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Review

The retinal pigment epithelium: Functions and roles in ocular diseases

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ABSTRACT

The retinal pigment epithelium (RPE) between retinal photoreceptors and choroidal capillaries is a single layer of cells that are of critical importance to the eye. RPE cells are derived from the anterior neural plate of neuroectodermal origin. Instructed by specific molecules and signaling pathways, the RPE undergoes formation and maturation to form a functional unit together with photoreceptors. The RPE plays crucial roles in maintaining normal retinal structure and functions, such as phagocytosis; barrier function; transportation of nutrients, ions, and water; resistance to oxidative damage; maintenance of visual cycle; and production of various important factors. RPE cells have an efficient metabolic machinery to provide sufficient energy to the retina. RPE dysfunction or atrophy can lead to many retinopathies, such as age-related macular degeneration and proliferative vitreoretinopathy. Here, we discuss RPE development, functions, and roles in various ocular diseases, and the mechanisms involved. A better understanding of the functions of the RPE and related regulatory pathways may help identify novel or better therapies for the treatment of many blinding diseases.

1. Introduction

The retinal pigment epithelium (RPE), located between retinal photoreceptors and choroidal capillaries, is a single layer of cells that are of critical importance for the eye. The RPE plays key roles in maintaining the structure and function of the retina; phagocytosis; barrier formation; transportation of nutrients, ions and water; absorption of light; protection of retinal cells against photo-oxidation; visual cycle maintenance; and production of various factors (Fig. 1). Impaired RPE structure or function plays important roles in many ocular pathologies, such as AMD, PVR, Stargardt disease, retinitis pigmentosa (RP), and diabetic retinopathy (DR). Here we will provide a comprehensive overview of the functions and underlying mechanisms of RPE during physiological and pathological processes. In addition, translational research on RPE cells in relationship to ocular diseases is also discussed.

2. The development of RPE

The RPE is a derivative of the anterior neural plate [1]. Early in embryonic development, a specific region of the anterior neuroectoderm co-expresses a number of transcription factors, such as Otx2, Pax6, Rx1, and Six3 [1], and is the starting point of eye development, laying the foundation for RPE development and visual function [1]. The initial establishment of the human eye prototype occurs on embryonic day 22 (E22). At this stage, the neural plates derived from the neuroectoderm on the either side of the forebrain invaginate and differentiate into two laminar optic vesicles. The outer layer of the neural plate develops into RPE cells [2]. By E36, the RPE in the human eye has largely differentiated with the appearance of RPE characteristics, including pigmentation. Thereafter, the RPE cells undergo further differentiation and specification. At 14 weeks of gestation, the RPE forms a functional unit together with photoreceptors [2].

RPE development can be divided into two stages of RPE formation and maturation [3]. During the RPE formation stage, the neuroectoderm-derived anterior neural plate begins to invaginate and differentiate into the RPE and neuroretinal layers [1]. Early in this stage, the interphotoreceptor matrix (IPM) begins to form at the center of the optic vesicle, dividing the vesicle into two opposing parts, with the dorsal side developing into RPE cells and the ventral side into the neuroretina. The formation of the IPM is determined by the expression of the transcription factors OTX2 and MITF [1,3]. As the IPM forms, the interphotoreceptor retinal binding protein (IRBP) begins to be expressed, which triggers the expression of a number of other genes related to early eye development and promotes RPE differentiation [3]. For example, the receptor for retinoic acid (RAR-β2) in RPE cells binds to retinoic acid (RA) in the retina to maintain early embryonic eye development [3]. When IRBP begins to be expressed, the interaction between these two genes drives RPE formation and the establishment of the visual cycle [3].

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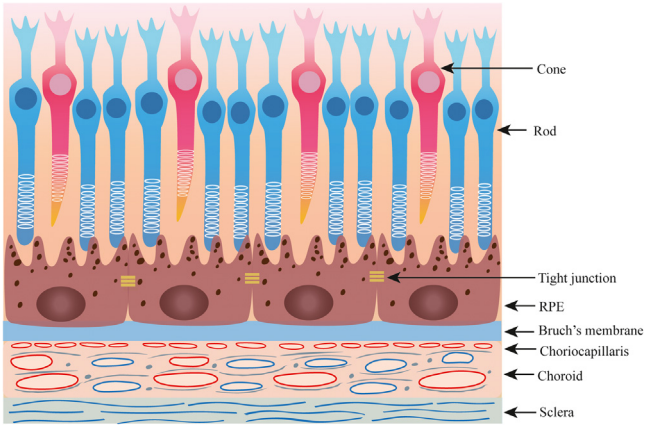

Fig. 1. The retinal pigment epithelium in the eye. The RPE is located between photoreceptors and the choroid. The RPE is composed of a single layer of hexagonal epithelial cells enriched in melanin granules. The RPE is arranged in a polarized pattern and is considered as "inverted" epithelium connecting the outer segments of the rod and cone cells through their microvilli on the apical side of RPE cells. RPE cells are connected to the Bruch's membrane and choroid on their basal side. RPE cells, together with the Bruch's membrane and choroidal capillaries, form the blood-retinal barrier (BRB).

In addition, the expression of multiple differentiation-related genes is required to ensure proper RPE differentiation. For instance, the Hedgehog signaling pathway is responsible for RPE development and the establishment of visual function [4]. Hes1 and Wnt4 signaling pathways are important for RPE differentiation and morphological development [5]. Vhl and Hif1α are essential for the maintenance of RPE morphology [6]. As RPE differentiation continues, RPE65, the hallmark gene of RPE, begins to be expressed and upregulated steadily from the IPM formation stage until the postnatal stage [3].

