Circulating microRNA expression in Age Related Macular Degeneration

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Background

- Age-related macular degeneration (AMD) is a progressive, degenerative retinal disease involving the RPE, Bruch's membrane, and the choriocapillaris, eventually resulting in irreversible central blindness. It is the most common cause of blindness in people over 60 years of age[1–3]. It can broadly be categorised into either dry AMD or wet AMD.
- AMD has an insidious clinical onset, and as of yet there are no effective means of screening for the disease[4,5]. Moreover, fundoscopy, imaging and self-monitoring for disease progression fall short of identifying patients who will go on to develop neovascular disease[4]. As such, there is a distinct need for the identification of useful biomarkers in AMD which could be used in the diagnosis and recognition of disease progression.
- MicroRNAs (miRNAs) are small noncoding RNA molecules involved in post-transcriptional regulation of gene expression through the targeting and silencing of complementary messenger RNA (mRNA). miRNAs have been shown to have a governing function in processes underpinning AMD such as inflammation, angiogenesis and in oxidative stress responses[6,7]. Their distinctive expression in disease-states and their relative stability in serum samples make miRNAs very promising diagnostic biomarkers, and potential therapeutic targets[4,8,9].
- This study aims to validate a number of promising serum miRNA biomarkers identified in AMD, and to characterise their expression in the context of Irish patients with AMD.

Methods

- This study involved total RNA isolation from sera specimens from patients with
  i. Dry AMD (n=12)
  ii. Wet AMD (n=14)
  iii. Age- and gender matched controls (n=10)
- 42 previously investigated miRNAs were co-analysed using miRCURY LNA miRNA SYBR Green PCR kit via quantitative real-time polymerase chain reaction (qRT-PCR) to validate their presence.
- Means and standard deviations of fold change in miRNA expression (C_T) were determined. The significance of difference in serum miRNA expression between cohorts (wet AMD, dry AMD, and control) was assessed using analysis of variance (one-way ANOVA) The Holm-Šídák method was applied for multiple comparisons tests.
- Post-hoc testing was used to determine the significance in difference between wet AMD vs. control and dry AMD vs. control.

Results

Differentially expressed miRNAs in AMD

- Hierarchical clustering analysis performed for the study (performed using Morpheus software) showed higher miRNA expression in wet and dry AMD patients groups compared with controls.
- Notably, miR-626 an miR-410-5p were not differentially expressed, indicating that they potentially may not be useful AMD biomarkers in Irish patients.

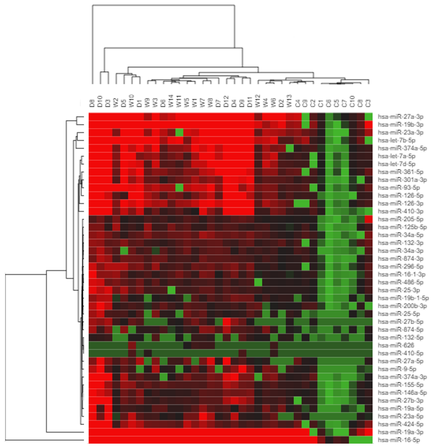


Figure 1: hierarchical clustering of miRNAs. Colours correspond to the relative abundance of miRNA expression in each sample, with green indicating a low level of miRNA expression and red indicating high.

MicroRNAs as candidate biomarkers for AMD

- Of the 42 miRNA candidates, 39 have been confirmed to be over expressed in the serum of Irish AMD patients.
- Notably, 8 miRNAs showed higher expression in the serum of the dry AMD patients than wet AMD compared with healthy controls; (hsa-let-7a-5p, hsa-let-7 d-5p, hsa-miR-23a-3p, hsa-miR-301a-3p, hsa-miR-361-5p, hsa-miR-27b-3p, hsa-miR-874-3p, hsa-miR-19b-1-5p).

