounds, thus mandating diffusion across the plasma membrane and cytosol (O_2, CO_2, lipophilic molecules with molecular masses <500 Da, e.g., ethanol [165]). Other molecules require transporters or paracellular channels. For endothelial cells of the BBB, this includes cerebral Na^+-uptake (indirectly that of water), extracellular HCO_3^- (pH), and the maintenance of interstitial K^+-concentration (Na^+, K^+-ATPase) in the brain [166]. Thus, the TJs allow for a number of electrochemical gradients to be established, and these provide the driving force for the transport of substances that maintain homoeostasis of the CNS. Special transporters also exist for hydrophilic substrates such as glucose, amino acids, lactate [167–169], neuropeptides [170,171], and biotransformed products [172]. Receptor-mediated transcytosis via the use of specific carrier proteins facilitates intake of macromolecules as insulin [173], LDL [174], transferrin [175], leptin [176], and others [177]. Additionally, TJs render efflux transporters barrier-effective by excluding small (400–600 Da) lipophilic/non-charged compounds from the brain, amounting to more than 300 metabolites, toxins, and drugs [178–181] (Figure 1C).

3.1. Claudins

Claudins 1, 5, 11, 12, 25, 27 (only human), and 20 (only mouse) are abundantly expressed in capillary endothelial cells of cerebral cryosections (mRNA). Claudins 2–4, 6, 9, 15, 17, 22, and 20 and 23 (both only human) and 14, 24, and 26 (only mouse) are less abundant [50] (Figure 5). The mRNA values roughly reflect the protein values [50,63]. Consequently, the highly abundant claudins 1, 5, 11, 12, and 25 are discussed below, while

the less-abundant claudin-3 is also discussed since a number of reports suggest that it plays a role in BBB pathology [50,53,78,104,105,182] (Table 2).

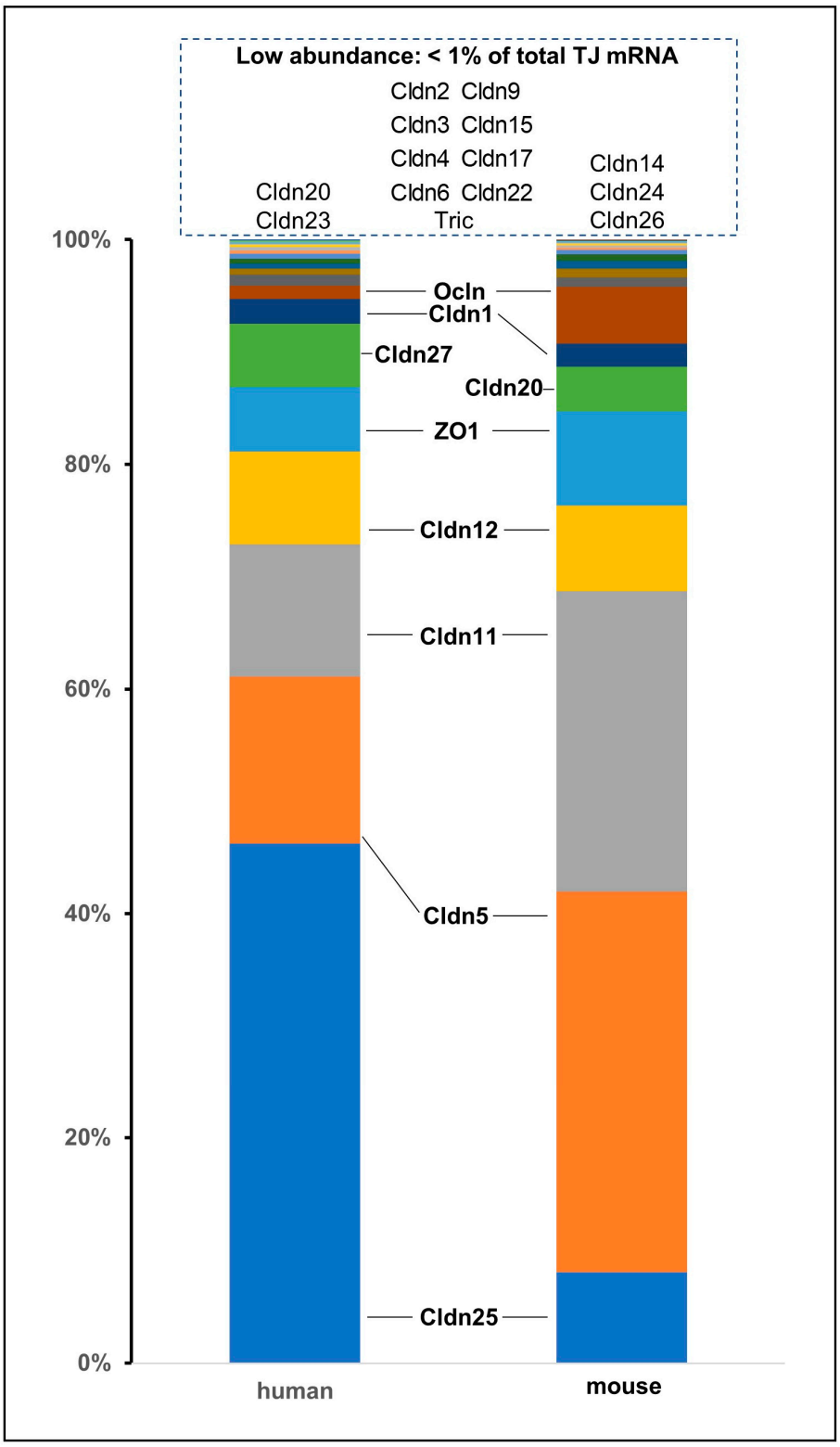

Figure 5. mRNA expression of tight junction (TJ) proteins in human and mouse brain endothelium. Proportions of individual proteins of the total expression of TJ protein mRNA. High abundance, >1%; low abundance, <1% of total mRNA ([50], modified). Cldn, claudin; Ocln, occludin; Tric, tricellulin.

Claudin-5, a tightening claudin and highly expressed [183,184], is most important for very dense TJs [158] in vertebrate BBBs [63,185]. It tightens the barrier (two-cell- or two cell(-like) contacts, Figure 2A, left) for molecules < 800 Da [63], which includes the majority of physiologically active substances. The paracellular cleft is closed via tight *cis* and *trans* interactions of the extracellular domains [115,186]. A hereditary mutation in loop 1 results in a severe channelopathy with the increased permeation of ions and small molecules [115]. These data support the great relevance of claudin-5 for the pharmacology and pathology of the BBB. Its knockdown up-regulates claudin-1 [50], pointing to a compensatory potential between sealing claudins. ZO-1 [40] and occludin [55] interact with claudin-5 and facilitate the formation of claudin-5 strands [41,55]. On the other hand, the clustering of ZO-1 is independent of Claudin-5 [114]. As visualised by freeze fracture electron microscopy, claudin-5 alone assembles in exoplasmic TJ strands [54,187]. Freeze fracture EM of mouse cerebral endothelium shows that these strands also appear on the protoplasmic surface [188] (Figure 3), which indicates that additional strand-building proteins are sufficiently expressed. In primary cultures of human brain endothelial cells, claudin-5 has a half-life of 13.8 h [189]. The trafficking is caveolin-dependent [26,27], and it is either recycled [27,30] or ubiquitinylated and degraded in the proteasome [42,111], indicating high protein dynamics.

Claudin-11, another sealing [50,130,190] protein, forms strands associated with the protoplasmic face [50], interacts only in a homophilic manner [55,133], and is almost exclusively detectable in vivo [50,133]. The protein level is comparable to that of claudin-5 [130]. Claudin-11 has a much higher homophilic affinity than claudin-5 [55], resulting in a considerably reduced membrane mobility. It can be found in distinct TJ segments being free of other claudins [50]. Molecular modelling indicates a relatively small extracellular binding domain (Supplementary Figure S1). This, combined with a strong capacity for oligomerisation, low junctional agility, and no interrelations with other claudins, suggests a very tight intercellular seal. Occludin indirectly modulates claudin-11 strand morphology [50]. Knockdown in brain endothelial cells decreases paracellular tightness [50,130]. Mild neurological deficits have been described in claudin-11 (also known as oligodendrocyte-specific protein) knockout mice, since its function in the myelin sheath is partly substituted by another structurally similar membrane protein [1,131]. Claudin-5 and -11 seem to act partly synergistically, and seem to compensate for each other; claudin-5 deficiency does not lead to a complete loss of sealing [63]. Their exoplasmic and protoplasmic strand orientations also complement each other.

Claudin-12 is well expressed in endothelial cell contacts both in brain sections [63] and in purified brain capillary endothelial cells [136]. Claudin-12 does not, however, form TJ strands [87], probably due to its lack of homophilic *cis* interaction and a lack of the C-terminal PDZ-binding motif that prevents association with the PDZ1 domain of ZO-1 [40], which usually supports TJ formation [191]. Additionally, this claudin shows weak homo- and heterophilic [55] *trans* oligomerisation with claudin-25 and occludin. These interactions could help to maintain the support of claudin-25 for TJ strand morphology [50] or to preserve the TJ regulation by occludin [192], and it appears that claudin-12 plays a role in the maintenance of the BBB as it has been shown to be down-regulated under pathological or toxicological conditions [50,94,107,139–141].

Similarly, claudin-25 is highly expressed and its contribution to barrier function is unclear; it does not show homophilic *trans* interaction, which limits its potential for direct barrier sealing and TJ formation. Claudin-25 does localise at cerebral endothelial cell contacts and indirectly supports a functional TJ morphology. This is probably due to *trans* interactions with occludin [50], a main regulator of BBB TJs [192] under normal [156] and pathological [193] conditions. Its importance in TJs is demonstrated by claudin-25 knockdown, leading to the hyperpermeability of small molecules and weakening the TJ strand network in an in vitro BBB model [50]. Since claudin-25 interacts with occludin but not with strand-forming claudins, it probably indirectly contributes to TJ function. N-Glycosylation at the extracellular loop 1 of claudin-25 [194] promotes its localisation

at the plasma membrane and can initiate signal transduction processes [195]. Hence, claudin-25 can be considered as a TJ modulator at the BBB.

Claudin-1 and -3 form strands associated with the protoplasmic face and are frequently involved in sealing of epithelial barriers. Their function at the BBB is not clear, their interaction potential with claudin-5, occludin, and tricellulin renders both as candidates for tightening the BBB. Claudin-1 is also considered in a developmental context [196]. Its immune reactivity is often observed as more cytosolic than junctional [50] and its deficiency does not show a cerebral phenotype [197], but overexpression has a tightening effect at the BBB [83]. The low mobility [55] correlates with strong homophilic affinity [86]. Application of claudin-1-derived peptides that block its interactions leads to higher permeability of cerebral endothelial barriers [198]. The high affinity to various interaction partners [87], including occludin [55] and its redox sensitivity [86,91,94], point to a modulatory role in corresponding pathologies.

The expression of claudin-3 at the adult BBB is rather low [50,94], and its significance for the barrier is questioned [102]. Claudin-3 knockout experiments, however, result in reduced claudin-5 expression, lower junctional occludin localisation, and increase branching of TJ strands. The strand network is weakened, and barrier permeability increases, which diminishes the infarct area and oedema formation in a stroke model [53]. This supports the idea that to attenuate ischemia-related damage (e.g., oedema) via TJ modulation using claudin inhibitors could open the BBB reversibly [199]. Changes of claudin-3 at the BBB are found in experimental ischemia/reperfusion [50,53], haemorrhage [104], and chronic inflammatory pain [182] in a multiple sclerosis model (experimental autoimmune encephalitis [130]) or in human glioblastoma multiforme [78]. Moreover, claudin-3 is involved in the development and maintenance of the BBB [105]. Thus, a role in barrier regulation is assumed, which would be supported by its high membrane mobility [87] and its interaction with other claudins and occludin [55].

In summary, claudin-5 is considered the major component of the TJs and, for small molecule tightness, the essential sealer of the BBB; claudin-11 also seals the barrier and partially compensates for claudin-5. Claudin-3 might have a limited contribution to TJ function and could be involved in pathological processes. Claudin-25 does not contribute to the structure and function of TJs directly but could modulate it indirectly. The function of claudin-12 remains unclear.

3.2. Tight Junction-Associated MARVEL Proteins

Table 3 characterises structure, function, and regulation of the TJ-associated MARVEL proteins (TAMPs) expressed at the BBB, i.e., occludin, tricellulin, and MarvelD3 (expression of the latter reported only once so far [200]). Occludin localises mainly in two-cell contacts [201]; it is found ubiquitously in TJs and is often used as a TJ marker protein [202]. Tricellulin (in particular, tricellulin a), is expressed with markedly lower total expression level (Figure 5, dashed box) [50] but is highly enriched in tricellular contacts [203] (Figure 2C). TAMPs show four transmembrane domains, cytosolic termini, one intra-, and two extracellular loops [22] (the second containing a conserved intramolecular disulfide bridge [133,204]. The MARVEL (MAL and related proteins for vesicle trafficking and membrane link) domain comprises all transmembrane domains and loops [205] (Figure 2D) that form cholesterol-rich microdomains in plasma membrane appositions [206]. The TAMPs are involved in the formation of TJs [207,208] by interacting with claudins, but occludin and tricellulin do not bind each other [55], although they support TJ-strand branching and stabilise epithelial barrier integrity [209]. Tricellulin may also occur at bicellular contacts [210] and can partially compensate for occludin [22], thus contributing to paracellular tightness [211]. Occludin and tricellulin are redox-sensitive and regulate bicellular and tricellular TJs under oxidative or reducing conditions [133,204].

The exact function of occludin is still unclear. There is no evidence for a direct barrier function [212,213], although heterophilic and homophilic oligomerisation do occur [55,204], the latter via its cytosolic C-terminal coiled-coil domain [214]. This domain is involved

in macromolecule flux through TJ barriers [215], ZO-1 association [215,216], and their proteins are targets of various protein kinases [217,218]. Multiple phosphorylation [202] is demonstrated via a molecular weight shift in electrophoresis [219]. These and other modifications [220] are mediated by different signalling pathways (relevant for normal and pathological conditions) and strongly point at a regulatory function, e.g., via interaction with claudins and/or ZO proteins (Figure 2D) [205]. The number of studies that exploit these pathways for new medical applications is increasing [141,221]. There is wide agreement that occludin is involved in the regulation of the TJ permeability [212,222].

Tricellulin fulfils its general function, sealing the gap at the contact points (Figure 2C) of three membranes. In the BBB, primarily single endothelial cells form distinct capillary segments by themselves; thus, three separated membrane patches from two different cells meet in one point (three-cell-like contacts, Figure 2A, right) [203,223]. The protein oligomerises homophilicly, creates tricellular TJs [133], and tightens this area (in epithelial cells) for molecules < 10 kDa without affecting ion permeability [211]. Comparing bi- and tricellular contacts, it is concluded that the expressed amount of sealing TJ proteins (claudin-5 and -11) in bicellular TJs is about two orders of magnitude higher than that of tricellulin [50]. This suggests that the BBB function is quantitatively determined by two-cell TJs, which prevent permeation of small and large molecular weight compounds [63], rather than by tricellular TJs that withhold larger molecules [211,224].

In three-cell(-like) contacts of brain endothelial cells, tricellulin specifically concentrates and colocalises with angulin-1/LSR (lipolysis-stimulated lipoprotein receptor) [203], a type I transmembrane protein with a single Ig-like domain [225]. Tricellulin and angulins form so-called central sealing elements [226] laterally in three-cell(-like) contacts (Figure 2C). For delivery of antisense oligonucleotides (5.3 kDa) through the BBB, these TJs can be modulated by recombinant angubindin-1, a *Clostridium perfringens* iota-toxin, which binds to angulin-1 [224]. Angulin-1 knockout mice exhibit embryonic lethality [227] with BBB failure [228], which is not known from tricellulin deficiency. These and other observations support the assumption that angulin-1, and not tricellulin, could be essential for sealing the three cell-like contacts in cerebral capillaries. In epithelial cells, angulin-1 forms tricellular contacts, even in tricellulin- or claudin-deficient cells [229].

Table 3. Tight junction-associated MARVEL-proteins (TAMPs) expressed at the blood-brain barrier.

Expression	Function	Structure/Interactions	Regulation/Signalling
Occludin			
- gene *OCLN*, chromosome 5 (human), -13 (mouse) - expression < Cldn5 [50] - increased expression in co-culture of BEC with astro-/pericytes [230], neurons [231] - half-life 6.2 h [189] - KO mouse: TJ-morphology unchanged, calcification of brain [212] - Ocln/Tric-double KO: lowers TJ-strand branches/ barrier integrity [209]	- TJ-regulation postulated [192] - redox sensor in TJs [204] - facilitates TJ branching/barrier tightness [55,209] - C-terminal CC-domain required for maintenance and regulation of macromolecule flux through TJs [215] - regulates centrosomes in cortex genesis [232] - regulates apoptosis via caspase-3 transcription [233] - controls HIV-transcription [234], glucose uptake/ATP-synthesis [235] of BBB pericytes - Ocln/caveolin-1/Alix-complex regulates HIV-permeation through BBB [192] - required for cytokine-mediated signal transduction [236]	- human: 522 aa; M.W. 59.1 kDa (polyphosphorylated $\leq$ 65 kDa [219]); pI 5.77 - cytosolic N-/C-termini 66/257 aa; ECL1 46 aa (11 Tyr, 19 Gly—potentially hydrophobic interactions, flexibility); ICL 11 aa; ECL2 48 aa (2 Cys, disulfide bridge, hypoxia-/redox-sensitive) [204] - interactions: homophilic *trans*, *cis* (CC domain dimerises [214]); heterophilic *cis* Cldn1, MD3/*trans* Cldn1, -5 [55], -25 [50] - 3D-structures: cytosolic C-terminal region [237], complex ZO-1 (PDZ3-SH3-U5-GuK)/ Ocln (CC-domain) [222] - CC-domain binds ZO-1 (SH3-hinge-GuK) [216], possibly interacts with ZO-1 [215] - peptide sequence of CC-domain associates PKC ζ, Tyr-kinase c-Yes, PI3K, connexin 26 [218] - MARVEL-domain: in membrane appositions, cholesterol-rich microdomains [206]; mediates interaction of TJs with membrane lipids, *cis*-oligomerisation via Cys and membrane insertion [238]	- Tyr398, Ser408: high-conserved phosphorylation sites for PKCs, CK2, Tyr-kinase Src [217] - thrombin: Tyr-phosphorylation, Ocln-ZO-1-/TJ disruption, BBB leakage; angiopoietin-1 inhibits this Tyr-phosphorylation, stabilises TJs [239] - VEGF-activated atypical PKC opens BBB [240] - VEGF/hypoxia activate PLCγ, PI3K/Akt, PKG: rearrange Ocln, ZO-1, -2; open BBB [241] - EGFR-activation: p38 MAPK/NFκB signal pathway reduce Ocln expression [242] - ubiquitinated by E3A Nedd4-2 [243]/Itch [244] (prevented by γ-secretase blockade [245] - KD of E3A MARCH3 tightens BEC barrier, induces Ocln-/Cldn5 by FoxO1 deactivation [246] - reduction: TGF-β via MMPs [93], IL-17 [247] - degradation: MMP [248,249], calpain (Zn^{2+}-dependent) [250], proteasome [244,251] - microwave radiation: reduced Ocln/Ocln-ZO-1 binding, TJ broadening/fracture, BBB opening (VEGF/Flk-1-ERK Tyr-phosphorylation mediated) [252] - ischemia/reperfusion: Ser490 phosphorylation/ ubiquitination via VEGFR2 [253] - Netrin-1 protects BBB, activates Kruppel-like factor 2/Ocln path (ischemia/reperfusion) [221] - hypoxia: MMP9 caused Ocln rearrangement in TJs, BBB leakage [254] - diet-induced diabetes: Ocln/ZO-1 down, BBB leak; lessened by carboanhydrase inhibitor [141] - autistic children: serum Ocln increase [126]

Table 3. *Cont.*

Expression	Function	Structure/Interactions	Regulation/Signalling
Tricellulin (*MARVEL domain-containing protein 2*)			
- gene *MARVELD2*, chromosome 5 (human), 13 (mouse) - particular isoform Tric a [50] - expression <Ocln, <<Cldn5 [50] - brain [223], retina [203,255] - membranal in tricellular [256], bicellular cell contacts [257], likewise nuclear, perinuclear localised [223] - KO mouse: hearing loss, degenerated cochlea hair cells [258] - Tric/Occl-double KO lowers TJ-strand branches/ barrier integrity [209]	- sealing of macromolecules but not ions in tricellular TJs [211] - regulates H_2O-permeability [259] - role in regulating blood-cerebrospinal fluid barrier [255] - facilitates TJ branching/barrier integrity [55,209] - redox-regulation in TJs [260]	- mouse: 558 aa, M.W. 64.2 kDa; pI 7.21 - cytosolic N-/C-terminal 194/196 aa; very short ECL1/ECL2 8/16 aa; ECL2: disulfide bridge, hypoxia-/redox-sensitive [260] - homophilic: *cis* interaction in 2- and 3-cell TJs; *trans* in 3-cellular TJs [55,260] - heterophilic interaction: *cis* Cldn1, -3, -5, MD3; *trans* Cldn1, -5 [55] - continous P-face strand network in 3-cell TJs [260]; intensifies Cldn1 TJs [55] - C-terminal CC-domain: crystal structure (2.2 Å), dimer with polar interface [261] - angulins bind/recruit Tric in TJs [225] - N-terminus associates dynamin-binding protein (=scaffold protein Tuba) [262], human plasminogen [263]	- ubiquitination by Itch [264] - MAPK-, PKC-activation causes nuclear localisation in weakly differentiated tissue [265] - toxin ESX-1 secretion-associated protein EspG1 reduces expression [266] - induction by mirRNA-203 (microRNA binding motif on Tric) inhibitor, weakening Pb-induced blood-cerebrospinal fluid barrier leak [255] - down-regulated: interleukin-13 (via IL-13-receptor α2) [267], choleratoxin [268] - degradation: by MMP2/3 [269] - apoptosis: degraded at Asp487, Asp 441 (C-terminal CC-domain, caspase cleavage) [270] - OGD: Tric down in BEC [200] - increase in cortex: autism spectrum disorders (Cldn3, -5, -12) [271]
MarvelD3 (*MARVEL domain-containing protein 3*)			
- gene *MARVELD3*, chromosome 16 (human) - KD retards TJ formation [22]	- may partially replace Ocln, Tric [22]	- human 401 aa, M.W. 44.9 Da; pI 8.84; ECL1/2 24/39, N-/C-terminal 226/39 aa - *cis* binding: MD3, Ocln, Tric, Cldn1, -5 [55]	- down-regulated by OGD in bovine BEC [55]

aa, amino acid; BBB, blood-brain barrier; BEC, brain endothelial cell; CC, cytosolic C-terminal coiled-coil (OCEL, ELL) domain; *cis* interaction, cf. Figure 4B; CK, casein kinase; Cldn, claudin; EGFR, epidermal growth factor receptor; ECL/ICL, extracellular/intracellular loop; E3A, E3 ubiquitin ligase; FoxO1, forkhead box O1; KO/KD, knockout/-down; MAPK, mitogen-activated protein kinase; MARVEL, MAL and related proteins for vesicle trafficking and membrane link; MARVEL-domain, transmembrane domain 1–4, ECL1, ICL, ECL2; MD3, MarvelD3; MMP, matrix metalloproteinase; M.W., molecular weight; NFκB, nuclear factor κB; Ocln, occludin; OGD, oxygen/glucose deprivation; P-face, protoplasmic face; pI, isoelectric point; PI3K, phosphoinositol 3-kinase; PK, proteinkinase (PKB/Act, PKC, PKG); PL, phospholipase; TGF, transforming growth factor; TJ, tight junction; *trans* interaction, cf. Figure 4B; Tric, tricellulin; VEGF(R), vascular endothelial growth factor (receptor); ZO-1, *zonula occludens* protein 1.

