CRB1 Mutation in Human Retinal Organoids Alters Photoreceptor Development

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Introduction: Inherited retinal diseases (IRDs) cause progressive and irreversible blindness in approximately two to five million people globally. Crumbs cell polarity complex component 1 (CRB1) mutations account for around 10% of all genotypes associated with two common IRDs, causing severe early vision loss in children and young adults with no treatment. This vision loss is caused by a decrease in the number of photoreceptors, and some animal studies report potential Hippo/YAP and NOTCH pathway involvement. However, detailed knowledge about the timing and mechanisms underlying PR loss in human CRB1-associated retinal disease (CD) is limited. As such, we hypothesize that CD leads to a prolonged proliferative state and increased cell death, lowering the photoreceptor population.

Methods: Human retinal organoids (ROs) were generated from induced pluripotent stem cell (iPSC) lines derived from a patient with homozygous CRB1 mutation (p.Q120X; p.Q120X) and a healthy donor. Every four weeks between weeks 8-12, immunofluorescent staining for proliferation, cell type-specific markers (progenitors: PAX6; photoreceptors: recoverin, CRX, OTX2; cones: ARR3; rods: NRL, rhodopsin; ganglion cells: BRN3a; Muller glia: SOX-9) and CRB1-related proteins (CRB1, ZO-1) was performed. RNA from bulk ROs were extracted and used in qRT-PCR to measure the expression of the NOTCH (NOTCH1, NOTCH2, Hes1, Hey1, Hey2) and Hippo/Yap (STK3, STK4, YAP1) pathways.

Results: Both healthy and diseased donor-derived ROs displayed sequential differentiation consistent with fetal development in vivo with positive staining for early retinal cell markers (ganglion cells: BRN3a, progenitor cells: PAX6, CHX10; photoreceptor/ bipolar cell progenitors: OTX2, CRX) at week 8, and positive staining for later markers (cones: ARR3, rods: NRL) at week 13. Compared to week 8, expression of CRB1, YAP1, NOTCH1, NOTCH2, and Hes1 significantly increased in healthy ROs at week 12. Initial observations of mature CRB1-mutant ROs showed less rhodopsin staining and shorter brush border compared to healthy ROs at week 34, which may suggest alterations to development of mature photoreceptors. Moreover, CRB1-mutated ROs showed more disruptions in outer neuroblastic layer compared to healthy ROs at week 8, which suggests structural disorganization at an early timepoint. Further analysis of CRB1-mutated ROs is ongoing.

Conclusions: Disease-specific retinal organoids derived from a CD patient showed differences in photoreceptor development compared to healthy ROs. Further study of protein and gene expression in an organoid model will clarify the effects of NOTCH1 and Hippo/Yap signaling in CD to facilitate the discovery of novel therapies.