Once the RPE and neuroretinal layers are established, they begin to consolidate and specialize, with the bridging structure of the IPM playing a key role. At this point, the activation of the tyrosinase promoter allows RPE cells to synthesize melanin, which marks RPE cell maturation [3]. Meanwhile, RPE cells begin to secrete extracellular matrix to form a basolateral membrane on the choroidal side, together with the extracellular matrix secreted by the ciliary epithelium and endothelial cells, forming the Bruch's membrane [7]. At this point, the RPE acquires basolateral polarity, short microvilli, and a small basolateral intra-membrane fold [3]. By this stage, ~75% of the RPE apical architecture has formed, and the areas of apical membrane are about three times of that of the basolateral membrane [3]. The completion of these structures allows for the localization and expression of ion transporters or other functional proteins in the apical membrane. For example, the expression of Na^+/K^+ adenosine triphosphatase (ATPase) pumps enables the RPE to transport ions [8]. The MRCKβ signal pathway enables the RPE to phagocytize the shed photoreceptor outer segments (POS) [9]. N–CAM-140, Ezrin and EBP50 are important for polar alignment, microvilli development and the communication between photoreceptors and RPE cells [3,10]. At the same time, tight junctions between RPE cells are established along with the expression of tight junctional proteins, such as zonula occludens 1 (ZO-1), junctional adhesion molecules (JAMs), occluding, and claudins, which are essential for RPE polarization, molecule transportation, and integrity of the blood-retinal barrier (BRB) [8]. The emergence of these proteins and signaling pathways establishes the unique structure and function of the RPE, preparing it to interact with the photoreceptors to form a functional unit in the next stage.

In the RPE maturation stage, the RPE further differentiates and forms a functional unit with the neural retina. The key step is the fusion of the RPE with photoreceptors. At this stage, the photoreceptors are driven by the transmembrane protein P/rds and extend towards the RPE to form outer segments [11]. Meanwhile, the RPE elongates its apical microvilli into the subretinal space under the guidance of certain molecules, such as CLIC4 [12]. There are two types of apical microvilli for RPE: long and short forms. Long microvilli express various optical proteins, such as EZRIN and EBP50 [3,12]. Together with photoreceptors, these proteins complete the optical cycle. The short microvilli express MRCKβ and MerTK [9], which form photoreceptor sheaths and perform phagocytosis and POS renewal. In addition to the apical membrane on the outer side of the RPE, deep basal infoldings on the basolateral membrane are required for barrier and substance exchange functions of the RPE [3]. The formation of microvilli and basement membranes is completed around the 14th week of human embryonic development [2,7].

The RPE cells in different regions of the retina vary in size, density, structure, and gene expression. For example, RPE cells in the macula are smaller (7–11 μm in diameter) but with a higher density and a more regular cell morphology than those in the periphery region [13]. By contrast, RPE cells in the peripheral region of the retina can be up to 60 μm in diameter and differ significantly in height [13]. In addition, RPE cells in the macula contain more melanin and a more complex apical membrane structure, and the connections between RPE cells and photoreceptors in this region are tighter than those in the peripheral region [13]. At the molecular level, RPE cells in the macula show higher expression levels of genes related to glucose and lipid metabolism, angiogenesis, inflammation, and the extracellular matrix. These RPE cells also display higher enzymatic activities of acid phosphatase and cathepsin D, which may be related to the functions of the macula. By contrast, peripheral RPE cells express higher levels of Na^+/K^+-ATPase [14], monocarboxylic acid, α-ketoglutarate, leucine, proline, and choline transporter proteins, suggesting an ion transport-related function for RPE cells in the peripheral region of the retina.

Recent single-cell sequencing analyses also revealed the heterogeneity of RPE cells in different regions of the retina. The development-related transcription factors IRX3, FOXP1, and KLF2 are more active in the RPE cells of the macular area, suggesting potential roles of these transcription factors in RPE and retinal development [15]. Additionally, RPE cells in different regions of the retina differ in their susceptibility to retinal degenerative diseases. For example, age-related macular degeneration primarily affects RPE cells in the macular region [14,15], whereas late-onset retinal degeneration (L-ORD) and choroideremia (CHM) mainly affect RPE cells in the midperipheral region of the retina [14]. Recently, an artificial intelligence-based analysis of human eye tissue images also showed that RPE subpopulations have distinct susceptibilities to different retinal degenerative diseases and aging [14].

3. RPE functions

3.1. Phagocytosis

RPE cells can phagocytize and degrade exfoliated POS and maintain the renewal of POS to ensure the normal function of photoreceptors, the visual cycle, and nutrient circulation (Fig. 2). Phagocytosis of POS by RPE cells can be divided into three stages: binding of the POS, endocytosis, and degradation of the POS. RPE cells recognize and bind to POS-exposed phosphatidylserine (PS) via MerTK/Gas6 and αVβ5-integrin/MFGE8 pathways to initiate phagocytosis. Once engulfed, the shed POS enters the phagosome and the Atg12-Atg5-Atg16L complex is recruited. The cytosolic form of LC3 (LC3-I) is then recruited to the phagosome [16], which subsequently fuses with the lysosome to form a phagolysosome, resulting in degradation of the ingested POS cargo [16].

The constant production of shed POS requires RPE cells to phagocytize and degrade POS daily. This heavy phagocytic process makes the RPE one of the most phagocytic cell types. It is worth noting that the phagocytosis of POS by RPE is not in a steady state but peaks 1–2 h after the onset of light, which is called the diurnal phagocytic peak (DPK).

