Review

MicroRNAs in Vascular Eye Diseases

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Received: 27 December 2019; Accepted: 16 January 2020; Published: 19 January 2020

Abstract: Since the discovery of the first microRNA (miRNA) decades ago, studies of miRNA biology have expanded in many biomedical research fields, including eye research. The critical roles of miRNAs in normal development and diseases have made miRNAs useful biomarkers or molecular targets for potential therapeutics. In the eye, ocular neovascularization (NV) is a leading cause of blindness in multiple vascular eye diseases. Current anti-angiogenic therapies, such as anti-vascular endothelial growth factor (VEGF) treatment, have their limitations, indicating the need for investigating new targets. Recent studies established the roles of various miRNAs in the regulation of pathological ocular NV, suggesting miRNAs as both biomarkers and therapeutic targets in vascular eye diseases. This review summarizes the biogenesis of miRNAs, and their functions in the normal development and diseases of the eye, with a focus on clinical and experimental retinopathies in both human and animal models. Discovery of novel targets involving miRNAs in vascular eye diseases will provide insights for developing new treatments to counter ocular NV.

Keywords: AMD; biomarker; eye disease; retinopathy; neovascularization; microRNA

1. Introduction

The Human Genome Project from 1990 to 2003 provided, for the first time, complete comprehensive information on human genome sequences with their entire complexity and thereby started the post-genomic era [1,2]. This era is marked in part by extensive studies on non-coding RNAs (ncRNAs), which do not encode for proteins yet still account for more than 98.5% of human genome transcripts [1,2]. While the housekeeping ncRNAs, such as transfer RNAs (tRNAs) and ribosomal RNAs (rRNAs), have already been shown to exhibit relatively clear functions, the regulatory ncRNAs including long non-coding RNAs (lncRNAs), microRNAs (miRNAs), and circular RNAs (circRNAs), once considered “junk” RNAs, have more recently been found to play important roles in a wide variety of physiological and pathological processes, and hence have become attractive targets in deciphering and treating human diseases. Among these regulatory ncRNAs, miRNAs are arguably one of the most widely studied ncRNAs in biomedical research.

First identified in 1993 [3], miRNAs are a class of naturally occurring small ncRNAs ranging in size from 19 to 25 nucleotides, and their major function is in regulating gene expression at the post-transcriptional level [4]. Since the initial discovery, thousands of miRNAs have been found in various species and the number of miRNAs continues to increase. In the human genome, expression of up to 60% of protein-coding genes may be regulated by miRNAs, indicating their pervasive role in multiple biological processes such as proliferation, apoptosis, differentiation, and development [5–8]. Importantly, dysregulation of miRNA is found to be involved in many diseases, such as various cancers, cardiovascular diseases, and neurodegenerative disorders. Genetic variation of miRNAs is also linked with several inherited diseases including hearing loss and growth defects. Given their
biological importance, miRNAs are currently recognized as novel disease biomarkers and potential therapeutic targets for developing new interventions [8–10].

2. miRNA Biogenesis and Function

The biogenesis of miRNAs starts in the nucleus, where miRNA genes are transcribed primarily by RNA polymerase II (Pol II) into long primary miRNA transcripts (pri-miRNAs), which contain one or more hairpin structures and can be more than 1000 nucleotides long [11]. Pri-miRNAs are then processed by the RNaseIII endonuclease, Drosha, along with its co-factor protein DGCR8 (DiGeorge syndrome critical region 8) into the resulting much shorter precursor miRNAs (pre-miRNAs), usually about 70 nucleotides long, which are then transported to the cytoplasm via exportin-5 [12–15]. In the cytoplasm, the pre-miRNAs are subsequently cleaved by another RNaseIII endonuclease, Dicer, with assistance from TAR RNA binding protein (TRBP), which together remove the terminal loop, and generate a miRNA duplex [16]. After double-strand RNA unwinding, the mature miRNA strand is selected by the argonaute (AGO) family protein, and assembled into an RNA-induced silencing complex (RISC). This complex may then bind downstream targets to act through translational repression or mRNA cleavage [17,18] (Figure 1). miRNAs function by base-paring to the complementary sequence in the 3′ untranslated region (3′ UTR) of target mRNAs to either induce mRNA degradation, promote deadenylation, or reduce translational efficacy [4,19]. Based on these features, miRNAs are considered key mediators of post-transcriptional regulation through fine-tuning gene expression.

Figure 1. MicroRNA (miRNA) biogenesis. This schematic diagram illustrates the canonical pathway of miRNA biogenesis. The miRNA gene is transcribed by RNA polymerase II (Poly II) to generate the primary miRNA (pri-miRNA) that forms hairpin structures. The long pri-miRNA is then processed by Drosha and DiGeorge syndrome critical region 8 (DGCR8) into the shorter precursor miRNA (pre-miRNA), which is then exported to the cytosol with the help of exportin-5. The pre-miRNA is further cleaved by Dicer and transactivation response element RNA-binding protein (TRBP), yielding the miRNA:miRNA* duplex molecule, which is loaded into argonaute (AGO) to unwind and form the functional RNA-induced silencing complex (RISC). The mature miRNA then binds to the seed sequences on the 3′ untranslated region (3′ UTR) of the target mRNA, leading to its translation repression or cleavage and thereby degradation.
3. miRNA as Clinical Biomarkers

Over the past two decades, numerous studies recognize the clinical value of miRNAs in the diagnosis of virtually all major diseases, including cancers, cardiovascular diseases, and neurodegenerative diseases. The deregulation of miRNAs in cancer was first reported in chronic lymphocytic leukemia in 2002 [20]. Since then, extensive evidence points to largely altered expression levels of miRNAs in various types of cancers; and suggests the immense diagnostic potential of miRNA alteration, specifically circulating miRNAs, as biomarkers [21]. The diagnostic value of many circulating miRNAs has been reported for both chronic and acute leukemia (e.g., miR-10, miR-29, miR-31, miR-34, miR-130b, miR-146a, miR-148, miR-150, miR-155, miR-181b-5p, miR-192, miR-203, miR-210, miR-212, miR-328, miR-335, miR-342, miR-375) [22–27], breast cancer (e.g., miR-9, miR-10b, miR-21, miR-155, miR-181a-5p and miR-192) [28–32], lung cancer (e.g., miR-7, miR-25, miR-193a-3p, miR-214, and miR-483-5p) [33–37], and other human cancers [38], to name just a few. Besides cancer, circulating miRNAs are also implicated in the diagnosis of cardiovascular diseases and neurodegenerative diseases. For instance, miR-1, miR-133a, miR-133b, miR-145, miR-208a, miR-208b, and miR-499, may have diagnostic potential for coronary heart disease [39]; whereas four decreased serum miRNAs, namely miR-141, miR-146b-5p, miR-193a-3p, and miR-214, are suggested as biomarkers to detect early stages of Parkinson’s disease [40]. In addition, mutations in miRNAs are also associated with inherited diseases, including miR-96 in progressive hearing loss [41], miR-184 in familial keratoconus with cataract [42], and the miR-17–92 cluster in skeletal and growth defects [43]. Together these findings strongly suggest the useful value of miRNAs as clinical biomarkers and diagnostic tools to identify diseases in multiple organs.

4. miRNA in the Eye

Just like elsewhere in the body, the function of miRNAs has shown increasing relevance in the eye. In the back of the eye, the retina is comprised of a thin layer of neuronal tissue of diverse cell types and is equipped with a highly specialized light-sensing capacity (Figure 2). Fine-tuning gene expression for cell differentiation and function is crucial for vertebrate retinal development and proper vision. Given its major impact on gene regulation, miRNA serves a vital role in the retina throughout development and in eye diseases [44–46]. Previous animal studies examining the effects of Dicer-deficiency in retinal development identified the differential expression patterns of many miRNAs in vertebrate neural retinas [47–56], and developed the ability to categorize retinal cells through specific miRNA signatures (Figure 2).

**Figure 2.** The anatomy of the eye and relevant miRNAs. (A) The schematic diagram illustrates the main structures of the human eye. (B) The schematic representation of the cell types in the neural retina depicts their cellular connections (including ganglion cells, amacrine cells, bipolar cells, horizontal cells, as well as rod and cone photoreceptors) and supporting cells (Müller cells and RPE). (C) A cross...
section of the eye shows the laminar organization of the nuclear layers (GCL, INL, and ONL), the retinal vasculature, and segments of photoreceptors (IS/OS). The RPE monolayer, with Bruch’s membrane underneath, is located between the neural retina and the choroid complex. miRNAs that regulate the physiological functions or pathological conditions related to each retinal neuronal and vessel layers, and RPE, are listed next to respective histological structure. Deep, deep layer of retinal vessels; GCL, ganglion cell layer; INL, inner nuclear layer; Int, intermediate layer of retinal vessels; IS/OS, inner/outer segments; ONL, outer nuclear layer; RPE, retinal pigment epithelium; Sup, superficial layer of retinal vessels. Figure adapted from “Animal models of ocular angiogenesis: from development to pathologies” by Liu et al. 2017, FASEB J, 31(11), p. 4665–4681 [57].

In the developing eye of several species including mammals, amphibians, and fish, miRNA expression patterns were analyzed using a variety of different approaches [48,52,54,58–61]. Although the expression profiles of miRNAs vary across species and also differ based on the methods of analysis, some specific miRNAs demonstrate their similar patterns and common roles in retinal development, and in both structural and functional maintenance [62–64]. For example, miR-204, one of the most abundant miRNAs in the retina, regulates multiple aspects of eye development [60,61]. Ablation of miR-204 results in abnormal lens formation, altered dorsoventral patterning of the retina, dedifferentiation of the retinal pigment epithelium (RPE), microphthalmia, and coloboma [62,63]. It is strongly expressed in the ganglion cell layer (GCL), the inner nuclear layer (INL), and the RPE [61,65]. In addition, miR-211, another member of the miR-204/211 subfamily, is highly similar to miR-204 in its sequence, target capability, and expression pattern. Loss of miR-211 in mice results in progressive cone dystrophy which is accompanied by the degeneration of cone cells and reduced visual responses detected from electroretinogram (ERG) [66].

Some miRNAs important for brain neural development also play crucial roles in the retina, as a part of the central nervous system (CNS). miR-124, enriched in the vertebrate CNS, is one of the most well studied miRNAs in the developing retina [51,52,54,61,64,67–74]. miR-124 targets Lhx2, a transcriptional factor essential for CNS development, and hence it is important in the maturation and survival of both dentate gyrus neurons and retinal cone photoreceptors. miR-124 is expressed in all retinal neuronal cell layers with the most prominent expression in photoreceptors [46,64]. Abolishing miR-124a in mice leads to mislocalization and apoptosis of cone photoreceptors, altered expression of cone-specific genes and reduced photopic ERG [64]. In addition to photoreceptors, miR-124 is expressed in other retinal neuronal cell layers and contributes to their functions. For instance, in donor eyes from patients with age-related macular degeneration (AMD) and in mouse models of retinal degeneration, miR-124 exhibits a time-dependent altered expression pattern from the outer nuclear layer (ONL) neurons to the Müller glia in the INL, followed by its eventual depletion at a later stage [75]. miR-132, another critical miRNA in brain neural synaptic growth [76], is also important for retinal neurons. As a member of the miR-132/212 cluster, miR-132 shapes brain synapse formation and influences visual cortex plasticity [77]. In the eye, miR-132 promotes axon formation of retinal ganglion cells (RGCs), and is expressed in GCL and INL under the control of brain-derived neurotrophic factor (BDNF) [78].

Given the crucial role of the aforementioned miRNAs in ocular and retinal development and their functional conservation across species, it is plausible that altered expression of miRNAs may lead to ocular disorders, including those vascular eye diseases that are characterized by primary abnormalities in retinal blood vessels which control metabolic availability of oxygen and nutrients to impact retinal neurons.

