Updates on RPE cell damage in diabetic retinopathy (Review)

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Abstract. Diabetic retinopathy (DR) is a microvascular complication of diabetes. The retinal pigment epithelium (RPE) forms the outer layer of the blood-retinal barrier and serves a role in maintaining retinal function. RPE cell injury has been revealed in diabetic animal models, and high glucose (HG) levels may cause damage to RPE cells by increasing the levels of oxidative stress, promoting pro-inflammatory gene expression, disrupting cell proliferation, inducing the endothelial-mesenchymal transition, weakening tight conjunctions and elevating cell death mechanisms, such as apoptosis, ferroptosis and pyroptosis. Non-coding RNAs including microRNAs, long non-coding RNAs and circular RNAs participate in RPE cell damage caused by HG levels, which may provide targeted therapeutic strategies for the treatment of DR. Plant extracts such as citrusin and hesperidin, and a number of hypoglycemic drugs, such as sodium-glucose co-transporter 2 inhibitors, metformin and glucagon-like peptide-1 receptor agonists, exhibit potential RPE protective effects; however, the detailed mechanisms behind these effects remain to be fully elucidated. An in-depth understanding of the contribution of the RPE to DR may provide novel perspectives and therapeutic targets for DR.

Contents

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1. Introduction

According to the 2021 data from the International Diabetes Federation, there are currently 537 million adults between 20 and 79 years old with diabetes mellitus (DM) worldwide (1). Diabetic retinopathy (DR) is a microvascular complication of DM and is the main cause of visual impairment in adults of working age (2). With the increase in life expectancy, the number of patients with DR continues to increase globally (3). The number of adults with DR globally was estimated to be 103.12 million in 2020, and this number is projected to increase to 160.50 million by 2045 (4).

DR, which has traditionally been considered as a blood-retinal barrier (BRB) disorder, is characterized by the vessel leakage in the retina (5). The retinal pigment epithelium (RPE) is a single layer of cells underlying the neural retina and forms the outer BRB, regulating the transport of materials such as ions across the BRB (6,7). The role of RPE cells in the pathogenesis of DR has been recognized since 1987 (8) and, in the last number of years, accumulating data has indicated that RPE injury is involved in the pathogenesis of DR (9). In streptozotocin (STZ)-induced DM mice, the barrier function of the RPE was revealed to be disrupted, leading to an increased leakage over the BRB (10). In a human study, an altered RPE proteome was observed in patients with diabetic pre-retinopathy, indicating that RPE alterations may contribute to the development of DR (11).

2. Detrimental effects caused by high glucose (HG) levels

HG causes RPE cell injury or dysfunction via various mechanisms; these are summarized in Fig. 1 and are described in detail in the following sections.

Increased levels of oxidative stress. Oxidative stress caused by HG serves a role in the pathogenesis of DR (12). Due to high metabolic activity, the RPE produces high levels of

physiological reactive oxygen species (ROS) (13). However, increased levels of oxidative stress caused by HG levels leads to cell damage or dysfunction (14,15), contributing to apoptosis (16), mitochondrial dysfunction (17) and altered cell behaviors, such as increased cell migration (18).

RPE cells contain numerous mitochondria and mitochondrial dysfunction leads to increased ROS production (13). Mitophagy is a dynamic autophagy process that eliminates excess or damaged mitochondria, maintaining the quality and quantity of the mitochondria (19). In previous studies, reduced mitophagy has been revealed in RPE cells under conditions of HG (20,21), which causes increased cellular levels of oxidative stress.

Increased inflammatory response. It has been revealed that RPE cells may produce and release various cytokines and chemokines, such as RANTES, monocyte chemoattractant protein-1, interleukin (IL)-6 and IL-8 (22,23), thus triggering the inflammatory response. There is evidence to indicate that HG can upregulate the expression of genes involved in the inflammatory response, including tumor necrosis factor-α, IL-6, IL-1β (15,24), as well as intercellular adhesion molecule-1 (25) and monocyte chemoattractant protein-1 (26) in RPE cells, which may consequently cause cell damage via increasing the production of ROS in the mitochondria (27).

