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Micro-RNAs and Their Roles in Eye Disorders

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Key Words
Eye disorders · Micro-RNA · Regulatory mechanism

Abstract
Micro-RNAs (miRNAs) are members of the family of noncoding RNA molecules that regulate gene expression by translational repression and mRNA degradation. Initial identification of miRNAs revealed them only as developmental regulators; later, their radiated roles in various cellular processes have been established. They regulate several pathways, including developmental timing, hematopoiesis, organogenesis, apoptosis, cell differentiation and proliferation. Their roles in eye disorders are being explored by biologists around the world. Eye physiology requires the perfect orchestration of all the regulatory networks; any defect in any of the networks leads to eye disorders. The dysregulation of miRNA expression has been reported in many eye disorders, which paves the way for new therapeutics. This review summarizes the biogenesis of miRNAs and their role in eye disorders. miRNA studies also have implications for the understanding of various complex metabolic pathways leading to disorders of the eye. The ultimate understanding leads to potential opportunities in evaluating miRNAs as molecular biomarkers, prognostic tools, diagnostic tools and therapeutic agents for eye disorders.

Introduction

For the past 2 decades, the most exciting new development in RNA biology has been the identification of micro-RNAs (miRNAs). miRNAs are members of the family of noncoding RNA molecules, genomically encoded, untranslated RNA molecules of approximately 18–24 nucleotides. They regulate protein-coding genes by interfering with the mRNA's original instructions. Expression of the miRNAs is spatially and temporally regulated and hence they were initially named small temporal RNAs. miRNAs are found in some viruses and in all multicellular eukaryotes such as species of algae, plants and animals. Many important biological processes are regulated by miRNAs, which includes cell growth, death, development and differentiation. Research findings suggest that miRNAs play an integral role in the genome-wide regulation of gene expression, and they provide an additional layer of complexity to the regulation of gene expression [1]. The mystery of gene regulation not only resides in genes and mRNAs themselves, but also in miRNAs. It is estimated that over a third of human protein-coding genes are regulated by miRNAs [2]. Recent research outcomes not only shed light onto the roles of miRNAs in eye disorders, but also explored the unexplored complex regulatory mechanisms involved in these processes. In this review, the basic knowledge of miRNAs, including their discovery, genome organization and biogenesis, has been summarized. In addition, the recent progress of miRNA involvements in eye disorders will be discussed.

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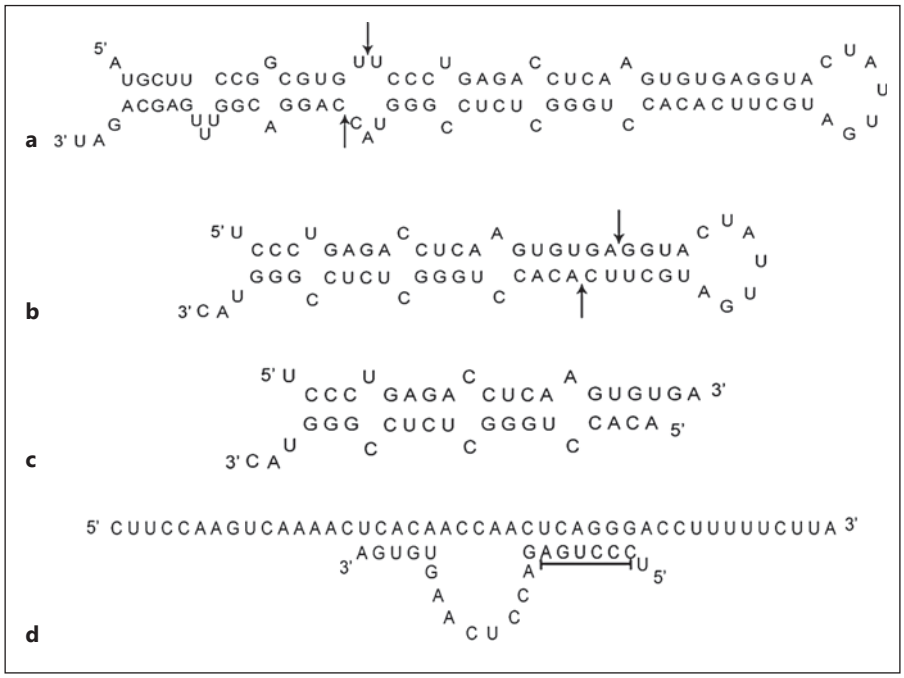

Fig. 1. *LIN-4* processing and its interaction with *LIN-4* transcript. **a** Primary miRNA transcript of *LIN-4*, the arrows show the cleaving site. **b** Precursor miRNA *LIN-4*, the arrows show the cleaving site. **c** *LIN-4* duplex. **d** *LIN-4* interaction with the 3′-UTR of *LIN-4* transcript, the underlined region is the seed (from Araud [7]).

Discovery of miRNA

It all started with the isolation of a mutant (e912) in *Caenorhabditis elegans* with the 'vulvaless' phenotype. This failure of temporal development was caused by this e912 mutation. Embryogenesis in *C. elegans* is divided into 4 distinct stages, from L1 to L4. *LIN-4* is essential for the transition of L1 to L2 during postembryonic development. In the e912 mutant, there is a loss of *LIN-4* gene and loss of the vulva in the female worm. One of *LIN-4*'s target genes, *LIN-14*, encodes a novel nuclear protein which is a putative transcription factor (fig. 1). LIN-14 is present at high levels in newly hatched L1 nematodes and decreases by the L2 stage. In the middle of the first larval stage (L1), *LIN-4* tiny transcripts were found to accumulate, thereby downregulating two genes, *LIN-14* and *LIN-28*, by binding to the 3′-untranslated regions (UTRs) [3]. This binding of abundant *LIN-4* tiny transcripts to *LIN-14* transcripts blocked LIN-14 protein synthesis. In a similar way *LIN-28* is also regulated by the *LIN-4* tiny transcripts. LIN-28 is a cold-shock protein located in the cytoplasm that initiates the developmental transition between stages L2 and L3. This temporal decline of LIN-14 and LIN-28 was considered an essential factor for the proper development of a larva [4]. It varied from the usual mode of gene regulation, and so this discovery was neglected and considered pertinent in the development of nematodes alone. Seven years later, the discovery of a second tiny RNA transcript, *LET-7*, in *C. elegans* was reported. *LET-7* regulates *LIN-41* and *LIN-57* very similarly to that of *LIN-4*. In the nematode *C. elegans*, at the late L3 and early L4 stages, *LET-7* RNA is expressed. The larval to adult transition in *C. elegans* development is controlled by downregulating *LIN-41*. It was only in the year 2001 that these tiny RNA transcripts were termed as 'micro-RNAs'. This discovery of miRNA propelled scientists around the world into the search of many such tiny noncoding RNAs and their functions also in other species. The miRBase database is a searchable online database which consists of published and annotated miRNA sequences [5]. As in the October 2013 release, version 20 of the miRNA database contained 24,521 entries representing hairpin precursor miRNAs, expressing 30,424 mature miRNA products, in 206 species (fig. 2). With the advent of new sequencing technologies in recent years, there will be an exponential increase in miRNA entries into the miRBase in the coming years.

Genome Organization of miRNAs

In the early days, it was found that the miRNAs were encoded in intergenic regions termed as intergenic miRNAs (e.g. miR-45) [6]. These are not found in known

