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MicroRNA regulation of critical retinal pigment epithelial functions

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Abstract

MicroRNAs are short, evolutionarily conserved noncoding RNAs that are critical for the control of normal cellular physiology. In the retina, photoreceptors are highly specialized neurons that transduce light into electrical signals. Photoreceptors, however, are unable to process visual stimuli without the support of the retinal pigment epithelium (RPE). The RPE performs numerous functions to aid the retina, including the generation of visual chromophore and metabolic support. Recent work has underscored how microRNAs enable vision through their contributions to RPE functions. This review focuses on the biogenesis and control of microRNAs in rodents and humans, the roles microRNAs play in RPE function and degeneration, and how microRNAs could serve as potential therapeutics and biomarkers for visual diseases.

The retinal pigment epithelium's critical functions

The RPE is a postmitotic epithelial monolayer that is located directly adjacent to the photoreceptor outer segments (POS) of the outer neural retina. Both the RPE and retina develop from the optic neuroepithelium, and the RPE is necessary for both proper retinal development and function throughout the human life span [1]. Furthermore, the retina is the only directly viewable portion of the central nervous system (CNS), and numerous noninvasive tools for investigating the structure, function, and physiology of the retina have been developed, allowing for direct assessment *in vivo* of neural function. Thus, study of the

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Declaration of interests

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RPE and retina is likely to yield valuable insights on the structure and function of the CNS more generally.

The RPE and photoreceptors are deeply intertwined, both physically and functionally. Multiple functions of the RPE directly support photoreceptor health, including critical steps in the visual (retinoid) cycle, transport of nutrients, maintenance of the blood–retinal barrier, absorption of stray light, and daily phagocytosis of shed POS [2]. Of note, the RPE secretes growth factors such as the pigment epithelium-derived factor (PEDF) that maintain normal neural function [3]. The RPE also has a glial-like role in the regulation of ion balances necessary for photoreceptor excitability [4]. Critically, the RPE and photoreceptors are metabolically coupled. As the outer retina is highly hypoxic, photoreceptors rely almost exclusively on glycolysis of glucose supplied by the choroidal vasculature via the RPE, while the RPE largely avoids the use of glucose, relying instead primarily on oxidative metabolism of spent metabolites and macromolecules from photoreceptors such as lactate, succinate, proline, and materials from phagocytosed POS [2,5,6]. The RPE is heavily dependent on mitochondrial metabolism, and RPE metabolic derangement from mitochondrial damage is detrimental to RPE and photoreceptor alike. Thus, persistent dysfunction of the RPE can lead to the decline of photoreceptors; indeed, RPE deficits are hypothesized to contribute to the early pathogenesis of **age-related macular degeneration (AMD**; see Glossary). One major contributor to RPE genetic and functional regulation is its system of microRNAs (miRs). In this review, we first provide a brief overview of miR biogenesis and physiology as well as summarize recent work in the field of rodent and human miR RPE biology in various contexts, from tissue development to normal function and disease. Lastly, we discuss methods for studying miR biology, offer perspective on the potential utilization of miR in diagnostics and therapeutics, and suggest potential new avenues for advancement of the field.

miR biogenesis and physiology

First discovered in *Caenorhabditis elegans*, miRs were identified to be noncoding RNAs that suppress mRNA expression through complementary base pairing [7,8]. Since their discovery, miRs have been found in a diverse array of organisms, from eukaryotes to viruses. Their nucleotide sequences and functions are often broadly conserved across species, supporting their hypothesized role in fine-tuning and controlling gene expression. This review focuses specifically on recent reports of miRs in the eyes of humans and in rodent models, and of the RPE in particular, though due to the focused scope of this review, we are unable to discuss several important reports of other eye-related miR findings.

In most instances, eukaryotic miRs are transcribed by RNA polymerase II in the nucleus to form a primary miR (pri-miR), which assumes a stem-loop structure (Figure 1). Pri-miRs are then usually processed by Drosha and DCGR8 to form a pre-miR and then exported to the cytoplasm by exportin 5 and RAN-GTP [9]. The pre-miR undergoes further processing by Dicer and forms an RNA complex composed of the miR and its passenger strand. The duplex is then loaded into an RNA-induced silencing complex (RISC), of which the main effectors are the Argonaute proteins, mainly Argonaute 2 (Ago2) [10]. The passenger strand is then removed from the RISC and the mature miR, which is roughly 20–22 nucleotides

long, remains bound to Ago2 [11]. Of note, either strand can be loaded into the RISC; the sequences closer to the 5′-end of the original pri-miR are called the 5p miRs, and the sequences closer to the 3′-end are called the 3p miRs. Once bound to Ago2, the miR–RISC complex is relatively stable, with reported half-lives on the order of days, though in some specialized compartments, such as the CNS, neuronal miR expression is tightly regulated by activity, and turnover happens more rapidly, on the order of hours [12].

miRs canonically repress mRNA expression through base pairing of the seed sequence to a complementary site in the 3′-untranslated region (3′-UTR) of the target mRNA molecule [8,10]. The seed sequence is a generally well-conserved 7–8-nucleotide region at the 5′-end of the miR, starting with the second nucleotide. Tight complementarity of the target mRNA to the seed sequence of the miR loaded into the RISC is thought to be the basis for the specificity of miR regulatory functions [13]. Accordingly, most miR target prediction algorithms heavily weight the presence of a seed sequence binding region [14,15]. Once bound to their target, vertebrate miRs generally induce downregulation of mRNA expression through two main mechanisms: translational repression or deadenylation and destabilization of the mRNA [16,17]. Although most miR interactions lead to decreased gene expression, there are limited reports of miR expression leading to increased translation and upregulation of target genes under specific conditions, such as cell cycle quiescence and altered metabolism [18,19]. Another layer of complexity is found in miR families, which share identical or similar seed sequences. These so-called iso-miRs are thought to offer redundant control over mRNAs, as they can target the same mRNAs, and some knockdown experiments only show an effect when multiple members of a miR family are disrupted [20].