3.3. Junctional Adhesion Molecules

The JAMs belong to the immunoglobulin superfamily [272], form one transmembrane domain, and are connected to the cytoskeleton via the binding of their short C-terminus to the PDZ3 domain of ZO-1 [273]. The N-terminal extracellular domain contains two immunoglobulin-like loops which can interact homo- and heterophilically in *cis* (with proteins of the same endothelial cell) or *trans* (e.g., with proteins on blood cell proteins) [272,274,275] (Figure 4B). Mainly JAM-A (JAM-1) [276], JAM-C (JAM-3) [277], and the endothelial-cell-selective adhesion molecule (ESAM) are found in TJs of the BBB [278,279] and support barrier features. JAM-A binds to integrin α-V β3 in *cis* [280] and integrin α-L of leukocytes in *trans* [281], and JAM-C binds to integrin α-M (*trans*, leukocyte) [282]). JAMs support the correct localisation of other junctional proteins (e.g., claudins) at the TJs [283] and stabilise cell barriers [284]. They are also involved in the regulation of cell contact formation, cellular migration, and mitosis, and, in this way, take part in barrier formation, angiogenesis and cerebral homoeostasis [285]. JAM-A regulates barrier properties by promoting the expression of C/EPB-α, a transcription factor regulating claudin-5 [125]. ESAM seems to be involved in the endothelial tube formation [286] (Figure 2A), as well as in the extravasation of white blood cells [287]; however, deactivation of the ESAM gene does not change the vascular permeability in mouse brain [288].

3.4. Cytosolic Tight Junction-Associated Proteins

The guanylate kinase homologous (MAGuKs [289]) ZO-1 (TJP1, 225 kDa [290]), as well as N-terminally truncated ZO-2 (TJP1, 160 kDa [291]) and ZO-3 (TJP3, 130 kDa [292]), are the most important membrane-associated proteins on the cytosolic TJ sides in the BBB. ZO proteins are involved in the formation and function of adherens- [293] and gap junctions [294]; consequently, ZO-1 is often used as cell contact marker [50]. In addition, ZO proteins are included in the regulation of cytoskeletal organisation, the establishment of cell polarity, and signalling to and from the nucleus [24]. They constitute the scaffold of TJs via multiple binding areas (in ZO-1: $_{NH2}$PDZ1–PDZ2–PDZ3–SH3–hinge region–GuK–acidic region–U6 region–proline rich region–ZU5 region$_{COOH}$, Figure 2D). These sections recruit transmembrane TJ-proteins and associate signalling- and structural proteins which, in turn, are involved in the formation, regulation, and/or stabilisation of TJs [295]. PDZ1 associates with claudins, and PDZ2 mediates the dimerisation of ZO proteins and may provide a structural basis for the association of claudins, namely, the formation of TJs [191,296]. PDZ3 and SH3-hinge-GuK bind JAMs and occludin, respectively [216]. SH3 interacts with ZONAB [297]. The hinge region (U5) attracts G-proteins [298], enabling a wide diversity of G-protein-coupled receptors to regulate the BBB [299]. Part of the proline-rich region (amino acids 1151–1371 of ZO-1) allows for the anchoring at the actin cytoskeleton [300]. Nuclear localisation of ZO proteins plays a further role in the signalling of TJ proteins [301,302] (Figure 2D). ZO-1 and ZO-2 can compensate for each other; only double knockdown leads to changes in the localisation of claudins and occludin, resulting in paracellular leakage [293,303,304].

Another scaffolding protein of the apical junctional complex is afadin (AF-6, gene AFDN, a multidomain protein with one PDZ, binds JAM-A). It interacts N-terminally with membranal adhesion- and signalling proteins while its C-terminus binds to the actin filament and to actin-binding proteins. Afadin can modulate signalling processes that influence cellular migration, invasion, and apoptosis [305]. Afadin is expressed at the cerebral endothelium [306] and contributes (in cooperation with PI3K/Akt signalling) to neovascularisation [307]. The cellular polarity at the TJs is maintained by the apical polarity complex, which includes the PAR complex (PAR3, PAR6, aPKC [308]) and the basolateral scribble complex (scribble, DLG, LGL). These complexes form networks via several signalling pathways (e.g., small GTPases as RhoA, RAC, and CDC42 [309]; Wnt/β-catenin [310]); their disbalance leads to perturbations in the barrier function [311]. aPKC and RhoA [283], as well as the PDZ-free scaffolding cingulin-like protein 1 (gene JACOP) [312], are involved in the regulation of the endothelial TJ conglomerate.

Summarising the data of the differentiated BBB, claudin-5 is proven to be the most prominent TJ component that bicellularly seals the barrier, probably assisted by claudin-11. The proper morphology and function of the TJ strand network is facilitated by occludin and tricellulin under support of ZO-1. Quantitatively, the sealing capacity is mainly accomplished by two cell (-like) TJs. The barrier function is subject of versatile regulation by signalling pathways relevant under normal and pathological conditions that particularly target claudin-5 and, to an even greater extent, occludin.

4. Tight Junctions and Pathologies of the Brain

In Table 4, disease states and diseases are compiled, which are accompanied by BBB impairment and TJ protein involvement. It is a widely held consideration that BBB damage is a consequence of many severe pathological processes of the brain. However, evidence is given that barrier disturbances can also be triggered by peripheral diseases [313] or by mutations of TJ proteins [115,314]. Leakage of the barrier is observed in the early stages of some brain diseases and might even contribute to disease progression [315,316]. Various cerebral diseases, such as ischemia and related disorders, tumours or, inflammatory processes, can lead to BBB disturbances, imbalances of ion-/molecular fluxes, increased extravasation of blood cells, and impaired TJ morphology [317]. Novel BBB models of human-induced pluripotent stem cells have been introduced to study diverse neurological diseases [318]. Details are given in Table 4.

Table 4. Pathologies, alterations of permeability and of tight junction proteins at the blood-brain barrier.

Type of Disorder	Leakage of Blood-Brain Barrier	Tight Junction Alteration
neurodegeneration	- m. Alzheimer mouse model [319,320]; iBEC layer, mutant transfected [321]	- ß-amyloid triggered angiogenesis → Cldn1, -5 down-regulated [319,320] → Cldn3, -5 up-regulated [321]
	- multiple sclerosis [322] - EAE [228]	→ down-regulated: Cldn3 (EAE) [78], Cldn11 (patient, EAE/mouse) [130] → angulin-1 down, 3-cell contacts [228]
	- amyotrophic lateral sclerosis [323]	- mouse model → BCSFB: loss of Cldn5, Ocln, ZO-1 [324]
	- m. Parkinson, extravasation in striatum [325]	- rat model → Cldn5, Ocln, ZO-1 up-regulated (*substantia nigra*) [326]
	- chorea Huntington, human and mouse model [327,328]	→ Occl, ZO-1 reduced in iBEC [328]
epilepsy	- cainic acid-induced seizures, temporal lope epilepsy [329]	- resected brain → Cldn5, Ocln, ZO-1 reduced [329]
psychiatric disorders	- schizophrenia, autism spectrum disorder (ADS), affective disorders [330] - ADS, cortex	→ Cldn12, Ocln, ZO-1 down-regulated [330] → Cldn3, -5, -12, Tric up [271]
	- schizophrenia associated with Cldn5 gene variant [314]	→ lessens Cldn5 in BEC; antipsychotic drug enhances Cldn5 [314]
brain tumours	- advanced tumour grades [78,93,331]	→ reduction of Cldn1, -3, -5, Ocln (glioblastoma) [78,80,332]
traumatic brain injury	- rat model [333] - patients [334] - Cldn5-si/shRNA enhanced leakage, reduced swelling [335]	→Ocln, ZO-1 reduced [333] → Cldn5 reversibly down-regulated [335] → ZO-1 degradation [336]

Table 4. *Cont.*

Type of Disorder	Leakage of Blood-Brain Barrier	Tight Junction Alteration
ischemia/stroke	- acute ischemic stroke, human [337] - ischemia/reperfusion, mouse [129]	- at clinical worsening → Cldn5, Cldn5:ZO-1 ratio increased in blood [127]
	- middle cerebral artery occlusion [228,338]	→ 3 h: Cldn1, -3, -12, Ocln dropped, but Cldn5 rose [50,53]; 5 d: Cldn5, Ocln, ZO-1 reduced [338] → angulin-1 down, 3-cell contact [228]
	- hypoxia/glucose lack, BEC [200]	→ Cldn5, Ocln, ZO-1 down [339]
	- haemorrhage [76,104]	→ Cldn25 down-regulated (BEC) [76] → Cldn3, -5 down; improvement/reduced leak by anti-malaria drug [104]
- reinforced by	- d. mellitus [340] - BRB leakage in murine diabetic retinopathy [341] - microangiopathy: small vessel disease, stroke imaging [342]; iBEC layer, mutant transfected [343]	→ Cldn5, Ocln depressed [341] → Cldn5 and ZO-1 expression reduced (autopsy samples) [344] → Cldn5-, Ocln-junctions affected [343]
high-fat diet	- diet-induced obese diabetic mice [141]; obesity [345]	→ Cldn12, Ocln, ZO-1 reduced [141]
inflammation	- thrombin-caused, BEC layer [239] - astrocyte-derived VEGF [346] - peripheral (CFA) [182,347,348] - pancreatitis [349]	→ Ocln-ZO-1 binding lost [239] → Cldn5 decrease in BEC [332] → Ocln down/Cldn3, -5 up (CFA) [182] → degradation of ZO-1, Cldn5 [349]
infection	- Zika virus in mice [350] - bacterial pertussis toxin [347] - *Plasmodium falciparum* [353] - *Neisseria meningitidis*, iBEC [354] - long COVID patients [355]	→ Cldn5 down [350], via miR-101-3p [351] → ZO-1 down (BCSFB) [352] → Cldn5, Ocln down (mouse) [353] → Cldn5, Ocln down (cell layer) [354] → Cldn5 down (mouse) [356]
hypertonia	- acute in rat [357]	→ Cldn3, -5, -12 depression [357]
alcohol abuse	- hippocampal IgG extravasation [358]	→ Cldn5 down [358]
microcephaly	- small molecule flux higher [115]	→ human Cldn5-G60R (ECL1) [115]

BCSFB, blood-cerebrospinal fluid barrier; BEC, brain endothelial cells; BRB, blood-retina barrier; CFA, complete Freund's adjuvant; Cldn, claudin; EAE, experimental autoimmune encephalitis; ECL, extracellular loop; iBEC, BEC induced from human embryonic stem cells; Ocln, occludin; VEGF, vascular endothelial growth factor; ZO-1, *zonula occludens* protein 1.

Similar alterations of the barrier are described for neurodegenerative [359] and psychiatric disorders (e.g., schizophrenia, autism spectrum disorder, affective disorders) [330]. In the context of Alzheimer's disease, angiogenic processes can be triggered by β-amyloid, which result in a loss of microvascular TJ proteins (claudin-1, -5) and increased permeability of the barrier in rodent brain [319,320]. In human stem cell models, claudin-3 and -5 are found up-regulated [321]. In patients with Alzheimer's disease, plasma claudin-5 levels are increased; the protein has been suggested as a potential biomarker for the diagnosis of AD [360].

Multiple sclerosis is characterised by decreased levels of claudin-11 at the BBB of patients [130] and of claudin-3 in a mouse model which exhibits lowered barrier tightness [78]. Another mouse study revealed down-regulation of angulin-1, suggesting that tricellular TJs could be disturbed during multiple sclerosis [228]. The inflammatory cytokine IFNγ safeguards tight junctions of the BBB in a mouse model of multiple sclerosis by up-regulating claudin-5, which could be inhibited by TNFα and IL17 [361], revealing a physiological protective mechanism of the BBB during inflammation.

In a model of amyotrophic lateral sclerosis, reduction of claudin-5, occludin, and ZO-1 in endothelial cells results in microhaemorrhages before motor neuron degeneration and neurovascular inflammatory markers occur, indicating a central contribution to disease

initiation [324]. For schizophrenia, a variant in the claudin-5 gene is reported, leading to suppression of claudin-5 [314]. Interestingly, loss of TJ proteins from a damaged BBB can be detected in the peripheral blood of autistic children (claudin-5, -11, occludin) [126], supporting earlier findings that claudin-5 is released from the brain endothelium during disease. Claudin-5 can be transferred to circulating leukocytes, which could support leukocyte transendothelial migration into the CNS [116].

The BBB in brain tumours is widely intact in early stages but breaks down during progression of the tumour [331] with a concomitant diminution of claudin-1, -3, -5, and occludin [78,80,93]. Many different types of brain tumours have been documented, showing large heterogeneity. They arise from primary tumours, such as glioblastoma or astrocytoma, and metastatic tumours frequently originate from breast or lung cancer [362].

Breakdown of the BBB is well documented for ischemic states [9,338], which lead to disorders such as stroke or oedema [363]. At the molecular level, multiple factors are discussed: oxidative/nitrosative stress, metabolic/ionic dysregulation, and/or inflammatory/neurodegenerative processes [364]. Experimental ischemia/reperfusion results in the loss of claudin-1, -3, -12, and occludin, whereas claudin-5 has been found to be up-regulated 3 h after occlusion [50,53]. After 120 h, similar effects have been observed for occludin and ZO-1, but this was also true for claudin-5 in the study in question, suggesting a biphasic time-course [338]. Claudin-5 has been also found in the serum of stroke patients [127]. Studies on protective approaches often report claudin-5 preservation, e.g., by a novel antioxidant, attenuating BBB breakdown via erythroid 2-like 2 nuclear translocation/haem oxygenase 1 signalling stimulation [129], by small molecules [365], or by mesenchymal stem cells [366]. Endothelial reduction of claudin-25 and the subsequent permeability increase for small molecules have been shown for cerebellar haemorrhage [76]. Disturbances of the cerebral circulation are fostered by obesity [345], diabetes mellitus [340], or microangiopathies [342]. BBB leakage is also accompanied by the down-regulation of claudin-12, occludin, and ZO-1 in obesity with concomitant type II diabetes [141], or of claudin-5 and occludin in diabetes [341]. Interestingly, $Tie2^{+}$ macrophages promote endogenous revascularisation in mouse brains after ischemic injury [367], which possibly leads to reconstruction of the BBB.

Infarct progression and oedema formation can be increased by a tight barrier [53]. Absence of claudin-3 and a reduced level of occludin can limit the infarcted and oedematous area. Consequently, TJ modulation has been postulated as an approach to treating stroke and related disorders at least early after onset. There is good evidence that mild trauma resulting in BBB disruption in rats opens a paracellular pathway of approximately 22 nm, consistent with disrupted TJs, leaving adherens junctions intact [368]. In this context, EphA4/Tie2/Akap12 signalling has been reported to limit the expression of claudin-5 and to mediate microvascular dysfunction [128]. On the other hand, trauma-associated brain oedema can be diminished via suppression of claudin-5 after administration of the corresponding siRNA in rats [335] or by a novel inhibitor of claudin-5 interactions [369], which also alleviates ZO-1 degradation [336]. In addition to its direct effect, claudin-5 siRNA can be applied to improve the pharmacokinetics of agents targeting brain diseases [185]. There are other modulators discussed in this context: claudin-5 shRNA [370], monoclonal anti-claudin antibodies [371], and peptides (disclosed by phage display [372–374] or derived from sequences of the extracellular loop 1 of claudins [190,199]), which exerted transient BBB opening. The last-mentioned approaches can be generalised to the extent that small agents can be developed according to this principle to open the BBB transiently and size-selectively and provide conditions allowing for the delivery of a wide range of compounds for the treatment of neurodegenerative, neuropsychiatric, and malignant diseases. For the delivery of larger molecules, such as antisense oligonucleotides, the angulin-1 binder angubindin-1 has been applied in vivo. The data demonstrate that not only bicellular but also tricellular TJs may be targeted to improve drug permeation through the BBB [375].

Cerebral inflammation is often induced by infections. Zika or meningitis viruses cause disruption of the mouse BBB and down-regulation of claudin-5 [350,354]. The

malaria pathogen *Plasmodium falciparum* elicits vascular permeability, fatal brain oedema, and down-regulation of claudin-5 and occludin [353]. Chronic inflammatory pain induced by peripheral injection of complete Freund's adjuvant suppressed occludin but up-regulated claudin-3 and -5 with simultaneous opening of the barrier [182]. Various mediators and pathways are involved in alterations of TJ proteins during inflammation. Vascular endothelial growth factor contributes to BBB opening [83,346] by down-regulating claudin-5 [332]. Increased permeability is also caused by pro-inflammatory stimuli such as thrombin [239] or microbial toxins down-regulating occludin and claudin-5 [347]. Barrier strengthening can be achieved via the administration of anti-inflammatory agents, e.g., of angiopoietin-1, which promotes occludin-ZO-1 interaction and stabilises TJs by inhibiting thrombin-induced Tyr-phosphorylation of occludin [239].

Reviewing the latest experimental data and the increasing number of clinical studies reveals more and more neurological disorders characterised by BBB involvement. In the vast majority of cases, disturbances of the TJs at the molecular level of claudin-5 are involved, with evidence of pathogenic significance. Modulation of three cellular TJ-proteins provides a novel approach for drug delivery. Human-induced stem cell models offer diagnostic potential in analysing various neurological diseases individually. These advances have led to new diagnostic approaches and will further encourage pharmacological studies.

5. Conclusions and Perspectives

Tight junctions are of crucial importance for the BBB, which both directly and indirectly controls the overwhelming majority of the exchange mechanisms between the brain and blood. On the other hand, a functional barrier may also cause problems such as reduced edema drainage in stroke or trauma or insufficient drug delivery to tumours. Further progress in understanding the BBB requires reliable BBB models; previous model investigations often applied non-human cells with dedifferentiated TJ proteins. Recent developments favour models using human primary and induced pluripotent stem cells. For in vivo studies, the prospects of genetically modified mice should be further exploited in the context of TJs. These approaches bear great potential for BBB-related research with high clinical relevance.

Elucidation of the molecular composition of TJs revealed that claudin-5 plays a central role in tightening the BBB, but the TJ does not solely depend on this claudin; it must be considered in concert with other TJ proteins. In particular, the impact of claudin-11 and tricellulin/angulin requires further experimental and clinical studies. Numerous signalling pathways relevant to the BBB have been clarified, and above all, we have described the role of these in pathologies, as is increasingly being shown clinically. Claudin-5 can be regulated directly, but more often, indirect regulation via occludin is observed. More insight into these processes will open up new diagnostic and therapeutic perspectives.