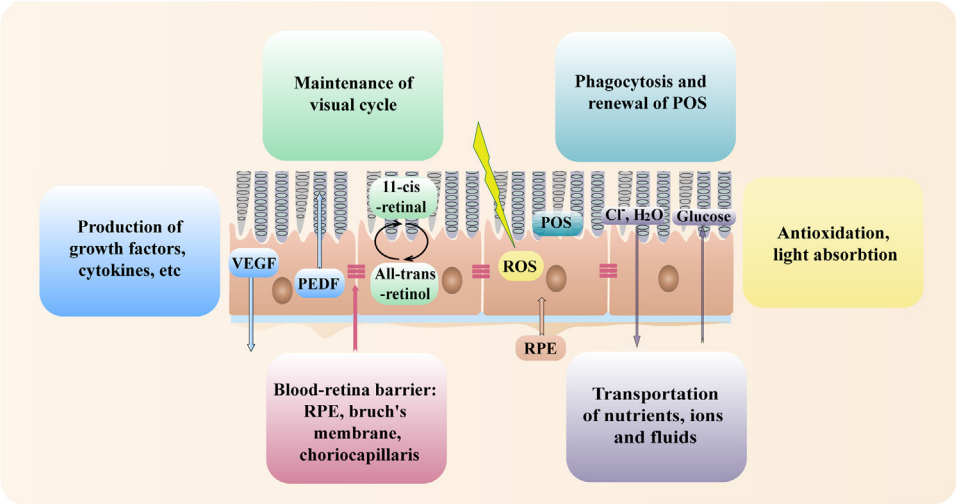

Fig. 2. Functions of the retinal pigment epithelium. RPE cells phagocytize and digest the shed photoreceptor outer segments (POS) to maintain the renewal of photoreceptor cells. Tight junctions between RPE cells play crucial roles in preventing plasma components and toxic molecules from entering the retina. RPE cells selectively transport nutrients, such as fatty acids and glucose, from the choroid membrane to the photoreceptors, and transport reversely the metabolic end products, ions, and excessive water. RPE cells are enriched with granules of melanin and can filter natural light and absorb light of different wavelengths, thus reducing the accumulation of reactive oxygen species (ROS) and oxidative damage to retinal cells. RPE cells isomerize the atRAL produced by photoreceptors after their irradiation to 11-*cis*-retinal. RPE cells synthesize and secrete numerous growth factors, cytokines and cellular structure related proteins, and play key roles in the regulation of the structures and functions of the retina and choroid.

DPK is a highly rhythmic activity of RPE cells controlled by a group of circadian clock genes that link the interaction between the RPE and the retina. For example, the deletion of the circadian clock gene *Bmal1* in RPE cells, but not in the retina, results in a significant decrease of DPK [17]. Mice deficient of the core circadian clock genes *Per1* and *Per2* lack RPE DPK under constant darkness, with altered gene expression in RPE cells [18].

Currently, it remains unclear whether dysregulated phagocytosis rhythm of RPE cells disrupts the normal functions of RPE and the retina. Some studies have shown that the destruction of phagocytic peaks affects the phagocytosis of POS by RPE cells, resulting in RPE and photoreceptor degeneration. For example, genetic deletion of the $\alpha v\beta 5$ receptor disrupts RPE phagocytosis, causing the accumulation of RPE lipofuscin and age-related loss of photoreceptors in mice [19]. On the other hand, transcriptome analysis of mice with conditional knockout of the circadian clock gene *Bmal1* in RPE cells revealed that approximately 830 protein-coding genes maintain normal rhythm and approximately 2696 non-rhythmic genes acquire rhythm after *Bmal1* deletion, which might compensate *Bmal1* deficiency to maintain the normal structure and function of the RPE and retina [17].

3.2. Barrier function

The BRB, which contains the RPE, Bruch's membrane, and choriocapillaris, is located between the choroidal blood supply and the neural retina. RPE polarization is an important prerequisite for BRB. Similar to other epithelial cells, RPE cells exhibit highly polarized characteristics with asymmetrically distributed proteins between the apical and basolateral membranes. The Na^+/K^+-ATPases that drive fluid transportation are located in the apical plasma membrane of RPE cells. The membrane polarity of RPE cells is the opposite of that of other types of epithelial cells. As such, RPE is considered as an "upside-down" epithelium. This special polar structure includes tight junctions between the RPE cells and the apical and basement membranes, all of which mediate the directional transportation of solutes and liquids to maintain the barrier function of the RPE cells. The tight junctions of RPE cells are formed by membrane, cytoskeletal, and cytoplasmic plaque proteins, including occludin, claudins, cell adhesion factors, tricellulin, and blocking proteins (as zonula occludens (ZO)). The RPE tight junctions permit the movement of fluid from the choroid to the retina but not the toxic molecules or plasma components. Claudins are the most important proteins involved in the formation of cellular tight junctions, intercellular adhesion, and cellular permeability [20,21]. Passive diffusion occurs through pores formed in the paracellular space and involves claudins [20,21]. In human fetal RPE cells, claudin-19 deficiency leads to elimination of transepithelial electrical resistance and damage to the BRB [22].

3.3. Transportation of nutrients, ions, and fluids

RPE cells selectively transport fatty acids, ascorbic acid, glucose, and other nutrients from choroids to photoreceptors. RPE cells also transport metabolic end products, ions, and excess water from photoreceptor cells to the choroid, mainly through membrane pumping, endocytosis, and passive diffusion. For adequate material exchange, RPE cells contain numerous infoldings that increase their membrane surface area for material transportation. GLUT1 transports glucose to photoreceptors from the choroid [23]. Lactate is then produced by photoreceptors utilizing glucose and cleared by the RPE cells via MCT1 on the apical-side [24]. Water transport is mainly driven by Cl^- channels from the subretinal space through the RPE to the bloodstream. K^+ and Cl^- channels are important for transepithelial ion transport. Ca^{2+} channels regulate the secretory function of RPE cells. Ligand-gated cation channels are important for RPE functions as they promote ion transportation or regulate intracellular Ca^{2+} homeostasis [25]. The activities of the K^+, Cl^-, and Ca^{2+} channels determine the properties of the RPE and its interaction with photoreceptors. Mutations in ion channel genes or alterations in the regulation of ion channel activity can lead to retinal degenerative diseases [25].

3.4. Protection of retinal cells against oxidative stress

The retina is often exposed to chronic oxidative stress, which can lead to or expedite many ocular diseases, such as AMD. The pigment particles in RPE cells can filter and absorb light of different wavelengths, reduce the reactive oxygen species (ROS), and decrease the harmful effects of ultraviolet rays on the retina. In addition, RPE cells are rich in

antioxidants, such as superoxide dismutase, catalase, and glutathione, which can decrease cellular damage due to oxidative stress. For example, the genetic deletion of the redox-sensitive protein DJ-1 in mice reduces the antioxidant capacity of RPE cells, thus rendering them more susceptible to low-dose sodium iodate ($NaIO_3$)-mediated oxidative damage [26]. In addition, conditional knockout of the transcription factor *REV-ERBα* in RPE cells exacerbates damage to RPE cells from oxidative stress in aged mice, whereas pharmacological activation of REV-ERBα decreases oxidative damage to RPE cells by activating NRF2 and the downstream antioxidant enzyme SOD1 [27].

