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Regulation of blood-retinal barrier cell-junctions in diabetic retinopathy

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Abstract

Loss of the blood-retinal barrier (BRB) integrity and subsequent damage to the neurovascular unit in the retina are the underlying reasons for diabetic retinopathy (DR). Damage to BRB eventually leads to severe visual impairment in the absence of prompt intervention. Diabetic macular edema and proliferative DR are the advanced stages of the disease where BRB integrity is altered. Primary mechanisms contributing to BRB dysfunction include loss of cell-cell barrier junctions, vascular endothelial growth factor, advanced glycation end products-induced damage, and oxidative stress. Although much is known about the involvement of adherens and tight-junction proteins in the regulation of vascular permeability in various diseases, there is a significant gap in our knowledge on the junctional proteins expressed in the BRB and how BRB function is modulated in the diabetic retina. In this review article, we present our current understanding of the molecular composition of BRB, the changes in the BRB junctional protein turnover in DR, and how BRB functional modulation affects vascular permeability and macular edema in the diabetic retina.

Keywords

Diabetic Retinopathy; Blood-retinal barrier; Macular Edema; Tight-junction; Claudins

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Conflict of interest

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Introduction

Diabetic retinopathy (DR) is one of the most common microvascular complications of diabetes (1). It is the significant cause of new cases of legal blindness among adults aged 20 to 74 years in the United States (2). Estimates by the Center for Disease Control and Prevention demonstrate that VCAM-1: Vascular cell adhesion molecule-1 the prevalence of DR will triple from 2005 to 2050 (3). The vascular dysfunction associated with the DR can contribute to diabetic macular edema (DME), pathological neovascularization, and hemorrhages, contributing to severe vision loss (4). Based on the clinical manifestations, DR has been broadly classified into an early non-proliferative and an advanced (severe) proliferative stage (5). While non-proliferative DR is characterized by retinal vascular dilation, tortuosity, capillary occlusion, and increased permeability of the retinal blood vessels, pathologic pre-retinal neovascularization exhibiting leaky and tuft-like vessels, frequent retinal exudation and hemorrhage is the hallmark of proliferative DR (5). Pathological neovascularization contributes to retinal damage and/or retinal detachment (6).

DR is recognized as a neurovascular disease, and the diabetic retina exhibits both neuronal and vascular abnormalities (7). The underlying mechanisms causing vascular dysfunction include alterations in the blood-retinal barrier (BRB), resulting in the increased vascular permeability and degeneration of retinal capillaries (8). It is well accepted that the breakdown of the BRB triggers the accumulation of fluid in the macula and plays a critical role in the development of DME and vision loss in DR patients (4, 5). As edema worsens, fluid-filled cysts become evident on retinal imaging by optical coherence tomography (9). DME is the major cause of blindness in diabetic patients and can develop at any stage of DR (10). The choice of intraocular treatment modalities for the diabetic eye that includes laser photocoagulation, intra-vitreous injections of steroids, and anti-vascular endothelial growth factor (VEGF) monoclonal antibodies such as Bevacizumab, as well as vitreoretinal surgery for treatment purely depends on clinical prognosis (11). Anti-VEGF monotherapy can be considered as the first-line for proliferative DR, provided there is prudent oversight on disease progression. It serves as a major alternative when pan-retinal photocoagulation is not ideal, due to dense cataract, or non-clearing vitreous hemorrhage (12). Nevertheless, adverse effects, close monitoring, and the unpredictability of long-term outcomes with anti-VEGF treatment remain a major concern.

Although current therapies are effective in improving the vision, a considerable percentage (40%–50%) of eyes with DME respond only partially to anti-VEGF treatment, demanding the need for novel therapies (4). Given the limitations of the current therapies available for DR, our understanding of the mechanisms regulating BRB function in the normal and diabetic retina will pave the way for the identification of novel targets of future therapeutic development. In this review, we will examine our current knowledge on the molecular composition of BRB, and detail the factors involved in the increased vascular permeability in retinal eye diseases, DR in particular. Finally, a possible link between changes in the adherens junction (AJ) and tight-junction (TJ) protein turnover induced by the VEGF and the advanced glycation end products (AGE) and unstable BRB in DR will be explored. Understanding the molecular changes in the BRB and its relationship to vascular

hyperpermeability and pathological angiogenesis in the proliferative DR will provide a foundation for the development of therapies for this leading cause of vision loss.

Modulation of BRB in the regulation of vascular permeability

The retina is made up of vascular cells, glia, and distinct groups of neurons comprised of photoreceptors, bipolar cells, ganglion cells, horizontal cells, and amacrine cells (13). Retinal oxygen demand is quite high, at the same time retinal vasculature is relatively sparse, which contributes to the vulnerability of the retina to vascular disease (14). The high metabolic rate of the neural retina demands the need for a distinct and regulated supply of nutrients mediated via the BRB (15). Further, the BRB regulates ion balance, the permeability of the water and plasma, delivery of amino acids and sugars, and prevents exposing the sensitive neural tissue to circulatory factors such as antibodies and immune cells (16, 17). This strict control of fluids, solutes, and cells that cross the BRB is achieved through two well-developed cell junctions known as the adherens junctions (AJs) and tight junctions (TJs) (18). Contiguous expression of these AJs and TJs along the apical or basal perimeter of cells forms a characteristic sealant ring, intended to restrict access through paracellular routes between the cells (19).

The BRB is made up of two distinct inner and outer barriers (20) (Figure 1A); the inner BRB (iBRB) is composed of the endothelium resting on the basal lamina and is enveloped by the processes of Müller cells (Figure 1B). These, along with pericytes, are considered to influence the activity of retinal endothelial cells and the iBRB by transmitting regulatory signals (20). The iBRB capillaries supply oxygen, glucose, and essential nutrients to the neurons but restrict the flux of other molecules and cells for protection (21). As depicted in Figure 1C, the outer BRB (oBRB), consists of retinal pigment epithelium (RPE) that controls transport between the choriocapillaris and the retina (22). The photoreceptor outer segments in the outer nuclear layer of the retina interact with RPE on its apical side. While the basolateral side of RPE interacts with the choroid, serving as a barrier to the highly perfused and fenestrated choriocapillaris (Figure 1A–C) (23). Though the breakdown of both the inner and outer BRB is implicated in the development of ocular disorders, breakdown of the iBRB especially is a hallmark of many degenerative retinal diseases, including DR, age-related macular degeneration, glaucoma, and cystoid macular edema (24).

Pathological neovascularization associated with vascular hyperpermeability in DR is partly because of the increased levels in growth factors such as VEGF, insulin-like growth factor I (IGFI), and placental growth factor (PIGF) (25). IGF-1 levels, though initially low in untreated diabetes, tend to rise with insulin therapy, increasing serum IGF-1 levels are likely to promote retinal neovascularization by supporting VEGF-driven endothelial cell proliferation (26). PIGF acts in synergism with VEGF under some conditions with significant impact on pathological angiogenesis (27, 28). Primarily, VEGF-A secreted by the retinal neurons and the glia activates the endothelium by binding to VEGFR2 to promote neovascularization (29). Increased VEGF expression has been reported in the post-mortem frozen eye sections of patients with proliferative DR (30) suggesting a potential link between increased VEGF and the pathological vascular permeability in DR (31). VEGF reduced the expression of AJ (*E.g.*, VE-Cadherin, and β -catenin) and TJ proteins (*E.g.* occludin and

claudins), in turn, destabilizing the cell-junctions (32) *via* increased phosphorylation of VE-cadherin, and inhibiting the expression of occludin, and zona occludens-1 (ZO-1) (33, 34).

Molecular mechanisms of BRB-breakdown

Various growth factors and cytokines play a role in induction of BRB breakdown through multiple signaling pathways (35). Molecules contributing to the BRB breakdown include VEGF, hypoxia-inducible factors-1 and -2 (HIF-1 and -2), placental growth factor (PIGF), TNF α , IL-1 β , platelet-derived growth factors A and B (PDGF-A and -B), insulin-like growth factor-1 (IGF-1), and adhesion molecules such as the ICAM-1, VCAM-1, P-selectin, and E-selectin (35–37). VEGF-A, a potent inducer of vascular permeability, binds to both VEGFR1 and VEGFR2 receptors (38). VEGFR1-mediated signaling appears to operate primarily through p38 MAPK, while VEGFR2 signaling may be mediated through the renin-angiotensin system, phosphoinositide3-kinases (PI3K)/Akt, or phospholipase C (PLC) γ (35).

Endothelial nitric oxide synthase activation and the nitric oxide formation also appear to be involved in VEGF-mediated vascular permeability, through the activation of Akt pathway (39). TNF α promotes diabetes-related leukostasis as well as increased ICAM-1 expression through distinct pathways independent of VEGF mechanism (40). IL-1 β stimulate the production of reactive oxygen species and can accelerate apoptosis of retinal capillary endothelial cells through the activation of nuclear factor kappa light-chain enhancer of activated B cells, which is exacerbated in high glucose conditions (41, 42). Hyperglycemia-induced oxidative stress also activates the transcriptional nuclear factor kappa B (NF- κ B), which in turn enhances the expression of pro-inflammatory genes, notably cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), IL-1 β , TNF- α (43).

Apart from these, the kallikrein-kinin system activation in DR has also been reported to contribute to neovascularization and retinal hemorrhage through B1 and B2 receptor activation, in turn, promoting vasodilation, vascular permeability, inflammation, and leukostasis (44, 45). B2 receptor specifically stimulates eNOS and PLA₂ leading to vasodilation by increasing the production of NO and prostacyclin (46). B2 receptor also activates the Src kinases, thereby promoting VE-cadherin phosphorylation, which contributes to the plasma leakage through reversible opening of the endothelial cell junctions (45). Concomitant activation of iNOS by B1 receptor can also cause peroxynitrite formation contributing to oxidative stress and leukostasis in the inflammatory diabetic retina (45, 46).

