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In silico and *in vitro* screening of rod opsin mutants and development of small chaperones with therapeutic potential

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1. Background

Inherited retinal diseases like autosomal dominant retinitis pigmentosa (adRP) [1-3], autosomal dominant congenital stationary night blindness (adCSNB) [4], and, more rarely, autosomal recessive RP [5, 6] can be linked to mutations in the *RHO* gene encoding for the G protein coupled receptor (GPCR) opsin specifically expressed in the rod photoreceptor cells (reviewed in Refs. [7]). Covalently linked to the retinal chromophore, the opsin apoprotein forms the visual pigment rhodopsin. Upon photon absorption and *cis*-*trans* isomerization of 11-*cis*-retinal, rhodopsin transits to the signaling active metarhodopsin II (MII) states that trigger the visual phototransduction cascade by coupling to the G protein transducin (Gt). The signal is terminated upon opsin phosphorylation by the GPCR kinase 1 (GRK1) and binding to visual arrestin (ARR1, whose encoding gene is known as *SAG*).
Over 200 different variants in *RHO*, listed in the human genome mutation database (HGMD), are linked to adRP, but only seven pathogenic variants have been found in adCSNB patients so far [8-12].
The vast majority of adRP *RHO* mutants is misfolded and either retained in the endoplasmic reticulum (ER) or degraded. As a conformational disease, adRP linked to *RHO* mutations is amenable to treatment with pharmacological chaperones, small molecules that bind specific sites within a misfolded protein and stabilize the native state.
We developed and implemented computational strategies to decipher the structural determinants of adRP linked to *RHO* mutations [13-15]. Significant insights in this regard were inferred from a study, in which molecular dynamics (MD) simulations were coupled to the graph-based protein-structure-network (PSN) analysis and combined with *in vitro* experiments to characterize the effects of 33 adRP-*RHO* mutations on the stability and subcellular localization of the protein in the absence and presence of the retinal ligand [14]. Based on structure-network perturbation, the adRP-*RHO* mutants were clustered into four groups, cluster 2 and 3 being the most responsive to small chaperones like 11-*cis*- or 9-*cis*-retinal. The study led to the discovery of a novel chaperone, 5,8-epoxy-13-*cis*-retinoic acid (13-*cis*-5,8-ERA), which was more effective than 9-*cis*-retinal in relieving ER retention and promoting membrane localization of three of the adRP-*RHO* mutants from clusters 2 and 3, *i.e.* T17M, P23H, and E181K [14]. In a more recent study, extensive mechanical unfolding simulations of WT and 33 adRP-*RHO* mutants with known degree of ER retention [14] were coupled with PSN analysis [15]. The computational model was able to predict ER retention of uncharacterized adRP-*RHO* mutants as well as the effect of small chaperones [15].

2. Methods and results

We pursued the combined *in silico*, *in vitro*, and *in vivo* approach to characterize the chaperone 13-*cis*-5,8-ERA and infer the determinants of adRP and adCSNB rod opsin mutants.
In vivo testing of the small chaperone targeted the P23H variant in the $Rho^{P23H/+}$ mouse model of adRP. 13-*cis*-5,8-ERA (200 μM of the compound in PBS+1% BSA and 4% EtOH) was topically delivered as eye drop for six days starting from post-natal day 14 (P14) to P19 (sacrifice day, when the peak of retinal degeneration occurs in the $Rho^{P23H/+}$ mouse model). The small chaperone could significantly reduce cell death by about 50% (as inferred by TUNEL assay) and preserve photoreceptors based on the measure of the outer nuclear layer (ONL) thickness. We also tested the ability of 13-*cis*-5,8-ERA to mitigate the tendency of the P23H mutant to form intracellular aggregates. Indeed, the formation of intracellular aggregates by P23H was observed in $Rho^{P23H/+}$ mice by ProteoStat® assay [16]. The dye labels aggregated proteins and allows recognizing intracellular accumulation of P23H in photoreceptor cells. Following administration of 13-*cis*-5,8-ERA as eye drops, the percentage of Proteostat-positive photoreceptor cells in the ONL decreased from almost 5% to 2%. These results suggest that 13-*cis*-5,8-ERA is able to reduce the aggregation of P23H mutant opsin in the ONL of $Rho^{P23H/+}$ mice. MD simulations of the four stereo-isomers of 13-*cis*-5,8-ERA in complex with wild type and P23H mutant rod opsin provided insights into the structural effects of the small chaperone. Ongoing structural optimization of the compound together with its formulation for controlled release to the retina is expected to lead to a therapeutic approach.
As for molecular characterization of pathogenic rod opsin variants, we reported two novel adCSNB mutants, A269V and W265R located in helix 6 (H6) [12]. The two new variants were characterized in comparison with G90D and T94I, CSNB variants located in H2 by combining MD simulations with BRET. While the mutants on H2 are characterized by altered kinetics of activation and deactivation of rhodopsin, leading to slower recycling of the receptor, the H6 variants share light-

independent coupling with the G protein suggesting constitutively active states. The constitutive activation of the G protein is exacerbated upon light stimulation by reduced desensitization due to impaired binding to ARR1. The study strengthens the evidence that CSNB can be caused by diverse structural mechanisms, offering a framework for precision therapy development.

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4. References

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