3.5. Maintenance of the visual cycle

The RPE is critical in maintaining the visual cycle. Phototransduction begins with the absorption of light by rhodopsin in photoreceptors. Rhodopsin is formed by the combination of opsin and 11-*cis*-retinal, and is reddish-purple in color as a result of the absorption of blue-green light. Light exposure isomerizes the rhodopsin-bound 11-*cis*-retinal to all-*trans*-retinal (atRAL), causing its separation from opsin. Photoreceptors cannot isomerize atRAL into 11-*cis*- retinal [3]. However, atRAL can be reduced to all-*trans*-retinol by retinoid dehydrogenases (RDH) and transported to the RPE cells, where it is enzymatically converted back to 11-*cis*-retinal. 11-*cis*- retinal returns to the outer segments of rod photoreceptors, where 11-*cis*- retinal binds to opsin to regenerate rhodopsin, thus completing the visual perception cycle.

Visual cycle defects cause the accumulation of large amounts of atRAL in the retina, resulting in the degeneration or death of photoreceptors and RPE cells. Simultaneously, atRAL accumulation produces bis-retinoids, which are the main components of lipofuscin. Both atRAL and bisretinoids are important causative factors of AMD and Stargardt disease. Studies have shown that the atRAL-dimer is less cytotoxic and phototoxic than atRAL, has strong photosensitivity, and can be quickly decomposed into non-toxic hydrophilic small molecular fragments under light and cleared from cells, reducing the chance of an abnormal cluster of atRAL-dimers in the retina [28]. The formation of the atRAL-dimer is a rapid detoxification process for the body to combat atRAL overload, thereby helping to maintain the normal functions of the visual system.

3.6. Production of various factors

Another important function of RPE cells is to synthesize and secrete a variety of growth factors, cytokines, and structure-related proteins, which are important for the cytoskeletal system, angiogenesis, neural protection, and nutrient supply. RPE cells play key roles in the morphological and functional regulation of the retina and choroid.

RPE cells produce VEGFs, PDGFs, FGFs, CNTF, TGF-β, and IGF-1. Levels of VEGFs are closely related to vascular growth and function. The conditional knockout of *Vegf-a* in RPE cells leads to chorionic capillary ablation and visual defects in mice [29], whereas excessive VEGFs induce vascular abnormalities. The RPE secretes the neurotrophic factor pigment epithelium-derived factor (PEDF) to retain retinal and choriocapillary structures by inhibiting endothelial cell proliferation. Transforming growth factor-β (TGF-β) generated by RPE is a potent multifunctional cytokine known to provide immunosuppressive actions in the eye. RPE-derived tissue inhibitor of metalloprotease-1 (TIMP-1) and TIMP-3 are important factors that stabilize endothelial cells and extracellular matrix. CNTF, bFGF, and PEDF generated by RPE cells protect photoreceptors from light-induced damage. In addition, RPE cells are involved in immunomodulation of the eye by secreting cytokines, chemokines, complement components, and MHC class I/II molecules. For example, RPE cells produce and secrete a variety of cytokines, such as IL-6 and IL-8, after stimulation with the allergic toxin C5a, resulting in complement activation, RPE epithelial-mesenchymal transition (EMT), infiltration of MHC-II$^+$ cells, and subretinal fibrosis [30].

The levels of various factors produced by the RPE are associated with various eye diseases. For instance, RPE cells from patients with AMD produce 2–3 folds more MMP2 and pigment epithelial-derived factor than normal RPE cells [31], while the level of cysteine-rich acid protein (SPARC) was 2-fold lower than that of normal RPE cells [31]. Single-cell RNA sequencing has revealed that in non-human primates, RPE cells are highly susceptible to aging in the retina [32]. Also, the ligand-receptor pairs between aged RPE and choroidal cells are mostly related to inflammatory response and chemotaxis, such as the “CCL8-CCR3” and “IL34-CSF1R” pathways [32], suggesting an increased pro-inflammatory status of RPE during aging.

4. Immunomodulation by RPE

RPE cells play important roles in retinal immunity by orchestrating both innate and adaptive immunity, including cytokine secretion, activation of Toll-like receptors (TLRs), complement activation, and regulation of antigen-presenting cell (APC) properties. The immunogenic properties of RPE cells are primarily mediated by MHC molecules. RPE produces cytokines such as TLRs, IL-6, IL-8, MCP-1, ICAM-1, CXCL9, and CXCL10. TLR3 is the most abundantly expressed TLR in RPE cells, and its depletion inhibits AMD neovascularization. Similarly, TLR2 deletion greatly reduces choroidal neovascularization (CNV) formation in CNV mouse models [33,34]. Moreover, the TLR2 blockade preserves RPE tight junctions and protects RPE cells from fragmentation by reducing macrophage infiltration and the formation of a terminal complement membrane attack complex. When retinal inflammation occurs, IFN-γ induces RPE cells to produce pro-inflammatory numerous cytokines, which in turn recruit neutrophils and T cells to the inflammation site.

RPE cells have immunosuppressive and anti-inflammatory effects that prevent ocular inflammation. For instance, RPE cells produce immunosuppressive cytokines, such as TGF-β, IL-11, and IFN-β, to reduce the immune response in the posterior segment of the eye and to protect the retina from damage. RPE cells also produce TGF-β and IL-11 to support Treg formation and reduce the migration capacity of activated T cells. Furthermore, RPE cells can interact with tissue-resident immune cells, such as microglia for immune regulation. For example, treatment of microglia with supernatants from cultured TLR-3-activated RPE cells induces microglia to produce IL-1β, IL-6, and COX-2, thereby exacerbating the inflammatory response [35]. Microglia, in turn, produce and secrete TNF-α, leading to a reduction in ZO-1 levels in RPE cells and the disruption of tight junctions, thereby impairing BRB integrity [36].

5. RPE metabolism

RPE cells have efficient metabolic machinery to ensure energy supply to retinal cells and maintain the normal structure and function of photoreceptors. Photoreceptors mainly depend on glucose transported by the RPE to generate energy through aerobic glycolysis. If the RPE consumes too much glucose for its own metabolism, it would not be able to supply enough glucose to photoreceptors, which may lead to retinal dysfunction. Therefore, to limit glucose consumption, the RPE uses various nutrients from the bloodstream and oxidative metabolic byproducts from the retina to fuel mitochondrial metabolism [37]. In addition, RPE cells are highly active in lipid metabolism [38]. Recent studies have shown a close relationship between dysregulated lipid metabolism in the RPE and AMD development [39–41]. In this section, we discuss the mitochondrial and lipid metabolism of the RPE.

