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miRNAs as potential therapeutic targets for age-related macular degeneration

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Abstract

Since their recent discovery, miRNAs have been shown to play critical roles in a variety of pathophysiological processes. Such processes include pathological angiogenesis, the oxidative stress response, immune response and inflammation, all of which have been shown to have important and interdependent roles in the pathogenesis and progression of age-related macular degeneration (AMD). Here we present a brief review of the pathological processes involved in AMD and review miRNAs and other noncoding RNAs involved in regulating these processes. Specifically, we discuss several candidate miRNAs that show promise as AMD therapeutic targets due to their direct involvement in choroidal neovascularization or retinal pigment epithelium atrophy. We discuss potential miRNA-based therapeutics and delivery methods for AMD and provide future directions for the field of miRNA research with respect to AMD. We believe the future of miRNAs in AMD therapy is promising.

Age-related macular degeneration (AMD) is a progressive and degenerative disease of the retina, and the leading cause of irreversible vision loss in the developed world in elderly populations [1]. Approximately 1.8 million Americans are afflicted by AMD and this number is estimated to increase by more than 50% by the year 2020, because of the projected increase in aging populations [2]. Early AMD is characterized by drusen (yellow spots) and pigmentary changes in the choroid/retinal pigment epithelium (RPE) layers in the macula (Figure 1A & 1B) [3,4]. Late AMD has interconvertible 'dry' and 'wet' forms. The advanced form of dry AMD, also called **geographic atrophy** (GA), is characterized by extensive loss of the RPE, its overlying photoreceptors (PRs) and, possibly, the underlying choriocapillaris. **Choroidal neovascularization** (CNV), which involves abnormal growth of blood vessels from the choroid, is a hallmark of wet (or neovascular) AMD (Figure 1C) [3].

Although the molecular underpinning of AMD remains unclear, it is postulated that oxidative stress, inflammation and angiogenesis play critical roles in AMD pathogenesis [5]. The retina is a highly metabolic tissue and is particularly susceptible to oxidative stress [6]. Consistent with a causative role for oxidative stress in AMD, smoking, which is linked to increased oxidative stress in the RPE, has been known to be an established risk factor for

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AMD [7,8]. RPE cells are critical to the integrity of the retina, as they are responsible for phagocytizing PR fragments and stabilizing the local extracellular environment. Oxidative damage can induce RPE cell death, local autoimmune responses and chronic inflammatory responses, which in turn may result in wide-spread GA and CNV [3,9]. GA is defined as a sharply circumscribed area of RPE atrophy within a parafoveal band, resulting in exposure of the underlying choroidal vessels [10]. By histology, GA typically presents as thinning or absence of the RPE, closure of the choriocapillaris and degeneration of the overlying PRs. During CNV, neovessels originate in the choroid and grow under and through the RPE and Bruch's membrane (BM), spreading beneath the retina and causing subretinal hemorrhage, RPE detachment and fibrotic scar formation. While dry AMD leads to progressive vision loss over the course of several years, neovascular AMD can result in legal blindness within months [3].

miRNAs (or miRs) are small, noncoding RNAs that negatively regulate gene expression post-transcriptionally [11,12]. miRNAs are transcribed by RNA polymerase II or III into a primary transcript (pri-miRNA). In the canonical pathway (Figure 2), Drosha, an RNase III-type endonuclease localized in the nucleus, processes the pri-miRNA into the pre-miRNA. The premiRNA is then exported to the cytoplasm, where Dicer processes it into a miRNA/miRNA* duplex. Finally, one strand of the duplex is incorporated into the miRNA-induced silencing complex, which guides sequence-specific post-transcriptional gene repression. Typically, a short sequence of approximately six to seven nucleotides near the 5´-end of the miRNA, called the 'seed region,' binds to sequence(s) on the target mRNAs, usually in the 3´ untranslated region and represses translation or induces target mRNA degradation. Over 1000 miRNAs have been identified in humans and have been predicted to target more than one third of the protein-coding genes. Because of the incomplete complementarity between miRNAs and their target sequences, one miRNA can target multiple mRNAs, and one mRNA can be targeted by multiple miRNAs, adding yet another layer of post-transcriptional gene regulation to the genome.

Since their discovery in 1993, miRNAs have been shown to contribute to the pathogenesis of numerous disease processes, such as pathological angiogenesis, oxidative stress and inflammation, and have become the focus of therapeutic research [13,14]. With regard to AMD, miRNAs have only recently become a focus of therapeutic research and no miRNAs have been directly associated with human AMD pathology. Nevertheless, several miRNAs have been shown to be directly associated with processes potentially involved in AMD by *in vivo* and *in vitro* studies. We hypothesize that miRNAs may be promising therapeutic targets for both wet and dry AMD.

miRNAs in angiogenesis & CNV

Pathological angiogenesis in the choroid underlies the pathogenesis of wet AMD. Apoptotic RPE and inflammatory cells are the major sources of angiogenic factors, which can induce neovascularization in the choroid. Anti-angiogenic therapy has been shown to be a viable therapeutic solution for wet AMD, and antibodies to VEGF, including bevacizumab and ranibizumab, have been approved by the US FDA and can slow CNV progression and, in some cases, improve visual outcome in wet AMD patients [15–17]. However, limited efficacy, short half-lives and potential systemic side effects of the current agents necessitate the development of combinational or novel therapies for wet AMD [18]. Recent studies have revealed important functions of miRNAs in angiogenesis and CNV [19–21]. As opposed to current agents, these miRNAs can target multiple components in the angiogenic pathways, representing a novel therapeutic solution for wet AMD.

