MicroRNA Expression Patterns Involved in Amyloid Beta–Induced Retinal Degeneration

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PURPOSE. Dry age-related macular degeneration (AMD) is characterized by the accumulation of drusen under Bruch’s membrane, and amyloid beta (Aβ) is speculated to be one of the key pathologic factors. While the detrimental effects of Aβ on retinas have been widely explored, Aβ-induced epigenetic regulatory changes have yet to be fully investigated. We therefore aimed to identify the microRNA (miRNA) expression profiles in an Aβ-induced mouse model of retinal degeneration.

METHODS. C57BL/6 mice were intravitreally injected with Aβ1-40 or PBS and the eye tissues were collected for hematoxylin and eosin (H&E) staining, apoptosis immunofluorescence staining, and miRNA profiling. After filtering, 10 miRNAs and their target genes were chosen for quantitative RT-PCR (qRT-PCR) confirmations. Pathway analyses were employed for further bioinformatic analyses.

RESULTS. Hematoxylin and cosin–stained sections of retinal pigment epithelium (RPE)/neural retina tissue demonstrated degenerative alterations, and immunofluorescence testing revealed apoptosis within the retina after Aβ treatments. MicroRNA profiling revealed 61 miRNAs that were differentially expressed between the model and the control group. Among these, 38 miRNAs were upregulated (fold change > 1.5, P < 0.05) and 23 miRNAs were downregulated (fold change < 0.667, P < 0.05). Figure of the 10 selected miRNAs (miR-142, miR-216, miR-155, miR-223, and miR-433) as well as several key target genes (CFH, IGF-IR, c-MET, and ABCA1) were confirmed by qRT-PCR analyses.

CONCLUSIONS. Our study is the first to profile the miRNA expression patterns and suggests that Aβ accumulation could lead to relevant biochemical alternations such as complement activation, barrier impairment, apoptosis, and positive feedback of Aβ production.

Keywords: amyloid beta, retina, microRNA, age-related macular degeneration

Age-related macular degeneration (AMD) is the leading cause of untreatable blindness in Western countries.1,2 Anti-VEGF therapy has been greatly effective for the wet/neovascular form of AMD; however, no effective therapies are available for the dry/atrophic form.3 Dry AMD is characterized by the accumulation of drusen under Bruch’s membrane,4 and amyloid beta (Aβ) is a main component of these drusen.5 Amyloid beta metabolic dysregulation is also hypothesized to contribute to Alzheimer’s disease (AD). Interestingly, several transgenic mouse models overexpressing Aβ, such as 5XFAD and nephrilysin−/− mice, develop retinal pigment epithelial (RPE) degeneration and basal deposits similar to dry AMD patients.6,7 In addition, several studies have reported on the potential of anti-Aβ therapeutic strategies and their impact on functional recovery in experimental AMD rat models, implying the vital role of Aβ in the retina.8,9

Recently, intravitreally injected Aβ1-40, instead of the more AD-specific Aβ1-42 peptide, has been used to produce an in vivo model of Aβ stimulation on the retina.10 Aβ1-40 is the principal form found in the ocular fluids11 and drusen.5 The gene expression profile in response to Aβ1-40 stimulation in hRPE cells has been reported.12 After intravitreal Aβ injection, Aβ was found across all neuropil and extracellular compartments of the neuroretina layers, as indicated by diffuse staining with the Aβ–specific antibody 4G8.13 Positive Aβ staining was also observed in the RPE as discrete intracellular vesicles because the RPE continuously phagocytoses the outer segment discs.13 This model could partially simulate how Aβ disturbs the normal
miRNA Profiles in Aβ-Induced Retinal Degeneration

physiological processes and influences the morphologic appearance of the retina and RPE/choroid in AMD eyes.

Studies have demonstrated the detrimental roles of Aβ on the structure, homeostasis, properties, and functions of the retina. It has been made clear that Aβ can accelerate cell stress and the aging process, vital pathologic processes in AMD initiation and progression. Furthermore, elevated Aβ deposits can induce a proinflammatory microenvironment and subsequently activate the complement system. Interestingly, Aβ can also induce the upregulation of angiogenic factors as well as break down the outer barrier of retinas, which might play a role in subtype conversion. However, the epigenetic changes caused by Aβ have not been fully investigated.

