Review

The role of microRNA in the pathogenesis of age-related macular degeneration: its pathophysiology and potential pharmacological aspects

Milosz Kawa\textsuperscript{a}, Anna Machalińska\textsuperscript{b,*}

\textsuperscript{a}Department of General Pathology, \textsuperscript{b}Department of Histology and Embryology
Pomeranian Medical University, Al. Powstancow Wilk. 72, 70-111 Szczecin, Poland
*Corresponding author: Email: annam@pum.edu.pl

(Received February 13, 2014; Revised March 15, 2014; Accepted March 15, 2014; Published online: March 22, 2014)

Abstract: Age-related macular degeneration (AMD) is the leading cause of vision loss in people over the age of 50 years in developed countries. The number of people affected is expected to increase over the next ten years because of the increase in the aging population. In recent years, a novel class of non-coding RNA, microRNA (miRNA), has emerged as an important regulator of numerous biological cell functions. Among these actions are the basic maintenance of cell signaling and metabolism. Disruption of physiological miRNA functions in retinal pigment epithelium cells can contribute to the development of the chronic atrophy observed in the early phases of AMD. Moreover, numerous studies indicate that miRNAs have a crucial role in regulating angiogenesis and vascular homeostasis; thus, miRNAs might play an important role in the choroid neovascularization typical of late AMD. The increasing relevance of miRNAs in retinal tissue has prompted investigations to evaluate their potential as therapeutic and diagnostic targets. In this review, we summarize the current findings on the potential involvement of miRNAs in AMD pathogenesis. We also review recent attempts to use miRNA to modulate biological cellular responses in retinal degeneration and we delineate new perspectives for applying miRNAs as new therapeutic targets and tools for therapeutic interventions.

Keywords: age-related macular degeneration, microRNA, neovascularization, retinal pigment epithelium cells

Introduction

Age-related macular degeneration (AMD) is the leading cause of irreversible visual impairment and disability among the elderly in developed countries. The number of people affected is expected to increase over the next ten years because of an increase in the aging population [1]. AMD is a degenerative and progressive condition involving the retinal pigment epithelium (RPE), Bruch’s membrane and the choriocapillaries. The most common AMD phenotype, called “dry AMD”, is characterized by an increase in the number and diameter of the extracellular sub-RPE deposits called “drusen”, pigmentary irregularities, and progressive atrophy of the RPE and retina. In advanced cases, AMD is often associated with subretinal choroidal neovascularization (CNV) in the macular region (“wet AMD”), which results in the rapid loss of visual acuity. The pathogenesis of AMD is still poorly understood, and treatment options remain limited. The molecular events responsible for AMD progression include altered cellular homeostasis, free radical generation, oxidative stress, necrosis, apoptosis, inflammation, DNA damage, and aberrant RNA processing, culminating in the degeneration of photoreceptors [2]. In addition, there is increasing evidence showing that constant
oxidative stress impairs autophagy clearance in the RPE cells, as well as increases protein aggregation and causes inflammasome activation that might lead to the pathological phenotype of AMD. Especially, various genetic and environmental risk factors associated with lysosomal damage, including accumulation of lipofuscin with subsequent drusen formation, and induction of chronic inflammation may cause the decreased autophagy in RPE cells resulting in AMD progression [3, 4]. Moreover, lysosomal dysfunction including autophagy flux disturbances may be a factor contributing not only to the pathogenesis of AMD in general, but, in particular, to the development of wet AMD via upregulation of vascular endothelial growth factor (VEGF) in retinal cells, most profoundly in the RPE [5]. In recent decades, AMD has become a major challenge in ophthalmology because of its enormous and growing social and economic implications in an aging society worldwide [6]. For the same reason, increasing research efforts have investigated the underlying molecular mechanisms of AMD to find a cure and to select

---

**Fig. 1. Basic miRNA synthesis pathway.** Primary miRNA (pri-miRNA) transcripts are synthetized by RNA polymerase II in the cell nucleus. Next, pri-miRNAs are processed by the microprocessor complex into precursor miRNA (pre-miRNA) molecules, which are transported into the cytoplasm using membrane protein exportin-5 to be further processed by Dicer1 into miRNA duplexes. After separating into two complementary strands, the complete miRNAs are loaded into RNA-induced silencing complexes (RISCs) to repress the synthesis of particular proteins or to activate mRNA degradation. Some miRNA might be exported into extracellular space and taken up by other cells, degraded by RNases, or excreted.
specific biomarkers of AMD development.

The identification of possible therapies for AMD may be facilitated by high-throughput system biological analyses, particularly at the proteome and transcriptome levels. Likewise, significant progress has been made in identifying the cytokines or growth factors that promote and inhibit angiogenesis in CNV [7]. Several other types of agents participate in CNV formation, including angiopoietins, complement proteins, interleukins and chemokines [8]. Similarly, previous studies assessing messenger RNA (mRNA) levels in normal human retina, RPE, and choroid revealed tissue-specific molecular signatures [9] and differences between macular and extramacular transcript expression [10, 11]. Moreover, systematic transcriptional profiling performed in AMD eyes at different stages of neurodegeneration revealed that in both the RPE/choroid and retina, the genes that were elevated across all AMD grades were especially enriched for regulators of cell-mediated immunity [12].