A growing number of specific miRNAs, dubbed 'angiomiRs', have been shown to play a significant role in angiogenesis [19]. Several miRNAs (i.e., the miR-15/107 group, the miR-17~92 cluster, miR-21, miR-132, miR-296, miR-378 and miR-519c) were shown to be involved in tumor angiogenesis, and may be potentially transferable to wet AMD research [22–29]. Among these miRNAs, the expression of pro-angiomiR miR-132 was undetectable in the normal endothelium, but was induced by VEGF and FGF in the endothelial cells (ECs), while pro-angiomiR miR-296 expression in ECs was upregulated by VEGF, EGF or conditioned medium from tumor cells [25–26]. These miRNAs function to modulate angiogenic signaling downstream of angiogenic factors.

Hypoxia and inflammatory stimuli have been heavily involved in AMD. In the presence of a malfunctional RPE, elevated PR metabolism can produce hypoxic conditions and stimulates angiogenesis through stabilizing of the hypoxia inducible factor-1α, which induces VEGF expression [3]. Hypoxia-regulated miRNAs have been identified in cancer cell lines and ECs by several groups [30–32]. Among these miRNAs, miR-210 was induced by hypoxia in all cell types tested and regulates hypoxia-dependent metabolism and angiogenesis [33]. Several miRNAs are downregulated by inflammation stimuli or ischemia conditions. MiR-222 expression was decreased in ECs by inflammatory stimuli, which led to enhancement of signal transducer and activator of transcription-5A and may contribute to inflammation-induced neovessel formation [34]. MiR-100 was downregulated by ischemia in the limb, and serves as an endogenous repressor of the serine/threonine protein kinase mammalian target of rapamycin, which is known to modulate vessel growth [35].

Specifically, several angiomiRs have been demonstrated to be specifically involved in retinal vascular development or CNV. MiR-126 has been shown to be an EC-specific miRNA that can promote MAPK and PI3K signaling in response to angiogenic factors [36–38]. MiR-126$^{-/-}$ mice showed significantly delayed retinal vascularization during postnatal retinal development [36,38]. Consistently, silencing of miR-126 impaired ischemia-induced angiogenesis in the mouse limb [39]. A recent study demonstrates that inhibition of miR-132 reduced postnatal retinal vascular development in mice [25]. It would be interesting to test whether inhibiting miR-126 and/or miR-132 in the adult mice has beneficial effects on repressing CNV. The laser-induced CNV mouse model is a reliable model to study CNV mechanism and test the effect of potential drugs in CNV [40]. Recently, a group of miRNAs has been shown to be substantially downregulated in a laser-induced CNV model [21]. Overexpression of miR-21, miR-31 and miR-150 each repressed laser-induced CNV, supporting a functional role for these miRNAs in neovascularization in the eye (Table 1) [21,41]. In addition, we recently found that members of the miR-23~27~24 clusters were upregulated in the retina/choroid in a laser-induced CNV mouse model [20]. Importantly, silencing of the miR-23~27~24 members miR-23 and miR-27 repressed laser-induced CNV by approximately half, suggesting that these two miRNAs may have therapeutic potential in neovascular AMD (Figure 3). MiR-23 and miR-27 regulate MAPK and VEGF receptor angiogenic pathways by targeting Sprouty2 and Sema6A, which are negative regulators of these angiogenic pathways (Table 1).

Overall, the study of miRNAs in neovascular AMD is still at its early stage, and a more systematic study of miRNA function in the disease processes is clearly warranted. miRNAs, possibly in combination with current anti-angiogenic agents, may target multiple angiogenic pathways and provide superior outcome in treating CNV in neovascular AMD patients.

miRNAs in oxidative stress

Oxidative stress has been implicated in the pathogenesis of AMD. The retina is particularly vulnerable to oxidative damage because of its intensive oxygen metabolism, continual

exposure to light, high concentrations of polyunsaturated fatty acids and the presence of photosensitizers, which increase the production of reactive oxygen species (ROS) in the retina [6]. ROS include highly reactive free radicals generated in mitochondria and peroxisomes, such as superoxide anions and hydroxyl radicals, and stable, freely diffusible substances such as hydrogen peroxide (H_2O_2). Oxidative stress is aggravated by the presence of lipofuscin, lipid–protein aggregates that accumulate with age in RPE cells. ROS overload can lead to DNA damage, RPE and PR cell death and chronic inflammation, which are responsible for AMD disease progression. Under normal conditions, endogenous oxidative stress is counteracted by the antioxidant defense system, which contains superoxide dismutase (SOD), catalase and glutathione peroxidase. In support of the role of oxidative damage in AMD, *Sod1*$^{-/-}$ and *Sod2* knockdown mice show pathologic changes in the retina reminiscent of AMD [42,43].