Modulation of AJ protein turnover in the BRB

AJs have a unique molecular composition enriched with cadherins, catenins, and nectin (47) that connect cells to facilitate tissue formation and morphogenesis in the embryo as well as the maintenance of solid tissues and blood-tissue barrier in the adult organism (48, 49). AJs bind to a continuous belt of actin filaments (adhesion belt) and function to hold the neighboring cells together through the homophilic interaction of a family of Ca²⁺-dependent cell-cell adhesion molecules called cadherins (50). Although cadherins exist in many forms, epithelial E-cadherin and vascular endothelial VE-cadherin are the best-characterized

members in the cadherin family (51, 52). Continued expression of the functionally active E-cadherin is essential for the tight association of the cells in the epithelium (53). Cadherins form complexes with cytoplasmic plaque proteins called catenins (α , β , and γ catenins) to connect to the actin cytoskeleton (54). The catenins are associated with several intracellular molecules, including cytoskeletal proteins and their regulators such as nectin and afadin to form the AJ protein complex (55). β -Catenin is a critical regulatory component of the AJs, and regulation of its tyrosine phosphorylation by the Src family of kinases correlates with diminished cell interaction (56).

In the oBRB, the AJs play a role in the maintenance of the polygonal shape of the RPE cells through the organization of their actin cytoskeleton (57). The expression of E-cadherin by RPE cells has been controversial. In comparison with other epithelial cells, RPE cells have been reported not to express E-cadherin and to express only P-cadherin (58). In contrast, an *in situ* analysis by Burke *et al.*, (59) revealed E-cadherin expression in human and bovine RPE cells. Besides, the study also reported that the post-confluent RPE cell cultures show a high E-cadherin/P-cadherin ratio associated with the decreased apical polarity of Na/K-ATPase. Agreeing to this observation, AJs have been demonstrated in the establishment of cell polarity and adhesion (60). Protein turnover in the AJs composed of cadherins and catenins as well as cytoplasmic protein complexes comprised of crumbs-homologue (CRB) and partitioning defective (PAR) complexes is associated with mild to severe retinal degeneration and visual impairment due to interference in the spatiotemporal aspects of retinogenesis (61, 62). TJs form the apical cell-junction complex with AJs lying just beneath it (63), together provide the mechanical strength to the bond between neighboring cells. A circumferential band of actin filaments underlies the apical junctional complex and applies tension to it in conjunction with associated myosin (64). Early studies suggested that TJs and AJs perform discrete roles of diffusion barrier and adhesion, but as per the current model, the apical junctional complex is an integrated signaling complex that works in conjunction to help regulate cell shape, polarity, and proliferation (65). Gap junctions (GJs) are the third component of the apical junctional complex (66). In most epithelia, GJs are spread along the lateral membrane and are proven to be highly expressed in the crystalline lens as well as the neural retina (67). In contrast to AJs and GJs, TJs form the connections between the lateral membranes of the neighboring cells (68).

Modulation of TJ protein turnover in the BRB

The TJs (occluding junctions) limit diffusion through the paracellular spaces between cells in the epithelium and the endothelium (69) and maintain membrane polarity, cell morphology, and cell growth (70, 71). Though TJs may not be present in most polarized cells, epithelial cells use TJs to anchor elements of their polarization apparatus (72). Consequently, TJs define the boundary between the apical and basolateral membranes (69). The structure of TJs is quite complex, composed of a series of integral and peripheral membrane proteins (73). Typically, each TJ consists of mainly three types of proteins, namely occludin, junctional adhesion molecule, and claudins (74), all of which possess tetraspan transmembrane domains with two extracellular loops, and their N-terminal and C-terminal ends localized in the cytoplasm (75). However, the TJs are reported to consist of over 40 individual support, structural, and transport proteins (76), including some that are

unique to these complexes such as tricellulin, a homolog of occludin that binds tri-cellular TJs (77). The asymmetric distribution of proteins amongst the plasma membrane domains confers to the regulation of membrane polarity by TJs (78). The difference in the composition of proteins in the leading edge of migrating cells or axons compared to the trailing edge or dendrites explains how the asymmetric distribution of proteins is crucial in the maintenance of cell polarity (79). The variation in electrical conductance, charge selectivity, non-charged solute permeability, and size selectivity in some tissues is attributed to a large degree of differences in the density of claudin proteins present in their TJs (80).

Distinct organization and the initial formation of TJs are governed by the peripheral proteins including ZO-1, ZO-2, and ZO-3, which act through multiple protein-protein interaction domains (63). The occludin, junctional adhesion molecule, and claudins extend into the paracellular space, creating the seal, which is characteristic of the TJs in any blood-tissue barriers (69, 81). Occludin was the first TJ protein to be identified and was solely implicated in TJ formation until claudins were discovered in the late 1990s (82). Occludin, a 62–64 kDa transmembrane protein in retinal endothelial cell junctions, has distinct extracellular and intracellular domains (83). Interestingly, targeted disruption of the occludin gene in embryonic stem cells did not prevent the formation of intact TJs (84). Though occludin by itself may not be required for TJ formation, evidence suggests a prominent role for occludin in the organization and stabilization of the TJ complex to coordinate its association with the actin cytoskeleton (85). Later, claudins were identified as the primary strand-forming proteins that join neighboring cells forming the anastomosing network of strands (86). Based on the physiological function, different claudin subsets are thought to be expressed in different species and during embryonic development (87). For example, humans have cation-selective RPE cells, predominantly expressing claudin-19 and claudin-3 (88). A decrease in epithelial transport of chloride ions has been reported to cause retinal degeneration (89). It is challenging to measure the function of retinal cell TJs and BRB *in vivo*. There are several assays reported in the literature, including the diffusion of tracers and dyes injected into the systemic circulation and monitoring the distribution of proteins of the TJ facilitate a brief understanding of their stability in physiology and disease (90). TJ function and cell-cell barrier tightness can also be measured *in vitro* using the electric cell-substrate impedance sensing technology (91, 92).

The role of TJ claudins in the regulation of vascular permeability

Even though the TJs are composed of several proteins, the components determining paracellular selectivity and permeability are members of the claudin family (86). Studies have shown that the 27-gene family of claudins configure the functional backbone of TJ pore and play a very active role in paracellular transport by forming ion-selective barriers and pores (86). Claudins possess cytoplasmic, intracellular NH₂– and COOH– termini and two extracellular loops (93). The ion selectivity property of claudin is determined by the amino acid charge at the first extracellular loop, while the second extracellular loop is involved in claudin-claudin interactions (94). Besides, their interaction with the actin cytoskeleton determines the localization of claudins in TJs. The localization is guided through the interaction between the carboxy-terminus of claudins and the first of three PDZ domains on ZO-1 (95).

Based on their charge and localization, claudins are either 'permeable' or 'non-permeable' to cations or anions (69). They can be categorized into 'sealing' (blocking passage) and pore-forming (facilitating passage) ion-specific paracellular channels (96). The barrier properties of various tissues are determined by the expression pattern of various claudin members, and individual cells express more than one type of claudin (97). The intrinsic ability of claudins to form TJs was observed when their expression in fibroblasts that do not have TJs was found to develop singular TJ strands (98). Claudins can polymerize by cis-interactions into homomeric or heteromeric strands and trans-interaction between claudins of adjacent cells in a homotypic and heterotypic fashion (99). These specific properties of claudins may provide a route to therapeutic manipulation of the TJ structure for the delivery of pharmacological agents.

Claudin mRNA analysis of mouse brain capillary endothelial cells revealed that claudins-1, -3, -5, -8, -10, -12, -15, -17, -19, -20, -22, and -23 are expressed in the blood-brain barrier (BBB) (100). Claudin-5 is the most abundant TJ protein in brain endothelial cells, which is expressed by more than 500-fold compared to the next highly expressed claudin subtype. Additionally, claudins-1, -3, and -12 have shown lower but inducible expression levels of mRNA (101). Intriguingly, a study on postnatal day 18 (P18) mouse retinas demonstrated the mRNA expression of claudin-1 to -5, -7, -9, -10 to -14, -17, -19, -20, -22, and -23 (102). Claudin-1 to -5, -12, -22, and -23 are reported to regulate retinal development shown by their increased expression, especially at P5, coinciding with the formation of the iBRB in retinal capillaries. The expression of these claudins gradually decreases as the mice get into adulthood, except for claudin-2 (21).

In the mouse embryo, retinal angiogenesis is initiated as early as postnatal day 8 (P8) by the formation of the superficial plexus that envelope the entire retinal surface followed by vascular sprouting into the deep and intermediate plexus layers between P7-P12 and P14-P21, respectively (21). The study by Yan Luo *et al.* compared the expression patterns of several claudins in the normal and oxygen-induced retinopathy (OIR) in the mouse retinas (102). The analysis of changes in claudin mRNA levels in the normal mouse embryos indicated that the mRNA levels of claudin-1 to -5, -12, -22, and -23 were developmentally altered by 2-folds in the retinas of mouse pups from P8 until P21 (102). While claudin-22 mRNA was increased throughout this period, others exhibited transient peaks. During P13–P15, mRNA levels of claudin-1, -3, -5, and -12 showed broad peaks with claudin-2 and -23 being high at P5, and the claudin-4 peak was observed during P15-P18 (102). On the other end, claudin-6, -8, -15, and -18 mRNA were undetected in the normal retina. Remarkably, claudin-1 and 5 remained high immunoblots when their mRNA levels decreased at P21, suggesting that cells regulated the steady-state level and intracellular distribution of claudins (102). However, the protein levels of claudin-2 paralleled the decrease in mRNA expression (102). Immunolocalization studies revealed the presence of claudin-1, 2, and 5 in the blood vessels localized in the retinal ganglion cell, and the inner and outer plexiform layers co-localized with occludin in the lateral membranes of retinal vascular endothelial cells (102). On the contrary, claudin-3, -4, -12, and -23 did not localize in the blood vessels but were expressed in distinct layers of the retina. While claudin-3, -4, and -23 were expressed in the retinal ganglion cell layer, claudin-12 was confined to the outer plexiform layer. Additionally, when claudin-4 was also found in outer plexiform layer, claudin-23 was

identified in the inner nuclear layer (102). Although TJs do occur in other cell types, including Schwann cells and neurons, the identities of these extravascular cells in the retina and the TJ proteins expressed in them need to be investigated further (103).

In the rodent OIR model of retinal vasoproliferative disease (104) characterized by pathological angiogenesis and neovascular tufts in the retina (55), both mRNA and protein levels of claudin-2 and -5 were upregulated by 2-folds at P18 compared to normal retinas (102). While claudin-1 expression remained unaffected, occludin exhibited punctate distribution with a similar expression as in normal retina (102). Interestingly, each claudin was mislocalized to the cytosolic compartment or distributed to non-junctional regions of the plasma membrane both in large and small vessels, suggesting a break in TJ strands of each cell, thus contributing to the formation of new vessels (105). With a significant difference, human data suggest that claudin-1 to -5 are expressed on the normal adult human retinal blood vessels (106). Among other claudins, claudin-12 was the most abundantly expressed, followed by claudin-1, -2, -3, -4, -5, and -22. Among others, claudin-6 to -11, -14 to -19, and -23 were not detected at the mRNA level (106).

