Type of presentation: Poster

ID-9-P-3451 Quantified phenotype analysis in a cell model for Autosomal Dominant Retinitis Pigmentosa

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Autosomal Dominant Retinitis Pigmentosa (ADRP) is an inherited eye disease, which can lead to photoreceptor cell death, and result in reduced vision and complete blindness. This disorder is estimated to affect one in 3000–5000 individuals worldwide. The main causes of ADRP are mutations in rhodopsin (the light pigment protein); this glycoprotein is responsible for vision under dim light conditions. There have been more than 140 mutations identified on the rhodopsin gene which are linked to ADRP. The cellular trafficking of rhodopsin to the outer segment of vertebrate rod cells is critical for normal function; misfolding interferes with the normal trafficking pathway and may eventually lead to photoreceptor degeneration.
We have developed inducible stable HEK293S cell lines expressing wild-type and mutant rhodopsin-GFP to gain greater insight into pathways triggered by its misfolding. Using a combination of experimental procedures, standardized optimal three-dimensional image acquisition, volumetric and super-resolved localisation analysis, we characterise the phenotypes of rhodopsin mutants and show subtle but significant differences.
Mutation P23H, well researched by biochemical means in previous studies, is used as a reference. Some cells expressing mutant rhodopsin were treated with the 11-cis-retinal cofactor to see if they responded with improved folding and plasma membrane localisation; these are called ‘rescued’ cells.
Live imaging of the cells was conducted to monitor formation of aggresomes and translocation of rhodopsin-GFP to the plasma membrane. In fixed cells, variable levels of GFP expression combined with immunofluorescence showed a considerable difference in dynamic range. Wild-type and rescued mutant cells shared similar localisation and volumes of rhodopsin-GFP. Calnexin, an endoplasmic reticulum chaperone important for assisting the folding of glycoproteins, was also quantified using monoclonal antibody (mAb) labelling. We discuss the unexpected finding of lower calnexin-mAb volumes found in P23H cells.
Performances of visual experts and algorithms for overlap measurement and object-based colocalisation with high measurement accuracy are compared. They were applied to a growing database of three-dimensional multichannel images of single HEK293S cells expressing various rhodopsin-GFP mutants.

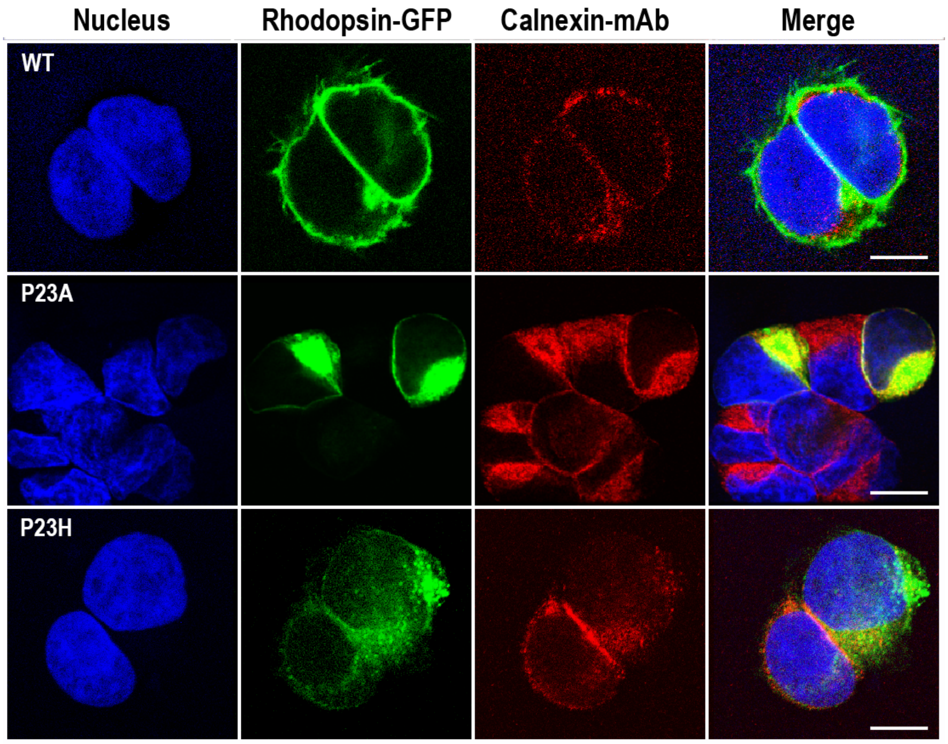

Fig. 1: Colocalisation of rhodopsin-GFP and calnexin-mAb in HEK293S cells expressing wildtype (WT) and mutant (P23A and P23H) rhodopsin-GFP (green) . Immunofluorescently labelled calnexin is shown in red and DAPI-stained nuclei in blue. Note poor plasma membrane localisation and aggresome formation of mutant P23H rhodopsin-GFP. Scalebars: 10µm.

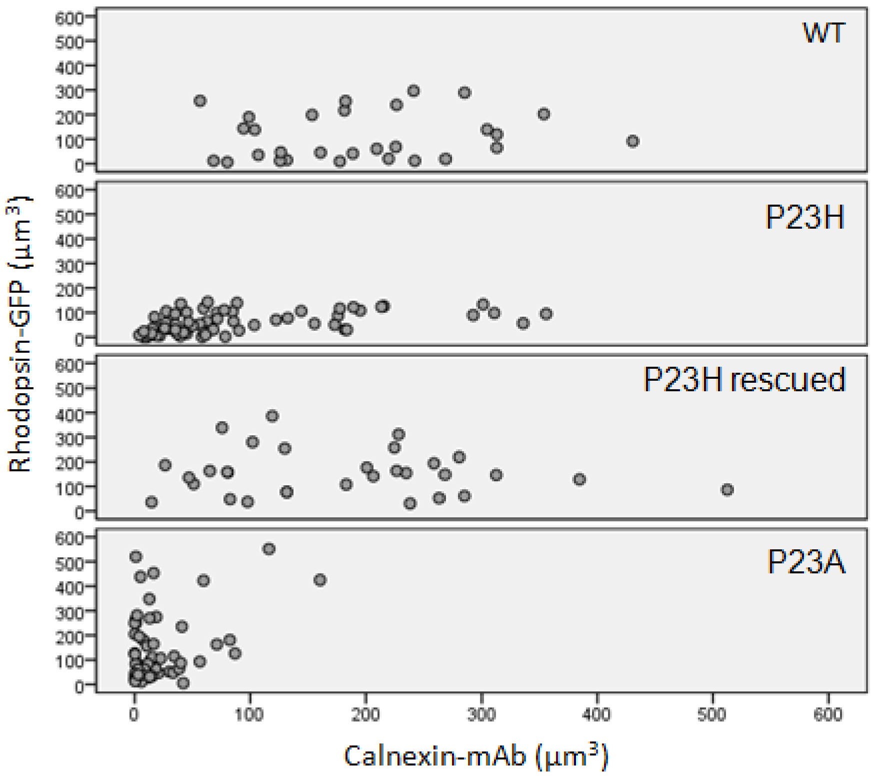

Fig. 2: Scatterplots of rhodopsin-GFP and calnexin-mAb volumetry in cells expressing wild-type (WT), mutant (P23H and P23A) and rescued mutant (P23H resc) rhodopsin.