Proliferation disorders. RPE cell proliferation has a role in maintaining the integrity and function of the BRB (28). A decreased RPE proliferation leads to reduced RPE cell numbers, resulting in a reduced metabolic support for photoreceptor cells. In a previous study reduced RPE cell proliferation was observed in rats with STZ-induced DM 5 weeks following the onset of DM (29). The evidence from *in vitro* studies has indicated that HG inhibits RPE cell proliferation by targeting different pathways such as the miR-338-3p/CARM1, ROS/PINK1/Parkin and miR-218/Runx2 pathways (15,21,30). By contrast, an increased RPE cell proliferation has been suggested to be involved in epiretinal membrane formation in proliferative diabetic retinopathy (31); there is also evidence to indicate that HG promotes RPE cell proliferation (32,33).

Epithelial-mesenchymal transition (EMT). EMT is a complex biological process through which epithelial cells acquire a mesenchymal phenotype, displaying cellular motility and contractile properties (34). The EMT in RPE cells has been revealed to be involved in certain types of retinopathy, such as proliferative vitreoretinopathy (35), AMD (36) and diabetic proliferative diabetic retinopathy (37). As previously demonstrated in *in vitro* studies, the gene expression levels of mesenchymal cell markers N-cadherin and Vimentin were induced by HG in ARPE-19 cells (38,39), suggesting that the EMT in RPE cells participates in the pathogenesis of DR.

Destruction of tight junctions. RPE cells are connected by tight junctions, which are located in the upper part of the lateral surface of the cells, thereby maintaining the permeability of the BRB (40). The breakdown of the outer BRB has been observed in diabetic mice, which was combined with reduced expression of tight-junction protein occludin in the RPE (10); this suggests that the destruction of tight junctions between RPE cells may serve a role in the development of DR. New blood vessels may grow through the destructed tight junction into the macula, resulting in diabetic macular edema, which is the leading cause of blindness in patients with DM (4).

Increased cell death

Apoptosis. It was considered that HG mainly causes RPE cell necrosis instead of apoptosis. However, a number of studies have revealed that RPE cell apoptosis is a characteristic process in DR (30,41,42). HG may induce RPE cell apoptosis via different signaling pathways, such as microRNA (miRNA/miR) associated pathways (30,41) and p38-mitogen-activated protein kinase pathways (42).

Ferroptosis. Ferroptosis is a novel form of cell death that was first reported in 2012 (43), and is characterized by intracellular iron overload and the accumulation of iron-dependent lipid peroxide (44). RPE cell ferroptosis has been previously revealed to be involved in the pathogenesis of AMD (45-47). Recently, in STZ-induced diabetic mice, iron overload (48) and ferroptosis were observed in retinal tissue (49). *In vitro* studies have revealed that HG may induce RPE cell ferroptosis via different signaling pathways, such as the miR-338-3p/solute carrier family 1 member 5 (50) and miR-138-5p/sirtuin 1/nuclear factor erythroid 2-related factor 2 (Nrf2) pathways (51), thus serving a role in the pathogenesis of DR.

Pyroptosis. Pyroptosis is a form of programmed cell death characterized by the rupture of the plasma membrane and the release of proinflammatory cytokines such as IL-1β and IL-1 (52). Recent *in vitro* studies have revealed that HG induces RPE cell pyroptosis (53) by targeting multiple signaling pathways, such as maternally expressed 3 (MEG3) (54), miR-192 (55), circular (circ)-zinc finger protein 532 (ZNF532) (56) and methyltransferase-like 3 (57), and their downstream signaling cascades. Huang *et al* (58) revealed that circFAT1 was downregulated in retinal proliferative fibrovascular membranes from patients with DR, which led to an increased HG-induced RPE pyroptosis. Additional investigation of the mechanisms underlying HG-induced RPE cell pyroptosis may provide further targeted therapeutic strategies for DR.

