Dysregulation of RPE immunosuppression during ageing: a RNA-seq study

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BACKGROUND

Age-related macular degeneration (AMD) is a leading cause of irreversible vision loss among around 10% of people over 65 years old in developed countries [1-3]. The major cell type linked with this disease is the retinal pigment epithelium (RPE). The ageing of the RPE, and loss of immune privilege in the subretinal space between the light-sensitive photoreceptor layer and the RPE are thought to be fundamental in the development of AMD [4-5]. However, the molecular mechanism which links RPE ageing and immune dysregulation to AMD is still unclear.

PURPOSE: To characterise the global transcriptomic changes associated with RPE immunosuppression during ageing using next-generation sequencing.

METHODS

Animal Model: C57BL6/J mice at 3 months (young) and 22 months (aged).

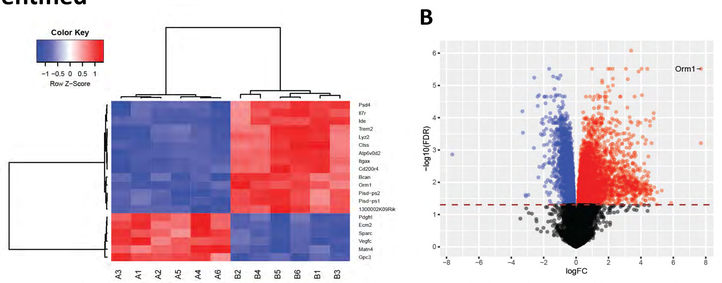
Transcriptome sequencing: High-quality total RNA was extracted from RPE-choroidal tissues of freshly enucleated eyes. mRNA sample libraries were prepared with the TruSeq Stranded mRNA kit. RNA-seq was performed on Illumina NovaSeq 6000 System at Australian Genome Research Facility.

Differential gene expression and gene ontology functional overrepresentation analyses: Differentially expressed genes (DEGs) analysis and functional overrepresentation analysis of DEGs were conducted using edgeR (v. 3.26.8) and clusterProfiler (v. 3.12.0) packages respectively in R.

Previous transcriptome data acquisition and reanalyses: Gene Expression Omnibus database was assessed for previous transcriptome work studying gene expression changes in RPE/choroid tissues from mice and human donors with ageing and AMD characteristics. Curated mouse microarray datasets were reanalysed for identifying DEGs via limma (v. 3.40.6) package in R.