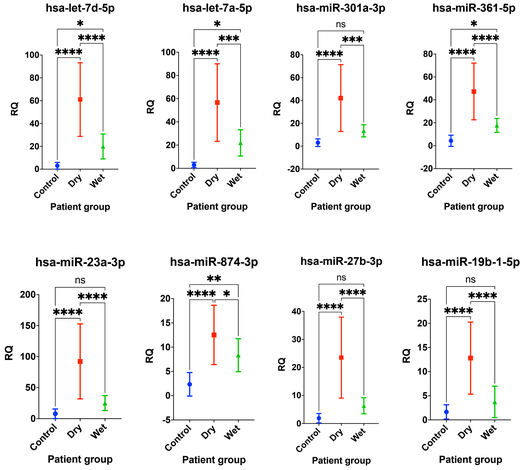


Figure 2: miRNA expression levels (Relative Quantification (RQ)) for dry AMD, wet AMD and control patient groups . Shapes represent mean values of each dataset. Whiskers represent ± 1 standard deviation

Conclusion

- We successfully validated the biomarker potential of a number of circulating miRNAs and characterised their expression in an Irish population. Additionally, 8 of the miRNAs identified showed significantly higher expression in the serum of the dry AMD patients than wet AMD compared with healthy controls. The serum miRNAs identified show promise as potential biomarkers for AMD. Prospective longitudinal studies are required to fully explore their usefulness as early indicators of disease and disease progression.
- Interestingly, miR-410-5p and miR-626 did not show any notable difference in expression levels between AMD patients and healthy controls, despite having been validated in AMD patients in previous studies. We hypothesise that these miRNAs might not be useful biomarkers in the context of Irish patients, perhaps showing that different populations might require different biomarker profiling. Further investigation with a larger sample size is needed.
- Novel drug delivery mechanisms for RNA-based therapies have been instrumental this past year in tackling the COVID-19 pandemic. There now exists the potential for an array of RNA-based therapeutic strategies in a variety of diseases using similar mechanisms. Pending the aforementioned studies, the miRNA identified may serve as potential therapeutic targets for AMD.

References

1. Wang L, Lee AYW, Wigg JP, Peshavariya H, Liu P, Zhang H. miRNA involvement in angiogenesis in age-related macular degeneration. J Physiol Biochem [Internet]. 2016 Dec 1;72(4):583–92.
2. Grassmann F, Schoenberger PGA, Brandl C, Schick T, Hasler D, Meister G, et al. A Circulating MicroRNA Profile Is Associated with Late-Stage Neovascular Age-Related Macular Degeneration. Sørensen TL, editor. PLoS One [Internet]. 2014 Sep 9;9(9):e107461.
3. Liu CH, Huang S, Britton WR, Chen J. MicroRNAs in vascular eye diseases [Internet]. Vol. 21, International Journal of Molecular Sciences. MDPI AG; 2020.
4. Ren C, Liu Q, Wei Q, Cai W, He M, Du Y, et al. Circulating miRNAs as Potential Biomarkers of Age-Related Macular Degeneration. Cell Physiol Biochem [Internet]. 2017 May 1;41(4):1413–23.
5. ElShelmani H, Wride MA, Saad T, Rani S, Kelly DJ, Keegan D. Identification of Novel Serum MicroRNAs in Age-Related Macular Degeneration. Transl Vis Sci Technol [Internet]. 2020 Mar 30;9(4):28.
6. Yao Q, Chen Y, Zhou X. The roles of microRNAs in epigenetic regulation. Vol. 51, Current Opinion in Chemical Biology. Elsevier Ltd; 2019. p. 11–7.
7. Litwińska Z, Sobuś A, Łuczkowska K, Grabowicz A, Mozolewska-Piotrowska K, Safranow K, et al. The Interplay Between Systemic Inflammatory Factors and MicroRNAs in Age-Related Macular Degeneration. Front Aging Neurosci [Internet]. 2019 Oct 22;11.
8. Romano GL, Platania CBM, Drago F, Salomone S, Ragusa M, Barbagallo C, et al. Retinal and circulating miRNAs in age-related macular degeneration: An in vivo animal and human study. Front Pharmacol [Internet]. 2017 Mar 30 ;8(MAR):168.
9. Glinge C, Clauss S, Boddum K, Jabbari R, Jabbari J, Risgaard B, et al. Stability of Circulating Blood-Based MicroRNAs – Pre-Analytic Methodological Considerations. Calin G, editor. PLoS One [Internet]. 2017 Feb 2 ;12(2):e0167969.

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