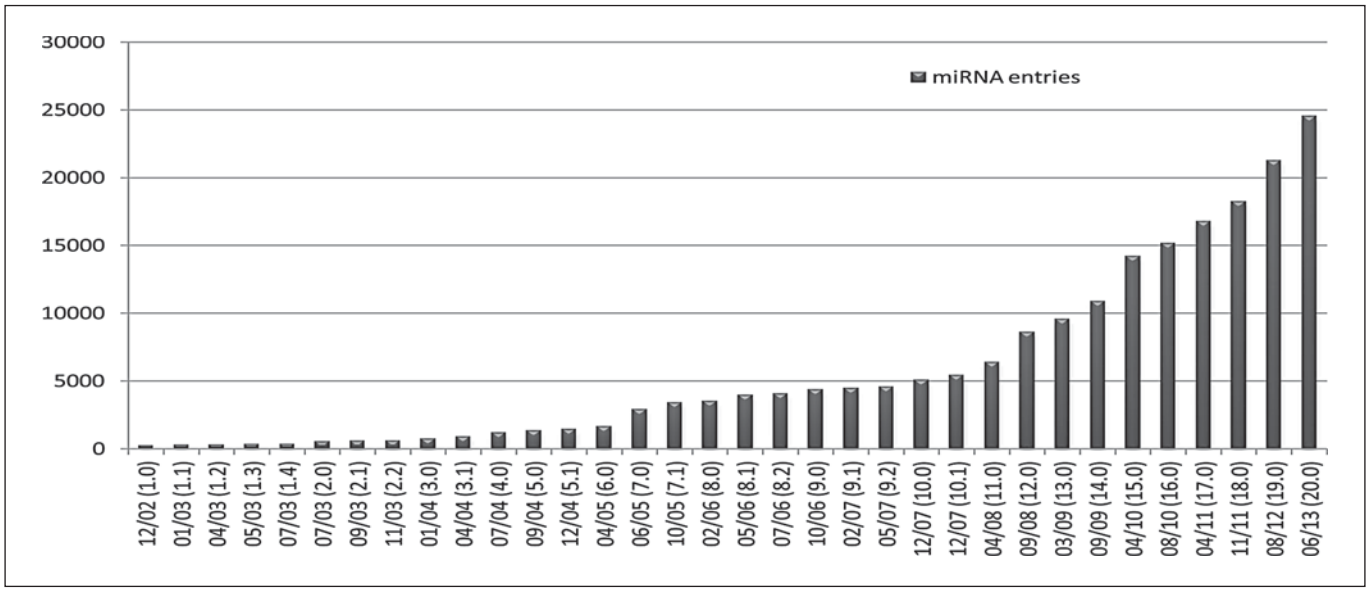

Fig. 2. miRNA entries in miRBase. The numbers outside the parentheses indicate month and year of release, and numbers inside the parentheses indicate the version of release.

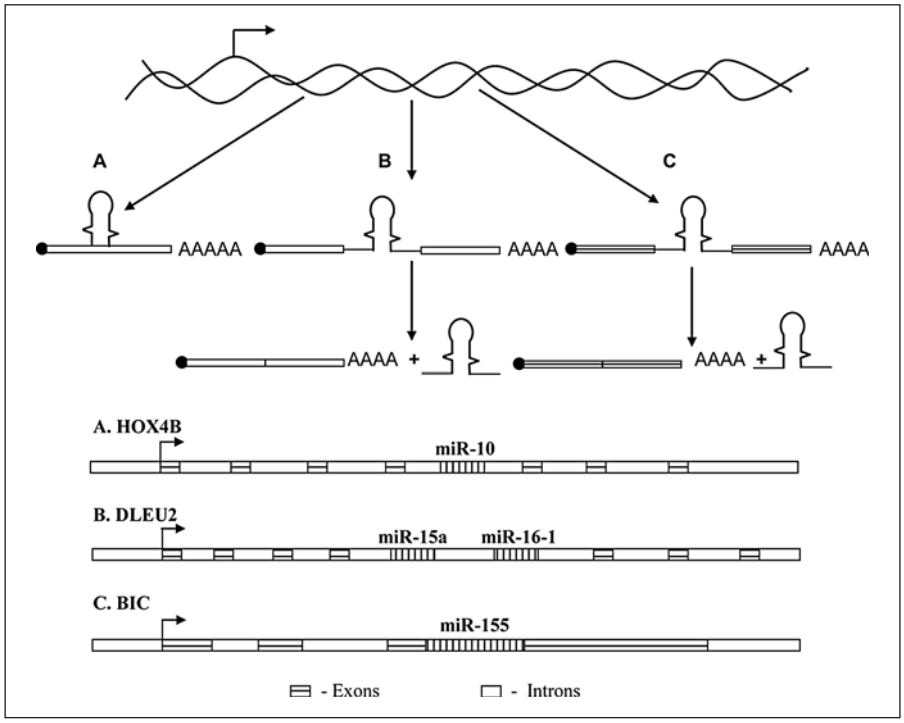

Fig. 3. Primary miRNAs and their location within the introns and exons. A = Intronic miRNA in protein-coding TUs; B = intronic miRNA in noncoding TUs; C = exonic miRNA in noncoding TUs (from Kim and Nam [8]; Araud [7]).

transcription units (TUs). Intergenic miRNAs can be monocistronic or polycistronic. However, recent studies revealed that about 50% of miRNAs are present in known TUs in mammalian genomes [7]. Many miRNA genes are located within the TUs of other genes, both intronic and exonic (fig. 3). Based on miRNA locations in the genome, miRNA genes can be classified into (i) exonic miRNA in noncoding TUs (e.g. *BIC* gene, miR-155), (ii) intronic miRNA in noncoding TUs (e.g. *DLEU2* gene, miR-15a and miR-16–1) and (iii) intronic miRNA in protein-cod-

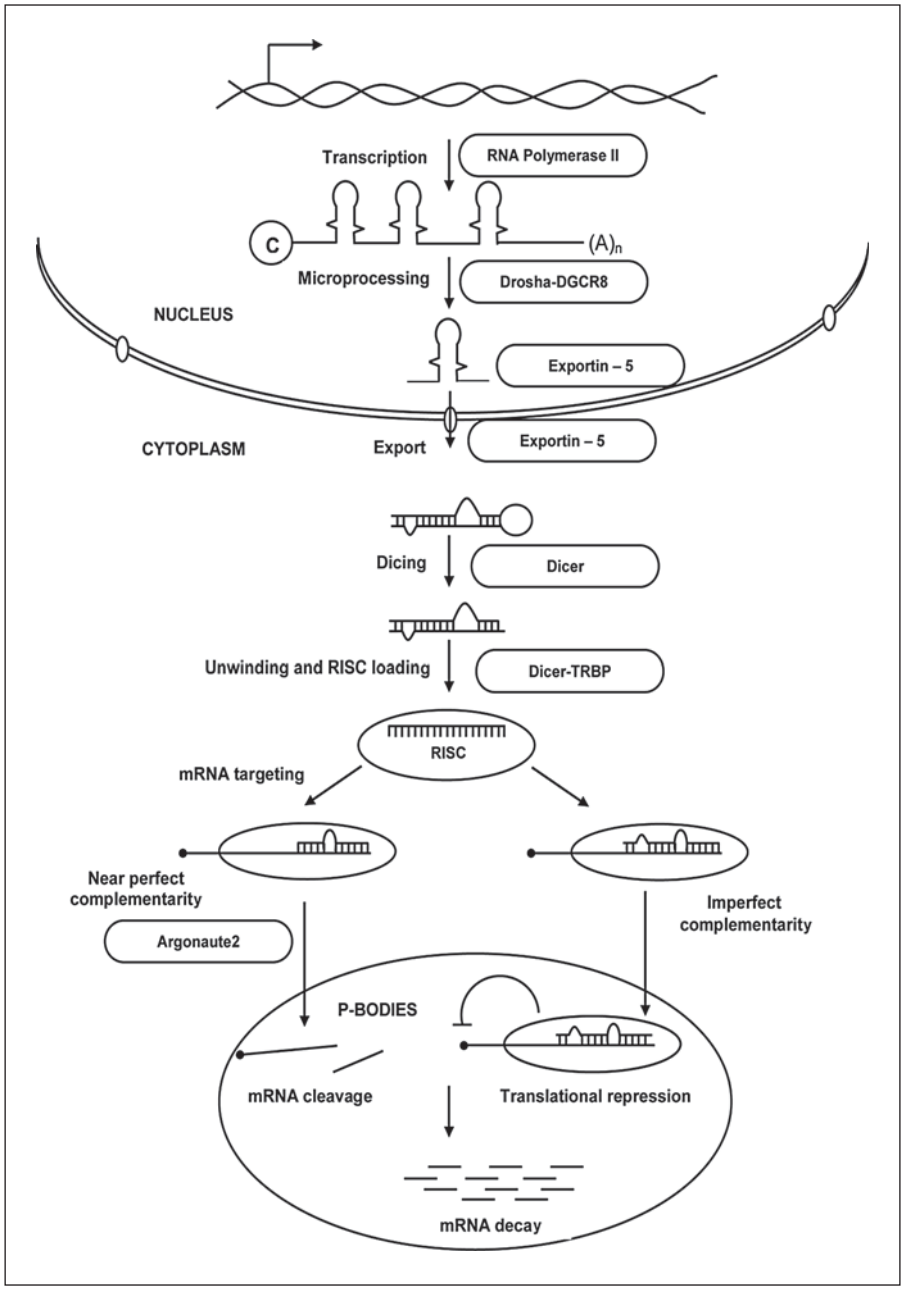

Fig. 4. miRNA biogenesis model. RNA polymerase II transcribes the miRNA genes to form primary miRNA. Microprocessing by Drosha-DGCR8 complex results in precursor miRNA, which is exported to cytoplasm by exportin-5. The processing by Dicer produces miRNA duplexes. Of the duplex the one which serves as mature miRNA is loaded onto the RISC complex, thus preventing protein synthesis.

