Check for updates

A new perspective on protecting the blood-retinal barrier against injury in diabetic retinopathy: mitophagy

Mengtian Li[1†], Liyang Yang[1,2†], Haoyu Zhai[3], Liping Qiao[4], Zhaobo Wang[5], Xuedong An[5*] and Jia Wang[1*]

[1]Department of General Internal Medicine, Guang'anmen Hospital, China Academy of Chinese Medical Sciences, Beijing, China, [2]Beijing University of Chinese Medicine, Beijing, China, [3]Department of Oncology, Guang'anmen Hospital, China Academy of Chinese Medical Sciences, Beijing, China, [4]Jiangsu Provincial Hospital of Traditional Chinese Medicine Affiliated to Nanjing University of Chinese Medicine, Nanjing, China, [5]Department of Endocrinology, Guang'anmen Hospital, China Academy of Chinese Medical Sciences, Beijing, China

The blood-retinal barrier (BRB) comprises the inner blood-retinal barrier (iBRB) and the outer blood-retinal barrier (oBRB). The integrity of the BRB is essential to maintaining stability of the retinal microenvironment. Mitophagy plays a crucial role in maintaining organellar integrity by regulating mitochondrial quality and quantity. High glucose-induced mitophagy dysfunction contributes to diabetic retinopathy (DR) by damaging the BRB. This review presents mitophagy mechanisms under physiological conditions and examines changes across different cell types under DR-related pathological conditions that damage the BRB. It also summarizes drugs and targets that regulate mitophagy to stabilize the BRB and alleviate DR, offering new therapeutic insights.

1 Introduction

In 2015, approximately 415 million people worldwide were affected by diabetes, and this number is expected to reach 642 million by 2040 (1). DR is the most common microvascular complication of diabetes, with a prevalence of approximately 34.6% among patients with diabetes (2). Diabetic macular edema (DME), a complication of DR, affects 6.8% of patients with diabetes mellitus (DM) and is a leading cause of visual impairment and blindness. Clinically, DR is classified as proliferative (PDR) or non-proliferative (NPDR). NPDR is characterized by retinal capillary basement membrane thickening, increased retinal vascular permeability, and tissue ischemia, whereas PDR involves pathological neovascularization, potentially leading to vitreous hemorrhage or retinal detachment (3). The potential mechanisms causing these vascular issues include the breakdown of BRB. The integrity of the BRB helps keep the retinal microenvironment

separate from the systemic circulation, reducing the impact of oxidative stress on this microenvironment, thereby maintaining its homeostasis under hyperglycemic conditions (4).

The BRB is composed of an inner layer and an outer layer. The iBRB mainly consists of a microvascular network that nourishes the retina's inner layer. Its primary structure is vascular endothelial cells. These endothelial cells interact with neurons, pericytes, and glial cells to form the neurovascular unit (NVU) (5). The oBRB consists of a monolayer of retinal pigment epithelial (RPE) cells that interact with the fenestrated choroidal capillaries and Bruch's membrane, whereas adjacent RPE cells are connected by tight junctions (6). The integrity of the BRB is essential for protecting the retina from harmful substances, clearing metabolic waste, regulating angiogenesis, and maintaining visual signal transmission.

Intravitreal anti-vascular endothelial growth factor (anti-VEGF) therapy is the established first-line treatment for center-involved DME (7). However, only 29% of patients show significant visual improvement after 2 years of treatment (8). Moreover, antibody-based therapies impose a significant economic burden on patients, making anti-VEGF therapy unlikely to be widely used for the routine treatment of NPDR. Given the limitations of current DR therapies, there is a need to explore new strategies targeting the core pathophysiological mechanisms. The disruption of the BRB, a critical event in the progression of DR, has molecular mechanisms that remain inadequately targeted by current therapies. Recent studies have shown that mitophagy dysfunction is a key factor in the destruction of the BRB during the progression of DR (9, 10). Focusing on the BRB, this review first analyzes the composition and molecular mechanisms underlying high glucose-induced BRB damage in DR, including mitochondrial dysfunction, inflammation, oxidative stress, and related pathways. Subsequently, it explores the regulatory mechanisms of mitophagy—a selective autophagy process that eliminates damaged mitochondria—and finally reviews potential drugs and targets aimed at ameliorating BRB damage through mitophagy modulation in DR, offering novel mechanistic insights and therapeutic strategies for targeting BRB lesions.

2 Composition and damage of the BRB

2.1 Composition of the BRB

The BRB consists of the iBRB and oBRB, which separate the systemic circulation from the retina (11). This unique structure not only helps transport nutrients and oxygen but also blocks large molecules, pathogens, and toxins from the blood. It also selectively regulates molecular flow between the systemic circulation and the retina, therefore maintaining homeostasis of the retinal microenvironment (12).

2.1.1 Composition of the iBRB

Retinal endothelial cells (RECs), the principal components of the iBRB, constitute the retinal microvascular endothelium. These cells form a selective barrier via tight junctions between adjacent cells, regulating the transport of fluids and macromolecules between the blood and neural retina (13). The formation, maturation, and stability of retinal microvessels depend on the interactions between pericytes and endothelial cells. Specifically, RECs are interconnected by tight junction proteins and ensheathed by pericytes and glial cells (e.g., Müller cells and astrocytes) (14). Furthermore, endothelial cells within the retinal microvasculature rely on vascular endothelial (VE)-cadherin-mediated, calcium-dependent adherens junctions between the cells, which are essential for maintaining barrier integrity (15). Although the iBRB specifically reflects the specialized properties of RECs, the integrated functions of all NVU components, including neurons (ganglion, bipolar, horizontal, and amacrine cells), glial cells (astrocytes and Müller cells), immune cells (microglia), and vascular cells (RECs and pericytes), are essential for maintaining iBRB integrity and dynamically coordinating local blood flow to meet the metabolic demands of retinal neurons (16). Bidirectional signaling among these NVU cells constitutes a complex, dynamic network. The retinal vascular network regulates blood flow, angiogenesis, and permeability in response to the dynamic demands of retinal neurons. By providing oxygen and nutrients, recycling neurotransmitters, and clearing metabolic waste, the retinal vasculature plays a crucial role in fine-tuning vascular function to maintain retinal homeostasis (17). The endothelial cell population, specifically RECs, plays an important role in retinal diseases. Early intervention targeting RECs can help reduce the progression of retinal lesions and visual impairment.

2.1.2 Composition of the oBRB

The oBRB consists of the choroid, Bruch's membrane (BM), and RPE cells. The RPE is closely associated with the oBRB and directly constitutes the foundation of the neural retina (11). The RPE contributes to barrier function by forming tight junction complexes and providing metabolic support to the neural retina (18). This cell layer is essential for maintaining visual function by creating a selective barrier between the choroidal capillaries and the neural retina and regulating bidirectional solute transport, including nutrient delivery and metabolic waste clearance (11). Therefore, understanding RPE function is vital for developing effective prevention and treatment strategies for DR.

However, in the diabetic state, persistent pathological factors, including chronic hyperglycemia, severely disrupt the integrity and precise regulatory functions of both the iBRB and oBRB. This BRB dysfunction is one of the core pathophysiological bases for the onset and progression of DR (19) Figure 1.

2.2 Damage to the BRB in DR

The damage to the BRB in DR results from the multimechanistic synergy of four core pathways: inflammatory activation, oxidative stress, dysregulation of vascular growth factors, and mitochondrial homeostatic imbalance. These mechanisms are intertwined and mutually reinforcing, collectively driving disease progression.
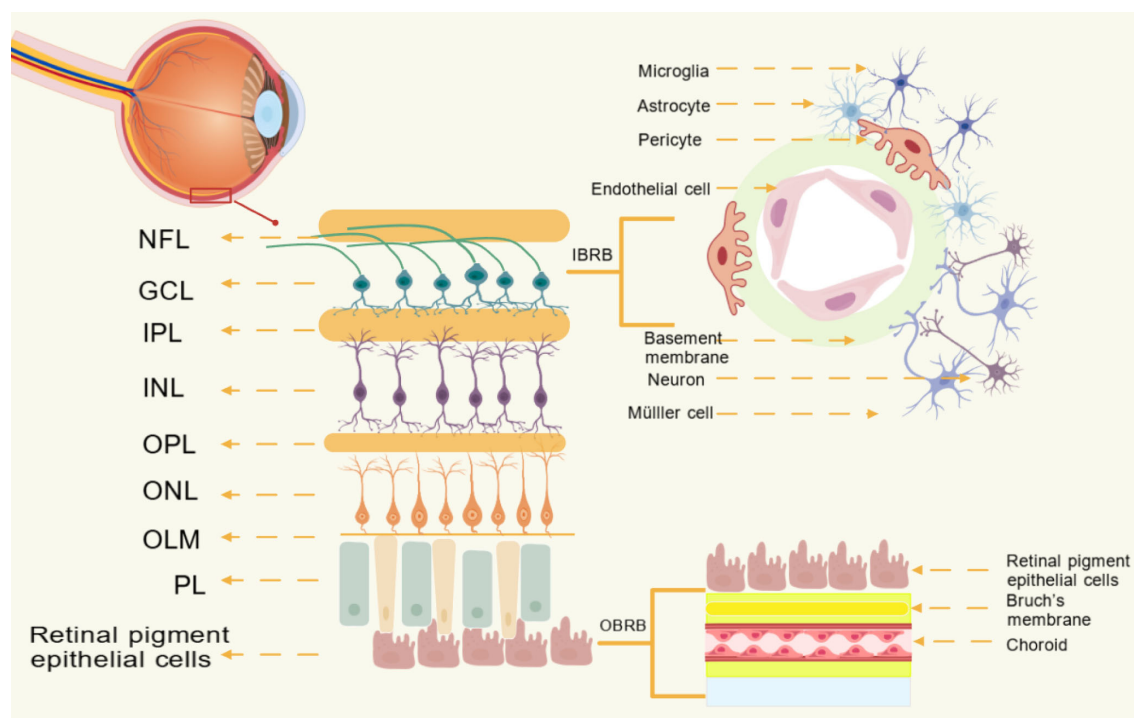
FIGURE 1
The image shows the structure of the blood-retinal barrier (BRB). The inner blood-retinal barrier (iBRB) is a functional barrier composed of retinal endothelial cells (RECs), which form retinal capillaries that traverse the inner layer of the retina. It is produced by vascular endothelial cells and features a double basement membrane, tight junctions between adjacent cells, and interactions with surrounding pericytes, microglia, and neurons. The retinal neurovascular unit (NVU), composed of vascular endothelial cells, pericytes, Müller glial cells, astrocytes, neurons, and microglial cells, contributes to the integrity of the iBRB. The outer blood-retinal barrier (oBRB) consists of the choroid, Bruch's membrane (BM), and retinal pigment epithelium (RPE), with the RPE being most relevant to the oBRB, directly forming the foundation of the neuroretina. PL, Photoreceptor Layer; OLM, Outer Limiting Membrane; ONL, Outer Nuclear Layer; OPL, Outer Plexiform Layer; INL, Inner Nuclear Layer; IPL, Inner Plexiform Layer; GCL, Ganglion Cell Layer; NFL, Nerve Fiber Layer.

2.2.1 Inflammation

DR is a chronic, low-grade inflammatory disease involving various inflammatory mediators and adhesion molecules, with chronic hyperglycemia serving as the main trigger (20). Studies have shown that levels of interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) are elevated in the serum and vitreous of DR patients (21). IL-1β and TNF-α act as inducers of adhesion molecule expression by binding to their respective receptors (IL-1 receptor and TNF receptor), thereby promoting the activation of nuclear factor kappa-B (NF-κB). This activation leads to increased expression of IL-6 and IL-8, and it also triggers caspase-1 activation (22). Furthermore, IL-1β activates NF-κB, which enhances the adhesion of retinal capillary cells to the endothelium and induces their apoptosis. These events, therefore, increase vascular permeability (22, 23). TNF-α promotes leukocyte adhesion to RECs, directly impairing BRB integrity (24, 25). These pro-inflammatory cytokines stimulate RECs to upregulate intercellular adhesion molecules, thereby facilitating leukocyte recruitment and capillary adhesion. Adherent leukocytes obstruct capillaries and disrupt tight endothelial junctions. Consequently, BRB dysfunction manifests as acellular capillary formation, vascular leakage, and DME (25). Furthermore, hyperglycemia (HG) activates the NLRP3 (NOD-, LRR-, and pyrin domain-containing protein 3)

inflammasome, contributing to inflammation. Activation of the NLRP3 inflammasome mediates cytokine secretion, which downregulates tight junction protein expression and exacerbates BRB damage (26).