Claudin-5 deficient mice showed no abnormal development or morphology of blood vessels and no abnormal bleeding or edema in other tissues except for a leaky BBB for smaller molecules with a molecular weight of less than 800 Da (107). Intriguingly, no BRB defects have been reported in claudin-5 deficient mice. However, ongoing studies focusing on the BRB in pathologies that model diabetes and ischemia have identified loss of claudin-5 and increased vascular permeability (108), thus supporting the important role of claudin-5 in BRB. Nevertheless, a study using siRNA targeting claudin-5 for clearance of edema from the optic nerve head by intravitreal injection was proven to be effective (109). While the passage of low molecular weight proteins across the BRB is prevented when claudin-5 is expressed, silencing of claudin-5 expression in the mouse BRB permitted the concurrent administration of low molecular weight therapeutic agents that markedly improved vision (110). Claudin-5 in the retina appears to be more dynamic in function rather than simply structural. Down-regulation of claudin-5 been reported in mice exposed to hypoxic conditions, which justifies the size-selective function claudin-5 in the iBRB, as it restricts the passage of small molecules into the retina, similar to the phenotype seen in claudin-5 deficient mice (111). On the other end, claudin-1 deficient mice die soon after birth caused by skin barrier disruption, however, no BBB or BRB alteration has been reported (112) proving that claudin-1 expression is not necessary for endothelial barrier formation or maintenance. However, some studies suggest that claudin-1 is expressed in the retinal vasculature of the adult mice, and its content was reported to be decreased in DR and experimental autoimmune uveoretinitis (EAU) in rats (113).

The RPE forms the oBRB by separating the neural retina from the fenestrated capillaries in the choroid (114). The barrier function of RPE depends upon TJs within the apical junctional complexes that bind neighboring cells (115). As the RPE functionally develops, permeability decreases, suggesting gradual maturation of the apical junctional complex (116). Latker and Beebe demonstrated that functional TJs are present as early as embryonic day 7 (E7) in the chick (117). In their study, chick embryos were injected with low concentrations of HRP for 5 min and surprisingly that junctions retarded diffusion of HRP

into the subretinal space suggesting their functional development as early as E7. Accumulating evidence suggests the existence of regional variations in the claudin expressions found in RPE TJs (118). Human RPE claudin analysis revealed that claudin-19 was expressed predominantly at both mRNA and protein levels, followed by significant amounts of claudin-3 (105). Notably, patients with a genetic defect in claudin-19 also suffered severe visual impairment (119). Reports on the expression of claudins and occludin in freshly isolated human fetal retinal pigment epithelial cells (hFRPE) suggested that the mRNA for claudin-19 was the most prominent, followed by claudin-3 (25% of claudin-19) and claudin-12 (7% of claudin-19) (88, 120). Additional claudin-mRNAs detected were 85 times less abundant than claudin-19. Surprisingly, the mRNA for claudin-16 was 3000 times less abundant than claudin-19 justifying the robust expression pattern of Claudin-19 in RPE (88). Interestingly, claudin-10b, but not claudin-10a, was expressed *in vivo* and *in vitro* culture of hFRPE pointing out isoform-specific expression in RPE (88). The same study reported a difference in expression of claudin-1, claudin-2, and claudin-10 at different culture conditions, except for claudin-19, which remained unaffected by variation in serum concentrations in the growth medium. Nonetheless, claudin-19 remained the dominant claudin, with mRNA levels 20 times higher than any other claudins (88). While claudin-12 mRNA was 3 times less than claudin-19, the expression of claudin-12 could not be confirmed at the protein level (88). Together, these studies describe the complexity and unique composition of TJs in RPE.

Regulation of TJ protein turnover in the diabetic retina

Recent studies suggest that growth factor-stimulated alterations in TJs contribute to permeability in a variety of disease states (121). Increased expression of VEGF may enhance vascular permeability in DR, leading to vision impairment and blindness (121). It is known that cytokines such as tumor necrosis factor- α and interleukin-1 contribute to retinal endothelial cell dysfunction through induction of oxidative stress and activation of P38 MAPK and NF- κ B signaling pathways (122). Growth factors, such as transforming growth factor- β increase permeability and mediate discontinuous cell border staining of ZO-1 and/or occludin in endothelial and epithelial cells (92, 123, 124). Disruption of the endothelial-barrier contributes to retinal vasculopathy associated with diabetes (125). Long-term hyperglycemia induces microvascular damage in the retina, which leads to non-perfusion and ischemia (4). These changes increase the expression of various hypoxia-driven cytokines and growth factors, which disrupt the BRB and enhance retinal vascular permeability (126).

The cellular localization of occludin is regulated by its phosphorylation, predominantly on its serine and threonine residues (127). Studies indicate that tyrosine phosphorylation of occludin may also play a role in both TJ assembly and disassembly (125). Of particular relevance to DR, activation of the signaling pathways downstream of VEGFR2 leads to elevated serine/threonine phosphorylation of occludin and tyrosine phosphorylation of ZO-1 (128), and chronic stimulation with VEGF results in degradation of occludin (129). Occludin phosphorylation may induce a conformational change in protein or may expose regions of occludin and subsequent modification promoting its internalization by

endocytosis. Internalized occludin may be recycled back to the plasma membrane or may be directed to a degradation pathway (121)

Kinases that could affect vascular permeability include Src family kinases and Akt (protein kinase B) (91, 92, 130–133). In DR, the formation of advanced glycation end products (AGE), as a consequence of hyperglycemia, increases vascular endothelial monolayer permeability and microvascular permeability by modifications in several proteins, including NADPH oxidase, VEGF, matrix metalloproteinases (MMPs), and VE-cadherin, etc. (134). AGE bind specifically to its receptor RAGE to activate Src through a time- and dose-dependent phosphorylation through tyrosine kinase (135). Studies have shown that Src contributed to AGE-induced phosphorylation of VE-cadherin and catenins, and AJ disassembly (136). Src may be an appropriate target for the prevention and therapy of AGEs associated microvasculopathy (135). In support of this, a recent study showed that the systemic and topical application of Src inhibitors suppressed the breakdown of the BRB and vascular leakage in the laser-injured ischemic retinal tissues (137) (Figure 2).

A-kinase anchor protein 12 (AKAP12) has been reported to mediate inner BRB maturation by downregulating HIF-1 α protein level, which in turn, leads to an increase in the secretion of Angiopoietin-1 (Ang-1) and a decrease in the secretion of VEGF in human astrocytes (138). Conditioned media from AKAP 12-overexpressing astrocytes induced barrierogenesis by upregulating the expression of TJ proteins such as claudin-1, occludin and ZO-2 in human retinal microvascular endothelial cells. Defects in this mechanism can lead to a loss of TJ proteins and contribute to the development of retinal pathologies such as retinopathy (138).

Akt1 enhances endothelial-barrier integrity by stabilizing TJ through claudin expression regulation and Akt1 deficiency resulted in a 2 to 50-fold reduction in the mRNA levels of several TJ claudins (91). VEGF induces the phosphorylation and inactivation of the forkhead box O (FoxO) transcription factors via a PI3K/Akt-dependent mechanism, in turn, regulating apoptosis, proliferation, and the control of oxidative stress (139). FoxOs are negatively regulated by the PI3K/Akt pathway, which induces phosphorylation and nuclear exclusion of FoxO, thereby inhibiting FoxO transcriptional activity (140). In conditions such as oxidative stress FoxO transcriptional activity is enhanced by its direct binding to β -catenin (141). The PI3K-Akt pathway has also been shown to phosphorylate and negatively regulate glycogen synthase kinase (GSK3 β) activity. Dephosphorylated GSK3 β has increased kinase activity and can target β -catenin for degradation by increased serine phosphorylation (142). However, the significance of the Akt-FoxO pathway regulated TJ protein expression modulation in DR has not yet been investigated (Figure 2).

Mitogen-activated protein (MAP) kinases are serine/threonine-specific protein kinases that play an important role in cell proliferation, differentiation, and survival (143). p38MAPK and extracellular signal-regulated protein kinase are two important MAP kinase subtypes that are reported to be activated in endothelial cells treated with VEGF (143). Activation of p38MAPK has also been observed in endothelial cells maintained in high glucose *in-vitro* (144) and the diabetic retinas (145). Inhibition of p38 was proven to hinder the generation of iNOS and nitrotyrosine and partly inhibit the death of retinal pigment epithelial cells

cultured in high glucose condition (146). Treatment with p38MAPK inhibitor has been shown to block the vascular permeability increase in experimental diabetes (147). MAP kinase inhibitors, in general, preserved the endothelial-barrier function, suppressed uPAR expression, blocked β -catenin transcriptional activation, and inhibited phosphorylation/deactivation of GSIG- β (148). Additionally, treatment with a p38 MAPK inhibitor was proven to suppress the increase in production or accumulation of superoxide in the retina of streptozotocin-induced diabetic rats, possibly through direct effects on NADPH oxidase or transcriptional regulation of inflammatory proteins and cytokines (149). Studies show that phosphorylation of p38 and ERK1 is increased upon insulin stimulation in retina of ob/ob or STZ mice emphasizing contribution of insulin in altered retinal vascular function in diabetes (145).

Increased expression of MMPs is mediated through NF- κ B upregulation in retinal microvascular endothelium (150). The proteolytic degradation of TJ proteins in response to hyperglycemia and increased VEGF levels may be facilitated by the elevated expression of specific extracellular proteinases. Studies have shown that MMP-2, MMP-9, and MMP-14 are upregulated in the retina of diabetic animals and certainly contribute to BRB permeability changes (83). Although MMP3 has been linked to TJ breakdown in the BBB (151) and blood-air barriers (133, 152), its importance in BRB has not yet been investigated. Apart from this limited information available in the literature, the precise role of claudins, a family of 27 members (153), and several MMPs, in the physiology and pathology of BRB in vision disorders is yet to be identified.