5. miRNA and Angiogenesis

To understand the roles of miRNAs in vascular eye diseases, here we first summarize the function of miRNAs in angiogenesis. Angiogenesis is the process of new vessels sprouting from existing vessels, which is orchestrated by various angiogenic stimulators and inhibitors, including miRNAs. Angiogenesis plays crucial roles in both physiological development and homeostasis, as the vascular system delivers nutrients to organs and tissues and removes catabolic products [79,80]. Dysregulation
of vascular growth is associated with many cardiovascular diseases, cancers, neurodegenerative disorders, and vascular eye diseases [81,82]. Abnormal angiogenesis disrupts the delivery of oxygen and nutrients, which can lead to an unbalanced metabolic status, and result in structural instability and functional loss of affected tissues. Many eye diseases with vascular components are marked by pathologic ocular neovascularization (NV), characterized by a leaky, fragile, and tuft-like appearance, which may cause retinal hemorrhage and lead to retinal damage and/or tractional retinal detachment, and ultimately result in vision loss in the most severe cases [83].

The importance of miRNAs in angiogenesis and endothelial function was first established by analysis of Dicer- and Drosha-deficient mice with defective miRNA biosynthesis [84–87]. Mouse embryos with a Dicer hypomorphic mutation have defective angiogenesis accompanied by reduced miRNA production and dysregulation of angiogenic genes [84,85]. In addition, the knockdown of Dicer and Drosha in human endothelial cells (ECs) suppresses angiogenic functions, including sprouting, tubular formation and migration [86]. Moreover, mice with conditional EC-specific loss of Dicer exhibit reduced vascular endothelial growth factor (VEGF)-driven angiogenesis postnatally, along with an altered set of angiogenic regulating miRNAs [87].

Several miRNAs are highly expressed in vascular endothelium, including miR-126, miR-210, miR-221/222, the miR-17-92 cluster, and the miR-23-27-24 cluster, as revealed by miRNA profiling in human ECs [88–93]. These miRNAs are also called “angiomiRs” for their angiogenesis-related targeting genes [89]. For example, miR-126 promotes angiogenesis and enhances VEGF signaling in ECs, as it suppresses sprouty-related protein-1 (Spred1), a negative factor of Ras-MAP kinase pathway involved in VEGF signaling [90–92]. miR-126 null mouse embryos have vascular abnormalities and a high mortality rate most likely due to SPRED1 induction, and subsequent diminished MAP kinase signaling in response to VEGF [91]. miR-210, another angiomiR and hypoxia-induced endothelial miRNA, regulates angiogenesis and cell survival in response to hypoxia [93,94]. Overexpression of miR-210 increases VEGF-driven EC migration and tube formation by targeting Ephrin-A3, an angiogenic receptor tyrosine kinase [93,95,96]. The miR-23-27-24 cluster is highly enriched in ECs and vascularized tissues [97,98]. Whereas miR-23 and miR-27 act as enhancers of angiogenesis in vascular development and pathological angiogenesis [97,99,100], miR-24 inhibits cardiac angiogenesis [98]. Similarly, miR-221/222 is identified as an anti-angiogenic miRNA by targeting c-Kit, the tyrosine kinase receptor of pro-angiogenic stem cell factor (SCF). Overexpressing miR-221/222 in HUVECs suppresses c-Kit and thereby inhibits SCF-mediated angiogenic abilities, such as tubular formation and migration [88]. In addition, the miR-17-92 cluster is a well-characterized polycistronic miRNA that functions as another intrinsic anti-angiogenic regulator in human ECs [101]. Individual miRNAs of this cluster have the ability to cooperate or work independently to modulate multiple signaling pathways, such as the VEGF, Wnt signaling, and PTEN pathways to impact angiogenesis [101–104]. Together these findings suggest that miRNAs play critical functions in developmental and pathological angiogenesis and are additionally implicated in vascular eye diseases.

6. miRNAs Dysregulation in Neovascular Eye Diseases

Increasing evidence indicates that miRNAs and their biogenesis machinery may be altered and dysregulated in neovascular eye diseases, such as diabetic retinopathy (DR), age-related macular degeneration (AMD), and retinopathy of prematurity (ROP), suggesting the potential of using miRNAs as biomarkers and targeting them for potential therapeutics. In this section, we discuss some prominent examples of clinical studies on miRNAs dysregulation in vascular eye diseases, focusing on miRNAs as potential biomarkers (Table 1).
Table 1. Selected miRNAs associated with DR, wet AMD, and ROP.

| Diseases | miRNAs | Effects | miRNA Targets | Reference |
|----------|--------|---------|---------------|-----------|
| DR       | miR-126| Downregulated in serum of T1DM and T2DM patients | SPRED-1, PIK3R2, VECAM-1 | [91,92,105–108] |
|          | miR-150| Downregulated in plasma of T1DM with DR | n/a | [109] |
|          | miR-155| Upregulated in blood samples of T2DM patients with DR | TGFβ | [110] |
|          | miR-200b| Downregulated in serum of patient with DR | ETS-1, VEGF-A | [111,112] |
|          | miR-221| Upregulated in serum of T2DM patients | n/a | [113–116] |
|          | miR-27b| Associated with incidence and progression of T1DM by analyzing serum miRNA | SEMA6a, THBS-1 | [117] |
|          | miR-320a| Associated with incidence and progression of T1DM by analyzing serum miRNA | NRP1 | [117] |
| Wet AMD  | Let-7  | Upregulated in blood samples of AMD patients | TIMP-1, TSP-1 | [84,86,118–120] |
|          | miR-126| Downregulated in blood samples of AMD patients | KDR, SPRED-1, VEGF-A | [89–92,118,121–123] |
|          | miR-146| Upregulated in retinal tissues of AMD patients | IRAK1, TNFA | [124,125] |
|          | miR-21 | Downregulated in blood samples of AMD patients | RHOB | [89,118,121,126] |
| ROP      | miR-23a| Upregulated in plasma of ROP patients | ISM1, SEMA6a, SEMA6D, SPRY2 | [97,127] |
|          | miR-200b| Upregulated in plasma of ROP patients | ETS-1, VEGF-A | [112,127,128] |
|          | miR-27b| Downregulated in plasma of ROP patients | VEGF-B, VEGF-C | [72,127,129,130] |
|          | miR-214| Downregulated in plasma of ROP patients | ANG, HIF1α, QKI | [72,127,131] |

AMD, age-related macular degeneration; DR, diabetes retinopathy; n/a, not applicable; ROP, retinopathy of prematurity; T1DM, Type 1 diabetes mellitus; T2DM, Type 2 diabetes mellitus.

6.1. miRNAs in DR

DR is one of the most common microvascular complications of diabetes mellitus (DM), which is now recognized as a global epidemic. In the Western world, DR is a leading cause of vision impairment, particularly among individuals of working-age, and poses a significant economic and life quality burden on patients and society [132–134]. To find ways to alleviate this burden, it is important to first understand the biochemical basis underlying DR pathogenesis. Hyperglycemia induces alteration in cellular metabolism and causes oxidative injury. Prolonged exposure to hyperglycemia and metabolic changes leads to microvasculature damage in the retinas of diabetic patients. Progressive retinal ischemia eventually stimulates the expression of hypoxia-induced growth factors, such as VEGF, that promote retinal NV [135]. Retinal NV can then cause vitreous hemorrhage or tractional retinal detachment to result in severe vision loss [135,136]. Other major events involved in DR pathogenesis are the breakdown of the blood-retinal barrier and the consequent vascular leakage and thickening of the retina [137]. DR develops in approximately 50% patients with Type 1 diabetes mellitus (T1DM) and over 40% of patients with Type 2 diabetes mellitus (T2DM) by the first decade of incidence [138].

In addition to diagnosing DR through histological examination of the fundus vasculature, circulating miRNAs were found to play critical roles in DR development, suggesting that miRNAs could also serve as new biomarkers in detecting or predicting the progress of retinopathy and...
furthermore, the overall progress of DM [139–141]. Analysis of circulating miRNAs from serum or plasma samples of DM patients both with and without DR showed altered expression levels of many miRNAs throughout different patient populations (e.g., ages, type of DM, years after onset, etc.) [142]. Among them, miR-27b and miR-320a are the two miRNAs mostly associated with the risks of DR in T1DM [117]. miR-221, an anti-angiogenic miRNA, is significantly altered in DM and is involved in the DM physiopathology and macrovascular complications associated with T2DM [113–116]. In addition, other circulating miRNAs including miR-126 [105–107], miR-150 [109], miR-155 [110], and miR-200b [111] are also dysregulated in DR patients, as well as in pre-clinical animal models of DR [143–152]. These findings indicate the complex regulation of miRNAs in DR and the potential of miRNAs as biomarkers and/or therapeutic targets for treating DR.

### 6.2. miRNAs in Neovascular Age-Related Macular Degeneration (wet AMD)

AMD is a leading cause of irreversible loss of central vision in the elderly. Approximately 10–18% of individuals between 65 and 75 will lose some central vision as a result of AMD, while this number increases to 30% for those aged 75 or older [153]. There are two major clinical types of AMD: Atrophic (dry form) AMD with photoreceptor and RPE atrophy; and neovascular (wet form) AMD which is characterized by pathologic subretinal vessels originating from the choroid, i.e., choroidal neovascularization (CNV), the hallmark of wet AMD. Although only 10–20% of AMD patients develop wet AMD, this form of the disease accounts for approximately 80% of severe visual loss in AMD cases [154]. Central vision loss occurs when pathological choroidal neovessels protrude into the subretinal space and subsequently leak blood and cause exudates and hemorrhagic detachment of the retina, thereby resulting in irreversible photoreceptor damage [155].

As a complex, multifactorial, and progressive disease, AMD is linked with both genetic (including complement) and environmental risk factors [155]. Certain miRNAs associated with the complement factor H (CFH)-mediated inflammatory degeneration and neovascularization are dysregulated in the circulating blood or ocular tissues isolated from AMD patients [118,121,156,157]. Two studies identified differential sets of miRNAs altered in plasma collected from wet AMD patients compared to healthy subjects [118,156]. In one study, 16 miRNAs were found to be dysregulated out of 384 miRNAs screened in wet AMD patient plasma samples using quantitative real-time PCR-based methods, with 10 of the 384 miRNAs being exclusively expressed in the wet AMD patient group [118]. Another similar study using next-generation sequencing identified that 3 out of 203 circulating miRNAs were significantly altered in plasma from wet AMD patients vs. non-AMD controls [156]. Moreover, miRNA microarray screening found 23 out of 337 miRNAs were upregulated in the serum from both dry and wet AMD patients vs. non-AMD cohorts. Among them, only 3 miRNAs were expressed at significantly higher levels in the serum of patients with wet AMD [119]. The difference in miRNA profiles from these studies may reflect the variation in their miRNA screening methodology, the nature of samples (plasma vs. serum), the diverse patient population and the different inclusion criteria.

Some miRNAs are altered in both AMD patients and pre-clinical models of AMD, including Let-7, miR-126, and miR-21, all of which are implicated in angiogenic pathways [118–121]. The Let-7 family, upregulated in AMD patients [118,119], is pro-angiogenic and acts through the inhibition of anti-angiogenic factors tissue inhibitor of metalloproteinase-1 (TIMP-1) and thrombospondin-1 (TSP-1) [84,86,120]. miR-126 and miR-21, both angiomiRs [89–92,126], are downregulated in the blood samples of AMD patients [118,121], suggesting the dysregulation of angiogenic effects in these patients. Additionally, in experimental models of CNV, miR-126 regulates CNV lesion size [122,123]. These studies revealed the emerging role of miRNAs in AMD and the possibility of targeting miRNAs for suppressing CNV in neovascular AMD.