Although the canonical pathway of miR biogenesis and function has been well described, knowledge of other mechanisms of miR-mediated regulation is still evolving. For example, miRs can be generated through pathways independent of Drosha/DCGR8 or Dicer [21–23]. Similarly, while seed sequence binding to the 3′-UTR is heavily weighted in predicting miR interactions, there are multiple reports of noncanonical binding events. These include interactions with an incomplete seed sequence, pairing with the 3′-end of the miR, and pairing with mRNAs in their 5′-untranslated region and open reading frames. Indeed, up to half of the miR–mRNA pairs isolated by various methods of cross-linking or copurifying with Argonaute show promiscuous and noncanonical binding [24,25]. The relevance of these interactions is unclear, suggesting that the RISC spends time transiently sampling and binding to potential targets. Hence, it seems reasonable to speculate that additional pathways controlling miR expression and function may be characterized, and these could potentially lead to the discovery of novel functions of these noncoding RNAs. Alternatively, the RISC could have additional regulatory functions in certain contexts that have not been fully explored and cannot be fully described by current models.

miRs in the RPE

As a nonrenewing tissue, the RPE must carefully coordinate and regulate its functions in support of photoreceptors. The additional layer of control offered by miRs allows the RPE to regulate its metabolism, phagocytosis, and other pathways in a coordinated manner. Recent work on RPE miRs, discussed later, has highlighted both the critical roles of miRs

in the RPE and how miRs may enable intercellular communication (Figure 2). Collectively, these studies have advanced current understanding of both the regulatory mechanisms of RPE physiology during healthy conditions and how dysregulation of the RPE contributes to retinal diseases.

There are several critical features of the miRs found in the eye. For example, miRs in photoreceptors and other CNS neurons were shown to be translated rapidly and degraded in a rapid and direct manner by light [12]. This transient, cyclical behavior contrasts with the longer half-lives shown for miRs in other tissues, suggesting unique functions and circadian regulation for miRs in photoreceptors and the RPE [26,27]. miRs are also indispensable for RPE and eye development and maintenance. In mature murine RPE, loss of Dicer1 or DCGR8 resulted in severe disorganization of the RPE and retinal dysfunction, and similar defects can be found in photoreceptors lacking Dicer1 and retinas lacking Ago2 [28–30]. Similarly, decreased expression of Dicer1 is correlated with both dry and wet AMD, though the role of Dicer1 in this pathology is disputed and may also be related to the processing of *Alus* and other short RNA species [31,32]. While the importance of miRs and their processing machinery in the RPE is not in doubt, their exact roles need further elucidation.

RPE development and tissue identity

miRs play a pivotal role in RPE development. For example, master transcription factors such as Pax6 and Mitf regulate RPE differentiation in part through miRs [33–36]. Several miRs are differentially regulated in ophthalmic development, such as miR-204 and miR-302 [37]. During human RPE development, miR-302 was shown to be highly downregulated, and overexpression of miR-302 induced dedifferentiation of RPE through the targeting of *CDKN1A* [38]. Recent work has identified other miRs thought to play roles during development, including miR-125n, let-7a, and miR-382 [39,40]. Not only do miRs regulate normal RPE development, they also regulate transdifferentiation. A major phenotype of RPE stress in AMD and other diseases is **epithelial–mesenchymal transition (EMT)**, where transdifferentiation of RPE cells results in a loss of epithelial phenotype and an increased ability to migrate and proliferate, to the detriment of the retina and its vasculature [41–43]. For example, *in vitro*, EMT was repressed in cultured human RPE through the expression of miR-27b-3p, which targeted HOXC6 and downregulated transforming growth factor (TGF)-β2 signaling [44]. By contrast, when EMT was induced by exogenous TGF-β2 or mechanical stretch in the transformed ARPE-19 human RPE line, miR-29b was downregulated [45]. In streptozotocin-induced diabetes, EMT of murine RPE was promoted by miR-195 through downregulation of Smurf2, resulting in increased vascular endothelial growth factor A (VEGF-A) expression [46]. Further support for this hypothesis can be found in animal model studies of EMT. A major focus of the study of EMT and RPE dysfunction is in proliferative vitreoretinopathy, where inflammatory and dedifferentiation signaling through molecules such as Yap1, galectin-1, TGF-β, and caveolin-1 drives RPE EMT and subretinal fibrosis [47–52]. Similarly, EMT in a mouse model of **diabetic retinopathy (DR)** was inhibited through either overexpression of miR-2094 or inhibition of its target, the long noncoding RNA (lncRNA) NEAT1 [53]. Further study of miRs in normal and aberrant development of the RPE will deepen our understanding of the basic processes that drive

differentiation and will identify targetable miRs and pathways that could be manipulated in RPE dysfunction in order to maintain cellular and tissue identity.