A recent study revealed a protective role for miR-23a in oxidative stress-induced RPE cell death (Table 1) [44]. MiR-23a, a member of the miR-23~27~24 cluster, was found to be downregulated in human RPE cells from AMD patients. In RPE cells, miR-23a was upregulated after low concentration of H_2O_2 treatment, but downregulated at higher H_2O_2 concentration. Overexpression of miR-23a attenuated H_2O_2-induced apoptosis in RPEs, but had no effect on cell viability under basal conditions. Fas, an important molecule involved in ROS-mediated apoptosis, was identified as a direct miR-23a target and H_2O_2-induced upregulation of Fas was attenuated by miR-23a overexpression. These results are consistent with increased Fas and FasL expression in the PR and the RPE monolayer of the choroidal neovascular membranes from patients with AMD, suggesting therapeutic potential for manipulating the miR-23a-Fas axis in RPE/PR cell death in AMD [45,46]. Interestingly, miR-27a* and miR-27b* in the miR-23~27~24 family were both downregulated by H_2O_2 in RAW264.7 macrophages and possibly function to protect against NFκB-dependent macrophage activation [47]. However, the functions of miR-27 and miR-27* in RPE are not yet known.

A number of miRNAs involved in the oxidative stress response have been identified in other cell types. Hypoxia-inducible miR-210 may enhance or repress ROS production depending on cell type and oxygen conditions [48–50]. MiR-210 overexpression in cardiomyocytes was protective against ROS production and apoptosis following hypoxia-reoxygenation treatment [48]. Pre-miR-21 treatment in cardiomyocytes decreased apoptosis in response to oxidative stress through repressing programmed cell death-4, while miR-21 inhibitor produced the opposite effects (Table 1) [51]. However, in angiogenic progenitor cells, miR-21 targets of SOD-2 and its overexpression led to inhibition of SOD-2 production, enhancing ROS production [52]. While conflicting, these findings strongly implicate miR-210 and miR-21 in oxidative stress. Thus, it would be worthwhile to investigate their roles in the oxidative stress response in the RPE or in PR.

Several other miRNAs have been shown to promote/inhibit oxidative stress-induced cell death. MiR-200c was upregulated by oxidative stress in ECs and induced apoptosis and senescence by inhibiting ZEB1 [53]. MiR-204 was upregulated by H_2O_2 in human trabecular meshwork cells [54]. Overexpression of miR-204 increased cell death in response to oxidative stress. However, in cardiomyocytes, miR-204 protected against autophagy induced by hypoxia-reoxygenation through a target gene encoding microtubule-associated protein [55]. MiR-24 has been shown to repress apoptosis in cardiomyocytes and the developing retina by repressing pro-apoptotic factors Bcl2l11 (Bim), Caspase-9 and Apaf-1 [56,57]. The functional significance of these miRNAs in AMD awaits future studies.

Heme oxygenases (HO) are important markers of iron-related oxidative stress and have been shown to be antioxidant, anti-inflammatory and cytoprotective [58]. Polymorphisms in

HO-1 and HO-2 have been associated with AMD [59]. Overexpression of miRs-155, -183 and -872 has been shown to repress HO-1 in adipocytes, while a combination of miR-377 and miR-217 was found to repress HO-1 in a hemin-dependent manner in human ECs [60,61]. It would be interesting to test the role of these miRNAs in HO-1 regulation and oxidative stress in the RPE or PR cells.

While oxidative stress is heavily implicated in the pathogenesis of AMD, the mechanisms of oxidative stress in these processes are not well characterized. Recent studies are starting to reveal a role for miRNAs in regulating oxidative stress response in the RPE and other cell types, pointing to a miRNA connection between oxidative stress and AMD pathogenesis. Future studies should focus on identifying miRNAs in oxidative stress AMD animal models and testing the function of the candidate miRNAs *in vivo*. For now, miR-23a seems to be a viable candidate for *in vivo* testing of miR-based therapy in dry AMD-related RPE cell death. To do this, a RPE-specific delivery strategy would be suggested, since recent work in our laboratory suggests that overexpression of miR-23 and miR-27 may enhance CNV [20].

miRNAs in immune response & inflammation related to AMD

The immune system and inflammation have been implicated in both dry and wet AMD [62]. Support for the involvement of immune response in AMD includes the identification of drusen, which contains complement and other inflammatory components and is deposited beneath the RPE in AMD patients [3,4]. The RPE and the underlying BM are essential for the maintenance of retinal homeostasis. The complement system of the innate immune system is crucial for the immune responses against pathogens or dying cells [63,64]. Activation of complement system leads to the production of membrane-attack complexes, which cause cell lysis, release of chemokines and generation of pro-inflammatory responses. Presumably, disruption of RPE/BM integrity caused by oxidative stress-induced RPE death can cause the activation of the complement system and, subsequently, chronic inflammation. Consistent with a role for complement system in AMD, mutations/polymorphisms in genes coding for the alternative complement pathway regulator (factor H) and complement pathway proteins (complement component C2, C3 and factor B) are associated with AMD [65–69].