ing TUs (e.g. *HOX4B* gene, miR-10) [8]. Exonic miRNAs are the least ones which cover part of an exon and an intron of a noncoding gene. The maturation of exonic miRNA from the primary miRNA transcript eliminates the host gene function. Intronic miRNAs are present in the introns of annotated genes. The transcription of intronic miRNA in protein-coding TUs may be coregulated with their host genes, which help in either positive or negative feedback loops. The miRNA and the protein are transcribed from the same promoter and processed from the introns of host gene transcripts. miR-186 (human and mouse) resides in the 2-kb intron 8 of zinc finger protein 5, and miR-208, a heart-specific miRNA, is encoded in intron 27 of human and mouse major histocompatibility complex [9]. In rare cases, the intron is the exact sequence of precursor (pre) miRNA termed mirtron with splice sites on either side; in such cases, microprocessor complex (Drosha-DGCR8/Pasha) is not needed in the matu-

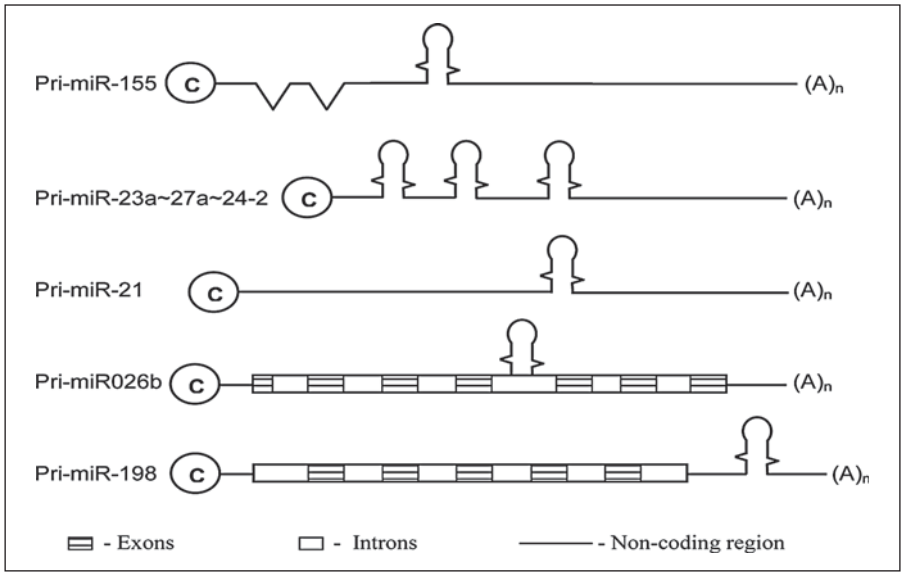

Fig. 5. Structure of primary miRNAs. The transcribed primary miRNA can either be monocistronic or polycistronic. The 5′-end is capped, and the 3′-end is polyadenylated (from Cullen [12]).

ration process. In many instances, miRNA genes are found in clusters suggesting gene duplication and therefore resulted in polycistronic primary transcripts during transcription. From 36 to 47% of miRNAs are present as clusters in the zebrafish, mouse and human. It is obvious that these miRNA clusters target the same gene or different genes in the same pathway. The miR-17-92 cluster in mice functions in the proliferation as well as limited differentiation of lung epithelial progenitors. It was evident from the several miRNA cluster sequences that RNA polymerase II transcribes the majority of miRNAs. As a result, the miRNAs are capped and polyadenylated, like other RNA polymerase II transcripts. The primary transcript of the majority of human miRNAs ranges from 3 to 4 kb in length with a distinct transcription start site and polyadenylation. This was evident from the study of transcript start sites, expressed sequencing tag matches, CpG island predictions, 5′- and 3′-end identifiers, transcription factor binding sites and polyadenylation signals of various intergenic human miRNAs.

miRNA Biogenesis Pathway

The well-coordinated multiple steps of the miRNA biogenesis model are overviewed (fig. 4).

Transcription and Nuclear Processing

The miRNA gene is transcribed by RNA polymerase II in most cases and in few other cases by other RNA polymerases (e.g. miRNA gene in mouse γ-herpes virus 68 by RNA Pol III). Analysis of the miRNA promoters revealed TATA box Pol II elements, and the α-amanitin sensitivity of miRNA transcription showed RNA polymerase II as the enzyme for transcription of miRNA genes [10]. Like a typical mRNA transcript, the transcripts from the miRNA gene possess the 7-methylguanosine cap and a polyadenylation tail. Transcription of miRNA genes yields nascent transcripts termed as 'primary miRNAs'. Primary miRNAs can be as long as several kilobases and contain one or more local foldback secondary stem-loop structures. They are monocistronic or polycistronic (fig. 5).

miRNA processing begins in the nucleus with the cleavage of the stem-loop structures by an RNase III-like enzyme, Drosha [11]. Drosha is a large protein of ~160 kDa, which is conserved in animals but not in plants and belongs to the class II of the RNase III family characterized by a tandem of RNase III domains (RIIID) and a double-stranded RNA binding domain (dsRBD) [12]. The 5′-strand of the stem-loop is cleaved by C-terminal RIIID, whereas the 3′-strand is cleaved by RIIIDa. This process of cleaving the stem-loop structure is termed as 'cropping', and the processing reaction releases an intermediate stem-loop structure of ~70 nucleotides with a 2-nucleotide overhang at its 3′-end termed as pre-miRNA [13]. Drosha alone cannot cleave the stem-loop structure but requires a cofactor, the DiGeorge syndrome critical region 8 (DGCR8) protein in humans (also called Pasha in flies and nematodes), and this complex is called

'microprocessor complex'. The microprocessor complex is ~650 kDa in humans and ~500 kDa in flies. This cropping process occurs cotranscriptionally and precedes the splicing of intron-encoded miRNAs. The cropping process by Drosha is not essential in the case of mirtrons, i.e. the spliced intron itself corresponds to a processed precursor miRNA.

Nuclear Export

At the end of the processing by the Drosha-DGCR8/Pasha complex or the excision as a mirtron, the resulting pre-miRNAs fold into mini-helical structures for trafficking from the nucleus into the cytoplasm. The mini helix motif consists of a more than 14-bp stem and a short 3′-overhang and has a capacity to bind dsRNA. The pre-miRNA is recognized by exportin-5, a Ran-dependent nuclear transport receptor, through the mini helix motif. Exportin-5 recognizes and binds the pre-miRNA inside the nucleus where the RanGTP level is high. And once outside the nucleus into the cytoplasm where the RanGTP level is low, exportin-5 releases the pre-miRNA for maturation into miRNA [14].

Cytoplasmic Processing

Once into the cytoplasm, pre-miRNAs are processed by a class III of RNase III called Dicer. Dicer is a multidomain protein of ~200 kDa highly conserved among all eukaryotic organisms. Dicer consists of an N-terminal DEXH-box RNA helicase domain, a DUF283 domain (unknown function), a PAZ domain (also found in the Argonate protein family), 2 RNase III domains and 3 dsRBD. The 2 RNase III domains of Dicer cleave pre-miRNA at a specific distance to produce ~22 nucleotide dsRNA products. The resulting miRNA duplex possesses 3′-overhangs of 2 nucleotides at each extremity. The miRNA duplex is composed of the paired mature miRNA; one is called guide strand, and the other is called the passenger strand. The passenger strand is occasionally termed as minor species and designated as miRNA*.

RNA-Induced Silencing Complex Assembly and Activation

The final step in miRNA biogenesis is the RNA-induced silencing complex (RISC) assembly. The RISC is an important player in the biological activity of miRNAs. The Dicer-cleaved products are not long-lived in the cells. The guide strand is incorporated into the effector complexes termed as miRNP (miRNA-containing ribonucleoprotein complex) or mirgonaute or miRISC (miRNA-induced silencing complex), while the other passenger strand is degraded [15]. R2D2, a dsRNA protein, senses the differences in the thermodynamic stabilities at each extremity, binding the more stable one, and thereby orienting the duplex. The human analog of R2D2, TRBP (HIV-transactivating response RNA-binding protein), is important for miRNA processing and incorporation into miRISC. The strand with lower stability base pairing (lower thermodynamic energy) of the 2–4 nucleotides at the 5′-end of the duplex preferentially associates with RISC and thus becomes the active miRNA [16]. There is equal probability of one of two strands to become the active miRNA when both have similar 5′-end stability. The fate of the other inactive miRNA (passenger strand) is either degraded, or it can act as the template for the synthesis of new dsRNA by the presence of RNA-dependent RNA polymerase, which again forms more miRNAs. The former hypothesis is evident from the 100-fold lower recovery rate of miRNAs* from endogenous tissues, and the latter is criticized as there is no RNA-dependent RNA polymerase so far in mammals or in flies, although homologs are found in worms, fungi and plants.