2.2.2 Oxidative stress

In the hyperglycemic state of diabetes, chronic hyperglycemia causes oxidative stress through four main pathways, generating reactive oxygen species (ROS) and exacerbating damage to the BRB. One such pathway is the activation of the polyol pathway of glucose metabolism (27), where aldose reductase (AR) converts glucose into sorbitol, which is then oxidized by sorbitol dehydrogenase into fructose. During this process, the cofactor nicotinamide adenine dinucleotide (NAD^+) is reduced to NADH (28, 29). This leads to an abnormal increase in the NADH/NAD+ ratio, with excess NADH acting as a substrate for NADPH oxidase, thereby promoting ROS generation within retinal cells (30). Products generated by fructose phosphorylation and degradation can serve as precursors for the formation of advanced glycation end products (AGEs) (31). HG-induced accumulation of diacylglycerol activates the protein kinase C (PKC) pathway and promotes the expression of VEGF in retinal tissues (32). The upregulation of VEGF contributes to endothelial dysfunction, increased vascular permeability, and pathological

neovascularization in DR (33). Additionally, PKC enhances the activity of NADPH oxidase, thereby promoting ROS production in various vascular cells (34). Prolonged hyperglycemia significantly increases the non-enzymatic glycation of proteins and lipids, ultimately leading to AGEs accumulation (35). The interaction of AGEs with their receptor RAGE promotes NF-κB activation, triggering retinal pericyte apoptosis and upregulating VEGF, which increases vascular endothelial permeability (36).Additionally, this AGEs-RAGE axis stimulates Müller cells to release VEGF and MCP-1 (monocyte chemoattractant protein-1), further disrupting the BRB and inducing inflammatory infiltration (37). Furthermore, AGEs-RAGE interaction activates NADPH oxidase, enhancing intracellular ROS generation (38, 39). Elevated ROS levels, in turn, contribute to AGEs formation, further amplifying AGEs-mediated damage (40). AGEs stimulation can also lead to the upregulation of proteases, which cleave VE-cadherin on RECs, resulting in BRB breakdown and increased vascular permeability (41). After activation of the hexosamine pathway, the resulting high concentrations of glucosamine accumulate, stimulating excessive ROS generation in mitochondria and damaging mitochondrial respiratory function. This process further exacerbates oxidative stress, increases vascular permeability, and promotes angiogenesis (42, 43).

ROS are continuously produced in all cells to support normal cellular functions. However, under hyperglycemic conditions, the activation of the four pathways mentioned above stimulates excessive production of mitochondrial ROS, further exacerbating oxidative stress. First, elevated ROS levels activate the NLRP3 inflammasome in RECs, as induced by AGEs in diabetic rats. This activation leads to the caspase-1-dependent release of the pro-inflammatory cytokine IL-1β (44). These inflammatory cytokines damage RECs, impairing their ability to maintain BRB integrity. Second, heightened oxidative stress potently activates NF-κB, which regulates the transcription of numerous genes. The expression of zonula occludens-1 (ZO-1), a critical tight junction protein, is suppressed by NF-κB, thereby disrupting the normal structure of the BRB (45). Furthermore, oxidative stress, which is exacerbated by excess ROS, contributes to mitochondrial dysfunction. Notably, mitochondrial DNA (mtDNA) is particularly vulnerable to extensive and persistent damage induced by oxidative stress (46). Damaged mtDNA impairs transcription and protein synthesis, resulting in further production of ROS.

2.2.3 Elevated vascular endothelial growth factor

Elevated VEGF levels are positively correlated with DR severity, particularly in PDR. Studies have demonstrated significantly higher serum VEGF concentrations in patients with PDR than in patients with NPDR and healthy controls (47). Similarly, the aqueous humor VEGF levels in patients with PDR substantially exceed those in non-DR individuals (48). Firstly, in a vitro co-culture model of the BRB based on primary RECs, pericytes and astrocytes, VEGF treatment disrupts the junctional assembly of tight junction proteins (occludin, claudin-5, ZO-1), adherens junction protein (VE-cadherin), directly compromising BRB structural integrity

(49).Elevated VEGF levels also induce the expression of urokinase plasminogen activator receptor (uPAR), leading to activation of matrix metalloproteinase-9 (MMP-9), which degrades the extracellular matrix and further disrupts junctional complexes (50). Secondly, VEGF significantly increases the expression of plasmalemma vesicle-associated protein (PLVAP), promoting endothelial cell vesicular transport activity. This facilitates plasma protein leakage into retinal tissue via the transcellular route (49, 51). Furthermore, VEGF induces RECs proliferation, promoting retinal neovascularization, which ultimately progresses to PDR (52).

2.2.4 Imbalance in mitochondrial dynamics and mitophagy

Mitochondria are key organelles responsible for energy production and serve as central hubs for nutrient metabolism. They play vital roles in metabolism, signal transduction and are essential for cellular processes including growth, differentiation, aging, and death. Within the mitochondrial inner membrane, NADH and flavin adenine dinucleotide ($FADH_2$) act as electron donors, transferring electrons to the electron transport chain (ETC). During electron transfer, protons (H^+) are pumped into the intermembrane space, generating an electrochemical gradient. This gradient drives ATP synthesis by ATP synthase, producing chemical energy stored in ATP molecules that fuel cellular processes. Maintaining mitochondrial homeostasis critically depends on the precise regulation of dynamic processes, namely mitochondrial fusion, fission, and mitophagy. Fusion preserves mitochondrial network integrity and facilitates the complementation of damaged mtDNA (53, 54). Fission segregates damaged or dysfunctional mitochondria, enabling their subsequent incorporation into autophagosomes (55). Furthermore, mitochondria that exhibit a reduced membrane potential following fission are selectively engulfed by autophagosomes and degraded via mitophagy (56), thereby ensuring mitochondrial quality control and homeostasis (57). Mitophagy specifically targets and degrades damaged mitochondria through a lysosome-dependent pathway (58, 59). This extensive clearance of damaged mitochondria prevents ROS generation and accumulation, halts the propagation of mitochondrial damage, and confers cytoprotection (60). Under conditions of cellular damage or stress, mitophagy reduces the burden of mitochondria harboring pro-death signals through targeted elimination, thereby delaying the onset of apoptosis. Furthermore, mitophagy regulates apoptotic cascades via cytochrome c release, which activates caspase-family cysteine proteases to initiate programmed cell death (61). Mitophagy also modulates inflammatory responses and oxidative stress. Inflammatory diseases are primarily mediated by inflammasome activation such as NLRP3 (62, 63). By controlling the release of mitochondrial-derived damage-associated molecular patterns (mtDNA and ROS), mitophagy regulates inflammasome activation. It also exhibits cell-intrinsic anti-inflammatory mechanisms by suppressing excessive production of IL-1β and IL-18. Therefore, mitochondrial fusion, fission, and mitophagy collectively maintain efficient ATP generation while constraining ROS accumulation (64).

Mitochondrial number, size, structure, and physiological functions are typically altered under pathological conditions, including ischemia, hypoxia, nutrient deficiency/imbalance, endotoxin exposure, and calcium overload (32). In DR, imbalanced mitochondrial dynamics manifest as enhanced fission and suppressed fusion, promoting the intracellular accumulation of damaged or dysfunctional mitochondria (65). Concurrently, hyperglycemia may initially elevate mitophagic flux; however, when mitophagic activity exceeds the lysosomal degradative capacity, lysosomal enlargement occurs, accompanied by diminished enzyme activity, resulting in impaired clearance of damaged mitochondria (66). In addition, other studies have indicated that sustained high-glucose conditions ultimately suppress mitophagy and cellular proliferation (67). This mitophagy inhibition amplifies ROS overproduction (68), induces mtDNA mutations, disrupts cellular architecture, impairs metabolic homeostasis, and potentiates apoptosis (69, 70). Oxidative stress and calcium overload can trigger mitochondrial permeability transition (mPT) through the mitochondrial permeability transition pore (mPTP), facilitating the efflux of cytochrome c and other mitochondrial components. This process initiates apoptotic cascades and inflammatory responses (71). Persistent mPTP opening depletes NADH reserves, damages respiratory chain complex I, and further exacerbates oxidative injury (72, 73). Notably, chronic hyperglycemia may drive TXNIP-mediated sustained ROS accumulation and progressive ATP depletion, ultimately causing excessive mitochondrial elimination and worsening cellular dysfunction (74).

In the aforementioned mitochondrial dynamics disorders and related pathological cascades, mitophagy is the core mechanism for clearing damaged mitochondria, and its function directly affects cellular homeostasis. Under normal circumstances, it maintains mitochondrial population homeostasis by degrading abnormal mitochondria; however, in the hyperglycemic environment of DR, mitophagy may become functionally imbalanced due to flux overload, pathway inhibition, or excessive activation, which not only fails to prevent the accumulation of damaged mitochondria but also amplifies oxidative stress and exacerbates BRB damage. Therefore, clarifying the molecular mechanisms and pathological changes of mitophagy is crucial for understanding the progression of DR and developing targeted therapeutic strategies.

3 Mechanisms of mitophagy

Under mitochondrial damage conditions, mitophagy becomes an essential cellular quality control mechanism and includes two main categories: the ubiquitin (Ub)-dependent and Ub-independent pathways.

Ub-dependent pathway: This primarily involves the PINK1/Parkin pathway (75). Parkin is a cytosolic E3 ubiquitin ligase that links Ub molecules to substrate proteins. The identification of cytosolic ubiquitinated substrates of Parkin has advanced, and studies have demonstrated that Parkin plays a protective role in stabilizing mitochondrial function and morphology (76). Early

studies regarded PINK1 as a protein homologous to PTEN, which is believed to be associated with tumors and Parkinson's disease (77). PINK1 is widely recognized as a highly conserved mitochondrial protein involved in various cellular and physiological processes, primarily regulating mitochondrial function (78). PINK1 is autophosphorylated in the outer mitochondrial membrane (OMM), which is crucial for its activation and accumulation in mitochondria (79). In healthy mitochondria, PINK1 is degraded by other proteins after entering the inner membrane, resulting in low detectable levels (80). However, when mitochondria are damaged, changes in membrane structure or potential ($\Delta\Psi$m) inhibit PINK1 degradation, blocking its entry into the inner membrane, leading to increased accumulation of PINK1 on the OMM (81). The accumulation of PINK1 in the OMM promotes the translocation of Parkin to the mitochondria, thereby inducing mitophagy in dysfunctional mitochondria (82). Additionally, PINK1 phosphorylates ubiquitin at the Ser65 site to activate Parkin's ubiquitin ligase activity, recruiting more autophagy receptors. The recruitment of receptors such as NDP52 and OPTN can facilitate mitophagy (83). Subsequently, light chain 3 (LC3) is recruited to damaged mitochondria through DFCP1, allowing OPTN to mediate the initiation of autophagosomes through LC3 interaction regions (LIR) (84, 85). Ubiquitinated mitochondria can be recognized by the ubiquitin-binding protein P62. Subsequently, P62 binds to microtubule-associated LC3, allowing damaged mitochondria to be engulfed by autophagosomes and ultimately degraded by lysosomes. This process promotes mitophagy (86).