Nevertheless, the exact role that the BBB plays is still unclear in many CNS diseases, especially the extent to which it itself can cause cerebral dysfunction. Generally, its opening is considered to be a consequence of disease progression, but there is evidence that the BBB disruption is pathogenetic early in the development of disorders. We confidently expect that further molecular mechanisms and protein structures will be disclosed to advance our understanding of TJ biology and to be translated into new therapeutic approaches.

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References

1. Rodriguez-Boulan, E.; Macara, I.G. Organization and execution of the epithelial polarity programme. *Nat. Rev. Mol. Cell Biol.* **2014**, *15*, 225–242. [CrossRef]
2. Farquhar, M.G.; Palade, G.E. Junctional Complexes in Various Epithelia. *J. Cell Biol.* **1963**, *17*, 375–412. [CrossRef] [PubMed]
3. Irie, K.; Shimizu, K.; Sakisaka, T.; Ikeda, W.; Takai, Y. Roles and modes of action of nectins in cell-cell adhesion. *Semin. Cell Dev. Biol.* **2004**, *15*, 643–656. [CrossRef] [PubMed]
4. Hartsock, A.; Nelson, W.J. Adherens and tight junctions: Structure, function and connections to the actin cytoskeleton. *Biochim. Biophys. Acta Biomembr.* **2008**, *1778*, 660–669. [CrossRef] [PubMed]
5. Baum, B.; Georgiou, M. Dynamics of adherens junctions in epithelial establishment, maintenance, and remodeling. *J. Cell Biol.* **2011**, *192*, 907–917. [CrossRef] [PubMed]
6. Schulze, C.; Firth, J.A. Immunohistochemical localization of adherens junction components in blood-brain-barrier microvessels of the rat. *J. Cell Sci.* **1993**, *104*, 773–782. [CrossRef] [PubMed]
7. Rouaud, F.; Sluysmans, S.; Flinois, A.; Shah, J.; Vasileva, E.; Citi, S. Scaffolding proteins of vertebrate apical junctions: Structure, functions and biophysics. *Biochim. Biophys. Acta Biomembr.* **2020**, *1862*, 183399. [CrossRef] [PubMed]
8. Nagasawa, K.; Chiba, H.; Fujita, H.; Kojima, T.; Saito, T.; Endo, T.; Sawada, N. Possible involvement of gap junctions in the barrier function of tight junctions of brain and lung endothelial cells. *J. Cell. Physiol.* **2006**, *208*, 123–132. [CrossRef]
9. Weiss, N.; Miller, F.; Cazaubon, S.; Couraud, P.O. The blood-brain barrier in brain homeostasis and neurological diseases. *Biochim. Biophys. Acta Biomembr.* **2009**, *1788*, 842–857. [CrossRef] [PubMed]
10. Staehelin, L.A. Further observations on fine-structure of freeze-cleaved tight junctions. *J. Cell Sci.* **1973**, *13*, 763–786. [CrossRef] [PubMed]
11. Shen, L.; Weber, C.R.; Raleigh, D.R.; Yu, D.; Tumer, J.R. Tight, Junction Pore and Leak Pathways: A Dynamic Duo. In *Annual Review of Physiology*; Julius, D., Clapham, D.E., Eds.; Annual Reviews: San Mateo, CA, USA, 2011; Volume 73, pp. 283–309.
12. Kirschner, N.; Rosenthal, R.; Furuse, M.; Moll, I.; Fromm, M.; Brandner, J.M. Contribution of Tight Junction Proteins to Ion, Macromolecule, and Water Barrier in Keratinocytes. *J. Investig. Dermatol.* **2013**, *133*, 1161–1169. [CrossRef] [PubMed]
13. Krause, G.; Winkler, L.; Mueller, S.L.; Haseloff, R.F.; Piontek, J.; Blasig, I.E. Structure and function of claudins. *Biochim. Biophys. Acta Biomembr.* **2008**, *1778*, 631–645. [CrossRef] [PubMed]
14. Anderson, J.M.; Van Itallie, C.M. Physiology and Function of the Tight Junction. *Cold Spring Harbor Perspect. Biol.* **2009**, *1*, a002584. [CrossRef] [PubMed]
15. Kooij, G.; Kopplin, K.; Blasig, R.; Stuiver, M.; Koning, N.; Goverse, G.; van der Pol, S.M.A.; Hof, B.V.; Gollasch, M.; Drexhage, J.A.R.; et al. Disturbed function of the blood-cerebrospinal fluid barrier aggravates neuro-inflammation. *Acta Neuropathol.* **2014**, *128*, 267–277. [CrossRef] [PubMed]
16. Gonzalez-Mariscal, L.; Namorado, M.C.; Martin, D.; Luna, J.; Alarcon, L.; Islas, S.; Valencia, L.; Muriel, P.; Ponce, L.; Reyes, J.L. Tight junction proteins ZO-1, ZO-2, and occludin along isolated renal tubules. *Kidney Int.* **2000**, *57*, 2386–2402. [CrossRef] [PubMed]
17. Cao, X.W.; Surma, M.A.; Simons, K. Polarized sorting and trafficking in epithelial cells. *Cell Res.* **2012**, *22*, 793–805. [CrossRef] [PubMed]
18. Cereijido, M.; Valdes, J.; Shoshani, L.; Contreras, R.G. Role of tight junctions in establishing and maintaining cell polarity. *Annu. Rev. Physiol.* **1998**, *60*, 161–177. [CrossRef] [PubMed]
19. Matter, K.; Aijaz, S.; Tsapara, A.; Balda, M.S. Mammalian tight junctions in the regulation of epithelial differentiation and proliferation. *Curr. Opin. Cell Biol.* **2005**, *17*, 453–458. [CrossRef] [PubMed]
20. Furuse, M.; Fujita, K.; Hiiragi, T.; Fujimoto, K.; Tsukita, S. Claudin-1 and -2: Novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. *J. Cell Biol.* **1998**, *141*, 1539–1550. [CrossRef] [PubMed]
21. Morita, K.; Furuse, M.; Fujimoto, K.; Tsukita, S. Claudin multigene family encoding four-transmembrane domain protein components of tight junction strands. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 511–516. [CrossRef] [PubMed]
22. Raleigh, D.R.; Marchiando, A.M.; Zhang, Y.; Shen, L.; Sasaki, H.; Wang, Y.M.; Long, M.Y.; Turner, J.R. Tight Junction-associated MARVEL Proteins MarvelD3, Tricellulin, and Occludin Have Distinct but Overlapping Functions. *Mol. Biol. Cell* **2010**, *21*, 1200–1213. [CrossRef] [PubMed]
23. Martin-Padura, I.; Lostaglio, S.; Schneemann, M.; Williams, L.; Romano, M.; Fruscella, P.; Panzeri, C.; Stoppacciaro, A.; Ruco, L.; Villa, A.; et al. Junctional adhesion molecule, a novel member of the immunoglobulin superfamily that distributes at intercellular junctions and modulates monocyte transmigration. *J. Cell Biol.* **1998**, *142*, 117–127. [CrossRef]
24. Guillemot, L.; Paschoud, S.; Pulimeno, P.; Foglia, A.; Citi, S. The cytoplasmic plaque of tight junctions: A scaffolding and signalling center. *Biochim. Biophys. Acta Biomembr.* **2008**, *1778*, 601–613. [CrossRef] [PubMed]
25. Ivanov, A.I.; Nusrat, A.; Parkos, C.A. Endocytosis of epithelial apical junctional proteins by a clathrin-mediated pathway into a unique storage compartment. *Mol. Biol. Cell* **2004**, *15*, 176–188. [CrossRef] [PubMed]
26. Zwanziger, D.; Staat, C.; Andjelkovic, A.V.; Blasig, I.E. Claudin-derived peptides are internalized via specific endocytosis pathways. In *Barriers and Channels Formed by Tight Junction Proteins I*; Fromm, M., Schulzke, J.D., Eds.; Wiley Online Library: Hoboken, NJ, USA, 2012; Volume 1257, pp. 29–37.

27. Stamatovic, S.M.; Keep, R.F.; Wang, M.M.; Jankovic, I.; Andjelkovic, A.V. Caveolae-mediated Internalization of Occludin and Claudin-5 during CCL2-induced Tight Junction Remodeling in Brain Endothelial Cells. *J. Biol. Chem.* **2009**, *284*, 19053–19066. [CrossRef] [PubMed]
28. Bruewer, M.; Utech, M.; Ivanov, A.I.; Hopkins, A.M.; Parkos, C.A.; Nusrat, A. Interferon-gamma induces internalization of epithelial tight junction proteins via a macropinocytosis-like process. *FASEB J.* **2005**, *19*, 923–933. [CrossRef]
29. Takahashi, S.; Iwamoto, N.; Sasaki, H.; Ohashi, M.; Oda, Y.; Tsukita, S.; Furuse, M. The E3 ubiquitin ligase LNX1p80 promotes the removal of claudins from tight junctions in MDCK cells. *J. Cell Sci.* **2009**, *122*, 985–994. [CrossRef] [PubMed]
30. Gehne, N.; Lamik, A.; Lehmann, M.; Haseloff, R.F.; Andjelkovic, A.V.; Blasig, I.E. Cross-over endocytosis of claudins is mediated by interactions via their extracellular loops. *PLoS ONE* **2017**, *12*, e0182106. [CrossRef] [PubMed]
31. Mineta, K.; Yamamoto, Y.; Yamazaki, Y.; Tanaka, H.; Tada, Y.; Saito, K.; Tamura, A.; Igarashi, M.; Endo, T.; Takeuchi, K.; et al. Predicted expansion of the claudin multigene family. *FEBS Lett.* **2011**, *585*, 606–612. [CrossRef]
32. Furuse, M.; Tsukita, S. Claudins in occluding junctions of humans and flies. *Trends Cell Biol.* **2006**, *16*, 181–188. [CrossRef] [PubMed]
33. Suzuki, H.; Nishizawa, T.; Tani, K.; Yamazaki, Y.; Tamura, A.; Ishitani, R.; Dohmae, N.; Tsukita, S.; Nureki, O.; Fujiyoshi, Y. Crystal Structure of a Claudin Provides Insight into the Architecture of Tight Junctions. *Science* **2014**, *344*, 304–307. [CrossRef] [PubMed]
34. Saitoh, Y.; Suzuki, H.; Tani, K.; Nishikawa, K.; Irie, K.; Ogura, Y.; Tamura, A.; Tsukita, S.; Fujiyoshi, Y. Structural insight into tight junction disassembly by Clostridium perfringens enterotoxin. *Science* **2015**, *347*, 775–778. [CrossRef] [PubMed]
35. Shinoda, T.; Shinya, N.; Ito, K.; Ohsawa, N.; Terada, T.; Hirata, K.; Kawano, Y.; Yamamoto, M.; Kimura-Someya, T.; Yokoyama, S.; et al. Structural basis for disruption of claudin assembly in tight junctions by an enterotoxin. *Sci. Rep.* **2016**, *6*, 33632. [CrossRef] [PubMed]
36. Vecchio, A.J.; Stroud, R.M. Claudin-9 structures reveal mechanism for toxin-induced gut barrier breakdown. *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 17817–17824. [CrossRef] [PubMed]
37. Gunzel, D.; Yu, A.S.L. Claudins and the modulation of tight junction permeability. *Physiol. Rev.* **2013**, *93*, 525–569. [CrossRef] [PubMed]
38. Tsukita, S.; Tanaka, H.; Tamura, A. The Claudins: From Tight Junctions to Biological Systems. *Trends Biochem. Sci.* **2019**, *44*, 141–152. [CrossRef] [PubMed]
39. Zhang, Y.N.; Yeh, S.; Appleton, B.A.; Held, H.A.; Kausalya, P.J.; Phua, D.C.Y.; Wong, W.L.; Lasky, L.A.; Wiesmann, C.; Hunziker, W.; et al. Convergent and divergent ligand specificity among PDZ domains of the LAP and zonula occludens (ZO) families. *J. Biol. Chem.* **2006**, *281*, 22299–22311. [CrossRef] [PubMed]
40. Itoh, M.; Furuse, M.; Morita, K.; Kubota, K.; Saitou, M.; Tsukita, S. Direct binding of three tight junction-associated MAGUKs, ZO-1, ZO-2 and ZO-3, with the COOH termini of claudins. *J. Cell Biol.* **1999**, *147*, 1351–1363. [CrossRef] [PubMed]
41. Ruffer, C.; Gerke, V. The C-terminal cytoplasmic tail of claudins 1 and 5 but not its PDZ-binding motif is required for apical localization at epithelial and endothelial tight junctions. *Eur. J. Cell Biol.* **2004**, *83*, 135–144. [CrossRef] [PubMed]
42. Liu, J.; Weaver, J.; Jin, X.C.; Zhang, Y.; Xu, J.; Liu, K.J.; Li, W.P.; Liu, W.L. Nitric Oxide Interacts with Caveolin-1 to Facilitate Autophagy-Lysosome-Mediated Claudin-5 Degradation in Oxygen-Glucose Deprivation-Treated Endothelial Cells. *Mol. Neurobiol.* **2016**, *53*, 5935–5947. [CrossRef]
43. Tanaka, M.; Kamata, R.; Sakai, R. EphA2 phosphorylates the cytoplasmic tail of claudin-4 and mediates paracellular permeability. *J. Biol. Chem.* **2005**, *280*, 42375–42382. [CrossRef] [PubMed]
44. Van Itallie, C.M.; Gambling, T.M.; Carson, J.L.; Anderson, J.M. Palmitoylation of claudins is required for efficient tight-junction localization. *J. Cell Sci.* **2005**, *118*, 1427–1436. [CrossRef] [PubMed]
45. Lohrberg, D.; Krause, E.; Schumann, M.; Piontek, J.; Winkler, L.; Blasig, I.E.; Haseloff, R.F. A strategy for enrichment of claudins based on their affinity to Clostridium perfringens enterotoxin. *BMC Mol. Biol.* **2009**, *10*, 61. [CrossRef] [PubMed]
46. Gow, A.; Southwood, C.M.; Li, J.S.; Pariali, M.; Riordan, G.P.; Brodie, S.E.; Danias, J.; Bronstein, J.M.; Kachar, B.; Lazzarini, R.A. CNS myelin and Sertoli cell tight junction strands are absent in Osp/Claudin-11 null mice. *Cell* **1999**, *99*, 649–659. [CrossRef]
47. Gow, A.; Davies, C.; Southwood, C.M.; Frolenkov, G.; Chrustowski, M.; Ng, L.; Yamauchi, D.; Marcus, D.C.; Kachar, B. Deafness in Claudin 11-null mice reveals the critical contribution of basal cell tight junctions to stria vascularis function. *J. Neurosci.* **2004**, *24*, 7051–7062. [CrossRef] [PubMed]
48. Gupta, I.R.; Ryan, A.K. Claudins: Unlocking the code to tight junction function during embryogenesis and in disease. *Clin. Genet.* **2010**, *77*, 314–325. [CrossRef] [PubMed]
49. Haseloff, R.F.; Dithmer, S.; Winkler, L.; Wolburg, H.; Blasig, I.E. Transmembrane proteins of the tight junctions at the blood-brain barrier: Structural and functional aspects. *Semin. Cell Dev. Biol.* **2015**, *38*, 16–25. [CrossRef] [PubMed]
50. Berndt, P.; Winkler, L.; Cording, J.; Breitkreuz-Korff, O.; Rex, A.; Dithmer, S.; Rausch, V.; Blasig, R.; Richter, M.; Sporbert, A.; et al. Tight junction proteins at the blood-brain barrier: Far more than claudin-5. *Cell. Mol. Life Sci.* **2019**, *76*, 1987–2002. [CrossRef] [PubMed]
51. Gregory, M.; Dufresne, J.; Hermo, L.; Cyr, D.G. Claudin-1 is not restricted to tight junctions in the rat epididymis. *Endocrinology* **2001**, *142*, 854–863. [CrossRef]
52. Inai, T.; Sengoku, A.; Hirose, E.; Iida, H.; Shibata, Y. Claudin-7 expressed on lateral membrane of rat epididymal epithelium does not form aberrant tight junction strands. *Anat. Rec. Adv. Integr. Anat. Evol. Biol.* **2007**, *290*, 1431–1438. [CrossRef]

53. Winkler, L.; Blasig, R.; Breitkreuz-Korff, O.; Berndt, P.; Dithmer, S.; Helms, H.C.; Puchkov, D.; Devraj, K.; Kaya, M.; Qin, Z.; et al. Tight junctions in the blood-brain barrier promote edema formation and infarct size in stroke—Ambivalent effects of sealing proteins. *J. Cereb. Blood Flow Metab.* **2020**, *41*, 132–145. [CrossRef] [PubMed]
54. Piontek, J.; Winkler, L.; Wolburg, H.; Müller, S.L.; Zuleger, N.; Piehl, C.; Wiesner, B.; Krause, G.; Blasig, I.E. Formation of tight junction: Determinants of homophilic interaction between classical claudins. *Faseb J.* **2008**, *22*, 146–158. [CrossRef] [PubMed]
55. Cording, J.; Berg, J.; Kading, N.; Bellmann, C.; Tscheik, C.; Westphal, J.K.; Milatz, S.; Gunzel, D.; Wolburg, H.; Piontek, J.; et al. In tight junctions, claudins regulate the interactions between occludin, tricellulin and marvelD3, which, inversely, modulate claudin oligomerization. *J. Cell Sci.* **2013**, *126*, 554–564. [CrossRef] [PubMed]
56. Inai, T.; Kobayashi, J.; Shibata, Y. Claudin-1 contributes to the epithelial barrier function in MDCK cells. *Eur. J. Cell Biol.* **1999**, *78*, 849–855. [CrossRef] [PubMed]
57. Furuse, M.; Furuse, K.; Sasaki, H.; Tsukita, S. Conversion of Zonulae occludentes from tight to leaky strand type by introducing claudin-2 into Madin-Darby canine kidney I cells. *J. Cell Biol.* **2001**, *153*, 263–272. [CrossRef]
58. Amasheh, S.; Meiri, N.; Gitter, A.H.; Schoneberg, T.; Mankertz, J.; Schulzke, J.D.; Fromm, M. Claudin-2 expression induces cation-selective channels in tight junctions of epithelial cells. *J. Cell Sci.* **2002**, *115*, 4969–4976. [CrossRef]
59. Milatz, S.; Krug, S.M.; Rosenthal, R.; Gunzel, D.; Muller, D.; Schulzke, J.D.; Amasheh, S.; Fromm, M. Claudin-3 acts as a sealing component of the tight junction for ions of either charge and uncharged solutes. *Biochim. Biophys. Acta Biomembr.* **2010**, *1798*, 2048–2057. [CrossRef] [PubMed]
60. Van Itallie, C.; Rahner, C.; Anderson, J.M. Regulated expression of claudin-4 decreases paracellular conductance through a selective decrease in sodium permeability. *J. Clin. Investig.* **2001**, *107*, 1319–1327. [CrossRef] [PubMed]
61. Hou, J.H.; Renigunta, A.; Yang, J.; Waldegger, S. Claudin-4 forms paracellular chloride channel in the kidney and requires claudin-8 for tight junction localization. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 18010–18015. [CrossRef]
62. Colegio, O.R.; Van Itallie, C.M.; McCrea, H.J.; Rahner, C.; Anderson, J.M. Claudins create charge-selective channels in the paracellular pathway between epithelial cells. *Am. J. Physiol. Cell Physiol.* **2002**, *283*, C142–C147. [CrossRef] [PubMed]
63. Nitta, T.; Hata, M.; Gotoh, S.; Seo, Y.; Sasaki, H.; Hashimoto, N.; Furuse, M.; Tsukita, S. Size-selective loosening of the blood-brain barrier in claudin-5-deficient mice. *J. Cell Biol.* **2003**, *161*, 653–660. [CrossRef] [PubMed]
64. Hou, J.H.; Gomes, A.S.; Paul, D.L.; Goodenough, D.A. Study of claudin function by RNA interference. *J. Biol. Chem.* **2006**, *281*, 36117–36123. [CrossRef] [PubMed]
65. Alexandre, M.D.; Lu, Q.; Chen, Y.H. Overexpression of claudin-7 decreases the paracellular Cl- conductance and increases the paracellular Na+ conductance in LLC-PK1 cells. *J. Cell Sci.* **2005**, *118*, 2683–2693. [CrossRef] [PubMed]
66. Sas, D.; Hu, M.C.; Moe, O.W.; Baum, M. Effect of claudins 6 and 9 on paracellular permeability in MDCK II cells. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2008**, *295*, R1713–R1719. [CrossRef] [PubMed]
67. Van Itallie, C.M.; Rogan, S.; Yu, A.; Vidal, L.S.; Holmes, J.; Anderson, J.M. Two splice variants of claudin-10 in the kidney create paracellular pores with different ion selectivities. *Am. J. Physiol. Renal Physiol.* **2006**, *291*, F1288–F1299. [CrossRef] [PubMed]
68. Abuazza, G.; Becker, A.; Williams, S.S.; Chakravarty, S.; Truong, H.T.; Lin, F.M.; Baum, M. Claudins 6, 9, and 13 are developmentally expressed renal tight junction proteins. *Am. J. Physiol. Renal Physiol.* **2006**, *291*, F1132–F1141. [CrossRef] [PubMed]
69. Yu, A.S.L.; Enck, A.H.; Lencer, W.I.; Schneeberger, E.E. Claudin-8 expression in Madin-Darby canine kidney cells augments the paracellular barrier to cation permeation. *J. Biol. Chem.* **2003**, *278*, 17350–17359. [CrossRef] [PubMed]
70. Krug, S.M.; Gunzel, D.; Conrad, M.P.; Rosenthal, R.; Fromm, A.; Amasheh, S.; Schulzke, J.D.; Fromm, M. Claudin-17 forms tight junction channels with distinct anion selectivity. *Cell. Mol. Life Sci.* **2012**, *69*, 2765–2778. [CrossRef] [PubMed]
71. Ben-Yosef, T.; Belyantseva, I.A.; Saunders, T.L.; Hughes, E.D.; Kawamoto, K.; Van Itallie, C.M.; Beyer, L.A.; Halsey, K.; Gardner, D.J.; Wilcox, E.R.; et al. Claudin 14 knockout mice, a model for autosomal recessive deafness DFNB29, are deaf due to cochlear hair cell degeneration. *Hum. Mol. Genet.* **2003**, *12*, 2049–2061. [CrossRef]
72. Hou, J.H.; Renigunta, A.; Konrad, M.; Gornes, A.S.; Schneeberger, E.E.; Paul, D.L.; Waldegger, S.; Goodenough, D.A. Claudin-16 and claudin-19 interact and form a cation-selective tight junction complex. *J. Clin. Investig.* **2008**, *118*, 619–628. [CrossRef] [PubMed]
73. McCabe, M.J.; Foo, C.F.H.; Dinger, M.E.; Smooker, P.M.; Stanton, P.G. Claudin-11 and occludin are major contributors to Sertoli cell tight junction function, in vitro. *Asian J. Androl.* **2016**, *18*, 620–626. [PubMed]
74. Jovov, B.; Van Itallie, C.M.; Shaheen, N.J.; Carson, J.L.; Gambling, T.M.; Anderson, J.M.; Orlando, R.C. Claudin-18: A dominant tight junction protein in Barrett's esophagus and likely contributor to its acid resistance. *Am. J. Physiol. Gastroint. Liver Physiol.* **2007**, *293*, G1106–G1113. [CrossRef] [PubMed]
75. Tanaka, H.; Yamamoto, Y.; Kashihara, H.; Yamazaki, Y.; Tani, K.; Fujiyoshi, Y.; Mineta, K.; Takeuchi, K.; Tamura, A.; Tsukita, S. Claudin-21 Has a Paracellular Channel Role at Tight Junctions. *Mol. Cell. Biol.* **2016**, *36*, 954–964. [CrossRef]
76. Ohnishi, M.; Ochiai, H.; Matsuoka, K.; Akagi, M.; Nakayama, Y.; Shima, A.; Uda, A.; Matsuoka, H.; Kamishikiryo, J.; Michihara, A.; et al. Claudin Domain Containing 1 Contributing to Endothelial Cell Adhesion Decreases in Presence of Cerebellar Hemorrhage. *J. Neurosci. Res.* **2017**, *95*, 2051–2058. [CrossRef] [PubMed]
77. Mahajan, S.D.; Aalinkeel, R.; Sykes, D.E.; Reynolds, J.L.; Bindukumar, B.; Adal, A.; Qi, M.; Toh, J.; Xu, G.; Prasad, P.N.; et al. Methamphetamine alters blood brain barrier permeability via the modulation of tight junction expression: Implication for HIV-1 neuropathogenesis in the context of drug abuse. *Brain Res.* **2008**, *1203*, 133–148. [CrossRef] [PubMed]