5.1. Mitochondrial metabolism

Mitochondrial metabolism is the primary mode of energy production in the RPE. The RPE mainly utilizes lactate, proline, and fatty acids as substrates for mitochondrial respiration. Large amounts of lactate are

produced during retinal glycolysis, inhibiting RPE glycolysis. Lactate serves as a substrate for RPE mitochondrial metabolism [42], in which ATP is generated by the oxidative phosphorylation of lactate [42]. Recent studies revealed that RPE uses proline as its major substrate [43]. Proline is synthesized in RPE cells from ornithine by ornithine aminotransferase (OAT). OAT deficiency causes RPE degeneration and gyrate atrophy of the choroid and retina [44]. Furthermore, proline is the only amino acid, whose levels increase during RPE differentiation in vitro [45]. Inhibition of mitochondrial oxidative phosphorylation by piericidin, antimycin, and oligomycin in RPE cells blocks this process, suggesting that proline catabolism is the major pathway for proline consumption during RPE differentiation [46]. In addition, the inhibition of proline catabolism can disrupt glucose metabolism and glutathione production in the RPE, whereas proline supplementation improves visual function in a $NaIO_3$-induced retinal degeneration mouse model [45]. Abnormal RPE proline metabolism is associated with the pathogenesis of several retinopathies, such as AMD and PVR [43]. However, whether dysregulated RPE proline metabolism is a direct cause of these pathologies is unknown [43]. The mitochondrial respiration of the RPE using fatty acids as substrates is discussed in Section 5.3.

Dysregulated mitochondrial metabolism can lead to mitochondrial damage or dysfunction, resulting in RPE and photoreceptor degeneration [47]. For example, genetic deletion of the mitochondrial phosphatase *PGAM5* increased mitochondrial fusion, decreased mitochondrial division, and enhanced mitochondrial ATP and ROS levels in RPE cells, resulting in RPE senescence by upregulating mTOR and IFN-β signal pathways [48]. The conditional deletion of *Sod2* (encoding the mitochondrial antioxidant enzyme MnSOD) in mouse RPE cells decreases RPE mitochondrial ATP levels and increases RPE glycolysis and ROS production, leading to RPE degeneration and impaired photoreceptor mitochondrial function [47].

5.2. Lipid metabolism

Normal lipid metabolism in the RPE is important for photoreceptor survival and function. RPE cells phagocytize and digest shed polyunsaturated fatty acid-rich photoreceptor outer segments via a non-canonical form of autophagy, and metabolize fatty acids to produce-hydroxybutyric acid, which is transported to the retina via MCT1 (on the apical processes) and MCT7 (in the retina) to be used as metabolic substrates [16,37]. RPE cells also transport lipids to fuel photoreceptors [49,50]. RPE cells take up lipoproteins in circulation via basal lipoprotein receptors, such as LDL-R, SR-BI, SR-B II, and CD36 [49]. RPE cells transfer these lipids to high-density lipoprotein (HDL)-like particles containing endogenous apoA-I and apoA-E [49]. These HDL-like particles are subsequently released from RPE cells via the ABCA1 protein in the apical aspects of RPE cells, and enter the photoreceptors via the SR-BI and SR-BII receptors on the outer segments of the photoreceptors [49]. RPE cells have an active cholesterol reverse transport system. Excessive cholesterol is removed by HDLs after binding to adenosine triphosphate-binding cassette (ABC) transporters, ultimately entering the liver, thus avoiding the secretion of large amounts of apoB100-containing lipoproteins by the lipid-overloaded RPE to Bruch's membrane [38].

Dysregulated lipid metabolism often leads to lipid accumulation in the RPE cells, resulting in RPE dysfunction [39,40]. For instance, treating cultured RPE cells with POS results in the accumulation of lipid droplets in RPE cells, indicating that daily phagocytosis of the photoreceptor outer segments by RPE cells may be one of the reasons for the accumulation of lipid droplets in RPE cells [40]. Chuang *et al.* found that RPE-specific knockdown of CLIC4 activated the key regulators of lipid metabolism and upregulated the expression of PLN2 and ApoE; and inhibited cholesterol transport through basal infolding of RPE cells [39]. These changes result in the accumulation of excessive cholesterol underneath the RPE to form drusen-like deposits, thereby inducing AMD phenotypes, such as infiltration of subretinal inflammatory cells, loss of RPE and photoreceptors, and impaired visual function [39]. Autophagy is critical for regulating RPE lipid metabolism regulation [16]. In mice with a genetic deletion of the lysosome-associated membrane protein LAMP2, RPE autophagy is reduced, leading to lipid accumulation in RPE cells and sub-RPE deposits (containing apolipoprotein E, clusterin, and vitronectin), with increased immune cell infiltration and other AMD-related phenotypes [51]. Overall, these studies suggest that abnormal RPE lipid metabolism may induce AMD pathogenesis. Therefore, restoring normal lipid metabolism in the RPE may be an important strategy for preventing and treating AMD. For example, upregulation of LXR in RPE cells reduces oxidative damage, inflammation, and subretinal lipid deposition in an *apoB100* mouse model [52]. Intracellular lipid droplet formation inhibits POS uptake by RPE cells, whereas the acyl-CoA synthetase inhibitor triacsin C displays the reverse effect [40]. Moreover, administration of the autophagy inducer flubendazole reduces intracellular lipid accumulation in human RPE cells [53].