### 6.3. miRNAs in Retinopathy of Prematurity (ROP)

ROP is an ocular disease associated with abnormal retinal vascular development that occurs in premature infants and contributes to 6–18% of blindness in children in the developed countries [158–160].
ROP is a two-phase disease, beginning with incomplete retinal vessel growth after premature birth, which results in a peripheral avascular zone. As the infant matures, increasing metabolic activities of the peripheral avascular retina cause tissue ischemia and hypoxia. This stimulates a second phase of hypoxia-driven pathological vessel proliferation. In severe cases, pathologic neovessels in the second phase can cause tractional retinal detachment, ultimately leading to blindness [161–163]. Current ablation treatments may substantially reduce the incidence of blindness by 25% and improve long-term outcomes in infants with severe ROP. However, these treatments do not address the underlying causes of ROP or other comorbidities, including the failure of normal neuronal and vascular development [163–165]. Whether miRNA dysregulation contributes to ROP development has been a subject of recent studies assessing the diagnostic and therapeutic potential miRNAs as novel ROP biomarkers.

Plasma miRNAs were evaluated in premature infants with ROP and compared to preterm infants without ROP in a recent study using high-throughput quantitative real-time PCR [127]. Four out of 46 plasma miRNAs were significantly altered in ROP patients, with miR-23a and miR-200b-3p being upregulated and miR-27b-3p and miR-214-3p being downregulated [127]. miR-23a represses anti-angiogenic genes, such as sprouty2 (Spry2) and semaphorin6A (Sema6A), and Sema6D [97], and hence might be pro-angiogenic in ROP pathogenesis. Expression of miR-200b correlates with VEGF expression [128], and is an angiogenic regulator targeting Ets-1 in ECs [112]. On the other hand, miR-27b and miR-214 are anti-angiogenic factors as they inhibit VEGF family protein expression [129–131]. Dysregulation of these miRNAs in ROP is consistent with their potential roles in mediating pathological angiogenesis in ROP development.

Profiles of miRNAs are evaluated in several pre-clinical animal models of ROP. The miRNA expression patterns in different models vary widely, and may depend on a number of factors including animal species (mice vs. rats), the oxygen condition, time point of tissue collection, and analysis methods. Some miRNAs exhibit dramatically varied expression patterns in different models, including miR-126 [144,166], miR-145 [167,168], miR-150 [72,167,169], and miR-155 [170]. The function of these miRNAs as ocular angiogenic regulators are discussed in detail in the next section.

7. Dysregulated miRNAs in Experimental Models of Pathological Ocular Angiogenesis

Expression patterns of miRNAs were investigated in several animal models of ocular NV that mimic pathological features of human vascular eye diseases. Of particular relevance to this review is the oxygen-induced retinopathy (OIR) model, mimicking pathological retinal NV in ROP and DR, and the laser-induced CNV model, mimicking wet AMD. By exposing the newborn experimental animals (rodents, in most cases) to continuous hyperoxic or cycling oxygen conditions, the OIR model reliably reproduces the phenotypes of ROP—characterized by an initial phase of vaso-obliteration and a subsequent phase of hypoxia-induced NV [171,172]. As the current diabetic models fail to consistently develop proliferative retinopathy in rodents, the OIR model also serves as a platform to facilitate the investigation of the ischemic angiogenesis aspect of DR [57]. For wet AMD, the rodent model of laser-induced CNV is the most standard and widely used animal model for investigating many aspects of choroidal angiogenesis. In this model an argon laser is used to induce rupture of the Bruch’s membrane, which increases pro-angiogenic and inflammatory factors and stimulates new choroidal vessels growth into the laser-injured subretinal areas to form CNV [173–175]. The pioneering work in the laser-induced CNV and the OIR models has laid the experimental foundation for establishing the therapeutic value of anti-VEGF therapies as these are useful models for investigating the mechanisms of NV and evaluating novel anti-neovascular therapies [176,177], including the role of miRNAs. Some examples of well-characterized miRNAs that regulate pathological ocular angiogenesis in experimental models are reviewed here.
7.1. miR-126

miR-126 is one of the angiomiRs implicated in the regulation of angiogenic factors including VEGF and FGF for vascular growth, and regulates embryonic angiogenesis and cardiac angiogenesis [91,92]. miR-126 exhibits significant downregulation in the choroids of mice with laser-induced CNV [123], as well as in rodent OIR retinas and choroids [144,166]. Moreover, miR-126 knockout mice have vascular lesions in the peripheral areas of choroids in mature adults, delayed choroidal vascular development, and focal choroidal vascular atrophy in aged mice [178]. In OIR mice, miR-126 supplementation inhibits retinal neovascularization and blood-retinal barrier breakdown [144,179]. Overall these findings indicate that miR-126 is required for maintaining ocular vasculature integrity in pathological conditions.

7.2. miR-132

In the mouse models, miR-132 plays a crucial role in promoting angiogenesis by targeting Rasa1 (encoding p120RasGAP) [180–183]. Inhibition of miR-132 in the mouse models of OIR and retinal angiomatous proliferation (RAP) promotes EC quiescence and prevents NV by enhancing the expression of p120RasGAP [181]. Inhibition of miR-132 reduces EC function and suppresses growth factor-mediated developmental retinal and tumor angiogenesis in vivo and in vitro [182]. Furthermore, silencing miR-132 also suppresses corneal angiogenesis after eye infection with herpes simplex virus [184]. Beyond vascular endothelium, miR-132 is also expressed in the eye by RGCs and is up-regulated in response to BDNF [78]. These findings suggest the angiogenic functions of miR-132 in neovascular ocular diseases and its additional function in retinal neuronal health.

7.3. miR-145

miR-145 is co-transcribed with miR-143 as a cluster, which is generally considered as a tumor suppressor cluster in cancer cells [185]. However, in the mouse model of lung adenocarcinoma, tumor-specific deletion of miR-143/145 resulted in diminished angiogenesis; whereas overexpression of miR-143/145 stimulated EC proliferation in the tumor mass [186], indicating a surprising pro-tumor and pro-angiogenic function of miR-143/145, likely reflecting its diverse role in a context-dependent manner. Mice with systemic knockout of miR-143/145 are viable and show no overt abnormalities in cardiac structure and vascular smooth muscle cell differentiation [187]. In the OIR model, miR-145 is significantly upregulated in the retinas at P17 when compared to the age-matched normoxic control mice [167]. Intravitreal injection of miR-145 inhibitors suppresses NV in OIR. Moreover, modulation of miR-145 in vitro alters human retinal microvascular endothelial cell (HRMEC) angiogenic functions by targeting tropomodulin 3 (Tmod3), an actin-capping protein. miR-145 may thereby influence angiogenesis in ocular neovascular diseases through the modulation of the cytoskeletal architecture dynamics, and EC morphological changes [168] (Figure 3). Other studies also showed that miR-145 in retinal ECs may attenuate oxidative stress and inflammation induced by high-glucose, further supporting its role in DR [188]. Within the eye, miR-143/145 cluster also regulates intraocular pressure through the regulation of actin dynamics and trabecular meshwork contractility [189]. miR-145 also promotes ganglion cell survival in DR [190]. These findings demonstrate multiple roles of miR-143/145 in various ocular cell types and eye diseases.
functions by targeting tropomodulin 3 (Tmod3) of 7.4. miR-146a mice [167]. Intravitreal injection of angiogenic disorders [191]. Samples from patients with wet AMD and mouse retinas with roles of [189]. in vitro alters human retinal microvascular endothelial cell (HRMEC) angiogenic influence angiogenesis in ocular neovascular diseases through the modulation of the cytoskeletal architecture dynamics, and EC morphological changes [168] (Figure 3). Other studies also showed downstream angiogenic genes, such as miR-146a, and Fzd4, leading to increased angiogenesis and formation of pathologic neovascularization. Retinal dysregulation of miR-145, leading to repression of Tmod3, releasing the capping of actin filaments. This alteration in actin dynamics and architecture leads to increased endothelial cell angiogenic function, and thereby enhanced pathological angiogenesis. Figure adapted from “Endothelial microRNA-150 is an intrinsic suppressor of pathologic ocular neovascularization” by Liu et al. 2015, PNAS, 112(39), p. 12163–12168 [169]; and “MicroRNA-145 Regulates Pathological Retinal Angiogenesis by Suppression of TMOD3” by Liu et al. 2019, Mol Ther Nucleic Acids, 16, p. 335–347 [168].

7.4. miR-146a

miR-146a has been linked to the innate immune response, inflammation, and age-related neurodegenerative disorders [191]. Samples from patients with wet AMD and mouse retinas with selective glial cell ablation showed an upregulation of miR-146a and an involvement in CFH-mediated inflammation [124,125]. In the pre-clinical models of DR, miR-146a is upregulated in the retinal ECs with transactivation by nuclear factor-kappaB (NF-κB). Upregulation of miR-146a exerts negative regulation in multiple pathways of NF-κB activation, which suggests its correlation to inflammatory responses in DR [148,192–197]. Specifically, miR-146a upregulates inflammatory cytokines in the diabetic retina and kidney [197], protects HRMECs [194], reduces retinal microvascular leakage, and improves visual function in diabetic rats [198]. Moreover, diabetes induces rhythmic dysregulation of miR-146a and its inflammatory genes in human retinal endothelial cells [195]. These findings all point to the potential implication of miR-146a in DR development.

7.5. miR-150

miR-150 is a well-studied miRNA which was initially identified by its regulatory effects in lymphocyte development and differentiation [199–201]. Monocytic-secreted miR-150 influences angiogenesis in cancer and diabetes by modulating target gene expression in recipient ECs [202,203]. In the retina, miR-150 is enriched in retinal ECs more than in any other nuclear layers [169]. In OIR,
miR-150 is substantially reduced in OIR mouse retinas with specific downregulation in OIR neovessels, and regulates expression of several angiogenic factors, such as Cxcr4 (C-X-C chemokine receptor type 4), Dll4 (Delta like ligand 4) and Fzd4 (Frizzled-4) [72,169] (Figure 3). Treatment of miR-150 in vivo, via intraocular injection into the OIR mice, or in vitro, by transfection into HRMECs, demonstrated that miR-150 reduces pathological NV and regulates EC angiogenic functions in a VEGF-independent manner by targeting CXCR4, DLL4, and FZD4 [169]. Furthermore, miR-150 knockout mice show increased size of laser-induced CNV lesion [169], suggesting the role of miR-150 as an intrinsic inhibitor of pathological ocular angiogenesis. Similarly, miR-150 deletion leads to increased pulmonary angiogenesis in a hyperoxia-induced lung injury model [204], and exacerbates high fat diet-induced retinal NV in diabetic mice [149], suggesting an overall protective role of miR-150 against pathological angiogenesis.

7.6. miR-155

miR-155 is significantly upregulated in retinas of several ocular disease models, including the mouse OIR model [167,170], the rat models of light-induced retinal degeneration [205] and streptozotocin (STZ)-induced diabetes [148], as well as in human patients with AMD [124]. miR-155 is a HIF-dependent miRNA and its deficiency results in the reduction of the avascular area and NV in the mouse OIR model [150,170]. By targeting CCN1—a cysteine-rich and integrin-binding matricellular protein, upregulated miR-155 disturbs the normal retinal vessel growth in mice [170]. In addition to regulation of angiogenesis, miR-155 is also involved in inflammatory and immunomodulatory signaling pathways, which are of crucial importance in pathological angiogenesis [206]. In the preclinical model of STZ-induced DR, miR-155 was identified as a NF-κB- and VEGF-responsive miRNA [148]. miR-155 has also been shown to regulate CFH in AMD [124], further supporting its role in ocular angiogenesis and inflammation in eye diseases.

