Drug screening and hit identification for night blindness with zebrafish

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OBJECTIVES/SPECIFIC AIMS: Retinitis pigmentosa (RP), also known as night blindness, is an incurable disease which affects ~1 in 4000 individuals globally. Since there are no effective treatment options for RP, the goal of this project is to identify novel drug treatments that can prevent or slow the disease progression. To this end, we optimized a behavioral assay, visual-motion-response (VMR) assay, to investigate rod function (Ganzen et al, ARVO, 2017; Ganzen et al., JMS, 2017). This was done utilizing a transgenic zebrafish RP model expressing human rhodopsin with the Q344X mutation. In this study, we used this model to perform a proof-of-concept screen for drugs which may improve the vision of the larvae. METHODS/STUDY POPULATION: To screen for beneficial drugs, the SCREEN-WE® REDox library was chosen for screening. This library was selected to identify a compound that may alleviate any excessive oxidative stress in the diseased retina. The Q344X zebrafish line suffers from significant rod degeneration by 7 days post-fertilization (dpf) and displayed deficits in VMR under scotopic conditions (Ganzen et al, ARVO, 2017). The Q344X larvae were drug treated beginning at 5 dpf at 10 μM. Compounds that were toxic at this concentration were retested at 1 μM. The 5 dpf stage was chosen as most of the rods are intact, and these concentrations were chosen to optimize the drug effect based on similar studies. Hits were identified by assays that provided a robust and reproducible enhancement in the Q344X VMR. The retinas of any drug hit tests were dissected from larvae crossed with a rod EGFP reporter line and whole-mounted to analyze rod survival via fluorescence. To determine if drug effects were exerted through the retina, eyeless chokh mutant zebrafish were exposed to the drug and tested with the same assay. RESULTS/ANTICIPATED RESULTS: Of the 84 compounds tested, we identified 1 drug that ameliorated the VMR of the Q344X scotopic VMR. Eyeless chokh mutant zebrafish larvae did not exhibit the same VMR when treated with the same drug. Histological analysis suggested increased rod survival in the drug-treated retina of Q344X mutations. DISCUSSION/SIGNIFICANCE OF IMPACT: These results indicate that the vision of the Q344X rod survival in the drug-treated retina of Q344X mutants. DISCUSSION/SIGNIFICANCE OF IMPACT: The genes and proteins determined from this study will provide future directions in order to determine whether obes Zucker rats are a valid model organism for the development of cardiac hypertrophy.

Exploring Müller cell-cone interactions in human fovea using 3-dimensional volume electron microscopy (3D-VM)

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OBJECTIVES/SPECIFIC AIMS: Muller cells, radial glial cells of the retinal, are the principal repository of xanthophyll pigment (lutein, zeaxanthin, meso-zeaxanthin), which are modifiable by diet and visible clinically by autofluorescence imaging. To understand the structural basis of xanthophyll visualization in vivo, we used 3-dimensional electron microscopic reconstruction of Muller cells surrounding one cone in a healthy human fovea. METHODS/STUDY POPULATION: From a 21-year-old male organ donor, dissected retinas were rejuvenated by oxygenated Ames medium then fixed in 4% glutaraldehyde. A tissue block 3.5 mm² centered on the fovea was prepared for Automated Tape Ultra-microtomy (Kasthuri et al., Cell 162, 648–661, 2015). From 1462 serial 65 nm horizontal sections, an area ~250 x 250 μm was imaged at 6 nm xy resolution. Images were stitched and aligned. TrackEM software on a pen display was used to trace, reconstruct, and display cone Ø (186) and its contacting Muller cells. RESULTS/ANTICIPATED RESULTS: Cone 5 is ensheathed by 2 types of Muller cells, outer and inner (Dacey, Arvo, 2016). The outer cell is first seen at the external limiting membrane (ELM) between cones 5 and 17. Moving inward from the ELM, it tightly wraps around cone 5’s fiber in a C-shape profile for 78 μm. This Muller cell also intermittently projects to neighboring cones, 2 of which were close to cone 5 at the ELM. As cone 5’s axon approaches the pedicle, it contours into a cross-screw. The outer cell fluidly molds to this changing shape. At this level, this Muller cell doubles in volume to encompass not only cone 5, but also cone 17 and another Muller cell. In the final 17 μm of the block the Muller cell’s volume quickly dissipates as it sends a small projection towards the internal limiting membrane, eventually encasing an OFF midget bipolar cell also associated with cone 5. In contrast to this outer cell, an inner Muller cell adjoining cone 5 spans only 19 μm, interacting directly with cone 5 and the outer cell for 3.9 μm. DISCUSSION/SIGNIFICANCE OF IMPACT: Neural-glial relationships in a human fovea are visible through 3-dimensional volume EM. The volume of Muller cells in the fovea is impressive, consistent with a pivotal role in the health of cone photoreceptors and xanthophyll homeostasis. It is possible that individual glia also enmesh the post-receptorial neurons in a cone-driven circuit, supporting the concept that xanthophylls contribute to neural efficiency in vision.