Action of miRNA/RISC Complex

The miRNA guides the miRNA/RISC complex to its target mRNA by base pairing. The specific actions depend on the complementarity between miRNA and mRNA. The nucleotides located at positions 2–8 relative to the 5′-end of the miRNA termed 'seeds' are essential for the target recognition. Three modes of action are possible by miRNA. They are as follows:

When there is perfect or near-perfect complementarity between the miRNA and target mRNA sequences, the mRNA is cleaved between nucleotides located at position 10 and 11 of the paired bases relative to the 5′-end of the miRNA and ultimately degraded. Ago 2, an important component of the RISC complex, possesses the cleavage activity of the mRNA target. When there is imperfect base pairing at the 3′-UTR in mRNA, the miRNA/RISC complex rests there, preventing the elongation process by ribosomes and thereby terminates the translation prematurely. This process is termed translational repression. The binding sites for miRNA on mRNA are spread over kilobases of the 3′-UTR which is usually longer than coding sequences. These binding sites are enriched with

AU-rich sequences near the termination site and polyA proximal regions. For strong repression to take place, multiple sites are required and there is some kind of cooperativity among many different miRNAs which have not been elucidated clearly. This distinctly conveys that one miRNA can control the activity of hundreds of mRNAs. Conversely, one mRNA can be a target of multiple different miRNAs. The miRNA/RISC complex silences mRNA transcripts by sequestering transcripts away from the translational machinery into cytoplasmic foci termed P bodies, thereby inhibiting protein accumulation without having any impact on the levels of mRNA transcripts.

The miRNA/RISC complex may also recognize homologous DNA and silences chromatin by histone and DNA methylation. The chromatin silence happens by the methylation of lysine 9 of the histone 3 (H3K9) which involves Swi6. This phenomenon is termed transcriptional gene silencing or posttranscriptional gene silencing in plants and quelling in fungi. This kind of regulation makes miRNAs suitable for networking and fine-tuning of gene expression because they can target several mRNAs in the same pathway. These miRNAs need not undergo transcriptional processing, translation and so on like proteins; thereby they can facilitate rapid switching of new developmental programs without undergoing any extensive processes. miRNAs can also act as on and off reversible switches.

miRNAs and Eye Disorders

Cataract

Cataract is the clinical term for reduced visual function resulting from optical disturbances in the crystalline lens. It is an impairment of the crystalline lens of the eye in which the lens hardens, becomes opaque and yellows. Trauma, exposure to sunlight and a variety of age-related physiological manifestations, including inflammatory diseases, diabetes and genetic predisposition, lead to cataracts. After birth, the central lens epithelium is maintained at a mitotic dormancy state by the optimal transforming growth factor β (TGF-β); when this exceeds the optimum level, the lens epithelial cells (LECs) direct themselves to epithelial-to-mesenchymal transition (EMT), ending differentiated into fibroblastic/myofibroblastic cells leading to the lens pathology. LECs and lens fiber cells behave differently on TGF-β exposure. TGF-β promotes deviation in the differentiation pathway on LECs to progress into cataract [17].

In patients with cataract, 110 miRs were identified from aqueous humor with abundance. Of the 110 miRs, miR-202, miR-193b, miR-135a, miR-365 and miR-376a were the most abundant. Many miRs have been identified so far in humans; only 264 were assayed, and 110 were detected out of those assayed [18]. miR-218, miR-195 and miR-452 were found in abundance in many body fluids, which includes aqueous humor, serum, urine, tears and saliva [19]. On target prediction these aqueous humor miRNAs revealed more than 1 target. These multiple targets show that they have multiple roles in many pathways. Validation of these miRs will give us more clues to their functions and can be utilized in diagnosis.

Differential miRNA expression was detected in cataractous and noncataractous LECs. *LET-7C*, miR-29a, miR-29c and miR126 were downregulated in Shumiya cataract rats with cataract, whereas *LET-7C*, miR-29a and miR-29c were upregulated in noncataractous lenses [20]. The downregulation of *LET-7C*, miR-29a, miR-29c and miR126 was found to be involved in the progression of cataract in Shumiya cataract rats. miRNAs target mostly redox homeostasis and growth factor genes in the lens development and cataractogenesis [21].

Such genes are essential for normal lens organogenesis, cell proliferation and apoptosis, and any modulation leads to cellular abnormalities and cataractogenesis. Deficiency of peroxiredoxin (PRDX6), a multifunctional protein necessary for cell proliferation, differentiation and protection, causes cataractogenesis. *PRDX6* is the target for miR-551b. Downregulation of *PRDX6* by miR-551b causes activation of TGF-β which in turn leads to extracellular matrix protein upregulation ultimately resulting in cataract.

Surgical therapy of capsular lens fiber removal and synthetic lens implantation can lead to secondary cataract also termed as posterior capsular opacification (PCO) in humans. The etiology of PCO includes the transdifferentiation of anterior capsule residual lens epithelial cells into mesenchymal myofibroblast cells with EMT that can migrate and expand into the posterior area of the lens capsule. EMT-associated changes in crystallin proteins, upregulation of cytoskeletal proteins such as α-smooth muscle actin and fibrotic extracellular matrix remodeling results in corresponding lens opacity. During surgery exogenous TGF-β_2 activates and senses the signal, and on binding to its receptor triggers the Smad proteins through its transmembrane kinases. The triggering happens through the phosphorylation of SMAD2 and SMAD3 by type I kinase of the TGF-β receptor [22]. This phosphorylation makes SMAD2 and SMAD3 to combine with

SMAD4 and the complex to translocate into the nucleus and activates TGF-β-dependent genes. SMAD3 controls the expression of the master transcription factor Snail essential for the EMT in the tissue fibrosis process and the synthesis of ECM components. The pathway thus leads to the accumulation of fibrous ECM [23].

A study on mouse cataract surgery model for PCO revealed the regulatory role of miRNAs on PCO etiology. miRNA expression profiles at different time points from regenerating lenses were made after cataract surgery. The profile using 627 mouse miRNAs showed a PCO-associated expression pattern of 55 regulatory miRNAs. A capsular bag culture model used to study PCO characteristics showed abundant expression of miR-184 and miR-204 with their potential role in regulation. They both exhibited differential expression patterns during lens differentiation, regeneration and cataract etiology. miR-184 and miR-204 target cataract-associated GTPase-binding protein bridging integrator 3, the homeobox transcription factor. Meis2 and canonical Wnt signaling associated transcription factor RUNX2, respectively. Hence the complex miRNA network interactions are necessary for the formation of PCO in mice [24]. It sheds light onto the use of anti-miRNAs that target the competitive miRNA network which can be a potential therapeutic for PCO and other diseases. EMT is regulated by miR-204-5p during the formation of human posterior capsule opacification. *SMAD4* was predicted to be the target for miR-204-5p through computational predictions. *SMAD4*, a direct target of miR-204-5p, was regulated by miR-204-5p in EMT. By targeting *SMAD4*, miR-204-5p inhibits EMT in the LECs of a human donor capsular bag model in vitro. Development of PCO involves miR-204-5p and *SMAD4* in the EMT, and hence miR-204-5p serves as a novel target for therapeutic intervention in PCO [25]. In addition to miR-204-5p, miR-26b was also found to target *SMAD4* mRNA. In LECs and SRA01/04 cells, miR-26b was downregulated even in the presence of TGF-β2 which substantiates the role of miR-26b in PCO. On miR-26b restoration, miR-26b targets and downregulates *SMAD4*. In PCO, *COX-2* also gets upregulated like *SMAD4* in LECs. miR-26b overexpression downregulates *COX-2*. When *SMAD4* and *COX-2* get downregulated, LEC proliferation, migration and EMT processes were inhibited. This silencing of *SMAD4* and *COX-2* by miR-26b paves the way for the therapeutics of PCO [26].

Myopia

Myopia is the eye defect in which distant objects appear blurred because images are focused in front of the retina instead of on the retina. *PAX6* is the master gene for the globe and also involved in nervous system development. It induces lens and retinal differentiation in globe development. It is a highly conserved family of transcription factors containing paired and homeobox DNA-binding domains. The reduction in PAX6 protein levels significantly increases the risk for myopia.

The interplay of miR-204, *MEIS2* and *PAX6* is essential for ocular development. The regulatory loop formed among them regulates the early lens development. In addition to other miRs, miR-204 regulates *PAX6,* and both of their sustained expressions are required for the proper ocular development. Any disruption in the regulatory loop may lead to deleterious effects ending up with an abnormal eye [27].