3. Role of non-coding RNAs (ncRNAs) in RPE damage in DR

ncRNAs, which account for ~98% of the human genome, are a type of RNA that cannot be translated into protein. ncRNAs mainly include miRNAs, circRNAs, long ncRNAs (lncRNAs) and small nucleolar RNAs, and participate in a number of physiological and pathophysiological processes (59) Accumulating evidence suggests that miRNAs, lncRNAs and circRNAs all participate in HG-induced RPE cell dysfunction by targeting different pathways (Table I).

miRNAs. miRNAs are single-stranded ncRNAs consisting of 20-24 nucleotides (60) that can interact with the 3'-untranslated region of targeted mRNAs and mediate gene silencing

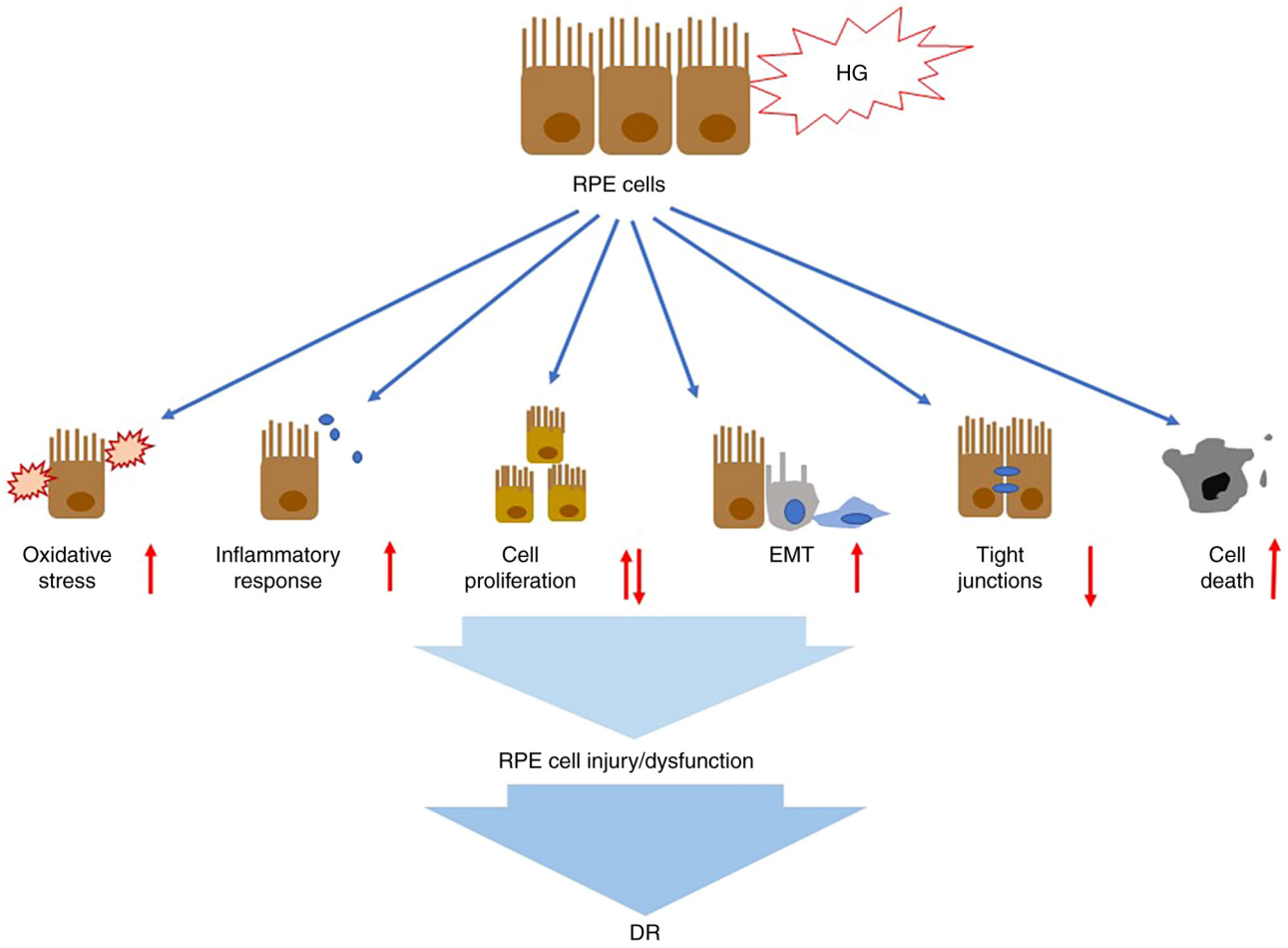

Figure 1. RPE cell injury caused by HG. HG may cause RPE cell damage by increasing the levels of oxidative stress, inducing the inflammatory response, disrupting cell proliferation, causing tight junction disorders and promoting the EMT and cell death, thus contributing to the pathogenesis of DR. HG, high glucose; RPE, retinal pigment epithelium; EMT, epithelial-mesenchymal transition; DR, diabetic retinopathy.

in cells (61). miRNAs exert complex effects on RPE cell development, differentiation, homeostasis and barrier function (62), and serve a role in the pathogenesis of DR (63). Various miRNAs exhibit potential protective effects against DR. A previous *in vitro* study on RPE cells under conditions of HG demonstrated that miRNA-451a reduced cell migration and proliferation, and protected mitochondrial function (31). Another study demonstrated that miRNA-27a reduced RPE cell apoptosis and the inflammatory response (24), while miRNA-125b was revealed to reduce cell death (64). Both miRNA-130a and miRNA-25-3p have been revealed to protect RPE cells from pyroptosis (53,57). A number of miRNAs, such as miRNA-219-5p (65), miRNA-217 (41) and miRNA-218 (30), have been revealed to exert detrimental effects by inducing apoptosis, the inflammatory response and inhibiting cell proliferation.

