CALIFORNIA STATE SCIENCE FAIR
2017 PROJECT SUMMARY

Name(s)
Kalyan Nath

Project Number
S0518

Project Title
Spectral Phasor Protein Characterization of Huntington's Disease for Early Onset Diagnosis

Abstract

Objectives/Goals
The goal of the study was to find a method to detect the onset of Huntington's disease, HD, in its earlier stage. I hypothesized that by using FLIM (Fluorescence Lifetime Imaging) and Spectral Phasor Analysis, I will be able to derive a range of emission values that differentiate the diseased cells from healthy rat neuronal (PC12) and human embryonic kidney (HEK 293) cells.

Methods/Materials
Materials: HEK 293 cells, PC12 cells, GFP, SimFCS computer tool, a two-photon femtosecond microscope, FBS-DMEM medium.
Procedure: Phase 1: A total of 61 PC12 and HEK 293 cells were transfected with either the diseased or healthy variant of the Huntington gene. The Huntingtin protein was tagged with GFP, whose emission wavelength varies based on its attachment to a diseased or healthy protein. The resulting fluorescent emission wavelengths were collected via FLIM and analyzed via Spectral Phasor Analysis. Phase 2: The emission wavelengths were grouped into four categories (diseased and healthy PC12, diseased and healthy HEK293) and data range for each category was created. Phase 3: The accuracy of these data ranges were tested with emission wavelength of seven cells whose disease state was unknown to me but known to my mentor.

Results
The data ranges provided here clearly distinguish healthy and diseased cells as was hypothesized. A. Data ranges HEK293 cells (Healthy: 514nm-516nm; Diseased: 516nm-531nm). PC12 cells (Healthy: 527nm-538nm; Diseased: 550nm-593nm). It shows that the ranges for diseased cells are higher than that of the healthy cells. B: The emission wavelengths of 7 unknown cells (4 HEK and 3 PC 12), correctly fitted into their respective cell categories validating the accuracy of the established data ranges.

Conclusions/Discussion
HD is marked by the aggregation of polyQ proteins in a cell which are not always visible even under an electron microscope. This study introduced a novel biological approach for early diagnosis of HD by doctors and researchers. Although proven to be a highly efficient method, FLIM and Spectral Phasor Analysis have not been applied to HD. I will extend my study to include different types of cell lines and a variety of dyes as well as focus on fluorescent lifetime values derived from the cellular membrane of diseased and healthy cells based on a recent discovery that HD also results in alteration of lipid metabolism within the cellular membrane. As a limitation, I do recognize that cells within a human patient cannot be fluorescently tagged with a dye easily. However, via the use of immunochemistry, the

Summary Statement
By measuring the fluorescent emission values of diseased and healthy huntingtin protein, I was able to detect the presence of Huntington's Disease in early-stage cells in minute quantities.

Help Received
I developed the idea of using FLIM and Spectral Phasor Analysis in Huntington's Disease. My mentor prepared the cells for me and I analyzed and interpreted the data.