The interaction of the *PAX6* mRNA with miR-328 is disrupted when there is a polymorphism in the 3′-UTR of the *PAX6* gene. A particular functional polymorphism is single nucleotide polymorphism (SNP) rs662702; the high-risk allele was found to be strongly downregulated by miR-328. This may be brought about by high stability interaction between the *PAX6* transcript-carrying polymorphism at the 3′-UTR and miR-328. Such a functional polymorphism was associated with extreme myopia. This study reveals the importance of mutations in the 3′-UTR of miRNA targets leading to a particular disease [28].

A study from Taiwan revealed that miRNA may play a role in the regulation of the *PAX6* gene [29]. miR-328 was predicted to bind to 3′-UTRs of *PAX6* transcripts. An interesting observation was made in the expression levels of miR-328 and *PAX6* in the retinal pigment epithelium (RPE) and in the sclera cells. When there was increased expression in miR-328, the expression of *PAX6* was lowered in the scleral cells, and the opposite happened in the case of RPE. The binding of miR-328 to the 3′-UTR of *PAX6* was validated by mutated forms of 3′-UTR of *PAX6*. Then the inhibition of *PAX6* expression by miR-328 in RPE cells was studied with different doses of a miR-328 mimic. A dose-dependent decrease in *PAX6* expression was observed, which revealed the negative regulation of *PAX6* expression by miR-328. RPE cell proliferation was also enhanced by transfection with different doses of miR-328. Increased retinoic acid expression has been reported during the development of myopia. The JASPAR database located retinoic acid-responsive elements in the 2-kb promoter region of miR-328 and predicted it to regulate miR-328 expression. RPE cells treated with different doses of retinoic acid showed a considerable increase in miR-328 levels which further led to the downregulation of *PAX6*. All these studies imply that miR-328-mediated

PAX6 downregulation plays a significant role in the development of myopia.

In another instance, *PAX6* was observed to be downregulated along with a set of eye field transcriptional factors – *ET, RX1, SIX3, ATH2, OPTX2* and *LHX2* – when miR-196a was ectopically expressed. Such a misexpression will lead to an abnormal phenotype affecting the size of the eye. This study highlights the importance of a new tool in analyzing the miRs and their networks in eye diseases [30].

Retinoblastoma

Different types of tumors may arise in the eye, but the most common primary malignant ocular tumors are retinoblastoma (RB) in children and uveal melanoma in adults. The incidence of RB is 1 out of 20,000 births in the USA [31]. RB had never been reported in rodents, and the development seems specific to humans and to some extent to other mammals. Apart from its significant role in eye development, *PAX6* plays a vital part not only in the development and progression of RB, but also many other cancer types. miR-365b-3p was downregulated and found to be a tumor suppressor gene in RB cells. They induce cell cycle arrest by the increased expression of P21 and P27 proteins and by the decreased levels of cdc2 and cyclin D1 by targeting *PAX6*. This inhibitory role of miR-365b-3p in cell cycle progression may provide a therapeutic strategy to RB [32]. In mammals, the retinoblastoma family of structurally and functionally related proteins, including pRB itself, as well as p107 and p130. These 3 family members are inhibited by the same cyclin/cyclin-dependent kinase (CDK) complexes, including E2F transcription factors, viral oncoproteins and chromatin remodeling enzymes. Mice with mutations in *RB* and *p107* or *RB* and *p130* in the developing retina were generated by many research groups and developed double-knockout mice that developed tumors closely resembling human RB. These double-knockout mice provided a novel system to study RB both in vivo and ex vivo which possessed metastatic potential. Of the 21 murine RB generated by the concomitant inactivation of *RB* and *p107*, 2 tumors showed focal amplification of a chromosomal region containing the miR-17-92 clusters. miR-17-92 copy numbers were increased in a panel of 32 primary RB samples [33]. A similar study in murine samples correlated well with the copy number. In human samples, 6 miRNAs were highly expressed by this cluster irrespective of copy number. More research revealed that the overexpression of miR-17-92 alone is not a factor for tumor induction, but pRB loss along with miR-17-92 overexpression resulted in RB. All these studies question further why the loss of pRB and p107 is required in addition to the miR-17-92 overexpression to lead to RB in mice but not in humans. Research from different laboratories on mouse models showed that miR-17-92 can accelerate tumorigenesis by suppressing apoptosis, increasing angiogenesis or suppressing TGF-β signaling [34, 35]. Experiments in RB cell lines demonstrated that only miR-17 and miR-20a play a role in cell proliferation but not miR-19a and miR-19b in the cluster. miR-17-92 – a polycistronic cluster – was named oncomir-1 specifically for acting as oncogene in a B-cell mouse lymphoma model, in vitro and in human cancers [36]. The cluster was upregulated in various types of tumors [37]. Dysregulation of certain miRs not only brings about RB but also other tumor types [38]. This can be evidenced from the role played by miR-17-92 clusters in multiple tumors [39]. These results open a new era of therapeutic implication where inactivation of miR-17-92 clusters might be a strategy for preventing RB without major side effects.

In contrast, miR-34b and miR-34c/p53 transcriptional targets repressed many targets involved in the proliferation of cells. Downregulation of such targets inhibited cell proliferation. Hence these 2 miRs act as tumor suppressors. Such inhibitory effects on cell proliferation shown by miR-34b and miR-34c have opened new avenues in therapeutics using miRs [40].

hsa-miR-494, hsa-let-7e, hsa-miR-513-1, hsa-miR-513-2, hsa-miR-518c*, hsa-miR-129-1, hsa-miR-129-2, hsa-miR-198, hsa-miR-492, hsa-miR-498, hsa-miR-320, hsa-miR-503 and hsa-miR-373* – other miR clusters – were found upregulated in RB in comparison with normal retina. Though these miRs were overexpressed in RB, none were reported in tumor development through functional studies so far [41].

A study on 2 RB cell lines revealed 39 miRs were differentially expressed: 22 and 17 miRs were found to upregulated in SNUOT-Rb1 and Y79 cells, respectively. Most of these miRs were found to have targets that had a relation with cell growth patterns from cell proliferation to cell death and were supposed to have a role in the progression of RB [42]. miR-34a expression was found to differ with different RB cells. miR-34a was upregulated in Y79 RB cells. Other transcriptional targets of miR-34a expression have not been found yet, which might open new signaling pathways into further studies [43].

In a Taqman low-density array study on 12 human RB and 3 normal human retinae, of the 377 miRNAs analyzed, 41 showed differential expression in RB when compared to the normal human retina. None of the miRNAs corre-

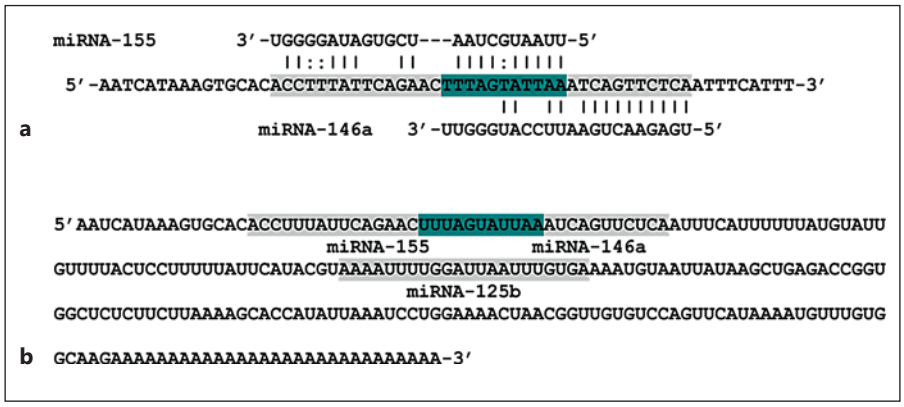

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Fig. 6. miR-155, miR-146a and miR-125b targeting CFH 3′-UTR. **a** miR-155 and miR-146a separately target 3′-UTR of CFH. **b** miR-125b, miR-146a and miR-155 target CFH mRNA 3′-UTR either individually or as a group (from Lukiw et al. [45]).

lated with optic nerve invasion or intraocular neovascularization. Within these 41 miRNAs, 13 were already reported in human or mouse RB. miR-139-3p was upregulated whereas miR-129-3p, miR-382, miR-504, miR-22 and miR-129-5p were downregulated in primary human RB as compared to normal human retinae [44]. In 2 human RB cell lines, Weri1 and Y79, miR-129-3p, miR-382, miR-504, miR-22, miR-874 and miR-129-5p were found to be downregulated whereas miR-139-3p showed no difference in expression. All these 7 miRNAs were found to be downregulated in the mouse RB cell lines SJmRBL3 and SJmRBL8. miR-129 downregulation was not only found in RB, but also in many different cancers where miRNAs play a key role in RB; further research will provide more clues on differentially expressed miRNAs in relation to RB.