Interestingly, based on the latest evidence, the deregulation of microRNA (miRNA) expression is emerging as a contributor to neurodegeneration by influencing most of the mechanisms responsible for this pathologic process [13]. The progress that has been made in recent years in miRNA expression screening using either microarray platforms or next-generation sequencing technology has facilitated numerous studies on alterations of miRNA levels in different neurodegenerative diseases [14 - 17]. Therefore, the aim of this review is to elucidate the broad implications of miRNAs in age-related macular neurodegeneration. We also report in detail what has been discovered regarding the involvement of diverse miRNAs in specific forms of AMD to evaluate their potential as therapeutic and diagnostic targets.

The biogenesis if microRNA

MicroRNAs (miRNAs) are small non-coding RNAs (~20–25 nt) that were first discovered in C. elegans [18]. MiRNAs may regulate gene expression at the post-transcriptional level by impairing target mRNA through either the translational inhibition or destabilization of target mRNAs [19, 20]. MiRNAs are initially transcribed as long primary miRNAs (pri-miRNAs) that are enlarged by several kilobases. These pri-miRNAs are enzymatically cleaved by a microprocessor complex composed of nuclear RNase-III, known as Drosha, and its binding partner, DiGeorge syndrome critical region gene 8 (DGCR8), a double-stranded RNA-binding protein [21]. The Drosha-cleaved sequence, known as a precursor miRNA (pre-miRNA), is long for 60–70 nucleotides; it is then transported to the cytoplasm, where it is cleaved by another RNase enzyme, Dicer1, to produce the mature double-stranded miRNA duplex of ~22 nucleotides [22]. After Dicer1 processing, one strand of the miRNA duplex is retained to become the mature miRNA, and it is loaded into RNA-induced silencing complexes (RISCs) to participate in mRNA expression modulation. The complementary strands, which are found at lower concentrations within the cell, sometimes also become active [23]. The classic pathway of miRNA biogenesis is displayed in Figure 1. The mature miRNAs within the RISCs bind target sequences of mRNA transcripts to mediate either translational repression or mRNA degradation [22]. MiRNAs repress target mRNA through an antisense mechanism, mainly by binding to the 3′-UTR (untranslated region) of mRNA and, thereby, inhibiting protein synthesis. Importantly, a recent role for miRNA in translational stimulation has also been proposed [24]. A high degree of sequence complementarity between the 5′ end of the miRNA, known as the ‘seed’ sequence, and the mRNA is an important determinant of miRNA targeting [25]. MiRNAs show partial complementarity to respective miRNAs, thereby allowing multiple mRNA locations to be targeted at the same time in a cell type-specific manner. This mechanism significantly supports the strong biological effects of miRNA. MiRNAs have numerous high- and low-affinity targets, averaging 300 conserved targets per miRNA family. Individual 3′-UTRs also contain binding sites for multiple miRNAs, allowing redundancy and cooperation between miRNAs [26]. One miRNA can target hundreds of downstream target mRNAs, while one mRNA can be targeted by multiple miRNAs. Indeed, miRNAs work as regulators of approximately 30–50% of the whole genome [26]. MiRNAs can be transcribed individually, as single transcriptional units, or together with genes in which they are located [26]. MiRNAs have been recognized as a major posttranscriptional regulation level in the fine-tuning of gene expression; they play important roles in cellular proliferation, differentiation, and cell death and are involved in all aspects of the biological processes investigated thus far. All these mechanisms permit a fine modulation of gene expression at posttranscriptional level in different cells.

MicroRNAs as potential modulators of retinal diseases

MiRNAs showed tissue-specific and developmental stage-specific expression patterns, suggesting potential unique functions in different locations. In the last few years, several groups have investigated miRNA expression in the retina and other ocular tissues [27]. It is known that more than 300 miRNAs are expressed in the rodent retina, and they are particularly important for the fine-tuning of gene expression networks in this ocular tissue [28]. Indeed, miRNAs represent a newly recognized level of gene expression regulation that may affect retinal development, function, and metabolism. For example, it was determined that 23 out of 138 miRNAs detected in the retina varied at some point between embryonic day 5 and day 50 of adult life by at least 4- or 8-fold, respectively [29]. Diurnal variation of expression was also reported for a set of retina-specific miRNAs, and their expression was mostly regulated by light [29]. Based on these studies, it appears that miRNAs are required for normal retinal development and function.
Consequently, miRNA dysregulation has been demonstrated in several animal models of different retinal diseases. Likewise, several groups reported that in different mouse models of retinitis pigmentosa (including both autosomal dominant and autosomal recessive forms), there were changes in the expression of six different retinal miRNAs [30, 31]. In these models, miRNA-96, -182, and -183 were suppressed, and miRNA-1, -133, and -142 were upregulated, when compared with wild-type animals. Next, it was determined that three of these miRNAs (miRNA-96, -182, and -183) appeared to promote the survival of rods in the light-damage mouse model of photoreceptor degeneration [32]. These results suggest the potential strong involvement of some of these miRNAs in the pathology of retinitis pigmentosa. Moreover, abnormal miRNA expression has been observed in animal models of retinoblastoma. The experimental data indicate that downregulation of let-7 may be associated with the progression of retinoblastoma [33]. In contrast, miRNA-17-92 overexpression may promote retinoblastoma, as this miRNA cluster is a potent microRNA-encoding oncopogene that promotes cell proliferation and tumorigenesis [34]. Similarly, several miRNAs were implicated in different inflammatory states of the retina, indicating that miRNAs may play also an important role in immune response and autoimmune. miRNAs were found to control cytokine production and inflammatory-related retinal conditions. In the animal model of experimental autoimmune uveoretinitis (EAU), Ishida et al. found that both miRNA-142-5p and miRNA-21 were significantly upregulated in EAU-affected eyes, whereas miRNA-182 was significantly downregulated [35]. Interestingly, increasing evidence has indicated that miRNAs play important roles in diabetes and its complications, including diabetic retinopathy. It was found that at least 86 and 120 miRNAs were differentially expressed in the retina and retinal endothelial cells of streptozotocin (STZ)-induced diabetic rats and controls, respectively [28]. Likewise, microRNA dysregulation has been linked to such processes as RPE degeneration, choroidal neovascularization, and oxidative stress, all of which are hallmarks in the pathogenesis of AMD.