Besides an indirect mechanism through the complement system, inflammation is also directly involved in AMD. Chemokines are required to direct monocyte recruitment into inflamed tissues. Monocyte chemotactic protein-1 (MCP-1 or CCL2) and the chemokine receptor CX3CR1 have been implicated in AMD. Loss-of-function mutations in *CX3CR1* have been associated with AMD [70]. Moreover, *Ccl-2*$^{-/-}$, *Ccr-2*$^{-/-}$and *CX3CR1*$^{-/-}$/ *Ccl-2*$^{-/-}$ mice develop AMD-like pathology [71,72]. Inflammatory cells, in particular macrophages and microglia cells, have been observed in the AMD lesions. Macrophages in CNV lesions have been shown to express angiogenic growth factors such as VEGF [73,74], which has been shown to play a pivotal role in the development of CNV [75]. Although chronic inflammation is believed to be causative in AMD, the role of inflammation in CNV is still ambiguous. For example, both depletion of macrophages by dichloromethylene diphosphonate-containing liposomes and increase of macrophage recruitment in *IL-10* knockout mice resulted in decreased susceptibility to laser-induced CNV [76–78].

Direct evidence for miRNAs regulating immune response and inflammation in AMD is still lacking. However, increasing numbers of miRNAs have been implicated in immune response and inflammation [79]. MiR-146a was shown to be induced by inflammatory agent lipopolysaccharide (LPS) and the inflammatory cytokines IL-1b and TNF-α, functioning to negatively regulate IL-6 and IL-8 expression [80–83]. MiR-146a was also shown to inhibit oxidized low-density lipoprotein (oxLDL)-induced lipid accumulation and inflammatory

response by targeting Toll-like receptor (TLR)-4 [84]. Interestingly, miR-146a expression is inversely correlated with its potential target CFH in human brain cells [85]. TLRs have critical roles in innate immunity and TLR-4 has also been implicated in the phagocytosis and handling of PR outer segments by RPE cells [86]. Despite these findings, the direct relationship between miR-146a and CFH and TLR-4 in AMD needs to be established (Table 1).

miRNA miR-155 was also upregulated by LPS or oxLDL in macrophages, and silencing of miR-155 in macrophages promoted the release of cytokines IL-6, IL-8 and TNF-α [87,88]. A recent study showed that miR-23~27~24 member miR-27b is induced by LPS in human macrophage cells and contributes to LPS-induced inflammation response by targeting peroxisome proliferator-activated receptor gamma [89]. In contrast, miR-125b was downregulated in LPS-treated macrophages and potentially targets TNF-α [90]. Many additional miRNAs have been shown to be linked to the inflammatory response, including miR-9, miR-21, miR-124, miR-214, miR-223, miR-224, miR-513 and miR-1224 [80,91–95].

Taken together, a systematic analysis of miRNAs involved in immune responses and inflammation in AMD is warranted. Of the miRNAs studied so far, miR-146a and miR-27 might be viable initial candidates for investigating the function of miRNAs in inflammation in AMD. MiR-146a targets or regulates multiple inflammatory cytokines, including IL-6 and IL-8, as well as CFH and TLR-4, which have been linked to AMD, while miR-27 may contribute to both angiogenesis and inflammation in CNV [20,89]. Therefore, these miRNAs have the potential to target multiple pathways involved in CNV and AMD.

Noncoding RNAs in GA

A recent study has implicated an important role for noncoding RNAs in GA [96]. In addition to its miRNA processing function, Dicer is also required for cutting long dsRNA into other small RNAs. Dicer-1 was shown to be specifically downregulated in the RPE of human donors with GA. When Dicer was selectively deleted in the RPE in mice, the RPE appeared severely degenerated. However, mice lacking other components of miRNA processing, including Drosha, DGCR8 or Argonaute proteins, failed to show degeneration of the RPE. This argues against the involvement of general miRNA pathways in GA. Nevertheless, accumulation of *Alu* RNAs in the RPEs of GA eyes was discovered. *Alu* RNAs are dsRNAs approximately 300 nucleotides in length and are transcribed from Alu elements, the most common noncoding, repetitive DNA sequence in the human genome. Direct injection of *Alu* RNAs into the eye of normal mice reduced RPE cell viability and caused degeneration of the tissue. Interestingly, Dicer functions to process the long, toxic *Alu* RNAs into shorter, nontoxic versions. Moreover, overexpression of Dicer blocked *Alu* RNA-induced RPE degeneration *in vivo*. These elegant studies indicate that *Alu* RNA accumulation caused by Dicer downregulation underlies RPE degeneration in GA. Although these findings implicate miRNA pathway-independent, ‘noncanonical’ Dicer function in dry AMD, the function of individual miRNAs in AMD should not be ignored. Dicer-independent miRNA biogenesis has been recently discovered [97,98]. Moreover, specific miRNAs may have contradictory but independent functions in AMD, which would not manifest after *Dicer* deletion. In addition, the mechanism whereby Dicer is downregulated in the RPEs in GA patients was not discovered. miRNA target prediction programs predict that numerous miRNAs theoretically target Dicer-1. Therefore, restoration of Dicer expression by inhibiting specific miRNAs might be a viable therapeutic strategy for GA in AMD.