Age-Related Macular Degeneration

Age-related macular degeneration (AMD) is a late-onset, multifactorial, progressive neurodegenerative disease of the human retina. It represents the leading cause of visual impairment in aging populations of the industrialized world. AMD causes nonreversible blindness worldwide. miR-9, miR-125b, miR-146a and miR-155 are shown to be involved in a comparable progressive, complement factor H (CFH)-mediated inflammatory degeneration characteristic of AMD [45–47]. CFH functions against unscheduled or spontaneous activation of innate immune system. The upregulation of miR-9, miR-125b, miR-146a and miR-155 has been shown to downregulate CFH expression in the aged degenerating retina [48]. This quartet of upregulated miRNAs not only downregulated *CFH*, but also other pathogenic genes, which included 15-lipoxygenase, synapsin-2 and tetraspanin-12. miR-125b, miR-146a and miR-155 target *CFH* mRNA 3′-UTR either individually or as a group (fig. 6). miR-9 also has a binding site in the *CFH* mRNA 3′-UTR. However, its elevation in AMD is different from that of the above 3 miRNAs. These studies speculate that this quartet of miRNAs coordinates innate immune and inflammatory signals across the retina-primary visual cortex pathway and their contribution to the aging and degenerative diseases [49, 50]. Further investigation is needed whether individual retinal cell types contribute to these miRNAs and *CFH* aberrations in AMD.

Two of the inflammatory process miRs – miR-146a and miR-146b – were found to negatively regulate the NF-κB pathway in AMD [51]. The pioneer clinical study on miR expression between AMD and healthy controls suggested changes in plasma miRNA levels [52]. Of the 384 miRNAs in plasma tested, 5 (miR-17-5p, miR-20a-5p, miR-24-3p, miR-106a-5p and miR-223-3p) showed increased expression, and 11 (miR-21-5p, miR-25-3p, miR-140-3p, miR-146b-5p, miR-192-5p, miR-335-5p, miR-342-3p, miR-374a-5p, miR-410, miR-574-3p and miR-660-5p) showed decreased expression in AMD. The most significant outcome from the study was that 10 miRs (miR-26b-5p, miR-27b-3p, miR-29a-3p, miR-130-3p, miR-212-3p, miR-324-3p, miR-324-5p, miR-532-3p, miR-744-5p and *LET-7C*) were specifically expressed in AMD patients. This unique expression of miRs in AMD has presented plasma miRs as promising biomarkers for the rapid diagnosis of AMD in the near future.

Oxidative stress leads to retinal degeneration [53, 54]. Hydrogen peroxide (H_2O_2) and t-butylhydroperoxide act as oxidant generators. Reactive oxygen species (ROS) induce cell death in RPE cells leading to the pathogenesis of AMD [55]. ROS-mediated oxidative damage is thought to play a crucial role in AMD [56, 57]. Oxidants activate the Fas death receptor and apoptotic pathway. The RPE monolayer of the choroidal neovascular membranes from

patients with AMD showed increased *FAS* and *FASL* expression [58, 59]. *FAS* acts as an apoptotic factor in ROS-mediated cell death. One member of the miR-23-27-24 clusters, miR-23a, was downregulated in RPE cells of AMD patients and might have a role on the pathogenesis of AMD in the early stages [60]. Cell death due to ROS is reduced by the overexpression of miR-23a. It has been found through computational analysis that miR-23a has the binding site in the 3′-UTR of the *FAS* mRNA. miR-23a downregulates the *FAS* thereby protects RPE cells from damage caused by ROS. RPE cells were resistant to apoptosis by oxidative stress with the overexpression of miR-23a. miR-23a inhibits the cell death pathways which are different/independent from that of the caspase activation. miR-23a has been shown to have an antiapoptotic effect only against oxidative injury, and hence it plays an important role in ROS-mediated cell death/survival. When the higher dose of H_2O_2 was supplied to primary cultured RPE and ARPE-19 cells, there was downregulation of miR-23a. Overexpression of miR-23a reduces the cell damage whereas its inhibition increases cell damage. miR-23a was found to be downregulated in RPE of AMD patients and thought to initiate AMD pathogenesis. Both the computational analysis and laboratory findings imply that *FAS*, a functional target of miR-23a, plays a part in the injury of RPE cells caused by H_2O_2 [61]. This study sheds light onto the diagnosis and treatment of eye diseases related to ROS, such as AMD. Differentially expressed miRs in AMD patients will pave the way for early diagnosis and the observation of AMD progression. Various miRs in AMD distinctly reveal the enormous potential to completely elucidate the physiology of RPE cells.

In recent studies on the retina, using the different mouse models of retinitis pigmentosa (RP), researchers found altered miRNA expression profiles. The members of miR-183/96/182 are downregulated in RP mice, whereas miR-1, miR-133 and miR-142 are upregulated. An expansive list of target mRNAs was found for these miRNAs. In order to know the mechanism how retinal degeneration is brought about by these miRNAs, more research has to be done in these areas. miRNAs have also played some role on hypoxia-induced retinal and choroidal neovascularization, AMD and diabetic retinopathy (DR). Injection of pre-miR-31, pre-miR-150 or pre-miR-184 and pre-miR-31 or pre-miR-150 significantly reduced retinal and choroidal neovascularization, respectively [62]. No clear evidence of possible target genes has been established in vivo so far. miR expression profiles between the wild-type retina and the retinas of RP transgenic mice showed that there was altered miR expression [63]. The miR expression profile was found to be changed in this transgenic mouse model [64]. Expression profiling studies like these using transgenic models will give us more clues on the role of miRs and their targets in RP. The identification and the validation of miRs related to RP will pave the way for a better understanding of the pathophysiology of RP. DR is the injury to the vasculature causing leakage and occlusion due to persistent hyperglycemia. There are numerous studies reporting on the role of miRNAs in DR. miRNAs in DR modulation were reviewed extensively [65]. In more than a study, it was reported that miR-93 plays a role in DR. Vascular endothelial growth factor expression is controlled by miR-93. miR-93 expression was found to be inhibited under hyperglycemic conditions [66].

Endothelial Dystrophy, Iris Hypoplasia, Congenital Cataract and Stromal Thinning Syndrome

EDICT is an autosomal dominant syndrome characterized by endothelial dystrophy, iris hypoplasia, congenital cataract and stromal thinning. Through linkage analysis, the *EDICT* gene was located to be on the long arm of chromosome 15 with a common linkage interval consisting of 75 annotated genes and 4 miRNAs (miR-184, miR-433a, miR-7-2 and miR-9-3). A single-base substitution in the seed region of miR-184 (+57C>T) was alone identified in sequencing of all the 4 miRNAs [67]. Pedigree analysis of a family through 4 generations revealed the substitutions segregating with the disease phenotype. This single-base-pair substitution miR-184 (+57C>T) is the causal mutation, for the EDICT syndrome has been evidenced through mutation segregation by pedigree analysis, absence of this variance in 1,000 genomes, 1,130 control chromosomes and in 28 nonhuman vertebrates and in silico stability prediction. This miR-184 (+57C>T) substitution brings about a conformation change. The number of potential targets and genes predicted through computations revealed that the wild-type and mutant miR-184 differed to a greater extent. Many of the targets were missed while predicted using mutant miR-184 compared with the targets of wild-type miR-184 [67]. Mutation in the seed region of the miR elicits the cause of the syndrome. Functional analysis of such mutations in the seed region of the miR will provide more insights into the novel molecular pathways of disease.

Pterygium

Pterygium is a noncancerous growth on the conjunctiva or mucous membrane that covers the white part of the eye. Pterygium obstructs vision by growing over the

cornea. EMT may play a role in the pathogeneis of pterygium. Comparative genome-wide miRNA and mRNA expression analysis of human primary pterygium demonstrated that the difference in expression patterns of various miRNAs and mRNAs was found to be associated with the pathogenesis of pterygium [68]. The miR-200 family has been considered as an important regulator of EMT. Seventy miRNAs were found to be differentially expressed in human pterygium samples when compared with the normal conjunctiva. Twenty-five miRNAs were found to vary by more than twofold, of which 14 showed increased and 11 decreased expressions. Of the miR-200 family members, miR-200a, miR-200b and miR-141 were downregulated in pterygium. miR-29b and miR-192, the regulators of tissue fibrosis, were found to be downregulated in pterygium. miR-31 was downregulated and miR-146, miR-199a and miR-451 were upregulated in pterygium; these were the putative regulators of ocular angiogenesis. Clustering analysis through the Euclidean, unsupervised, hierarchical clustering of the 25 miRNA probe sets showed a similar expression profile as said above across all pterygium samples. When subjected to biological functional analysis, these miRNA data sets were found to be involved in cellular development, growth, proliferation and movement. The differential expression of multiple miRNAs was found to be involved in the pathogenesis of pterygium. The miR-200 family and other miRs coordinately regulate the EMT process; alteration in the expression leads to pterygium. Multiple miRs regulating the EMT process may control the vast repertoire of proteins. The role of individual miRs of the miR-200 family and their combined effects still have to be explored. The deregulated miRs that lead to pterygium may be targeted to combat its development.