Phagocytosis and endolysosomal function

One critical role for the RPE is the circadian phagocytosis of POS. Up to 10% of POS are shed each day, necessitating their daily removal and processing [54]. miRs regulate the phagocytosis of POS and their subsequent breakdown in the lysosomal pathway. For instance, overexpression of miR-302d suppressed RPE phagocytosis [38]. Other miRs such as miR-184, miR-29, and miR-1273g modulate autophagy, a process through which the RPE breaks down and recycles POS [55–57]. Further understanding of autophagy could lead to a new targetable pathway for AMD treatment [58]. Integration of our knowledge of the general phagocytic pathway and identification of miRs that regulate various steps in that pathway have resulted in the identification of numerous miR–mRNA pairs in the RPE, such as miR-204 targeting Rab22a, a regulator of vesicle trafficking [59]. Additionally, mice that lack miR-211 globally exhibit defects in RPE-mediated phagocytosis and endolysosomal function; miR-211 was shown to downregulate the cytoskeletal protein Ezrin, which is highly expressed in the RPE as a coordinator of phagocytosis. Confirming the role of Ezrin in RPE phagocytic function, pharmacological inhibition of Ezrin with the small molecule NSC668394 by blocking Ezrin actin binding through phosphorylation at T567 rescued the lysosomal dysfunction phenotype in miR-211-knockout mice [60–62]. Further identification of miR regulators of phagocytosis can point to novel risk factors for RPE dysfunction in the context of AMD and suggest potential targets for treatment or augmentation of RPE function.

Exosome-derived miRs

A large proportion of cell and tissue types secrete exosomes and **extracellular vesicles (EVs)**. These EVs are theorized to enable intercellular communication and can contain DNA, RNA, proteins, and other metabolites [63]. A growing body of literature has investigated the EVs released by the RPE in both homeostasis and disease [64–66]. EVs frequently carry miRs as cargo, and EVs from RPE may help regulate retinal and RPE physiology.

In AMD, inflammation is often implicated in RPE and retinal stress, and signaling from the immune system plays a role. Retinal and RPE degeneration often leads to the migration of macrophages and microglia into the outer retina. To investigate the role of EV secretion between the RPE and macrophages, human macrophages and iPSC-derived human RPE were cocultured in transwell plates. EV communication between the two cell types was associated with the secretion of proinflammatory cytokines such as interleukin (IL)-6 and VEGF and miR-494-3p from the RPE [67]. Another study reported that RPE samples from elderly humans released miR-21 from EVs that were then taken up by microglia, leading to a change in expression of genes in the p53 signaling pathway, implicating microglia in regulation of aging and AMD [68].

EMT can also be induced by exosomes that carry miRs. In one model, EMT was induced in ARPE-19 cells via administration of TGF-β. When EVs were isolated from these induced ARPE-19 cells, the EVs were themselves able to induce EMT in naive ARPE-19 cells, and miR-543 was identified as the most significantly enriched miR in those EVs [64]. Similarly, exosomes derived from ARPE-19 cells in high-glucose conditions which contained miR-202-5p were able to suppress vascular proliferation and EMT through direct targeting of TGF-βR2 [69]. While these preliminary results indicate that EV-derived miRs could modulate EMT, further work in primary cultures and animal models are needed to confirm these findings. Nonetheless, the identification of critical miRs contained in EVs could be leveraged as a treatment platform for a variety of RPE diseases and offer a potential delivery system for miRs.

Inflammation, oxidative stress, and neovascularization

As a postmitotic monolayer, the RPE must last a lifetime to provide support to photoreceptors. Inflammation and oxidative stress can result in RPE deterioration, and miRs can help the RPE respond to such stressors. For instance, in ARPE-19 cells, inflammation caused by the *Nlrp3* inflammasome is directly counteracted by targeting of NLRP3 mRNA by miR-22-3p or miR-191-5p [70,71]. In primary human RPE cultures, apoptosis triggered through the Fas receptor can be abrogated by targeting of Fas receptor by miR-374 [72].

Oxidative stress and lipid dysregulation in the RPE potentially contribute to AMD and other RPE and retinal disorders. This can be through direct modulation of mitochondria or through interaction with downstream effectors [73,74]. miR-33 was recently shown to modulate expression of ABCA1, a cholesterol pump in the RPE, and suppression of miR-33 via antisense oligonucleotides in mouse and non-human primate models alleviated the dry AMD phenotype [75]. Similarly, the H-RPE cell line treated with oxidized low-density lipoproteins exhibited altered expression of miRs and genes known to cause retinitis pigmentosa [76]. Additionally, hyperglycemia is thought to cause oxidative and metabolic stress in the RPE, leading to apoptosis. Preliminary data from the ARPE-19 and RPE-1 cell lines suggest that protection from high-glucose conditions is mediated in part through miRs such as miR-27a, which inhibits Toll-like receptor (TLR) 4, and miR-25, which inhibits PTEN and Akt signaling [39,77,78]. Two studies independently concluded that miR-125b inhibits oxidative stress through binding to hexokinase 2, a key enzyme in the glycolysis pathway, though they reached opposite conclusions about the role of hexokinase 2 in the ARPE-19 cell line. While one study argued that inhibition of hexokinase 2 resulted in decreased oxidative stress from hyperglycemia, according to the second study, disordered glucose metabolism was restored and protective against hydrogen peroxide treatment [79,80]. Further work in human and animal samples is necessary to reconcile these opposing ideas. Other identified mediators of oxidative stress and their miR regulators include Nrf2, regulated by miR-626, miR-141, and miR-144 [81–84], α_V integrin (IGTAV) and PEDF through miR-25 [85], and sirtuin 1 (SIRT1) through miR-204 [86].