MicroRNAs (miRNAs) have emerged as a new therapeutic direction for several diseases, including neurodegenerative disorders. MicroRNAs are single-stranded short (21–23 nucleotides long) noncoding RNAs that are involved in posttranscriptional modulation. Each miRNA can specifically inhibit its target mRNA's function by binding to the complementary site. MicroRNAs are crucial in normal biological development and metabolism. In disease states, their expression levels change. Balancing the level of miRNAs is a new area of therapeutic targeting. Furthermore, the miRNA metabolism in neurons, such as photoreceptors, is higher than in other cell types. Prior studies showed differential levels of secreted miRNAs (miR-301-3p, -361-5p, and -424-5p) in wet AMD patients, and neurovascularization could be repressed by certain miRNA targets, including microRNA-24. Kaneko et al. found that miRNA processing of the enzyme Dicer1 was reduced in the RPE of humans with geographic atrophy (GA) and that conditional Dicer1 ablation induced RPE degeneration. However, studies on the role of miRNA regulation in Aβ stimulation in the retina are limited.

MicroRNA microarrays facilitate the analysis of miRNA expression patterns and quantification of the levels in a high-throughput screening manner. We therefore aimed to profile the miRNA expression changes in Aβ-induced retinal degeneration using microarray chips, information that has not been previously reported. The observations of our study may provide clues for the pathologic regulation of miRNAs in the Aβ1-40-induced retina degeneration model and provide potential interventional targets for further research.

**MATERIALS AND METHODS**

**Amyloid Preparation**
Aβ1-40 (GL Biochemistry, Shanghai, China) was prepared as previously described. Briefly, Aβ peptides were dissolved in double-distilled H2O at a concentration of 28 μg/μL and then immediately added to phosphate-buffered saline (PBS) to obtain a final concentration of 2.8 μg/μL. The mixture was incubated at 37°C for 7 days prior to use. The aggregated states were checked by electron microscopy.

**Animal Model and Treatment**
Two-month-old male C57BL/6 mice were supplied by the Laboratory Animal Center at the Shanghai First People’s Hospital, raised on a standard rodent diet, and housed with a 12-hour light/dark cycle. The experiments strictly conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. All animal experiments were approved by the Ethics Committee of Jiao Tong University, Shanghai, China.

To explore the Aβ-induced miRNA expression profile, six mice were anesthetized with 1.5% sodium pentobarbital (100 μL/20 g intraperitonally) and underwent a single unilateral intravitreal injection (IVL) with Aβ1-40 peptides (14 μg/5 μL) in PBS, which is comparable to other in vivo studies. Briefly, the IVL was administered under a dissecting microscope (stereo dissection microscope, SMZ 1000; Nikon, Tokyo, Japan) using a 32-gauge Hamilton needle and syringe (Hamilton, Reno, NV, USA) to deliver 5 μL oligomeric Aβ1-40 peptides. Six age-matched controls received 5 μL PBS in the same manner. The mice were kept for 48 hours, and then their eyes were enucleated and preserved in 4% paraformaldehyde in Dulbecco’s PBS (DPBS; Invitrogen, Carlsbad, CA, USA) under intraperitoneal anesthesia.

**Hematoxylin and Eosin (H&E) Staining**
Tissue sections were embedded in paraffin and then cut to a thickness of 4 μm as previously described. Sections from within 200 μm of the optic disc were chosen for processing because they were assumed to have a similar thickness. Briefly, after deparaffinization and rehydration, sections were treated with sodium citrate buffer (pH 6.0, 10 minutes) and blocking buffer (10% normal goat serum, 0.05% Triton X-100 in PBS; 1 hour, room temperature). Then, the specimens were stained with H&E. The slides were dehydrated and placed on a coverslip.

**Annexin V-FITC/PI Assay**
Enucleated eyes were immediately fixed with 10% formalin and embedded in paraffin. Apoptosis was evaluated using an Annexin V-FITC kit (Beyotime Institute of Biotechnology, Haimen, Jiangsu, China) according to the manufacturer’s protocols. Annexin V-FITC was used to reveal the level of apoptosis using a fluorescence microscope. The sections were stained with propidium iodide (PI) (1:3000; Molecular Probes, Eugene, OR, USA) for 30 minutes to determine the retinal cell distribution.

**Total RNA Extraction**
Total RNA extraction and quantification were performed as previously described. Briefly, total RNA was extracted from tissue using the TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA). The RNA samples were treated with DNase I (Sigma-Aldrich Corp., St. Louis, MO, USA) and then quantified on a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA).