Unlike the PINK1/Parkin pathway, the BNIP3/Nix pathway mediates mitophagy independently of ubiquitination.Bcl-2 and adenovirus E1B 19-kDa interacting protein 3 (BNIP3) and BNIP3-like protein (BNIP3L, also known as Nix) are homologous members of the Bcl-2 family of proteins (87). They are located on the OMM and were initially classified as apoptotic proteins, functioning as engulfment receptors under cellular developmental or pathological conditions.BNIP3 is present at low abundance in cells but is upregulated under hypoxia. Hypoxia-inducible factor-1α (HIF-1α), an important transcription factor affecting mitophagy, can upregulate BNIP3 and NIX under hypoxic conditions, thereby clearing excessive mitochondria and maintaining cell viability (88). BNIP3 acts as a hypoxia-induced mitochondrial connector that directly targets mitochondrial structures (89). The NIX protein directly binds LC3 through its BH3 domain and induces mitophagy (90, 91). Both BNIP3 and NIX contain LC3 interaction regions (LIRs) at their N-termini, which bind to the homolog of autophagy-related gene 8 and induce mitophagy (92). NIX and BNIP3 also interact with Bcl-2, thereby increasing the cytoplasmic levels of free Beclin-1 and promoting the activation of autophagy-related gene 5, ultimately initiating mitophagy (92). Fun14 domain-containing protein 1 (FUNDC1) is another protein located on the OMM that, similar to BNIP3/NIX, can induce mitophagy by binding to LC3 through its LIR under hypoxic conditions (93) (Figure 2).

Mitophagy plays a central role in clearing damaged mitochondria through both ubiquitin-dependent (PINK1/Parkin

pathway) and -independent (BNIP3/Nix and FUNDC1 pathways) mechanisms, maintaining cellular homeostasis and regulating apoptosis and inflammation. In DR, metabolic disorders induced by hyperglycemia directly interfere with the normal functioning of these mechanisms in cells, leading to mitophagy dysfunction and subsequent BRB damage. This article specifically elaborates on the pathological changes in mitophagy in the core constituent cells of the BRB (such as RPE cells, RECs, pericytes, and Müller cells) under DR conditions and their impact on barrier integrity.

4 Mitophagy dysfunction in BRB constituent cells: a mechanism underlying barrier injury in DR

Increasing evidence suggests that mitochondrial dysfunction plays a key role in the pathogenesis of DR. Specifically, excessive mtROS production, mtDNA damage, ETC damage, and inefficient mtDNA repair mechanisms caused by mitochondrial dynamic imbalance all contribute to DR (94). In particular, mitophagy, as the core mechanism for clearing damaged mitochondria, is crucial for maintaining the homeostasis of BRB constituent cells. Under high-glucose conditions, mitophagy in cells may undergo bidirectional changes. Excessive mitophagy can remove essential organelles and proteins, leading to a loss of compensatory capacity and ultimately resulting in apoptosis (95). Conversely, a decrease in

mitophagy can lead to the accumulation of damaged mitochondria, resulting in BRB degradation (10).

4.1 BRB damage caused by excessive activation of mitophagy

RECs are the main components of the iBRB, and their dysfunction plays an important role in the pathogenesis of DR. Hyperglycemia disrupts mitochondrial homeostasis in RECs, leading to their dysfunction. The destruction of connections between adjacent RECs and the apoptosis of RECs are the primary drivers of the acellular capillary formation and the subsequent destruction of the iBRB during NPDR (96). Studies on HG intervention in rat RECs revealed that HG upregulates dynamin-related protein 1 (Drp1). Following its activation, Drp1 induces excessive mitochondrial fission, generating numerous fragmented mitochondria. These fragmented mitochondria are prone to damage, releasing ROS and triggering mitophagy, which ultimately leads to an elevated apoptosis rate in RECs (97). This directly disrupts the BRB, increasing vascular permeability. Additionally, oxidative stress further damages intercellular tight junctions, exacerbating vascular leakage of the BRB. Similarly, another study showed that mitophagy increased in retinal Müller cells cultured under HG conditions. The possible mechanism is that TXNIP induces mitochondrial oxidative stress and dysfunction,
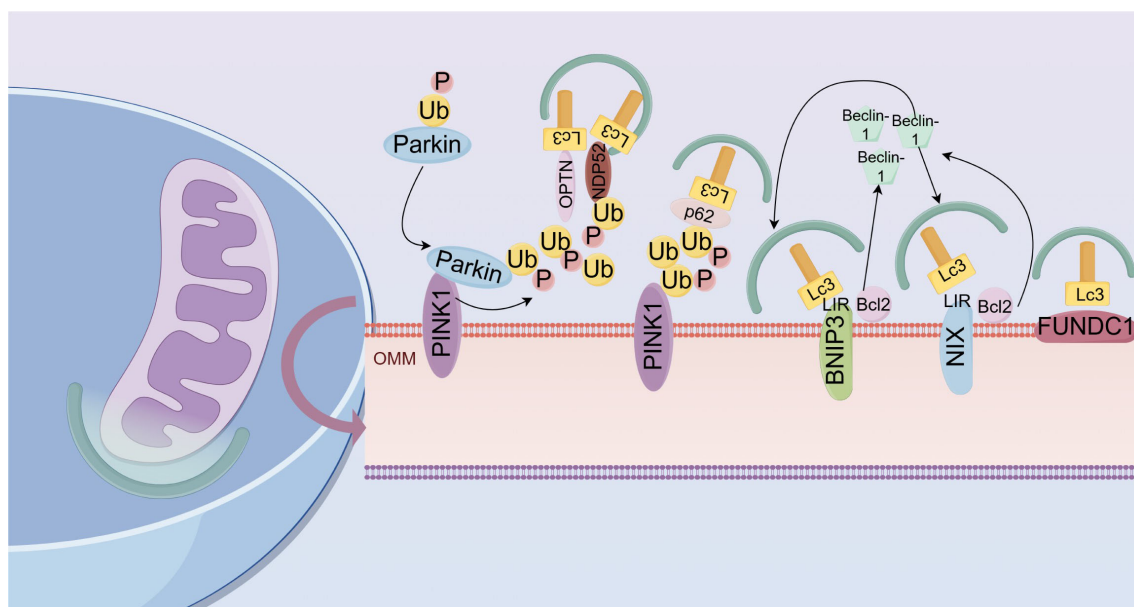

FIGURE 2
Schematic diagram of the mechanisms of mitophagy: Mitophagy is regulated through both ubiquitin (Ub)-dependent and -independent pathways. The Ub-dependent pathway is dominated by the PINK1/Parkin pathway: When mitochondria are damaged, PINK1 accumulates on the outer mitochondrial membrane (OMM) and phosphorylates Ub, activating Parkin's ubiquitin ligase activity, promoting the ubiquitination of OMM proteins; the ubiquitination signal recruits autophagy receptors (NDP52, OPTN), connecting to autophagosomes through LC3 interaction regions (LIR), which are ultimately degraded by lysosomes. The Ub-independent pathway includes BNIP3/Nix and FUNDC1 pathways: BNIP3 and NIX interact with Atg8 on autophagosomes through their LIR, thereby inducing mitophagy. NIX and BNIP3 can also interact with Bcl-2, increasing the cytoplasmic levels of free Beclin-1 and promoting the activation of Atg5, ultimately initiating mitophagy. Furthermore, FUNDC1 is another protein located on the OMM that can induce mitophagy by binding to LC3 under hypoxic conditions.

promoting Drp1-mediated fission and Parkin-mediated ubiquitination. Simultaneously, TXNIP inhibits ATG4B, enhancing autophagosome formation and inducing excessive mitophagy (98),ultimately leading to Müller cell dysfunction. As critical BRB supporting cells, their dysfunction impairs the metabolic and trophic support to RECs and retinal neurons, further compromising BRB integrity.

4.2 BRB damage caused by inhibition of mitophagy

However, many scholars have observed the inhibitory effects of mitophagy pathways in DR. HG induces mitochondrial fission by activating PKCδ/Drp1 in RECs, resulting in damaged mitochondria. Additionally, HG phosphorylates Drp1 via PKCδ, triggering the dissociation of HK-II from the outer mitochondrial membrane and blocking HK-II-mediated activation of the PINK1/Parkin pathway. As a result, HK-II-mediated PINK1/Parkin mitophagy is inhibited, leading to RECs apoptosis and subsequent damage to the barrier (99). Moreover, in microglia treated with HG (BV2 cell model), HG upregulated Poldip2 expression, promoting the ubiquitination degradation of Pink1 and inhibiting mitophagy, leading to the accumulation of dysfunctional mitochondria that cannot be cleared (100). Damaged mitochondria accumulated in microglia trigger oxidative stress and endoplasmic reticulum stress. These stress responses activate microglia to polarize toward the pro-inflammatory M1 type, releasing large amounts of inflammatory cytokines including IL-6 and TNF-α. These cytokines damage the tight junctions of the BRB, increasing vascular permeability and leading to pathological changes such as BRB dysfunction, vascular leakage, and neovascularization. Research using AGEs to simulate the diabetic microenvironment in rat Müller cells observed similar trends in mitophagy changes. Specifically, levels of TOM20, LC3II/LC3I, PINK1, and Parkin proteins were significantly downregulated, whereas P62 levels were elevated. These changes indicate that AGEs inhibit the mitophagy function of Müller cells (101). Excessive ROS production induces apoptosis and glial activation in Müller cells. As key supporting cells of the BRB, Müller cell glial activation disrupts the physical connections and functional synergy of retinal vascular endothelial cells, weakening the structural stability of the BRB. Increased oxidative stress and the release of inflammatory cytokines enhance retinal vascular leakage, ultimately leading to BRB functional collapse.

Treatment with HG (50 mM) reduces PINK1/Parkin signaling in the RPE by elevating ROS levels, leading to increased apoptosis of the RPE, reduced proliferation, and exacerbated oxidative stress, ultimately compromising the integrity of the BRB (67). Sirtuin 3 (SIRT3) is a mitochondrial NAD+-dependent deacetylase that plays a key role in mitochondrial metabolic regulation (102). Studies have found that HG leads to decreased SIRT3 expression, inhibiting the AMPK/mTOR/ULK1 pathway and resulting in reduced mitophagy (103). Inhibition of mitophagy triggers ROS accumulation and apoptosis in RPE, potentially compromising the integrity of the BRB. HG causes the accumulation of Telomeric Repeat-Binding

Factor 1 (TRF1)-interacting protein 2 (TIN2) in mitochondria in RPE cells. The accumulation of TIN2 reduces the expression of mitophagy-related proteins Microtubule-associated protein 1 light chain 3 beta(LC3B), PINK1, and Parkin, thereby inhibiting mitophagy. The inhibition of mitophagy disrupts the tight junctions of RPE cells, leading to structural damage of the BRB (104). The accumulation of AGEs caused by hyperglycemia is a major factor in the development of DR, and Methylglyoxal(MGO) is a precursor of AGEs (105). MGO levels in diabetic patients are higher than those in healthy controls (106), potentially leading to impaired retinal blood flow regulation in patients with DR (107) MGO suppresses protein and gene expression of mitochondrial fusion protein-1, peroxisome proliferator-activated receptor gamma coactivator 1-alpha, and mitochondrial transcription factor A, thereby reducing mitochondrial biogenesis and fusion. Concurrently, MGO inhibits AMP-activated protein kinase (AMPK) activity, decreases LC3-II accumulation, and impairs mitophagy in RPE cells, ultimately leading to RPE cells death (108).