The RPE is thought to play an important role in the development of vascular complications such as **choroidal neovascularization (CNV)** and DR. Dysregulated RPE miRs and other signals can lead to the promotion of angiogenesis, in opposition to the RPE's normal

antiangiogenic interactions [87]. Similarly, though DR has classically been considered as a vascular disease, the RPE is thought to modulate DR, in part through the decreased secretion of antiangiogenic factors such as PEDF, increased secretion of proinflammatory cytokines, barrier dysfunction, production of reactive oxygen species, potential modulation of the visual cycle, and various other mechanisms [88]. For in-depth coverage of the role of the RPE in DR, we refer the readers to several reviews that cover this topic [88–92]. As inflammation and oxidative stress are major mechanisms of RPE stress and damage, augmentation of the ability of the RPE to withstand such stressors can offer avenues to stop RPE and retinal degeneration. Similarly, vascular abnormalities found in the retina and choroid can be modulated by the RPE and offer new tools to target vascularization in a vessel-independent manner.

Other noncoding RNAs and their interactions with miRs

miRs are not the only type of noncoding RNA present in the RPE. Other RNA species, such as lncRNAs and circular RNAs (circRNAs), can dampen the activity of miRs by providing complementary binding sites that divert miRs from their mRNA targets [93]. For instance, the lncRNA NEAT1 expressed in the RPE in one study was shown to interact with miR-148a-3p to suppress PTEN and macrophage polarization in a mouse model of CNV, and in another study, it was shown to decrease the development of DR in a streptozotocin-induced mouse model of diabetes [53,94]. Another study of PTEN signaling in the RPE identified the circRNA NR3C1 as a miR-382-5p modulator in human AMD samples, protecting iPSC-derived human RPE from stress and dysfunction [39]. Similarly, the lncRNA LINC00167 was downregulated in human AMD samples and played an important role in modulating SOCS3 signaling by binding miR-203a-3p to maintain RPE differentiation and function [95]. Lastly, a study in a mouse CNV model identified 100 differentially regulated circRNAs and explored their regulatory networks and interactions with miRs and mRNAs [96]. In all, these results emphasize the continuing need to further characterize interactions between miRs and noncoding RNAs in the RPE. The ongoing identification of other noncoding RNA species that may modulate RPE physiology promises to be an exciting new frontier in RNA biology.

Novel methods to investigate miRs

Computational tools used for the prediction of miR–mRNA interactions rely on finding seed sequence homology in mRNA 3′-UTRs. Results from these predictive algorithms imply that a single miR could regulate hundreds, if not thousands, of genes [14,15]. Similarly, many genes could potentially be regulated by numerous miRs. However, not every predicted interaction is a *bona fide* interaction, and as such must be verified by orthogonal techniques such as the luciferase assay [10]. Additionally, the local composition and concentration of miRs and mRNA targets varies widely by tissue type and conditions, further adding complexity and nuance to the interpretation of predicted interactions.

However, with the advent of next-generation sequencing (NGS) platforms, the ability to interrogate the composition of miRs in specific tissues has been greatly advanced. Numerous techniques to capture miR–mRNA and Argonaute–miR interactions have been

pioneered, which do not rely on prepredicted targets. These approaches often rely on the immunoprecipitation of Argonaute ribonucleoprotein complexes and cross-linking of Argonaute and miRs [often referred to as cross-linking immunoprecipitation sequencing (CLIP-seq)] [97,98]. Common pitfalls of these techniques include high background, incomplete UV cross-linking, dependence on high-quality immunoprecipitation antibodies, reliance on cell lines, and artificial manipulation of Argonaute or miR levels. Thus, the development of more physiologically relevant methods is necessary for tissue- and disease-specific miR profiling. For example, the newly described Halo-enhanced Ago2 pull-down (HEAP) protocol sidesteps many of the aforementioned limitations [99]. In the HEAP mouse model, a conditional Ago2 allele covalently tagged with the 33-kDa HaloTag is expressed from the endogenous *Ago2* locus only upon expression of Cre recombinase in a particular tissue. This technique allows for the specific isolation of Ago2-containing RISCs from tissues collected from an appropriate tissue-specific Cre mouse line, although it still relies on UV cross-linking and has a downstream workflow similar to previously described CLIP-seq workflows. Combined with the generation of new RPE-specific Cre lines, such as the recently described RPE65-ERT2-cre line [100], an RPE-specific unbiased analysis of miRs and their mRNA binding is now easier and more feasible. Another recent innovation has been the use of RNA immunoprecipitation sequencing (RIP-seq) for the *in vivo* unbiased isolation of Ago2–miR complexes [101]. Using an Argonaute-binding antibody, RIP-seq takes advantage of the direct pull down of these complexes by digesting unprotected and unbound RNA before elution and library construction for NGS, though this approach retains some of the specific limitations described earlier. As the investigation of the networks of miRs and their target genes is critical for a fuller understanding of ophthalmic genetic regulation, development of new tools to further probe miR biology will be needed for uncovering novel miRs and their targets in a wide variety of ophthalmic contexts.