Role of microRNA in RPE damage

The retinal pigment epithelium (RPE) is a monolayer of pigmented cuboidal cells that perform highly specialized and unique functions essential for the homeostasis of the neural retina. It provides multiple functions that support normal photoreceptor activity. These include the phagocytosis of outer segments of photoreceptors, the directional transport of nutrients, the removal of waste products from photoreceptor cells, and visual pigment transport and regeneration. Thus, RPE damage represents a key event leading to the degeneration of the retina, visual impairment, and blindness [36]. Central geographic atrophy (GA) develops as a result of progressive apoptosis of the RPE, which consequently leads to damage to the photoreceptors and the underlying choriocapillaries [37]. In patients with diagnosed GA, Kaneko and colleagues found that the RPE cells have a decreased level of Dicer1, and this produced the specific accumulation of Alu element transcripts and subsequent RPE toxicity. Concurrently, RPE-specific Dicer1 deletion in mice also led to widespread RPE cell degeneration, similar to the situation in human geographic atrophy [38]. These data therefore imply that the reduced Dicer1 levels might be important in the pathogenesis of the dry form of AMD. Interestingly, in mice that lack two other enzymes (Drosha and DGCR8) that along with Dicer1 are required for microRNA processing, there was no degeneration of the RPE, even though these animals showed severely impaired miRNA function. Kaneko et al. proposed the following model for the pathogenesis of geographic atrophy in course of AMD. In the RPE, long double-stranded Alu RNA is constantly produced. In healthy individuals, Dicer1 processes these toxic RNAs into shorter, nontoxic versions. In patients with geographic atrophy, however, Dicer1 levels are greatly reduced, allowing the long Alu RNAs to cause cell death and RPE degeneration [38]. Tarallo and colleagues investigated the mechanism of Alu RNA-induced cytotoxicity and found that Dicer1 deficit or Alu RNA exposure activates the NLRP-3 inflammasome and triggers MyD88-dependent signaling via interleukin-18 (IL-18) in the RPE. Importantly, the genetic or pharmacological inhibition of inflammasome components (NLRP3, Pycard, Caspase-1), MyD88, or IL-18 prevents RPE degeneration induced by Dicer1 loss [39].

On the other hand, the RPE is at high risk for oxidative stress because it is exposed to high levels of phototoxic blue light and high oxygen tension [40]. Therefore, the RPE has evolved an intrinsic antioxidant defense network that consists of a variety of antioxidant enzymes, such as superoxide dismutases, glutathione peroxidases, glutathione S-transferases, and catalase. Accumulated oxidative damage in the largely nonmitotic RPE monolayer is likely to cause RPE cell degeneration, which may contribute to the pathogenesis of AMD [41]. Recently, Haque et al. found in a human retinal pigment epithelial cell line (ARPE-19) that miRNA-30b, a member of the miRNA-30 family, regulates the expression of catalase. They reported that under in vitro conditions of oxidative stress to ARPE-19 cells, the expression of this miRNA molecule was upregulated, which in turn inhibited the expression of endogenous catalase both at the transcript and protein levels [42]. Interestingly, the same group declared that using the antisense of miRNA-30b produced a spectacular increase in the expression of catalase in the RPE, even in an oxidant environment [42]. This observation indicates that a miRNA antisense approach could enhance cytoprotective mechanisms against oxidative stress by increasing the antioxidant defense system [42]. Importantly, a quantitative analysis of the miRNA-30b expression level might work as a sensitive sensor of the degree of oxidative stress in human tissues, including ocular ones. In contrast, antioxidant substances that prominently
protect RPE cells against oxidative stress, such as curcumin, significantly altered the expression profile of H$_2$O$_2$-modulated miRNA expression in ARPE-19 cells. In this experiment, five miRNAs (miRNA-20a, -126, -146, -150, and -155) that target vascular-endothelial growth factor-A (VEGF-A), platelet-derived growth factor β (PDGFβ), nuclear factor-κB (NF-κB), endothelin-1, p53, and AT1R appeared to be significantly upregulated compared with the controls. Moreover, curcumin significantly induced nine other miRNAs (miRNA-9, -23a, -23b, -122, -223, -191, -376c, -424, and let-7d) compared with the controls [43]. Of note, the miRNA-30b mentioned above together with other members of the miR-30 family (miRNA-30a-e) were significantly downregulated after ARPE-19 cells were treated with curcumin [43]. These results indicate that a pharmacological antioxidative approach could enhance cytoprotective mechanisms against oxidative stress by modulating miRNA expression in RPE cells.