7.7. miR-21

miR-21 plays an important role in the regulation of angiogenesis, tumor growth and metastasis, as well as in cardiac hypertrophy [126,148,207,208]. This miRNA is downregulated in the plasma of wet AMD patients [118]. As such, miR-21 may play an important role in AMD pathogenesis for its involvement in the regulation of vascular growth, as exhibited by its high expression in retinal ECs [126]. This notion is supported by the fact that overexpression of miR-21 reduces CNV lesions in the laser-induced CNV mice. In addition, stimulated expression of miR-21 inhibits cultured EC proliferation and migration by targeted inhibition of RhoB, which controls the dynamics of actin-filament and thereby affects the EC function [126]. However, in the rat diabetic model, miR-21, as well as miR-146 and miR-155, are upregulated in the retinas and retinal ECs along with NF-κB and/or VEGF activation [148]. In the retinas of leptin receptor-deficient (db/db) mice, miR-21 was also significantly upregulated while its target gene PPARα (peroxisome proliferator-activated receptor-α), was downregulated [209]. These different findings from several animal models may reflect an underlying difference among various eye disease models or assay conditions, yet together indicate a potential disease-modifying effect of miR-21.

8. Conclusions

In summary, miRNAs are potent effectors in the post-transcriptional regulation of gene activity and play an important role in the modulation of retinal homeostasis and diseases including vascular eye diseases. Although the miRNA expression profiles from different experimental models of ocular angiogenesis differ in a disease- and model-dependent manner, these studies provide valuable clues to understanding the functions of dysregulated miRNAs in retinopathies. Furthermore, dysregulation of specific miRNAs can be utilized to identify potential miRNA candidates for therapeutic intervention. With expanding knowledge of miRNA profiles and their molecular mechanisms in eye development and ocular diseases, miRNAs can be harnessed for their capacities as biomarkers and their potential to be targeted for treating neovascular ocular diseases. In fact, the emerging miRNA therapeutics with its ability to target multiple pathological target genes may likely yield one of the most exciting
breakthroughs in the current treatment options for ocular diseases. With the current surge in omics research providing vast amounts of datasets, identification of critical miRNA targets for drug development presents considerable potential for generating such novel therapies for vascular eye diseases.

Funding: This work was supported by NIH grants (R01 EY028100 and EY024963), BrightFocus Foundation, Boston Children’s Hospital Ophthalmology Foundation, Massachusetts Lions Eye Research Fund Inc. (to JC), and the Knights Templar Eye Foundation under the Pediatric Ophthalmology Career-Starter Research Grant (to CHL).

Conflicts of Interest: The authors declare no conflict of interest.

References
1. Lander, E.S.; Linton, L.M.; Birren, B.; Nusbaum, C.; Zody, M.C.; Baldwin, J.; Devon, K.; Dewar, K.; Doyle, M.; FitzHugh, W.; et al. Initial sequencing and analysis of the human genome. *Nature* 2001, 409, 860–921. [PubMed]
2. International Human Genome Sequencing Consortium. Finishing the euchromatic sequence of the human genome. *Nature* 2004, 431, 931–945. [CrossRef] [PubMed]
3. Lee, R.C.; Feinbaum, R.L.; Ambros, V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* 1993, 75, 843–854. [CrossRef]
4. He, L.; Hannon, G.J. MicroRNAs: Small RNAs with a big role in gene regulation. *Nat. Rev. Genet.* 2004, 5, 522–531. [CrossRef] [PubMed]
5. Friedman, R.C.; Farh, K.K.; Burge, C.B.; Bartel, D.P. Most mammalian mRNAs are conserved targets of microRNAs. *Genome Res.* 2009, 19, 92–105. [CrossRef] [PubMed]
6. Brennecke, J.; Hipfner, D.R.; Stark, A.; Russell, R.B.; Cohen, S.M. bantam encodes a developmentally regulated microRNA that controls cell proliferation and regulates the proapoptotic gene hid in Drosophila. *Cell* 2003, 113, 25–36. [CrossRef]
7. Chang, T.C.; Mendell, J.T. microRNAs in vertebrate physiology and human disease. *Annu. Rev. Genom. Hum. Genet.* 2007, 8, 215–239. [CrossRef]
8. Im, H.I.; Kenny, P.J. MicroRNAs in neuronal function and dysfunction. *Trends Neurosci.* 2012, 35, 325–334. [CrossRef]
9. Du, L.; Pertsemlidis, A. Cancer and neurodegenerative disorders: Pathogenic convergence through microRNA regulation. *J. Mol. Cell Biol.* 2011, 3, 176–180. [CrossRef] [PubMed]
10. Nicoli, S.; Standley, C.; Walker, P.; Hurlstone, A.; Fogarty, K.E.; Lawson, N.D. MicroRNA-mediated integration of haemodynamics and Vegf signalling during angiogenesis. *Nature* 2010, 464, 1196–1200. [CrossRef]
11. Lee, Y.; Kim, M.; Han, J.; Yeom, K.H.; Lee, S.; Baek, S.H.; Kim, V.N. MicroRNA genes are transcribed by RNA polymerase II. *EMBO J.* 2004, 23, 4051–4060. [CrossRef] [PubMed]
12. Han, J.; Lee, Y.; Yeom, K.H.; Kim, Y.K.; Jin, H.; Kim, V.N. The Drosha-DGCR8 complex in primary microRNA processing. *Genes Dev.* 2004, 18, 3016–3027. [CrossRef] [PubMed]
13. Han, J.; Lee, Y.; Yeom, K.H.; Nam, J.W.; Heo, I.; Rhee, J.K.; Sohn, S.Y.; Cho, Y.; Zhang, B.T.; Kim, V.N. Molecular basis for the recognition of primary microRNAs by the Drosha-DGCR8 complex. *Cell* 2006, 125, 887–901. [CrossRef] [PubMed]
14. Lund, E.; Guttinger, S.; Calado, A.; Dahlberg, J.E.; Kutay, U. Nuclear export of microRNA precursors. *Science* 2004, 303, 95–98. [CrossRef]
15. Yi, R.; Qin, Y.; Macara, I.G.; Cullen, B.R. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. *Genes Dev.* 2003, 17, 3011–3016. [CrossRef]
16. Hutvagner, G.; McLachlan, J.; Pasquinelli, A.E.; Balint, E.; Tuschi, T.; Zamore, P.D. A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. *Science* 2001, 293, 834–838. [CrossRef]
17. Khvorova, A.; Reynolds, A.; Jayasena, S.D. Functional siRNAs and miRNAs exhibit strand bias. *Cell* 2003, 115, 209–216. [CrossRef]
18. Schwarz, D.S.; Hutvagner, G.; Du, T.; Xu, Z.; Aronin, N.; Zamore, P.D. Asymmetry in the assembly of the RNAi enzyme complex. *Cell* 2003, 115, 199–208. [CrossRef]
19. Krol, J.; Loedige, I.; Filipowicz, W. The widespread regulation of microRNA biogenesis, function and decay. Nat. Rev. Genet. 2010, 11, 597–610. [CrossRef]

20. Calin, G.A.; Dumitru, C.D.; Shimizu, M.; Bichi, R.; Zupo, S.; Noch, E.; Aldler, H.; Rattan, S.; Keating, M.; Rai, K.; et al. Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proc. Natl. Acad. Sci. USA 2002, 99, 15524–15529. [CrossRef]

21. Pogribny, I.P. MicroRNAs as biomarkers for clinical studies. Exp. Biol. Med. 2018, 243, 283–290. [CrossRef]

22. Rashed, W.M.; Hamza, M.M.; Matboli, M.; Salem, S.I. MicroRNA as a prognostic biomarker for survival in childhood acute lymphoblastic leukemia: A systematic review. Cancer Metastasis Rev. 2019. [CrossRef]

23. Zhang, Y.; Li, X.; Bai, L.; Li, L.; Li, D.; Ding, X.; Wang, B.; Li, C. MicroRNA-31 is a potential biomarker for screening B-lymphoblastic leukemia in children. Oncol. Lett. 2018, 18, 4939–4945. [CrossRef]

24. Guo, Y. Clinical significance of serum MicroRNA-203 in patients with acute myeloid leukemia. Bioengineered 2019, 10, 345–352. [CrossRef] [PubMed]

25. Fathullahzadeh, S.; Mirzaei, H.; Honardoost, M.A.; Sahebkar, A.; Salehi, M. Circulating microRNA-192 as a diagnostic biomarker in human chronic lymphocytic leukemia. Cancer Gene Ther. 2016, 23, 327–332. [CrossRef]

26. Yeh, C.H.; Moles, R.; Nicot, C. Clinical significance of microRNAs in chronic and acute human leukemia. Mol. Cancer 2016, 15, 37. [CrossRef]

27. Giza, D.E.; Calin, G.A. microRNA and Chronic Lymphocytic Leukemia. Adv. Exp. Med. Biol. 2015, 889, 23–40.

28. Tavakolian, S.; Goudarzi, H.; Torfi, F.; Faghihloo, E. Evaluation of microRNA-9 and -192 expression levels as biomarkers in patients suffering from breast cancer. Biomed. Rep. 2020, 12, 30–34. [CrossRef]

29. Al-Othman, N.; Ahram, M.; Alqaraleh, M. Role of androgen and microRNA in triple-negative breast cancer. J. Exp. Clin. Cancer Res. 2014, 33, 287–314. [CrossRef]

30. Liu, B.; Su, F.; Lv, X.; Zhang, W.; Shang, X.; Zhang, Y.; Zhang, J. Serum microRNA-21 predicted treatment outcome and survival in HER2-positive breast cancer patients receiving neoadjuvant chemotherapy combined with trastuzumab. Cancer Chemother. Pharmacol. 2019, 84, 1039–1049. [CrossRef] [PubMed]

31. Al-Othman, N.; Ahram, M.; Alqaraleh, M. Role of androgen and microRNA in triple-negative breast cancer. J. Exp. Clin. Cancer Res. 2014, 33, 287–314. [CrossRef]

32. Tahiri, A.; Aure, M.R.; Kristensen, V.N. MicroRNA Networks in Breast Cancer Cells. Methods Mol. Biol. 2018, 1711, 55–81. [PubMed]

33. Kang, S.M.; Lee, H.J. MicroRNAs in human lung cancer. Exp. Biol. Med. 2014, 239, 1505–1513. [CrossRef] [PubMed]

34. Jeong, H.C. Clinical Aspect of MicroRNA in Lung Cancer. Tuber. Respir. Dis. 2014, 77, 60–64. [CrossRef]

35. Wang, Y.; Zhang, X.; Liu, L.; Li, H.; Yu, J.; Wang, C.; Ren, X. Clinical implication of microRNA in lung cancer. Cancer Biother. Radiopharm. 2013, 28, 261–267. [CrossRef]

36. Markou, A.; Sourvinou, I.; Vorkas, P.A.; Yousef, G.M.; Lianidou, E. Clinical evaluation of microRNA expression profiling in non small cell lung cancer. Lung Cancer 2013, 81, 388–396. [CrossRef]

37. Lee, J.H.; Voortman, J.; Dingemans, A.M.; Voeller, D.M.; Pham, T.; Wang, Y.; Giacone, G. MicroRNA expression and clinical outcome of small cell lung cancer. PLoS ONE 2011, 6, e21300. [CrossRef]

38. Di Leva, G.; Garofalo, M.; Croce, C.M. MicroRNAs in cancer. Annu. Rev. Pathol. 2014, 9, 287–314. [CrossRef]

39. Navickas, R.; Gal, D.; Laucevicius, A.; Taparauskaite, A.; Zdanyte, M.; Holvoet, P. Identifying circulating microRNAs as biomarkers of cardiovascular disease: A systematic review. Cardiovasc. Res. 2016, 111, 322–337. [CrossRef]

40. Dong, H.; Wang, C.; Lu, S.; Yu, C.; Huang, L.; Feng, W.; Xu, H.; Chen, X.; Zen, K.; Yan, Q.; et al. A panel of four decreased serum microRNAs as a novel biomarker for early Parkinson’s disease. Biomark. Biochem. Indic. Exp. Response Susceptibility Chem. 2016, 21, 129–137. [CrossRef]