Potential applications of miRs as therapeutics and biomarkers

Because they play a critical role in the regulation of RPE physiology and disease, miRs are an attractive target for therapeutic and biomarker development [102]. A careful understanding of miR genetics and mutations can lead to a better understanding of risk factors and biomarker identification. For instance, a familial mutation in miR-204 was described as the cause of an inherited retinal dystrophy and defective iris development, highlighting the contributions of miR-204 both to ophthalmic development and eye disease [103]. This mutation is one of the first identified miR mutations that were found to lead to a syndromic inherited retinal disease, suggesting that other miR variants could lead to both inherited disease and susceptibility to other pathologies. For other ophthalmic diseases, various serum miRs can serve as accessible biomarkers (Figure 3). For example, miR-19a, miR-126, and miR-410 are differentially expressed in the serum of dry AMD patients [104]. miRs also have been described as serum markers for CNV and DR. These include miR-486a-5p and miR-92a-3p in murine laser-induced CNV [105]; miR-34-p, miR-126-3p, miR-145-5p, and miR-205-5p, which are correlated with VEGF-A expression in human wet AMD [106]; and miR-423-5p in human DR [107].

Because of their role in regulating disease, miRs could be exploited to target various pathologies. Antisense oligonucleotides and other interfering nucleic acid molecules are

already being tested in clinical and preclinical studies for their efficacy in treating various disorders, suggesting that miRs could also potentially be employed to treat retinal and RPE diseases [108]. For example, subretinal injection of lentiviruses expressing antiangiogenic miRs in mice suppressed CNV [109]. Similarly, an adeno-associated virus (AAV) encoding miR-204 was shown to counteract retinal degeneration and microglial activation in the rhodopsin P347S and the AIPL1-knockout mouse models, while intraocular injection of a miR-126-expressing plasmid suppressed VEGF-A expression in a rat model of retinopathy of prematurity [110,111]. We hypothesize that the engineered and targeted expression of miRs could prove particularly beneficial as a gene therapy or augmentation modality, as miRs that target multiple genes and pathways can be administered to a broader patient pool, in contrast to other gene therapy treatments that must be tailored to specific gene mutations. Intriguingly, miRs have even been shown *in vitro* to induce the transdifferentiation of RPE into photoreceptors through miR-124 and the miR-183/96/182 cluster, potentially offering another route to regenerate photoreceptors and other neural cell types through the targeted reprogramming of RPE via various miRs [112,113]. Further preclinical development of miR therapy is needed through comparison of preformed precursor or mature miRs or constructs that express miRs. Additional refinement of both delivery modalities, such as viral or lipid nanoparticle systems, and stabilizing or regulatory modifications of RNA species must be accomplished before widespread application to clinical needs can be implemented (Figure 3) [114]. Alternatively, careful identification of miR targets or agents that modify miR expression in common degenerative or pathological pathways could lead to development of biologics or small molecules that target either miRs or their downstream targets [115].

Concluding remarks and future perspectives

miRs regulate and influence the development and function of the RPE. They are also intricately involved in the development of diseases of the RPE and, by extension, diseases of the neural retina, such as AMD, DR, and CNV. Accordingly, miRs could also serve as potential targets in the design of treatments for these diseases. While the broad strokes of miR–mRNA cross talk have thus far been outlined, the specific roles of individual miRs and their targets remain more elusive, as most studies focus on one miR and its most important putative target. Moreover, studies typically address a specific context, and in a different context, another target of the same miR might be the more relevant one. Many identified miR–mRNA pairs have also not been validated in other studies, pointing to the need for further work to confirm these interactions.

A number of preliminary studies on miR roles in the RPE have thus far been conducted in immortalized cell lines, such as the widely used ARPE-19 line. However, the clinical relevance of these studies must be corroborated with rigorous studies in primary cultured RPE, iPSC-derived RPE cultures, animal models, and human samples before these findings can be more generally studied for application in the clinic. In addition, while miRs have so far been studied with regard to their targeting of specific genes, in our view, they should also be studied more broadly using bioinformatics and systems biology approaches to dissect their basic biology and effects on broader signaling pathways and gene regulation (see Outstanding questions). Considering redundancy in miR systems and various homologous members of miR families that could have overlapping functions, we opine that care should

be taken to validate the effects of miR deletion and overexpression. Furthermore, given that many miRs are not tissue or cell specific, the generation and interpretation of animal models for the effect of miRs on the RPE and other tissues must carefully consider the critical roles of miRs in development and tissue homeostasis; ideally, suppression or overexpression of various miRs should be done in a temporally and spatially controlled manner.

