Interrogation of Rhodopsin Structural Stability and Cytotoxic Chromophore Release

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Introduction: Degenerative retinal diseases, like retinitis pigmentosa (RP), affect 1 in 4000 people worldwide. RP is most often caused by non-synonymous mutations in RHO, the gene encoding rhodopsin, the light-sensing receptor of rod cells in the retina. Since rods are critical to conferring dim-light and peripheral vision, changes to rhodopsin function can have significant impacts on quality of life. Previous investigations have attempted to classify various RHO disease mutations based on in vitro rhodopsin surface expression and misfolding. These investigations have been useful for classifying many variants that were of severe pathogenicity but do not explain how changes in protein function may be associated with milder disease progression. Recent studies of rhodopsin biochemistry suggest that light-activated rhodopsin stability plays a critical role in preventing cytotoxic effects associated with the dissociated all-trans-retinal (ATR) chromophore. Since changes to rhodopsin structure can impact the stability of the active state, we hypothesized that cytotoxic effects associated with accelerated rates of ATR release may be related to some cases of mild RP.

Specifically, we predicted that rhodopsin mutants nearby the Activation Switch 1 domain may be associated with mild RP. Activation Switch 1 is centred around a critical E122-H211-W126 interaction, which is known to maintain the stability of the dark-state and light-activated structures of rhodopsin. Additionally, there are a number of mutation sites in rhodopsin associated with degenerative visual disease in humans that are proximal to Activation Switch 1. "

Methods: Thus, we have investigated variants associated with mild disease in the Activation Switch 1 region that do not cause significant protein misfolding, as well as other natural variants in humans. Proteins have been heterologously expressed and purified to be assayed in the laboratory using spectroscopic approaches that measure the stability of light-activated rhodopsin. Light-activated stability was monitored through fluorescence spectroscopy, to observe the rates of chromophore release associated with the active state decay.

Results: Our preliminary results have shown that some of these variants, including M163T, have significantly accelerated retinal release which is believed to be associated with higher cytotoxicity. Homology modeling of these variants has suggested that the variants with accelerated retinal release have a widened chromophore exit pore, which may explain the observed changes in kinetics.

Conclusion: As the results of these variants are being interpreted in light of the known clinical phenotype, this study will expand our understanding of the molecular basis of retinal degeneration and human visual disease associated with mutations in the rhodopsin gene.