78. Wolburg, H.; Wolburg-Buchholz, K.; Kraus, J.; Rascher-Eggstein, G.; Liebner, S.; Hamm, S.; Duffner, F.; Grote, E.H.; Risau, W.; Engelhardt, B. Localization of claudin-3 in tight junctions of the blood-brain barrier is selectively lost during experimental autoimmune encephalomyelitis and human glioblastoma multiforme. *Acta Neuropathol.* **2003**, *105*, 586–592. [CrossRef] [PubMed]
79. Hanske, S.; Dyrna, F.; Bechmann, I.; Krueger, M. Different segments of the cerebral vasculature reveal specific endothelial specifications, while tight junction proteins appear equally distributed. *Brain Struct. Funct.* **2017**, *222*, 1179–1192. [CrossRef]
80. Liebner, S.; Fischmann, A.; Rascher, G.; Duffner, F.; Grote, E.H.; Kalbacher, H.; Wolburg, H. Claudin-1 and claudin-5 expression and tight junction morphology are altered in blood vessels of human glioblastoma multiforme. *Acta Neuropathol.* **2000**, *100*, 323–331. [CrossRef] [PubMed]
81. Furuse, M.; Hata, M.; Furuse, K.; Yoshida, Y.; Haratake, A.; Sugitani, Y.; Noda, T.; Kubo, A.; Tsukita, S. Claudin-based tight junctions are crucial for the mammalian epidermal barrier: A lesson from claudin-1-deficient mice. *J. Cell Biol.* **2002**, *156*, 1099–1111. [CrossRef] [PubMed]
82. McCarthy, K.M.; Francis, S.A.; McCormack, J.M.; Lai, J.; Rogers, R.A.; Skare, I.B.; Lynch, R.D.; Schneeberger, E.E. Inducible expression of claudin-1-myc but not occludin-VSV-G results in aberrant tight junction strand formation in MDCK cells. *J. Cell Sci.* **2000**, *113*, 3387–3398. [CrossRef] [PubMed]
83. Pfeiffer, F.; Schafer, J.; Lyck, R.; Makrides, V.; Brunner, S.; Schaeren-Wiemers, N.; Deutsch, U.; Engelhardt, B. Claudin-1 induced sealing of blood-brain barrier tight junctions ameliorates chronic experimental autoimmune encephalomyelitis. *Acta Neuropathol.* **2011**, *122*, 601–614. [CrossRef] [PubMed]
84. Mailly, L.; Baumert, T.F. Hepatitis C virus infection and tight junction proteins: The ties that bind. *Biochim. Biophys. Acta Biomembr.* **2020**, *1862*, 183296. [CrossRef] [PubMed]
85. Milatz, S.; Piontek, J.; Schulzke, J.D.; Blasig, I.E.; Fromm, M.; Gunzel, D. Probing the cis-arrangement of prototype tight junction proteins claudin-1 and claudin-3. *Biochem. J.* **2015**, *468*, 449–458. [CrossRef] [PubMed]
86. Dabrowski, S.; Staat, C.; Zwanziger, D.; Sauer, R.S.; Bellmann, C.; Guenther, R.; Krause, E.; Haseloff, R.F.; Rittner, H.; Blasig, I.E. Redox-Sensitive Structure and Function of the First Extracellular Loop of the Cell-Cell Contact Protein Claudin-1: Lessons from Molecular Structure to Animals. *Antioxid. Redox Signal.* **2015**, *22*, 1–14. [CrossRef]
87. Piontek, J.; Fritzsche, S.; Cording, J.; Richter, S.; Hartwig, J.; Walter, M.; Yu, D.; Turner, J.R.; Gehring, C.; Rahn, H.P.; et al. Elucidating the principles of the molecular organization of heteropolymeric tight junction strands. *Cell. Mol. Life Sci.* **2011**, *68*, 3903–3918. [CrossRef] [PubMed]
88. Rajapakse, H.E.; Gahlaut, N.; Mohandessi, S.; Yu, D.; Turner, J.R.; Miller, L.W. Time-resolved luminescence resonance energy transfer imaging of protein-protein interactions in living cells. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 13582–13587. [CrossRef] [PubMed]
89. Akimoto, T.; Takasawa, A.; Takasawa, K.; Aoyama, T.; Murata, M.; Osanai, M.; Saito, T.; Sawada, N. Estrogen/GPR30 Signaling Contributes to the Malignant Potentials of ER-Negative Cervical Adenocarcinoma via Regulation of Claudin-1 Expression. *Neoplasia* **2018**, *20*, 1083–1093. [CrossRef] [PubMed]
90. Upmanyu, N.; Bulldan, A.; Papadopoulos, D.; Dietze, R.; Malviya, V.N.; Scheiner-Bobis, G. Impairment of the Gn alpha 11-controlled expression of claudin-1 and MMP-9 and collective migration of human breast cancer MCF-7 cells by DHEAS. *J. Steroid Biochem. Mol. Biol.* **2018**, *182*, 50–61. [CrossRef]
91. Saeedi, B.J.; Kao, D.J.; Kitzenberg, D.A.; Dobrinskikh, E.; Schwisow, K.D.; Masterson, J.C.; Kendrick, A.A.; Kelly, C.J.; Bayless, A.J.; Kominsky, D.J.; et al. HIF-dependent regulation of claudin-1 is central to intestinal epithelial tight junction integrity. *Mol. Biol. Cell* **2015**, *26*, 2252–2262. [CrossRef]
92. Ishizaki, T.; Chiba, H.; Kojima, T.; Fujibe, M.; Soma, T.; Miyajima, H.; Nagasawa, K.; Wada, I.; Sawada, N. Cyclic AMP induces phosphorylation of claudin-5 immunoprecipitates and expression of claudin-5 gene in blood-brain-barrier endothelial cells via protein kinase A-dependent and -independent pathways. *Exp. Cell Res.* **2003**, *290*, 275–288. [CrossRef]
93. Ishihara, H.; Kubota, H.; Lindberg, R.L.P.; Leppert, D.; Gloor, S.M.; Errede, M.; Virgintino, D.; Fontana, A.; Yonekawa, Y.; Frei, K. Endothelial cell barrier impairment induced by glioblastomas and transforming growth factor beta(2) involves matrix metalloproteinases and tight junction proteins. *J. Neuropathol. Exp. Neurol.* **2008**, *67*, 435–448. [CrossRef] [PubMed]
94. Wang, J.; Chen, J.Q.; Tang, Z.X.; Li, Y.; Hu, L.M.; Pan, J.Q. The Effects of Copper on Brain Microvascular Endothelial Cells and Claudin Via Apoptosis and Oxidative Stress. *Biol. Trace Elem. Res.* **2016**, *174*, 132–141. [CrossRef] [PubMed]
95. Burek, M.; Konig, A.; Lang, M.; Fiedler, J.; Oerter, S.; Roewer, N.; Bohnert, M.; Thal, S.C.; Blecharz-Lang, K.G.; Woitzik, J.; et al. Hypoxia-Induced MicroRNA-212/132 Alter Blood-Brain Barrier Integrity Through Inhibition of Tight Junction-Associated Proteins in Human and Mouse Brain Microvascular Endothelial Cells. *Transl. Stroke Res.* **2019**, *10*, 672–683. [CrossRef]
96. Velandia-Romero, M.L.; Calderon-Pelaez, M.A.; Castellanos, J.E. In Vitro Infection with Dengue Virus Induces Changes in the Structure and Function of the Mouse Brain Endothelium. *PLoS ONE* **2016**, *11*, e0157786. [CrossRef] [PubMed]
97. Blackmon, A.M.; Como, C.N.; Bubak, A.N.; Mescher, T.; Jones, D.; Nagel, M.A. Varicella Zoster Virus Alters Expression of Cell Adhesion Proteins in Human Perineurial Cells via Interleukin 6. *J. Infect. Dis.* **2019**, *220*, 1453–1461. [CrossRef] [PubMed]
98. Yumine, N.; Matsumoto, Y.; Ohta, K.; Fukasawa, M.; Nishio, M. Claudin-1 inhibits human parainfluenza virus type 2 dissemination. *Virology* **2019**, *531*, 93–99. [CrossRef] [PubMed]
99. Tian, T.; Zi, X.; Peng, Y.; Wang, Z.; Hong, H.; Yan, Y.; Guan, W.; Tan, K.S.; Liu, J.; Ong, H.H.; et al. H3N2 influenza virus infection enhances oncostatin M expression in human nasal epithelium. *Exp. Cell Res.* **2018**, *371*, 322–329. [CrossRef]

100. Kast, J.I.; McFarlane, A.J.; Globinska, A.; Sokolowska, M.; Wawrzyniak, P.; Sanak, M.; Schwarze, J.; Akdis, C.A.; Wanke, K. Respiratory syncytial virus infection influences tight junction integrity. *Clin. Exp. Immunol.* **2017**, *190*, 351–359. [CrossRef]
101. Wachter, B.; Schurger, S.; Schmid, A.; Groger, A.; Sadler, R.; Speidel, A.; Rolinger, J.; Pichler, B.J.; Berg, D.; Wagner, H.J.; et al. 6-Hydroxydopamine leads to T2 hyperintensity, decreased claudin-3 immunoreactivity and altered aquaporin 4 expression in the striatum. *Behav. Brain Res.* **2012**, *232*, 148–158. [CrossRef]
102. Dias, M.C.; Coisne, C.; Lazarevic, I.; Baden, P.; Hata, M.; Iwamoto, N.; Francisco, D.M.F.; Vanlandewijck, M.; He, L.; Baier, F.A.; et al. Publisher Correction: Claudin-3-deficient C57BL/6J mice display intact brain barriers. *Sci. Rep.* **2019**, *9*, 10702. [CrossRef]
103. Hashimoto, K.; Oshima, T.; Tomita, T.; Kim, Y.; Matsumoto, T.; Joh, T.; Miwa, H. Oxidative stress induces gastric epithelial permeability through claudin-3. *Biochem. Biophys. Res. Commun.* **2008**, *376*, 154–157. [CrossRef] [PubMed]
104. Zuo, S.L.; Ge, H.F.; Li, Q.; Zhang, X.; Hu, R.; Hu, S.L.; Liu, X.; Zhang, J.H.; Chen, Y.J.; Feng, H. Artesunate Protected Blood-Brain Barrier via Sphingosine 1 Phosphate Receptor 1/Phosphatidylinositol 3 Kinase Pathway After Subarachnoid Hemorrhage in Rats. *Mol. Neurobiol.* **2017**, *54*, 1213–1228. [CrossRef] [PubMed]
105. Liebner, S.; Corada, M.; Bangsow, T.; Babbage, J.; Taddei, A.; Czupalla, C.J.; Reis, M.; Felici, A.; Wolburg, H.; Fruttiger, M.; et al. Wnt/beta-catenin signaling controls development of the blood-brain barrier. *J. Cell Biol.* **2008**, *183*, 409–417. [CrossRef] [PubMed]
106. Furuse, M.; Sasaki, H.; Tsukita, S. Manner of interaction of heterogeneous claudin species within and between tight junction strands. *J. Cell Biol.* **1999**, *147*, 891–903. [CrossRef] [PubMed]
107. Markov, A.G.; Fedorova, A.A.; Kravtsova, V.V.; Bikmurzina, A.E.; Okorokova, L.S.; Matchkov, V.V.; Cornelius, V.; Amasheh, S.; Krivoi, I.I. Circulating Ouabain Modulates Expression of Claudins in Rat Intestine and Cerebral Blood Vessels. *Int. J. Mol. Sci.* **2020**, *21*, 16. [CrossRef] [PubMed]
108. Ohtsuki, S.; Ikeda, C.; Uchida, Y.; Sakamoto, Y.; Miller, F.; Glacial, F.; Decleves, X.; Scherrmann, J.M.; Couraud, P.O.; Kubo, Y.; et al. Quantitative Targeted Absolute Proteomic Analysis of Transporters, Receptors and Junction Proteins for Validation of Human Cerebral Microvascular Endothelial Cell Line hCMEC/D3 as a Human Blood-Brain Barrier Model. *Mol. Pharm.* **2013**, *10*, 289–296. [CrossRef] [PubMed]
109. Ek, C.J.; Dziegielewska, K.M.; Stolp, H.; Saunders, N.R. Functional effectiveness of the blood brain barrier to small water-soluble molecules in developing and adult opossum (*Monodelphis domestica*). *J. Comp. Neurol.* **2006**, *496*, 13–26. [CrossRef] [PubMed]
110. Luissint, A.C.; Federici, C.; Guillonneau, F.; Chretien, F.; Camoin, L.; Glacial, F.; Ganeshamoorthy, K.; Couraud, P.O. Guanine nucleotide-binding protein G alpha i2: A new partner of claudin-5 that regulates tight junction integrity in human brain endothelial cells. *J. Cereb. Blood Flow Metab.* **2012**, *32*, 860–873. [CrossRef] [PubMed]
111. Mandel, I.; Paperna, T.; Volkowich, A.; Merhav, M.; Glass-Marmor, L.; Miller, A. The Ubiquitin-Proteasome Pathway Regulates Claudin 5 Degradation. *J. Cell. Biochem.* **2012**, *113*, 2415–2423. [CrossRef]
112. Honda, M.; Nakagawa, S.; Hayashi, K.; Kitagawa, N.; Tsutsumi, K.; Nagata, I.; Niwa, M. Adrenomedullin improves the blood-brain barrier function through the expression of claudin-5. *Cell. Mol. Neurobiol.* **2006**, *26*, 109–118. [CrossRef] [PubMed]
113. Wen, H.J.; Watry, D.D.; Marcondes, M.C.G.; Fox, H.S. Selective decrease in paracellular conductance of tight junctions: Role of the first extracellular domain of claudin-5. *Mol. Cell. Biol.* **2004**, *24*, 8408–8417. [CrossRef] [PubMed]
114. Sasson, E.; Anzi, S.; Bell, B.; Yakovian, O.; Zorsky, M.; Deutsch, U.; Engelhardt, B.; Sherman, E.; Vatine, G.; Dzikowski, R.; et al. Nano-scale architecture of blood-brain barrier tight-junctions. *eLife* **2021**, *10*, e63253. [CrossRef] [PubMed]
115. Hashimoto, Y.; Poirier, K.; Boddaert, N.; Hubert, L.; Aubart, M.; Kaminska, A.; Alison, M.; Desguerre, I.; Munnich, A.; Campbell, M. Recurrent de novo mutations in CLDN5 induce an anion-selective blood-brain barrier and alternating hemiplegia. *Brain* **2022**, *145*, 3374–3382. [CrossRef] [PubMed]
116. Krajewski, D.; Paul, D.; Ge, S.; Jellison, E.; Pachter, J.S. Appearance of claudin-5(+) leukocyte subtypes in the blood and CNS during progression of EAE. *J. Neuroinflamm.* **2021**, *18*, 296. [CrossRef] [PubMed]
117. Soma, T.; Chiba, H.; Kato-Mori, Y.; Wada, T.; Yamashita, T.; Kojima, T.; Sawada, N. Thr(207) of claudin-5 is involved in size-selective loosening of the endothelial barrier by cyclic AMP. *Exp. Cell Res.* **2004**, *300*, 202–212. [CrossRef] [PubMed]
118. Watabe, T.; Nishihara, A.; Mishima, K.; Yamashita, J.; Shimizu, K.; Miyazawa, K.; Nishikawa, S.; Miyazono, K. TGF-beta receptor kinase inhibitor enhances growth and integrity of embryonic stem cell-derived endothelial cells. *J. Cell Biol.* **2003**, *163*, 1303–1311. [CrossRef] [PubMed]
119. Taddei, A.; Giampietro, C.; Conti, A.; Orsenigo, F.; Breviario, F.; Pirazzoli, V.; Potente, M.; Daly, C.; Dimmeler, S.; Dejana, E. Endothelial adherens junctions control tight junctions by VE-cadherin-mediated upregulation of claudin-5. *Nat. Cell Biol.* **2008**, *10*, 923–934. [CrossRef] [PubMed]
120. Sadowska, G.B.; Malaeb, S.N.; Stonestreet, B.S. Maternal glucocorticoid exposure alters tight junction protein expression in the brain of fetal sheep. *Am. J. Physiol. Heart Circ. Physiol.* **2010**, *298*, H179–H188. [CrossRef] [PubMed]
121. Felinski, E.A.; Cox, A.E.; Phillips, B.E.; Antonetti, D.A. Glucocorticoids induce transactivation of tight junction genes occludin and claudin-5 in retinal endothelial cells via a novel cis-element. *Exp. Eye Res.* **2008**, *86*, 867–878. [CrossRef]
122. Burek, M.; Arias-Loza, P.A.; Roewer, N.; Forster, C.Y. Claudin-5 as a Novel Estrogen Target in Vascular Endothelium. *Arterioscler. Thromb. Vasc. Biol.* **2010**, *30*, 298–304. [CrossRef] [PubMed]
123. Zhao, Y.L.; Li, W.M.; Song, J.N.; Zhang, M.; Huang, T.Q.; Wei, X. High expression of EphA2 led to secondary injury by destruction of BBB integrity though the ROCK pathway after diffuse axonal injury. *Neurosci. Lett.* **2020**, *736*, 10. [CrossRef] [PubMed]