The inconsistencies in cellular mitophagy changes in DR may be owing to the level of mitophagy depending on the degree of hyperglycemia. Zhang et al. observed that in cultured retinal pigment epithelial cells, a slight increase in glucose concentration (15 mM) induced upregulation of mitophagy, whereas a significant increase (50 mM) inhibited mitophagy, leading to apoptosis (67). The corresponding mechanism may be that mild hyperglycemia induces a stress response, prompting cells to clear damaged mitochondria through mitophagy. Conversely, severe or persistent hyperglycemia causes cellular damage, leading to mitophagy dysfunction. Additionally, the duration of diabetes appears to play a crucial role, as studies on human retinas, mice, and primary Müller cells have demonstrated that prolonged diabetes progressively suppresses Pink1-dependent mitophagy, causing the accumulation of damaged mitochondria and eventually leading to BRB breakdown (10). Furthermore, as the duration of diabetes increases, aging may also affect mitophagy, as late-stage DR retinas show increased activity of senescence-associated β-galactosidase (SA-β-Gal). *In vitro* studies have shown that in continuously aged Müller cells, high-glucose levels, hyperosmolarity, or starvation fail to activate autophagy (10) Figure 3.

5 Therapeutic strategies targeting mitophagy dysfunction in BRB constituent cells: protecting against BRB injury in DR

Intensified control of blood pressure and blood glucose levels reduces the incidence or slows the progression of microvascular complications in diabetic patients (e.g., DR), thereby preserving visual stability (109). Furthermore, a meta-analysis demonstrated that maintaining adequate glycemic control may mitigate the risk of progression to PDR and other vision-threatening complications, irrespective of baseline DR severity (110). Notably, DME, which is characterized by BRB disruption during DR progression, represents
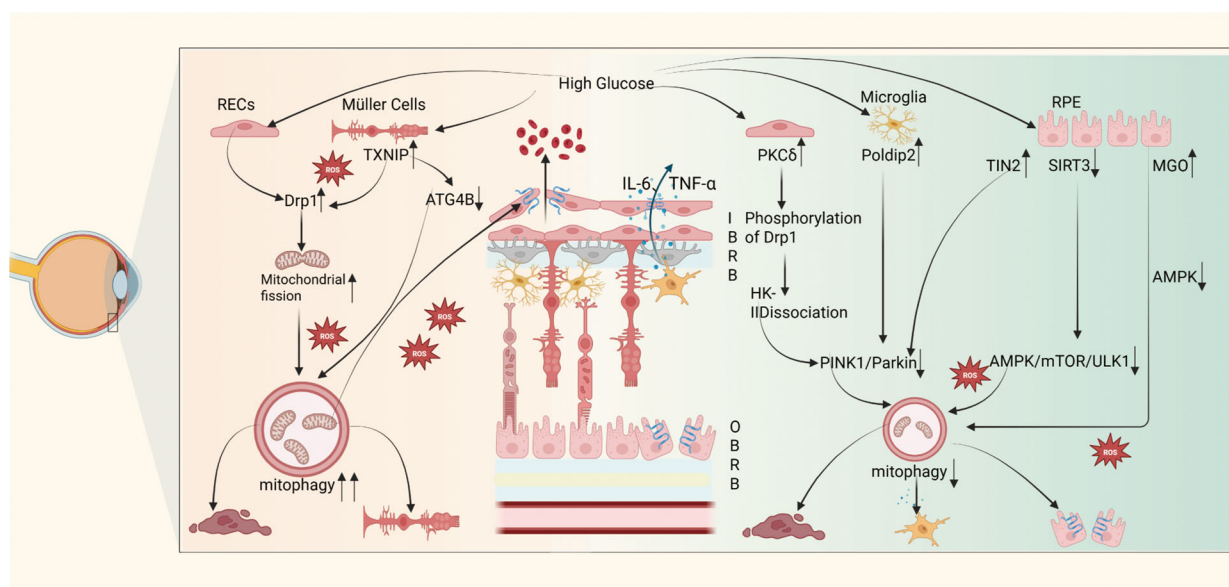
FIGURE 3

HG induces impairment of mitophagy in BRB constituent cells, contributing to BRB disruption in DR. The left panel depicts HG-promoted mitochondrial fission, resulting in oxidative stress and excessive mitophagy activation, ultimately culminating in RECs apoptosis and Müller cell death. In RECs, HG phosphorylates Drp1 via PKCδ, triggering HK-II dissociation from mitochondria and blockade of the PINK1/Parkin pathway, thereby suppressing mitophagy, inducing damaged mitochondrial accumulation, and promoting REC apoptosis. In microglia, HG promotes pro-inflammatory M1 polarization, inducing substantial secretion of inflammatory cytokines (e.g., IL-6, TNF-α), thereby compromising BRB tight junctions and increasing vascular permeability. In RPE cells, HG inhibits mitophagy through suppression of both PINK1/Parkin and AMPK/mTOR/ULK1 pathways, driving RPE apoptosis and tight junction disruption. Collectively, these HG-driven impairments compromise BRB integrity and increase vascular permeability.

the leading cause of vision loss in patients with DR (111). Currently, several new methods for treating DME have been introduced, with anti-VEGF drugs being the first-line treatment for center-involved DME. Although anti-VEGF drugs maintain the integrity of the BRB by inhibiting VEGF-A and/or placental growth factor, some patients show a poor response to this treatment in clinical practice. Studies have shown that 31.6% to 65.6% of DME patients still exhibit persistent edema symptoms after receiving at least four regular intravitreal injections within 24 weeks (112). Furthermore, repeated injections are often required for many patients, which may pose economic burdens. Real-world evidence suggests that the mean annual injection frequency is approximately 3.1 sessions per patient, with about 68.6% of patients receiving ≤3 injections, generally below the dosing schedules used in clinical trials (typically 9–12 sessions) (113). For individuals with PDR, laser photocoagulation continues to be widely utilized as a therapeutic modality (114). It should be noted that laser treatment can sometimes be associated with discomfort, and extensive applications might potentially affect peripheral visual fields, particularly when involving central macular areas (115). Given the limitations of the aforementioned treatment methods, identifying new approaches to treat DR is particularly important. The previous discussion highlighted the key role of the BRB in retinal stability and the damage caused by mitophagy dysregulation in DR. The following section introduces existing research on maintaining cellular homeostasis by regulating mitophagy, aiming to provide new strategies for DR treatment from the perspective of preserving BRB integrity (Table 1).

5.1 Inhibiting mitophagy in BRB constituent cells to improve BRB damage in DR

Melatonin, the primary hormone of the pineal gland, is also secreted by RECs (116). Functioning as an intracellular antioxidant and modulator of mitochondrial bioenergetic function, it traverses mitochondrial membranes, supporting its potential as a therapeutic agent for mitochondrial dysfunction-related diseases such as DR (117). In an *in vitro* study of a DME model, melatonin was found to reduce the expression of HIF-1α, HIF-1β, VEGF, and VEGF receptor genes, thereby preventing increased cell permeability and damage to the oBRB (118). Additionally, melatonin inhibits the expression of mitophagy-related genes (PINK, BNIP3, and NIX), thereby preventing excessive activation of mitophagy and maintaining mitochondrial homeostasis (119), reducing RPE cells apoptosis and alleviating BRB leakage. HG upregulates TXNIP in both rMC-1 cells and Müller cells of diabetic rats, leading to excessive activation of mitophagy. Knocking out TXNIP using CRISPR/Cas9 or intravitreal injection of TXNIP siRNA can inhibit this excessive activation. This intervention reduces Müller cell apoptosis and mitigates gliosis mediated by Glial Fibrillary Acidic Protein (GFAP) overexpression, potentially helping to maintain the supportive function of Müller cells for the BRB and mitigate structural damage to the BRB in diabetic retinopathy (98). HG also promotes apoptosis in ARPE-19 cells through upregulation of TXNIP. Knocking out TXNIP using TXNIP short hairpin RNA can significantly inhibit the excessive enhancement of mitophagy flux induced by high-glucose and alleviate mitochondrial fragmentation. This intervention restores

TABLE 1 Protective effects of targeting mitophagy in BRB constituent cells on the BRB in DR.

Drug/targets	Models (cells or animals)/intervention measures	Influence pathways	Effects on mitophagy	Findings	Reference
Inhibition of mitophagy					
Melatonin	ARPE-19 (pretreated with HG+ deferoxamine mesylate, followed by melatonin treatment for 48 hours)	HIF-1α, HIF-1β, VEGF ↓; DRP1↓;PINK, BNip3, NIX↓; PGC-1α, NRF2↑	Inhibition of overactivated mitophagy	Melatonin reduces RPE cells apoptosis, alleviates BRB leakage, and enhances BRB integrity.	(118)
TXNIP	rMC1/STZ-induced SD rats (TXNIP knockout cell line/ intravitreal injection of TXNIP siRNA)	ROS↓;DRP1↓;LC3BII puncta , Parkin↓; co - localization of COXIV and LAMP2A↓, OPTN, p62↓;GFAP↓	Inhibition of overactivated mitophagy	Knocking out TXNIP reduces apoptosis and gliosis in Müller cells, thereby BRB integrity and mitigating structural damage to the BRB in DR.	(98)
TXNIP	ARPE-19 (TXNIP knockout strain shTXNIP3 + 4)	ROS↓;Restore Trx1/Trx2 function, inhibit mitochondrial fission and TBK1-mediated phosphorylation of autophagy adaptors	Reduced mitophagic flux impairs autophagic degradation of damaged mitochondria	Knocking out TXNIP reduces RPE cells apoptosis, restores proliferative capacity, maintains RPE layer integrity, and protects the barrier function of BRB.	(66)
WIF1	ARPE-19/STZ-induced diabetic C57BL/6J mice (treated with WIF1/intravitreal injection of WIF1)	AMPK/mTOR, PINK1/ Parkin↓;LC3-II/LC3-I , p62↓; ROS, MDA↓;SOD, GPX↑	Inhibition of overactivated mitophagy	WIF1 restores the function of RPE cells, reduces the formation of RECs tube, protects the integrity of BRB, and thickens all retinal layers	(120)
Promotion of mitophagy					
MSC-derived small extracellular vesicles (containing miR-125a-5p)	Rat Müller cells/STZ-induced SD rats (co-cultured with MSC-sEVs/intravitreally injected with MSC-sEVs)	PINK1/Parkin ↑;LC3II/LC3I , TOM20↑;P62 ↓; GFAP↓;occludin↑	Promotion of mitophagy	MSC-sEVs-miR-125a-5p reduce Müller cells apoptosis, restore their proliferative capacity, decrease retinal vascular leakage, and improve BRB integrity.	(101)
Leflunomide	HRECs(leflunomide added when normal blood glucose is restored after HG exposure)	Mfn2 ↑;ROS↓;LC3II/LC3I ↑	Promotion of mitophagy	Leflunomide reduces RECs apoptosis, improves their proliferative capacity, and protects BRB integrity.	(65)
Drp1	RMECs/STZ-induced SD rats (pretreated with Mdivi-1/ intravitreally injected with Mdivi-1)	HK-II↑; PINK1/Parkin↑;LC3B-II↑, p62↓	Promotion of mitophagy	Inhibiting Drp1 can reduce RMECs apoptosis and retinal vascular leakage, decrease the number of acellular capillaries, restore retinal thickness, and protect BRB integrity.	(99)
TIN2	ARPE-19/STZ-induced C57BL/6J diabetic mice (transfected with sh-TIN2/intravitreally injected with aav-shTIN2)	mTOR↓;PINK1/Parkin↑;LC3B-II↑, p62↓;SA-β-gal positive cells↓;ZO-1↑	Promotion of mitophagy	Knocking out TIN2 inhibits RPE cells senescence; alleviates oxidative stress, protects BRB integrity, and increases retinal thickness.	(104)
Sirt3	ARPE-19(Transfection withLV-Sirt3)	AMPK ↑, mTOR↓;ULK1↑, LC3B-II/LC3B-I ↑;ROS↓	Promotion of mitophagy	Sirt3 overexpression reduces RPE cells ROS and apoptosis, protects RPE cells integrity, and maintains BRB barrier function.	(103)
Poldip2	BV2 cells/STZ-induced diabetic SD rats (Transfection with Poldip2-siRNA/intravitreally injected with AAV9-Poldip2-shRNA)	AMPK/ULK1/Pink1/Parkin↑; IL-6, TNF-α ↓;VEGFR ↓;LC3B-II/LC3B-I ↑;p62 ↓	Promotion of mitophagy	Inhibition of Poldip2 increases microglial M2 polarization, reduces cytokines factors, decreases retinal vascular leakage, inhibits neovascularization, and protects BRB integrity.	(100)