The role of protein kinase-C (PKC) in TJ protein turnover

VEGF-induced BRB breakdown in DR has been linked to the activation of the protein kinase-C (PKC) pathway (154), a serine/threonine-kinase that exists in 3 subfamilies based on their mechanisms of activation (155) (Figure 3). Although VEGF-induced PKC activation results in the increased phosphorylation of occludin leading to its internalization and BRB breakdown, it is unclear whether PKC directly phosphorylates occludin or it mediates this *via* secondary signaling pathways (154). Experiments *in vitro*, however, demonstrated that the C-terminal region of mouse occludin was phosphorylated by a purified mixture of the PKC isoforms α , β I, β II, and γ suggesting such a possibility also *in vivo* (156). A study using the “Ca²⁺ switch” procedure on epithelial cells revealed that cells placed in low Ca²⁺ medium lose their intercellular contacts (157) and apical-basolateral polarity (158). Functional TJs were restored and cell polarity was developed within a few hours once the cell monolayers were switched back to normal Ca²⁺ medium (157).

Isozymes of PKC (PKC- β , PKC- δ , and PKC- ζ) are activated by hyperglycemia-induced increased DAG, through the AGE/RAGE pathways, and/or oxidative stress in DR (159, 160). Hyperglycemia primarily activates PKC- β to induce VEGF expression (161). PKC- β has also been implicated in damaging the retinal capillaries by activating NAD(P)H-oxidase (NOX), in turn, producing O₂^{•-} that interact with NO to form peroxynitrite resulting in eNOS uncoupling and endothelial dysfunction (162, 163). The stimulation of PKC- δ signaling by high glucose has been reported to promote loss of pericyte coverage in retinal capillaries (164) by activating the p38MAPK-Scr homology-2 domain that contains

phosphatase-1 (SHP-1) pathway (165). The pericytic death altered retinal microvessel integrity, leading to microaneurysms and neovascularization (166). PKC-Chas been implicated in the hyperpermeability induced by TNF- α and thrombin (167, 168) (Figure 3).

Activation of PKC triggers the translocation of TJ proteins ZO-1 and ZO-2 to the cell borders (169). PKC isozymes have also been demonstrated to regulate the cellular translocation of several TJ proteins such as ZO-1, ZO-2, claudin-5, and occludin in epithelial cells (170). There are also reports on the differential regulation of TJs by PKC isoforms. Whereas cPKC participates in TJ disassembly, the novel isoforms (nPKC; isoforms PKC δ , PKC ϵ , PKC η , and PKC θ) promote TJ formation (155) (Figure 3). Studies have revealed that the conventional and novel PKC inhibitors like staurosporine, Gö6850, and CGP41251, as well as the pan-PKC inhibitors GF-109203X and H7, attenuate the opening of TJs (171). Although the initial clinical trials revealed potential benefits of PKC β inhibitor, ruboxistaurin, in the treatment of DR (172), more studies will be necessary on the mechanisms linking PKC isozymes and the cell-junction modulation in the diabetic retina as the global benefit of ruboxistaurin was minimum.

Conclusions and future directions

Several studies have discussed a wide range of mechanisms contributing to vascular permeability changes in DR and ways to target them. Additionally, it is important to be aware of the changes in the AJ and TJ on proteins that have been reported to be seen in a wide variety of retinal diseases associated with the loss of the BRB. Alteration in the content of junctional proteins and also their redistribution and phosphorylation can contribute to the difference in permeability patterns. Our understanding of the complexity of the TJ complex in the BRB is quite naive. The relative contribution of the various junctional proteins to BRB properties and the changes in permeability in disease states are critical areas for future studies. Future studies may provide insight as to whether these TJ proteins serve to be the potential molecule to target vascular permeability changes contributing to DR.

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ABBREVIATIONS

DR	Diabetic retinopathy
BRB	Blood-retinal barrier
BBB	Blood-brain barrier
VEGF	Vascular endothelial growth factor
PLCγ	Phospholipase-C γ
HIF1	Hypoxia-Inducible Factor

COX-2	Cyclooxygenase-2
PlGF	Placental growth factorIGFI: Insulin-like growth factor I
bFGF	Basic fibroblast growth factor
PDGF	Platelet derived growth factor
TNFα	Tumor necrosis factor- α
IL-1β	Inteleukin-1 β
RPE	Retinal pigment epithelium
DME	diabetic macular edema
AJ	Adherens junction
TJ	Tight junction
GJ	Gap junction
ZO	Zona occludens
OIR	Oxygen-induced retinopathy
hFRPE	human fetal retinal pigment epithelium cells
TER	Trans-epithelial resistance
AGE	Advanced glycation end products
RAGE	Receptor for advanced glycation end products
MMP	Matrix metalloproteinases
GSK3β	Glycogen synthase kinase-3 β
FoxO	Forkhead box O
NOS	Nitric oxide synthase
ICAM-1	Inter-cellular adhesion molecule-1
VCAM-1	Vascular cell adhesion molecule-1

References

1. Antonetti DA, Klein R, Gardner TW. Diabetic Retinopathy. New England Journal of Medicine. 2012;366(13):1227–39.
2. Group* TEDPR. The Prevalence of Diabetic Retinopathy Among Adults in the United States. Archives of Ophthalmology. 2004;122(4):552–63. [PubMed: 15078674]
3. Shah AR, Gardner TW. Diabetic retinopathy: research to clinical practice. Clinical Diabetes and Endocrinology. 2017;3(1):9. [PubMed: 29075511]
4. Duh EJ, Sun JK, Stitt AW. Diabetic retinopathy: current understanding, mechanisms, and treatment strategies. JCI Insight. 2017;2(14):e93751.

5. Wang W, Lo ACY. Diabetic Retinopathy: Pathophysiology and Treatments. *Int J Mol Sci*. 2018;19(6):1816.
6. Al-Latayfeh M, Silva PS, Sun JK, Aiello LP. Antiangiogenic therapy for ischemic retinopathies. *Cold Spring Harb Perspect Med*. 2012;2(6):a006411-a. [PubMed: 22675660]
7. Rossino MG, Dal Monte M, Casini G. Relationships Between Neurodegeneration and Vascular Damage in Diabetic Retinopathy. *Frontiers in Neuroscience*. 2019;13(1172).
8. Shin ES, Sorenson CM, Sheibani N. Diabetes and retinal vascular dysfunction. *J Ophthalmic Vis Res*. 2014;9(3):362–73. [PubMed: 25667739]
9. Trichonas G, Kaiser PK. Optical coherence tomography imaging of macular oedema. *Br J Ophthalmol*. 2014;98 Suppl 2(Suppl 2):ii24–ii9. [PubMed: 24934220]
10. Das A Diabetic Retinopathy: Battling the Global Epidemic. *Invest Ophthalmol Vis Sci*. 2016;57(15):6669–82. [PubMed: 27936469]
11. Mansour SE, Browning DJ, Wong K, Flynn HW Jr., Bhavsar AR. The Evolving Treatment of Diabetic Retinopathy. *Clin Ophthalmol*. 2020;14:653–78. [PubMed: 32184554]
12. Zhao Y, Singh RP. The role of anti-vascular endothelial growth factor (anti-VEGF) in the management of proliferative diabetic retinopathy. *Drugs Context*. 2018;7:212532-. [PubMed: 30181760]
13. Gupta MP, Herzlich AA, Sauer T, Chan CC. Retinal Anatomy and Pathology.
14. Yu D-Y, Cringle SJ. Oxygen Distribution and Consumption within the Retina in Vascularised and Avascular Retinas and in Animal Models of Retinal Disease. *Progress in Retinal and Eye Research*. 2001;20(2):175–208. [PubMed: 11173251]
15. Runkle E, Antonetti D. The Blood-Retinal Barrier: Structure and Functional Significance. *Methods in molecular biology* (Clifton, NJ). 2011;686:133–48.
16. Antonetti DA, Barber AJ, Bronson SK, Freeman WM, Gardner TW, Jefferson LS, et al. Diabetic Retinopathy. *Diabetes*. 2006;55(9):2401. [PubMed: 16936187]
17. Cunha-Vaz J, Bernardes R. Blood-retinal barrier. *Eur J Ophthalmol*. 2011;21:S3–S9. [PubMed: 23264323]
18. Campbell M, Humphries P. The blood-retina barrier: Tight junctions and barrier modulation. *Advances in experimental medicine and biology*. 2012;763:70–84. [PubMed: 23397619]
19. Peng S, Rahner C, Rizzolo LJ. Apical and Basal Regulation of the Permeability of the Retinal Pigment Epithelium. *Investigative Ophthalmology & Visual Science*. 2003;44(2):808–17. [PubMed: 12556417]
20. Hosoya K-i, Tachikawa M. The inner blood-retinal barrier: molecular structure and transport biology. *Advances in experimental medicine and biology*. 2012;763:85–104. [PubMed: 23397620]
21. Díaz-Coránguez M, Ramos C, Antonetti DA. The inner blood-retinal barrier: Cellular basis and development. *Vision Res*. 2017;139:123–37. [PubMed: 28619516]
22. Strauss O The Retinal Pigment Epithelium in Visual Function. *Physiological Reviews*. 2005;85(3):845–81. [PubMed: 15987797]
23. Marneros AG, Fan J, Yokoyama Y, Gerber HP, Ferrara N, Crouch RK, et al. Vascular endothelial growth factor expression in the retinal pigment epithelium is essential for choriocapillaris development and visual function. *Am J Pathol*. 2005;167(5):1451–9. [PubMed: 16251428]
24. Cunha-Vaz J, Bernardes R, Lobo C. Blood-Retinal Barrier. *European Journal of Ophthalmology*. 2010;21(6_suppl):3–9.
25. Antonetti D, Lieth E, Barber A, Gardner T. Molecular Mechanisms of Vascular Permeability in Diabetic Retinopathy. *Seminars in ophthalmology*. 2000;14:240–8.
26. Smith LE, Shen W, Perruzzi C, Soker S, Kinose F, Xu X, et al. Regulation of vascular endothelial growth factor-dependent retinal neovascularization by insulin-like growth factor-1 receptor. *Nat Med*. 1999;5(12):1390–5. [PubMed: 10581081]
27. Carmeliet P, Moons L, Luttun A, Vincenti V, Compernelle V, De Mol M, et al. Synergism between vascular endothelial growth factor and placental growth factor contributes to angiogenesis and plasma extravasation in pathological conditions. *Nat Med*. 2001;7(5):575–83. [PubMed: 11329059]

