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Authors

Woodard, DaNae R

Ayyagari, Radha

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13 MFRP in Early Onset Retinal Degeneration: Clinical and Molecular Perspectives

DaNae R. Woodard and Radha Ayyagari

Abstract

The membrane-frizzled related protein (*MFRP*) is a retinal pigment epithelium (RPE) and ciliary epithelium-expressed gene of unknown function. Interest in *MFRP* stems from clinical manifestations that range from acute-angle closure glaucoma to microphthalmia and Retinitis pigmentosa in patients with *MFRP* mutations. Furthermore, the genetic ablation of *Mfrp* in mice results in an early-onset retinal disease with visible degeneration around 1mo of age and primary rod photoreceptor loss. Yet, little is known regarding the exact role of *MFRP* in the RPE, its underlying contribution to pathology, and the impacted mechanisms at the early stages of *MFRP*-related disease. This review presents a current overview of *MFRP* studies and poses outstanding questions that are crucial for understanding the involvement of *MFRP* in early-onset retinal degeneration. Such insight could pave the way for deciphering the molecular mechanisms associated with *MFRP* that are impacted during early stages in the retina, offering the potential to translate this knowledge into determining similarities and differences in mechanisms involved in late-stage retinal diseases.

Keywords

AMD · MFRP · C1QTNF5/CTRP5 · Photoreceptors · Retinal degeneration · Retinitis pigmentosa · RPE

13.1 Introduction

Despite significant advancements in the field, understanding the molecular underpinnings of common age-related diseases that present a global burden, such as with age-related macular degeneration (AMD), remains complex and therefore challenging (and costly) to treat (Gehrs et al. 2006). AMD currently affects approximately 20 million older individuals in the United States (Rein et al. 2022) and is a late-stage macular degenerative disease whereby progressive degeneration of the RPE and photoreceptors severely compromise vision in the macula (Bhutto and Lutty 2012). Countless studies over the last few decades have focused on AMD etiology by implementing genetic approaches (i.e., mouse models, GWAS), studying AMD donor eyes, and even learning from monogenic inherited retinal diseases (IRDs) that share similar clinical phenotypes to that of AMD. Of these, many genes implicated in the most common IRD,

D. R. Woodard · R. Ayyagari (✉)
Department of Ophthalmology, Shiley Eye Institute, University of California San Diego, San Diego, CA, USA
e-mail: rayyagari@health.ucsd.edu

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Retinitis pigmentosa (RP) have been useful for interrogating retinal changes during pathology (Ferrari et al. 2011). The investigation of RPE-expressed genes that lead to RP, such as with autosomal recessive mutations in *MERTK* (Al-Khersan et al. 2017), have been invaluable for attempting to understand mechanisms of RPE degeneration. Similarly, autosomal recessive mutations in the *MFRP* gene have also been implicated in RP (Pauer et al. 2005; Ayala-Ramirez et al. 2006; Dinculescu et al. 2012; Kannabiran et al. 2012; Morillo Sanchez et al. 2019; Ren et al. 2022; Kovacs et al. 2023) and insight its molecular profile over the last 20 years leaves much to discover.

13.2 The Molecular Profile of MFRP

Initially cloned in 2001, MFRP was designated as a type II transmembrane protein because it contains cubilin (CUB) and a frizzled (FZ)/cysteine-rich domain (CRD) based on its shared sequence homology with other transmembrane proteins (i.e., Corin and SFRPs) (Katoh 2001). Structurally, the C-terminal portion of MFRP consists of a short 69 amino acid region (1–69 amino acids) in the cytoplasm, and the N-terminus comprises an extracellular region of a CUB-LDLA repeat domain followed by the FZ/CRD domain (101–579 amino acids) (Katoh 2001; Sharmila et al. 2014). The estimated molecular weight of MFRP is 65 kDa; however, a few studies have detected MFRP protein at ~100–120 kda (Mandal et al. 2006; Won et al. 2008; Dinculescu et al. 2012), which may indicate post-translational modifications that are currently unknown for MFRP. Human tissues have been used to gage the expression profile of *MFRP* using northern blot analysis (Katoh 2001). *MFRP* was detected in the brain, whereas its expression was absent in heart, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, colon, and leukocytes (Katoh 2001). In a separate study, in situ hybridization not only confirmed the expression of *Mfrp* in the brains of C57BL/6J mice, but also revealed that *Mfrp* is highly expressed in the RPE and ciliary epithelium (Kameya et al. 2002). A separate study also confirmed this phenomenon (Mandal et al. 2006). In contrast, *Mfrp* was not detected in the neural retina (Kameya et al. 2002). In the RPE, MFRP is predominantly expressed apically as demonstrated by colocalization with ezrin, an RPE microvilli marker (Mandal et al. 2006). To a lesser extent, MFRP is also found in the basal infoldings of RPE (Mandal et al. 2006).