**miRNA Microarray and Analysis**
Neuroretina and RPE/choroid tissues were all collected from multiple eyes for total RNA extraction. RNA labeling and hybridization were performed using a miRNA microarray chip from KangChen Bio-Tech (Shanghai, China) following the manufacturer’s protocol. Briefly, the total RNA samples from both the Aβ-injected and vehicle groups were labeled using a miRCURY HY3 Power labeling kit (Exiqon, Vedbaek, Denmark). Hybridization was performed on a miRCURY LNA Array Manual (Exiqon). Finally, images of the microarray were scanned on an array scanner (Axon GenePix 4000B; Molecular Devices, Sunnyvale, CA, USA) and the signal intensity values were quantified.

The results were subsequently analyzed using GenePix Pro 6.0 software (Axon Instruments, Sunnyvale, CA, USA). Volcano plot filtering was employed to identify the significantly differentially expressed miRNAs between the control and treatment groups. The thresholds we used to identify up- or
downregulated miRNAs were fold change greater than 1.5 and P value less than 0.05.

Quantitative Real-Time PCR (qRT-PCR) Validation of miRNA Expression

MicroRNAs were chosen for further qRT-PCR analysis based on the following criteria: an up- or downregulated fold change greater than 1.5 as screened by the microarray; miRNA star forms were excluded; and the top five up- or downregulated miRNAs were chosen after filtering. Total RNA was isolated using the miRcute miRNA Isolation Kit (Tiangen, Beijing, China). The poly(A) tail was added to the 5' terminal of the miRNA, and the first-strand cDNA was synthesized using a miRcute miRNA First-strand cDNA Synthesis Kit (Tiangen). The target cDNA was amplified using a miRcute miRNA qPCR Detection Kit (SYBR Green, Tiangen) according to the manufacturer’s protocol. The primers used for the 18S sequence were 5'-GCTGTCAACGATAAGCTACCTA-3' (forward) and 5'-GGCCATGT-GTA-3' (reverse).

The RT-PCR reactions for 18S and the 10 miRNAs were performed using a real-time PCR detection system (Eppendorf, Germany) with 40 PCR cycles (denaturation at 95°C and then annealing at 57°C for 10 seconds). SYBR Green RealTime PCR Master Mix (Toyobo, Osaka, Japan) was used to demonstrate any change in the fluorescence intensity. The relative expression for each mRNA was calculated using the 2^(-ΔΔCt) method. The miRNA primers used are listed in Table 1.

Biologic Functional Analyses

All of the miRNAs confirmed by PCR were included in the biologic functional analyses. We used three databases—miRBase (http://www.mirbase.org/, in the public domain), miRanda (http://www.microrna.org/, in the public domain), and TargetScan (http://www.targetscan.org/, in the public domain) to specify the potential miRNA target genes. The targets were then subjected to functional analysis via Kyoto Encyclopedia Genes and Genomes (KEGG) pathways. A P value less than 0.05 was considered to be statistically significant.

Quantitative Real-Time PCR Validation of mRNA Expression

Quantitative RT-PCR was conducted according to our former published studies.3,2 Briefly, the first-strand cDNA was synthesized from 2 μg total RNA in a 20-μg mixture according to the protocol for the RT Master Mix (Takara Bio, Inc., Dalian, China). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used to normalize all of the samples. Respectively, the specific sense and antisense primer sequences used were as follows: EF1, 5'-CTCCGTGTTGAGGCGCTTCTT-3' and 5'-GTATGTAACCTCTCCTGGGTTG-3'; ABCA1, 5'-CAGAAAGGAGGTCTGCAGAGAT-3' and 5'-GGGGCCAGCCCGTCACTGATT-3'; c-MET, 5'-ACCTCAGCAATGTCAGCACCA-3' and 5'-GGCCATGATGTGATCTTGGG-3'; IGF-1R, 5'-GGGAGGTCCCTGGAGGACAGA-3' and 5'-GGATACCTCAGGACAGAGAGA-3' (reverse).

The RT-PCR reactions for 18S and the 10 miRNAs were performed using a real-time PCR detection system (Eppendorf; 40 cycles of 95°C for 15 seconds, 58°C for 30 seconds, and 60°C for 45 seconds). The SYBR Green RealTime PCR Master mix (Toyobo) was used to demonstrate any change in the fluorescence intensity. The relative expression for each mRNA was calculated using the expression 2^(-ΔΔCt) method.