41. Mencia, A.; Modamio-Hoybjørn, S.; Redshaw, N.; Morin, M.; Mayo-Merino, F.; Olavarrieta, L.; Aguirre, L.A.; del Castillo, I.; Steel, K.P.; Dalmay, T.; et al. Mutations in the seed region of human miR-96 are responsible for nonsyndromic progressive hearing loss. Nat. Genet. 2009, 41, 609–613. [CrossRef] [PubMed]

42. Hughes, A.E.; Bradley, D.T.; Campbell, M.; Lehner, J.; Dash, D.P.; Simpson, D.A.; Willoughby, C.E. Mutation altering the miR-184 seed region causes familial keratoconus with cataract. Am. J. Hum. Genet. 2011, 89, 628–633. [CrossRef] [PubMed]
43. De Pontual, L.; Yao, E.; Callier, P.; Faivre, L.; Drouin, V.; Cariou, S.; Van Haeringen, A.; Genevieve, D.; Goldenberg, A.; Oufadem, M.; et al. Germline deletion of the miR-17 approximately 92 cluster causes skeletal and growth defects in humans. Nat. Genet. 2011, 43, 1026–1030. [CrossRef] [PubMed]

44. Reh, T.A.; Hindges, R. MicroRNAs in Retinal Development. Annu Rev. Vis. Sci. 2018, 4, 25–44. [CrossRef]

45. Karali, M.; Banfi, S. Non-coding RNAs in retinal development and function. Hum. Genet. 2019, 138, 957–971. [CrossRef]

46. Zuzic, M.; Rojo Arias, J.E.; Wohl, S.G.; Busskamp, V. Retinal miRNA Functions in Health and Disease. Genes 2019, 10, 377. [CrossRef]

47. Akhtar, S.; Patnaik, S.R.; Kotapati Raghupathy, R.; Al-Mubrad, T.M.; Craft, J.A.; Shu, X. Histological Characterization of the Dicer1 Mutant Zebrafish Retina. J. Ophthalmol. 2015, 2015, 309510. [CrossRef]

48. La Torre, A.; Georgi, S.A.; Reh, T.A. Dicer is required for the transition from early to late progenitor state in the developing mouse retina. J. Neurosci. 2008, 28, 4878–4887. [CrossRef]

50. Damiani, D.; Alexander, J.J.; O’Rourke, J.R.; McManus, M.; Jadhav, A.P.; Cepko, C.L.; Hauswirth, W.W.; Harfe, B.D.; Strettoi, E. Dicer inactivation leads to progressive functional and structural degeneration of the mouse retina. J. Neurosci. 2008, 1125–1134. [CrossRef] [PubMed]

51. Georgi, S.A.; Reh, T.A. Dicer plays essential roles for retinal development by regulation of survival and differentiation. Invest. Ophthalmol. Vis. Sci. 2011, 52, 3008–3017. [CrossRef] [PubMed]

52. Hackler, L., Jr.; Wan, J.; Swaroop, A.; Qian, J.; Zack, D.J. MicroRNA profile of the developing mouse retina. J. Neurosci. 2008, 28, 4878–4887. [CrossRef]

54. Georgi, S.A.; Reh, T.A. Dicer is required for the transition from early to late progenitor state in the developing mouse retina. J. Neurosci. 2010, 30, 4048–4061. [CrossRef] [PubMed]

55. Liu, C.H.; Wang, Z.; Sun, Y.; Chen, J. Animal models of ocular angiogenesis: From development to pathologies. FASEB J. 2017, 31, 4665–4681. [CrossRef]

56. Iida, A.; Shinoe, T.; Baba, Y.; Mano, H.; Watanabe, S. Dicer plays essential roles for retinal development via Meis2 targeting. Proc. Natl. Acad. Sci. USA 2013, 110, E2362–E2370. [CrossRef]

57. Liu, C.H.; Wang, Z.; Sun, Y.; Chen, J. Animal models of ocular angiogenesis: From development to pathologies. FASEB J. 2017, 31, 4665–4681. [CrossRef]

58. Pinter, R.; Hindges, R. Perturbations of microRNA function in mouse dicer mutants produce retinal defects and lead to aberrant axon pathfinding at the optic chiasm. PLoS ONE 2010, 5, e10021. [CrossRef]

59. Liu, C.H.; Wang, Z.; Sun, Y.; Chen, J. Animal models of ocular angiogenesis: From development to pathologies. FASEB J. 2017, 31, 4665–4681. [CrossRef]

60. Arora, A.; Guturic-Fuchs, J.; Harwood, L.; Dellett, M.; Cogliati, T.; Simpson, D.A. Prediction of microRNAs affecting mRNA expression during retinal development. BMC Dev. Biol. 2010, 10, 1. [CrossRef]

61. Liu, C.H.; Wang, Z.; Sun, Y.; Chen, J. Animal models of ocular angiogenesis: From development to pathologies. FASEB J. 2017, 31, 4665–4681. [CrossRef]

62. Aiello, L.P.; Barmada, M.M.; Beeler, R.; Boulton, A.J.; Broman, K.O.; Cinti, S.; et al. The Comprehensive Retinal Degeneration Database (CRDD): A biomedical resource for retinal degeneration. Nat. Genet. 2011, 43, 1125–1134. [CrossRef]

63. Conte, I.; Carrella, S.; Avellino, M.; Caralli, M.; Marco-Ferreres, R.; Bovolenta, P.; Banfi, S. miR-204 is required for lens and retinal development via Meis2 targeting. Proc. Natl. Acad. Sci. USA 2010, 107, 15491–15496. [CrossRef] [PubMed]

64. Sanuki, R.; Onishi, A.; Koike, C.; Muramatsu, R.; Watanabe, S.; Muranishi, Y.; Irie, S.; Uneo, S.; Koyasu, T.; Matsui, R.; et al. miR-124a is required for hippocampal axogenesis and retinal cone survival through Lhx2 suppression. Nat. Neurosci. 2011, 14, 1125–1134. [CrossRef]

65. Karali, M.; Persico, M.; Mutarelli, M.; Carissimo, A.; Pizzo, M.; Singh Marwah, V.; Ambrosio, C.; Pinelli, M.; Carrella, D.; Ferrari, S.; et al. High-resolution analysis of the human retina miRNAome reveals isomiR variations and novel microRNAs. Nucleic Acids Res. 2016, 44, 1525–1540. [CrossRef]
66. Barbato, S.; Marrocco, E.; Intartaglia, D.; Pizzo, M.; Asteriti, S.; Naso, F.; Falanga, D.; Bhat, R.S.; Meola, N.; Carissimo, A.; et al. MiR-211 is essential for adult cone photoreceptor maintenance and visual function. *Sci. Rep.* 2017, 7, 17004. [CrossRef]

67. Landgraf, P.; Rusu, M.; Sheridan, R.; Sewer, A.; Iovino, N.; Aravin, A.; Pfeffer, S.; Rice, A.; Kamphorst, A.O.; Landthaler, M.; et al. A mammalian microRNA expression atlas based on small RNA library sequencing. *Cell 2007*, 129, 1401–1414. [CrossRef]

68. Arora, A.; McKay, G.J.; Simpson, D.A. Prediction and verification of miRNA expression in human and rat retinas. *Investig. Opthalmol. Vis. Sci.* 2007, 48, 3962–3967. [CrossRef]

69. Kapsimali, M.; Kloosterman, W.P.; de Bruijn, E.; Rosa, F.; Plasterk, R.H.; Wilson, S.W. MicroRNAs show a wide diversity of expression profiles in the developing and mature central nervous system. *Genome Biol. 2007*, 8, R173. [CrossRef]

70. Makarev, E.; Spence, J.R.; Del Rio-Tsonis, K.; Tsonis, P.A. Identification of microRNAs and other small RNAs from the adult newt eye. *Mol. Vis.* 2006, 12, 1386–1391.

71. Ryan, D.G.; Oliveira-Fernandes, M.; Lavker, R.M. MicroRNAs of the mammalian eye display distinct and overlapping tissue specificity. *Mol. Vis.* 2006, 12, 1175–1184. [PubMed]

72. Shen, J.; Yang, X.; Xie, B.; Chen, Y.; Swaim, M.; Hackett, S.F.; Campochiaro, P.A. MicroRNAs regulate ocular neovascularization. *Mol. Ther. J. Am. Soc. Gene Ther.* 2008, 16, 1208–1216. [CrossRef] [PubMed]

73. Wohl, S.G.; Reh, T.A. The microRNA expression profile of mouse Muller glia in vivo and in vitro. *Sci. Rep.* 2016, 6, 35423. [CrossRef] [PubMed]

74. Xu, S.; Wittmer, P.D.; Lumayag, S.; Kovacs, B.; Valle, D. MicroRNA (miRNA) transcriptome of mouse retina and identification of a sensory organ-specific miRNA cluster. *J. Biol. Chem.* 2007, 282, 25053–25066. [CrossRef]

75. Chu-Tan, J.A.; Rutar, M.; Saxena, K.; Aggio-Brice, R.; Essex, R.W.; Valter, K.; Jiao, H.; Fernando, N.; Woff, Y.; Madigan, M.C.; et al. MicroRNA-124 Dysregulation is Associated with Retinal Inflammation and Photoreceptor Death in the Degenerating Retina. *Investig. Opthalmol. Vis. Sci.* 2018, 59, 4094–4105. [CrossRef]

76. Qian, Y.; Song, J.; Ouyang, Y.; Han, Q.; Chen, W.; Zhao, X.; Xie, Y.; Chen, Y.; Yuan, W.; Fan, C. Advances in Roles of miR-132 in the Nervous System. *Front. Pharmacol.* 2017, 8, 770. [CrossRef]

77. Min, S.W.; Cho, S.H.; Zhou, Y.; Schroeder, S.; Haroutunian, V.; Seeley, W.W.; Huang, E.J.; Shen, Y.; Masliah, E.; Mukherjee, C.; et al. Acetylation of tau inhibits its degradation and contributes to tauopathy. *Neuron 2010*, 67, 953–966. [CrossRef]

78. Marler, K.J.; Suetterlin, P.; Dopplapudi, A.; Rubikaite, A.; Adnan, J.; Maiorano, N.A.; Lowe, A.S.; Thompson, I.D.; Puthania, M.; Bordey, A.; et al. BDNF promotes axon branching of retinal ganglion cells via miRNA-132 and p250GAP. *J. Neurosci. 2014*, 34, 969–979. [CrossRef]

79. Folkman, J. Angiogenesis: An organizing principle for drug discovery? *Nat. Rev. Drug Discov.* 2007, 6, 273–286. [CrossRef]

80. Sholley, M.M.; Ferguson, G.P.; Seibel, H.R.; Montour, J.L.; Wilson, J.D. Mechanisms of neovascularization. Vascular sprouting can occur without proliferation of endothelial cells. *Lab. Invest.* 1984, 51, 624–634.