Uveitis

Uveitis is an inflammation of the uvea which includes the iris, ciliary body and choroid and is the most common cause of blindness in the world. Of the many forms of uveitis, the role of miRs in Behçet's disease (BD) and Vogt-Koyanagi-Harada disease (VKH) have been studied so far. BD is an inflammatory disease showing the following symptoms: intraocular inflammation, oral aphthous ulcers, skin lesions and genital ulcers. VKH is an autoimmune disorder with meningismus, cerebrospinal fluid pleocytosis, auditory findings and integumentary findings. In a study to investigate the role of miRNAs in the pathogenesis of uveitis, miR-155, an immunologically relevant miRNA, was found to be expressed less in peripheral-blood mononuclear cells and dendritic cells of BD patients than of healthy controls [69]. No such difference was detected between VKH patients and healthy controls. TGF-β-activated protein kinase 1-binding protein 2 *(TAB2)* is important in the TLR/IL-1 signal transduction cascade and has been tested as target for miR-155. A luciferase reporter study and Western blotting showed *TAB2* as the target for miR-155. In addition to TAB2, *IL-6* and *IL-1β* were found to be the targets for miR-155. In contrast to the decreased expression of miR-155, *TAB2* expression was found to be increased in BD patients with active uveitis when compared to healthy controls [69]. miR-155 expression is downregulated in the pathogenesis of BD but not in VKH. BD pathogenesis may be exerted by miR-155 through changing the levels of dendritic cell cytokines. The cytokines IL-6 and IL-1β are involved in autoimmune and inflammatory disease pathogenesis.

Disease susceptibility increases with SNPs either on the pre-miRs or on the miRNA targets. The association of 3 miR-146a SNPs (rs2910164, rs57095329 and rs6864584) and 2 ets-1 SNPs (rs1128334 and rs10893872) with pediatric uveitis, BD and VKH was investigated in a Chinese Han population. miR-146a plays a role in the development of autoimmune disease. *ETS-1* regulates miR-146a expression. Individuals carrying the CC genotype of miR-146a rs2910164 not only had the lower miR-146a expression, but also had a lower risk of developing BD. All these 5 SNPs showed no association with VKH [69]. The SNP rs2910164 of miR-146a and the SNP rs10893872 were shown to have an association with pediatric uveitis [70]. In another study, the association of SNP rs76481776 of miR-182 was assessed. The CC genotype frequency of miR-182/rs786481776 was found to be significantly decreased in both BD and VKH cases [71]. Genetic polymorphisms in miRs which are involved in an inflammatory process may offer genetic predisposition to various eye diseases.

As a model of human uveitis, experimental autoimmune uveoretinitis (EAU) is induced in animals by immunization with different proteins [72]. During the development of EAU using peptides of interphotoreceptor retinoid binding protein, IL-17A, IL-17F and IFN-γ were upregulated whereas IL-12p35 mRNA was downregulated in the eye. Upregulation of *IL-17* was shown with a decrease in the level of miR-182 and with changes in miR-142-5p and miR-21 expression levels [73]. The downregulation of miR-182 expression by EAU induction may be related to the destruction of retinal structure. Although the study reveals the influence of miRNAs in the patho-

logical changes before the onset of inflammatory conditions, further studies will unravel the complete role of miRNAs in the development of EAU.

Uveal Melanoma

The expression pattern of miR-124a in 6 human uveal melanoma specimens and in 3 uveal melanoma cell lines (M17, M23 and SP6.5) showed downregulation when normal uveal tissues were used as controls. When miR-124a was transfected, there was inhibition of M23 and SP6.5 cell growth. miR-124a transfection not only inhibits cell proliferation with arrest in the G_1 phase of the cell cycle, but also suppresses the migration and invasion of uveal melanoma. *CDK4*, *CDK6*, cyclin D2 and enhancer of zeste homolog 2 *(EZH2)* were the targets of miR-124a. *CDK4*, *CDK6*, cyclin D2 and *EZH2* were found to be upregulated in uveal melanoma cells. Ectopic expression of miR-124a downregulated *CDK4*, *CDK6*, cyclin D2 and *EZH2* along with other cell cycle regulatory proteins suppressing cell proliferation. The most conspicuous hypermethylation status of miR-124a was observed in both the uveal melanoma cell lines and the clinical samples. These epigenetic changes may in part be responsible for the downregulation of miR-124a in uveal melanoma cells [74]. miR-124a can be a potential tumor suppressor in uveal melanoma.

In a study, using cell lines M23 and SP6.5, it was observed that the induction of miR-182 is dependent on p53 activation. As the p53 expression increased in the M23 and SP6.5 cells so was that of miR-182. After activation by p53, the induction of miR-182 functioned as a tumor suppressor by inhibiting the cell proliferation, migration and invasion in both the M23 and SP6.5 cells. miR-182 was also found to enhance apoptosis through caspase 3/7 activity. miR-182 targeted and suppressed *MITF*, *BCL2* and cyclin D2. miR-182 induction upon p53 activation downregulated the expression of *MITF* and *c-MET* in a sequential fashion. *c-MET*, being the target of *MITF*, was found to activate Akt and ERK1/2 pathways. Thus, induction of miR-182 downregulated multiple cell signaling pathways. Downregulation of *c-MET* by miR-182 was shown to inhibit uveal melanoma cell proliferation, migration and invasion [75]. Both in vitro and in vivo studies demonstrated that the overexpression of miR-182 suppresses the growth of uveal melanoma.

Immunoregulatory miRs in circulation were found to be altered in uveal melanoma [76]. The levels of immunoregulatory miRs such as miR-20a, -125b, -146a, -155, -181a, -223 and -17-92 complexes in plasma were higher in uveal melanoma patients. The levels of the immunoregulatory miRs except miR-181a were elevated in metastasis compared to primarily diagnosed uveal melanoma. In contrast to the other immunoregulatory miRs, the miR-181a level was lower in metastasized stages compared to primary diagnosis. In addition, these miRs showed varied levels in CD3+, CD15+ and CD56+ cells. All 3 populations showed increased miR-146a, CD15+ and CD56+ cells showed decreased miR-155 and CD3+ cells showed decreased miR-181. The difference in the immunoregulatory miR levels at various stages of uveal melanoma may help develop a blood biomarker in the near future.

Glaucoma

Glaucoma is characterized by elevated pressure in the eye; unrelenting pressure causes optic nerve damage starting with peripheral vision loss and ending up with blindness. Intraocular pressure is critical for the normal globe physiology. miRs have been found to play roles in glaucoma pathogenesis either directly or indirectly. The role of miRNAs in the molecular mechanisms of ECM synthesis offers great hope for therapies of glaucoma. miR-183 was found to alter the intergrin-β_1 expression and thereby to affect trabecular meshwork (TM) physiology ending up in glaucoma [77]. The miR-29 family regulates the ECM synthesis in the TM [78]. The alterations in the ECM proteins by the miR-29 family bring about changes in intraocular pressure. It was found that miR-29b negatively regulates ECM proteins and its regulators under chronic oxidative stress in human TM cells affecting the ECM homeostasis. miR-29b expression decreased significantly under chronic oxidative stress resulting in the upregulation of ECM genes. This increased expression of ECM genes changes the outflow pathway causing increased ECM deposition and cell loss. All these events got reversed when an miR-29b mimic was transfected during chronic oxidative stress. Such an expression of miR-29b offers great therapeutic potential for mimics in the future. TGF-β_2 differentially regulates the miR-29 family in the ECM synthesis [79]. miR-29a and miR-29b were found to be increased and decreased significantly on TGF-β_2 induction, respectively, with no effect on the miR-29c. This differential expression of miR-29a and miR-29b through TGF-β_2 may be essential for the ECM synthesis and deposition while miR-29c was indirectly involved in ECM production. The upregulation of miR-29b suppresses the expression of ECM components. Apart from SMAD3, SMAD7 and p38, recent research suggests that there may be other players involved in the modulation of miR-29b [80, 81]. Such complexities involving the