We believe that miRs could serve as effective biomarkers of disease, and engineered expression of miRs could be a powerful approach to therapeutic intervention in RPE diseases. A deeper understanding of miRs in the RPE and retina will also lead to insights into the regulation of neural function and health and how miRs serve to modulate important RPE roles such as phagocytosis. Indeed, deeper understanding of miR regulation of RPE phagocytosis may even lead to more efficient and selective RPE uptake of lipid nanoparticles and other gene transfer modalities that could enable transformative gene editing and gene delivery for inherited diseases. Further study of miRs will hopefully lead to insights that allow for early detection of and intervention for RPE and retinal diseases.

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Glossary

Age-related macular degeneration (AMD)
the macula is an area of the eye with an overrepresentation of cones; it is responsible for high-acuity vision necessary for activities such as reading, facial recognition, and driving. Degeneration of the macula in AMD leads to significant visual impairment. Risk factors include advanced age, smoking, genetics, and diet.

Choroidal neovascularization (CNV)
a pathology found in 'wet'/neovascular age-related macular degeneration, CNV is the abnormal growth of vasculature in the choroid, which is found under the RPE. Major complications of CNV include vascular leakage and hemorrhage, which can lead to vision loss. Though the RPE plays a role in this pathology, it is thought that the choriocapillaris initiates CNV through blood vessel signaling.

Diabetic retinopathy (DR)
a late-stage complication of diabetes mellitus is DR, a disease of the vasculature of the retina due to dysfunction of the retinal endothelial cells, and it is divided into nonproliferative and proliferative disease. It is a major cause of acquired blindness. Current treatments include anti-VEGF therapy, laser photocoagulation, steroid treatment, and surgery. Recent work has uncovered an important role of the RPE and neural retina in contributing to the progression of this vascular disease.

Epithelial–mesenchymal transition (EMT)

epithelial cells under stress can deprogram away from healthy cells through mechanisms such as downregulation of adhesion molecule expression, detachment from their basement membranes, and loss of apical–basal polarization. These mesenchymal-like cells can proliferate and invade and play a role in numerous pathologies, such as cancer and tissue degeneration.

Extracellular vesicle (EV)

usually 40–160 nm in diameter, is formed by invagination of intracellular cellular membranes to form multivesicular bodies, which are subsequently released extracellularly upon fusion with the plasma membrane. They can contain DNA, protein, RNA, and other cargoes and are hypothesized to enable intercellular and long-distance communication and regulation.

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Outstanding questions

How does the RPE sense changes in light, and what signals are required to up- and downregulate microRNA (miR) expression in response to light? Are these miR-regulatory signals intrinsic to the RPE or do they originate in the neural retina, and what other purposes do these signals serve? What drives the circadian rhythm in the RPE? Does the RPE have circadian-independent light sensitivity?

Is iso-miR expression in the eye tissue specific? Do iso-miRs have tissue-specific roles and targets? Do they define tissue specificity?

Does the RPE utilize miRs to communicate with photoreceptors to mediate phagocytosis, metabolism, and other interconnected functions? If so, how? Is it through exosomes, through the regulation of miR target proteins, or through other mechanisms?

What are the molecular regulators of miR expression in RPE development?

Do complementary miR networks within the RPE cross-compensate for changes in miR expression?

How do miRs interact with circular RNAs and other noncoding RNAs in the RPE?

How does the miR content of the RPE influence gene expression and translation in photoreceptors and the neural retina?

Highlights

The retinal pigment epithelium (RPE) is crucial for maintaining photoreceptor and neural retinal health. Dysregulation of this postmitotic monolayer leads to retinal degeneration in various diseases such as age-related macular degeneration.

MicroRNAs have been shown to regulate the gene expression of many vital RPE pathways. These include oxidative stress and metabolism, phagocytosis and endolysosomal function, exosomes and intercellular signaling, and vascular homeostasis.

RPE development has been shown to be heavily dependent on the correct expression of microRNAs and their processing machinery, and altered microRNA levels can suppress differentiation or promote dedifferentiation of the RPE.

Perturbations in the levels or composition of RPE microRNAs lead to RPE dysfunction and the progression of disease, revealing potential targets for therapeutic and biomarker development.