5.3. The interplay between mitochondrial and lipid metabolism of RPE

Normal lipid metabolism is important for maintaining RPE mitochondrial metabolism. Fatty acids from shed photoreceptor outer segments are metabolized in the mitochondria of RPE by β-oxidation to produce NADH, FADH2, and acetyl coenzyme A [54–56]. Acetyl coenzyme A is synthesized by HMG-CoA synthase to form ketone bodies, which are transported to the retina via the monocarboxylate transporter protein at the apical processes of RPE cells and are eventually oxidized into CO_2 [54]. Dysregulation of lipid metabolism can lead to mitochondrial dysfunction in the RPE. Zhang *et al.* found that CYP4V2 mutations cause mitochondrial dysfunction by affecting fatty acid hydroxylation, resulting in the accumulation of polyunsaturated fatty acids in RPE cells, which subsequently increases ROS production in the mitochondria, disrupts mitochondrial respiratory chains, and triggers RPE apoptosis [57]. Restoration of CYP4V2 expression significantly reduces lipid accumulation in RPE cells and prevents apoptosis [57]. Deletion of Elovl2, an essential gene for the synthesis of polyunsaturated fatty acids, causes fatty acid accumulation and disrupts mitochondrial metabolism in both mouse and human RPE cells, which in turn induces chronic endoplasmic reticulum stress and mitochondrial dysfunction, eventually leading to RPE cell senescence and AMD. By contrast, vitamin B3 supplementation markedly ameliorated RPE senescence caused by ELOVL2 deficiency [41].

Mitochondrial metabolism also affects lipid metabolism in RPE cells. Mitochondria are key sites for the oxidative metabolism of fatty acid [41]. Compared to the peroxisomal-mediated fatty acid-oxidation pathway, which does not produce ATP, fatty acids within RPE cells mainly use oxidative catabolism via the mitochondria-mediated-oxidation pathway [50]. Deficiency of long-chain 3-hydroxyacyl coenzyme A dehydrogenase (LCHAD) induces pigmentary retinopathy, a disorder of mitochondrial fatty acid-oxidation with the accumulation of 3-hydroxyacyl intermediates in RPE cells [56]. Activation of the LXR pathway in RPE cells by its agonist GW3965 activates mitochondrial autophagy, reduces the accumulation of the lipid metabolite 7-ketocholesterol, suppresses the mTOR and P62 pathways, and ameliorates 7-ketocholesterol-induced apoptosis of RPE [58]. Moreover, overexpression of the mitochondrial regulator PGC-1α in RPE cells induces mitochondrial respiration and promotes fatty acid-oxidation, thereby protecting RPE cells from oxidative damage-induced cell death [59].

5.4. Other types of metabolism in RPE cells

Apart from mitochondrial and lipid metabolism, reductive carboxylation is also crucial to maintain RPE homeostasis. Cultured RPE cells display a high level of reductive carboxylation, a reverse tricarboxylic acid (TCA) cycle, which is fueled by proline [45]. Reductive carboxylation can confer RPE cells resistance against oxidative stress by the supplementation of NAD^+ precursors or its substrate α-ketoglutarate, or by the treatment with a poly (ADP ribose) polymerase inhibitor [60].

5.5. Metabolic interplay between RPE and photoreceptors

Photoreceptors and RPE cells have close metabolic interactions. For instance, photoreceptors are the primary cells in the retina using glucose. Photoreceptors are capable of converting glucose into lactate, which suppresses glycolysis in RPE cells, thereby ensuring more glucose to reach the retina [42]. On the other hand, conditional deletion of the mitochondrial antioxidant enzyme *Sod2* in mouse RPE cells caused a compensatory increase in RPE glycolytic metabolism and disrupted RPE function, and led to severe damage to photoreceptor mitochondria and metabolism [47].

6. RPE dysfunction/atrophy-related pathologies

Normal structure and function of RPE cells are essential for the maintenance of ocular homeostasis and vision. Oxidative stress, inflammation, aging, and other factors may cause RPE degeneration or atrophy, thus triggering or exacerbating many eye diseases, such as AMD, PVR, Stargardt disease, RP, and DR.

6.1. Age-related macular degeneration

AMD is the third leading cause of visual loss globally, occurring in two forms: dry and wet AMD [61]. Dry AMD accounts for 85%–90% of total AMD and is characterized by the accumulation of subretinal drusen deposits and degeneration of RPE and photoreceptors. Oxidative stress-induced loss of RPE plays a key role in dry AMD. Wet AMD, characterized by CNV and accounting for approximately 10% of the total AMD cases, is the main cause of vision loss in patients with AMD.

Necroptosis is the major form of oxidative stress-induced RPE cell death. RPE cell necroptosis (cell swelling and vacuolization) have been observed in both dry AMD mouse models and patients with geographic atrophy (GA), with no features of RPE cell apoptosis (caspase-3 activation or chromatin condensation). In a $NaIO_3$-induced dry AMD mouse model, the loss of RPE cells occurred via necroptosis in an RIPK1/RIPK 3-dependent manner, which was ameliorated by the administration of a RIPK1 inhibitor [62,63]. Ferroptosis and pyroptosis can lead to RPE cell death. In a $NaIO_3$-induced dry AMD mouse model, heme oxygenase-1 (HO-1) knockdown or administration of the HO-1 inhibitor ZnPP significantly reduced RPE ferroptosis and improved retinal structure and visual function [64]. Accumulation of atRAL in RPE cells induces RPE pyroptosis through the activation of the NLRP3 inflammasome [65].

Additionally, impaired RPE autophagy promotes AMD pathogenesis. The autophagic activity of RPE cells in patients with AMD is lower than that in healthy RPE cells. Mice with a genetic deletion of lysosome-associated membrane protein-2 (LAMP2) exhibit decreased autophagy in RPE cells and many dry AMD pathologies, such as basal laminar deposits (BLamDs) and accumulation of APOE, APOA1, clusterin, and vitronectin adjacent to BLamDs [51]. Conditional knockout of the autophagy-inducing protein RB1CC1 in RPE cells disrupts RPE autophagy in mice, leading to AMD phenotypes, such as RPE degeneration, loss of photoreceptors, and CNV [66].

Under pathological conditions, such as in hypoxia or oxidative stress, RPE cells are induced to produce and secrete excessive VEGF, which promotes abnormal angiogenesis and results in CNV, a hallmark of wAMD [61]. Therefore, inhibiting VEGF produced by RPE cells is a useful strategy for wAMD treatment. For instance, intravitreal injection of a RPE-specific AAV-mediated soluble fms-like tyrosine kinase-1 (sFlt-1) expression reduced laser-induced CNV in mice [67].