Another study shows a downregulation of miRNA-23a, a member of the miRNA-23-27-24 cluster, in RPE cells harvested directly from AMD patients compared with healthy age-matched controls [44]. Based on these results, the authors have postulated that miRNA-23a might be particularly important in the initial mechanisms of AMD pathogenesis, as this miRNA is associated with the cellular metabolism of reactive oxygen species. In addition, miRNA-23a regulates retinoic acid-induced neuronal differentiation through the transcription factor Hes-1, which is a target for this miRNA and is required for the differentiation and longevity of neuronal cells [45]. Another miRNA that was reported to be regulated by both retinoic acid and oxidative stress is miRNA-9. Kutty and colleagues found that there was a twofold increase in the expression of miRNA-9 when ARPE-19 cells were incubated with a retinoic acid derivative that produces oxidative stress conditions in vitro [46]. In addition, the potential binding sites for the transcription factors encoded by CEBPA and CEBPB genes were found to be present in the putative promoter regions of genes encoding miRNA-9. The induced miRNA-9 overexpression was associated with parallel increases in the expression of both transcription factor genes [46]. They also observed the increased expression of miRNA-16, miRNA-26b, miRNA-23a, and miRNA-15b in ARPE-19 cells, although these increases might also be related to the apoptosis induced in these cells, as it was confirmed by the increased expression of two pro-apoptotic HMOXI and GADD153 genes [46].

Interestingly, data accumulated in the last decade suggest that AMD may involve chronic inflammatory processes [47]. Specifically, the RPE cells orchestrate both innate and adaptive immunity because they express complement components, MHC class I and II molecules and Toll-like receptors and serve as an antigen-presenting cells [48]. Therefore, the analysis of inflammation-related miRNA in human RPE cells and their response to proinflammatory cytokines could have great value. Such an investigation was performed by Kutty and colleagues, who found that at least two miRNAs, miRNA-146a and miRNA-146b, could play a role in inflammatory processes underlying AMD, and other retinal degenerative diseases through their ability to negatively regulate the NF-κB pathway in human RPE [49]. The cultured ARPE-19 cells responded to a cocktail of proinflammatory cytokines (IFN-γ + TNF-α + IL-1β) by greatly increasing the expression of both miRNA-146a and miRNA-146b. Furthermore, posttranscriptional gene silencing by these two closely related miRNAs is known to play an important role in regulating inflammatory responses in other immune-related cells, such as human monocytes [50]. The same group of Kutty et al. reported that the inflammatory cytokines increase miRNA-155 expression in human RPE cells by activating the JAK/STAT signaling pathway [51]. A recent study has shown that the NF-κB-regulated miRNAs miRNA-9, miRNA-125b, miRNA-146a, and miRNA-155 are upregulated in AMD, and that miRNA-146a and miRNA-155 target the 3′-UTR of complement factor H (CFH), thereby downregulating CFH expression [52]. CFH deficiency in turn activates immune-related signaling that drives inflammatory neurodegeneration in the brain and retinal tissue. These upregulated miRNAs in turn appear to downregulate multiple target miRNAs and thus regulate a family of potentially pathogenic genes, including 15-lipoxygenase (crucial for neurotrophic support), synapsin-2 (important for synaptogenesis), tetraspanin-12 (significant for amyloidogenesis), and CFH (responsible for innate immune signaling and inflammation) [52].

Taken together, these data clearly indicate that miRNAs have tremendous potential to contribute to many interrelated aspects of RPE cell functions and their dysfunction, including physiological growth/regeneration and nonphysiological degeneration.

**Role of microRNA in neovascular phase of AMD**

Interestingly, the increasing evidence indicates that angiogenesis-related miRNAs are emerging as pivotal modulators of pathologic vascular development in the course of the neovascular form of AMD. Numerous endothelium-related miRNAs have been shown to be involved in the dynamic regulation of neovascularization, suggesting their crucial role at this stage of AMD [53]. Although the pathogenic mechanisms underlying neovascular AMD are still largely unknown, blood vessel formation depends on the highly coordinated actions of a variety of angiogenic regulators, e.g. VEGF, PDGF-B, and Angiopoietin-1 (Ang-1). Importantly, it was found that miRNA-126 strongly regulates Ang-1 signaling as well as vessel maturation by targeting p85β, and thus may indirectly influence the angiogenesis [54]. The molecular analysis revealed that miRNA-31 may target and downregulate the expression of hypoxia-inducible factor-1α (HIF-1α) and PDGF-B, while miRNA-150 may target and downregulate the expression of...
Table 1. The selected miRNA with potential role in pathogenesis of AMD.

