Production of Recombinant Cataract-Inducing Alpha-B Crystallin With E. coli

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Congenital cataracts are present in 20,000-40,000 births each year and are among the leading causes of childhood blindness. Surgery is currently the only available treatment due to a poor understanding of the biochemical relationship between genetic mutation and cataract formation. Missense mutations in the eye lens crystallin alpha-B (CRYAB) have previously been shown to lower CRYAB's ability to "chaperone" the stability of the eye lens. This allows denatured proteins to aggregate and form cataracts. Cook et al. trained a machine learning (ML) model on bioinformatic data of documented CRYAB mutations to predict potentially pathogenic undocumented mutations. This tool has demonstrated up to 96.50% accuracy but must be experimentally validated in-vitro due to the poorly-understood prediction mechanisms of such an algorithm. This project investigates several ML-predicted mutations via recombinant DNA and measures their in-vitro pathogenic characteristics in comparison to documented control mutations. Wild-type CRYAB DNA were amplified by polymerase chain reaction and the 24 mutant DNA were obtained via site-directed mutagenesis. Proteins were expressed in E. coli and purified via ammonium sulfate precipitation and anion exchange chromatography. Characterization methods were selected to reflect the CRYAB pathogenic pathway. Cytotoxicity was measured by yield and purity, which were obtained by UV-vis and SDS-PAGE. The protein aggregation response to heat-shock and addition of Zn2+ was measured by dynamic light scattering. Chaperone activity was measured by tubulin aggregation assays. Current results have neither refuted nor validated the ML model with statistical significance but ongoing work holds promise for revealing the pathogenic mechanism of congenital cataracts.