circRNAs. circRNAs were first revealed to be expressed in the cytoplasm of mammalian cells by Hsu and Coca-Prados in 1979 (66). circRNAs are generated from linear precursor mRNAs by non-canonical back-splicing reactions. circRNAs are more stable compared with the linear transcripts (67) due to their closed-loop structure, which reduces degradation by nucleases. circRNAs perform multiple functions in cells, serving mainly as miRNA sponges, protein regulators and translation templates (68).

Evidence obtained thus far has indicated that various circRNAs, such as circ0000615 (14), circADAM9 (15) and circ0084043 (69), can be induced by HG in RPE cells, thereby causing cell damage by promoting inflammation, oxidative stress and apoptosis. A number of circRNAs are involved in HG-induced RPE cell death; for example the upregulation of circ-presenilin 1 and ZNF532 induced by HG has been revealed in RPE cells, and have thus been suggested to be involved in cell ferroptosis (70) and pyroptosis (56), respectively.

lncRNAs. lncRNAs are non-coding protein transcripts composed of >200 nucleotides, which can regulate gene expression by stabilizing mRNAs, remodeling chromatin architecture and regulating transcriptions (71). The role of lncRNAs in DR has been studied previously. A number of lncRNAs have been suggested to be protective and are downregulated by HG conditions in RPE cells. For example, maternally expressed 3 has been demonstrated to inhibit the inflammatory response (54), while brain-derived neurotrophic factor antisense (72) and B-Raf proto-oncogene, serine/threonine kinase-activated non-protein coding RNA (73) may promote apoptosis induced by HG.

Table I. Expressions and functions of miRNAs, circRNAs and lncRNAs in retinal pigment epithelial cells under high glucose.

A, miRNAs

Name	Functions	Possible signaling pathways	Dysregulation	(Refs.)
miR-451a	Reduce migration and protect mitochondrial function	miR-451a/ATF2, CyclinA1, CyclinD1 and MMP2	Downregulated	(31)
miR-27a	Inhibit inflammation and apoptosis	TLR4	Downregulated	(24)
miR-125b	Attenuate cell death	Hexokinase 2	Downregulated	(64)
miR-130a	Alleviate pyroptosis	TNF-α/SOD1/ROS	Downregulated	(53)
miR-25-3p	Alleviate pyroptosis	miR-25-3p/PTEN/Akt	Downregulated	(57)
miR-219-5p	Induce apoptosis	LRH-1/Wnt/β-Catenin	Upregulated	(65)
miR-217	Induce inflammation and apoptosis	SIRT1	Upregulated	(41)
miR-218	Inhibit proliferation and induce apoptosis	RUNX2	Upregulated	(30)