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Change in expression level</th>
<th>Effect/significance</th>
<th>Target</th>
<th>Species/Model/Tissue (cell) type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dicer1</td>
<td>downregulation</td>
<td>specific accumulation of Alu element transcripts and subsequent RPE toxicity</td>
<td>Alu</td>
<td>Human/In vivo/RPE of humans with GA [38]</td>
<td></td>
</tr>
<tr>
<td>Dicer1</td>
<td>RPE-specific deletion</td>
<td>specific accumulation of Alu element transcripts and subsequent RPE degeneration</td>
<td>Alu</td>
<td>Mouse/In vivo/RPE [38]</td>
<td></td>
</tr>
<tr>
<td>Dicer1</td>
<td>downregulation</td>
<td>specific activation of the NLRP-3 inflammasome and subsequent RPE degeneration</td>
<td>MyD88 via IL-18</td>
<td>Mouse/In vivo/RPE [39]</td>
<td></td>
</tr>
<tr>
<td>miRNA-30b</td>
<td>upregulation</td>
<td>inhibition of catalase expression at the transcript and protein levels</td>
<td>catalase</td>
<td>Human/In vitro/ARPE-19 under an oxidant environment [42]</td>
<td></td>
</tr>
<tr>
<td>miRNA-20a, miRNA-126, miRNA-146, miRNA-150, miRNA-155;</td>
<td>upregulation</td>
<td>protection of RPE cells against oxidative stress</td>
<td>VEGF-A, PDGFß, NF-κB, endothelin-1, p53, AT1R</td>
<td>Human/In vitro/ARPE-19 incubated with antioxidant substances, i.e. curcumin [43]</td>
<td></td>
</tr>
<tr>
<td>miRNA-23a</td>
<td>downregulation</td>
<td>protection of RPE cells against oxidative damage</td>
<td>Fas</td>
<td>Human/In vivo/RPE of donor eyes with AMD [44]</td>
<td></td>
</tr>
<tr>
<td>miRNA-9</td>
<td>upregulation</td>
<td>importance in maintaining RPE cell function</td>
<td>CEBPA, CEBPB</td>
<td>Human/In vitro/ARPE-19 cells treated with retinoic acid derivatives to induce oxidative stress conditions [46]</td>
<td></td>
</tr>
<tr>
<td>miRNA-146a, miRNA-146b</td>
<td>upregulation</td>
<td>role in inflammatory processes underlying AMD, and other retinal degenerative diseases</td>
<td>NF-κB signaling pathway</td>
<td>Human/In vitro/ARPE-19 cells treated with the proinflammatory cytokines [49]</td>
<td></td>
</tr>
<tr>
<td>miRNA-155</td>
<td>upregulation</td>
<td>role in inflammatory processes underlying AMD, and other retinal degenerative diseases</td>
<td>JAK/STAT signaling pathway</td>
<td>Human/In vitro/ARPE-19 cells obtained from adult donor eyes exposed to inflammatory cytokine mix [51]</td>
<td></td>
</tr>
<tr>
<td>miRNA-31, miRNA-150</td>
<td>downregulation</td>
<td>dynamic regulation of neovascularization through downregulation of expression of angiogenic growth factors, i.e. VEGF, HIF-1α and PDGF-B</td>
<td>HIF-1α, VEGF, PDGF-B</td>
<td>Mouse/In vivo Model of ischemia-induced retinal neovascularization [55]</td>
<td></td>
</tr>
<tr>
<td>miRNA-23, miRNA-27</td>
<td>upregulation</td>
<td>induction of sprouting angiogenesis by promoting angiogenic signaling</td>
<td>Sprouty2, Sema6A, Sema6D</td>
<td>Mouse/In vivo Laser-induced retinal neovascularization [61]</td>
<td></td>
</tr>
<tr>
<td>miRNA-21, miRNA-24</td>
<td>upregulation</td>
<td>inhibition of angiogenesis by targeting multiple members downstream of Rho signaling</td>
<td>Pak-4, Limk-2, Diaph-1</td>
<td>Mouse/In vitro/Ischemia-induced retinal neovascularization [62, 63]</td>
<td></td>
</tr>
<tr>
<td>miRNA-126</td>
<td>upregulation</td>
<td>regulation of the response of endothelial cells to VEGF</td>
<td>SPRED-1</td>
<td>Mouse/In vitro/Endothelial cells [64]</td>
<td></td>
</tr>
</tbody>
</table>
contrast, miRNA-24 was able to repress angiogenesis by simultaneously regulating multiple components in the actin-related cytoskeleton pathways of endothelial cells (ECs). In the complex study, the authors found that miRNA-24 inhibits angiogenesis by targeting multiple members downstream of Rho signaling, including Pak-4, Limk-2, and Diaph-1 proteins. Moreover, the overexpression of miRNA-24 in ECs blocks fiber and lamellipodia formation and repressed EC migration, proliferation, and tube formation in vitro and angiogenesis in an ex vivo aortic ring assay [62].