Statistical Analysis

Each experiment was performed in triplicate, and the data are expressed as the mean ± SEM, where applicable. The statistical significance of differences was evaluated using Student’s t-test. A P value less than 0.05 was considered statistically significant.

RESULTS

Aβ-Induced Retinal Degeneration and Mild Apoptosis in the Outer Retina

To investigate the pathologic effects of Aβ1-40 on the retina, H&E-stained sections of RPE/nerial retinal tissue were examined (Fig. 1). The PBS-injected group showed normal-appearing retinas (Fig. 1A), while the Aβ1-40-injected mice showed retinal degenerative changes (Fig. 1B). These alterations included hyperpigmentation (red triangles), hypopigmentation (white arrows), and obvious disorganization of the outer nuclear layer (ONL) (Fig. 1D).

We further explored the effect of Aβ1-40 on retinal apoptosis. Immunofluorescence assays revealed double-positive staining (Annexin V and PI) in the inner nuclear layer (INL) and ONL (Fig. 2). Apoptosis (Annexin V+) was found in the INL and ONL only in the experimental group compared with the control group 48 hours after Aβ injection (indicated by white arrows in Fig. 2H). It was spatially consistent with the strong immunoreactivity of Aβ1-40 found in a previous study.10

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**Table 1. MiRNA qRT-PCR Primers Used in the Study**

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<td>MIMAT0005376</td>
<td>mmu-miR-298-5p</td>
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* Reverse transcription primer: 5'-GCTGTCAACGATAAGCTACCTA-3', according to the cDNA synthesis kit.
**FIGURE 1.** Aβ-induced morphologic changes of the retina. Hematoxylin and eosin (H&E)-stained paraffin sections of the retina and RPE layer from control mice (A) and Aβ1-40 injected mice (B). (C, D) Magnified portions of images (A, B), respectively (enclosed in the black boxes). Disorganization of the outer retina and thinning of the IS/OS layer, especially the OS layer, were found. RPE changes such as hypopigmentation (white arrows), hyperpigmentation (red triangles), and irregular shapes were also found. GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; IS, inner segment; OS, outer segment. Scale bars: 50 μm (A–D).

**FIGURE 2.** Aβ-induced apoptosis within the retina. Immunofluorescence assay via an Annexin V-FITC (green) and PI (red) staining method was employed to test apoptosis on day 2 in the control (A–D) and experimental groups (E–H). Images (D, H) are magnified portions of images (C, G), respectively (enclosed in the white box). Apoptosis (Annexin V⁺) was found only in the ONL and INL in the experimental group compared with the control group 48 hours after Aβ injection (white arrows). Scale bars: 50 μm (A–H).
miRNA Profiling

The volcano plot (Fig. 3) aimed to visualize the significantly differentially expressed miRNAs (red point). The details of the significant data of the differentially expressed miRNAs on the scatter plot demonstrates the differential expression between Aβ-injected mice and the PBS control groups. The horizontal green line and the vertical green lines represent the cutoff of 1.5-fold change up- or downregulated, and the horizontal green line represents the cutoff of P value significance (P < 0.05). In this manner, the red points represent the significantly differentially expressed miRNAs.

miRNA Profiling

To verify the chip results for the miRNAs of interest, RT-PCR was employed. According to the included criteria described in the Methods section, 10 miRNAs underwent confirmation, including the top 5 miRNAs that were overexpressed (miR-142, miR-183-3p, miR-216, miR-155, miR-223) and the top 5 miRNAs that were underexpressed (miR-744, miR-433, miR-1224, miR-2136, miR-298). Finally, five miRNAs (miR-142, miR-216, miR-155, miR-223, and miR-433) were confirmed by qRT-PCR. However, the remaining five miRNAs showed nonsignificant differences (Fig. 4).