124. Lee, E.C.; Hong, D.Y.; Lee, D.H.; Park, S.W.; Lee, J.Y.; Jeong, J.H.; Kim, E.Y.; Chung, H.M.; Hong, K.S.; Park, S.P.; et al. Inflammation and Rho-Associated Protein Kinase-Induced Brain Changes in Vascular Dementia. *Biomedicines* **2022**, *10*, 446. [CrossRef] [PubMed]
125. Kakogiannos, N.; Ferrari, L.; Giampietro, C.; Scalise, A.A.; Maderna, C.; Rava, M.; Taddei, A.; Lampugnani, M.G.; Pisati, F.; Malinverno, M.; et al. JAM-A Acts via C/EBP-alpha to Promote Claudin-5 Expression and Enhance Endothelial Barrier Function. *Circ. Res.* **2020**, *127*, 1056–1073. [CrossRef] [PubMed]
126. Bilgic, A.; Ferahkaya, H.; Karagoz, H.; Kilinc, I.; Energin, V.M. Serum claudin-5, claudin-11, occludin, vinculin, paxillin, and beta-catenin levels in preschool children with autism spectrum disorder. *Nord. J. Psychiatry* **2023**, *77*, 506–511. [CrossRef] [PubMed]
127. Kazmierski, R.; Michalak, S.; Wencel-Warot, A.; Nowinski, W.L. Serum tight-junction proteins predict hemorrhagic transformation in ischemic stroke patients. *Neurology* **2012**, *79*, 1677–1685. [CrossRef] [PubMed]
128. Cash, A.; de Jager, C.; Brickler, T.; Soliman, E.; Ladner, L.; Kaloss, A.M.; Zhu, Y.; Pridham, K.J.; Mills, J.; Ju, J.; et al. Endothelial deletion of EPH receptor A4 alters single-cell profile and Tie2/Akap12 signaling to preserve blood-brain barrier integrity. *Proc. Natl. Acad. Sci. USA* **2023**, *120*, e2204700120. [CrossRef] [PubMed]
129. Qiao, N.; An, Z.; Fu, Z.; Chen, X.; Tong, Q.; Zhang, Y.; Ren, H. Kinsenoside alleviates oxidative stress-induced blood-brain barrier dysfunction via promoting Nrf2/HO-1 pathway in ischemic stroke. *Eur. J. Pharmacol.* **2023**, *949*, 175717. [CrossRef] [PubMed]
130. Uchida, Y.; Sumiya, T.; Tachikawa, M.; Yamakawa, T.; Murata, S.; Yagi, Y.; Sato, K.; Stephan, A.; Ito, K.; Ohtsuki, S.; et al. Involvement of Claudin-11 in Disruption of Blood-Brain, -Spinal Cord, and -Arachnoid Barriers in Multiple Sclerosis. *Mol. Neurobiol.* **2019**, *56*, 2039–2056. [CrossRef] [PubMed]
131. Chow, E.; Mottahedeh, J.; Prins, M.; Ridder, W.; Nusinowitz, S.; Bronstein, J.M. Disrupted compaction of CNS myelin in an OSP/claudin-11 and PLP/DM20 double knockout mouse. *Mol. Cell. Neurosci.* **2005**, *29*, 405–413. [CrossRef] [PubMed]
132. Ruan, Z.; Cao, G.; Qian, Y.; Fu, L.; Hu, J.; Xu, T.; Wu, Y.; Lv, Y. Single-cell RNA sequencing unveils Lrg1's role in cerebral ischemia—reperfusion injury by modulating various cells. *J. Neuroinflamm.* **2023**, *20*, 285. [CrossRef] [PubMed]
133. Cording, J.; Gunther, R.; Vigolo, E.; Tscheik, C.; Winkler, L.; Schlattner, I.; Lorenz, D.; Haseloff, R.F.; Schmidt-Ott, K.M.; Wolburg, H.; et al. Redox Regulation of Cell Contacts by Tricellulin and Occludin: Redox-Sensitive Cysteine Sites in Tricellulin Regulate Both Tri- and Bicellular Junctions in Tissue Barriers as Shown in Hypoxia and Ischemia. *Antioxid. Redox Signal.* **2015**, *23*, 1035–1049. [CrossRef] [PubMed]
134. Tezuka, K.; Suzuki, M.; Sato, R.; Kawarada, S.; Terasaki, T.; Uchida, Y. Activation of Annexin A2 signaling at the blood-brain barrier in a mouse model of multiple sclerosis. *J. Neurochem.* **2022**, *160*, 662–674. [CrossRef] [PubMed]
135. Nagano, H.; Ogata, S.; Ito, S.; Masuda, T.; Ohtsuki, S. Knockdown of Podocalyxin Post-Transcriptionally Induces the Expression and Activity of ABCB1/MDR1 in Human Brain Microvascular Endothelial Cells. *J. Pharm. Sci.* **2022**, *111*, 1812–1819. [CrossRef] [PubMed]
136. Ohtsuki, S.; Yamaguchi, H.; Katsukura, Y.; Asashima, T.; Terasaki, T. mRNA expression levels of tight junction protein genes in mouse brain capillary endothelial cells highly purified by magnetic cell sorting. *J. Neurochem.* **2008**, *104*, 147–154. [CrossRef] [PubMed]
137. Castro Dias, M.; Coisne, C.; Baden, P.; Enzmann, G.; Garrett, L.; Becker, L.; Holter, S.M.; German Mouse Clinic, C.; Hrabe de Angelis, M.; Deutsch, U.; et al. Claudin-12 is not required for blood-brain barrier tight junction function. *Fluids Barriers CNS* **2019**, *16*, 30. [CrossRef] [PubMed]
138. Furuse, M.; Nakatsu, D.; Hempstock, W.; Sugioka, S.; Ishizuka, N.; Furuse, K.; Sugawara, T.; Fukazawa, Y.; Hayashi, H. Reconstitution of functional tight junctions with individual claudin subtypes in epithelial cells. *Cell Struct. Funct.* **2023**, *48*, 1–17. [CrossRef] [PubMed]
139. Kanoski, S.E.; Zhang, Y.S.; Zheng, W.; Davidson, T.L. The Effects of a High-Energy Diet on Hippocampal Function and Blood-Brain Barrier Integrity in the Rat. *J. Alzheimers Dis.* **2010**, *21*, 207–219. [CrossRef] [PubMed]
140. Belanger, M.; Asashima, T.; Ohtsuki, S.; Yamaguchi, H.; Ito, S.; Terasaki, T. Hyperammonemia induces transport of taurine and creatine and suppresses claudin-12 gene expression in brain capillary endothelial cells in vitro. *Neurochem. Int.* **2007**, *50*, 95–101. [CrossRef] [PubMed]
141. Salameh, T.S.; Mortell, W.G.; Logsdon, A.F.; Butterfield, D.A.; Banks, W.A. Disruption of the hippocampal and hypothalamic blood-brain barrier in a diet-induced obese model of type II diabetes: Prevention and treatment by the mitochondrial carbonic anhydrase inhibitor, topiramate. *Fluids Barriers CNS* **2019**, *16*, 17. [CrossRef] [PubMed]
142. Hao, N.; Lee, K.L.; Furness, S.G.B.; Bosdotter, C.; Poellinger, L.; Whitelaw, M.L. Xenobiotics and Loss of Cell Adhesion Drive Distinct Transcriptional Outcomes by Aryl Hydrocarbon Receptor Signaling. *Mol. Pharmacol.* **2012**, *82*, 1082–1093. [CrossRef]
143. Matsuoka, H.; Shima, A.; Uda, A.; Ezaki, H.; Michihara, A. The retinoic acid receptor-related orphan receptor alpha positively regulates tight junction protein claudin domain-containing 1 mRNA expression in human brain endothelial cells. *J. Biochem.* **2017**, *161*, 441–450. [PubMed]
144. Shima, A.; Matsuoka, H.; Yamaoka, A.; Michihara, A. Transcription of CLDND1 in human brain endothelial cells is regulated by the myeloid zinc finger 1. *Clin. Exp. Pharmacol. Physiol.* **2021**, *10*, 260–269. [CrossRef] [PubMed]
145. Matsuoka, H.; Tamura, A.; Kinehara, M.; Shima, A.; Uda, A.; Tahara, H.; Michihara, A. Levels of tight junction protein CLDND1 are regulated by microRNA-124 in the cerebellum of stroke-prone spontaneously hypertensive rats. *Biochem. Biophys. Res. Commun.* **2018**, *498*, 817–823. [CrossRef] [PubMed]

146. Abbott, N.J.; Patabendige, A.A.K.; Dolman, D.E.M.; Yusof, S.R.; Begley, D.J. Structure and function of the blood-brain barrier. *Neurobiol. Dis.* **2010**, *37*, 13–25. [CrossRef] [PubMed]
147. Mathiisen, T.M.; Lehre, K.P.; Danbolt, N.C.; Ottersen, O.P. The Perivascular Astroglial Sheath Provides a Complete Covering of the Brain Microvessels: An Electron Microscopic 3D Reconstruction. *Glia* **2010**, *58*, 1094–1103. [CrossRef] [PubMed]
148. Readnower, R.D.; Chavko, M.; Adeeb, S.; Conroy, M.D.; Pauly, J.R.; McCarron, R.M.; Sullivan, P.G. Increase in Blood-Brain Barrier Permeability, Oxidative Stress, and Activated Microglia in a Rat Model of Blast-Induced Traumatic Brain Injury. *J. Neurosci. Res.* **2010**, *88*, 3530–3539. [CrossRef]
149. Zhao, Y.N.; Wang, F.; Fan, Y.X.; Ping, G.F.; Yang, J.Y.; Wu, C.F. Activated microglia are implicated in cognitive deficits, neuronal death, and successful recovery following intermittent ethanol exposure. *Behav. Brain Res.* **2013**, *236*, 270–282. [CrossRef] [PubMed]
150. Abbott, N.J.; Ronnback, L.; Hansson, E. Astrocyte-endothelial interactions at the blood-brain barrier. *Nat. Rev. Neurosci.* **2006**, *7*, 41–53. [CrossRef] [PubMed]
151. Begley, D.J. Understanding and circumventing the blood-brain barrier. *Acta Paediatr.* **2003**, *92*, 83–91. [CrossRef] [PubMed]
152. Cecchelli, R.; Berezowski, V.; Lundquist, S.; Culot, M.; Renftel, M.; Dehouck, M.P.; Fenart, L. Modelling of the blood-brain barrier in drug discovery and development. *Nat. Rev. Drug Discov.* **2007**, *6*, 650–661. [CrossRef] [PubMed]
153. Risau, W.; Wolburg, H. Development of the blood-brain-barrier. *Trends Neurosci.* **1990**, *13*, 174–178. [CrossRef] [PubMed]
154. Wolburg, H.; Lippoldt, A. Tight junctions of the blood-brain barrier: Development, composition and regulation. *Vasc. Pharmacol.* **2002**, *38*, 323–337. [CrossRef] [PubMed]
155. Liebner, S.; Dijkhuizen, R.M.; Reiss, Y.; Plate, K.H.; Agalliu, D.; Constantin, G. Functional morphology of the blood-brain barrier in health and disease. *Acta Neuropathol.* **2018**, *135*, 311–336. [CrossRef] [PubMed]
156. Kimelberg, H.K. Water homeostasis in the brain: Basic concepts. *Neuroscience* **2004**, *129*, 851–860. [CrossRef] [PubMed]
157. Fraser, P.A.; Dallas, A.D.; Davies, S. Measurement of filtration coefficient in single cerebral microvessels of the frog. *J. Physiol.* **1990**, *423*, 343–361. [CrossRef] [PubMed]
158. Butt, A.M.; Jones, H.C.; Abbott, N.J. Electrical resistance across the blood-brain-barrier in anesthetized rats—A developmental-study. *J. Physiol.* **1990**, *429*, 47–62. [CrossRef]
159. Lauschke, K.; Frederiksen, L.; Hall, V.J. Paving the Way toward Complex Blood-Brain Barrier Models Using Pluripotent Stem Cells. *Stem Cells Dev.* **2017**, *26*, 857–874. [CrossRef] [PubMed]
160. Villegas, J.C.; Broadwell, R.D. Transcytosis of protein through the mammalian cerebral epithelium and endothelium. II. Adsorptive transcytosis of WGA-HRP and the blood-brain and brain blood barriers. *J. Neurocytol.* **1993**, *22*, 67–80. [CrossRef]
161. Lochhead, J.J.; Yang, J.Z.; Ronaldson, P.T.; Davis, T.P. Structure, Function, and Regulation of the Blood-Brain Barrier Tight Junction in Central Nervous System Disorders. *Front. Physiol.* **2020**, *11*, 17. [CrossRef]
162. Mark, K.S.; Davis, T.P. Cerebral microvascular changes in permeability and tight junctions induced by hypoxia-reoxygenation. *Am. J. Physiol. Heart Circ. Physiol.* **2002**, *282*, H1485–H1494. [CrossRef] [PubMed]
163. van der Goes, A.; Wouters, D.; van der Pol, S.M.A.; Huizinga, R.; Ronken, E.; Adamson, P.; Greenwood, J.; Dijkstra, C.D.; de Vries, H.E. Reactive oxygen species enhance the migration of monocytes across the blood-brain barrier in vitro. *FASEB J.* **2001**, *15*, 1852–1854. [CrossRef] [PubMed]
164. El-Bacha, R.S.; Minn, A. Drug metabolizing enzymes in cerebrovascular endothelial cells afford a metabolic protection to the brain. *Cell. Mol. Biol.* **1999**, *45*, 15–23. [PubMed]
165. Lipinski, C.A.; Lombardo, F.; Dominy, B.W.; Feeney, P.J. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* **1997**, *23*, 3–25. [CrossRef]
166. Hladky, S.B.; Barrand, M.A. Fluid and ion transfer across the blood-brain and blood-cerebrospinal fluid barriers; a comparative account of mechanisms and roles. *Fluids Barriers CNS* **2016**, *13*, 19. [CrossRef] [PubMed]
167. Tsuji, A. Small molecular drug transfer across the blood-brain barrier via carrier-mediated transport systems. *NeuroRx* **2005**, *2*, 54–62. [CrossRef]
168. Nalecz, K.A. Solute Carriers in the Blood-Brain Barier: Safety in Abundance. *Neurochem. Res.* **2017**, *42*, 795–809. [CrossRef] [PubMed]
169. Morris, M.E.; Rodriguez-Cruz, V.; Felmlee, M.A. SLC and ABC Transporters: Expression, Localization, and Species Differences at the Blood-Brain and the Blood-Cerebrospinal Fluid Barriers. *AAPS J.* **2017**, *19*, 1317–1331. [CrossRef] [PubMed]
170. Gao, P.; Stieger, B.; Noe, B.; Fritschy, J.M.; Meier, P.J. Localization of the organic anion transporting polypeptide 2 (Oatp2) in capillary endothelium and choroid plexus epithelium of rat brain. *J. Histochem. Cytochem.* **1999**, *47*, 1255–1263. [CrossRef] [PubMed]
171. Thompson, B.J.; Sanchez-Covarrubias, L.; Slosky, L.M.; Zhang, Y.F.; Laracuente, M.L.; Ronaldson, P.T. Hypoxia/reoxygenation stress signals an increase in organic anion transporting polypeptide 1a4 (Oatp1a4) at the blood-brain barrier: Relevance to CNS drug delivery. *J. Cereb. Blood Flow Metab.* **2014**, *34*, 699–707. [CrossRef] [PubMed]
172. Hagenbuch, B.; Meier, P.J. The superfamily of organic anion transporting polypeptides. *Biochim. Biophys. Acta Biomembr.* **2003**, *1609*, 1–18. [CrossRef] [PubMed]
173. Prasad, S.; Sajja, R.K.; Naik, P.; Cucullo, L. Diabetes Mellitus and Blood-Brain Barrier Dysfunction: An Overview. *J. Pharmacovigil.* **2014**, *2*, 125. [PubMed]
174. Zhao, Y.H.; Li, D.D.; Zhao, J.J.; Song, J.N.; Zhao, Y.L. The role of the low-density lipoprotein receptor-related protein 1 (LRP-1) in regulating blood-brain barrier integrity. *Rev. Neurosci.* **2016**, *27*, 623–634. [CrossRef] [PubMed]

175. Zhang, Y.; Pardridge, W.M. Rapid transferrin efflux from brain to blood across the blood-brain barrier. *J. Neurochem.* **2001**, *76*, 1597–1600. [CrossRef] [PubMed]
176. Bjorbaek, C.; Elmquist, J.K.; Michl, P.; Ahima, R.S.; van Bueren, A.; McCall, A.L.; Flier, J.S. Expression of leptin receptor isoforms in rat brain microvessels. *Endocrinology* **1998**, *139*, 3485–3491. [CrossRef]
177. Moura, R.P.; Martins, C.; Pinto, S.; Sousa, F.; Sarmento, B. Blood-brain barrier receptors and transporters: An insight on their function and how to exploit them through nanotechnology. *Expert Opin. Drug Deliv.* **2019**, *16*, 271–285. [CrossRef] [PubMed]
178. Wang, Q.; Yang, H.; Miller, D.W.; Elmquist, W.F. Effect of the P-Glycoprotein Inhibitor, Cyclosporine-A, on the Distribution of Rhodamine-123 to the Brain—An in-vivo Microdialysis Study in Freely Moving Rats. *Biochem. Biophys. Res. Commun.* **1995**, *211*, 719–726. [CrossRef] [PubMed]
179. Sparreboom, A.; vanAsperen, J.; Mayer, U.; Schinkel, A.H.; Smit, J.W.; Meijer, D.K.F.; Borst, P.; Nooijen, W.J.; Beijnen, J.H.; vanTellingen, O. Limited oral bioavailability and active epithelial excretion of paclitaxel (Taxol) caused by P-glycoprotein in the intestine. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 2031–2035. [CrossRef] [PubMed]
180. Ohnishi, T.; Tamai, I.; Sakanaka, K.; Sakata, A.; Yamashima, T.; Yamashita, J.; Tsuji, A. In vivo and in vitro evidence for ATP dependency of P-glycoprotein-mediated efflux of doxorubicin at the blood-brain barrier. *Biochem. Pharmacol.* **1995**, *49*, 1541–1544. [CrossRef]
181. Loscher, W.; Potschka, H. Drug resistance in brain diseases and the role of drug efflux transporters. *Nat. Rev. Neurosci.* **2005**, *6*, 591–602. [CrossRef] [PubMed]
182. Brooks, T.A.; Hawkins, B.T.; Huber, J.D.; Egleton, R.D.; Davis, T.P. Chronic inflammatory pain leads to increased blood-brain barrier permeability and tight junction protein alterations. *Am. J. Physiol. Heart Circ. Physiol.* **2005**, *289*, H738–H743. [CrossRef] [PubMed]
183. Morita, K.; Sasaki, H.; Furuse, M.; Tsukita, S. Endothelial claudin: Claudin-5/TMVCF constitutes tight junction strands in endothelial cells. *J. Cell Biol.* **1999**, *147*, 185–194. [CrossRef] [PubMed]
184. Amasheh, S.; Schmidt, T.; Mahn, M.; Florian, P.; Mankertz, J.; Tavalali, S.; Gitter, A.; Schulzke, J.D.; Fromm, M. Contribution of claudin-5 to barrier properties in tight junctions of epithelial cells. *Cell Tissue Res.* **2005**, *321*, 89–96. [CrossRef] [PubMed]
185. Campbell, M.; Kiang, A.S.; Kenna, P.F.; Kerskens, C.; Blau, C.; O'Dwyer, L.; Tivnan, A.; Kelly, J.A.; Brankin, B.; Farrar, G.J.; et al. RNAi-mediated reversible opening of the blood-brain barrier. *J. Gene. Med.* **2008**, *10*, 930–947. [CrossRef] [PubMed]
186. Berselli, A.; Alberini, G.; Benfenati, F.; Maragliano, L. The impact of pathogenic and artificial mutations on Claudin-5 selectivity from molecular dynamics simulations. *Comput. Struct. Biotechnol. J.* **2023**, *21*, 2640–2653. [CrossRef] [PubMed]
187. Rossa, J.; Ploeger, C.; Vorreiter, F.; Saleh, T.; Protze, J.; Gunzel, D.; Wolburg, H.; Krause, G.; Piontek, J. Claudin-3 and Claudin-5 Protein Folding and Assembly into the Tight Junction Are Controlled by Non-conserved Residues in the Transmembrane 3 (TM3) and Extracellular Loop 2 (ECL2) Segments. *J. Biol. Chem.* **2014**, *289*, 7641–7653. [CrossRef] [PubMed]
188. Wolburg, H.; Noell, S.; Mack, A.; Wolburg-Buchholz, K.; Fallier-Becker, P. Brain endothelial cells and the glio-vascular complex. *Cell Tissue Res.* **2009**, *335*, 75–96. [CrossRef]
189. Ramirez, S.H.; Fan, S.S.; Dykstra, H.; Rom, S.; Mercer, A.; Reichenbach, N.L.; Gofman, L.; Persidsky, Y. Inhibition of Glycogen Synthase Kinase 3 beta Promotes Tight Junction Stability in Brain Endothelial Cells by Half-Life Extension of Occludin and Claudin-5. *PLoS ONE* **2013**, *8*, e55972. [CrossRef]
190. Bocsik, A.; Walter, F.R.; Gyebrovszki, A.; Fulop, L.; Blasig, I.; Dabrowski, S.; Otvos, F.; Toth, A.; Rakhely, G.; Veszelka, S.; et al. Reversible Opening of Intercellular Junctions of Intestinal Epithelial and Brain Endothelial Cells With Tight Junction Modulator Peptides. *J. Pharm. Sci.* **2016**, *105*, 754–765. [CrossRef] [PubMed]
191. Wu, J.W.; Yang, Y.S.; Zhang, J.H.; Ji, P.; Du, W.J.; Jiang, P.; Xie, D.H.; Huang, H.D.; Wu, M.; Zhang, G.Z.; et al. Domain-swapped dimerization of the second PDZ domain of ZO2 may provide a structural basis for the polymerization of claudins. *J. Biol. Chem.* **2007**, *282*, 35988–35999. [CrossRef]
192. Torices, S.; Roberts, S.A.; Park, M.; Malhotra, A.; Toborek, M. Occludin, caveolin-1, and Alix form a multi-protein complex and regulate HIV-1 infection of brain pericytes. *Faseb J.* **2020**, *34*, 16319–16332. [CrossRef]
193. Koval, M. Claudin Heterogeneity and Control of Lung Tight Junctions. *Ann. Rev. Physiol.* **2013**, *75*, 551–567.
194. Wollscheid, B.; Bausch-Fluck, D.; Henderson, C.; O'Brien, R.; Bibel, M.; Schiess, R.; Aebersold, R.; Watts, J.D. Mass-spectrometric identification and relative quantification of N-linked cell surface glycoproteins. *Nat. Biotechnol.* **2009**, *27*, 378–386. [CrossRef] [PubMed]
195. Moremen, K.W.; Tiemeyer, M.; Nairn, A.V. Vertebrate protein glycosylation: Diversity, synthesis and function. *Nat. Rev. Mol. Cell Biol.* **2012**, *13*, 448–462. [CrossRef] [PubMed]
196. Wang, X.H.; Matsumoto, H.; Zhao, X.M.; Das, S.K.; Paria, B.C. Embryonic signals direct the formation of tight junctional permeability barrier in the decidualizing stroma during embryo implantation. *J. Cell Sci.* **2004**, *117*, 53–62. [CrossRef] [PubMed]
197. Paganelli, M.; Stephenne, X.; Gilis, A.; Jacquemin, E.; Henrion-Caude, A.; Girard, M.; Gonzales, E.; Revencu, N.; Reding, R.; Wanty, C.; et al. Neonatal Ichthyosis and Sclerosing Cholangitis Syndrome: Extremely Variable Liver Disease Severity from Claudin-1 Deficiency. *J. Pediatr. Gastroenterol. Nutr.* **2011**, *53*, 350–354. [CrossRef] [PubMed]
198. Staat, C.; Coisne, C.; Dabrowski, S.; Stamatovic, S.M.; Andjelkovic, A.V.; Wolburg, H.; Engelhardt, B.; Blasig, I.E. Mode of action of claudin peptidomimetics in the transient opening of cellular tight junction barriers. *Biomaterials* **2015**, *54*, 9–20. [CrossRef] [PubMed]