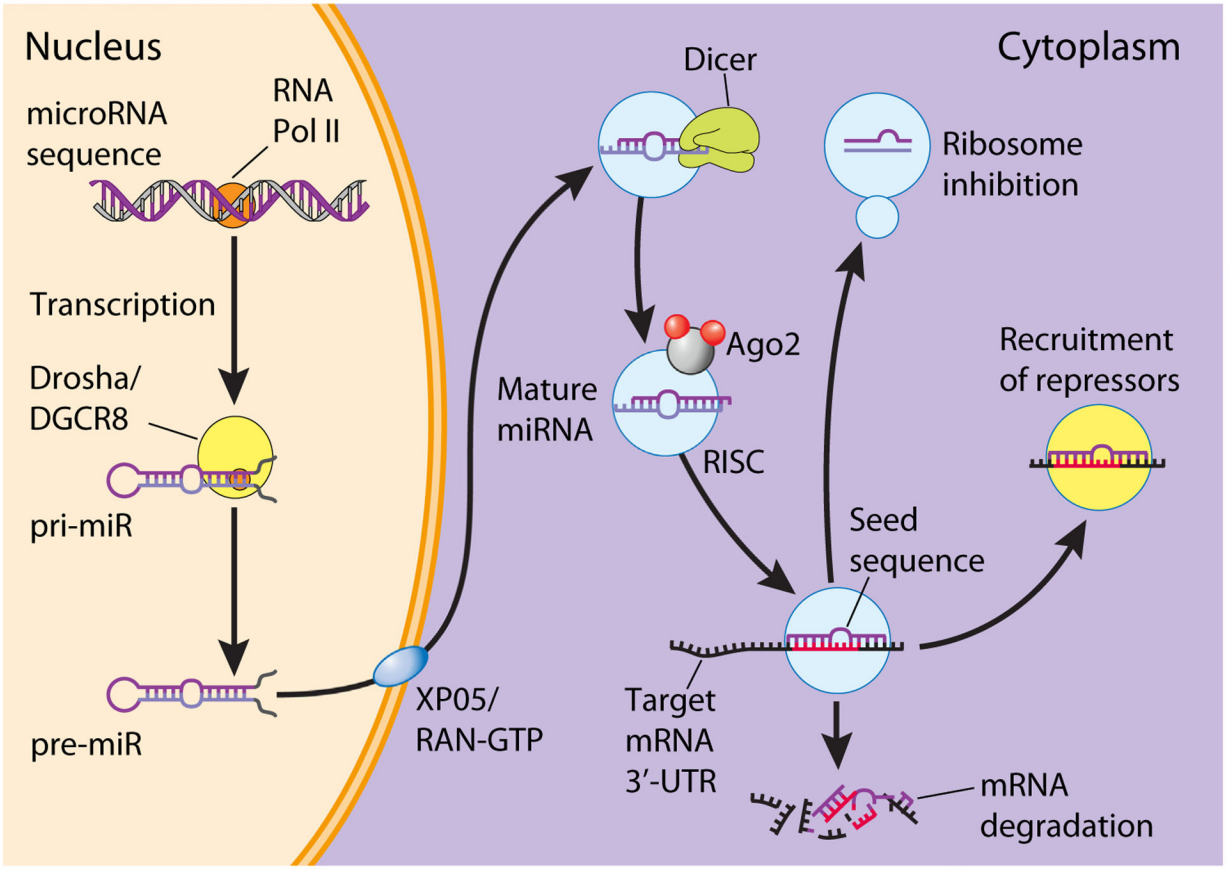

Figure 1. Canonical microRNA biogenesis and function.

Most microRNAs (miRs) are transcribed by RNA polymerase II in the nucleus to form a primary miR (pri-miR). The pri-miR is processed by Drosha/DGCR8 to form a pre-miR, which is then exported to the cytoplasm by exportin 5 (XPO5) and RAN-GTP. The pre-miR is cleaved by Dicer and loaded into the RNA-induced silencing complex (RISC), where a mature miR pairs to the 3′-UTR of its target mRNA through complementary base pairing to the seed sequence. Expression of the target mRNA is suppressed by the RISC through either mRNA degradation or translational repression, mediated through recruitment of repressors or ribosomal inhibition. Abbreviations: 3′-UTR, 3′-untranslated region; Ago2, Argonaute 2.