28. Sheibani N Placental growth factor inhibition for choroidal neovascularization. *J Ophthalmic Vis Res.* 2013;8(1):1–3. [PubMed: 23825705]
29. Penn JS, Madan A, Caldwell RB, Bartoli M, Caldwell RW, Hartnett ME. Vascular endothelial growth factor in eye disease. *Progress in retinal and eye research.* 2008;27(4):331–71. [PubMed: 18653375]
30. Luty GA, McLeod DS, Merges C, Diggs A, Plouët J. Localization of Vascular Endothelial Growth Factor in Human Retina and Choroid. *Archives of Ophthalmology.* 1996;114(8):971–7. [PubMed: 8694733]
31. Feeney SA, Simpson DAC, Gardiner TA, Boyle C, Jamison P, Stitt AW. Role of Vascular Endothelial Growth Factor and Placental Growth Factors During Retinal Vascular Development and Hyaloid Regression. *Invest Ophthalmol Vis Sci.* 2003;44(2):839–47. [PubMed: 12556420]
32. Antonetti D, Barber A, Hollinger LA, Wolpert E, Gardner T. Vascular endothelial growth factor induces rapid phosphorylation of tight junction proteins occludin and zonula occluden 1. A potential mechanism for vascular permeability in diabetic retinopathy and tumors. *The Journal of biological chemistry.* 1999;274:23463–7. [PubMed: 10438525]
33. Witmer AN, Vrensen GFJM, Van Noorden CJF, Schlingemann RO. Vascular endothelial growth factors and angiogenesis in eye disease. *Progress in Retinal and Eye Research.* 2003;22(1):1–29. [PubMed: 12597922]
34. Antonetti DA, Barber AJ, Hollinger LA, Wolpert EB, Gardner TW. Vascular endothelial growth factor induces rapid phosphorylation of tight junction proteins occludin and zonula occluden 1. A potential mechanism for vascular permeability in diabetic retinopathy and tumors. *J Biol Chem.* 1999;274(33):23463–7. [PubMed: 10438525]
35. Viores SA. Breakdown of the Blood–Retinal Barrier. *Encyclopedia of the Eye.* 2010:216–22.
36. Xu H, Forrester JV, Liversidge J, Crane JJ. Leukocyte Trafficking in Experimental Autoimmune Uveitis: Breakdown of Blood–Retinal Barrier and Upregulation of Cellular Adhesion Molecules. *Investigative Ophthalmology & Visual Science.* 2003;44(1):226–34. [PubMed: 12506079]
37. Luna J, Chan C-C, Derevjani N, L M, Chiu C, Peng B, et al. Blood-retinal barrier (BRB) breakdown in experimental autoimmune uveoretinitis: comparison with vascular endothelial growth factor, tumor necrosis factor alpha, and interleukin-1beta-mediated breakdown. *Journal of neuroscience research.* 1997;49:268–80. [PubMed: 9260738]
38. Peach CJ, Mignone VW, Arruda MA, Alcobia DC, Hill SJ, Kilpatrick LE, et al. Molecular Pharmacology of VEGF-A Isoforms: Binding and Signalling at VEGFR2. *Int J Mol Sci.* 2018;19(4):1264.
39. Ahmad S, Hewett PW, Wang P, Al-Ani B, Cudmore M, Fujisawa T, et al. Direct Evidence for Endothelial Vascular Endothelial Growth Factor Receptor-1 Function in Nitric Oxide–Mediated Angiogenesis. *Circulation Research.* 2006;99(7):715–22. [PubMed: 16946136]
40. Huang H, Gandhi JK, Zhong X, Wei Y, Gong J, Duh EJ, et al. TNFalpha is required for late BRB breakdown in diabetic retinopathy, and its inhibition prevents leukostasis and protects vessels and neurons from apoptosis. *Investigative ophthalmology & visual science.* 2011;52(3):1336–44. [PubMed: 21212173]
41. Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB. Reactive oxygen species in inflammation and tissue injury. *Antioxid Redox Signal.* 2014;20(7):1126–67. [PubMed: 23991888]
42. Liu Y, Biarnés Costa M, Gerhardinger C. IL-1 β is upregulated in the diabetic retina and retinal vessels: cell-specific effect of high glucose and IL-1 β autostimulation. *PLoS One.* 2012;7(5):e36949-e. [PubMed: 22615852]
43. Kowluru RA, Chan P-S. Oxidative stress and diabetic retinopathy. *Exp Diabetes Res.* 2007;2007:43603-. [PubMed: 17641741]
44. Liu J, Feener EP. Plasma kallikrein-kinin system and diabetic retinopathy. *Biol Chem.* 2013;394(3):319–28. [PubMed: 23362193]
45. Bhat M, Pouliot M, Couture R, Vaucher E. The kallikrein-kinin system in diabetic retinopathy. *Prog Drug Res.* 2014;69:111–43. [PubMed: 25130041]
46. Kuhr F, Lowry J, Zhang Y, Brovkovich V, Skidgel RA. Differential regulation of inducible and endothelial nitric oxide synthase by kinin B1 and B2 receptors. *Neuropeptides.* 2010;44(2):145–54. [PubMed: 20045558]

47. Niessen CM, Gottardi CJ. Molecular components of the adherens junction. *Biochimica et Biophysica Acta (BBA) - Biomembranes*. 2008;1778(3):562–71. [PubMed: 18206110]
48. Gumbiner B. Cell Adhesion: The Molecular Basis of Tissue Architecture and Morphogenesis. *Cell*. 1996;84:345–57. [PubMed: 8608588]
49. Ebnet K. Organization of multiprotein complexes at cell–cell junctions. *Histochemistry and Cell Biology*. 2008;130(1):1–20. [PubMed: 18365233]
50. McKay BS, Irving PE, Skumatz CMB, Burke JM. Cell–Cell Adhesion Molecules and the Development of an Epithelial Phenotype in Cultured Human Retinal Pigment Epithelial Cells. *Experimental Eye Research*. 1997;65(5):661–71. [PubMed: 9367646]
51. Niessen CM, Leckband D, Yap AS. Tissue organization by cadherin adhesion molecules: dynamic molecular and cellular mechanisms of morphogenetic regulation. *Physiological reviews*. 2011;91(2):691–731. [PubMed: 21527735]
52. Coopman P, Djiane A. Adherens Junction and E-Cadherin complex regulation by epithelial polarity. *Cellular and Molecular Life Sciences*. 2016;73(18):3535–53. [PubMed: 27151512]
53. Capaldo CT, Macara IG. Depletion of E-Cadherin Disrupts Establishment but Not Maintenance of Cell Junctions in Madin-Darby Canine Kidney Epithelial Cells. *Molecular Biology of the Cell*. 2007;18(1):189–200. [PubMed: 17093058]
54. Stepniak E, Radice GL, Vasioukhin V. Adhesive and signaling functions of cadherins and catenins in vertebrate development. *Cold Spring Harb Perspect Biol*. 2009;1(5):a002949-a. [PubMed: 20066120]
55. Zhang S, Leske DA, Holmes JM. Neovascularization Grading Methods in a Rat Model of Retinopathy of Prematurity. *Investigative Ophthalmology & Visual Science*. 2000;41(3):887–91. [PubMed: 10711709]
56. Adam AP. Regulation of Endothelial Adherens Junctions by Tyrosine Phosphorylation. *Mediators Inflamm*. 2015;2015:272858-. [PubMed: 26556953]
57. Thumann G, Dou G, Wang Y, Hinton DR. Chapter 16 - Cell Biology of the Retinal Pigment Epithelium In: Ryan SJ, Sadda SR, Hinton DR, Schachat AP, Sadda SR, Wilkinson CP, et al., editors. *Retina (Fifth Edition)*. London: W.B. Saunders; 2013 p. 401–14.
58. Yang X, Chung J-Y, Rai U, Esumi N. Cadherins in the retinal pigment epithelium (RPE) revisited: P-cadherin is the highly dominant cadherin expressed in human and mouse RPE in vivo. *PLoS One*. 2018;13(1):e0191279-e. [PubMed: 29338041]
59. Burke JM, Cao F, Irving PE, Skumatz CMB. Expression of E-Cadherin by Human Retinal Pigment Epithelium: Delayed Expression In Vitro. *Invest Ophthalmol Vis Sci*. 1999;40(12):2963–70. [PubMed: 10549658]
60. Baum B, Georgiou M. Dynamics of adherens junctions in epithelial establishment, maintenance, and remodeling. *J Cell Biol*. 2011;192(6):907–17. [PubMed: 21422226]
61. Alves CH, Pellissier LP, Wijnholds J. The CRB1 and adherens junction complex proteins in retinal development and maintenance. *Progress in Retinal and Eye Research*. 2014;40:35–52. [PubMed: 24508727]
62. Alves CH, Pellissier LP, Wijnholds J. The CRB1 and adherens junction complex proteins in retinal development and maintenance. *Prog Retin Eye Res*. 2014;40:35–52. [PubMed: 24508727]
63. Zihni C, Mills C, Matter K, Balda MS. Tight junctions: from simple barriers to multifunctional molecular gates. *Nature Reviews Molecular Cell Biology*. 2016;17(9):564–80. [PubMed: 27353478]
64. Hartsock A, Nelson WJ. Adherens and tight junctions: structure, function and connections to the actin cytoskeleton. *Biochim Biophys Acta*. 2008;1778(3):660–9. [PubMed: 17854762]
65. Schneeberger EE, Lynch RD. The tight junction: a multifunctional complex. *American Journal of Physiology-Cell Physiology*. 2004;286(6):C1213–C28. [PubMed: 15151915]
66. Miyoshi J, Takai Y. Structural and functional associations of apical junctions with cytoskeleton. *Biochimica et Biophysica Acta (BBA) - Biomembranes*. 2008;1778(3):670–91. [PubMed: 18201548]
67. Vaney DI, Weiler R. Gap junctions in the eye: evidence for heteromeric, heterotypic and mixed-homotypic interactions. *Brain Research Reviews*. 2000;32(1):115–20. [PubMed: 10751660]