RT-PCR Confirmation of the miRNA Microarray Results

Table 2. The miRNAs Displaying 1.5-Fold or More Change in Expression in Aβ-Injected Mice After 48 Hours as Determined by Microarray Analysis

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<th>P Value</th>
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Table 2. The miRNAs Displaying 1.5-Fold or More Change in Expression in Aβ-Injected Mice After 48 Hours as Determined by Microarray Analysis

**Legend:**
- **ID:** miRNA ID
- **miRNAs Upregulated:** miRNAs upregulated
- **miRNAs Downregulated:** miRNAs downregulated
- **Fold Change:** fold change
- **P Value:** P value

**Functional Analysis of miRNAs and Target Gene Confirmation**

Five miRNAs with the highest fold changes among the increased and decreased miRNAs were chosen for further bioinformatics analysis. We employed three databases to conduct the analyses to improve the accuracy of our predictions. In the miRanda, miRBase, and TargetScan databases, 11,968, 3886, and 1057 target genes were identified, respectively; 174 genes were common to the three databases and were subjected to further pathway analyses (Fig. 5).

Pathway analysis is a functional analysis mapping genes to KEGG pathways. The P value (Fisher P value) denotes the significance of the pathway correlated with the conditions. The lower the P value, the more significant the pathway. The pathway analysis demonstrated the top 10 most related pathways and the targeted genes of the regulated miRNAs in Table 3. From the existing literature, we found that the ubiquitin-mediated proteolysis pathway (UPP) and the mitogen...
quantitative real-time PCR. P1224, miR-200, and miR-298 expression levels remained nonsignificantly changed. * 

Genes and Genomes pathway analysis indicated the top 10 differentially expressed by qRT-PCR. Kyoto Encyclopedia miRNAs and their target genes were identified as being 

38 upregulated and 23 downregulated miRNAs (CFH, c-MET, ABCA1, and ATP-binding cassette transporter A1 (ABCA1) were the opposite of the expression levels of the corresponding miRNAs, consistent with the reported research on other tissues and experimental models.

**DISCUSSION**

This is the first study to demonstrate the differentially expressed miRNA pattern in early AMD pathogenesis in an Aβ1-40-induced retinal degeneration mouse model. In this study, we identified 38 upregulated and 23 downregulated miRNAs using a profiling microarray. In total, 5 of the 10 selected miRNAs and their target genes were identified as being differentially expressed by qRT-PCR. Kyoto Encyclopedia Genes and Genomes pathway analysis indicated the top 10 most related pathways in this Aβ-induced retinal degeneration model.

Age-related macular degeneration is a multifactorial disease involving a range of complex genetic and environmental influences. Reflecting this complexity, there is currently no single animal model that develops all of the signs of AMD in a progressive manner, including ours. Intravitreally injected Aβ is an acute retinal degeneration model rather than a progressive, gradual pathologic change like AMD. Nevertheless, our model has contributed to our understanding of how Aβ affects the miRNA profiling and target genes and their contribution to the etiology/pathogenesis of AMD.

In our preliminary experiment, we found that the levels of Aβ in the retina and RPE were most abundant on day 2, as were the levels of apoptosis and inflammation activation. This phenomenon is consistent with previous research and published studies. Liu et al. showed that the immunoreactivity of Aβ1-40 undertaken with 4G8 revealed immunoreactivity in the neuropil and extracellular compartments throughout all retinal layers on day 1 and less on day 4. For the RPE, there were discrete intracellular vesicles possibly representing phagocytosed outer segment discs in the Aβ group compared with the reverse peptide group on day 1, while there were no vesicles on day 4. This time point is also consistent with Anderson’s and Walsh’s studies, which indicated that neural cell death occurred subsequent to the 48-hour window. In the study by Bruban et al., genes related to inflammation, oxidative stress, and apoptosis were mostly changed on day 3. In a study over a longer period of observation, Liu et al. found that Aβ1-40 could stimulate the upregulation of IL-6 genes in the RPE/choroid and the neuroretina on day 1 and 4, but the changes became nonsignificant by later time points on days 14 and 49. Interleukin-6 is an inducer of acute-phase protein production, and its serum level has been associated with the progression of AMD, as has the prototypic cytokine TNF-a and proapoptotic gene XAF1. Therefore, we assumed that the changes in miRNA expression were probably significant by 2 days after the Aβ1-40 induced retinal degeneration model based on the above evidence. In our further research, we will perform longer observations to confirm our assumptions.

For the selection of the drug supply, most similar experiments adopted intravitreal or subretinal injection of Aβ instead of intravenous injection. Intravitreal or subretinal injection can stimulate local pathogenesis to AMD more effectively than intravenous injection. In a study by Howlette et al., diffuse staining of Aβ across several layers of
the retina extending as far as the outer plexiform layer was noted after intravitreal injection. Thus, there is no doubt that intravitreally injected Aβ can reach the whole of the retina and RPE/choroid layer and induce downstream changes. Therefore, this may be a useful mouse model with which to study local miRNA expression changes after Aβ stimulation.