B, circRNAs

Name	Functions	Possible signaling pathways	Dysregulation	(Refs.)
0000615	Promote apoptosis, inflammation and oxidative stress	mir-646/yap1	Upregulated	(14)
ADAM9	Inhibit proliferation, promote inflammation, apoptosis and oxidative stress	mir-338-3p/carm1	Upregulated	(15)
0084043	Inhibit cell viability, promote apoptosis and inflammation	mir-128-3p/txnip-mediated Wnt/β-catenin	Upregulated	(69)
PSEN1	Promote ferroptosis	mir-200b-3p/cofilin-2	Upregulated	(70)
ZNF532	Promote apoptosis and pyroptosis	mir-20b5p/stat3	Upregulated	(56)

C, lncRNAs

Name	Functions	Possible signaling pathways	Dysregulation	(Refs.)
MEG3	Promote proliferation, inhibit apoptosis and inflammation	mir-93/nrf2	Downregulated	(54)
BDNF-AS	Inhibit apoptosis	-	Downregulated	(72)
BANCR	Inhibit apoptosis	-	Downregulated	(73)
NEAT1	Promote proliferation and EMT	miR-204/SOX4	Upregulated	(39)
IGF2-AS	Promote apoptosis	IGF2/AKT	Upregulated	(74)

miRNA/miR, microRNA; circRNA, circular RNA; lncRNA, long non-coding RNA; TLR4, toll-like receptor 4; SOD, superoxide dismutase; ROS, reactive oxygen species; LRH, liver receptor homologue; SIRT1, sirtuin 1; RUNX2, runt-related transcription factor 2; yap1, yes-associated protein 1; carm1, coactivator associated arginine methyltransferase 1; txnip, thioredoxin-interacting protein; nrf2, nuclear factor erythroid 2-related factor 2;SOX4, SRY-Box transcription factor 4; IGF2, insulin-like growth factor 2; ADAM9, a disintegrin and metalloprotease domain 9; PSEN1, presenilin 1; ZNF532, zinc finger protein 532; MEG3, maternally expressed 3; BDNF-AS, brain-derived neurotrophic factor-antisense RNA; BANCR, BRAF-activated non-coding RNA; NEAT1, nuclear enriched abundant transcript 1; IGF2-AS, IGF2-antisense RNA.

Furthermore, a number of lncRNAs have been revealed to be upregulated by HG conditions, participating in the EMT of RPE cells and in the inhibition of proliferation, such as nuclear enriched abundant transcript 1 (39), as well as in promoting apoptosis, such as insulin growth factor 2 antisense (74).

4. Effects of drugs, plant extracts and DR treatment on RPE cells

Hypoglycemic drugs

Sodium-glucose co-transporter 2 inhibitors (SGLT2i). As glucose-lowering agents, SGLT2i have been receiving attention for their cardiovascular and renal benefits. Previously, their roles in the treatment of DR have been recognized (75,76). SGLT2 expression has been revealed to be increased in the lens epithelial tissue from patients with DM (77), in the whole eye tissue of diabetic mice (78) and in human retinal microvascular endothelial cells when under conditions of HG (79). However, to date, to the best of our knowledge, there is not data available on the SGLT2 expression levels in RPE cells.

A number of clinical studies (80) and animal experiments (78,79,81) have established the retinoprotective effects of SGLT2i by attenuating retinal oxidative stress, apoptosis and downregulating inflammation-related genes TNF-α and IL-6 (79,81), which was suggested to be independent of its hypoglycemic effects. However, evidence of whether SGLT2i may achieve similar effects to protect REP cells from HG-induced damage remains limited. A previous study by Gong *et al* (81), using db/db mice, revealed that treatment with empagliflozin, a SGLT2i, recovered tight-junction proteins in the retina, a process in which the RPE layer cells also appeared to be involved. Therefore, further evidence of the protective effects of SGLT2i on the REP cells is required.