miRNA therapeutics

With the increasing understanding of miRNAs in numerous diseases, the field of miRNA therapeutics is starting to emerge. miRNA-based therapeutics involves modulation of the functions of disease-associated miRNAs by miRNA antagonists or mimics [99,100]. Recent studies have shown powerful therapeutic effects of miRNAs in several disease models [13,14,101]. For example, an miR-122 inhibitor showed efficacy *in vivo* in suppressing virus infection in primates [13]. The unique strength of miRNA-based therapy is that an individual miRNA can regulate multiple genes in a pathway or several pathways, simultaneously. Although the effect of a single miRNA on a single gene is mild, simultaneous regulation of multiple targets may have far-reaching biological outcomes. miRNA antagonists are, typically, chemically modified single-stranded oligonucleotides with sequences complementary to the miRNAs of interest, which competitively bind to the endogenous miRNAs. The commonly used chemical modifications include cholesterol-conjugated 2´-*O*-methyl modification (so-called 'antagomiRs') and locked-nucleic acid modification [14,101]. miRNA 'sponges' contain complementary binding sites to miRNAs of interest and have the advantages of blocking a whole family of related miRNAs or achieving cell-type specific miRNA silencing [102]. miRNA mimics can be chemically modified double-stranded miRNAs or miRNAs expressed by viral vectors, including retrovirus, lentivirus and adeno-associated virus (AAV) virus.

miR-based therapeutics for AMD is still on the horizon. However, several miRNAs have shown promise for the future treatment of both wet and dry AMD. Overexpression of miR-21, miR-31 or miR-150, or silencing of miR-23/27, are each potential approaches for treating CNV in wet AMD [20,21,41]. Meanwhile, overexpression of miR-23a may be used for preventing GA [44]. However, efficacy and safety profiles of these miRNAs should be rigorously tested using animal models. A combination of miRNAs and existing drugs is also worth considering. As to the delivery methods for miRNA therapeutics to the posterior eye, an invasive local delivery by intravitreal, periocular or subconjunctival injection, or intravitreally inserted implants, may be preferable, since crossing the blood–brain barrier seems to be challenging for systemically delivered miRNA inhibitors [103]. Delivery can be assisted by conjugation with other molecular structures or encapsulation with carriers such as liposomes or nanoparticles. Delivery using viral vectors has the benefit of achieving sustained gene expression, but may cause adverse immune response. Among the viral vectors, AAV-mediated gene therapy seems to be well tolerated in humans and has been shown to improve vision in patients [104]. Since miRNAs (e.g., miR-23) may have cell type-specific functions in the eye, cell- or tissue-specific delivery of miRNA inhibitors or mimics may improve the therapeutic outcome while avoiding potential adverse effects. In this regard, the AAV may have an advantage in miRNA therapeutics [105]. For example, miRNAs or miRNA-sponge inhibitors could potentially be expressed in tissue-specific manners using RPE cell- or EC-specific AAV vectors.

Concluding remarks

The field of miRNA-based therapies has rapidly gained momentum over the last few years, but miR-based therapies for AMD are still on the horizon. Recent work has implicated important roles for miRNAs in pathological angiogenesis, oxidative stress, immune response and inflammation, which are the processes critical for AMD pathogenesis. Specifically, several miRNAs, including miR-21, miR-23, miR-27, miR-31 and miR-150, have been shown to directly regulate CNV, and miR-23a may be beneficial in protecting RPE cell death from oxidative stress in AMD. Apart from miRNAs, noncoding *Alu* RNAs are critical for RPE degeneration in GA. Targeting the aforementioned miRNAs and/or Alu noncoding RNAs holds great promise in AMD therapeutics. Future research should focus on

systematically identifying miRNAs directly involved in CNV, oxidative stress and inflammation in AMD, with a particular emphasis on elucidating their functional mechanisms. Optimally, dysregulation of these miRNAs should also be explored in human AMD patients. In the meantime, a robust, safe and cell- or tissue-specific system to deliver miRNA inhibitors and mimics merits establishment for future AMD therapy.