13.3 The Mysterious Role of MFRP in the RPE

One of the simplest, yet defining questions when studying any gene of interest is: What is its function? *MFRP* is no exception and little is known regarding its definitive role in the RPE. Few studies have speculated that because of its FZ/CRD domain, MFRP likely participates in Wnt signaling (Katoh 2001; Kameya et al. 2002). Furthermore, various frizzled and frizzled-related proteins function as receptors for Wnt signaling (Huang and Klein 2004). Wnt signaling has also been a contributing factor in several ocular diseases (de Iongh et al. 2006). The only study that directly suggests an association of MFRP to Wnts found that while Wnt-1 and Wnt-10b expression was apparent in the apical RPE of wildtype (Wt) C57BL/6 J mice, expression was absent in mice that lacked MFRP (*rd6* mice, discussed below) (Kameya et al. 2002). However, follow-up studies that directly assess the influence of MFRP on Wnt activity and signaling pathways are lacking. If speculation that MFRP participates in Wnt signaling is based on its CRD/FZD domain, shouldn't the CUB and LDLA domains also point to clues regarding its function? The LDLA domain is of particular interest since it is known to regulate cholesterol homeostasis through receptor-mediated endocytosis of lipoprotein particles (Rudenko et al. 2002). Is it possible that MFRP plays a role in lipid metabolism? Aside from its structure, biochemical approaches to uncover interacting partners of MFRP may also provide insight into its function. To date, the only other experimentally confirmed binding partner

of *MFRP* is its dicistronic partner, *C1QTNF5/CTRP5* (Kameya et al. 2002; Mandal et al. 2006). MFRP and CTRP5 colocalize and co-express in the RPE and ciliary epithelium, and CTRP5 interacts with the CUB domain of MFRP (Mandal et al. 2006). However, the function of CTRP5 also remains a mystery, and more focus has been on its infamous Ser163Arg (S163R) mutation, which causes late-onset retinal degeneration (L-ORD) (Hayward et al. 2003; Ayyagari et al. 2005). A few studies have hinted at a possible relationship between *MFRP* and *ADIPOR1*, an apical RPE-expressed adiponectin receptor (Yamauchi et al. 2014), due to their identical retinal disease phenotypes and the loss of ADIPOR1 expression in the RPE of *rd6* mice (Sluch et al. 2018; Gogna et al. 2022). It is currently unknown whether MFRP levels also decrease in *Adipor1*$^{-/-}$ mice. Yet, while insight into the function of ADIPOR1 has been investigated, MFRP function remains a mystery.

13.4 *MFRP*-Related Ocular Diseases and Clinical Features

Clinical manifestations reported in patients with mutations in *MFRP* include acute-angle closure glaucoma, hyperopia, microphthalmia, nanophthalmia, foveal schisis, optic disc drusen, retinal cysts, retinal folds, RPE atrophy, and RP (Ayala-Ramirez et al. 2006; Crespi et al. 2008; Wang et al. 2008; Zenteno et al. 2009; Mukhopadhyay et al. 2010). Furthermore, a combination of these phenotypes have been observed in patients. The majority of patients also develop symptoms at early ages (<30 years). *MFRP* mutations reported in patients lead to either complete loss or truncated MFRP protein (Chekuri et al. 2019). Clinically, a number of sequence variants in *MFRP* that comprise missense (343), frameshift (24), nonsense (14), and splice site (9) changes have been discovered, and most are classified with uncertain significance (386), followed by likely benign (244) and pathogenic (66) (ClinVar 2023). Given the heterogeneity with which *MFRP* mutations lead to disease, utilizing genetic models to study *MFRP*, especially as an IRD model of RP, has paved the way for understanding the ocular consequences of MFRP loss in the retina.