(Continued)

TABLE 1   Continued

Drug/targets	Models (cells or animals)/intervention measures	Influence pathways	Effects on mitophagy	Findings	Reference
Promotion of mitophagy					
TGR5	RMECs/STZ-induced SD rats (pretreated with INT-777/ intravitreally injected with INT-777)	PKCδ/Drp1 ↓;HK2/PINK1/ Parkin↑;LC3B-II/LC3B-I ↑; p62 ↓	Promotion of mitophagy	Activating TGR5 reduces retinal vascular leakage, RECs apoptosis and acellular capillaries, restores retinal thickness, and maintains BRB integrity.	(121)
VDAC1	HRCECs (Transfection with VDAC1- overexpressing adenovirus)	PINK1/Parkin↑; mtROS ↓; NLRP3 ↓	Promotion of mitophagy	VDAC1 overexpression reduces HRCECs proliferation, migration and tube formation, promotes apoptosis, and protects BRB integrity.	(122)
NGR1	rMC-1 cells/db/db mice (NGR1 pretreatment/oral administration of 30 mg/kg NGR1 for 12 weeks)	PINK1/Parkin↑; LC3-II/LC3-I ↑;VEGF↓;PEDF↑	Promotion of mitophagy	NGR1 reduces Müller cells apoptosis, inhibits inflammatory cytokines release, increases retinal thickness, alleviates vascular leakage, and protects BRB integrity.	(123)
Alc	High-fat diet + STZ-induced SD rats (Alc, 16 mg/kg/day)	PINK1/Parkin↑; MDA↓;SOD, GPx↑;NLRP3↑	Promotion of mitophagy	Alc restores retinal layer thickness, alleviates vascular lesions, reduces retinal ganglion cell degeneration, protects BRB structural integrity, and decreases permeability.	(124)
Heyingwuzi formulation	HRCECs/STZ-induced C57BL/6 diabetic mice (treated with 10% HYWZF serum/administered with 12 g/kg or 24 g/ kg HYWZF)	HIF-1α/BNIP3/NIX↑;LC3II/ LC3I ↑;P62↓;claudin-5↑;VEGF↓	Promotion of mitophagy	HYWZF reduces RECs apoptosis, restores their function, enhances tight junctions, reduces BRB permeability, restores retinal thickness, and decreases acellular capillaries.	(126)

the antioxidant function of thioredoxins (Trx1, Trx2), reduces ROS accumulation, alleviates increased lysosomal membrane permeability, and prevents the inactivation of tissue cathepsin L. Consequently, decreased RPE cells apoptosis and enhanced cell viability may contribute to maintained BRB structural integrity (66). Wnt inhibitory factor 1 (WIF1) is a gene reported to inhibit the Wnt/β-catenin signaling pathway. Initially discovered in human retinas, it is involved in regulating cell proliferation and tissue homeostasis. HG reduces the expression of WIF1, leading to excessive activation of mitophagy. Recombinant WIF1 protein downregulates the expression of mitophagy-related proteins in STZ-induced diabetic mice, including Parkin, PINK1, and the LC3-II/LC3-I ratio, inhibiting excessive activation of mitophagy (120). This helps restore RPE cells function, downregulate VEGFA expression, reduce tube formation in retinal endothelial cells, and maintain the integrity of the BRB.

5.2 Increasing mitophagy in BRB constituent cells to improve BRB damage in DR

In AGEs-induced Müller cells and STZ rat models, small extracellular vesicles from MSCs (which contain miR-125a-5p)

activate PINK1/Parkin-mediated mitophagy by inhibiting PTP1B expression, significantly increasing the LC3-II/LC3-I ratio while decreasing levels of p62 protein and the tight junction protein occludin. Ultimately, these changes alleviate glial cell activation and reduce vascular leakage (101). Leflunomide acts on human retinal endothelial cells under high-glucose conditions *in vitro*, promoting the expression of the mitochondrial fusion protein mitofusin 2 (Mfn2), reversing Drp1-mediated excessive fission, restoring mitophagy flow, and increasing the expression of the tight junction protein ZO-1, which helps maintain the structural integrity of the BRB (65). Studies have shown that HG promotes Drp1 phosphorylation, leading to reduced mitochondrial fission, separation of HK-II from mitochondria, and inhibition of PINK1/Parkin-mediated mitophagy. Notably, Mdivi-1 (a Drp1 inhibitor) and rapamycin (an autophagy agonist) can reverse the above phenomena. Pretreatment with Mdivi-1 or rapamycin can reduce mitochondrial fission, enhance PINK1/Parkin-mediated mitophagy, decrease RMEC apoptosis and retinal vascular leakage, and reduce the number of acellular capillaries, thereby protecting BRB integrity (99). Research has shown that both in diabetic mice and under hyperglycemic conditions *in vitro*,TIN2 exacerbates the aging of RPE cells, TIN2 overexpression activates the mTOR signaling pathway and suppresses PINK1/Parkin-mediated mitophagy in ARPE-19 cells

under high-glucose conditions. Conversely, knocking out TIN2 or using rapamycin reduced RPE cells aging, restored ZO-1 expression, increased retinal thickness, alleviated oxidative stress, and preserved BRB integrity (104).

Additionally, the decreased expression of SIRT3 caused by HG inhibits mitophagy. *In vitro* studies, overexpression of SIRT3 enhanced mitophagy in ARPE-19, reduced ROS production, decreased RPE cells apoptosis, and potentially maintained the barrier function (103). HG conditions *in vitro* induces the upregulation of Poldip2 expression in microglia, which directly obstructs the initiation and execution of mitophagy, leading to the accumulation of damaged mitochondria that cannot be cleared in time. Interventions targeting Poldip2 (such as knocking out Poldip2) can restore the activity of the AMPK/ULK1/PINK1 pathway, enhance mitophagy to clear damaged mitochondria, increase M2 polarization of microglia, reduce inflammatory cytokines, decrease retinal vascular leakage, and inhibit ocular neovascularization (lowering VEGFR) (100). Similarly, research teams have found that the membrane G protein-coupled bile acid receptor 5 (TGR5) enhances mitophagy and inhibits mitochondrial fission by regulating the PKCδ/Drp1-HK2 signaling pathway in STZ-induced SD rat models and HG-induced human retinal endothelial cells (RMEC). This mechanism reduces retinal vascular leakage, decreases the number of acellular capillaries, restores retinal thickness, reduces endothelial cell apoptosis, and maintains BRB integrity (121). Subsequently, in HG-induced human retinal capillary endothelial cells (HRCECs), increased expression of Drp1, decreased expression of MFN2, increased mtROS, and reduced expression of PINK1, Parkin, and VDAC1 proteins were observed. Further studies indicated that overexpression of VDAC1 could promote PINK1 expression and inhibit NLRP3 activation. Therefore, it is concluded that VDAC1 may be a potential target for the prevention and treatment of DR (122).

Ginsenoside R1 (NGR1) is a novel saponin extracted from Panax notoginseng with pharmacological properties. NGR1 pretreatment upregulates the levels of PINK1 and Parkin in db/db mouse retinas. It also increases the LC3-II/LC3-I ratio and downregulates the levels of p62/SQSTM1. These changes collectively enhance mitophagy via the PINK1/Parkin pathway (123). In these models, NGR1 reduces Müller cell apoptosis, lowers VEGF levels, increases PEDF expression, and inhibits the release of inflammatory cytokines. These preclinical findings demonstrate that NGR1 improves retinal function, increases retinal thickness, and attenuates vascular leakage under experimental conditions, thus potentially exerting protective effects on BRB integrity. Allicin (Alc) is a natural compound found in garlic that is gaining attention for its antioxidant and anti-inflammatory properties. Studies have shown that Alc promotes the expression of mitophagy-related proteins such as PINK1 and Parkin in DR rats, enhances mitophagy, reduces pro-inflammatory cytokines levels, and alleviates oxidative stress (124). These effects restore the thickness of various retinal layers, reduce vascular lesions, and decrease degeneration of retinal ganglion cells, thereby lowering BRB permeability. Recent studies have indicated that the HIF-1α/BNIP3/NIX pathway is associated with restoring

autophagy in degenerated retinas, alleviating oxidative stress, and preventing diabetic retinopathy (125, 126). The traditional Chinese medicine Heyingwuzi formulation (HYWZF) inhibits excessive ROS production, cell apoptosis, tube formation, and invasion in HG-induced HRCECs by promoting mitophagy. After HYWZF treatment, the expression levels of the tight junction protein claudin-5, HIF-1α, Beclin1, BNIP3, and BNIP3L in mice were significantly higher than in the model group. The results indicate that HYWZF increases mitophagy through the HIF-1α/BNIP3/NIX axis, reduces apoptosis of retinal endothelial cells, increases tight junction protein levels, downregulates VEGF, decreases the number of acellular capillaries, reduces BRB permeability, and alleviates retinal tissue damage (126).

6 Discussion

Mitophagy is a selective degradation mechanism targeting dysfunctional mitochondria. It participates in mitochondrial quality control and maintains cellular homeostasis. Current evidence indicates that dysfunctional mitophagy is associated with multiple diseases, including DR. Therefore, targeting the mitophagy pathway may hold therapeutic potential.

We summarize the effects of targeting mitophagy on BRB in DR. The results show that under high-glucose conditions, mitophagy in cells exhibits bidirectional changes, rather than simply increasing or decreasing. Mitophagy may be excessively activated, leading to the clearance of necessary organelles and proteins, causing cells to lose compensatory capacity and ultimately undergo apoptosis. Conversely, a decrease in mitophagy can lead to the accumulation of damaged mitochondria. Whether through excessive activation or inhibition, either condition can ultimately lead to BRB degradation. This difference may be owing to variations in induction methods and the use of different animal or cell models across various studies, leading to inconsistent results. Mild hyperglycemia can induce a stress response that excessively activates mitophagy, leading to the clearance of damaged mitochondria. Conversely, severe or persistent hyperglycemia leads to cellular damage, resulting in mitophagy dysfunction. Additionally, the duration of diabetes affects mitophagy function. With prolonged diabetes duration, mitophagy gradually decreases, which may be related to aging, as aging inhibits its activation.

Mitophagy acts as a key regulator in DR. Existing drug and target interventions can improve BRB damage in DR by promoting or inhibiting mitophagy, providing an effective strategy for the precise regulation of DR. Current research has confirmed this feasibility through cellular and animal models (such as rats and mice), demonstrating high translational value. In particular, MSC-EVs therapy has reached the *in vitro* validation phase, with preliminary evidence demonstrating its ability to penetrate the BRB and deliver miR-125a-5p to Müller cells, potentially circumventing systemic drug side effects. Leflunomide, an FDA-approved anti-rheumatic drug, has been shown in animal studies to activate Mfn2 and ameliorate DR pathology, suggesting potential

for accelerated clinical translation. Moreover, preclinical studies suggest that targeting molecules such as TXNIP, Drp1, and TIN2, or applying bioactive natural compounds like NGRI and Alc, may exert therapeutic potential by modulating mitophagic imbalance in BRB cells. These strategies merit further mechanistic and efficacy validation in subsequent research. Future research should focus on several aspects. First, retinal imaging technologies (such as OCT-A) should be combined to assess the state of mitophagy in patients and guide drug selection. Second, BRB-specific drug delivery systems should be developed, such as nanoparticles targeting Müller or RPE cells. Third, integrating multi-omics technologies to screen for new targets, such as compounds derived from the TXNIP pathway, can enhance treatment specificity and reduce side effects to support the development of DR treatment.

Author contributions

ML: Writing – original draft, Conceptualization. LY: Writing – original draft, Conceptualization. HZ: Writing – original draft. LQ: Writing – review & editing. ZW: Writing – review & editing. XA: Writing – review & editing, Conceptualization. JW: Conceptualization, Writing – review & editing.