Similarly, anti-angiogenic function, via the targeting of Rho signaling in ECs has been found in case of miRNA-21. Here, RhoB silencing through miRNA-21 overexpression impairs EC migration and tubulogenesis, thus providing a possible mechanism by which miRNA-21 can inhibit angiogenesis in course of CNV [63]. In another report, the endothelial-specific miRNA-126 inhibited sprout-related protein-1 (SPRED1), a negative inhibitor of VEGF signaling, and thus permits to regulate the response of endothelial cells to VEGF [64]. In contrast, Bonauer et al. determined that the miRNA 17–92 cluster controls angiogenesis via several anti-angiogenic miRNAs, including miRNA-92a, which block angiogenesis and impair the functional recovery of ischemic tissues [65]. Importantly, such anti-angiogenic miRNAs can also be targeted to induce angiogenesis at the site of ischemia.

Additionally, a role for Dicer1 in several angiogenic processes, including proliferation, migration, capillary sprouting, and the formation of endothelial cell networks, has been clearly demonstrated in experiments with cultured mammalian endothelial cells [66]. For example, via miRNA profiling of ECs, Fish et al. identified a group of endothelium-enriched miRNAs, including miRNA-126, -146, -197, and -625 [64]. Nicoli et al. found that miRNA-221 is required for endothelial tip cell behaviors during vascular development [67]. In contrast, another novel potential alteration found in neovascular AMD may involve senescent retinal macrophages. A recently published report by Sene et al. indicates that aged local macrophages increase miRNA-33 levels. This increase leads to decreased expression of its target gene, the cholesterol transporter ABCA1, which impairs the export of intracellular cholesterol and polarizes these cells to create a proangiogenic, macular degeneration-promoting phenotype [68]. Importantly, the age-related decrease of macrophage cholesterol efflux is therapeutically reversible by employing synthetic miRNA-33 inhibitors.

Taken together, these findings widely document that several broadly expressed miRNAs may regulate endothelial cell behavior, including proliferation, migration and the

<table>
<thead>
<tr>
<th>miRNA modification type</th>
<th>Change of expression level</th>
<th>Effect/significance</th>
<th>Target</th>
<th>Species/Model/Tissue (cell type)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>miRNA-30b antisense</td>
<td>upregulation</td>
<td>increase in catalase expression to enhance cytoprotective mechanisms against oxidative stress</td>
<td>Catalase</td>
<td>Human/In vitro/ ARPE-19 under an oxidant environment</td>
<td>[42]</td>
</tr>
<tr>
<td>miRNA-23a mimics</td>
<td>upregulation</td>
<td>the protection of RPE cells against oxidative damage through decrease of cell death and apoptosis</td>
<td>Fas</td>
<td>Human/In vivo/ARPE-19</td>
<td>[44]</td>
</tr>
<tr>
<td>miRNA-23 antisense, miRNA-27 antisense</td>
<td>downregulation</td>
<td>decreased angiogenesis via increase in the expression of Sprouty2, Sema6A, Sema6D</td>
<td>Sema6A, Sema6D</td>
<td>Mouse/In vitro/ Model of laser-induced retinal neovascularization</td>
<td>[61]</td>
</tr>
<tr>
<td>miRNA-24 mimics</td>
<td>upregulation</td>
<td>inhibition of angiogenesis by targeting multiple members downstream of Rho signaling</td>
<td>Pak-4, Limk-2, Diaph-1</td>
<td>Mouse/In vivo/ Model of ischemia-induced retinal neovascularization</td>
<td>[62]</td>
</tr>
<tr>
<td>pre-miRNA-31 mimics, pre-miRNA-150 mimics, pre-miRNA-104 mimics;</td>
<td>upregulation</td>
<td>inhibition of HIF-1α, VEGF, and PDGF-B, expression resulting in decreased retinal neovascularization</td>
<td>HIF-1α, VEGF, PDGF-B</td>
<td>Mouse/In vivo/ Model of ischemic retina</td>
<td>[75]</td>
</tr>
<tr>
<td>pre-miRNA-31 mimics, pre-miRNA-150 mimics;</td>
<td>upregulation</td>
<td>inhibition of HIF-1α, VEGF, and PDGF-B, expression resulting in decreased choroidal neovascularization</td>
<td>HIF-1α, VEGF, PDGF-B</td>
<td>Mouse/In vivo/ Model of laser-induced retinal neovascularization</td>
<td>[75]</td>
</tr>
<tr>
<td>miRNA-21 mimics</td>
<td>upregulated</td>
<td>decreased angiogenesis via inhibition of RhoB expression</td>
<td>RhoB</td>
<td>Mouse/In vivo/ Model of choroidal retinal neovascularization</td>
<td>[76]</td>
</tr>
</tbody>
</table>
ability to form capillary networks in retinal tissue, and thus may be important for neovascular AMD pathogenesis (Table 1).