68. Remington LA. Chapter 1 - Visual System In: Remington LA, editor. Clinical Anatomy and Physiology of the Visual System (Third Edition). Saint Louis: Butterworth-Heinemann; 2012 p. 1–9.
69. Anderson JM, Van Itallie CM. Physiology and function of the tight junction. Cold Spring Harb Perspect Biol. 2009;1(2):a002584-a. [PubMed: 20066090]
70. Zahraoui A, Louvard D, Galli T. Tight Junction, a Platform for Trafficking and Signaling Protein Complexes. Journal of Cell Biology. 2000;151(5):F31–F6.
71. Förster C Tight junctions and the modulation of barrier function in disease. Histochemistry and cell biology. 2008;130:55–70. [PubMed: 18415116]
72. Rodriguez-Boulán E, Macara IG. Organization and execution of the epithelial polarity programme. Nat Rev Mol Cell Biol. 2014;15(4):225–42. [PubMed: 24651541]
73. Furuse M Molecular basis of the core structure of tight junctions. Cold Spring Harb Perspect Biol. 2010;2(1):a002907-a. [PubMed: 20182608]
74. Matter K, Aijaz S, Tsapara A, Balda MS. Mammalian tight junctions in the regulation of epithelial differentiation and proliferation. Current Opinion in Cell Biology. 2005;17(5):453–8. [PubMed: 16098725]
75. Chiba H, Osanai M, Murata M, Kojima T, Sawada N. Transmembrane proteins of tight junctions. Biochimica et Biophysica Acta (BBA) - Biomembranes. 2008;1778(3):588–600. [PubMed: 17916321]
76. Heinemann U, Schuetz A. Structural Features of Tight-Junction Proteins. Int J Mol Sci. 2019;20(23):6020.
77. Van Itallie CM, Anderson JM. Architecture of tight junctions and principles of molecular composition. Semin Cell Dev Biol. 2014;36:157–65. [PubMed: 25171873]
78. Wang Q, Margolis B. Apical junctional complexes and cell polarity. Kidney International. 2007;72(12):1448–58. [PubMed: 17914350]
79. Mellman I, Nelson WJ. Coordinated protein sorting, targeting and distribution in polarized cells. Nature Reviews Molecular Cell Biology. 2008;9(11):833–45. [PubMed: 18946473]
80. Tsukita S, Yamazaki Y, Katsuno T, Tamura A. Tsukita S, Yamazaki Y, Katsuno T, Tamura A.. Tight junction-based epithelial microenvironment and cell proliferation. Oncogene 27: 6930–6938. [PubMed: 19029935] Oncogene. 2008;27:6930–8. [PubMed: 19029935]
81. Naylor A, Hopkins A, Hudson N, Campbell M. Tight Junctions of the Outer Blood Retina Barrier. Int J Mol Sci. 2019;21(1):211.
82. Tsukita S, Furuse M. Occludin and claudins in tight-junction strands: leading or supporting players? Trends in Cell Biology. 1999;9(7):268–73. [PubMed: 10370242]
83. Giebel SJ, Menicucci G, McGuire PG, Das A. Matrix metalloproteinases in early diabetic retinopathy and their role in alteration of the blood–retinal barrier. Laboratory Investigation. 2005;85(5):597–607. [PubMed: 15711567]
84. Saitou M, Fujimoto K, Doi Y, Itoh M, Fujimoto T, Furuse M, et al. Occludin-deficient embryonic stem cells can differentiate into polarized epithelial cells bearing tight junctions. J Cell Biol. 1998;141(2):397–408. [PubMed: 9548718]
85. Van Itallie CM, Fanning AS, Holmes J, Anderson JM. Occludin is required for cytokine-induced regulation of tight junction barriers. Journal of Cell Science. 2010;123(16):2844. [PubMed: 20663912]
86. Günzel D, Yu ASL. Claudins and the modulation of tight junction permeability. Physiological reviews. 2013;93(2):525–69. [PubMed: 23589827]
87. González-Mariscal L, Betanzos A, Ávila-Flores A. MAGUK proteins: structure and role in the tight junction. Semin Cell Dev Biol. 2000;11(4):315–24. [PubMed: 10966866]
88. Peng S, Rao VS, Adelman RA, Rizzolo LJ. Claudin-19 and the barrier properties of the human retinal pigment epithelium. Invest Ophthalmol Vis Sci. 2011;52(3):1392–403. [PubMed: 21071746]
89. Jentsch TJ, Stein V, Weinreich F, Zdebek AA. Molecular Structure and Physiological Function of Chloride Channels. Physiological Reviews. 2002;82(2):503–68. [PubMed: 11917096]

90. Xia T, Rizzolo LJ. Effects of diabetic retinopathy on the barrier functions of the retinal pigment epithelium. *Vision Res.* 2017;139:72–81. [PubMed: 28347688]
91. Gao F, Artham S, Sabbineni H, Al-Azayzih A, Peng X-D, Hay N, et al. Akt1 promotes stimuli-induced endothelial-barrier protection through FoxO-mediated tight-junction protein turnover. *Cell Mol Life Sci.* 2016;73(20):3917–33. [PubMed: 27113546]
92. Gao F, Sabbineni H, Artham S, Somanath PR. Modulation of long-term endothelial-barrier integrity is conditional to the cross-talk between Akt and Src signaling. *J Cell Physiol.* 2017;232(10):2599–609. [PubMed: 28075016]
93. Günzel D, Yu A. Claudins and the Modulation of Tight Junction Permeability. *Physiological reviews.* 2013;93:525–69. [PubMed: 23589827]
94. Krause G, Winkler L, Mueller SL, Haseloff RF, Piontek J, Blasig IE. Structure and function of claudins. *Biochimica et Biophysica Acta (BBA) - Biomembranes.* 2008;1778(3):631–45. [PubMed: 18036336]
95. Van Itallie CM, Tietgens AJ, Anderson JM. Visualizing the dynamic coupling of claudin strands to the actin cytoskeleton through ZO-1. *Molecular biology of the cell.* 2017;28(4):524–34. [PubMed: 27974639]
96. Shen L, Weber CR, Raleigh DR, Yu D, Turner JR. Tight junction pore and leak pathways: a dynamic duo. *Annu Rev Physiol.* 2011;73:283–309. [PubMed: 20936941]
97. Förster C Tight junctions and the modulation of barrier function in disease. *Histochemistry and cell biology.* 2008;130(1):55–70. [PubMed: 18415116]
98. Furuse M, Sasaki H, Fujimoto K, Tsukita S. A single gene product, claudin-1 or -2, reconstitutes tight junction strands and recruits occludin in fibroblasts. *J Cell Biol.* 1998;143(2):391–401. [PubMed: 9786950]
99. Koval M Differential pathways of claudin oligomerization and integration into tight junctions. *Tissue Barriers.* 2013;1(3):e24518-e. [PubMed: 24665398]
100. 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. *Journal of Neurochemistry.* 2008;104(1):147–54. [PubMed: 17971126]
101. Liebnier S, Kniesel U, Kalbacher H, Wolburg H. Correlation of tight junction morphology with the expression of tight junction proteins in blood-brain barrier endothelial cells. *European Journal of Cell Biology.* 2000;79(10):707–17. [PubMed: 11089919]
102. Luo Y, Xiao W, Zhu X, Mao Y, Liu X, Chen X, et al. Differential Expression of Claudins in Retinas during Normal Development and the Angiogenesis of Oxygen-Induced Retinopathy. *Invest Ophthalmol Vis Sci.* 2011;52(10):7556–64. [PubMed: 21862644]
103. Alanne MH, Pummi K, Heape AM, Grønman R, Peltonen J, Peltonen S. Tight junction proteins in human Schwann cell autotypic junctions. *J Histochem Cytochem.* 2009;57(6):523–9. [PubMed: 19153196]
104. Smith LE, Wesolowski E, McLellan A, Kostyk SK, D'Amato R, Sullivan R, et al. Oxygen-induced retinopathy in the mouse. *Investigative Ophthalmology & Visual Science.* 1994;35(1):101–11. [PubMed: 7507904]
105. Gonçalves A, Ambrósio AF, Fernandes R. Regulation of claudins in blood-tissue barriers under physiological and pathological states. *Tissue Barriers.* 2013;1(3):e24782. [PubMed: 24665399]
106. Xiao W, Luo Y, Chen X, Li J, Ding X, Zeng M, et al. Expression of Claudins in the Normal Adult Human Retinas. *Investigative Ophthalmology & Visual Science.* 2010;51(13):40–.
107. Nitta T, Hata M, Gotoh S, Seo Y, Sasaki H, Hashimoto N, et al. Size-selective loosening of the blood-brain barrier in claudin-5-deficient mice. *J Cell Biol.* 2003;161(3):653–60. [PubMed: 12743111]
108. Argaw AT, Gurfein BT, Zhang Y, Zameer A, John GR. VEGF-mediated disruption of endothelial CLN-5 promotes blood-brain barrier breakdown. *Proc Natl Acad Sci U S A.* 2009;106(6):1977–82. [PubMed: 19174516]
109. Campbell M, Cassidy PS, O'Callaghan J, Crosbie DE, Humphries P. Manipulating ocular endothelial tight junctions: Applications in treatment of retinal disease pathology and ocular hypertension. *Progress in Retinal and Eye Research.* 2018;62:120–33. [PubMed: 28951125]

