Editorial

The potential for microRNA-based therapeutics in retinal disorders

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Mammalian vision is entirely dependent on the unique methods our visual system has developed to focus light onto the retina. These photons of light are processed by the retina into a language that our brain's visual cortex can understand, and are subsequently portrayed as the images we see. The retina plays an important role in this as the neurological interconnection point sensing environmental changes and feeding that visual input into the central nervous system. Therefore, retinal degenerations or disorders often result in major visual disruptions causing significant alterations to the affected individual's lifestyle and a substantial increase in healthcare-related costs. Coupled with the inability of current medical technologies to ameliorate many retinal disorders, it is crucial that research into effective treatments for a variety of retinal maladies be conducted for both preventative and therapeutic measures. With our steadily increasing understanding of the pathophysiology of retinal disorders, we are beginning to gain more of an understanding into a particular facet underpinning many of them: the complex interplay between retinal pathology and gene regulation.

MicroRNA (miRNA) are small, endogenous, single-stranded, non-coding RNAs typically 16–24 nucleotides in length. They act as post-transcriptional regulators of messenger RNA (mRNA) mediated by the binding of the miRNA seed region (6–8 nucleotide region) to the 3'untranslated region (3'UTR) of the mRNA. Their strong therapeutic potential lies in the fact that single miRNAs frequently have vast regulatory networks which can span up to 200 different mRNA targets (1). Often these targets also work within similar biological and physiological pathways providing wide-ranging regulation at the molecular level (1-3). The importance of miRNA in development cannot be understated, nor can their role in the support, maintenance and homeostasis of biological systems as a molecular buffer (4). The dysregulation of miRNA expression has been shown to contribute to various common pathological conditions including cancer, diabetes, cardiovascular disease and neurodegenerative diseases (5). This includes disorders of the retina where it has been postulated that miRNA expression changes may underlie various problems arising in the tissue (6). Their highly conserved nature across multiple model systems has expedited their use as potential gene therapeutics for complex diseases relative to others in the same realm.

The retina acts as an ideal organ model to test out potential gene therapies for CNS disorders due to the fact that the blood-retinal barrier renders it a closed system allowing for ease of access, visualisation and prevention of systemic side effects. Additionally, its small and compartmentalised nature theoretically allows for low doses of drug administration to be effective. In fact, the first ever FDA-approved gene therapy, LUXTURNA (Spark Therapeutics), was released for the treatment of an inherited retinal disorder. The retina as a model organ, is leading the gene therapy revolution of 21st century medicine.

In the majority of studies regarding retinal miRNAs, the same recurring characters seem to appear, indicating significant roles in maintaining retinal homeostasis and

combatting or contributing to retinal disorders. The photoreceptor cluster miR-183/96/182 remains one of the most heavily studied miRNA families in the retina with their expression being exclusively localised to the light-sensing photoreceptor cells (7,8). This cluster has been strongly linked to a neuroprotective role in the retina (9,10) and their overexpression in the retinal pigmented epithelium (RPE) cells (cells underlying the photoreceptors and responsible for their maintenance to a high degree) have also been shown to trigger reprogramming into neurons (11). miR-124 is another heavily studied miRNA in the central nervous system (CNS) with high enrichment in neurons, including in the retina (12,13). We have demonstrated that intravitreal administration of miR-124 has been shown to ameliorate progression of retinal degeneration with rescuing of inflammation, photoreceptor cell death and preservation of retinal function, presumably through regulation of highly inflammatory molecules such as *Ccl2* which has been heavily implicated in retinal disorders such as age-related macular degeneration (AMD) and retinitis pigmentosa (RP) (14). Other recurrent retinal microRNAs include miR-204/211, and the seemingly connected miR-155 and miR-146 that have consistently been shown to play heavily within the retinal inflammation space (15). While much is known about a small group of miRNAs in the retina, little is known about miR-194 in the retina, which is the subject of the manuscript that this commentary aims to address.

The recent study conducted by Cui *et al.* (in 2019), investigated the role of miR-194 as a therapeutic in proliferative vitreoretinopathy (PVR) (16). PVR is currently still not completely understood with no preventative measures being effective (17). PVR is a complication that arises in both non-treated and post-surgery treated rhegmatogenous retinal detachment (RRD) cases and is characterised by the formation of an abnormal epiretinal membrane (ERM). The complication has been touted as the primary cause of retinal detachment surgery failure (18). The RPE forms a major part of the composition of the ERM and can directly be involved in the pathogenesis of PVR through a process called EMT, or epithelial mesenchymal transition, which describes a process during which the RPE cells undergo dedifferentiation and migrate through breaks in the retina resulting in proliferation on the retinal surface and eventually the vitreous body (19). This can eventually lead to secondary retinal detachment. All in all, PVR is detrimental to the vision of a patient. Despite the presence of previous studies implicating key retinal miRNAs as potential therapeutic targets of PVR such as miR-204/211 and miR-124, the authors decided to aim their research at the more controversial miR-194 (20,21). The controversy comes with the "see-saw" evidence pertaining to its role in EMT where support has been shown both for inhibitory and promoter roles of miR-194 in the process (22,23).