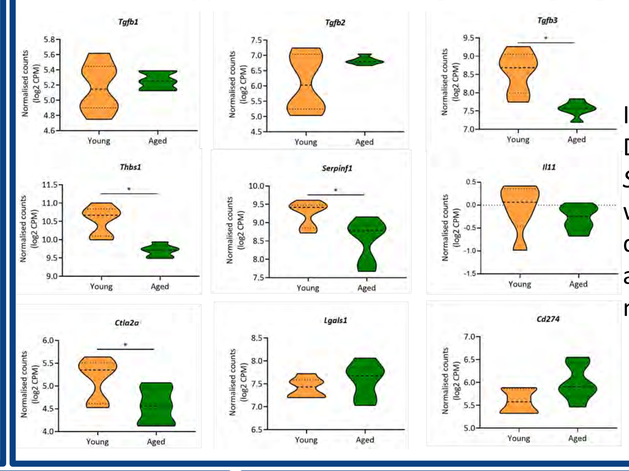
RESULTS

1. Genes differentially expressed between young and aged RPE-choroid are identified



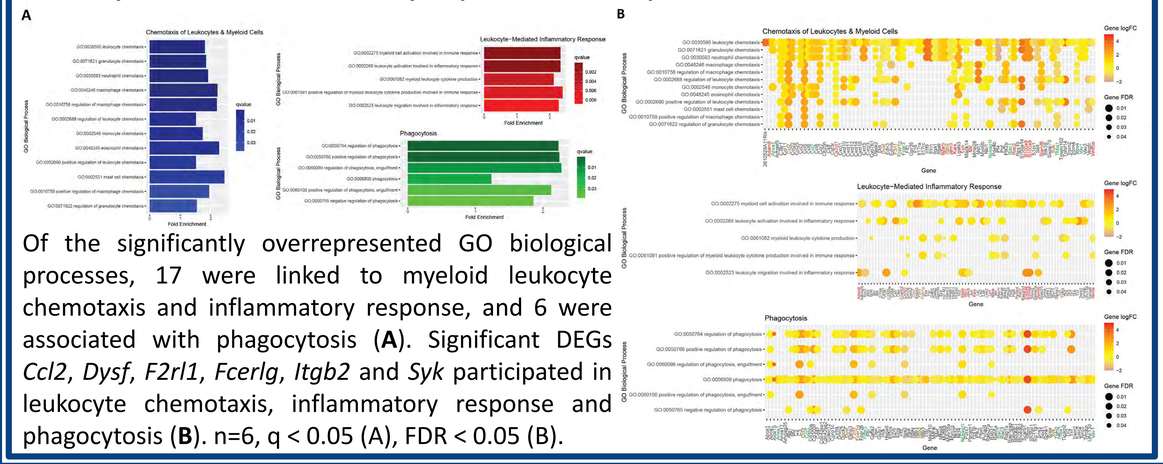
EdgeR analysis identified 5,967 DEGs at FDR < 0.05, including ageing-related *Il7r* as one of the top 20 significant DEGs (**A**). Among those 904 showed an absolute log2 fold change > 1.5 in expression, e.g. the most dysregulated immune-modulating *Orm1* (**B**). A1-A6: young RPE-choroidal samples; B1-B6: aged RPE-choroidal samples.

2. Immunosuppression of RPE changes with ageing



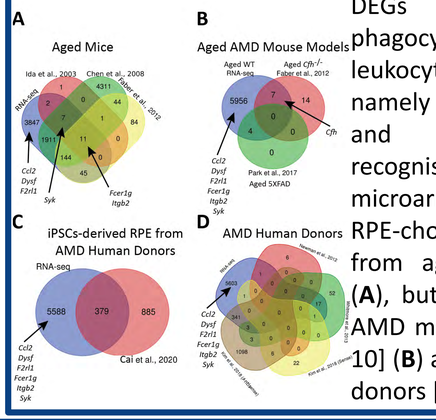
Immunosuppressive DEGs *Tgfb3*, *Thbs1*, *Serpinf1* and *Ctla2a* were significantly downregulated in aged RPE-choroid. n=6, *FDR < 0.05.

3. Gene Ontology (GO) biological pathways related to chemotaxis of myeloid leukocytes and leukocyte-mediated inflammatory response are overrepresented



Of the significantly overrepresented GO biological processes, 17 were linked to myeloid leukocyte chemotaxis and inflammatory response, and 6 were associated with phagocytosis (**A**). Significant DEGs *Ccl2*, *Dysf*, *F2rl1*, *Fcerlg*, *Itgb2* and *Syk* participated in leukocyte chemotaxis, inflammatory response and phagocytosis (**B**). n=6, q < 0.05 (A), FDR < 0.05 (B).

4. Comparison with previous ageing and AMD transcriptome studies reveals overlapping DEGs related to leukocyte chemotaxis and phagocytosis



DEGs involved in phagocytosis and leukocyte chemotaxis, namely *Fcerlg*, *Itgb2* and *Syk*, were recognised in previous microarray studies using RPE-choroid tissues from aged mice [6-8] (**A**), but not from aged AMD mouse models [8-10] (**B**) and AMD human donors [11-13] (**C, D**).

CONCLUSIONS

1. An age-related loss of RPE immunosuppression and immune privilege was confirmed.
2. *Fcerlg*, *Itgb2* and *Syk* may underlie the molecular mechanism for recruiting subretinal phagocytic leukocytes with age.
3. Since excess recruitment of leukocytes to the subretinal space and the dysfunctional RPE is a common feature in both ageing and AMD, these genes may potentially account for the immune dysregulation leading to AMD.

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