Funding

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Ogurtsova K, da Rocha Fernandes JD, Huang Y, Linnenkamp U, Guariguata L, Cho NH, et al. IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes Res Clin Pract*. (2017) 128:40–50. doi: 10.1016/j.diabres.2017.03.024

2. Yau JW, Rogers SL, Kawasaki R, Lamoureux EL, Kowalski JW, Bek T, et al. Global prevalence and major risk factors of diabetic retinopathy. *Diabetes Care*. (2012) 35:556–64. doi: 10.2337/dc11-1909

3. Kang Q, Yang C. Oxidative stress and diabetic retinopathy: Molecular mechanisms, pathogenetic role and therapeutic implications. *Redox Biol*. (2020) 37:101799. doi: 10.1016/j.redox.2020.101799

4. Shin ES, Sorenson CM, Sheibani N. Diabetes and retinal vascular dysfunction. *J Ophthalmic Vis Res*. (2014) 9:362–73. doi: 10.4103/2008-322x.143378

5. Díaz-Coránguez M, Ramos C, Antonetti DA. The inner blood-retinal barrier: Cellular basis and development. *Vision Res*. (2017) 139:123–37. doi: 10.1016/j.visres.2017.05.009

6. Yang X, Yu XW, Zhang DD, Fan ZG. Blood-retinal barrier as a converging pivot in understanding the initiation and development of retinal diseases. *Chin Med J (Engl)*. (2020) 133:2586–94. doi: 10.1097/cm9.0000000000001015

7. Tan TE, Wong TY. Diabetic retinopathy: Looking forward to 2030. *Front Endocrinol (Lausanne)*. (2022) 13:1077669. doi: 10.3389/fendo.2022.1077669

8. Gonzalez VH, Campbell J, Holekamp NM, Kiss S, Loewenstein A, Augustin AJ, et al. Early and long-term responses to anti-vascular endothelial growth factor therapy in diabetic macular edema: analysis of protocol I data. *Am J Ophthalmol*. (2016) 172:72–9. doi: 10.1016/j.ajo.2016.09.012

9. D'Amico AG, Maugeri G, Magrì B, Bucolo C, D'Agata V. Targeting the PINK1/Parkin pathway: A new perspective in the prevention and therapy of diabetic retinopathy. *Exp Eye Res*. (2024) 247:110024. doi: 10.1016/j.exer.2024.110024

10. Hombrebueno JR, Cairns L, Dutton LR, Lyons TJ, Brazil DP, Moynagh P, et al. Uncoupled turnover disrupts mitochondrial quality control in diabetic retinopathy. *JCI Insight*. (2019) 4:e129720. doi: 10.1172/jci.insight.129760

11. O'Leary F, Campbell M. The blood-retina barrier in health and disease. *FEBS J*. (2023) 290:878–91. doi: 10.1111/febs.16330

12. Yemanyi F, Bora K, Blomfield AK, Wang Z, Chen J. Wnt signaling in inner blood-retinal barrier maintenance. *Int J Mol Sci*. (2021) 22:11877. doi: 10.3390/ijms222111877

13. Gardiner TA, Archer DB, Curtis TM, Stitt AW. Arteriolar involvement in the microvascular lesions of diabetic retinopathy: implications for pathogenesis. *Microcirculation*. (2007) 14:25–38. doi: 10.1080/10739680601072123

14. Liu L, Liu X. Roles of drug transporters in blood-retinal barrier. *Adv Exp Med Biol*. (2019) 1141:467–504. doi: 10.1007/978-981-13-7647-4_10

15. Iyer S, Ferreri DM, DeCocco NC, Minnear FL, Vincent PA. VE-cadherin-p120 interaction is required for maintenance of endothelial barrier function. *Am J Physiol Lung Cell Mol Physiol*. (2004) 286:L1143–1153. doi: 10.1152/ajplung.00305.2003

16. Nian S, Lo ACY, Mi Y, Ren K, Yang D. Neurovascular unit in diabetic retinopathy: pathophysiological roles and potential therapeutical targets. *Eye Vis (Lond)*. (2021) 8:15. doi: 10.1186/s40662-021-00239-1

17. Ji L, Tian H, Webster KA, Li W. Neurovascular regulation in diabetic retinopathy and emerging therapies. *Cell Mol Life Sci*. (2021) 78:5977–85. doi: 10.1007/s00018-021-03893-9

18. Naylor A, Hopkins A, Hudson N, Campbell M. Tight junctions of the outer blood retina barrier. *Int J Mol Sci*. (2019) 21:211. doi: 10.3390/ijms21010211

19. Guerra MH, Yumnamcha T, Singh LP, Ibrahim AS. Relative contribution of different mitochondrial oxidative phosphorylation components to the retinal pigment epithelium barrier function: implications for RPE-related retinal diseases. *Int J Mol Sci*. (2021) 22:8130. doi: 10.3390/ijms22158130

20. Semeraro F, Morescalchi F, Cancarini A, Russo A, Rezzola S, Costagliola C. Diabetic retinopathy, a vascular and inflammatory disease: Therapeutic implications. *Diabetes Metab*. (2019) 45:517–27. doi: 10.1016/j.diabet.2019.04.002

21. Demircan N, Safran BG, Soylu M, Ozcan AA, Sizmaz S. Determination of vitreous interleukin-1 (IL-1) and tumour necrosis factor (TNF) levels in proliferative diabetic retinopathy. *Eye (Lond)*. (2006) 20:1366–9. doi: 10.1038/sj.eye.6702138

22. Kowluru RA, Odenbach S. Role of interleukin-1beta in the development of retinopathy in rats: effect of antioxidants. *Invest Ophthalmol Vis Sci*. (2004) 45:4161–6. doi: 10.1167/iovs.04-0633

23. Bamforth SD, Lightman SL, Greenwood J. Ultrastructural analysis of interleukin-1 beta-induced leukocyte recruitment to the rat retina. *Invest Ophthalmol Vis Sci*. (1997) 38:25–35.

24. Ben-Mahmud BM, Mann GE, Datti A, Orlacchio A, Kohner EM, Chibber R. Tumor necrosis factor-alpha in diabetic plasma increases the activity of core 2 GlcNAc-T and adherence of human leukocytes to retinal endothelial cells: significance of core 2 GlcNAc-T in diabetic retinopathy. *Diabetes*. (2004) 53:2968–76. doi: 10.2337/diabetes.53.11.2968

25. Saishin Y, Saishin Y, Takahashi K, Melia M, Vinores SA, Campochiaro PA. Inhibition of protein kinase C decreases prostaglandin-induced breakdown of the blood-retinal barrier. *J Cell Physiol*. (2003) 195:210–9. doi: 10.1002/jcp.10238

26. Xiang XH, Wei J, Wang XF, Xu Q, Yu CL, He CL, et al. Lychee seed polyphenol ameliorates DR via inhibiting inflammasome/apoptosis and angiogenesis in hRECs and db/db mice. *BioMed Pharmacother*. (2023) 167:115478. doi: 10.1016/j.biopha.2023.115478

27. Dagher Z, Park YS, Asnaghi V, Hoehn T, Gerhardinger C, Lorenzi M. Studies of rat and human retinas predict a role for the polyol pathway in human diabetic retinopathy. *Diabetes*. (2004) 53:2404–11. doi: 10.2337/diabetes.53.9.2404

28. Yang S, Zhao Y, Yu J, Fan Z, Gong ST, Tang H, et al. Sugar alcohols of polyol pathway serve as alarmins to mediate local-systemic innate immune communication in drosophila. *Cell Host Microbe*. (2019) 26:240–251.e248. doi: 10.1016/j.chom.2019.07.001

29. Yuan T, Yang T, Chen H, Fu D, Hu Y, Wang J, et al. New insights into oxidative stress and inflammation during diabetes mellitus-accelerated atherosclerosis. *Redox Biol*. (2019) 20:247–60. doi: 10.1016/j.redox.2018.09.025

30. Lassègue B, Clempus RE. Vascular NAD(P)H oxidases: specific features, expression, and regulation. *Am J Physiol Regul Integr Comp Physiol*. (2003) 285:R277–297. doi: 10.1152/ajpregu.00758.2002

31. Li C, Miao X, Li F, Wang S, Liu Q, Wang Y, et al. Oxidative stress-related mechanisms and antioxidant therapy in diabetic retinopathy. *Oxid Med Cell Longev*. (2017) 2017:9702820. doi: 10.1155/2017/9702820

32. Wu Y, Zou H. Research progress on mitochondrial dysfunction in diabetic retinopathy. *Antioxid (Basel)*. (2022) 11:2250. doi: 10.3390/antiox11112250

33. Donnelly R, Idris I, Forrester JV. Protein kinase C inhibition and diabetic retinopathy: a shot in the dark at translational research. *Br J Ophthalmol*. (2004) 88:145–51. doi: 10.1136/bjo.88.1.145

34. George A, Pushkaran S, Konstantinidis DG, Koochaki S, Malik P, Mohandas N, et al. Erythrocyte NADPH oxidase activity modulated by Rac GTPases, PKC, and plasma cytokines contributes to oxidative stress in sickle cell disease. *Blood*. (2013) 121:2099–107. doi: 10.1182/blood-2012-07-441188

35. Julius A, Hopper W. A non-invasive, multi-target approach to treat diabetic retinopathy. *BioMed Pharmacother*. (2019) 109:708–15. doi: 10.1016/j.biopha.2018.10.185

36. Yamagishi S, Nakamura K, Matsui T, Inagaki Y, Takenaka K, Jinnouchi Y, et al. Pigment epithelium-derived factor inhibits advanced glycation end product-induced retinal vascular hyperpermeability by blocking reactive oxygen species-mediated vascular endothelial growth factor expression. *J Biol Chem*. (2006) 281:20213–20. doi: 10.1074/jbc.M602110200

37. Zong H, Ward M, Madden A, Yong PH, Limb GA, Curtis TM, et al. Hyperglycaemia-induced pro-inflammatory responses by retinal Müller glia are regulated by the receptor for advanced glycation end-products (RAGE). *Diabetologia*. (2010) 53:2656–66. doi: 10.1007/s00125-010-1900-z

38. Guimarães EL, Empsen C, Geerts A, van Grunsven LA. Advanced glycation end products induce production of reactive oxygen species via the activation of NADPH oxidase in murine hepatic stellate cells. *J Hepatol*. (2010) 52:389–97. doi: 10.1016/j.jhep.2009.12.007

39. Zhang M, Kho AL, Anilkumar N, Chibber R, Pagano PJ, Shah AM, et al. Glycated proteins stimulate reactive oxygen species production in cardiac myocytes: involvement of Nox2 (gp91phox)-containing NADPH oxidase. *Circulation*. (2006) 113:1235–43. doi: 10.1161/circulationaha.105.581397

40. Moldogazieva NT, Mokhosoev IM, Mel'nikova TI, Porozov YB, Terentiev AA. Oxidative stress and advanced lipoxidation and glycation end products (ALEs and AGEs) in aging and age-related diseases. *Oxid Med Cell Longev*. (2019) 2019:3085756. doi: 10.1155/2019/3085756

41. Navaratna D, McGuire PG, Menicucci G, Das A. Proteolytic degradation of VE-cadherin alters the blood-retinal barrier in diabetes. *Diabetes*. (2007) 56:2380–7. doi: 10.2337/db06-1694

42. Kaneto H, Xu G, Song KH, Suzuma K, Bonner-Weir S, Sharma A, et al. Activation of the hexosamine pathway leads to deterioration of pancreatic beta-cell

function through the induction of oxidative stress. *J Biol Chem*. (2001) 276:31099–104. doi: 10.1074/jbc.M104115200