The authors first isolated retinal layers through laser capture microdissection (LCM), which allowed for the isolation of the retinal nuclear layers. Through this, they determined that miR-194 was most heavily expressed in the RPE. To specifically investigate the RPE cells, they overexpressed miR-194 in ARPE-19 cells, an immortalised RPE cell line, before performing RNAseq. Upon gene ontology analysis, some interesting pathways appeared to be enriched following miR-194 overexpression including the NF-κβ pathway, tumor necrosis factor (TNF) and NOD-like receptor signalling, which are all pathways involved in inflammation. A number of these inflammatory-related pathways seemed to be up-regulated following miR-194 overexpression, however, the authors continued to speculate that miR-194 may have a protective role in EMT.

Whilst there were many targets that appeared in their sequencing analysis, the authors analysed the "intersection between all downregulated [differentially expressed genes] in the miR-194 overexpression group and the predicted targets by TargetScan" and only chose to move forward with zinc-finger E-box binding homeobox-1 (ZEB1) as their main target of study. ZEB1 has been postulated to play a role in EMT via regulation of inflammatory pathways such as TGF-β and NF-κβ (24). They used an *in vitro* model of TGF-B1-induced EMT on ARPE-19 cells to show that the expression of various EMT-related proteins could be effectively regulated with the introduction of a miR-194 hairpin construct. This was corroborated with immunohistochemical, western blot, wound healing and cell migration assay analyses. A major issue with miRNA research up until recently, is the reliance on *in silico* predicted binding partners and targets simply based on the conserved seed binding regions of the miRNA. This has the tendency to generate false-positive results that must be taken with a "grain of salt" before appropriate validation. However, whilst the authors utilised the availability of online prediction tools such as TargetScan and PicTar to show seed region complementarity to ZEB1, they also used a dual-luciferase assay to successfully validate ZEB1 as a target of miR-194.

In vivo, Cui *et al.* intravitreally injected miR-194 in a rat PVR model and with this intervention observed

a subsequent decrease in ZEB1 levels compared to the untreated controls in both western blot and immunostaining analyses. They also provided fundus imagery detailing the supposed amelioration in retinal appearance in miR-194 treated eyes where a retinal fold was absent and blood vessels appeared straight. However, it is not clear how efficient the intravitreal injections were as there were no results indicating the efficiency of the transfection of miR-194 following the injections. This is vital information as the retina is notoriously difficult to transfect due to its multi-laminar structure.

Whilst the results of this study together are interesting, it is becoming increasingly obvious that concentrating on a single target of a single miRNA may not prove an effective search for therapeutics and elucidation of biological mechanism. This is due to the wide-ranging regulatory network of miRNAs. Techniques such as argonaute (AGO) HITS-CLIP have allowed researchers to identify binding sites and entire networks of miRNAs that are actively contributing to various tissue types (25). Argonaute is a family of essential proteins in the RNA-induced silencing complex (RISC) that miRNAs must bind with in order to perform post-transcriptional regulation. AGO HITS-CLIP is a technique where the tissue at hand is cross-linked with ultraviolet (UV) light creating covalent bonds between the argonaute protein, the active miRNA and the bound mRNA. This entire complex can then be immunoprecipitated allowing for the isolation of both binary AGO: miRNA and ternary AGO: miRNA: mRNA complexes. High-throughput sequencing will allow for the identification of the true miRNA targets in the respective tissue of interest. Though a difficult technique, it is currently the "gold standard" in miRNA research to understand and elucidate both the miRnome and targetome of any given biological system. Luciferase assays such as those used by the authors in their study, remain an effective assay to use in order to validate binding of the miRNA to the 3'UTR of an mRNA target therefore indicating a potential target. However, one cannot immediately assume a positive result to remain true in a biological system that is not synthetically created such as those done in *in vitro* conditions during the assay.

The issue of delineating all the targets of a single miRNA in a biological system has proved to be a major roadblock in its potential use as a therapeutic. Using a miRNA as a therapy either by itself or in combination with other miRNAs remains a promising avenue for treatment but, again, a holistic view at what they are targeting is key to their relative success. It is therefore vital that all functional targets of miRNAs be elucidated before proceeding, as failure to do so may result in negative effects arising due to its potential "Janus-faced" attributes as already seen in miR-194.

Although miR-194 has not typically been in the conversation in retinal miRNA studies, Cui *et al.* present solid evidence into its potential role in maintaining the health and integrity of RPE cells specifically. In saying this, there is a theme in miRNA studies that is gaining in popularity. This is the notion that despite a large number of miRNAs being detectable in any given system, only a small subset of these miRNAs account for the majority of functions. In the retina, these candidates seem to be the ones aforementioned in this commentary, which have been shown to account for approximately 90% of the retinal miRnome (13). As miR-194 is not among this list of 20 or so miRNA, as the authors themselves have stated in their conclusions, an extensive list of miR-194 targets in the retina must be elucidated in order for its mechanism and role to be further established as a miRNA for retinal therapeutic use.