Metformin. Metformin is a widely used anti-diabetic medicine. It has previously been revealed in *in vitro* studies that metformin protects RPE cells against glyoxal (82) or H2O2 (83)-induced oxidative stress via the activation of autophagy, suggesting that metformin may exert anti-oxidative effects. However, similar results have not been described in HG-treated-RPE cells or in diabetic animal models. In the study by Kim *et al* (84), treatment with metformin attenuated the increases in the gene expression levels of O-linked β-N-acetylglucosamine transferase, carbohydrate-responsive element-binding protein, thioredoxin-interacting protein and NF-κB in the retinal tissue of mice with STZ-induced diabetes and in RPE cells treated with HG, contributing to reduced apoptosis and thus an attenuation of the retinal damage in DR.

Glucagon-like peptide-1 (GLP-1) receptor (GLP-1R) agonist (GLP-1RA). As a glucose-lowering drug, the multiple benefits of GLP-1RA, including reducing body weight and cardio- and renal-protective effects, have been widely studied. The expression of GLP-1R in ARPE-19 cells was first described by Puddu *et al* (85) in 2013, which indicated the potential beneficial effects of GLP-1 treatment against DR. The expression of GLP-1R has been revealed to be downregulated in the retinal RPE cells of STZ-exposed mice and in HG-treated ARPE-19 cells, leading to increased levels of ROS and apoptosis; however, these cell injuries induced by HG were attenuated by the GLP-1RA, exendin-4 (86). Nrf2 has been revealed to serve a role in HG-induced RPE cell injury (51,87,88); in the study by Cui *et al* (89), exendin-4 was demonstrated to attenuate H_2O_2-induced oxidative stress in ARPE-19 cells by activating the Nrf2 signaling pathway.

Other drugs and plant extracts. A number of drugs have also been revealed to alleviate the negative effects of HG on RPE cells. For example, triptolide has been demonstrated to reduce the levels of oxidative stress by regulating the miR-29b/PTEN pathway (90). In addition, dexmedetomidine (91) and ferulic acid (92) have been revealed to reduce apoptosis, while sodium tanshinone IIA sulfonate has been demonstrated to attenuate the inflammatory response (93). Furthermore, fenofibric acid has been demonstrated to improve retinal permeability by downregulating the expression of fibronectin and type IV collagen in RPE cells (94).

There is evidence to indicate that various plant extracts can also protect RPE cells from HG-induced injury; for example, citrusin (95), astaxanthin (96) and shikonin (97) have been demonstrated to attenuate RPE cell inflammation. *Polygonatum sibiricum* polysaccharides (88), hesperidin (98), lutei (99) and eucalyptol (100) have also been revealed to alleviate cellular oxidative stress and apoptosis. *Astragalus* polysaccharides (101) have been demonstrated to improve mitochondrial function by regulating miR-195.

DR treatments. Retinal laser photocoagulation, photodynamic therapy, intravitreal steroids and anti-VEGF therapy are used to treat advanced DR. Limited evidence has described the effects of a number of these therapies on RPE. In STZ-induced diabetic mice, the proliferation of RPE cells was revealed to be impaired after laser photocoagulation (102). Intravitreal steroid treatment could reduce the breakdown of the BRB and the proliferation of RPE cells in proliferative vitreoretinopathy (103). In a clinical study performed in India, anti-VEGF therapy was revealed to be associated with the improvement in the grades of topographic alterations of RPE in diabetic macular edema (104). Furthermore, an *in vitro* study indicated that anti-VEGF compounds ranibizumab and pegaptanib sodium caused increased RPE permeability (105). However, further studies are still required.

5. Conclusions and future perspectives

There is evidence to indicate that HG can cause RPE cell disorders by triggering cell pathophysiological processes and disrupting the homeostasis of ncRNA gene expressions, contributing to the pathogenesis of DR. Various medications can exert protective effects on the RPE; however, these effects require further verification. Additional in-depth investigations into the underlying mechanisms of RPE injury under conditions of HG may provide new perspectives and therapeutic targets for DR.

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Availability of data and materials

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Authors' contributions

ML, MT, XJ and YL developed the manuscript concept and composed the initial draft. YW, HM and YZ contributed valuable comments on the first draft. ML, MT, YW, HM, YZ, XJ and YL critically revised the manuscript for intellectual content. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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