Novel microRNA-based therapeutics

The distinct connection between aberrant expression of miRNAs and retinal degeneration suggests that miRNAs could function as therapeutic targets. Because many miRNAs are expressed in a tissue-specific manner, they might be subjected to specific therapeutic modulation in tissues that are affected by disease processes. Two different therapies based on miRNA mimics and anti-miRNAs are now being developed to repress pathological miRNAs or overexpress protective miRNAs. miRNA mimics are small RNA molecules that resemble miRNA precursors; they are used to downregulate the expression of specific target proteins. Here, the target protein can be any gene involved in disease pathogenesis or the disease gene itself with a gain-of-function pathogenic mutation. The goal of this strategy is based on decreasing the abnormal protein level, thus providing a protective therapeutic effect. In the second approach, anti-miRNA molecules are created to inactivate the endogenous miRNA of interest [69]. Likewise, anti-miRNA-related and miRNA overexpression-based studies demonstrating efficacy in rodent animal models have already been reported [70]. Furthermore, Krützfeld et al. have designed anti-miRNAs, called “antagomirs”, which are conjugated to cholesterol molecules to help the miRNA enter to the cells. Intravenous administration of anti-miRNAs resulted in an apparent decline of corresponding miRNA levels in many organs, such as the liver, lung, kidney, heart, intestine, fat, skin, bone marrow, muscle, ovaries and adrenals. The silencing of endogenous miRNAs with this method was specific, efficient and long-lasting [71]. Recently, antagomirs have also been investigated in models of neurodegeneration. In the ALS disease mouse model SOD-1, the anti-miRNA to miRNA-155 has been used to prolong animals’ survival by 38% [72]. Importantly, there are also several clinical trials currently ongoing to test the therapeutic efficacy of miRNA modulation in selected diseases, such as cancer and chronic hepatitis C viral infection [73, 74].

Such innovative drugs may work as cellular mediators of disease and provide the possibilities for the therapeutic modulation of miRNA-regulated processes in the retina. They may also provide a potential therapeutic strategy for specific forms of AMD, such as choroidal neovascularization. Such experimental therapy was performed by Schen et al., who intraocularly injected pre-miRNA-31, -150, and -184 separately or mixed together [75]. Importantly, the in vivo intraocular injection of pre-miRNA-31, -150, or -184 into ischemic eyes caused significant reduction in the expression levels of HIF-1α, VEGF, and PDGF-B, resulting in decreased retinal neovascularization (NV) in a model of the ischemic retina. Moreover, the in vivo injection of pre-miRNA-31 or -150 also significantly reduced choroidal NV in a model of laser photocoagulation-induced CNV [75]. Likewise, miRNA-21 acts in endothelial cells as a negative regulator of angiogenesis; in contrast, miRNA-21 inhibition leads to opposite effects [76]. Sabatel et al. showed the upregulation of miRNA-21 in endothelial cells led to a reduction of EC migration and tubulogenesis through the impaired organization of actin into stress fibers and a decrease in RhoB expression, thus providing a possible mechanism for miRNA-21 to inhibit angiogenesis. Furthermore, the therapeutic potential of miRNA-21 as an angiogenesis inhibitor was demonstrated by the same group in vivo in a mouse model of CNV [76]. Concurrently, subretinal delivery of miR-24 mimics repressed laser-induced CNV in vivo [62]. Interestingly, similar results were obtained by employing a distinct approach based on synthetic anti-miRNA. Here, the inhibition of miRNA-23 and -27 function by complementary anti-miRNA repressed angiogenesis in an in vitro model and repressed postnatal retinal vascular development in vivo, what indicates that manipulating miRNA-23 and -27 levels may have important therapeutic implications in neovascular AMD or diabetic retinopathy [61]. All the presented results suggest that selected miRNAs may inhibit neovascularization in vivo and may potentially be used to treat neovascularization in the course of AMD and other vascular disorders. Similarly, novel miRNA-based therapeutics may be designed and applied for treatment of early AMD phase, when primary RPE cell damage play an important role in the pathogenesis of AMD. In this notion, Lin et al. found that ARPE-19 cell death and apoptosis were decreased by an miRNA-23a mimic through regulation of Fas expression [44].

The design of effective miRNA-based AMD therapeutics, however, faces several challenges because of the complexity of neurodegenerative process and the specific localization of the retina. One of the major therapeutic challenges is the off-target nonphysiological effect of miRNA, which strongly limits the potential for effortless manipulation of the levels or functions of a specific miRNA. Each miRNA can target several hundred mRNAs; therefore, knowledge about the effects of unwanted interactions between the miRNA and endogenous RNAs is a crucial pharmacological issue. Although synthetic miRNA therapeutics have been studied frequently in vitro (e.g. in cultures of retinal cell populations: RPE or endothelial cells), the efficacy of miRNA under in vivo conditions has not been thoroughly studied. The second major challenge for this novel therapeutic approach is the delivery of the miRNA molecules to the selected cells in the human organs. Delivery across the blood-retina barrier poses a particular technical hurdle in retinal disorders. The conjugation of antagomirs or miRNA mimics to selected molecules, such as aptamers, monoclonal antibodies (e.g. surface receptor-specific antibodies), peptides or other bioactive compounds to target specific cell-surface receptors might enrich their
uptake by retinal tissue [77]. Likewise, the encapsulation of synthetic miRNA with carriers, such as liposomes or nanoparticles, to escape local immune responses is another potential therapeutic option [78]. Specifically, the direct administration of miRNA therapeutics to the eyeball, which is a highly isolated environment, may improve the tissue-specific uptake of miRNA molecules. To extend the time of expression of miRNA mimics in the desired cells, they can be expressed by different vectors, including such long-term expression viral vectors as lentiviruses and adeno-associated viruses, which are quite convenient for viral-based therapeutics in ophthalmology [79]. Vector-based miRNA expression technology may also avoid the repeated administration of the miRNA mimic that is required to create sustained biological effects from such synthetic molecules. Interestingly, another experimental strategy developed in recent years to inhibit miRNA function is the mRNA-based synthetic sponge, which contains complementary binding sites for the miRNA of interest [80]. When the sponge is expressed, it specifically hampers the activity of a family of miRNAs with a common seed sequence, resulting in the sequestration and blocking of a whole family of related miRNAs.