110. Campbell M, Nguyen ATH, Kiang A-S, Tam LCS, Gobbo OL, Kerskens C, et al. An experimental platform for systemic drug delivery to the retina. *Proceedings of the National Academy of Sciences*. 2009;106(42):17817–22.
111. Koto T, Takubo K, Ishida S, Shinoda H, Inoue M, Tsubota K, et al. Hypoxia disrupts the barrier function of neural blood vessels through changes in the expression of claudin-5 in endothelial cells. *Am J Pathol*. 2007;170(4):1389–97. [PubMed: 17392177]
112. Furuse M, Hata M, Furuse K, Yoshida Y, Haratake A, Sugitani Y, et al. Claudin-based tight junctions are crucial for the mammalian epidermal barrier: a lesson from claudin-1-deficient mice. *J Cell Biol*. 2002;156(6):1099–111. [PubMed: 11889141]
113. Yu Z, Gong C, Lu B, Yang L, Sheng Y, Ji L, et al. *Dendrobium chrysotoxum* Lindl. alleviates diabetic retinopathy by preventing retinal inflammation and tight junction protein decrease. *J Diabetes Res*. 2015;2015:518317-. [PubMed: 25685822]
114. Williams CD, Rizzolo LJ. Remodeling of junctional complexes during the development of the outer blood-retinal barrier. *The Anatomical Record*. 1997;249(3):380–8. [PubMed: 9372172]
115. Vogelmann R, Amieva MR, Falkow S, Nelson WJ. Breaking into the epithelial apical-junctional complex--news from pathogen hackers. *Current opinion in cell biology*. 2004;16(1):86–93. [PubMed: 15037310]
116. Rizzolo L, Williams CD. Remodeling of apical junctions during development of the retinal neuroepithelium and blood-retinal barrier. 1996;37:S693.
117. Latker CH, Beebe DC. Developmental changes in the blood-ocular barriers in chicken embryos. *Experimental Eye Research*. 1984;39(4):401–14. [PubMed: 6499958]
118. Liu Z, Kocaoglu O, Miller D. 3D Imaging of Retinal Pigment Epithelial Cells in the Living Human Retina. *Investigative Ophthalmology & Visual Science*. 2016;57:OCT533.
119. Rizzolo LJ, Peng S, Luo Y, Xiao W. Integration of tight junctions and claudins with the barrier functions of the retinal pigment epithelium. *Progress in Retinal and Eye Research*. 2011;30(5):296–323. [PubMed: 21704180]
120. Peng S, Gan G, Rao V, Adelman R, Rizzolo L. Effects of Proinflammatory Cytokines on the Claudin-19 Rich Tight Junctions of Human Retinal Pigment Epithelium. *Investigative ophthalmology & visual science*. 2012;53:5016–28. [PubMed: 22761260]
121. Harhaj NS, Antonetti DA. Regulation of tight junctions and loss of barrier function in pathophysiology. *The International Journal of Biochemistry & Cell Biology*. 2004;36(7):1206–37. [PubMed: 15109567]
122. Palenski TL, Sorenson CM, Sheibani N. Inflammatory cytokine-specific alterations in retinal endothelial cell function. *Microvasc Res*. 2013;89:57–69. [PubMed: 23806781]
123. Harhaj NS, Barber AJ, Antonetti DA. Platelet-derived growth factor mediates tight junction redistribution and increases permeability in MDCK cells. *Journal of Cellular Physiology*. 2002;193(3):349–64. [PubMed: 12384987]
124. Fairaq A, Goc A, Artham S, Sabbineni H, Somanath PR. TNFalpha induces inflammatory stress response in microvascular endothelial cells via Akt- and P38 MAP kinase-mediated thrombospondin-1 expression. *Mol Cell Biochem*. 2015;406(1–2):227–36. [PubMed: 25963668]
125. Klaassen I, Van Noorden CJF, Schlingemann RO. Molecular basis of the inner blood-retinal barrier and its breakdown in diabetic macular edema and other pathological conditions. *Progress in Retinal and Eye Research*. 2013;34:19–48. [PubMed: 23416119]
126. Noma H, Yasuda K, Shimura M. Cytokines and the Pathogenesis of Macular Edema in Branch Retinal Vein Occlusion. *J Ophthalmol*. 2019;2019:5185128-. [PubMed: 31191997]
127. Dörfel MJ, Huber O. Modulation of Tight Junction Structure and Function by Kinases and Phosphatases Targeting Occludin. *Journal of Biomedicine and Biotechnology*. 2012;2012:807356. [PubMed: 22315516]
128. Antonetti DA, Lieth E, Barber AJ, Gardner TW. Molecular Mechanisms of Vascular Permeability in Diabetic Retinopathy. *Seminars in Ophthalmology*. 1999;14(4):240–8. [PubMed: 10758225]
129. Chapter 31 - Intercellular Junctions In: Pollard TD, Earnshaw WC, Lippincott-Schwartz J, Johnson GT, editors. *Cell Biology* (Third Edition): Elsevier; 2017 p. 543–53.
130. Miyahara T, Hamanaka K, Weber D, Drake D, Anghelescu M, Parker J. Phosphoinositide 3-kinase, Src, and Akt modulate acute ventilation-induced vascular permeability increases in mouse

lungs. American journal of physiology Lung cellular and molecular physiology. 2007;293:L11–21. [PubMed: 17322282]

131. Patel A, Sabbineni H, Clarke A, Somanath PR. Novel roles of Src in cancer cell epithelial-to-mesenchymal transition, vascular permeability, microinvasion and metastasis. Life Sci. 2016;157:52–61. [PubMed: 27245276]
132. Alwhaibi A, Verma A, Adil MS, Somanath PR. The unconventional role of Akt1 in the advanced cancers and in diabetes-promoted carcinogenesis. Pharmacol Res. 2019;145:104270. [PubMed: 31078742]
133. Artham S, Gao F, Verma A, Alwhaibi A, Sabbineni H, Hafez S, et al. Endothelial stromelysin1 regulation by the forkhead box-O transcription factors is crucial in the exudative phase of acute lung injury. Pharmacol Res. 2019;141:249–63. [PubMed: 30611853]
134. Basta G, Schmidt AM, De Caterina R. Advanced glycation end products and vascular inflammation: implications for accelerated atherosclerosis in diabetes. Cardiovascular Research. 2004;63(4):582–92. [PubMed: 15306213]
135. Zhang W, Xu Q, Wu J, Zhou X, Weng J, Xu J, et al. Role of Src in Vascular Hyperpermeability Induced by Advanced Glycation End Products. Sci Rep. 2015;5:14090-. [PubMed: 26381822]
136. Dejana E, Orsenigo F, Lampugnani MG. The role of adherens junctions and VE-cadherin in the control of vascular permeability. Journal of Cell Science. 2008;121(13):2115. [PubMed: 18565824]
137. Schepke L, Aguilar E, Gariano RF, Jacobson R, Hood J, Doukas J, et al. Retinal vascular permeability suppression by topical application of a novel VEGFR2/Src kinase inhibitor in mice and rabbits. J Clin Invest. 2008;118(6):2337–46. [PubMed: 18483622]
138. Choi YK, Kim JH, Kim WJ, Lee HY, Park JA, Lee S-W, et al. AKAP12 Regulates Human Blood–Retinal Barrier Formation by Downregulation of Hypoxia-Inducible Factor-1 α . The Journal of Neuroscience. 2007;27(16):4472–81. [PubMed: 17442832]
139. Abid MR, Guo S, Minami T, Spokes KC, Ueki K, Skurk C, et al. Vascular Endothelial Growth Factor Activates PI3K/Akt/Forkhead Signaling in Endothelial Cells. Arteriosclerosis, Thrombosis, and Vascular Biology. 2004;24(2):294–300.
140. Wang Y, Zhou Y, Graves DT. FOXO transcription factors: their clinical significance and regulation. Biomed Res Int. 2014;2014:925350-. [PubMed: 24864265]
141. Essers MA. Functional interaction between beta-catenin and FOXO in oxidative stress signaling. Science. 2005;308(1):181–184.
142. Beurel E, Grieco SF, Jope RS. Glycogen synthase kinase-3 (GSK3): regulation, actions, and diseases. Pharmacol Ther. 2015;148:114–31. [PubMed: 25435019]
143. Rousseau S, Houle F, Landry J, Huot J. p38 MAP kinase activation by vascular endothelial growth factor mediates actin reorganization and cell migration in human endothelial cells. Oncogene. 1997;15(18):2169–77. [PubMed: 9393975]
144. Srinivasan S, Bolick D, Hatley M, Natarajan R, Reilly K, Yeh M, et al. Glucose Regulates Interleukin-8 Production in Aortic Endothelial Cells through Activation of the p38 Mitogen-activated Protein Kinase Pathway in Diabetes. The Journal of biological chemistry. 2004;279:31930–6. [PubMed: 15145956]
145. Kondo T, Kahn C. Altered Insulin Signaling in Retinal Tissue in Diabetic States. The Journal of biological chemistry. 2004;279:37997–8006. [PubMed: 15201286]
146. Yuan Z, Feng W, Hong J, Zheng Q, Shuai J, Ge Y. p38MAPK and ERK promote nitric oxide production in cultured human retinal pigmented epithelial cells induced by high concentration glucose. Nitric Oxide. 2009;20(1):9–15. [PubMed: 18854222]
147. Poulaki V, Qin W, Jousen AM, Hurlbut P, Wiegand SJ, Rudge J, et al. Acute intensive insulin therapy exacerbates diabetic blood-retinal barrier breakdown via hypoxia-inducible factor-1 α and VEGF. J Clin Invest. 2002;109(6):805–15. [PubMed: 11901189]
148. Yang J, Duh EJ, Caldwell RB, Behzadian MA. Antipermeability function of PEDF involves blockade of the MAP kinase/GSK/beta-catenin signaling pathway and uPAR expression. Investigative ophthalmology & visual science. 2010;51(6):3273–80. [PubMed: 20089873]