199. Dithmer, S.; Staat, C.; Muller, C.; Ku, M.C.; Pohlmann, A.; Niendorf, T.; Gehne, N.; Fallier-Becker, P.; Kittel, A.; Walter, F.R.; et al. Claudin peptidomimetics modulate tissue barriers for enhanced drug delivery. *Ann. N. Y. Acad. Sci.* **2017**, *1397*, 169–184. [CrossRef] [PubMed]
200. Tornabene, E.; Helms, H.C.C.; Pedersen, S.F.; Brodin, B. Effects of oxygen-glucose deprivation (OGD) on barrier properties and mRNA transcript levels of selected marker proteins in brain endothelial cells/astrocyte co-cultures. *PLoS ONE* **2019**, *14*, e0221103. [CrossRef] [PubMed]
201. Furuse, M.; Hirase, T.; Itoh, M.; Nagafuchi, A.; Yonemura, S.; Tsukita, S.; Tsukita, S. Occludin—A novel integral membrane-protein localizing at tight junctions. *J. Cell Biol.* **1993**, *123*, 1777–1788. [CrossRef] [PubMed]
202. Wong, V. Phosphorylation of occludin correlates with occludin localization and function at the tight junction. *Am. J. Physiol.* **1997**, *273*, C1859–C1867. [CrossRef] [PubMed]
203. Iwamoto, N.; Higashi, T.; Furuse, M. Localization of Angulin-1/LSR and Tricellulin at Tricellular Contacts of Brain and Retinal Endothelial Cells in vivo. *Cell Struct. Funct.* **2014**, *39*, 1–8. [CrossRef] [PubMed]
204. Bellmann, C.; Schreivogel, S.; Gunther, R.; Dabrowski, S.; Schumann, M.; Wolburg, H.; Blasig, I.E. Highly Conserved Cysteines Are Involved in the Oligomerization of Occludin-Redox Dependency of the Second Extracellular Loop. *Antioxid. Redox Signal.* **2014**, *20*, 855–867. [CrossRef] [PubMed]
205. Blasig, I.E.; Bellmann, C.; Cording, J.; del Vecchio, G.; Zwanziger, D.; Huber, O.; Haseloff, R.F. Occludin Protein Family: Oxidative Stress and Reducing Conditions. *Antioxid. Redox Signal.* **2011**, *15*, 1195–1219. [CrossRef] [PubMed]
206. Bosse, F.; Hasse, B.; Pippirs, U.; Greiner-Petter, R.; Muller, H.W. Proteolipid plasmolipin: Localization in polarized cells, regulated expression and lipid raft association in CNS and PNS myelin. *J. Neurochem.* **2003**, *86*, 508–518. [CrossRef] [PubMed]
207. Balda, M.S.; Whitney, J.A.; Flores, C.; Gonzalez, S.; Cereijido, M.; Matter, K. Functional dissociation of paracellular permeability and transepithelial electrical resistance and disruption of the apical-basolateral intramembrane diffusion barrier by expression of a mutant tight junction membrane protein. *J. Cell Biol.* **1996**, *134*, 1031–1049. [CrossRef] [PubMed]
208. Hirase, T.; Staddon, J.M.; Saitou, M.; AndoAkatsuka, Y.; Itoh, M.; Furuse, M.; Fujimoto, K.; Tsukita, S.; Rubin, L.L. Occludin as a possible determinant of tight junction permeability in endothelial cells. *J. Cell Sci.* **1997**, *110*, 1603–1613. [CrossRef] [PubMed]
209. Saito, A.C.; Higashi, T.; Fukazawa, Y.; Otani, T.; Tauchi, M.; Higashi, A.Y.; Furuse, M.; Chiba, H. Occludin and tricellulin facilitate formation of anastomosing tight-junction strand network to improve barrier function. *Mol. Biol. Cell* **2021**, *32*, 722–738. [CrossRef] [PubMed]
210. Kojima, T.; Ninomiya, T.; Konno, T.; Kohno, T.; Taniguchi, M.; Sawada, N. Expression of tricellulin in epithelial cells and non-epithelial cells. *Histol. Histopath.* **2013**, *28*, 1383–1392.
211. Krug, S.M.; Amasheh, S.; Richter, J.F.; Milatz, S.; Gunzel, D.; Westphal, J.K.; Huber, O.; Schulzke, J.D.; Fromm, M. Tricellulin Forms a Barrier to Macromolecules in Tricellular Tight Junctions without Affecting Ion Permeability. *Mol. Biol. Cell* **2009**, *20*, 3713–3724. [CrossRef] [PubMed]
212. Saitou, M.; Furuse, M.; Sasaki, H.; Schulzke, J.D.; Fromm, M.; Takano, H.; Noda, T.; Tsukita, S. Complex phenotype of mice lacking occludin, a component of tight junction strands. *Mol. Biol. Cell* **2000**, *11*, 4131–4142. [CrossRef] [PubMed]
213. Schulzke, J.D.; Gitter, A.H.; Mankertz, J.; Spiegel, S.; Seidler, U.; Amasheh, S.; Saitou, M.; Tsukita, S.; Fromm, M. Epithelial transport and barrier function in occludin-deficient mice. *Biochim. Biophys. Acta Biomembr.* **2005**, *1669*, 34–42. [CrossRef] [PubMed]
214. Walter, J.K.; Rueckert, C.; Voss, M.; Mueller, S.L.; Piontek, J.; Gast, K.; Blasig, I.E. The oligomerization of the coiled coil-domain of occludin is redox sensitive. *Ann. N. Y. Acad. Sci* **2009**, *1165*, 19–27. [CrossRef] [PubMed]
215. Buschmann, M.M.; Shen, L.; Rajapakse, H.; Raleigh, D.R.; Wang, Y.T.; Wang, Y.M.; Lingaraju, A.; Zha, J.M.; Abbott, E.; McAuley, E.M.; et al. Occludin OCEL-domain interactions are required for maintenance and regulation of the tight junction barrier to macromolecular flux. *Mol. Biol. Cell* **2013**, *24*, 3056–3068. [CrossRef] [PubMed]
216. Müller, S.L.; Portwich, M.; Schmidt, A.; Utepbergenov, D.I.; Huber, O.; Blasig, I.E.; Krause, G. The tight junction protein occludin and the adherens junction protein alpha-catenin share a common interaction mechanism with ZO-1. *J. Biol. Chem.* **2005**, *280*, 3747–3756. [CrossRef] [PubMed]
217. Dorfel, M.J.; Huber, O. A phosphorylation hotspot within the occludin C-terminal domain. In *Barriers and Channels Formed by Tight Junction Proteins I*; Fromm, M., Schulzke, J.D., Eds.; Blackwell Science Publishing: Oxford, UK, 2012; Volume 1257, pp. 38–44.
218. Nusrat, A.; Chen, J.A.; Foley, C.S.; Liang, T.W.; Tom, J.; Cromwell, M.; Quan, C.; Mrsny, R.J. The coiled-coil domain of occludin can act to organize structural and functional elements of the epithelial tight junction. *J. Biol. Chem.* **2000**, *275*, 29816–29822. [CrossRef] [PubMed]
219. Andreeva, A.Y.; Krause, E.; Muller, E.C.; Blasig, I.E.; Utepbergenov, D.I. Protein kinase C regulates the phosphorylation and cellular localization of occludin. *J. Biol. Chem.* **2001**, *276*, 38480–38486. [CrossRef] [PubMed]
220. Reiche, J.; Huber, O. Post-translational modifications of tight junction transmembrane proteins and their direct effect on barrier function. *Biochim. Biophys. Acta Biomembr.* **2020**, *1862*, 183330. [CrossRef] [PubMed]
221. Li, Y.; Liu, C.; Chen, Z.; Lin, H.; Li, X. Netrin-1 protects blood-brain barrier (BBB) integrity after cerebral ischemia-reperfusion by activating the Kruppel-like factor 2 (KLF2)/occludin pathway. *J. Biochem. Mol. Toxicol.* **2024**, *38*, e23623. [CrossRef] [PubMed]
222. Tash, B.R.; Bewley, M.C.; Russo, M.; Keil, J.M.; Griffin, K.A.; Sundstrom, J.M.; Antonetti, D.A.; Tian, F.; Flanagan, J.M. The occludin and ZO-1 complex, defined by small angle X-ray scattering and NMR, has implications for modulating tight junction permeability. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 10855–10860. [CrossRef] [PubMed]

223. Mariano, C.; Palmela, I.; Pereira, P.; Fernandes, A.; Falcao, A.S.; Cardoso, F.L.; Vaz, A.R.; Campos, A.R.; Goncalves-Ferreira, A.; Kim, K.S.; et al. Tricellulin expression in brain endothelial and neural cells. *Cell Tissue Res.* **2013**, *351*, 397–407. [CrossRef]
224. Tachibana, K.; Kondoh, M. A Method to Prepare a Bioprobe for Regulatory Science of the Drug Delivery System to the Brain: An Angulin Binder to Modulate Tricellular Tight Junction-Seal. *Methods Mol. Biol.* **2021**, *2367*, 291–304. [PubMed]
225. Higashi, T.; Tokuda, S.; Kitajiri, S.; Masuda, S.; Nakamura, H.; Oda, Y.; Furuse, M. Analysis of the 'angulin' proteins LSR, ILDR1 and ILDR2-tricellulin recruitment, epithelial barrier function and implication in deafness pathogenesis. *J. Cell Sci.* **2013**, *126*, 966–977. [CrossRef] [PubMed]
226. Furuse, M.; Izumi, Y.; Oda, Y.; Higashi, T.; Iwamoto, N. Molecular organization of tricellular tight junctions. *Tissue Barriers* **2014**, *2*, e28960. [CrossRef] [PubMed]
227. Mesli, S.; Javorschi, S.; Berard, A.M.; Landry, M.; Priddle, H.; Kivlichan, D.; Smith, A.J.; Yen, F.T.; Bihain, B.E.; Darmon, M. Distribution of the lipolysis stimulated receptor in adult and embryonic murine tissues and lethality of LSR−/− embryos at 12.5 to 14.5 days of gestation. *Eur. J. Biochem.* **2004**, *271*, 3103–3114. [CrossRef]
228. Sohet, F.; Lin, C.; Munji, R.N.; Lee, S.Y.; Ruderisch, N.; Soung, A.; Arnold, T.D.; Derugin, N.; Vexler, Z.S.; Yen, F.T.; et al. LSR/angulin-1 is a tricellular tight junction protein involved in blood-brain barrier formation. *J. Cell Biol.* **2015**, *208*, 703–711. [CrossRef]
229. Sugawara, T.; Furuse, K.; Otani, T.; Wakayama, T.; Furuse, M. Angulin-1 seals tricellular contacts independently of tricellulin and claudins. *J. Cell Biol.* **2021**, *220*, e202005062. [CrossRef] [PubMed]
230. Hori, S.; Ohtsuki, S.; Hosoya, K.; Nakashima, E.; Terasaki, T. A pericyte-derived angiopoietin-1 multimeric complex induces occludin gene expression in brain capillary endothelial cells through Tie-2 activation in vitro. *J. Neurochem.* **2004**, *89*, 503–513. [CrossRef] [PubMed]
231. Savettieri, G.; Di Liegro, I.; Catania, C.; Licata, L.; Pitarresi, G.L.; D'Agostino, S.; Schiera, G.; De Caro, V.; Giandalia, G.; Giannola, L.I.; et al. Neurons and ECM regulate occludin localization in brain endothelial cells. *Neuroreport* **2000**, *11*, 1081–1084. [CrossRef] [PubMed]
232. Bendriem, R.M.; Singh, S.; Aleem, A.A.; Antonetti, D.A.; Ross, M.E. Tight junction protein occludin regulates progenitor Self-Renewal and survival in developing cortex. *eLife* **2019**, *8*, 26. [CrossRef] [PubMed]
233. Kuo, W.T.; Shen, L.; Zuo, L.; Shashikanth, N.; Ong, M.; Wu, L.C.; Zha, J.M.; Edelblum, K.L.; Wang, Y.T.; Wang, Y.M.; et al. Inflammation-induced Occludin Downregulation Limits Epithelial Apoptosis by Suppressing Caspase-3 Expression. *Gastroenterology* **2019**, *157*, 1323–1337. [CrossRef] [PubMed]
234. Castro, V.; Bertrand, L.; Luethen, M.; Dabrowski, S.; Lombardi, J.; Morgan, L.; Sharova, N.; Stevenson, M.; Blasig, I.E.; Toborek, M. Occludin controls HIV transcription in brain pericytes via regulation of SIRT-1 activation. *FASEB J.* **2016**, *30*, 1234–1246. [CrossRef] [PubMed]
235. Castro, V.; Skowronska, M.; Lombardi, J.; He, J.; Seth, N.; Velichkovska, M.; Toborek, M. Occludin regulates glucose uptake and ATP production in pericytes by influencing AMP-activated protein kinase activity. *J. Cereb. Blood Flow Metab.* **2018**, *38*, 317–332. [CrossRef] [PubMed]
236. Van Itallie, C.M.; Fanning, A.S.; Holmes, J.; Anderson, J.M. Occludin is required for cytokine-induced regulation of tight junction barriers. *J. Cell Sci.* **2010**, *123*, 2844–2852. [CrossRef] [PubMed]
237. Li, Y.H.; Fanning, A.S.; Anderson, J.M.; Lavie, A. Structure of the conserved cytoplasmic C-terminal domain of occludin: Identification of the ZO-1 binding surface. *J. Mol. Biol.* **2005**, *352*, 151–164. [CrossRef] [PubMed]
238. Yaffe, Y.; Shepshelovitch, J.; Nevo-Yassaf, I.; Yeheskel, A.; Shmerling, H.; Kwiatek, J.M.; Gaus, K.; Pasmanik-Chor, M.; Hirschberg, K. The MARVEL transmembrane motif of occludin mediates oligomerization and targeting to the basolateral surface in epithelia. *J. Cell Sci.* **2012**, *125*, 3545–3556. [CrossRef] [PubMed]
239. Siddiqui, M.R.; Mayanil, C.S.; Kim, K.S.; Tomita, T. Angiopoietin-1 Regulates Brain Endothelial Permeability through PTPN-2 Mediated Tyrosine Dephosphorylation of Occludin. *PLoS ONE* **2015**, *10*, e0130857. [CrossRef] [PubMed]
240. Titchenell, P.M.; Lin, C.M.; Keil, J.M.; Sundstrom, J.M.; Smith, C.D.; Antonetti, D.A. Novel atypical PKC inhibitors prevent vascular endothelial growth factor-induced blood-retinal barrier dysfunction. *Biochem. J.* **2012**, *446*, 455–467. [CrossRef] [PubMed]
241. Fischer, S.; Wiesnet, M.; Marti, H.H.; Renz, D.; Schaper, W. Simultaneous activation of several second messengers in hypoxia-induced hyperpermeability of brain derived endothelial cells. *J. Cell. Physiol.* **2004**, *198*, 359–369. [CrossRef] [PubMed]
242. Chen, F.; Hori, T.; Ohashi, N.; Baine, A.M.; Eckman, C.B.; Nguyen, J.H. Occludin Is Regulated by Epidermal Growth Factor Receptor Activation in Brain Endothelial Cells and Brains of Mice with Acute Liver Failure. *Hepatology* **2011**, *53*, 1294–1305. [CrossRef] [PubMed]
243. Raikwar, N.S.; Vandewalle, A.; Thomas, C.P. Nedd4-2 interacts with occludin to inhibit tight junction formation and enhance paracellular conductance in collecting duct epithelia. *Am. J. Physiol. Renal Physiol.* **2010**, *299*, F436–F444. [CrossRef] [PubMed]
244. Traweger, A.; Fang, D.; Liu, Y.C.; Stelzhammer, W.; Krizbai, I.A.; Fresser, F.; Bauer, H.C.; Bauer, H. The tight junction-specific protein occludin is a functional target of the E3 ubiquitin-protein ligase itch. *J. Biol. Chem.* **2002**, *277*, 10201–10208. [CrossRef] [PubMed]
245. Zhang, G.S.; Tian, Y.; Huang, J.Y.; Tao, R.R.; Liao, M.H.; Lu, Y.M.; Ye, W.F.; Wang, R.; Fukunaga, K.; Lou, Y.J.; et al. The g-Secretase Blocker DAPT Reduces the Permeability of the Blood-Brain Barrier by Decreasing the Ubiquitination and Degradation of Occludin During Permanent Brain Ischemia. *CNS Neurosci. Ther.* **2013**, *19*, 53–60. [CrossRef] [PubMed]