Table 1. miRNAs and their targets in eye disorders

Disorder	miRNAs	Site of expression	Targets	Action	References
Cataract	*let-7c*, miR-29a, miR-29c and miR-126	Lens epithelial cells (LECs) in Shumiya cataract rats	*TGF-β*, *FGF* and *PDGF*	Downregulation leads to cataract	Kubo et al. [20], 2013
Cataract	miR-551b	Lens epithelial cells in Shumiya cataract rats	*Prdx6*	Upregulation leads to cataract	Kubo et al. [20], 2013
Cataract	miR-218, miR-195 and miR-452 in abundance	Aqueous humor	Multiple targets in many pathways	Multifunction	Dunmire et al. [18], 2013
Secondary cataract	miR-184 and miR-204	Mouse capsular bag culture model	*Bin3*, *Meis2* and *Runx2*	Lens differentiation, regeneration and cataract etiology	Hoffmann et al. [24], 2012
Secondary cataract	miR-204-5p	Human capsular bag model	*Smad4*	Human posterior capsule opacification	Wang et al. [25], 2013
Secondary cataract	miR-26b	LECs/SRA01/04 cells	*Smad4*	LEC proliferation, migration and EMT processes	Dong et al. [26], 2014
Myopia	miR-328	Retinal pigment epithelial cells (RPE) and sclera cells	*Pax6*	Regulation of PAX6	Chen et al. [29], 2012
Retinoblastoma	miR-17-92 cluster	Human and murine retinoblastomas and retinoblastoma cell lines	Unknown	Tumorigenesis	Conkrite et al. [33], 2011; Sage and Ventura [34], 2011
Retinoblastoma	miR-365b-3p	Human RB cell lines	*Pax6*	Tumor suppressor	Wang et al. [32], 2013
Retinoblastoma	Hsa-miR-494, -let-7e, -513-1, -513-2, -518c*, -129-1, -129-2, -198, -492, -498, -320, -503, -373*	Human retinoblastoma	Unknown	Upregulated in retinoblastoma	Zhao et al. [41], 2009
Age-related macular degeneration	miRNA-9, miRNA-125b, miRNA-146a and miRNA-155	Retina	*CFH*, *15-LOX*, *SYN-2* and *TSPAN12*	Coordinate innate immune and inflammatory signals	Lukiw et al. [45], 2012
Age-related macular degeneration	miR-146a and miR-146b-5p	Human RPE	Unknown	Regulation of NF-ƙB pathway	Kutty et al. [51], 2013
Age-related macular degeneration	miR-23a	RPE	*Fas*	Early pathogenesis of AMD, prevents oxidative damage	Lin et al. [61], 2011
Pterygium	miR-200 family	Conjunctiva	*FN1*	Regulation of EMT	Engelsvold et al. [68], 2013
Uveitis (Behçet's disease and Vogt-Koyanagi-Harada disease)	miR-155	Uvea	*TAB2*, *IL-6* and *IL-1β*	Pathogenesis of uveitis	Zhou et al. [69], 2012
Uveal melanoma	miR-124a	Uvea	*CDK4*, *CDK6*, cyclin D2 and *EZH2*	Tumor suppressor	Chen et al. [74], 2013
Posterior uveal melanoma	miR-182	Uvea	*MITF*, *Bcl2* and cyclin D2	Tumor suppressor	Yan et al. [75], 2012
Experimental autoimmune uveoretinitis	miR-142-5p and miR-21	Ocular tissues	*IL-17*	Regulates the gene expression in immune system	Ishida et al. [73], 2011

Table 1 (continued)

Disorder	miRNAs	Site of expression	Targets	Action	References
Glaucoma	miR-183	Trabecular meshwork	Integrin-β_1	Physiology of trabecular meshwork	Li et al. [77], 2010
Glaucoma	miR-29 family	Trabecular meshwork	ECM proteins	ECM homeostasis	Luna et al. [78], 2009; Villarreal et al. [79], 2011
Glaucoma	miR-24	Trabecular meshwork	*Furin*	Flow pathway	Luna et al. [82], 2011
Fuchs' endothelial corneal dystrophy	miR-29 family	Cornea	ECM proteins	ECM homeostasis	Matthaei et al. [86], 2014; Wang et al. [87], 2012

miR-29 family in the ECM pathways necessitated further studies in the various pathophysiology models of glaucoma in the development of glaucoma therapeutics. In human TM cells, cyclic mechanical stress alters the TGF-β_1 levels. miR-24 plays an important role in the flow pathway [82]. miR-24 was found to be upregulated by TGF-β_1. As TGF-β_1 was found to increase, the outflow pathway gets triggered and leads to increased intraocular pressure. The increased level of miR-24 by TGF-β_1 is essential to contain the TGF-β_1 levels during cyclic mechanical stress which is brought about by the targeting of furin by miR-24. Yet another study identified many miRNAs in glaucoma with Hsa-let-7b-3p as the most prevalent in aqueous humor [83]. miRNA profiles not only reveal the specific pathophysiology, but also offer the diagnosis. Further deep understanding will aid in the diagnosis of glaucoma subtypes, pathophysiological stages and specific drugs for those stages.

Fuchs' Endothelial Corneal Dystrophy

Fuchs' endothelial corneal dystrophy (FECD) is the degenerative changes that happen in the innermost endothelial layer of the cornea progressing to corneal edema and cause blindness [84, 85]. It is a primary corneal disease with a reduced number of endothelial cells. miRNAs were found to be differentially expressed in FECD when compared with normal endothelium [86]. Eighty-seven miRNAs were significantly downregulated but none got upregulated in FECD. Of the miR-29 family, an ECM homeostatic modulator, 3 miRs – miR-29a-3p, miR-29b-2-5p and miR-29c-5p – were downregulated to a greater extent, and miR-29a-3p was the most deregulated in FECD endothelium. Decreased miR-29 expression increased the collagen I, collagen IV and laminin mRNA and protein levels in FECD [87]. This increase in ECM proteins may cause the increased Descemet membrane thickness. In addition to the miR-29 family, miR-184 was found to have a role in FECD when mutated or downregulated. In particular, when miR-184 was mutated in the seed region, it imparted damage to the endothelial layer of the cornea leading to FECD and EDICT syndrome. In a study on renal fibrosis, the ρ-associated kinase inhibitor fasudil and the angiotensin receptor blocker losartan increased miR-29 expression and brought beneficial effects [88]. A case study on FECD treatment by a ρ-associated kinase inhibitor administered in eyedrops resulted in corneal clarity with improved vision in the follow-up after treatment. It is plausible that the ρ-associated kinase inhibitor acts through miR-29, and this needs further investigation to be confirmed. Hence this inhibitor and the blocker were found to increase miR-29 expression and can be used as therapeutics in FECD as evidenced in a renal fibrosis study. The miRNAs and their targets involved in eye disorders are summarized in table 1.

Conclusion and Perspectives

Since the discovery of the first miRNA, almost 2 decades have gone by; there is a huge accumulation of published information on miRNAs. Although research on miRNA biology in the eye is still in its infancy, this aggregation of a huge volume of data on miRNAs is successfully advancing the field of eye miRNA biology. With the vast array of published research works done on the eye in various organisms, we found that miRNAs influence numerous events in disease processes of the eye. These new findings shed light onto the complex system of miRNA in eye disorders. These studies provided the immense insight that the miRNA agonists and antagonists could be the future therapeutics in eye disorders even though there

is a lack of efficient delivery methods. More miRNAs will emerge in future studies, and these have to be validated in detail. Though a lot of research work has been done on miRNA for the past 2 decades, still lots of challenges remain ahead of us. Some of them are: identification of mRNA targets/miRNA functions in eye diseases, understanding miRNA function at the organismal and tissue levels, regulation of miRNA biogenesis, function and turnover and mechanism of inhibition of protein synthesis. The complexity of these processes increases with the accumulation of new findings. Still there are many gaps to be bridged, e.g. in characterizing the functions of miRNAs in the visual system. Various studies accentuate the demand to combine miRNA expression studies on cellular processes in order to thoroughly understand eye diseases. There are lots of big challenges ahead of us to unravel the complex regulatory mechanisms of miRNA in the disorders of the eye. We now possess well-developed modern technologies with which we can reach this end. Discovering ever more miRNA regulatory circuits involved in normal cellular processes and disease development will help us to eventually develop diagnostic and therapeutic strategies for various eye diseases. Although miRNA offer both diagnostic and therapeutic opportunities, we are still in search of suitable methods that are affordable and simple.

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Disclosure Statement

The authors have no conflicts of interest to disclose.

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