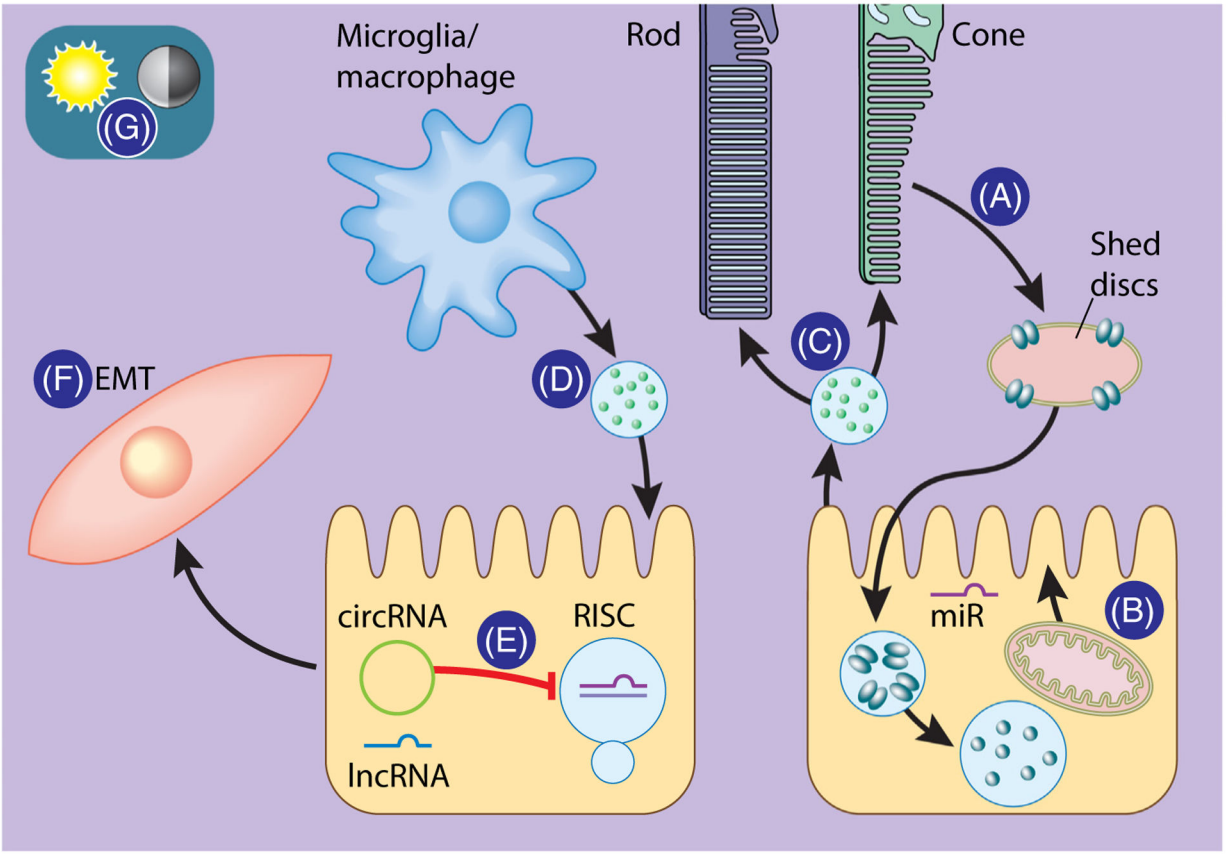

Figure 2. MicroRNA interactions and functions in the RPE and retina.

MicroRNAs (miRs) can facilitate RPE-intrinsic functions and intercellular communication within the eye. The daily release of photoreceptor outer segments (POS) as shed discs from rods and cones is regulated by RPE miRs (A). miRs contribute to the RPE response to oxidative and mitochondrial stress (B). Extracellular vesicles (EVs) from the RPE can influence the function and gene expression in rod and cone photoreceptors (C). The RPE, in turn, can be modulated by EVs from microglia and macrophages found in the retina (D). The RPE can also regulate miR levels through the expression of other noncoding RNAs, such as long noncoding RNAs (lncRNAs) and circular RNAs (circRNAs), which contain miR binding sites (E). Lastly, miR expression can induce or suppress epithelial–mesenchymal transition (EMT), a feature of RPE pathology and degeneration (F). Expression of miRs in the retina is tightly controlled by diurnal and circadian patterns (G). Abbreviations: RISC, RNA-induced silencing complex; RPE, retinal pigment epithelium.

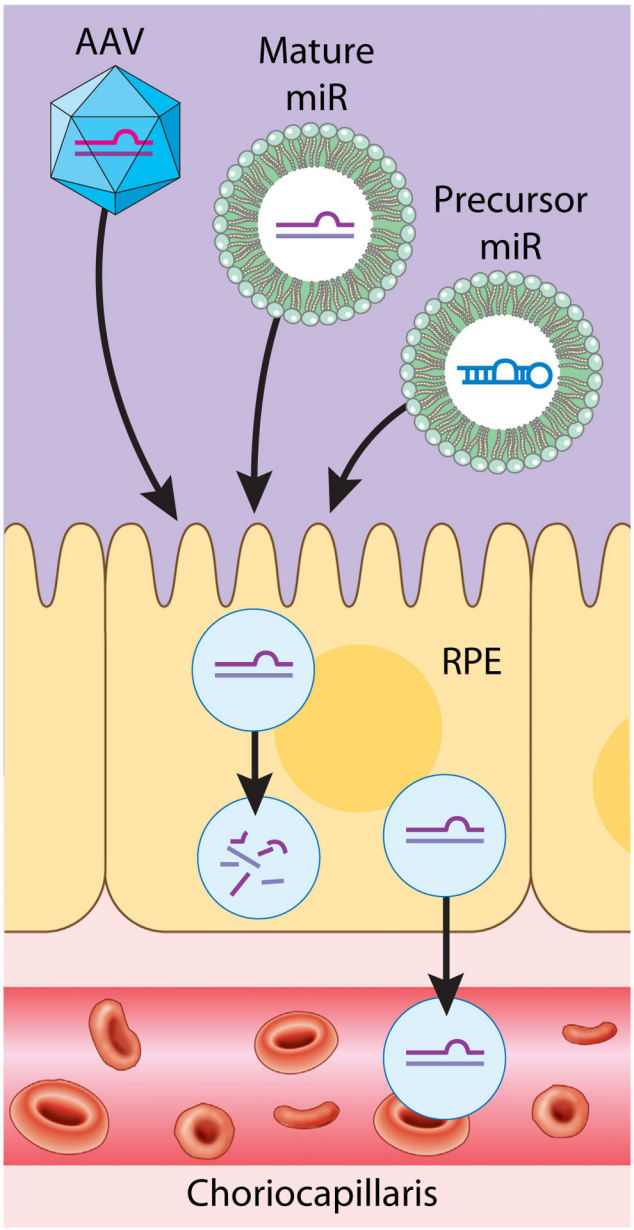

Figure 3. MicroRNAs for potential clinical applications.

MicroRNAs (miRs) can be delivered to the RPE for therapeutic purposes through different modalities, such as through viral vectors (AAV, lentivirus, etc.) or through lipid nanoparticles. These can be delivered as mature miRs or as precursor miRs. miRs that are released into the systemic circulation in exosomes and other extracellular vesicles can be detected in blood and could potentially serve as biomarkers of diseases such as AMD or CNV. Abbreviations: AAV, adeno-associated virus; AMD, age-related macular degeneration; CNV, choroidal neovascularization; RPE, retinal pigment epithelium.