43. Mi Y, Qi G, Gao Y, Li R, Wang Y, Li X, et al. (-)-epigallocatechin-3-gallate ameliorates insulin resistance and mitochondrial dysfunction in hepG2 cells: involvement of bmal1. *Mol Nutr Food Res*. (2017) 61:1700440. doi: 10.1002/mnfr.201700440

44. Chen Y, Liu C, Tong J, He C, Ling X, Xiang J, et al. Inhibition of cullin3 neddylation alleviates diabetic retinopathy by activating nrf2 signaling to combat ROS-induced oxidative stress and inflammation. *Inflammation*. (2025). doi: 10.1007/s10753-025-02259-8

45. Deissler HL, Deissler H, Lang GK, Lang GE. VEGF but not PlGF disturbs the barrier of retinal endothelial cells. *Exp Eye Res*. (2013) 115:162–71. doi: 10.1016/j.exer.2013.07.018

46. Chen XJ, Butow RA. The organization and inheritance of the mitochondrial genome. *Nat Rev Genet*. (2005) 6:815–25. doi: 10.1038/nrg1708

47. Abu-Yaghi NE, Abu Tarboush NM, Abojaradeh AM, Al-Akily AS, Abdo EM, Emoush LO. Relationship between serum vascular endothelial growth factor levels and stages of diabetic retinopathy and other biomarkers. *J Ophthalmol*. (2020) 2020:8480193. doi: 10.1155/2020/8480193

48. Zhou L, Sun H, Xu J, Kang J. Level of vascular endothelial growth factor and interleukin-6 in aqueous humor in diabetic retinopathy patients. *Yan Ke Xue Bao*. (2010) 25:26–30. doi: 10.3969/g.issn.1000-4432.2010.01.007

49. Wisniewska-Kruk J, Hoeben KA, Vogels IM, Gaillard PJ, Van Noorden CJ, Schlingemann RO, et al. A novel co-culture model of the blood-retinal barrier based on primary retinal endothelial cells, pericytes and astrocytes. *Exp Eye Res*. (2012) 96:181–90. doi: 10.1016/j.exer.2011.12.003

50. El-Remessy AB, Franklin T, Ghaley N, Yang J, Brands MW, Caldwell RB, et al. Diabetes-induced superoxide anion and breakdown of the blood-retinal barrier: role of the VEGF/uPAR pathway. *PLoS One*. (2013) 8:e71868. doi: 10.1371/journal.pone.0071868

51. Klaassen I, Hughes JM, Vogels IM, Schalkwijk CG, Van Noorden CJ, Schlingemann RO. Altered expression of genes related to blood-retina barrier disruption in streptozotocin-induced diabetes. *Exp Eye Res*. (2009) 89:4–15. doi: 10.1016/j.exer.2009.01.006

52. Yang Y, Liu Y, Li Y, Chen Z, Xiong Y, Zhou T, et al. MicroRNA-15b targets VEGF and inhibits angiogenesis in proliferative diabetic retinopathy. *J Clin Endocrinol Metab*. (2020) 105:3404–15. doi: 10.1210/clinem/dgaa538

53. Zorov DB, Vorobjev IA, Popkov VA, Babenko VA, Zorova LD, Pevzner IB, et al. Lessons from the discovery of mitochondrial fragmentation (Fission): A review and update. *Cells*. (2019) 8:175. doi: 10.3390/cells8020175

54. Gottlieb RA, Piplani H, Sin J, Sawaged S, Hamid SM, Taylor DJ, et al. At the heart of mitochondrial quality control: many roads to the top. *Cell Mol Life Sci*. (2021) 78:3791–801. doi: 10.1007/s00018-021-03772-3

55. Twig G, Shirihai OS. The interplay between mitochondrial dynamics and mitophagy. *Antioxid Redox Signal*. (2011) 14:1939–51. doi: 10.1089/ars.2010.3779

56. Twig G, Elorza A, Molina AJ, Mohamed H, Wikstrom JD, Walzer G, et al. Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. *EMBO J*. (2008) 27:433–46. doi: 10.1038/sj.emboj.7601963

57. Ng MYW, Wai T, Simonsen A. Quality control of the mitochondrion. *Dev Cell*. (2021) 56:881–905. doi: 10.1016/j.devcel.2021.02.009

58. Ryter SW, Cloonan SM, Choi AM. Autophagy: a critical regulator of cellular metabolism and homeostasis. *Mol Cells*. (2013) 36:7–16. doi: 10.1007/s10059-013-0140-8

59. Ryter SW, Bhatia D, Choi ME. Autophagy: A lysosome-dependent process with implications in cellular redox homeostasis and human disease. *Antioxid Redox Signal*. (2019) 30:138–59. doi: 10.1089/ars.2018.7518

60. Pan R, Ryan J, Pan D, Wucherpfennig KW, Letai A. Augmenting NK cell-based immunotherapy by targeting mitochondrial apoptosis. *Cell*. (2022) 185:1521–1538.e1518. doi: 10.1016/j.cell.2022.03.030

61. Obeng E. Apoptosis (programmed cell death) and its signals - A review. *Braz J Biol*. (2021) 81:1133–43. doi: 10.1590/1519-6984.228437

62. Pétrilli V, Dostert C, Muruve DA, Tschopp J. The inflammasome: a danger sensing complex triggering innate immunity. *Curr Opin Immunol*. (2007) 19:615–22. doi: 10.1016/j.coi.2007.09.002

63. Agostini L, Martinon F, Burns K, McDermott MF, Hawkins PN, Tschopp J. NALP3 forms an IL-1beta-processing inflammasome with increased activity in Muckle-Wells autoinflammatory disorder. *Immunity*. (2004) 20:319–25. doi: 10.1016/s1074-7613(04)00046-9

64. Wai T, Langer T. Mitochondrial dynamics and metabolic regulation. *Trends Endocrinol Metab*. (2016) 27:105–17. doi: 10.1016/j.tem.2015.12.001

65. Kowluru RA, Alka K. Mitochondrial quality control and metabolic memory phenomenon associated with continued progression of diabetic retinopathy. *Int J Mol Sci*. (2023) 24:8076. doi: 10.3390/ijms24098076

66. Devi TS, Yumnamcha T, Yao F, Somayajulu M, Kowluru RA, Singh LP. TXNIP mediates high glucose-induced mitophagic flux and lysosome enlargement in human retinal pigment epithelial cells. *Biol Open*. (2019) 8:bio038521. doi: 10.1242/bio.038521

67. Zhang Y, Xi X, Mei Y, Zhao X, Zhou L, Ma M, et al. High-glucose induces retinal pigment epithelium mitochondrial pathways of apoptosis and inhibits mitophagy by regulating ROS/PINK1/Parkin signal pathway. *BioMed Pharmacother*. (2019) 111:1315–25. doi: 10.1016/j.biopha.2019.01.034

68. Brooks CD, Kodati B, Stankowska DL, Krishnamoorthy RR. Role of mitophagy in ocular neurodegeneration. *Front Neurosci*. (2023) 17:1299552. doi: 10.3389/fnins.2023.1299552

69. Malaviya P, Kowluru RA. Homocysteine and mitochondrial quality control in diabetic retinopathy. *Eye Vis (Lond)*. (2024) 11:5. doi: 10.1186/s40662-023-00362-1

70. Smith RO, Ninchoji T, Gordon E, André H, Dejana E, Vestweber D, et al. Vascular permeability in retinopathy is regulated by VEGFR2 Y949 signaling to VE-cadherin. *Elife*. (2020) 9:e54056. doi: 10.7554/eLife.54056

71. Bonora M, Giorgi C, Pinton P. Molecular mechanisms and consequences of mitochondrial permeability transition. *Nat Rev Mol Cell Biol*. (2022) 23:266–85. doi: 10.1038/s41580-021-00433-y

72. Dumas JF, Argaud L, Cottet-Rousselle C, Vial G, Gonzalez C, Detaille D, et al. Effect of transient and permanent permeability transition pore opening on NAD(P)H localization in intact cells. *J Biol Chem*. (2009) 284:15117–25. doi: 10.1074/jbc.M900926200

73. Kent AC, El Baradie KBY, Hamrick MW. Targeting the mitochondrial permeability transition pore to prevent age-associated cell damage and neurodegeneration. *Oxid Med Cell Longev*. (2021) 2021:6626484. doi: 10.1155/2021/6626484

74. Devi TS, Lee I, Hüttemann M, Kumar A, Nantwi KD, Singh LP. TXNIP links innate host defense mechanisms to oxidative stress and inflammation in retinal Muller glia under chronic hyperglycemia: implications for diabetic retinopathy. *Exp Diabetes Res*. (2012) 2012:438238. doi: 10.1155/2012/438238

75. Lu Y, Li Z, Zhang S, Zhang T, Liu Y, Zhang L. Cellular mitophagy: Mechanism, roles in diseases and small molecule pharmacological regulation. *Theranostics*. (2023) 13:736–66. doi: 10.7150/thno.79876

76. Greene JC, Whitworth AJ, Kuo I, Andrews LA, Feany MB, Pallanck LJ. Mitochondrial pathology and apoptotic muscle degeneration in Drosophila parkin mutants. *Proc Natl Acad Sci U.S.A.* (2003) 100:4078–83. doi: 10.1073/pnas.0737556100

77. Clark IE, Dodson MW, Jiang C, Cao JH, Huh JR, Seol JH, et al. Drosophila pink1 is required for mitochondrial function and interacts genetically with parkin. *Nature*. (2006) 441:1162–6. doi: 10.1038/nature04779

78. Wang N, Zhu P, Huang R, Wang C, Sun L, Lan B, et al. PINK1: The guard of mitochondria. *Life Sci*. (2020) 259:118247. doi: 10.1016/j.lfs.2020.118247

79. Aerts L, Craessaerts K, De Strooper B, Morais VA. PINK1 kinase catalytic activity is regulated by phosphorylation on serines 228 and 402. *J Biol Chem*. (2015) 290:2798–811. doi: 10.1074/jbc.M114.620906

80. Deas E, Plun-Favreau H, Gandhi S, Desmond H, Kjaer S, Loh SH, et al. PINK1 cleavage at position A103 by the mitochondrial protease PARL. *Hum Mol Genet*. (2011) 20:867–79. doi: 10.1093/hmg/ddq526

81. Riley BE, Lougheed JC, Callaway K, Velasquez M, Brecht E, Nguyen L, et al. Structure and function of Parkin E3 ubiquitin ligase reveals aspects of RING and HECT ligases. *Nat Commun*. (2013) 4:1982. doi: 10.1038/ncomms2982

82. Narendra DP, Jin SM, Tanaka A, Suen DF, Gautier CA, Shen J, et al. PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. *PloS Biol*. (2010) 8:e1000298. doi: 10.1371/journal.pbio.1000298

83. Vargas JNS, Wang C, Bunker E, Hao L, Maric D, Schiavo G, et al. Spatiotemporal control of ULK1 activation by NDP52 and TBK1 during selective autophagy. *Mol Cell*. (2019) 74:347–362.e346. doi: 10.1016/j.molcel.2019.02.010

84. Qiu Y, Wang J, Li H, Yang B, Wang J, He Q, et al. Emerging views of OPTN (optineurin) function in the autophagic process associated with disease. *Autophagy*. (2022) 18:73–85. doi: 10.1080/15548627.2021.1908722

85. Wong YC, Holzbaur EL. Optineurin is an autophagy receptor for damaged mitochondria in parkin-mediated mitophagy that is disrupted by an ALS-linked mutation. *Proc Natl Acad Sci U.S.A.* (2014) 111:E4439–4448. doi: 10.1073/pnas.1405752111

86. Geisler S, Holmström KM, Skujat D, Fiesel FC, Rothfuss OC, Kahle PJ, et al. PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. *Nat Cell Biol*. (2010) 12:119–31. doi: 10.1038/ncb2012

87. Imazu T, Shimizu S, Tagami S, Matsushima M, Nakamura Y, Miki T, et al. Bcl-2/E1B 19 kDa-interacting protein 3-like protein (Bnip3L) interacts with bcl-2/Bcl-xL and induces apoptosis by altering mitochondrial membrane permeability. *Oncogene*. (1999) 18:4523–9. doi: 10.1038/sj.onc.1202722