Executive summary

Background

- Age-related macular degeneration (AMD) is a pervasive disorder and a leading cause of blindness in the developed world, affecting millions of elderly patients worldwide.
- The ability of miRNAs to target multiple messenger RNAs simultaneously makes them attractive targets for AMD therapy.

miRNAs in angiogenesis & choroidal neovascularization

- A growing number of miRNAs have been shown to be essential in normal and pathological angiogenesis.
- Choroidal neovascularization (CNV) underlies wet AMD pathology. Overexpression of miRs-21, −31 or −150, or silencing of miRs-23 and −27, each attenuated CNV in a laser-induced model of CNV.
- The role of miRNAs in CNV merits further elucidation, and these miRNAs should be correlated to human AMD pathology.

miRNAs in oxidative stress

- Oxidative stress has been implicated in AMD pathogenesis. Elevated concentration of reactive oxygen species in the retina can lead to DNA damage, chronic inflammation and cell death in retinal pigment epithelial cells and photoreceptors. These processes are known to be involved in AMD progression.
- Many miRNAs, particularly miR-23a, are involved in regulating the oxidative stress response and may be involved in AMD progression. Direct relationships between AMD and these miRNAs should be investigated.

miRNAs in immune response & inflammation related to AMD

- The immune system and inflammation have been implicated in both dry and wet AMD.
- Multiple miRNAs are involved in various inflammatory processes, but precise relationships with AMD pathology await further studies.

Noncoding RNAs in geographic atrophy

- *Alu* RNA accumulation caused by Dicer downregulation underlies retinal pigment epithelium degeneration in geographic atrophy.

miRNA therapeutics

- Several options for targeting miRNA pathways exist, including miRNA mimics, antagomiRs, locked nucleic acid anti-miRs and miRNA sponges.
- Delivery options include intravitreal injection or implants, in combination with nanoparticles or viral vectors.

- Development of therapeutic modalities should first await validation of miRNA targets *in vivo* and should emphasize cell-type specificity.

Concluding remarks

- miRNAs are involved in a number of AMD-related processes and show promise as targets in AMD therapy.
- AMD therapies are still on the horizon, and future research should focus on systematically investigating miRNA roles in AMD pathogenesis and validating them in animal models and human patients.

Key Terms

Age-related macular degeneration	Chronic medical condition in the elderly that causes vision loss in the center of the vision field (macula). It occurs in 'dry' and 'wet' forms, and is a major cause of blindness and vision impairment in older adults.
Geographic atrophy	Advanced form of 'dry' age-related macular degeneration, which involves the degeneration of retinal pigment epithelial cells and the overlying photoreceptor cells, and causes loss of visual function.
Choroidal neovascularization	Generation of abnormal blood vessels, which originate in the choroidal layer of the eye and are invaded into the retina. It is a common symptom of neovascular (or 'wet') age-related macular degeneration.
miRNA	A short (~22 nucleotides long) RNA molecule that exists in eukaryotic cells. Through binding to complementary sequences in the target mRNAs, miRNAs regulate gene expression post-transcriptionally by repressing translation or inducing target mRNA degradation.

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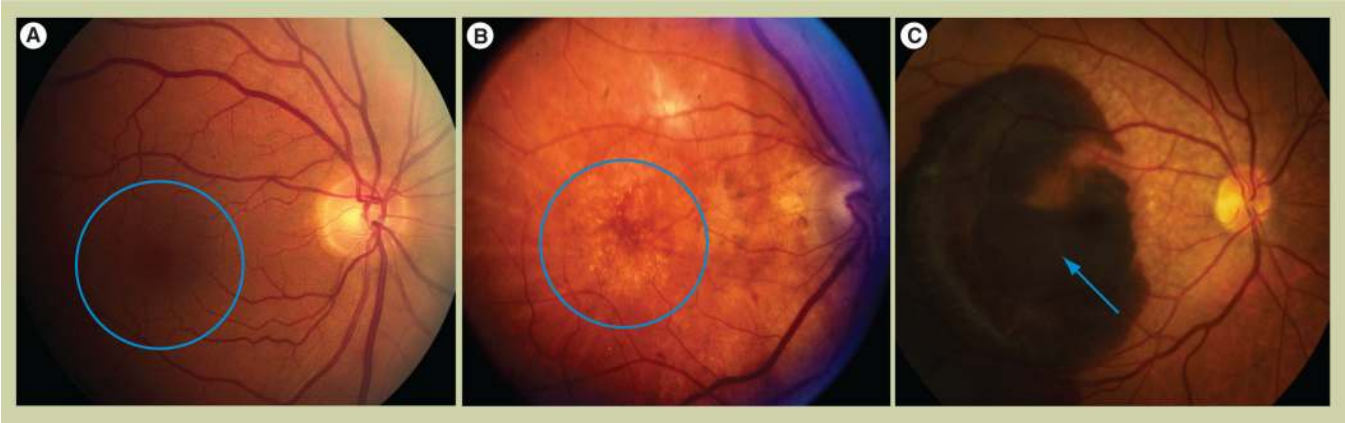

Figure 1. Fundus photographs in age-related macular degeneration patients

(A) Photograph of a normal fundus. The macular region is marked by the blue circle. **(B)** Photograph of a fundus showing drusen and pigmentary abnormalities in dry age-related macular degeneration. **(C)** Photograph of a fundus showing severe subretinal hemorrhage in neovascular age-related macular degeneration.