13.5 *Mfrp* Mouse Models

The contribution of *MFRP* to retinal degeneration has been studied utilizing three unique mouse models reported in the literature: *rd6*, *rdx/Mfrp*^{174delG}, and *Mfrp*$^{KI/KI}$. The *rd6* model naturally arose from a spontaneous mutation in the C3HfB/GaCas1b mouse strain and results in a 4 bp deletion in a splice site of intron 4, which causes exon 4 to be skipped (Hawes et al. 2000). Skipping of exon 4 leads to short *Mfrp* transcripts and the deletion of 58 amino acids in MFRP, predicting the formation of a truncated protein (Hawes et al. 2000; Kameya et al. 2002). Originally designated as *rdx* mice, *Mfrp*^{174delG} also results from a spontaneous mutation that causes a base-pair deletion in exon 3 of *Mfrp*, resulting in a frameshift and premature stop codon and thus loss of MFRP expression (Fogerty and Besharse 2011). The most recent *Mfrp* mouse model, *Mfrp*$^{KI/KI}$, is the first to encompass a mutation found in patients (Chekuri et al. 2019). *Mfrp*$^{KI/KI}$ harbors a homozygous c.498_499insC mutation in exon 5 of *MFRP* that causes a frameshift and predicts a truncated and nonfunctional protein (Ayala-Ramirez et al. 2006; Chekuri et al. 2019). Observed clinical features of patients with the c.498_499insC mutation include posterior microphthalmos, retinitis pigmentosa, foveoschisis, and optic disc drusen (Ayala-Ramirez et al. 2006; Chekuri et al. 2019). Interestingly, varying levels of mRNA expression were observed among the three mouse models, with increased *Mfrp* mRNA in *rd6* and *rdx/Mfrp*^{174delG} mice but decreased expression in *Mfrp*$^{KI/KI}$ mice. A recent study identified that loss of the miR-149-3p microRNA, which binds to the 3' UTR of *Mfrp* and regulates its expression, may be responsible for the increased *Mfrp* (and *Ctrp5*) transcripts in *rd6* mice (Tian et al. 2023).

13.6 Future Approaches for MFRP

While MFRP is crucial for RPE integrity, many questions remain regarding its fundamental role in the RPE. Before we can begin to fully understand its contribution to retinal pathology, efforts to determine the function of MFRP should be strongly considered. Along with definitively exploring the involvement of MFRP in Wnt signaling, studies to determine whether there is a relationship between MFRP and lipid metabolism (due to the presence of the LDLA domain) would be relevant for more common later-stage diseases like AMD, where lipids play an important role in pathology (van Leeuwen et al. 2018). Discovery of additional binding partners of MFRP would also be applicable not only for determining MFRP function but also for deciphering pathways that are most impacted during disease when MFRP is ablated. Perhaps generating a homozygous *Mfrp* knockout mouse model could be used as a tool for understanding how complete loss of MFRP contributes to early-onset retinal degeneration. This model could be used to study the transcriptional consequences of *Mfrp*-associated retinal degeneration through the use of single-cell RNA-sequencing and spatial transcriptomic tools. Insight from retinal gene models that display early-onset retinal degeneration, including *MFRP*, would allow for (i) deciphering the postnatal consequences that may precede visible retina and RPE degeneration and (ii) leveraging this knowledge to design therapies that significantly slow disease progression. The latter point could also impact the timeframe with which late-stage retinal diseases (i.e., L-ORD and AMD) could be treated by pinpointing early biomarkers implicated at onset. Imagine if we found a way to significantly delay the onset of AMD in patients.

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