81. Carmeliet, P. Angiogenesis in life, disease and medicine. *Nature 2005*, 438, 932–936. [CrossRef]

82. Apte, R.S.; Chen, D.S.; Ferrara, N. VEGF in Signaling and Disease: Beyond Discovery and Development. *Cell 2019*, 176, 1248–1264. [CrossRef]

83. Afzal, A.; Shaw, L.C.; Ljubimov, A.V.; Boulton, M.E.; Segal, M.S.; Grant, M.B. Retinal and choroidal microangiopathies: Therapeutic opportunities. *Microvasc. Res.* 2007, 74, 131–144. [CrossRef] [PubMed]

84. Otsuka, M.; Zheng, M.; Hayashi, M.; Lee, J.D.; Yoshino, O.; Lin, S.; Han, J. Impaired microRNA processing causes corpus luteum insufficiency and infertility in mice. *J. Clin. Invest. 2008*, 118, 1944–1954. [CrossRef] [PubMed]

85. Yang, W.J.; Yang, D.D.; Na, S.; Sandusky, G.E.; Zhang, Q.; Zhao, G. Dicer is required for embryonic angiogenesis during mouse development. *J. Biol. Chem.* 2005, 280, 9330–9335. [CrossRef] [PubMed]

86. Kuehbacher, A.; Urbich, C.; Zeiher, A.M.; Dimmeler, S. Role of Dicer and Drosha for endothelial microRNA expression and angiogenesis. *Circ. Res.* 2007, 101, 59–68. [CrossRef] [PubMed]

87. Suarez, Y.; Fernandez-Hernando, C.; Yu, J.; Gerber, S.A.; Harrison, K.D.; Pober, J.S.; Iruela-Arispe, M.L.; Merkenschlager, M.; Sessa, W.C. Dicer-dependent endothelial microRNAs are necessary for postnatal angiogenesis. *Proc. Natl. Acad. Sci. USA 2008*, 105, 14082–14087. [CrossRef]
88. Poliseno, L.; Turolli, A.; Mariani, L.; Evangelista, M.; Citti, L.; Woods, K.; Mercatanti, A.; Hammond, S.; Rainaldi, G. MicroRNAs modulate the angiogenic properties of HUVECs. Blood 2006, 108, 3068–3071. [CrossRef]
89. Wang, S.; Olson, E.N. AngiomiRs—Key regulators of angiogenesis. Curr. Opin. Genet. Dev. 2009, 19, 205–211. [CrossRef]
90. Wakioka, T.; Sasaki, A.; Kato, R.; Shouda, T.; Matsumoto, A.; Miyoshi, K.; Tsunoeoka, M.; Komiya, S.; Baron, R.; Yoshimura, A. SpreD is a Sprouty-related suppressor of Ras signalling. Nature 2001, 412, 647–651. [CrossRef]
91. Wang, S.; Aurora, A.B.; Johnson, B.A.; Qi, X.; McAnally, J.; Hill, J.A.; Richardson, J.A.; Bassel-Duby, R.; Olson, E.N. The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis. Dev. Cell 2008, 15, 261–271. [CrossRef] [PubMed]
92. Fish, J.E.; Santoro, M.M.; Morton, S.U.; Yu, S.; Yeh, R.F.; Wythe, J.D.; Ivey, K.N.; Bruneau, B.G.; Stainier, D.Y.; Srivastava, D. miR-126 regulates angiogenic signaling and vascular integrity. Dev. Cell 2008, 15, 272–284. [CrossRef]
93. Fasanaro, P.; D’Alessandra, Y.; Di Stefano, V.; Melchionna, R.; Romani, S.; Pompilio, G.; Capogrossi, M.C.; Martelli, F. MicroRNA-210 modulates endothelial cell response to hypoxia and inhibits the receptor tyrosine kinase ligand Ephrin-A3. J. Biol. Chem. 2008, 283, 15878–15883. [CrossRef] [PubMed]
94. Fasanaro, P.; Greco, S.; Lorenzi, M.; Pescatori, M.; Brioschi, M.; Kulshreshtha, R.; Banfi, C.; Stubbs, A.; Calin, G.A.; Ivan, M.; et al. An integrated approach for experimental target identification of hypoxia-induced miR-210. J. Biol. Chem. 2009, 284, 35134–35143. [CrossRef] [PubMed]
95. Miao, H.; Wang, B. EphA receptor signaling—Complexity and emerging themes. Semin. Cell Dev. Biol. 2012, 23, 16–25. [CrossRef]
96. Larson, J.; Schomberg, S.; Schroeder, W.; Carpenter, T.C. Endothelial EphA receptor stimulation increases lung vascular permeability. Am. J. Physiol. Lung Cell Mol. Physiol. 2008, 295, L431-9. [CrossRef] [PubMed]
97. Zhou, Q.; Gallagher, R.; Ufret-Vincenty, R.; Li, X.; Olson, E.N.; Wang, S. Regulation of angiogenesis and choroidal neovascularization by members of microRNA-23~27~24 clusters. Proc. Natl. Acad. Sci. USA 2011, 108, 8287–8292. [CrossRef]
98. Fiedler, J.; Jazbutyte, V.; Kirchmaier, B.C.; Gupta, S.K.; Lorenzen, J.; Hartmann, D.; Galuppo, P.; Nuebmi, S.; Pena, J.T.; Sohn-Lee, C.; et al. MicroRNA-24 regulates vascularity after myocardial infarction. Circulation 2011, 124, 720–730. [CrossRef]
99. Oikawa, S.; Wada, S.; Lee, M.; Maeda, S.; Akimoto, T. Role of endothelial microRNA-23 clusters in angiogenesis in vivo. Am. J. Physiol. Heart Circ. Physiol. 2018, 315, H838–H846. [CrossRef]
100. Li, J.; Zhao, Y.; Lu, Y.; Ritchie, W.; Grau, G.; Vadas, M.A.; Gamble, J.R. The Poly-cistronic miR-23-27-24 Complexes Target Endothelial Cell Junctions: Differential Functional and Molecular Effects of miR-23a and miR-23b. Mol. Ther. Nucleic Acids 2016, 5, e354. [CrossRef]
101. Bonauer, A.; Carmona, G.; Iwasaki, M.; Koyanagi, M.; Fischer, A.; Burchfield, J.; Fox, H.; Doebel, C.; Ohtani, K.; et al. MicroRNA-92a controls angiogenesis and functional recovery of ischemic tissues in mice. Science 2009, 324, 1710–1713. [CrossRef]
102. Chamorro-Jorganes, A.; Lee, M.Y.; Araldi, E.; Landskroner-Eiger, S.; Fernandez-Fuertes, M.; Sahraei, M.; Quiles Del Rey, M.; van Solingen, C.; Yu, J.; Fernandez-Hernando, C.; et al. VEGF-Induced Expression of the miR-17-92 Cluster in Endothelial Cells Is Mediated by ERK1 Activation and Regulates Angiogenesis. Circ. Res. 2016, 118, 38–47. [CrossRef] [PubMed]
103. Landskroner-Eiger, S.; Qiu, C.; Perrotta, P.; Siragusa, M.; Lee, M.Y.; Ulrich, V.; Luciano, A.; Zhuang, Z.W.; Corti, F.; Simons, M.; et al. Endothelial miR-17 approximately 92 cluster negatively regulates arteriogenesis via miRNA-19 repression of WNT signaling. Proc. Natl. Acad. Sci. USA 2015, 112, 12812–12817. [CrossRef] [PubMed]
104. Hua, Z.; Lv, Q.; Ye, W.; Xiong, C.K.; Cai, G.; Gu, D.; Ji, Y.; Zhao, C.; Wang, J.; Yang, B.B.; et al. Mrna-directed regulation of VEGF and other angiogenic factors under hypoxia. PLoS ONE 2006, 1, e116. [CrossRef] [PubMed]
105. Qin, L.L.; An, M.X.; Liu, Y.L.; Xu, H.C.; Lu, Z.Q. MicroRNA-126: A promising novel biomarker in peripheral blood for diabetic retinopathy. Int. J. Ophthalmol. 2017, 10, 530–534. [PubMed]
106. Barutta, F.; Bruno, G.; Matulio, G.; Chaturvedi, N.; Grimaldi, S.; Schalkwijk, C.; Stehouwer, C.D.; Fuller, J.H.; Gruden, G. MicroRNA-126 and micro-macrovacular complications of type 1 diabetes in the EURODIAB Prospective Complications Study. Acta Diabetol. 2017, 54, 133–139. [CrossRef]
107. Rezk, N.A.; Sabbah, N.A.; Saad, M.S. Role of MicroRNA 126 in screening, diagnosis, and prognosis of diabetic patients in Egypt. *IUBMB Life* 2016, 68, 452–458. [CrossRef]

108. Kuhnert, F.; Mancuso, M.R.; Hampton, J.; Stankunas, K.; Asano, T.; Chen, C.Z.; Kuo, C.J. Attribution of vascular phenotypes of the murine Egfl7 locus to the microRNA miR-126. *Development* 2008, 135, 3989–3993. [CrossRef]

109. Mazzeo, A.; Beltramo, E.; Lopatina, T.; Gai, C.; Trento, M.; Porta, M. Molecular and functional characterization of circulating extracellular vesicles from diabetic patients with and without retinopathy and healthy subjects. *Exp. Eye Res.* 2018, 176, 77–87. [CrossRef]

110. Yang, T.T.; Song, S.J.; Xue, H.B.; Shi, D.F.; Liu, C.M.; Liu, H. Regulatory T cells in the pathogenesis of type 2 diabetes mellitus retinopathy by miR-155. *Eur. Rev. Med. Pharmacol. Sci.* 2015, 19, 2010–2015.

111. Li, E.H.; Huang, Q.Z.; Li, G.C.; Xiang, Z.Y.; Zhang, X. Effects of miRNA-200b on the development of diabetic retinopathy by targeting VEGFA gene. *Biosci. Rep.* 2017, 37. [CrossRef] [PubMed]

112. Chan, Y.C.; Khanna, S.; Roy, S.; Sen, C.K. miR-200b targets Ets-1 and is down-regulated by hypoxia to induce angiogenic response of endothelial cells. *J. Biol. Chem.* 2011, 286, 2047–2056. [CrossRef] [PubMed]

113. Liu, H.N.; Li, X.; Wu, N.; Tong, M.M.; Chen, S.; Zhu, S.S.; Qian, W.; Chen, X.L. Serum microRNA-221 as a biomarker for diabetic retinopathy in patients associated with type 2 diabetes. *Int J. Ophthalmol.* 2018, 11, 1889–1894. [PubMed]

114. Fiorentino, L.; Cavalera, M.; Mavilio, M.; Conserva, F.; Menghini, R.; Gesualdo, L.; Federici, M. Regulation of TIMP3 in diabetic nephropathy: A role for microRNAs. *Acta Diabetol.* 2013, 50, 965–969. [CrossRef] [PubMed]

115. Costantino, S.; Paneni, F.; Luscher, T.F.; Cosentino, F. MicroRNA profiling unveils hyperglycaemic memory in the diabetic heart. *Eur. Heart J.* 2016, 37, 572–576. [CrossRef] [PubMed]

116. Lightell, D.J., Jr.; Moss, S.C.; Woods, T.C. Upregulation of miR-221 and -222 in response to increased extracellular signal-regulated kinases 1 activity exacerbates neointimal hyperplasia in diabetes mellitus. *Atherosclerosis* 2018, 269, 71–78. [CrossRef] [PubMed]

117. Zampetaki, A.; Willeit, P.; Burr, S.; Yin, X.; Langley, S.R.; Kiechl, S.; Klein, R.; Rossing, P.; Chaturvedi, N.; Mayr, M. Angiogenic microRNAs Linked to Incidence and Progression of Diabetic Retinopathy in Type 1 Diabetes. *Diabetes* 2016, 65, 216–227. [CrossRef]

118. Ertekin, S.; Yildirim, O.; Dinc, E.; Ayaz, L.; Fidanci, S.B.; Tamer, L. Evaluation of circulating miRNAs in wet age-related macular degeneration. *Mol. Vis.* 2014, 20, 1057–1066.