6.2. Proliferative vitreoretinopathy (PVR)

PVR, a complication of rhegmatogenous retinal detachment, is characterized by the formation of a proliferative membrane within the vitreous cavity and on both sides of the retinal surface [68]. Currently, there is no pharmacological approaches available for PVR treatment [68]. The epithelial-mesenchymal transition (EMT) of RPE is a key pathological mechanism of PVR [68]. When RPE cells undergo EMT, they lose their epithelial polarity and tight junction proteins, which promote their migration into the vitreous cavity, leading to the growth and contraction of cellular membranes, tractional retinal detachment, and PVR.

Several regulatory factors, such as TGF-β and FXa, are involved in RPE EMT. Recently, METTL3, a methyltransferase that catalyzes RNA m6A modification, has been found to have lower expression levels in the RPE of patients with PVR than in normal RPE [69]. METTL3 depletion promotes RPE EMT, whereas its overexpression had the opposite effect [69]. The intravitreal injection of RPE cells overexpressing METTL3 into the eyes of rats with experimental PVR delayed PVR development [69]. Genetic deletion of death-associated protein-like 1 (*DAPL1*) in mice promotes RPE migration, growth, and EMT, whereas DAPL1 overexpression restores tight junctions and hexagonal morphology of RPE cells. Injection of AAV9-DAPL1 reduced subretinal proliferative membranes in a retinal detachment mouse model, indicating that DAPL1 inhibits RPE EMT [68].

6.3. Stargardt disease

Stargardt disease is an inherited macular dystrophy characterized by lipofuscin deposition in RPE cells and death of photoreceptors [70]. STGD1 is the most common Stargardt disease caused by mutations in the ATP binding box A4 (*ABCA4*) gene [70]. Stem cell therapy, gene therapy, visual cycle modulators (VCMs), and complement inhibitors are considered as potential treatments for STGD1 [71,72].

Embryonic stem cells (ESCs) hold great promise for the treatment of Stargardt disease because of their unlimited capacity for self-renewal and ability to differentiate into numerous types of cells. A clinical trial revealed that subretinal injection of human embryonic stem cell-derived retinal pigment epithelium (hESC-RPE) cells improved visual acuity in two patients with Stargardt disease by 12 and 19 letters [73]. However, in another clinical trial of 12 patients diagnosed with severe Stargardt disease, subretinal transplantation of hESC-RPE cells did not significantly improve visual acuity, even though there were no obvious side effects [72]. Further investigation of the efficacy and safety of hESC-RPE transplantation in more patients are needed.

ABCA4 gene therapy is another potential therapeutic strategy for Stargardt disease. Subretinal injection of equine infectious anemia lentivirus (EIAV)-*ABCA4* into *ABCA4* knockout mice significantly reduced the concentration of A2E, the main component of lipofuscin. Moreover, subretinal injections of EIAV-*ABCA4* did not show obvious toxicity in rabbits and macaques [71], suggesting the potential efficacy and safety of EIAV-*ABCA4* therapy for Stargardt disease.

Treatment with remofuscin, a potent and reversible inhibitor of H^+/K^+ ATPase, reduced lipofuscin levels in both an aged STGD1 mouse model (*Abca4*$^{-/-}$ mice) and human RPE cells with no obvious toxic effects, indicating that remofuscin could be a potential therapeutic agent for the treatment of Stargardt disease [74].

6.4. Retinitis pigmentosa (RP)

RP is an inherited degenerative disease characterized by the progressive loss of photoreceptors and RPE cells, which can eventually lead to blindness. Mutations in more than 80 genes can cause RP, including *RHODOPSIN, PRPFs, RPGR,* and *MERTK* [75]. Gene therapy and antioxidant agents are potential therapeutic strategies for RP.

Mutations in pre-mRNA processing factors *(PRPFs)* are also found in inherited RP, among which *PRPF31* mutations are the most common [75]. However, the underlying pathogenic mechanisms and affected cells are not fully understood. The features of *PRPF31*-related RP include the incomplete penetrance and unaffected carriers of mutations. Similarly, no apparent RP phenotype has been observed in *Prpf31*$^{+/-}$ mice [75]. A recent study generated iPSCs from the fibroblasts of patients with RP with *PRPF31* mutations and induced iPSC differenti-

ation into RPE cells and retinal organoids [75]. These iPSC-derived RPE cells display structural abnormalities and functional defects, such as the loss of tight junctions and impaired polarity [75]. The iPSC-derived retinal organoids exhibit progressive photoreceptor degeneration and an RP-like phenotype [75]. Correction of *PRPF31* mutations using CRISPR/Cas9 showed that *PRPF31* haploinsufficiency is one of the causes of RP-like phenotypes [75]. Moreover, AAV-driven *PRPF31* overexpression improved the survival of photoreceptors [75]. Nrf2 is a transcription factor that inhibits oxidative stress. AAV-driven NRF2 overexpression improved cone cell survival and vision in the three RP mouse models [76]. Moreover, AAV-driven NRF2 overexpression in RPE cells rescued the loss of cone receptors and vision impairment in rd1 and rd10 mice by upregulating multiple anti-oxidation pathways, thereby improving RPE integrity and connections between the RPE and photoreceptors [76].

6.5. Diabetic retinopathy (DR)

DR, a severe complication of diabetes, is a major cause of vision loss worldwide. DR can be classified as non-proliferative (NPDR) or proliferative (PDR). NPDR is characterized by microaneurysms and vascular abnormalities of the retina, whereas PDR is characterized by retinal neovascularization that invades the vitreous body, vessel leakage, fibrosis, and vision impairment. BRB disruption or autophagy dysfunction in RPE promotes DR progression [36]. IL-6 expression is elevated in DR patients. In high-fat diet-induced obesity and streptozotocin (STZ)-induced hyperglycemia mouse models, intravitreal injection of IL-6 increased microglial adhesion to the RPE by stimulating VEGF-A secretion by RPE cells. Moreover, IL-6 induced TNFα secretion in microglia and reduced ZO-1 expression in RPE cells, resulting in BRB disruption and DR progression [36]. Dysregulated RPE autophagy leads to DR [77]. For example, HMGB1 inhibits lysosomal membrane permeabilization (LMP) in a CTSB-dependent manner [77]. In contrast, *HMGB1* knockdown rescued LMP in RPE cells, restores RPE autophagy, decreases the expression of proinflammatory factors and VEGF-A, and prevents RPE cell apoptosis in the early stages of DR [77].

