Understanding Factor H Related Protein 1 (FHR1) role in Age-related Macular Degeneration using Next Generation Sequencing

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1. Main Text

AMD is a multifactorial disease as it's development and progress depend on a combination of genetic factors, environment and life-style choices. Despite being a leading cause of blindness in the Western world, effective treatments remain limited, placing a burden on patients and healthcare system. AMD pathophysiology involves the progressive macula damage, partly due to compromised immune barrier function of the retinal pigment epithelium (RPE) and subsequent monocytes infiltration into sub-retinal space. Early genetic studies[1-3] were the first to implicate complement cascade in AMD, but understanding of how complement proteins contribute remains elusive. Recent advances, such as next-generation sequencing, have revealed allele-specific expression of CFH, chromatic accessibility and regulatory networks involved in AMD[4,5]. System biological approaches in conjunction with human and animal research helps to identify pathology relevant cells.

In this study, we focus on FHR1 (whose deletion protects against developing AMD later in life [6] using single nuclei RNA sequencing and how they translate to in vivo observations. Latest studies in antibody-associated vasculitis and atherosclerosis showed that after binding to deposits considering of necrotic-type cells, FHR1 triggers sterile inflammation by activating monocytes via EMR2[7,8]. Drusen, hallmarks of AMD, represent a similar type of deposits of extracellular debris found in the outer retina. With both approaches we were able to discover that FHR1 has a prominent role of FHR1 in AMD by contributing to cellular and para-inflammation. It mitigates from the serum to the degenerative area, where, upon binding, activates RPE cells to pro-inflammatory gene expression. Additionally, FHR1-binding facilitates the contact of RPE cells and mononuclear phagocytes, allowing them to infiltrate. Furthermore, FHR1 targets not only necrotic, but already stressed RPE cells that could express pre-degenerative markers.

2. Methods and results

Using single nuclei RNAseq (snRNAseq) we investigated muFHR1 effect and compared gene expression between WT and *muFHR1*$^{-/-}$ 14 days after CNV induction. We then compared the results from snRNAseq to collected data that consisted of classical "wet lab" techniques: immunohistochemistry of examined choroids that we have stained against muFHR1, Emr1 (receptor activated by muFHR1) and Iba1 (stains mononuclear phagocytes); in vitro experiments including western blots, Ca^{2+} imaging and qPCR.

Comparing the results from different approaches allowed us to confirm our observations and hypothesis: (i) no local FHR1 expression within the RPE/choroid confirming the restricted liver production; (ii) FHR1 not only affects RPE gene expression, but also every other cell type found in the choroid; (iii) in absence of FHR1, number of invading mononuclear phagocytes reduced; (iv) RPE sub-cluster heterogeneity effect varies suggesting different functional phenotypes. On the other hand, we have also observed inconsistencies within the data, including the enrichment pathway analysis and EMR1 expression on RPE cells.

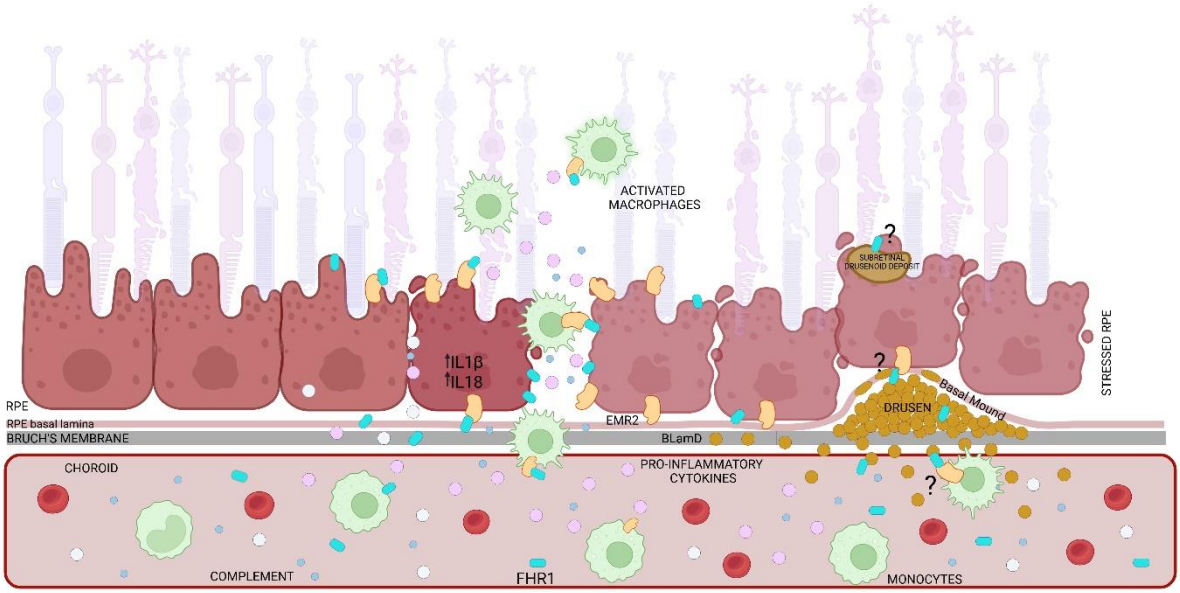

Fig. 1. Mechanism-of-action: FHR1 – FHR1 binds to degenerating RPE cells and subsequently: (i) changes the RPE phenotype into a pro-inflammatory one; (ii) allows interaction between RPE and MPs and as such (iii) contributes to the MPs accumulation in the sub-retinal space. FHR1 further over-activates MPs to additionally contribute to the parainflammation. We further hypothesize that FHR1 binds to cholesterol and oxidized lipids within drusen (hallmark of AMD) and subretinal drusenoid deposits to further aggravate inflammation in AMD.

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