119. Szemraj, M.; Bielecka-Kowalska, A.; Oszajca, K.; Krzewska, M.; Gos, R.; Jurowski, P.; Kowalski, M.; Szemraj, J. Serum MicroRNAs as Potential Biomarkers of AMD. *Med. Sci. Monit.* 2015, 21, 2734–2742. [CrossRef]

120. Chen, Z.; Lai, T.C.; Jan, Y.H.; Lin, F.M.; Wang, W.C.; Xiao, H.; Wang, Y.T.; Sun, W.; Cui, X.; Li, Y.S.; et al. Hypoxia-responsive miRNAs target argonaute 1 to promote angiogenesis. *J. Clin. Invest.* 2013, 123, 1057–1067. [CrossRef]

121. Romano, G.L.; Platania, C.B.M.; Drago, F.; Salomone, S.; Ragusa, M.; Barbagallo, C.; Di Pietro, C.; Purrello, M.; Reibaldi, M.; Avitabile, T.; et al. Retinal and Circulating miRNAs in Age-Related Macular Degeneration: An In vivo Animal and Human Study. *Front. Pharmacol.* 2017, 8, 168. [CrossRef] [PubMed]

122. Zhou, Q.; Anderson, C.; Hanus, J.; Zhao, F.; Ma, J.; Yoshimura, A.; Wang, S. Strand and Cell Type-specific Function of microRNA-126 in Angiogenesis. *Mol. Ther. J. Am. Soc. Gene Ther.* 2016, 24, 1823–1835. [CrossRef] [PubMed]

123. Wang, L.; Lee, A.Y.; Wigg, J.P.; Peshavarieha, H.; Liu, P.; Zhang, H. miR-126 Regulation of Angiogenesis in Age-Related Macular Degeneration in CNV Mouse Model. *Int. J. Mol. Sci.* 2016, 17, 895. [CrossRef] [PubMed]

124. Lukiw, W.J.; Surjyadipita, B.; Dua, P.; Alexandrov, P.N. Common micro RNAs (miRNAs) target complement factor H (CFH) regulation in Alzheimer’s disease (AD) and in age-related macular degeneration (AMD). *Int. J. Biochem. Mol. Biol.* 2012, 3, 105–116.

125. Kutty, R.K.; Nagineni, C.N.; Samuel, W.; Vijayasarathy, C.; Jaworski, C.; Duncan, T.; Cameron, J.E.; Flemington, E.K.; Hooks, J.J.; Redmond, T.M. Differential regulation of microRNA-146a and microRNA-146b-5p in human retinal pigment epithelial cells by interleukin-1beta, tumor necrosis factor-alpha, and interferon-gamma. *Mol. Vis.* 2013, 19, 737–750.
126. Sabatell, C.; Malvaux, L.; Bovy, N.; Deroanne, C.; Lambert, V.; Gonzalez, M.L.; Colige, A.; Rakic, J.M.; Noel, A.; Martial, J.A.; et al. MicroRNA-21 exhibits antiangiogenic function by targeting RhoB expression in endothelial cells. *PLoS ONE* **2011**, *6*, e16979. [CrossRef]

127. Metin, T.; Dinc, E.; Gorur, A.; Erdogan, S.; Ertel, K.; Sari, A.A.; Tamer, L.; Celik, Y. Evaluation of the plasma microRNA levels in stage 3 premature retinopathy with plus disease: Preliminary study. *Eye* **2018**, *32*, 415–420. [CrossRef]

128. Zhao, R.; Qian, L.; Jiang, L. Identification of retinopathy of prematurity related miRNAs in hyperoxia-induced neonatal rats by deep sequencing. *Int. J. Mol. Sci.* **2014**, *16*, 840–856. [CrossRef]

129. Ye, J.; Wu, X.; Wu, D.; Wu, P.; Ni, C.; Zhang, Z.; Chen, Z.; Qiu, F.; Xu, J.; Huang, J. miRNA-27b targets vascular endothelial growth factor C to inhibit tumor progression and angiogenesis in colorectal cancer. *PLoS ONE* **2013**, *8*, e60687. [CrossRef]

130. Liu, H.T.; Xing, A.Y.; Chen, X.; Ma, R.R.; Wang, Y.W.; Shi, D.B.; Zhang, H.; Li, P.; Chen, H.F.; Li, Y.H.; et al. MicroRNA-27b, microRNA-101 and microRNA-128 inhibit angiogenesis by down-regulating vascular endothelial growth factor C expression in gastric cancers. *Oncotarget* **2015**, *6*, 37458–37470. [CrossRef]

131. Van Mil, A.; Grundmann, S.; Goumans, M.J.; Lei, Z.; Oerlemans, M.I.; Jakubi, S.; Doevendans, P.A.; Sluijter, J.P. MicroRNA-214 inhibits angiogenesis by targeting Quaking and reducing angiogenic growth factor release. *Cardiovasc. Res.* **2012**, *93*, 655–665. [CrossRef] [PubMed]

132. Morello, C.M. Etiology and natural history of diabetic retinopathy: An overview. *Am. J. Health-Syst. Pharm.* **2007**, *64* (Suppl. 12), 53–57. [CrossRef] [PubMed]

133. Paulus, Y.M.; Gariano, R.F. Diabetic retinopathy: A growing concern in an aging population. *Geriatrics* **2009**, *64*, 16–20. [PubMed]

134. De La Cruz, J.P.; Gonzalez-Correa, J.A.; Guerrero, A.; de la Cuesta, F.S. Pharmacological approach to diabetic retinopathy. *Diabetes Metab. Res. Rev.* **2004**, *20*, 91–113. [CrossRef]

135. Fong, D.S.; Aiello, L.P.; Ferris, F.L., 3rd; Klein, R. Diabetic retinopathy. *Diabetes Care* **2004**, *27*, 2540–2553. [CrossRef]

136. Stitt, A.W.; Curtis, T.M.; Chen, M.; Medina, R.J.; McKay, G.J.; Jenkins, A.; Gardiner, T.A.; Lyons, T.J.; Hammes, H.P.; Simo, R.; et al. The progress in understanding and treatment of diabetic retinopathy. *Prog. Retin. Eye Res.* **2016**, *51*, 156–186. [CrossRef]

137. Qazi, Y.; Maddula, S.; Ambati, B.K. Mediators of ocular angiogenesis. *J. Genet.* **2009**, *88*, 495–515. [CrossRef]

138. Sivaprasad, S.; Gupta, B.; Crosby-Nwaobi, R.; Evans, J. Prevalence of diabetic retinopathy in various ethnic groups: A worldwide perspective. *Surv. Ophthalmol.* **2012**, *57*, 347–370. [CrossRef]

139. Jimenez-Lucena, R.; Camargo, A.; Alcala-Diaz, J.F.; Romero-Baldonado, C.; Luque, R.M.; Colige, A.; Rakic, J.M.; Noel, A.; Martial, J.A.; et al. MicroRNA-214 inhibits angiogenesis by targeting angiogenic growth factors. *Exp. Mol. Pathol.* **2012**, *93*, 840–856. [CrossRef]

140. Jimenez-Lucena, R.; Rangel-Zuniga, O.A.; Alcala-Diaz, J.F.; Lopez-Moreno, J.; Roncero-Ramos, I.; Camargo, A.; Alcala-Diaz, J.F.; Romero-Baldonado, C.; Luque, R.M.; Colige, A.; et al. Circulating microRNAs as Predictive Biomarkers of Type 2 Diabetes Mellitus Development in Coronary Heart Disease Patients from the CORDIOPREV Study. *Mol. Ther. Nucleic Acids* **2018**, *6*, 840–856. [CrossRef]

141. Barutta, F.; Bellini, S.; Mastrocola, R.; Bruno, G.; Gruden, G. MicroRNA and Microvascular Complications of Diabetes. *Int. J. Endocrinol.* **2018**, *2018*, 6890501. [CrossRef] [PubMed]

142. Ye, P.; Liu, J.; He, F.; Xu, W.; Yao, K. Hypoxia-induced deregulation of miR-126 and its regulative effect on VEGF and MMP-9 expression. *Int. J. Med. Sci.* **2014**, *11*, 17–23. [CrossRef] [PubMed]

143. Bai, Y.; Bai, X.; Wang, Z.; Zhang, X.; Ruan, C.; Miao, J. MicroRNA-126 inhibits ischemia-induced retinal neovascularization via regulating angiogenic growth factors. *Exp. Mol. Pathol.* **2011**, *91*, 471–477. [CrossRef] [PubMed]

144. Wang, Y.; Yan, H. MicroRNA-126 contributes to Niaspan treatment induced vascular restoration after diabetic retinopathy. *Sci. Rep.* **2016**, *6*, 26909. [CrossRef] [PubMed]

145. McArthur, K.; Feng, B.; Wu, Y.; Chen, S.; Chakrabarti, S. MicroRNA-200b regulates vascular endothelial growth factor-mediated alterations in diabetic retinopathy. *Diabetes* **2011**, *60*, 1314–1323. [CrossRef]
151. Gomaa, A.R.; Elsayed, E.T.; Moftah, R.F. MicroRNA-200b Expression in the Vitreous Humor of Patients with Diabetic Retinopathy. *Int. J. Mol. Sci.* 2020, 21, 19 of 21. [CrossRef]

152. Murray, A.R.; Chen, Q.; Takahashi, Y.; Zhou, K.K.; Park, K.; Ma, J.X. MicroRNA-200b downregulates oxidation resistance 1 (Oxr1) expression in the retina of type 1 diabetes model. *Mol. Ther. Nucleic Acids* 2019, 20, 1173–1184. [CrossRef]

153. Friedman, E. The pathogenesis of age-related macular degeneration. *Am. J. Ophthalmol.* 2008, 146, 348–349. [CrossRef]

154. Jager, R.D.; Mieler, W.F.; Miller, J.W. Age-related macular degeneration. *N. Engl. J. Med.* 2008, 358, 2606–2617. [CrossRef]

155. Coleman, H.R.; Chan, C.C.; Ferris, F.L., 3rd; Chew, E.Y. Age-related macular degeneration. *Clin. Perinatal.* 2012, 39, 297–310. [CrossRef]

156. Desjarlais, M.; Rivera, J.C.; Lahaie, I.; Cagnone, G.; Wirt, M.; Omri, S.; Chemtob, S. MicroRNA expression profile in retina and choroid in oxygen-induced retinopathy model. *Mol. Vis.* 2001, 7, 133–140. [CrossRef] [PubMed]

157. Tsai, J.; Wang, Z.; Sun, Y.; SanGiovanni, J.P.; Chen, J. Retinal expression of small non-coding RNAs in a murine model of proliferative retinopathy. *Sci. Rep.* 2016, 6, 33947. [CrossRef]

158. Liu, C.H.; Wang, Z.; Huang, S.; Sun, Y.; Chen, J. MicroRNA-145 Regulates Pathological Retinal Angiogenesis by Suppression of TMOD3. *Mol. Ther. Nucleic Acids* 2019, 16, 335–347. [CrossRef]

159. Liu, C.H.; Sun, Y.; Li, J.; Gong, Y.; Tian, K.T.; Evans, L.P.; Mori, P.C.; Fredrick, T.W.; Saba, N.J.; Chen, J. Endothelial microRNA-150 is an intrinsic suppressor of pathologic ocular neovascularization. *Proc. Natl. Acad. Sci. USA* 2015, 112, 12163–12168. [CrossRef]

160. Yan, L.; Lee, S.; Lazzaro, D.R.; Aranda, J.; Grant, M.B.; Chaqour, B. Single and Compound Knock-outs of MicroRNA (miRNA)-155 and Its Angiogenic Gene Target CCN1 in Mice Alter Vascular and Neovascular Growth in the Retina via Resident Microglia. *J. Biol. Chem.* 2015, 290, 23264–23281. [CrossRef]
171. Penn, J.S.; Tolman, B.L.; Lowery, L.A. Variable oxygen exposure causes preretal neovascularization in the newborn rat. *Investig. Ophthalmol. Vis. Sci.* 1993, 34, 576–585.

172. Smith, L.E.; Wesolowski, E.; McLellan, A.; Kostyk, S.K.; D’Amato, R.; Sullivan, R.; D’Amore, P.A. Oxygen-induced retinopathy in the mouse. *Investig. Ophthalmol. Vis. Sci.* 1994, 35, 101–111.