88. Nguyen-Dien GT, Townsend B, Kulkarni PG, Kozul KL, Ooi SS, Eldershaw DN, et al. PPTC7 antagonizes mitophagy by promoting BNIP3 and NIX degradation via SCF(FBXL4). *EMBO Rep*. (2024) 25:3324–47. doi: 10.1038/s44319-024-00181-y

89. Madhu V, Hernandez-Meadows M, Boneski PK, Qiu Y, Guntur AR, Kurland IJ, et al. The mitophagy receptor BNIP3 is critical for the regulation of metabolic homeostasis and mitochondrial function in the nucleus pulposus cells of the intervertebral disc. *Autophagy*. (2023) 19:1821–43. doi: 10.1080/15548627.2022.2162245

90. Novak I, Kirkin V, McEwan DG, Zhang J, Wild P, Rozenknop A, et al. Nix is a selective autophagy receptor for mitochondrial clearance. *EMBO Rep*. (2010) 11:45–51. doi: 10.1038/embor.2009.256

91. Schweers RL, Zhang J, Randall MS, Loyd MR, Li W, Dorsey FC, et al. NIX is required for programmed mitochondrial clearance during reticulocyte maturation. *Proc Natl Acad Sci U.S.A.* (2007) 104:19500–5. doi: 10.1073/pnas.0708818104

92. Liu L, Sakakibara K, Chen Q, Okamoto K. Receptor-mediated mitophagy in yeast and mammalian systems. *Cell Res*. (2014) 24:787–95. doi: 10.1038/cr.2014.75

93. Chen M, Chen Z, Wang Y, Tan Z, Zhu C, Li Y, et al. Mitophagy receptor FUNDC1 regulates mitochondrial dynamics and mitophagy. *Autophagy*. (2016) 12:689–702. doi: 10.1080/15548627.2016.1151580

94. Kowluru RA, Mishra M. Oxidative stress, mitochondrial damage and diabetic retinopathy. *Biochim Biophys Acta*. (2015) 1852:2474–83. doi: 10.1016/j.bbadis.2015.08.001

95. Van Houten B, Hunter SE, Meyer JN. Mitochondrial DNA damage induced autophagy, cell death, and disease. *Front Biosci (Landmark Ed)*. (2016) 21:42–54. doi: 10.2741/4375

96. Liao YL, Fang YF, Sun JX, Dou GR. Senescent endothelial cells: a potential target for diabetic retinopathy. *Angiogenesis*. (2024) 27:663–9. doi: 10.1007/s10456-024-09943-7

97. Wu H, Li G, Chen W, Luo W, Yang Z, You Z, et al. Drp1 knockdown represses apoptosis of rat retinal endothelial cells by inhibiting mitophagy. *Acta Histochem*. (2022) 124:151837. doi: 10.1016/j.acthis.2021.151837

98. Devi TS, Somayajulu M, Kowluru RA, Singh LP. TXNIP regulates mitophagy in retinal Müller cells under high-glucose conditions: implications for diabetic retinopathy. *Cell Death Dis*. (2017) 8:e2777. doi: 10.1038/cddis.2017.190

99. Zhang MY, Zhu L, Bao X, Xie TH, Cai J, Zou J, et al. Inhibition of Drp1 ameliorates diabetic retinopathy by regulating mitochondrial homeostasis. *Exp Eye Res*. (2022) 220:109095. doi: 10.1016/j.exer.2022.109095

100. Lin S, Ji Z, Gao J, Fan J, Hou J, Liu S, et al. Poldip2 Aggravates inflammation in diabetic retinopathy by impairing mitophagy via the AMPK/ULK1/Pink1 pathway. *Life Sci*. (2025) 373:123681. doi: 10.1016/j.lfs.2025.123681

101. Liu C, Xiang J, Chen Y, He C, Tong J, Liao Y, et al. MiR-125a-5p in MSC-derived small extracellular vesicles alleviates Müller cells injury in diabetic retinopathy by modulating mitophagy via PTP1B pathway. *Cell Death Discov*. (2025) 11:226. doi: 10.1038/s41420-025-02439-3

102. Hebert AS, Dittenhafer-Reed KE, Yu W, Bailey DJ, Selen ES, Boersma MD, et al. Calorie restriction and SIRT3 trigger global reprogramming of the mitochondrial protein acetylome. *Mol Cell*. (2013) 49:186–99. doi: 10.1016/j.molcel.2012.10.024

103. Yang W, Qiu C, Lv H, Zhang Z, Yao T, Huang L, et al. Sirt3 protects retinal pigment epithelial cells from high glucose-induced injury by promoting mitophagy through the AMPK/mTOR/ULK1 pathway. *Transl Vis Sci Technol*. (2024) 13:19. doi: 10.1167/tvst.13.3.19

104. Zhang S, Chen S, Sun D, Li S, Sun J, Gu Q, et al. TIN2-mediated reduction of mitophagy induces RPE senescence under high glucose. *Cell Signal*. (2024) 119:111188. doi: 10.1016/j.cellsig.2024.111188

105. van der Bruggen MM, Spronck B, Delhaas T, Reesink KD, Schalkwijk CG. The putative role of methylglyoxal in arterial stiffening: A review. *Heart Lung Circ*. (2021) 30:1681–93. doi: 10.1016/j.hlc.2021.06.527

106. Kong X, Ma MZ, Huang K, Qin L, Zhang HM, Yang Z, et al. Increased plasma levels of the methylglyoxal in patients with newly diagnosed type 2 diabetes 2. *J Diabetes*. (2014) 6:535–40. doi: 10.1111/1753-0407.12160

107. Akagawa M, Mori A, Sakamoto K, Nakahara T. Methylglyoxal impairs β(2)-adrenoceptor-mediated vasodilatory mechanisms in rat retinal arterioles. *Biol Pharm Bull*. (2018) 41:272–6. doi: 10.1248/bpb.b17-00861

108. Sekar P, Hsiao G, Hsu SH, Huang DY, Lin WW, Chan CM. Metformin inhibits methylglyoxal-induced retinal pigment epithelial cell death and retinopathy via AMPK-dependent mechanisms: Reversing mitochondrial dysfunction and upregulating glyoxalase 1. *Redox Biol*. (2023) 64:102786. doi: 10.1016/j.redox.2023.102786

109. Zhu CH, Zhang SS, Kong Y, Bi YF, Wang L, Zhang Q. Effects of intensive control of blood glucose and blood pressure on microvascular complications in patients with type II diabetes mellitus. *Int J Ophthalmol*. (2013) 6:141–5. doi: 10.3980/j.issn.2222-3959.2013.02.06

110. Perais J, Agarwal R, Evans JR, Loveman E, Colquitt JL, Owens D, et al. Prognostic factors for the development and progression of proliferative diabetic retinopathy in people with diabetic retinopathy. *Cochrane Database Syst Rev*. (2023) 2:Cd013775. doi: 10.1002/14651858.CD013775.pub2

111. Kang K, Lee H, Jang M, Kim HC, Chung H. Diabetic macular edema with pachychoroid features. *BMC Ophthalmol*. (2020) 20:392. doi: 10.1186/s12886-020-01663-y

112. Bressler NM, Beaulieu WT, Glassman AR, Blinder KJ, Bressler SB, Jampol LM, et al. Persistent macular thickening following intravitreous aflibercept, bevacizumab, or ranibizumab for central-involved diabetic macular edema with vision impairment: A secondary analysis of a randomized clinical trial. *JAMA Ophthalmol*. (2018) 136:257–69. doi: 10.1001/jamaophthalmol.2017.6565

113. Holekamp NM, Campbell J, Almony A, Ingraham H, Marks S, Chandwani H, et al. Vision outcomes following anti-vascular endothelial growth factor treatment of diabetic macular edema in clinical practice. *Am J Ophthalmol*. (2018) 191:83–91. doi: 10.1016/j.ajo.2018.04.010

114. Stitt AW, Curtis TM, Chen M, Medina RJ, McKay GJ, Jenkins A, et al. The progress in understanding and treatment of diabetic retinopathy. *Prog Retin Eye Res*. (2016) 51:156–86. doi: 10.1016/j.preteyeres.2015.08.001

115. Moutray T, Evans JR, Lois N, Armstrong DJ, Peto T, Azuara-Blanco A. Different lasers and techniques for proliferative diabetic retinopathy. *Cochrane Database Syst Rev*. (2018) 3:Cd012314. doi: 10.1002/14651858.CD012314.pub2

116. Genova ML, Pich MM, Bernacchia A, Bianchi C, Biondi A, Bovina C, et al. The mitochondrial production of reactive oxygen species in relation to aging and pathology. *Ann N Y Acad Sci*. (2004) 1011:86–100. doi: 10.1007/978-3-662-41088-2_10

117. Acuna-Castroviejo D, Escames G, Rodriguez MI, Lopez LC. Melatonin role in the mitochondrial function. *Front Biosci*. (2007) 12:947–63. doi: 10.2741/2116

118. Doğanlar ZB, Doğanlar O, Kurtdere K, Güçlü H, Chasan T, Turgut E. Melatonin prevents blood-retinal barrier breakdown and mitochondrial dysfunction in high glucose and hypoxia-induced *in vitro* diabetic macular edema model. *Toxicol In Vitro*. (2021) 75:105191. doi: 10.1016/j.tiv.2021.105191

119. Crooke A, Huete-Toral F, Colligris B, Pintor J. The role and therapeutic potential of melatonin in age-related ocular diseases. *J Pineal Res*. (2017) 63:12430. doi: 10.1111/jpi.12430

120. Zou J, Tan W, Liu K, Chen B, Duan T, Xu H. Wnt inhibitory factor 1 ameliorated diabetic retinopathy through the AMPK/mTOR pathway-mediated mitochondrial function. *FASEB J*. (2022) 36:e22531. doi: 10.1096/fj.202200366RR

121. Zhang MY, Zhu L, Zheng X, Xie TH, Wang W, Zou J, et al. TGR5 activation ameliorates mitochondrial homeostasis via regulating the PKCδ/drp1-HK2 signaling in diabetic retinopathy. *Front Cell Dev Biol*. (2021) 9:759421. doi: 10.3389/fcell.2021.759421

122. Xie J, Cui Y, Chen X, Yu H, Chen J, Huang T, et al. VDAC1 regulates mitophagy in NLRP3 inflammasome activation in retinal capillary endothelial cells under high-glucose conditions. *Exp Eye Res*. (2021) 209:108640. doi: 10.1016/j.exer.2021.108640

123. Zhou P, Xie W, Meng X, Zhai Y, Dong X, Zhang X, et al. Notoginsenoside R1 ameliorates diabetic retinopathy through PINK1-dependent activation of mitophagy. *Cells*. (2019) 8:213. doi: 10.3390/cells8030213

124. Xu Y, Yu J. Allicin mitigates diabetic retinopathy in rats by activating phosphatase and tensin homolog-induced kinase 1/parkin-mitophagy and inhibiting oxidative stress-mediated NOD-like receptor family pyrin domain containing 3 inflammasome. *J Physiol Investig*. (2024) 67:215–24. doi: 10.4103/ejpi.EJPI-D-24-00039

125. Hao XN, Zhao N, Huang JM, Li SY, Wei D, Pu N, et al. Intravitreal injection of ZYAN1 restored autophagy and alleviated oxidative stress in degenerating retina via the HIF-1α/BNIP3 pathway. *Antioxid (Basel)*. (2023) 12:1914. doi: 10.3390/antiox12111914

126. Wu JJ, Zhang SY, Mu L, Dong ZG, Zhang YJ. Heyingwuzi formulation alleviates diabetic retinopathy by promoting mitophagy via the HIF-1α/BNIP3/NIX axis. *World J Diabetes*. (2024) 15:1317–39. doi: 10.4239/wjd.v15.i6.1317