Of note, the disorganization of the outer retina in the model group was more severe in our study compared to other similar studies. Studies using Long-Evans rats instead of C57BL/6 mice showed no morphologic changes or retinal cell apoptosis on retinal neurons in contrast to ours. Interestingly, in studies using Sprague-Dawley rats there were no morphologic changes, but retinal cell apoptosis appeared as early as day 2. We attributed the varied morphologic changes and apoptosis state mainly to the diverse Aβ responses between different species or strains. In the studies using C57BL/6 the retina showed similar morphologic changes like disorganization of the photoreceptor nuclei and alteration of the inner segment (IS) and outer segment (OS) in the model group but these were still weaker than ours. Dinet et al. reported that very few apoptotic photoreceptors observed by day 3 became numerous in the retinas on day 7. In our study, neuron apoptosis appeared on day 2. We attributed the differences to the different drug supply. In all these studies, the investigators conducted subretinal other than intravitreal injections with Aβ peptide, and well-organized neural retina tissues were analyzed a 300-μm distance from the center of the injection site to avoid confusion between the effects induced by the syringe itself and those induced by the injected Aβ.

The concentration of Aβ in the observation site was unavoidably decreased as it is 300 μm away from the injection site. As for our model, intravitreal injections were delivered and changes were observed on day 2, a time point at which the Aβ levels were most abundant. So the effect of Aβ on retina might be higher in our model. In conclusion, our model showed generally more severe changes of morphologic and retina cell apoptosis than the former studies, and we think this is mostly attributed to the different species and drug delivery methods.

Our chip results and subsequent confirmation runs provided insights into the miRNA dysregulation focused on dry AMD instead of on the wet type. In our study, we found that Aβ could upregulate miR-155 levels in the retina. These observations were consistent with the findings in AMD retinas. The previous authors found that the progressive, pathogenic increases in specific miR-155 could bind to the entire 232-nucleotide 5′-untranslated region (5′-UTR) of CFH and downregulate its expression. The deficiency of CFH could drive inflammatory neurodegeneration. In our study, we found for the first time that Aβ can induce the upregulation of miR-155, and the expression of CFH was correspondingly downregulated, implying a vital role of Aβ in complement system dysregulation by specific miRNA differential expression.

**Table 3. Top 10 Pathways of the 10 Most Dysregulated miRNA Targets**

<table>
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<th>Definition of the Pathway, Mus musculus, Mouse</th>
<th>Fisher P Value</th>
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<td>FGF7/FGFR1/</td>
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<td>Small cell lung cancer</td>
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**Figure 6.** miRNA expression levels of downstream genes of selective miRNA RT-PCR data in Aβ-induced retinal degeneration and PBS-injected controls. Values are expressed as the fold change ± SEM relative to that of the control group. (A) Expression of c-MET, CFH, IGF-1R, and ABCA1 in the neuroretina. (B) Expression levels of c-MET, CFH, IGF-1R, and ABCA1 in the RPE/choroid. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001; n = 5 per group. miRNA, microRNA, qRT-PCR, quantitative real-time PCR.
It has been proven that Aβ can induce RPE barrier impairment. In our study, we found one possible way for barrier dysregulation by Aβ through the downregulation of miR-433. Its downregulation could induce an epithelial-mesenchymal transition that impairs RPE barrier function. Studies have also shown that miR-433 can activate c-MET/AKT/GSK-3β/Snail pathway, resulting in reduced cell-cell junctions. We also confirmed that the expression of c-MET is elevated in the RPE/choroid tissue. The RPE barrier functions as the outer barrier of the eyes. Our study indicated that overexpression of miR-433 might be a possible way to alleviate the impairment of the RPE barrier.

We found that the level of miR-233 increased after Aβ stimulation. Studies have reported that increasing miR-233 levels can induce apoptosis and activate the unfolded protein response (UPR). Specifically, miR-233 can target IGF-1R, which has a protective effect on Fas-induced hepatocyte apoptosis and liver injury. Although these studies were performed in other tissues, they have important implications in pathologic progress including apoptosis and UPR response. In our study, we confirmed regulated miRNAs and their important functions related to AMD pathologies are summarized in Figure 7.