246. Leclair, H.M.; Andre-Gregoire, G.; Treps, L.; Azzi, S.; Bidere, N.; Gavard, J. The E3 ubiquitin ligase MARCH3 controls the endothelial barrier. *FEBS Lett.* **2016**, *590*, 3660–3668. [CrossRef] [PubMed]
247. Kebir, H.; Kreymborg, K.; Ifergan, I.; Dodelet-Devillers, A.; Cayrol, R.; Bernard, M.; Giuliani, F.; Arbour, N.; Becher, B.; Prat, A. Human T(H)17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. *Nat. Med.* **2007**, *13*, 1173–1175. [CrossRef] [PubMed]
248. Lohmann, C.; Krischke, M.; Wegener, J.; Galla, H.J. Tyrosine phosphatase inhibition induces loss of blood-brain barrier integrity by matrix metalloproteinase-dependent and -independent pathways. *Brain Res.* **2004**, *995*, 184–196. [CrossRef] [PubMed]
249. Liu, J.; Jin, X.C.; Liu, K.J.; Liu, W.L. Matrix Metalloproteinase-2-Mediated Occludin Degradation and Caveolin-1-Mediated Claudin-5 Redistribution Contribute to Blood-Brain Barrier Damage in Early Ischemic Stroke Stage. *J. Neurosci.* **2012**, *32*, 3044–3057. [CrossRef] [PubMed]
250. Miyoshi, Y.; Tanabe, S.; Suzuki, T. Cellular zinc is required for intestinal epithelial barrier maintenance via the regulation of claudin-3 and occludin expression. *Am. J. Physiol. Gastroint. Liver Physiol.* **2016**, *311*, G105–G116. [CrossRef] [PubMed]
251. Li, R.R.; Qi, Y.N.; Jiang, M.; Zhang, T.H.; Wang, H.W.; Wang, L.G.; Han, M.Y. Primary tumor-secreted VEGF induces vascular hyperpermeability in premetastatic lung via the occludin phosphorylation/ubiquitination pathway. *Mol. Carcinog.* **2019**, *58*, 2316–2326. [CrossRef] [PubMed]
252. Wang, L.F.; Li, X.; Gao, Y.B.; Wang, S.M.; Zhao, L.; Dong, J.; Yao, B.W.; Xu, X.P.; Chang, G.M.; Zhou, H.M.; et al. Activation of VEGF/Flk-1-ERK Pathway Induced Blood-Brain Barrier Injury after Microwave Exposure. *Mol. Neurobiol.* **2015**, *52*, 478–491. [CrossRef] [PubMed]
253. Muthusamy, A.; Lin, C.M.; Shanmugam, S.; Lindner, H.M.; Abcouwer, S.F.; Antonetti, D.A. Ischemia-reperfusion injury induces occludin phosphorylation/ubiquitination and retinal vascular permeability in a VEGFR-2-dependent manner. *J. Cereb. Blood Flow Metab.* **2014**, *34*, 522–531. [CrossRef] [PubMed]
254. Bauer, A.T.; Burgers, H.F.; Rabie, T.; Marti, H.H. Matrix metalloproteinase-9 mediates hypoxia-induced vascular leakage in the brain via tight junction rearrangement. *J. Cereb. Blood Flow Metab.* **2010**, *30*, 837–848. [CrossRef] [PubMed]
255. Su, P.; Zhao, F.; Cao, Z.P.; Zhang, J.B.; Aschner, M.; Luo, W.J. Mir-203-mediated tricellulin mediates lead-induced in vitro loss of blood-cerebrospinal fluid barrier (BCB) function. *Toxicol. Vitr.* **2015**, *29*, 1185–1194. [CrossRef] [PubMed]
256. Ikenouchi, J.; Furuse, M.; Furuse, K.; Sasaki, H.; Tsukita, S.; Tsukita, S. Tricellulin constitutes a novel barrier at tricellular contacts of epithelial cells. *J. Cell Biol.* **2005**, *171*, 939–945. [CrossRef] [PubMed]
257. Ikenouchi, J.; Sasaki, H.; Tsukita, S.; Furuse, M.; Tsukita, S. Loss of Occludin Affects Tricellular Localization of Tricellulin. *Mol. Biol. Cell* **2008**, *19*, 4687–4693. [CrossRef] [PubMed]
258. Kamitani, T.; Sakaguchi, H.; Tamura, A.; Miyashita, T.; Yamazaki, Y.; Tokumasu, R.; Inamoto, R.; Matsubara, A.; Mori, N.; Hisa, Y.; et al. Deletion of Tricellulin Causes Progressive Hearing Loss Associated with Degeneration of Cochlear Hair Cells. *Sci. Rep.* **2015**, *5*, 12. [CrossRef] [PubMed]
259. Ayala-Torres, C.; Krug, S.M.; Schulzke, J.D.; Rosenthal, R.; Fromm, M. Tricellulin Effect on Paracellular Water Transport. *Int. J. Mol. Sci.* **2019**, *20*, 15. [CrossRef] [PubMed]
260. Cording, J.; Arslan, B.; Staat, C.; Dithmer, S.; Krug, S.M.; Kruger, A.; Berndt, P.; Gunther, R.; Winkler, L.; Blasig, I.E.; et al. Trictide, a tricellulin-derived peptide to overcome cellular barriers. *Ann. N. Y. Acad. Sci.* **2017**, *1405*, 89–101. [CrossRef] [PubMed]
261. Schuetz, A.; Radusheva, V.; Krug, S.M.; Heinemann, U. Crystal structure of the tricellulin C-terminal coiled-coil domain reveals a unique mode of dimerization. *Ann. N. Y. Acad. Sci.* **2017**, *1405*, 147–159. [CrossRef] [PubMed]
262. Oda, Y.; Otani, T.; Ikenouchi, J.; Furuse, M. Tricellulin regulates junctional tension of epithelial cells at tricellular contacts through Cdc42. *J. Cell Sci.* **2014**, *127*, 4201–4212. [PubMed]
263. Sumitomo, T.; Nakata, M.; Higashino, M.; Yamaguchi, M.; Kawabata, S. Group A Streptococcus exploits human plasminogen for bacterial translocation across epithelial barrier via tricellular tight junctions. *Sci. Rep.* **2016**, *6*, 13. [CrossRef] [PubMed]
264. Jennek, S.; Mittag, S.; Reiche, J.; Westphal, J.K.; Seelk, S.; Dorfel, M.J.; Pfirrmann, T.; Friedrich, K.; Schutz, A.; Heinemann, U.; et al. Tricellulin is a target of the ubiquitin ligase Itch. *Ann. N. Y. Acad. Sci.* **2017**, *1397*, 157–168. [CrossRef] [PubMed]
265. Takasawa, A.; Murata, M.; Takasawa, K.; Ono, Y.; Osanai, M.; Tanaka, S.; Nojima, M.; Kono, T.; Hirata, K.; Kojima, T.; et al. Nuclear localization of tricellulin promotes the oncogenic property of pancreatic cancer. *Sci. Rep.* **2016**, *6*, 12. [CrossRef] [PubMed]
266. Morampudi, V.; Graef, F.A.; Stahl, M.; Dalwadi, U.; Conlin, V.S.; Huang, T.; Vallance, B.A.; Yu, H.B.; Jacobson, K. Tricellular Tight Junction Protein Tricellulin Is Targeted by the Enteropathogenic Escherichia coli Effector EspG1, Leading to Epithelial Barrier Disruption. *Infect. Immun.* **2017**, *85*, 20. [CrossRef] [PubMed]
267. Krug, S.M.; Bojarski, C.; Fromm, A.; Lee, I.M.; Dames, P.; Richter, J.F.; Turner, J.R.; Fromm, M.; Schulzke, J.D. Tricellulin is regulated via interleukin-13-receptor alpha 2, affects macromolecule uptake, and is decreased in ulcerative colitis. *Mucosal Immunol.* **2018**, *11*, 345–356. [CrossRef] [PubMed]
268. Markov, A.G.; Vishnevskaya, O.N.; Okorokova, L.S.; Fedorova, A.A.; Kruglova, N.M.; Rybalchenko, O.V.; Aschenbach, J.R.; Amasheh, S. Cholera toxin perturbs the paracellular barrier in the small intestinal epithelium of rats by affecting claudin-2 and tricellulin. *Pflugers Arch.* **2019**, *471*, 1183–1189. [CrossRef] [PubMed]
269. Eum, S.Y.; Jaraki, D.; Bertrand, L.; Andras, I.E.; Toborek, M. Disruption of epithelial barrier by quorum-sensing N-3-(oxododecanoyl)-homoserine lactone is mediated by matrix metalloproteinases. *Am. J. Physiol. Gastroint. Liver Physiol.* **2014**, *306*, G992–G1001. [CrossRef] [PubMed]
270. Janke, S.; Mittag, S.; Reiche, J.; Huber, O. Apoptotic Fragmentation of Tricellulin. *Int. J. Mol. Sci.* **2019**, *20*, 15. [CrossRef] [PubMed]

271. Fiorentino, M.; Sapone, A.; Senger, S.; Camhi, S.S.; Kadzielski, S.M.; Buie, T.M.; Kelly, D.L.; Cascella, N.; Fasano, A. Blood-brain barrier and intestinal epithelial barrier alterations in autism spectrum disorders. *Mol. Autism* **2016**, *7*, 49. [CrossRef]
272. Severson, E.A.; Jiang, L.; Ivanov, A.I.; Mandell, K.J.; Nusrat, A.; Parkos, C.A. Cis-dimerization mediates function of junctional adhesion molecule A. *Mol. Biol. Cell* **2008**, *19*, 1862–1872. [CrossRef] [PubMed]
273. Itoh, M.; Sasaki, H.; Furuse, M.; Ozaki, H.; Kita, T.; Tsukita, S. Junctional adhesion molecule (JAM) binds to PAR-3: A possible mechanism for the recruitment of PAR-3 to tight junctions. *J. Cell Biol.* **2001**, *154*, 491–497. [CrossRef] [PubMed]
274. Bazzoni, G.; Martinez-Estrada, O.M.; Mueller, F.; Nelboeck, P.; Schmid, G.; Bartfai, T.; Dejana, E.; Brockhaus, M. Homophilic interaction of junctional adhesion molecule. *J. Biol. Chem.* **2000**, *275*, 30970–30976. [CrossRef]
275. Lamagna, C.; Meda, P.; Mandicourt, G.; Brown, J.; Gilbert, R.J.C.; Jones, E.Y.; Kiefer, F.; Ruga, P.; Imhof, B.A.; Aurrand-Lions, M. Dual interaction of JAM-C with JAM-B and alpha(M)beta(2) integrin: Function in junctional complexes and leukocyte adhesion. *Mol. Biol. Cell* **2005**, *16*, 4992–5003. [CrossRef] [PubMed]
276. Yeung, D.; Manias, J.L.; Stewart, D.J.; Nag, S. Decreased junctional adhesion molecule-A expression during blood-brain barrier breakdown. *Acta Neuropathol.* **2008**, *115*, 635–642. [CrossRef] [PubMed]
277. Aurrand-Lions, M.; Johnson-Leger, C.; Wong, C.; Du Pasquier, L.; Imhof, B.A. Heterogeneity of endothelial junctions is reflected by differential expression and specific subcellular localization of the three JAM family members. *Blood* **2001**, *98*, 3699–3707. [CrossRef] [PubMed]
278. Jia, W.; Martin, T.A.; Zhang, G.B.; Jiang, W.G. Junctional Adhesion Molecules in Cerebral Endothelial Tight Junction and Brain Metastasis. *Anticancer Res.* **2013**, *33*, 2353–2359. [PubMed]
279. Kummer, D.; Ebnet, K. Junctional Adhesion Molecules (JAMs): The JAM-Integrin Connection. *Cells* **2018**, *7*, 12. [CrossRef] [PubMed]
280. Naik, M.U.; Naik, U.P. Junctional adhesion molecule-A-induced endothelial cell migration on vitronectin is integrin alpha(v)beta(3) specific. *J. Cell Sci.* **2006**, *119*, 490–499. [CrossRef]
281. Ostermann, G.; Weber, K.S.C.; Zernecke, A.; Schroder, A.; Weber, C. JAM-1 is a ligand of the beta(2) integrin LFA-1 involved in transendothelial migration of leukocytes. *Nat. Immunol.* **2002**, *3*, 151–158. [CrossRef] [PubMed]
282. Santoso, S.; Sachs, U.J.H.; Kroll, H.; Linder, M.; Ruf, A.; Preissner, K.T.; Chavakis, T. The junctional adhesion molecule 3 (JAM-3) on human platelets is a counterreceptor for the leukocyte integrin Mac-1. *J. Exp. Med.* **2002**, *196*, 679–691. [CrossRef] [PubMed]
283. Tornavaca, O.; Chia, M.; Dufton, N.; Almagro, L.O.; Conway, D.E.; Randi, A.M.; Schwartz, M.A.; Matter, K.; Balda, M.S. ZO-1 controls endothelial adherens junctions, cell-cell tension, angiogenesis, and barrier formation. *J. Cell Biol.* **2015**, *208*, 821–838. [CrossRef]
284. Bazzoni, G. The JAM family of junctional adhesion molecules. *Curr. Opin. Cell Biol.* **2003**, *15*, 525–530. [CrossRef] [PubMed]
285. Ebnet, K. Junctional adhesion molecules (JAMs): Cell adhesion receptors with pleiotropic functions in cell physiology and development. *Physiol. Rev.* **2017**, *97*, 1529–1554. [CrossRef] [PubMed]
286. Ishida, T.; Kundu, R.K.; Yang, E.; Hirata, K.; Ho, Y.D.; Quertermous, T. Targeted disruption of endothelial cell-selective adhesion molecule inhibits angiogenic processes in vitro and in vivo. *J. Biol. Chem.* **2003**, *278*, 34598–34604. [CrossRef] [PubMed]
287. Wegmann, F.; Petri, B.; Khandoga, A.G.; Moser, C.; Khandoga, A.; Volkery, S.; Li, H.; Nasdala, I.; Brandau, O.; Fassler, R.; et al. ESAM supports neutrophil extravasation, activation of Rho, and VEGF-induced vascular permeability. *J. Exp. Med.* **2006**, *203*, 1671–1677. [CrossRef] [PubMed]
288. Duong, C.N.; Nottebaum, A.F.; Butz, S.; Volkery, S.; Zeuschner, D.; Stehling, M.; Vestweber, D. Interference with ESAM (Endothelial Cell-Selective Adhesion Molecule) Plus Vascular Endothelial-Cadherin Causes Immediate Lethality and Lung-Specific Blood Coagulation. *Arter. Thromb. Vasc. Biol.* **2020**, *40*, 378–393. [CrossRef] [PubMed]
289. Stevenson, B.R.; Siliciano, J.D.; Mooseker, M.S.; Goodenough, D.A. Identification of ZO-1—A high-molecular-weight polypeptide associated with the tight junction (*Zonula occludens*) in a variety of epithelia. *J. Cell Biol.* **1986**, *103*, 755–766. [CrossRef] [PubMed]
290. Anderson, J.M.; Stevenson, B.R.; Jesaitis, L.A.; Goodenough, D.A.; Mooseker, M.S. Characterization of ZO-1, a Protein-Component of the Tight Junction from Mouse Liver and Madin-Darby Canine Kidney Cells. *J. Cell Biol.* **1988**, *106*, 1141–1149. [CrossRef] [PubMed]
291. Jesaitis, L.A.; Goodenough, D.A. Molecular characterization and tissue distribution of ZO-2, a tight junction protein homologous to ZO-1 and the drosophila disks-large tumor-suppressor protein. *J. Cell Biol.* **1994**, *124*, 949–961. [CrossRef] [PubMed]
292. Haskins, J.; Gu, L.J.; Wittchen, E.S.; Hibbard, J.; Stevenson, B.R. ZO-3, a novel member of the MAGUK protein family found at the tight junction, interacts with ZO-1 and occludin. *J. Cell Biol.* **1998**, *141*, 199–208. [CrossRef] [PubMed]
293. Ikenouchi, J.; Umeda, K.; Tsukita, S.; Furuse, M.; Tsukita, S. Requirement of ZO-1 for the formation of belt-like adherens junctions during epithelial cell polarization. *J. Cell Biol.* **2007**, *176*, 779–786. [CrossRef] [PubMed]
294. Giepmans, B.N.G.; Moolenaar, W.H. The gap junction protein connexin43 interacts with the second PDZ domain of the zona occludens-1 protein. *Curr. Biol.* **1998**, *8*, 931–934. [CrossRef] [PubMed]
295. Fanning, A.S.; Anderson, J.M. Zonula Occludens-1 and-2 Are Cytosolic Scaffolds That Regulate the Assembly of Cellular Junctions. In *Molecular Structure and Function of the Tight Junction: From Basic Mechanisms to Clinical Manifestations*; Fromm, M., Schulzke, J.D., Eds.; Wiley Online Library: Hoboken, NJ, USA, 2009; Volume 1165, pp. 113–120.
296. Fanning, A.S.; Lye, M.F.; Anderson, J.M.; Lavie, A. Domain swapping within PDZ2 is responsible for dimerization of ZO proteins. *J. Biol. Chem.* **2007**, *282*, 37710–37716. [CrossRef] [PubMed]

297. Balda, M.S.; Matter, K. The tight junction protein ZO-1 and an interacting transcription factor regulate ErbB-2 expression. *EMBO J.* **2000**, *19*, 2024–2033. [CrossRef] [PubMed]
298. Bal, M.S.; Castro, V.; Piontek, J.; Rueckert, C.; Walter, J.K.; Shymanets, A.; Kurig, B.; Haase, H.; Nurnberg, B.; Blasig, I.E. The hinge region of the scaffolding protein of cell contacts, zonula occludens protein 1, regulates interacting with various signaling proteins. *J. Cell. Biochem.* **2012**, *113*, 934–945. [CrossRef] [PubMed]
299. Ye, X.; Wang, Y.; Cahill, H.; Yu, M.; Badea, T.C.; Smallwood, P.M.; Peachey, N.S.; Nathans, J. Norrin, frizzled-4, and Lrp5 signaling in endothelial cells controls a genetic program for retinal vascularization. *Cell* **2009**, *139*, 285–298. [CrossRef] [PubMed]
300. Fanning, A.S.; Ma, T.Y.; Anderson, J.M. Isolation and functional characterization of the actin-binding region in the tight junction protein ZO-1. *Faseb J.* **2002**, *16*, 1–13. [CrossRef] [PubMed]
301. Gottardi, C.J.; Arpin, M.; Fanning, A.S.; Louvard, D. The junction-associated protein, zonula occludens-1, localizes to the nucleus before the maturation and during the remodeling of cell-cell contacts. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 10779–10784. [CrossRef]
302. Traweger, A.; Fuchs, R.; Krizbai, I.A.; Weiger, T.M.; Bauer, H.C.; Bauer, H. The tight junction protein ZO-2 localizes to the nucleus and interacts with the heterogeneous nuclear ribonucleoprotein scaffold attachment factor-B. *J. Biol. Chem.* **2003**, *278*, 2692–2700. [CrossRef] [PubMed]
303. Umeda, K.; Ikenouchi, J.; Katahira-Tayama, S.; Furuse, K.; Sasaki, H.; Nakayama, M.; Matsui, T.; Tsukita, S.; Furuse, M.; Tsukita, S. ZO-1 and ZO-2 independently determine where claudins are polymerized in tight-junction strand formation. *Cell* **2006**, *126*, 741–754. [CrossRef] [PubMed]
304. Fanning, A.S.; Van Itallie, C.M.; Anderson, J.M. Zonula occludens-1 and-2 regulate apical cell structure and the zonula adherens cytoskeleton in polarized epithelia. *Mol. Biol. Cell* **2012**, *23*, 577–590. [CrossRef] [PubMed]
305. Huxham, J.; Tabaries, S.; Siegel, P.M. Afadin (AF6) in cancer progression: A multidomain scaffold protein with complex and contradictory roles. *Bioessays* **2020**, *17*, e2000221. [CrossRef] [PubMed]
306. Vasileva, E.; Sluysmans, S.; Bochaton-Piallat, M.L.; Citi, S. Cell-specific diversity in the expression and organization of cytoplasmic plaque proteins of apical junctions. *Ann. N. Y. Acad. Sci.* **2017**, *1405*, 160–176. [CrossRef] [PubMed]
307. Zhai, X.; Li, Y.L.; Liang, P.; Li, L.S.; Zhou, Y.D.; Zhang, W.D.; Wang, D.F.; Wei, G.H. PI3K/AKT/Afadin signaling pathway contributes to pathological vascularization in glioblastomas. *Oncol. Lett.* **2018**, *15*, 1893–1899. [CrossRef] [PubMed]
308. Coureuil, M.; Mikaty, G.; Miller, F.; Lecuyer, H.; Bernard, C.; Bourdoulous, S.; Dumenil, G.; Mege, R.M.; Weksler, B.B.; Romero, I.A.; et al. Meningococcal Type IV Pili Recruit the Polarity Complex to Cross the Brain Endothelium. *Science* **2009**, *325*, 83–87. [CrossRef] [PubMed]
309. Worzfeld, T.; Schwaninger, M. Apicobasal polarity of brain endothelial cells. *J. Cereb. Blood Flow Metab.* **2016**, *36*, 340–362. [CrossRef] [PubMed]
310. Cho, C.; Wang, Y.; Smallwood, P.M.; Williams, J.; Nathans, J. Dlg1 activates beta-catenin signaling to regulate retinal angiogenesis and the blood-retina and blood-brain barriers. *eLife* **2019**, *8*, e45542. [CrossRef] [PubMed]
311. Sewduth, R.N.; Kovacic, H.; Jaspard-Vinassa, B.; Jecko, V.; Wavasseur, T.; Fritsch, N.; Pernot, M.; Jeaningros, S.; Roux, E.; Dufourcq, P.; et al. PDZRN3 destabilizes endothelial cell-cell junctions through a PKC zeta-containing polarity complex to increase vascular permeability. *Sci. Signal.* **2017**, *10*, eaag3209. [CrossRef] [PubMed]
312. Chrifi, I.; Hermkens, D.; Brandt, M.M.; van Dijk, C.G.M.; Burgisser, P.E.; Haasdijk, R.; Pei, J.Y.; van de Kamp, E.H.M.; Zhu, C.B.; Blonden, L.; et al. Cgnl1, an endothelial junction complex protein, regulates GTPase mediated angiogenesis. *Cardiovasc. Res.* **2017**, *113*, 1776–1788. [CrossRef] [PubMed]
313. Huber, J.D.; Witt, K.A.; Hom, S.; Egleton, R.D.; Mark, K.S.; Davis, T.P. Inflammatory pain alters blood-brain barrier permeability and tight junctional protein expression. *Am. J. Physiol. Heart Circ. Physiol.* **2001**, *280*, H1241–H1248. [CrossRef] [PubMed]
314. Greene, C.; Kealy, J.; Humphries, M.M.; Gong, Y.; Hou, J.; Hudson, N.; Cassidy, L.M.; Martiniano, R.; Shashi, V.; Hooper, S.R.; et al. Dose-dependent expression of claudin-5 is a modifying factor in schizophrenia. *Mol. Psychiatr.* **2018**, *23*, 2156–2166. [CrossRef] [PubMed]
315. Montagne, A.; Zhao, Z.; Zlokovic, B.V. Alzheimer's disease: A matter of blood-brain barrier dysfunction? *J. Exp. Med.* **2017**, *214*, 3151–3169. [CrossRef] [PubMed]
316. Zlokovic, B.V. Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders. *Nat. Rev. Neurosci.* **2011**, *12*, 723–738. [CrossRef] [PubMed]
317. Rosenberg, G.A. Neurological diseases in relation to the blood-brain barrier. *J. Cereb. Blood Flow Metab.* **2012**, *32*, 1139–1151. [CrossRef] [PubMed]
318. Al-Ahmad, A.J. Human-Induced Pluripotent Stem Cell-Based Model of the Blood-Brain at 10 Years: A Retrospective on Past and Current Disease Models. *Handb. Exp. Pharmacol.* **2023**, *281*, 141–156. [PubMed]
319. Biron, K.E.; Dickstein, D.L.; Gopaul, R.; Jefferies, W.A. Amyloid Triggers Extensive Cerebral Angiogenesis Causing Blood Brain Barrier Permeability and Hypervascularity in Alzheimer's Disease. *PLoS ONE* **2011**, *6*, e23789. [CrossRef] [PubMed]
320. Hartz, A.M.S.; Bauer, B.; Soldner, E.L.B.; Wolf, A.; Boy, S.; Backhaus, R.; Mihaljevic, I.; Bogdahn, U.; Klunemann, H.H.; Schuierer, G.; et al. Amyloid-beta Contributes to Blood-Brain Barrier Leakage in Transgenic Human Amyloid Precursor Protein Mice and in Humans With Cerebral Amyloid Angiopathy. *Stroke* **2012**, *43*, 514–523. [CrossRef] [PubMed]
321. Oikari, L.E.; Pandit, R.; Stewart, R.; Cuni-Lopez, C.; Quek, H.; Sutharsan, R.; Rantanen, L.M.; Oksanen, M.; Lehtonen, S.; de Boer, C.M.; et al. Altered Brain Endothelial Cell Phenotype from a Familial Alzheimer Mutation and Its Potential Implications for Amyloid Clearance and Drug Delivery. *Stem Cell Rep.* **2020**, *14*, 924–939. [CrossRef] [PubMed]