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Footnote

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References

1. Bartel DP, Chen CZ. Micromanagers of gene expression: the potentially widespread influence of metazoan microRNAs. Nat Rev Genet 2004;5:396-400.
2. Krol J, Loedige I, Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. Nat Rev Genet 2010;11:597-610.
3. Kutsche LK, Gysi DM, Fallmann J, et al. Combined Experimental and System-Level Analyses Reveal the Complex Regulatory Network of miR-124 during Human Neurogenesis. Cell Syst 2018;7:438-52.e8.
4. Ebert MS, Sharp PA. Roles for microRNAs in conferring robustness to biological processes. Cell 2012;149:515-24.
5. Kantharidis P, Wang B, Carew RM, et al. Diabetes complications: the microRNA perspective. Diabetes 2011;60:1832-7.
6. Sundermeier TR, Palczewski K. The impact of microRNA gene regulation on the survival and function of mature cell types in the eye. FASEB J 2016;30:23-33.
7. Zhu Q, Sun W, Okano K, et al. Sponge transgenic mouse model reveals important roles for the microRNA-183 (miR-183)/96/182 cluster in postmitotic photoreceptors of the retina. J Biol Chem 2011;286:31749-60.
8. Busskamp V, Krol J, Nelidova D, et al. miRNAs 182 and 183 are necessary to maintain adult cone photoreceptor outer segments and visual function. Neuron 2014;83:586-600.
9. Lumayag S, Haldin CE, Corbett NJ, et al. Inactivation of the microRNA-183/96/182 cluster results in syndromic retinal degeneration. Proc Natl Acad Sci U S A 2013;110:E507-16.
10. Krol J, Busskamp V, Markiewicz I, et al. Characterizing light-regulated retinal microRNAs reveals rapid turnover as a common property of neuronal microRNAs. Cell 2010;141:618-31.
11. Davari M, Soheili ZS, Samiei S, et al. Overexpression of miR-183/-96/-182 triggers neuronal cell fate in Human Retinal Pigment Epithelial (hRPE) cells in culture. Biochem Biophys Res Commun 2017;483:745-51.
12. Lagos-Quintana M, Rauhut R, Yalcin A, et al. Identification of tissue-specific microRNAs from mouse. Curr Biol 2002;12:735-9.
13. Karali M, Peluso I, Marigo V, et al. Identification and characterization of microRNAs expressed in the mouse eye. Invest Ophthalmol Vis Sci 2007;48:509-15.
14. Chu-Tan JA, Rutar M, Saxena K, et al. MicroRNA-124 Dysregulation is Associated With Retinal Inflammation and Photoreceptor Death in the Degenerating Retina. Invest Ophthalmol Vis Sci 2018;59:4094-105.
15. Zuzic M, Rojo Arias JE, Wohl SG, et al. Retinal miRNA Functions in Health and Disease. Genes (Basel) 2019;10. doi: 10.3390/genes10050377.
16. Cui L, Lyu Y, Jin X, et al. miR-194 suppresses epithelial-mesenchymal transition of retinal pigment epithelial cells by directly targeting ZEB1. Ann Transl Med 2019;7:751.
17. Nemet A, Moshiri A, Yiu G, et al. A Review of Innovations in Rhegmatogenous Retinal Detachment Surgical Techniques. J Ophthalmol 2017;2017:4310643.
18. Kwon OW, Song JH, Roh MI. Retinal Detachment and Proliferative Vitreoretinopathy. Dev Ophthalmol 2016;55:154-62.
19. Kim IK, Arroyo JG. Mechanisms in proliferative vitreoretinopathy. Ophthalmol Clin North Am 2002;15:81-6.
20. Jun JH, Joo CK. MicroRNA-124 Controls Transforming Growth Factor beta1-Induced Epithelial-Mesenchymal Transition in the Retinal Pigment Epithelium by Targeting RHOG. Invest Ophthalmol Vis Sci 2016;57:12-22.
21. Kaneko H, Terasaki H. Biological Involvement of MicroRNAs in Proliferative Vitreoretinopathy. Transl Vis Sci Technol 2017;6:5.
22. Das R, Gregory PA, Fernandes RC, et al. MicroRNA-194 Promotes Prostate Cancer Metastasis by Inhibiting SOCS2. Cancer Res 2017;77:1021-34.
23. Zhang X, Wei C, Li J, et al. MicroRNA-194 represses glioma cell epithelialtomesenchymal transition by targeting Bmi1. Oncol Rep 2017;37:1593-600.
24. Vandewalle C, Van Roy F, Berx G. The role of the ZEB family of transcription factors in development and disease. Cell Mol Life Sci 2009;66:773-87.
25. Chi SW, Zang JB, Mele A, et al. Argonaute HITS-CLIP decodes microRNA-mRNA interaction maps. Nature 2009;460:479-86.

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