In summary, our results provide the first foundation for global miRNA expression pattern analysis in Aβ-related retinal.

Pathway analyses indicated that the miRNA target genes might be primarily involved in the ubiquitin-mediated proteolysis and the MAPK signaling pathways based on our chip results. These two pathways play important roles in AMD pathogenesis. Proper function of the UPP is crucial for protein-selective degradation. In contrast, age- or stress-induced impairment of UPP function may lead to the accumulation of abnormal proteins, such as drusen, in the retina. In addition, oxidative stress is considered to be a very important factor in dry AMD that can activate downstream pathways, including the MAPK pathway.

Interestingly, although the inflammatory pathway was not implicated in informatics analyses, genes in the pathway such as RELA, CSF1R, IKKBE, and RRAS all appeared in the predictions (Table 3). Chronic inflammation caused by Aβ has been widely reported. RelA is a subunit of nuclear factor-kappa B (NF-kB), a key regulator of the inflammation response. The IKK kinase inhibitor of kβ kinase epsilon (IKKBE) is an IkB kinase family member that can also activate the AKT and NF-kB pathways; IKKBE is also involved in the interleukin-1 (IL-1) signaling pathway. Our prior study confirmed that IL-1β could mediate RPE inflammation in an A2E-induced cell model. Colony-stimulating factor-1 receptor (CSF-1R) plays an important role in innate immunity and inflammatory processes in which CSF-1 and interleukin-34 (IL-34) compete for binding. Recent studies have revealed that CSF-1R signaling directly controls retinal microglia inflammation and might play a relevant role in neurodegenerative diseases such as AMD.

Cancer in pathways ranked first in the informatics prediction. This result might be attributable to common pathways shared with Aβ-induced changes such as apoptosis, angiogenesis, vascular homeostasis, regeneration, cell adhesion, and neuronal axon guidance. Some pathways, such as hepatitis B and salmonella infection, seemed not to be correlated with our model at first glance. We propose that they were suggested for several possible reasons. First, certain similar miRNA changes are shared after hepatitis B (HBV) or salmonella infection and in our model. As reported, miR-27b-5p and mir-23b-3p were upregulated after HBV infection, and t-26b-5p, mir-534-3p, and mir-27b were upregulated after salmonella infection. These miRNAs show the same tendency of changes after Aβ stimulation. Secondly, colonizing HBV infection, the host genome integrates the HBV DNA and produces viral proteins such as HBx, hepatitis B surface antigen (HBsAg), and HBeAg. These viral proteins can trigger cancer-related genes and motivate several signaling pathways including genetic instability, inflammatory, and host immune responses. These pathways, especially the proliferation pathways and apoptosis pathways, were included in our model. Thirdly, as we made predictions with limited confirmed miRNA data, some possible pathways might be missing as an inevitable undesired result. This is also a limitation of our study.

Our data partially explain the dysregulation of miRNAs induced by Aβ and how the drusen component Aβ affected the retina by regulating miRNAs. Furthermore, the confirmed up- or downregulated miRNAs might act as therapeutic targets, but this possibility requires further confirmation. MicroRNA regulation was deemed to be species specific. Hence, great caution should be exercised when the results obtained from mice are translated to human disease. Nevertheless, our preliminary results are valuable, as they indicate several directions with great promise.

In summary, our results provide the first foundation for global miRNA expression pattern analysis in Aβ-related retinal degeneration.
degeneration to provide novel therapeutic targets for diseases such as AMD. We identified 61 miRNAs that were differentially expressed between the Aβ-injected and the control groups, including 38 upregulated and 23 downregulated miRNAs. The confirmed miRNAs showed great functional roles in biochemistry processes such as complement activation, barrier impairment, apoptosis, and positive feedback for Aβ accumulation. According to the KEGG pathway analyses, we found that the target genes were primarily involved in ubiquitin-mediated proteolysis and the MAPK signaling pathways in this model, which play important roles in the initiation and progression of dry AMD. Although inflammatory pathways were not listed, several important preinflammatory factors or regulators were also suggested. Because miRNAs are endogenous molecules with a very low nonspecific immune response, balancing abnormal miRNA expression levels might be a relatively safe way to alleviate the detrimental effects of drusen deposits of Aβ on the retina.

Acknowledgments

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References