322. Alvarez, J.I.; Cayrol, R.; Prat, A. Disruption of central nervous system barriers in multiple sclerosis. *Biochim. Biophys. Acta Mol. Basis Dis.* **2011**, *1812*, 252–264. [CrossRef] [PubMed]
323. Henkel, J.S.; Beers, D.R.; Wen, S.; Bowser, R.; Appel, S.H. Decreased mRNA expression of tight junction proteins in lumbar spinal cords of patients with ALS. *Neurology* **2009**, *72*, 1614–1616. [CrossRef] [PubMed]
324. Zhong, Z.; Deane, R.; Ali, Z.; Parisi, M.; Shapovalov, Y.; O'Banion, M.K.; Stojanovic, K.; Sagare, A.; Boillee, S.; Cleveland, D.W.; et al. ALS-causing SOD1 mutants generate vascular changes prior to motor neuron degeneration. *Nat. Neurosci.* **2008**, *11*, 420–422. [CrossRef] [PubMed]
325. Gray, M.T.; Woulfe, J.M. Striatal blood-brain barrier permeability in Parkinson's disease. *J. Cereb. Blood Flow Metab.* **2015**, *35*, 747–750. [CrossRef]
326. Wu, X.L.; Wang, P.; Liu, Y.H.; Xue, Y.X. Effects of poly (ADP-ribose) polymerase inhibitor 3-aminobenzamide on blood-brain barrier and dopaminergic neurons of rats with lipopolysaccharide-induced Parkinson's disease. *J. Mol. Neurosci.* **2014**, *53*, 1–9. [CrossRef] [PubMed]
327. Drouin-Ouellet, J.; Sawiak, S.J.; Cisbani, G.; Lagace, M.; Kuan, W.L.; Saint-Pierre, M.; Dury, R.J.; Alata, W.; St-Amour, I.; Mason, S.L.; et al. Cerebrovascular and blood-brain barrier impairments in Huntington's disease: Potential implications for its pathophysiology. *Ann. Neurol.* **2015**, *78*, 160–177. [CrossRef] [PubMed]
328. Linville, R.M.; Nerenberg, R.F.; Grifno, G.; Arevalo, D.; Guo, Z.; Searson, P.C. Brain microvascular endothelial cell dysfunction in an isogenic juvenile iPSC model of Huntington's disease. *Fluids Barriers CNS* **2022**, *19*, 54. [CrossRef] [PubMed]
329. Greene, C.; Hanley, N.; Reschke, C.R.; Reddy, A.; Mae, M.A.; Connolly, R.; Behan, C.; O'Keeffe, E.; Bolger, I.; Hudson, N.; et al. Microvascular stabilization via blood-brain barrier regulation prevents seizure activity. *Nat. Commun.* **2022**, *13*, 2003. [CrossRef] [PubMed]
330. Kealy, J.; Greene, C.; Campbell, M. Blood-brain barrier regulation in psychiatric disorders. *Neurosci. Lett.* **2020**, *726*, 14. [CrossRef]
331. On, N.H.; Mitchell, R.; Savant, S.D.; Bachmeier, C.J.; Hatch, G.M.; Miller, D.W. Examination of blood-brain barrier (BBB) integrity in a mouse brain tumor model. *J. Neuro Oncol.* **2013**, *111*, 133–143. [CrossRef] [PubMed]
332. Wen, L.J.; Tan, Y.A.; Dai, S.H.; Zhu, Y.; Meng, T.T.; Yang, X.Q.; Liu, Y.P.; Liu, X.; Yuan, H.; Hu, F.Q. VEGF-mediated tight junctions pathological fenestration enhances doxorubicin-loaded glycolipid-like nanoparticles traversing BBB for glioblastoma-targeting therapy. *Drug Deliv.* **2017**, *24*, 1843–1855. [CrossRef] [PubMed]
333. Higashida, T.; Kreipke, C.W.; Rafols, J.A.; Peng, C.Y.; Schafer, S.; Schafer, P.; Ding, J.Y.; Dornbos, D.; Li, X.H.; Guthikonda, M.; et al. The role of hypoxia-inducible factor-la, aquaporin-4, and matrix metalloproteinase-9 in blood-brain barrier disruption and brain edema after traumatic brain injury Laboratory investigation. *J. Neurosurg.* **2011**, *114*, 92–101. [CrossRef] [PubMed]
334. Jungner, M.; Siemund, R.; Venturoli, D.; Reinstrup, P.; Schalen, W.; Bentzer, P. Blood-brain barrier permeability following traumatic brain injury. *Minerva Anestesiol.* **2016**, *82*, 525–533. [CrossRef] [PubMed]
335. Campbell, M.; Hanrahan, F.; Gobbo, O.L.; Kelly, M.E.; Kiang, A.S.; Humphries, M.M.; Nguyen, A.T.H.; Ozaki, E.; Keaney, J.; Blau, C.W.; et al. Targeted suppression of claudin-5 decreases cerebral oedema and improves cognitive outcome following traumatic brain injury. *Nat. Commun.* **2012**, *3*, 849. [CrossRef] [PubMed]
336. Huang, J.; Cao, Y.; Chang, S. An inhibitor of claudin-5 interactions, M01, alleviates neuroinflammation and vasogenic edema after blood-spinal cord barrier dysfunction. *NeuroReport* **2023**, *34*, 512–520. [CrossRef] [PubMed]
337. Merali, Z.; Huang, K.; Mikulis, D.; Silver, F.; Kassner, A. Evolution of blood-brain-barrier permeability after acute ischemic stroke. *PLoS ONE* **2017**, *12*, e0171558. [CrossRef] [PubMed]
338. Jiao, H.X.; Wang, Z.H.; Liu, Y.H.; Wang, P.; Xue, Y.X. Specific Role of Tight Junction Proteins Claudin-5, Occludin, and ZO-1 of the Blood-Brain Barrier in a Focal Cerebral Ischemic Insult. *J. Mol. Neurosci.* **2011**, *44*, 130–139. [CrossRef] [PubMed]
339. Ku, J.M.; Taher, M.; Chin, K.Y.; Grace, M.; McIntyre, P.; Miller, A.A. Characterisation of a mouse cerebral microvascular endothelial cell line (bEnd.3) after oxygen glucose deprivation and reoxygenation. *Clin. Exp. Pharmacol. Physiol.* **2016**, *43*, 777–786. [CrossRef] [PubMed]
340. Venkat, P.; Chopp, M.; Chen, J. Blood-Brain Barrier Disruption, Vascular Impairment, and Ischemia/Reperfusion Damage in Diabetic Stroke. *J. Am. Heart Assoc.* **2017**, *6*, e005819. [CrossRef] [PubMed]
341. Yan, Z.; Wang, C.; Meng, Z.; Gan, L.; Guo, R.; Liu, J.; Bond Lau, W.; Xie, D.; Zhao, J.; Lopez, B.L.; et al. C1q/TNF-Related Protein 3 Prevents Diabetic Retinopathy via AMPK-Dependent Stabilization of Blood-Retinal Barrier Tight Junctions. *Cells* **2022**, *11*, 779. [CrossRef] [PubMed]
342. Arba, F.; Leigh, R.; Inzitari, D.; Warach, S.J.; Luby, M.; Lees, K.R.; Collaboration, S.V.I. Blood-brain barrier leakage increases with small vessel disease in acute ischemic stroke. *Neurology* **2017**, *89*, 2143–2150. [CrossRef] [PubMed]
343. Al-Thani, M.; Goodwin-Trotman, M.; Bell, S.; Patel, K.; Fleming, L.K.; Vilain, C.; Abramowicz, M.; Allan, S.M.; Wang, T.; Cader, M.Z.; et al. A novel human iPSC model of COL4A1/A2 small vessel disease unveils a key pathogenic role of matrix metalloproteinases. *Stem Cell Rep.* **2023**, *18*, 2386–2399. [CrossRef] [PubMed]
344. El-Khouly, F.E.; Haumann, R.; Breur, M.; Veldhuijzen van Zanten, S.E.M.; Kaspers, G.J.L.; Hendrikse, N.H.; Hulleman, E.; van Vuurden, D.G.; Bugiani, M. The neurovascular unit in diffuse intrinsic pontine gliomas. *Free Neuropathol.* **2021**, *2*, 2–17. [PubMed]
345. Li, C.; Jiang, Z.; Lu, W.; Arrick, D.; McCarter, K.; Sun, H. Effect of obesity on early blood-brain barrier disruption following transient focal cerebral ischemia. *Obes. Sci. Pract.* **2016**, *2*, 58–68. [CrossRef]

346. Argaw, A.T.; Asp, L.; Zhang, J.Y.; Navrazhina, K.; Pham, T.; Mariani, J.N.; Mahase, S.; Dutta, D.J.; Seto, J.; Kramer, E.G.; et al. Astrocyte-derived VEGF-A drives blood-brain barrier disruption in CNS inflammatory disease. *J. Clin. Investig.* **2012**, *122*, 2454–2468. [CrossRef] [PubMed]
347. Schellenberg, A.E.; Buist, R.; Del Bigio, M.R.; Toft-Hansen, H.; Khorooshi, R.; Owens, T.; Peeling, J. Blood-brain barrier disruption in CCL2 transgenic mice during pertussis toxin-induced brain inflammation. *Fluids Barriers CNS* **2012**, *9*, 10. [CrossRef] [PubMed]
348. Xie, H.; Xue, Y.X.; Liu, L.B.; Liu, Y.H. Endothelial-monocyte-activating polypeptide II increases blood-tumor barrier permeability by down-regulating the expression levels of tight junction associated proteins. *Brain Res.* **2010**, *1319*, 13–20. [CrossRef] [PubMed]
349. Guo, P.; Liu, L.; Yang, X.; Li, M.; Zhao, Q.; Wu, H. Irisin improves BBB dysfunction in SAP rats by inhibiting MMP-9 via the ERK/NF-kappaB signaling pathway. *Cell. Signal.* **2022**, *93*, 110300. [CrossRef]
350. Leda, A.R.; Bertrand, L.; Andras, I.E.; El-Hage, N.; Nair, M.; Toborek, M. Selective Disruption of the Blood-Brain Barrier by Zika Virus. *Front. Microbiol.* **2019**, *10*, 2158. [CrossRef] [PubMed]
351. Bhardwaj, U.; Singh, S.K. Zika Virus NS1 Suppresses VE-Cadherin and Claudin-5 via hsa-miR-101-3p in Human Brain Microvascular Endothelial Cells. *Mol. Neurobiol.* **2021**, *58*, 6290–6303. [CrossRef] [PubMed]
352. Shrestha, B.; Paul, D.; Pachter, J.S. Alterations in tight junction protein and IgG permeability accompany leukocyte extravasation across the choroid plexus during neuroinflammation. *J. Neuropathol. Exp. Neurol.* **2014**, *73*, 1047–1061. [CrossRef] [PubMed]
353. Huggins, M.A.; Johnson, H.L.; Jin, F.; N'Songo, A.; Hanson, L.M.; LaFrance, S.J.; Butler, N.S.; Harty, J.T.; Johnson, A.J. Perforin Expression by CD8 T Cells Is Sufficient To Cause Fatal Brain Edema during Experimental Cerebral Malaria. *Infect. Immun.* **2017**, *85*, e00985-16. [CrossRef] [PubMed]
354. Endres, L.M.; Jungblut, M.; Divyapicigil, M.; Sauer, M.; Stigloher, C.; Christodoulides, M.; Kim, B.J.; Schubert-Unkmeir, A. Development of a multicellular in vitro model of the meningeal blood-CSF barrier to study Neisseria meningitidis infection. *Fluids Barriers CNS* **2022**, *19*, 81. [CrossRef] [PubMed]
355. Greene, C.; Connolly, R.; Brennan, D.; Laffan, A.; O'Keeffe, E.; Zaporojan, L.; O'Callaghan, J.; Thomson, B.; Connolly, E.; Argue, R.; et al. Blood-brain barrier disruption and sustained systemic inflammation in individuals with long COVID-associated cognitive impairment. *Nat. Neurosci.* **2024**, *27*, 421–432. [CrossRef] [PubMed]
356. Amruta, N.; Ismael, S.; Leist, S.R.; Gressett, T.E.; Srivastava, A.; Dinnon, K.H., III; Engler-Chiurazzi, E.B.; Maness, N.J.; Qin, X.; Kolls, J.K.; et al. Mouse Adapted SARS-CoV-2 (MA10) Viral Infection Induces Neuroinflammation in Standard Laboratory Mice. *Viruses* **2022**, *15*, 114. [CrossRef] [PubMed]
357. Mohammadi, M.T.; Dehghani, G.A. Acute hypertension induces brain injury and blood-brain barrier disruption through reduction of claudins mRNA expression in rat. *Pathol. Res. Pract.* **2014**, *210*, 985–990. [CrossRef] [PubMed]
358. Rubio-Araiz, A.; Porcu, F.; Perez-Hernandez, M.; Garcia-Gutierrez, M.S.; Aracil-Fernandez, M.A.; Gutierrez-Lopez, M.D.; Guerri, C.; Manzanares, J.; O'Shea, E.; Colado, M.I. Disruption of blood-brain barrier integrity in postmortem alcoholic brain: Preclinical evidence of TLR4 involvement from a binge-like drinking model. *Addict. Biol.* **2017**, *22*, 1103–1116. [CrossRef] [PubMed]
359. Costea, L.; Meszaros, A.; Bauer, H.; Bauer, H.C.; Traweger, A.; Wilhelm, I.; Farkas, A.E.; Krizbai, I.A. The Blood-Brain Barrier and Its Intercellular Junctions in Age-Related Brain Disorders. *Int. J. Mol. Sci.* **2019**, *20*, 28. [CrossRef] [PubMed]
360. Tachibana, K.; Hirayama, R.; Sato, N.; Hattori, K.; Kato, T.; Takeda, H.; Kondoh, M. Association of Plasma Claudin-5 with Age and Alzheimer Disease. *Int. J. Mol. Sci.* **2024**, *25*, 13. [CrossRef] [PubMed]
361. Ni, C.; Wang, C.; Zhang, J.; Qu, L.; Liu, X.; Lu, Y.; Yang, W.; Deng, J.; Lorenz, D.; Gao, P.; et al. Interferon-gamma safeguards blood-brain barrier during experimental autoimmune encephalomyelitis. *Am. J. Pathol.* **2014**, *184*, 3308–3320. [CrossRef] [PubMed]
362. Nayak, L.; Lee, E.Q.; Wen, P.Y. Epidemiology of Brain Metastases. *Curr. Oncol. Rep.* **2012**, *14*, 48–54. [CrossRef] [PubMed]
363. Michinaga, S.; Koyama, Y. Pathogenesis of Brain Edema and Investigation into Anti-Edema Drugs. *Int. J. Mol. Sci.* **2015**, *16*, 9949–9975. [CrossRef] [PubMed]
364. Sandoval, K.E.; Witt, K.A. Blood-brain barrier tight junction permeability and ischemic stroke. *Neurobiol. Dis.* **2008**, *32*, 200–219. [CrossRef]
365. Tian, Y.; Milic, J.; Monasor, L.S.; Chakraborty, R.; Wang, S.; Yuan, Y.; Asare, Y.; Behrends, C.; Tahirovic, S.; Bernhagen, J. The COP9 signalosome reduces neuroinflammation and attenuates ischemic neuronal stress in organotypic brain slice culture model. *Cell. Mol. Life Sci.* **2023**, *80*, 262. [CrossRef]
366. Cheng, Z.; Wang, L.; Qu, M.; Liang, H.; Li, W.; Li, Y.; Deng, L.; Zhang, Z.; Yang, G.Y. Mesenchymal stem cells attenuate blood-brain barrier leakage after cerebral ischemia in mice. *J. Neuroinflamm.* **2018**, *15*, 135. [CrossRef] [PubMed]
367. Sheng, Y.; Duan, X.; Liu, Y.; Li, F.; Ma, S.; Shang, X.; Wang, X.; Liu, Y.; Xue, R.; Qin, Z. Tie2-expressing monocytes/macrophages promote cerebral revascularization in peri-infarct lesions upon ischemic insult. *Signal Transduct. Target. Ther.* **2021**, *6*, 295. [CrossRef] [PubMed]
368. Easton, A.S.; Fraser, P.A. Variable restriction of albumin diffusion across inflamed cerebral microvessels of the anaesthetized rat. *J. Physiol.* **1994**, *475*, 147–157. [CrossRef] [PubMed]
369. Breitkreuz-Korff, O.; Tscheik, C.; Del Vecchio, G.; Dithmer, S.; Walther, W.; Orthmann, A.; Wolburg, H.; Haseloff, R.F.; Schroder, L.; Blasig, I.E.; et al. M01 as a novel drug enhancer for specifically targeting the blood-brain barrier. *J. Control. Release* **2021**, *338*, 137–148. [CrossRef] [PubMed]

370. Campbell, M.; Humphries, M.M.; Kiang, A.S.; Nguyen, A.T.; Gobbo, O.L.; Tam, L.C.; Suzuki, M.; Hanrahan, F.; Ozaki, E.; Farrar, G.J.; et al. Systemic low-molecular weight drug delivery to pre-selected neuronal regions. *EMBO Mol. Med.* **2011**, *3*, 235–245. [CrossRef] [PubMed]
371. Hashimoto, Y.; Shirakura, K.; Okada, Y.; Takeda, H.; Endo, K.; Tamura, M.; Watari, A.; Sadamura, Y.; Sawasaki, T.; Doi, T.; et al. Claudin-5-Binders Enhance Permeation of Solutes across the Blood-Brain Barrier in a Mammalian Model. *J. Pharmacol. Exp. Ther.* **2017**, *363*, 275–283. [CrossRef] [PubMed]
372. Johnson, P.H.; Quay, S.C. Advances in nasal drug delivery through tight junction technology. *Expert Opin. Drug Deliv.* **2005**, *2*, 281–298. [CrossRef] [PubMed]
373. Herman, R.E.; Makienko, E.G.; Prieve, M.G.; Fuller, M.; Houston, M.E.; Johnson, P.H. Phage display screening of epithelial cell monolayers treated with EGTA: Identification of peptide FDFWITP that modulates tight junction activity. *J. Biomol. Screen* **2007**, *12*, 1092–1101. [CrossRef] [PubMed]
374. Chen, S.C.; Eiting, K.; Cui, K.Y.; Leonard, A.K.; Morris, D.; Li, C.Y.; Farber, K.; Sileno, A.P.; Houston, M.E.; Johnson, P.H.; et al. Therapeutic utility of a novel tight junction modulating peptide for enhancing intranasal drug delivery. *J. Pharm. Sci.* **2006**, *95*, 1364–1371. [CrossRef] [PubMed]
375. Zeniya, S.; Kuwahara, H.; Daizo, K.; Watari, A.; Kondoh, M.; Yoshida-Tanaka, K.; Kaburagi, H.; Asada, K.; Nagata, T.; Nagahama, M.; et al. Angubindin-1 opens the blood-brain barrier in vivo for delivery of antisense oligonucleotide to the central nervous system. *J. Control. Release* **2018**, *283*, 126–134. [CrossRef] [PubMed]