7. Translational research

RPE impairment or defects are primary causes of many blindness diseases. Cell therapy to replace damaged RPE cells represents an attractive therapeutic strategy for such diseases. Currently, several clinical trials using pluripotent stem cells (PSCs)-derived RPE cells have been conducted. These studies differ in that they used different types of PSC (embryonic stem cells or induced PSCs), various delivery methods (cell suspension or RPE patch) in combination with different biomaterials [78]. Although most of such clinical trials are still undergoing, some of them have provided preliminary evidence of safety and efficacy of RPE cell therapy. For instance, subretinal transplantation of hESC-derived RPE was performed in 9 dry AMD patients and 9 Stargardt macular dystrophy patients. The results were encouraging since improved visual acuity in 10 of 18 patients was observed one year after cells transplantation, and no adverse event related to the transplanted cells was found after 22 months [79]. Another study on iPSCs-derived RPE monolayer sheet transplantation in a wAMD patient proved to be safe after 1 year, although no improvement or deterioration of visual acuity was observed [80]. In addition, numerous preclinical studies on cell therapy using PSC-derived RPE cells are conducted. A chemical reprogramming method was used to generate iPSCs. Efficient and reproducible experimental processes to obtain clinical-grade RPE cells were tested. Biocompatible materials were generated to support the transplantation of PSC-derived RPE cells [78]. And surgical devices were developed to facilitate RPE patch transplantation. The results of these studies will help accelerate RPE-based cell therapy for the treatment of various ocular diseases.

8. Conclusion and perspectives

The RPE is essential for maintaining retinal homeostasis. RPE cells are specialized phagocytes responsible for the clearance and turnover of POS, thereby preventing the accumulation of toxic waste that can lead to photoreceptor dysfunction. RPE cells can isomerize all-trans retinal derived from photoreceptors into 11-*cis* retinal and transport it back to the photoreceptors to ensure the integrity of the visual cycle. The RPE is an important component of the BRB that regulates the movement of nutrients, ions, and fluid between the choroid and neural retina. Moreover, the RPE can absorb light and protect retinal cells from oxidation, thereby preventing retinal oxidative damage. RPE cells secrete various growth factors, cytokines, and immune factors to maintain the retinal structural integrity. Therefore, RPE cell degeneration or dysfunction is often an initiating factor in many blinding diseases, such as AMD, PVR, and Stargardt disease.

The mechanisms underlying the RPE function are not fully understood. For example, RPE cell subpopulations exist in different regions of the retina. Whether and how such heterogeneity of RPE cells affects RPE development, the interaction between the RPE and photoreceptors, and susceptibility to retinal diseases remain to be better understood. In addition, many other questions remain unanswered. For example, does the rhythmic phagocytic activity of the RPE (the diurnal phagocytic peak) affect the function of the RPE or retina? What are the molecular mechanisms underlying the interactions between RPE and immune cells in the retina? What molecules/pathways determine the pro- or anti-inflammatory functions of RPE under different conditions? Accumulating evidence has shown that RPE metabolism is important for its function. However, our understanding of the regulation of RPE metabolism is still limited, and more in-depth investigations, such as clinical studies, are required to verify whether RPE metabolism could be a potential therapeutic target for the treatment of eye diseases. Clarifying the above questions may help gain a better understanding of RPE function, the underlying molecular mechanisms, and the pathogenesis of numerous eye diseases.

Abbreviations

ATPase, adenosine triphosphatase; ABC, adenosine triphosphate-binding cassette; AMD, age-related macular degeneration; atRAL, all-trans-retinal; APC, antigen-presenting cell; ABCA4, ATP binding box A4; BLamDs, basal laminar deposits; BRB, blood-retinal barrier; CNV, choroidal neovascularization; CHM, choroideremia; CNTF, ciliary neurotrophic factor; DAPL1, death-associated protein-like 1; DR, diabetic retinopathy; DPK, diurnal phagocytic peak; ESCs, embryonic stem cells; EMT, epithelial-mesenchymal transition; EIAV, equine infectious anemia lentivirus; GA, geographic atrophy; HO-1, heme oxygenase-1; HDL, high-density lipoprotein; hESC-RPE, human embryonic stem cell-derived retinal pigment epithelium; IGF-1, insulin-like growth factor-1; IPM, interphotoreceptor matrix; IRBP, interphotoreceptor retinal binding protein; L-ORD, late-onset retinal degeneration; LXR, liver X receptor; LCHAD, long-chain 3-hydroxyacyl coenzyme A dehydrogenase; LMP, lysosomal membrane permeabilization; LAMP2, lysosome-associated membrane protein-2; NPDR, non-proliferative diabetic retinopathy; OAT, ornithine aminotransferase; PPAR, peroxisome proliferator-activated receptors; PS, phosphatidylserine; POS, photoreceptor outer segments; PDGFs, platelet-derived growth factors; PRPFs, pre-mRNA processing factors; PDR, proliferative diabetic retinopathy; PVR, proliferative vitreoretinopathy; ROS, reactive oxygen species; RPE, retinal pigment epithelium; RP, retinitis pigmentosa; RA, retinoic acid; RXR, retinoid X receptor; STZ, streptozotocin; TGF-β, transforming growth factor-β; VEGFs, vascular endothelial growth factors; VCMs, visual cycle modulators; ZO, zonula occludens.

CRediT authorship contribution statement

Shasha Wang: Data curation, Writing – original draft. **Wanhong Li:** Writing – original draft. **Min Chen:** Data curation, Writing – original draft. **Yihai Cao:** Writing – original draft. **Weisi Lu:** Data curation, Writing – original draft. **Xuri Li:** Writing – original draft.

Declaration of competing interest

The authors declare that they have no conflicts of interest in this work.

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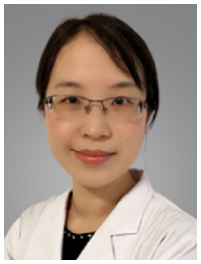

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