In conclusion, the potential of particular miRNAs to modulate complex disease pathways by targeting several components of regulatory networks enables these molecules to modulate biological cellular responses in a manner that is different from that of classical drugs (Table 2).

**MicroRNA as the diagnostic biomarkers**

In contrast, identifying the signature patterns of miRNAs associated with different degenerative retinopathies has generated opportunities for miRNA-based diagnostics. The identification of dysregulated miRNAs in patients affected by AMD might allow earlier diagnosis and the monitoring of disease progress. MiRNA profiling could also discriminate among patients with specific forms of AMD. In contrast to using the proteins as targets for routine diagnostics—in which case they show relatively low sensitivity, reproducibility, and specificity—RNA molecules, including miRNA, provide higher or equal sensitivity, reproducibility and specificity as diagnostic biomarkers, even when compared to DNA [13]. New methods of RNA isolation, purification and stabilization have recently been developed for routine diagnostics, making the RNA very well suited as a biomarker [81]. Different RNAs can be identified in most bodily fluids, including blood, urine, saliva and cerebrospinal fluid, and have been adopted for use as diagnostic biomarkers for diseases [82, 83]. Microarray technology and multiplex amplification techniques have shown that RNA is indeed a valid target for routine molecular diagnostics and testing [84]. If present, miRNAs provide potential biomarkers for identifying populations and patients at high risk for systemic diseases, and recently, they have been used to detect cancers and different brain disorders [85]. Particularly, several reports now implicate miRNAs and altered miRNA processing in neurodegeneration [86-88]. In general, these studies have taken one of two approaches: 1) studying the impact of global miRNA synthesis on neuronal function and neurodegeneration, or 2) identifying alterations in the expression of particular miRNAs in models of neurodegenerative diseases. Recent studies have also revealed important roles for miRNAs in vascular diseases because of their role in modulating angiogenic signals and vascular remodeling [89]. As mentioned above, several upregulated or downregulated miRNAs have been detected in endothelial cells and RPE cells collected from patients with different forms of AMD [90]. However, the limited numbers of human retinal tissue samples in these studies also need to be addressed to attain better identification of miRNA types. Whether abnormally expressed miRNAs are diagnostic for AMD, and macular failure remains to be determined. The multiplicity of cell types and the diverse miRNA expression patterns in different cell populations within the retina also hamper the interpretation of studies on retinal samples. Furthermore, it must also be determined whether these miRNA might be detected in plasma, as in case of several cardiovascular diseases [91, 92], and specifically in the vitreous fluid. Nevertheless, the cellular secretion of particular miRNAs by exosomes suggests specificity in the miRNA secretion process [93].

Altogether, we expect that further in-depth study of the specific patterns and expression profiles of miRNA with the above-discussed biologic activity in AMD will ultimately result in the identification of new diagnostic markers. The initial expression-profiling studies of RNA and protein molecules have shown the potential of this approach. Some studies have validated changes in miRNA concentrations in body fluids in the context of cardiovascular disorders and cancer [94, 95], which suggests that further work with different types of AMD might also be successful.

**Conclusions**

In summary, currently available evidence clearly points to a significant role for miRNAs in age-related macular degeneration. The emerging data support pivotal functions for miRNAs in retinal degeneration through their important roles in regulating the survival and metabolic function of RPE, endothelial cells, photoreceptors, and other retinal cells. The involvement of miRNAs in the pathogenesis of AMD presents a new opportunity for elucidating previously unrecognized molecular mechanisms that underlie specific forms of this disease and for these molecules to be used as a potential therapeutic intervention for these incurable disorders. Therefore, the development of tools for the delivery, stable expression and controlled modulation of miRNA levels and action in vivo will be valuable for enhancing the therapeutic value of miRNAs. In addition, exploring the possibility of unique patterns of miRNA
expression in the vitreous fluid or peripheral blood of patients with different stages of AMD could yield biomarkers of disease or targets to actual therapeutic strategies.

Acknowledgements

This work was supported by the National Science Center grant Number. DEC-2013/09/B/NZ7/04031 (to AM).

Conflict of interests

The authors declare no conflict of interest.

References


http://www.researchpub.org/journal/jbpr/jbpr.html