173. Takahashi, T.; Nakamura, T.; Hayashi, A.; Kamei, M.; Nakabayashi, M.; Okada, A.A.; Tomita, N.; Kaneda, Y.; Tano, Y. Inhibition of experimental choroidal neovascularization by overexpression of tissue inhibitor of metalloproteinases-3 in retinal pigment epithelium cells. *Am. J. Ophthalmol.* 2000, 130, 774–781. [CrossRef]

174. Yanagi, Y.; Tamaki, Y.; Obata, R.; Muranaka, K.; Homma, N.; Matsuoka, H.; Mano, H. Subconjunctival administration of bucillamine suppresses choroidal neovascularization in rat. *Investig. Ophthalmol. Vis. Sci.* 2002, 43, 3495–3499.

175. Kwak, N.; Okamoto, N.; Wood, J.M.; Campochiaro, P.A. VEGF is major stimulator in model of choroidal neovascularization. *Investig. Ophthalmol. Vis. Sci.* 2000, 41, 3158–3164.

176. Aiello, L.P.; Pierce, E.A.; Foley, E.D.; Takagi, H.; Chen, H.; Riddle, L.; Ferrara, N.; King, G.L.; Smith, L.E. Suppression of retinal neovascularization in vivo by inhibition of vascular endothelial growth factor (VEGF) using soluble VEGF-receptor chimeric proteins. *Proc. Natl. Acad. Sci. USA* 1995, 92, 10457–10461. [CrossRef]

177. Zambaraki, H.J.; Nakazawa, T.; Connolly, E.; Lane, A.M.; Mallemadugu, S.; Kaplan, M.; Michaud, N.; Hafezi-Moghadam, A.; Gragoudas, E.S.; Miller, J.W. Dose-dependent effect of pitavastatin on VEGF and angiogenesis in a mouse model of choroidal neovascularization. *Investig. Ophthalmol. Vis. Sci.* 2006, 47, 2623–2631. [CrossRef]

178. Zhao, F.; Anderson, C.; Karnes, S.; Zhou, Q.; Ma, J.; Jin, Z.G.; Bhattacharjee, P.S.; Wang, S. Expression, regulation and function of miR-126 in the mouse choroid vasculature. *Exp. Eye Res.* 2018, 170, 169–176. [CrossRef]

179. Bai, X.; Luo, J.; Zhang, X.; Han, J.; Wang, Z.; Miao, J.; Bai, Y. MicroRNA-126 Reduces Blood-Retina Barrier Breakdown via the Regulation of VCAM-1 and BCL2L11 in Ischemic Retinopathy. *Ophthalmic Res.* 2017, 57, 173–185. [CrossRef]

180. Hartmann, D.; Thum, T. MicroRNAs and vascular (dys)function. *Vasc. Pharmacol.* 2011, 55, 92–105. [CrossRef]

181. Westenskow, P.D.; Kurihara, T.; Aguilar, E.; Scheppke, L.; Marchetti, V.; Michael, L.P.; Anand, S.; Nagy, A.; et al. Ras pathway inhibition prevents neovascularization by repressing endothelial cell sprouting. *J. Clin. Invest.* 2013, 123, 4900–4908. [CrossRef] [PubMed]

182. Anand, S.; Majeti, B.K.; Acevedo, L.M.; Murphy, E.A.; Mukthavaram, R.; Scheppke, L.; Huang, M.; Shields, D.J.; Lindquist, J.N.; Lapinski, P.E.; et al. MicroRNA-132-mediated loss of p120RasGAP activates the endothelium to facilitate pathological angiogenesis. *Nat. Med.* 2010, 16, 909–914. [CrossRef] [PubMed]

183. Anand, S.; Cheresh, D.A. MicroRNA-mediated regulation of the angiogenic switch. *Curr. Opin. Hematol.* 2011, 18, 171–176. [CrossRef] [PubMed]

184. Mulik, S.; Xu, J.; Reddy, P.B.; Rajasagi, N.K.; Gimenez, F.; Sharma, S.; Lu, P.Y.; Rouse, B.T. Role of miR-132 in angiogenesis after ocular infection with herpes simplex virus. *Am. J. Pathol.* 2012, 181, 525–534. [CrossRef]

185. Kent, O.A.; McCall, M.N.; Cornish, T.C.; Halushka, M.K. Lessons from miR-143/145: The importance of cell-type localization of miRNAs. *Nucleic Acids Res.* 2014, 42, 7528–7538. [CrossRef]

186. Dimitrova, N.; Gocheva, V.; Bhutkar, A.; Resnick, R.; Jong, R.M.; Miller, K.M.; Bendor, J.; Jacks, T. Stromal Expression of miR-143/145 Promotes Neoangiogenesis in Lung Cancer Development. *Cancer Discov.* 2016, 6, 188–201. [CrossRef]

187. Xin, M.; Small, E.M.; Sutherland, L.B.; Qi, X.; McAnally, J.; Plato, C.F.; Richardson, J.A.; Bassel-Duby, R.; Olson, E.N. MicroRNAs miR-143 and miR-145 modulate cytoskeletal dynamics and responsiveness of smooth muscle cells to injury. *Genes Dev.* 2009, 23, 2166–2178. [CrossRef]

188. Hui, Y.; Yin, Y. MicroRNA-145 attenuates high glucose-induced oxidative stress and inflammation in retinal endothelial cells through regulating TLR4/NF-kappaB signaling. *Life Sci.* 2018, 207, 212–218. [CrossRef]

189. Li, X.; Zhao, F.; Xin, M.; Li, G.; Li, G.; Zhou, Q.; He, Y.; Yu, B.; Olson, E.; et al. Regulation of intraocular pressure by microRNA cluster miR-143/145. *Sci. Rep.* 2017, 7, 915. [CrossRef]

190. Zhang, J.; Cui, X.; Xu, H. Downregulation of miR-145-5p elevates retinal ganglion cell survival to delay diabetic retinopathy progress by targeting FGF5. *Biocyt. Biotechnol. Biochem.* 2019, 83, 1655–1662. [CrossRef]

191. Alexandrov, P.N.; Dua, P.; Lukiw, W.J. Up-Regulation of miRNA-146a in Progressive, Age-Related Inflammatory Neurodegenerative Disorders of the Human CNS. *Front. Neurol.* 2014, 5, 181. [CrossRef] [PubMed]
Kowluru, R.A.; Koppolu, P.; Chakrabarti, S.; Chen, S. Diabetes-induced activation of nuclear transcriptional factor in the retina, and its inhibition by antioxidants. Free Radic. Res. 2003, 37, 1169–1180. [CrossRef] [PubMed]

Cowan, C.; Muraleedharan, C.K.; O’Donnell, J.J., 3rd; Singh, P.K.; Lum, H.; Kumar, A.; Xu, S. MicroRNA-146 inhibits thrombin-induced NF-kappaB activation and subsequent inflammatory responses in human retinal endothelial cells. Investig. Ophthalmol. Vis. Sci. 2014, 55, 4944–4951. [CrossRef] [PubMed]

Ye, E.A.; Steinle, J.J. miR-146a Attenuates Inflammatory Pathways Mediated by TLR4/NF-kappaB and TNFalpha to Protect Primary Human Retinal Microvascular Endothelial Cells Grown in High Glucose. Mediators Inflamm. 2016, 2016, 3958453. [CrossRef]

Wang, Q.; Bozack, S.N.; Yan, Y.; Boulton, M.E.; Grant, M.B.; Busik, J.V. Regulation of retinal inflammation by rhythmic expression of MiR-146a in diabetic retina. Investig. Ophthalmol. Vis. Sci. 2014, 55, 3986–3994. [CrossRef]

Yan, Y.; Salazar, T.E.; Dominguez, J.M., 2nd; Nguyen, D.V.; Li Calzi, S.; Bhatwadekar, A.D.; Qi, X.; Busik, J.V.; Boulton, M.E.; Grant, M.B. Dicer expression exhibits a tissue-specific diurnal pattern that is lost during aging and in diabetes. PLoS ONE 2013, 8, e80029. [CrossRef]

Chen, S.; Feng, B.; Thomas, A.A.; Chakrabarti, S. miR-146a regulates glucose induced upregulation of inflammatory cytokines extracellular matrix proteins in the retina and kidney in diabetes. PLoS ONE 2017, 12, e0173918. [CrossRef]

Zhuang, P.; Muraleedharan, C.K.; Xu, S. Intraocular Delivery of miR-146 Inhibits Diabetes-Induced Retinal Functional Defects in Diabetic Rat Model. Invest. Ophthalmol. Vis. Sci. 2017, 58, 1646–1655. [CrossRef]

Bender, T.P.; Kremer, C.S.; Kraus, M.; Bach, T.; Rajewsky, K. Critical functions for c-Myb at three checkpoints during thymocyte development. Nat. Immunol. 2004, 5, 721–729. [CrossRef]

Thomas, M.D.; Kremer, C.S.; Ravichandran, K.S.; Rajewsky, K.; Bender, T.P. c-Myb is critical for B cell development and maintenance of follicular B cells. Immunity 2005, 23, 275–286. [CrossRef]

Xiao, C.; Calado, D.P.; Galler, G.; Thai, T.H.; Patterson, H.C.; Wang, J.; Rajewsky, N.; Bender, T.P.; Rajewsky, K. MiR-150 controls B cell differentiation by targeting the transcription factor c-Myb. Cell 2007, 131, 146–159. [CrossRef] [PubMed]

Li, J.; Zhang, Y.; Liu, Y.; Dai, X.; Li, W.; Cai, X.; Yin, Y.; Wang, Q.; Xue, Y.; Wang, C.; et al. Microvesicle-mediated transfer of microRNA-150 from monocytes to endothelial cells promotes angiogenesis. J. Biol. Chem. 2013, 288, 23586–23596. [CrossRef] [PubMed]

Zhang, Y.; Liu, D.; Chen, X.; Li, J.; Li; L.; Bian, Z.; Sun, F.; Lu, J.; Yin, Y.; Cai, X.; et al. Secreted monocytic miR-150 enhances targeted endothelial cell migration. Mol. Cell 2010, 39, 133–144. [CrossRef] [PubMed]

Narasaraju, T.; Shukla, D.; More, S.; Huang, C.; Zhang, L.; Xiao, X.; Liu, L. Role of microRNA-150 and glycoprotein nonmetastatic melanoma protein B in angiogenesis during hyperoxia-induced neonatal lung injury. Am. J. Respir. Cell Mol. Biol. 2015, 52, 253–261. [CrossRef] [PubMed]

Pilakka-Kanthikeel, S.; Raymond, A.; Afturi, V.S.; Sagar, V.; Saxena, S.K.; Diaz, P.; Chevelon, S.; Concepcion, M.; Nair, M. Sterile alpha motif and histidine/aspartic acid domain-containing protein 1 (SAMHD1)-facilitated HIV restriction in astrocytes is regulated by miRNA-181a. J. Neuroinflamm. 2015, 12, 66. [CrossRef] [PubMed]

O’Connell, R.M.; Kahn, D.; Gibson, W.S.; Round, J.L.; Scholz, R.L.; Chaudhuri, A.A.; Kahn, M.E.; Rao, D.S.; Baltimore, D. MicroRNA-155 promotes autoimmune inflammation by enhancing inflammatory T cell development. Immunity 2010, 33, 607–619. [CrossRef]

Krichevsky, A.M.; Gabriely, G. miR-21: A small multi-faceted RNA. J. Cell Mol. Med. 2009, 13, 39–53. [CrossRef]

Thum, T.; Gross, C.; Fiedler, J.; Fischer, T.; Kissler, S.; Bussen, M.; Galuppo, P.; Just, S.; Rottbauer, W.; Frantz, S.; et al. MicroRNA-21 contributes to myocardial disease by stimulating MAP kinase signalling in fibroblasts. Nature 2008, 456, 980–984. [CrossRef]

Chen, Q.; Qiu, F.; Zhou, K.; Matlock, H.G.; Takahashi, Y.; Rajala, R.V.S.; Yang, Y.; Moran, E.; Ma, J.X. Pathogenic Role of microRNA-21 in Diabetic Retinopathy Through Downregulation of PPARalpha. Diabetes 2017, 66, 1671–1682. [CrossRef]