Adeno-Associated Virus (AAV) Mediated Expression of Bcl-xL Attenuates. Apoptosis in Human Corneal Endothelial Cells

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Introduction: A significant effort in biomedical research has been to identify mutations responsible for diseases from leukemia to visual degeneration. Yet, even characterizing all possible single mutations underlying a disease is insufficient for understanding its progression. How does pathogenicity arise as mutations accumulate in the soma throughout life or in the germline over generations? This question has remained elusive, even for studies focused on single genes, given the immense space of possible mutational combinations and trajectories that need to be tested. Previous attempts at mapping genotype-phenotype-fitness landscapes have focused primarily on adaptive mutational trajectories inferred from genome sequence data. However, disease-causing mutations are difficult to detect and are not amenable to such approaches, as they are often non-adaptive, transient, and rarely establish at high frequencies in the population.

Method: To investigate how disease phenotypes may arise from vast mutational space, we developed a high-throughput platform in yeast capable of assaying two crucial aspects of protein function for the visual protein rhodopsin: light-dependent activation and stability. We achieved this by engineering a fluorescence-based transcriptional reporter for measuring light-dependent rhodopsin activation, and a separate fluorescent protein fusion with distinct absorbance and emission spectra for measuring rhodopsin stability.

Results: Anti-apoptotic BclxL2/5 and control ssAAV2/5 were both able to transduce FECD CECs with high efficiency, with transduction rates of 70.47% ± 8.28 and 65.86% ± 3.54, respectively. Infection with BclxL2/5 resulted in significantly lower levels of etoposide-induced apoptosis (23.96% ± 11.54) compared to control ssAAV2/5 (70.43% ± 14.35; p=0.000) and non-transduced FECD CECs (71.68% ± 18.70; p=0.000). In untreated FECD CECs, Bcl-xL2/5 resulted in lower levels of apoptosis (9.98% ± 5.95) compared to control ssAAV2/5 (21.08% ± 16.43; p=0.264), and non-transduced FECD CECs (9.61% ± 7.87; p=0.999). All experiments were done using n = 3.

Conclusion: Anti-apoptotic BclxL2/5 can transduce FECD cells with high efficiency and protect against etoposide-induced cell death. Future studies could explore using BclxL2/5 to extend the corneal transplantation window past 14 days following donor death to increase the number of donor corneas available for transplantation.