149. Du Y, Tang J, Li G, Berti-Mattera L, Lee CA, Bartkowski D, et al. Effects of p38 MAPK inhibition on early stages of diabetic retinopathy and sensory nerve function. *Investigative ophthalmology & visual science*. 2010;51(4):2158–64. [PubMed: 20071676]
150. Xu J, Chen LJ, Yu J, Wang HJ, Zhang F, Liu Q, et al. Involvement of Advanced Glycation End Products in the Pathogenesis of Diabetic Retinopathy. *Cellular Physiology and Biochemistry*. 2018;48(2):705–17. [PubMed: 30025404]
151. Hafez S, Abdelsaid M, El-Shafey S, Johnson MH, Fagan SC, Ergul A. Matrix Metalloprotease 3 Exacerbates Hemorrhagic Transformation and Worsens Functional Outcomes in Hyperglycemic Stroke. *Stroke*. 2016;47(3):843–51. [PubMed: 26839355]
152. Artham S, Verma A, Newsome AS, Somanath PR. Patients with acute respiratory distress syndrome exhibit increased stromelysin1 activity in the blood samples. *Cytokine*. 2020;131:155086. [PubMed: 32272349]
153. Liu F, Koval M, Ranganathan S, Fanayan S, Hancock W, Lundberg E, et al. Systems Proteomics View of the Endogenous Human Claudin Protein Family. *Journal of proteome research*. 2015;15.
154. Harhaj NS, Felinski EA, Wolpert EB, Sundstrom JM, Gardner TW, Antonetti DA. VEGF activation of protein kinase C stimulates occludin phosphorylation and contributes to endothelial permeability. *Invest Ophthalmol Vis Sci*. 2006;47(11):5106–15. [PubMed: 17065532]
155. Andreeva AY, Piontek J, Blasig IE, Utepbergenov DI. Assembly of tight junction is regulated by the antagonism of conventional and novel protein kinase C isoforms. *Int J Biochem Cell Biol*. 2006;38(2):222–33. [PubMed: 16257565]
156. Andreeva AY, Krause E, Muller EC, Blasig IE, Utepbergenov DI. Protein kinase C regulates the phosphorylation and cellular localization of occludin. *J Biol Chem*. 2001;276(42):38480–6. [PubMed: 11502742]
157. Gonzalez-Mariscal L, Chavez de Ramirez B, Cereijido M. Tight junction formation in cultured epithelial cells (MDCK). *J Membr Biol*. 1985;86(2):113–25. [PubMed: 4032460]
158. Vega-Salas DE, Salas PJ, Rodriguez-Boulton E. Modulation of the expression of an apical plasma membrane protein of Madin-Darby canine kidney epithelial cells: cell-cell interactions control the appearance of a novel intracellular storage compartment. *J Cell Biol*. 1987;104(5):1249–59. [PubMed: 3553208]
159. Koya D, King GL. Protein kinase C activation and the development of diabetic complications. *Diabetes*. 1998;47(6):859–66. [PubMed: 9604860]
160. Zong H, Ward M, Stitt AW. AGEs, RAGE, and diabetic retinopathy. *Curr Diab Rep*. 2011;11(4):244–52. [PubMed: 21590515]
161. Clarke M, Dodson PM. PKC inhibition and diabetic microvascular complications. *Best Pract Res Clin Endocrinol Metab*. 2007;21(4):573–86. [PubMed: 18054736]
162. Chalupsky K, Cai H. Endothelial dihydrofolate reductase: critical for nitric oxide bioavailability and role in angiotensin II uncoupling of endothelial nitric oxide synthase. *Proc Natl Acad Sci U S A*. 2005;102(25):9056–61. [PubMed: 15941833]
163. Kowluru RA, Abbas SN. Diabetes-induced mitochondrial dysfunction in the retina. *Invest Ophthalmol Vis Sci*. 2003;44(12):5327–34. [PubMed: 14638734]
164. Park J, Kim H, Park SY, Lim SW, Kim YS, Lee DH, et al. Tonicity-responsive enhancer binding protein regulates the expression of aldose reductase and protein kinase C delta in a mouse model of diabetic retinopathy. *Exp Eye Res*. 2014;122:13–9. [PubMed: 24631337]
165. Geraldes P, Hiraoka-Yamamoto J, Matsumoto M, Clermont A, Leitges M, Marette A, et al. Activation of PKC-delta and SHP-1 by hyperglycemia causes vascular cell apoptosis and diabetic retinopathy. *Nat Med*. 2009;15(11):1298–306. [PubMed: 19881493]
166. Devi TS, Hosoya K, Terasaki T, Singh LP. Critical role of TXNIP in oxidative stress, DNA damage and retinal pericyte apoptosis under high glucose: implications for diabetic retinopathy. *Exp Cell Res*. 2013;319(7):1001–12. [PubMed: 23353834]
167. Avelaira CA, Lin CM, Abcouwer SF, Ambrosio AF, Antonetti DA. TNF-alpha signals through PKCzeta/NF-kappaB to alter the tight junction complex and increase retinal endothelial cell permeability. *Diabetes*. 2010;59(11):2872–82. [PubMed: 20693346]
168. Minshall RD, Vandenbroucke EE, Holinstat M, Place AT, Tiruppathi C, Vogel SM, et al. Role of protein kinase C ζ in thrombin-induced RhoA activation and inter-endothelial gap formation of

- human dermal microvessel endothelial cell monolayers. *Microvasc Res.* 2010;80(2):240–9. [PubMed: 20417648]
169. Balda MS, Gonzalez-Mariscal L, Matter K, Cereijido M, Anderson JM. Assembly of the tight junction: the role of diacylglycerol. *J Cell Biol.* 1993;123(2):293–302. [PubMed: 8408213]
170. Gonzalez-Mariscal L, Tapia R, Chamorro D. Crosstalk of tight junction components with signaling pathways. *Biochim Biophys Acta.* 2008;1778(3):729–56. [PubMed: 17950242]
171. Fasano A, Fiorentini C, Donelli G, Uzzau S, Kaper JB, Margaretten K, et al. Zonula occludens toxin modulates tight junctions through protein kinase C-dependent actin reorganization, in vitro. *J Clin Invest.* 1995;96(2):710–20. [PubMed: 7635964]
172. Deissler HL, Lang GE. The Protein Kinase C Inhibitor: Ruboxistaurin. *Dev Ophthalmol.* 2016;55:295–301. [PubMed: 26501476]
173. Urias EA, Urias GA, Monickaraj F, McGuire P, Das A. Novel therapeutic targets in diabetic macular edema: Beyond VEGF. *Vision Res.* 2017;139:221–7. [PubMed: 28993218]
174. Kim SR, Suh W. Beneficial effects of the Src inhibitor, dasatinib, on breakdown of the blood-retinal barrier. *Arch Pharm Res.* 2017;40(2):197–203. [PubMed: 27988882]
175. Scott IU, Jackson GR, Quillen DA, Klein R, Liao J, Gardner TW. Effect of doxycycline vs placebo on retinal function and diabetic retinopathy progression in mild to moderate nonproliferative diabetic retinopathy: a randomized proof-of-concept clinical trial. *JAMA Ophthalmol.* 2014;132(9):1137–42. [PubMed: 24969516]
176. Scott IU, Jackson GR, Quillen DA, Larsen M, Klein R, Liao J, et al. Effect of doxycycline vs placebo on retinal function and diabetic retinopathy progression in patients with severe nonproliferative or nonhigh-risk proliferative diabetic retinopathy: a randomized clinical trial. *JAMA Ophthalmol.* 2014;132(5):535–43. [PubMed: 24604308]
177. Hidalgo M, Eckhardt SG. Development of matrix metalloproteinase inhibitors in cancer therapy. *J Natl Cancer Inst.* 2001;93(3):178–93. [PubMed: 11158186]
178. Sadiq MA, Hanout M, Sarwar S, Hassan M, Agarwal A, Sepah YJ, et al. Platelet-Derived Growth Factor Inhibitors: A Potential Therapeutic Approach for Ocular Neovascularization. *Dev Ophthalmol.* 2016;55:310–6. [PubMed: 26501397]

Figure 1: The blood-retinal barrier.

A) Simplified representation of the retinal neurovascular unit, comprising vascular cells, glia, and distinct groups of neurons. B) Inner BRB is mainly composed of endothelial cells, pericytes, and Muller cells. TJs serve to be an integral part of iBRB. C) The outer BRB consists of RPE cells that control transport between the choriocapillaris and the retina and serves to retard the transepithelial diffusion through the paracellular spaces in between the RPE with the help of TJs and AJs. NFL: Nerve fiber layer; GCL: Ganglion cell layer; IPL: inner plexiform layer; INL: inner nuclear layer; OPL: outer plexiform layer; ONL: Outer nuclear layer, RPE: Retinal pigment epithelium; Choroid: iBRB: Inner Blood retinal barrier, oBRB: Outer Blood retinal barrier. A part of this figure was created using the Servier Medical Art (<https://smart.servier.com/>).

Figure 2: Regulation of BRB in physiology and diabetes.

Ang-1/Tie-2 signaling maintains systemic vascular quiescence by negatively regulating endothelial hyperpermeability and inflammation through the stabilization of TJs via the Akt pathway. In diabetes, there is overt production of VEGF and accumulation of AGE contributing to inflammation, which in turn activates several downstream kinases such as Src, p38MAPK, and Akt pathways. Each of these pathway activations leads to discrete effects on TJ stability. TJ protein turnover through changes in the expression and phosphorylation are the major contributing factors of increased vascular permeability observed in several vascular disorders. Ang-1: Angiopoietin-1, Ang-2: Angiopoietin-2, VEGF: Vascular endothelial Growth Factor, AGEs: Advanced glycation end products, RAGE: Receptor for AGE, TNFα: Tumor necrosis factor α, FoxO1/3a: Forkhead box O family of transcription factors 1/3a, p38MAPK: p38 mitogen-activated protein kinase.

Figure 3:

PKC isozymes are activated in the diabetic retina by hyperglycemia through the activation of DAG and AGE/RAGE pathways. This, in turn, activates NOX to produce reactive oxygen species such as O₂^{•-} and NO to generate peroxynitrites thus uncoupling eNOS leading endothelial dysfunction. High glucose-induced activation of PKC- δ signaling promotes the p38MAPK-induced expression of SHP-1 contributing to pericyte loss affecting retinal microvessel integrity and leading to microaneurysms and neovascularization.

Table 1:

Expression pattern of several claudins in eye

Junctional Protein	Expression	Method	Reference
Adherens Junction	E-Cadherin P-Cadherin	In-vitro (Human and bovine RPE cells)	(59)
Tight Junctions			
Claudins	<p>Mice:</p> <p>P18: Claudin 1 to 5, 7, 9, 10 to 14, 17, 19, 20, 22, and 23</p> <p>P15: Claudin 1 to 5, 12, 22, and 23</p> <ul style="list-style-type: none"> • 1, 2, 5 – Blood vessels localized in RGC, IPL and OPL • 3, 4, 23 – RGC • 12 – OPL • 23 – INL <p>Claudin 2, 5 (two-fold increase at P18)</p> <p>Human:</p> <ul style="list-style-type: none"> • Claudin 1, 2, 3, 4, 5, 12 and 22 • Claudin 3, 19-RPE 	<p>Retinal mRNA analysis</p> <p>Immunolocalization</p> <p>OIR model</p> <p>Human Retinal mRNA analysis</p>	<p>(102)</p> <p>(102)</p> <p>(102)</p> <p>(106)</p>

Table 2:

Potential therapeutic modalities to target the increased vascular permeability in DR

Anti-VEGF (173) • Ranibizumab • Bevacizumab • Pegaptanib • Aflibercept	p38MAPK inhibitors (148) • SB202190
Src Inhibitors • Dasatinib (174)	MMP inhibitors • Doxycycline (175, 176) • Bisphosphonates (177)
Angiopoietin-2 inhibitor (173) • Monoclonal antibody (R06867461)	Inhibitors of kallikrein-kinin (KK) system (173) • KVD001
Integrin inhibitors (173) • α - β integrin inhibitor • VAP-1 inhibitor	PDGF inhibitors (178) • E10030