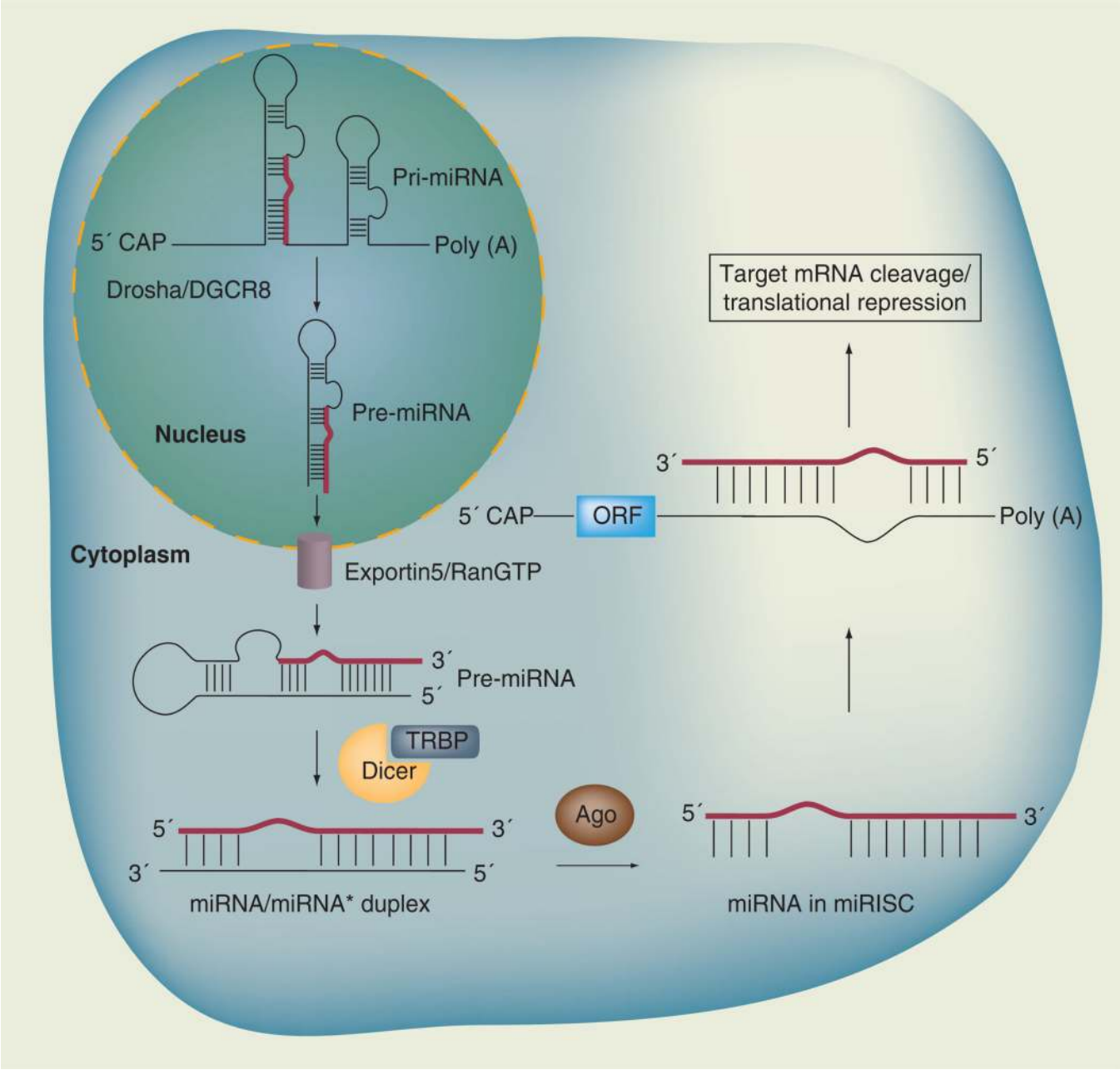

Figure 2. Canonical miRNA biogenesis pathway

The mature miRNA is highlighted in red. Primary (pri)-miRNAs are transcribed by RNA Polymerase II or III, and are processed by Drosha/DGCR8 into precursor (pre)-miRNAs. The pre-miRNA is transported from the nucleus to the cytoplasm by Exportin-5 and Ran-GTP, where it is cut by a Dicer complex (Dicer/TRBP) into a miRNA/miRNA* duplex. One strand of the duplex is preferably incorporated into miRISC-containing Ago proteins. The miRISC mediates sequence-specific gene repression by binding to the 3'-untranslated region of the target mRNAs and repressing translational or inducing mRNA degradation.

Ago: Argonaute; DGCR8: DiGeorge syndrome critical region 8; miRISC: miRNA-induced silencing complex; ORF: Open reading frame; TRBP: TAR RNA-binding protein.

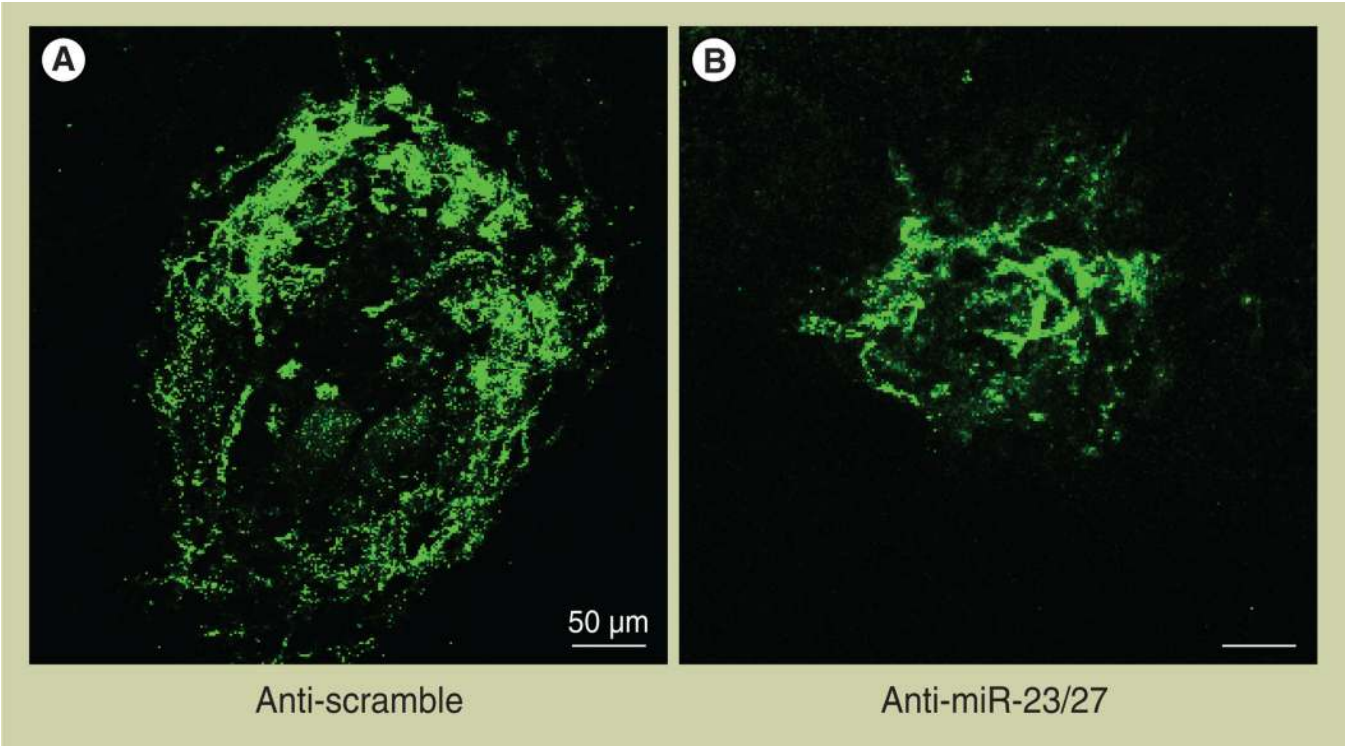

Figure 3. Choroidal flat mount pictures showing repression of laser-induced choroidal neovascularization by silencing of miR-23 and miR-27 using locked nucleic acid modified anti-miRs relative to anti-scramble controls (modified random RNAs lacking mRNA sequence complementarity)

Choroidal neovascularization was visualized by staining with anti-VCAM2 antibody. **(A)** Anti-scramble control, **(B)** Anti-miR-23/27.

Table 1

Implication of miRNAs in age-related macular degeneration.

miRNA	Context	Regulation	Targets	Functions	Ref.
miR-21	Choroid/retina, cardiomyocyte		RhoB, PDCD-4	Represses CNV and prevents apoptosis induced by oxidative stress	[38,49]
miR-23 and miR-27	Choroid/retina	Upregulated in CNV	Sprouty2 and Sema6A	Silencing of miR-23/27 represses CNV	[39]
miR-23a	RPE	Downregulated in the RPE of AMD patients	Fas	Protects against oxidative stress-induced apoptosis	[42]
miR-31 and miR-150	Choroid/retina	Downregulated in CNV	PDGF-B, HIF-1α and VEGF	Suppresses CNV	[37]
miR-146a	Various	Induced by LPS, IL-1β, TNF-α and NFκB	TLR4 and IRAK-1	Suppresses inflammatory response	[77–82]

AMD: Age-related macular degeneration; CNV: Choroidal neovascularization; IRAK: IL-1 receptor-associated kinase; LPS: Lipopolysaccharide; PDCD: Programmed cell death; RPE: Retinal pigment epithelium; Sema: Semaphorin; TLR: Toll-like receptor.