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The use of lysotracker to label lysosomes in attached cell cultures

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Our overall goal is to develop stem cell therapies to alleviate neurodegenerative diseases. We are focused on a class of hereditary diseases called the neuronal ceroid-lipofuscinoses (NCLs; often grouped together under the name Batten Disease). The NCLs are a group of autosomal, recessively inherited, progressive neurodegenerative disorders characterized by the accumulation of autofluorescent lipopigment within storage bodies of the lysosomes of cells in various tissues, including the retina and CNS. Children with NCLs are normal at birth but undergo progressive brain and retinal atrophy, and patients with early onset forms of NCL seldom survive past their teenage years. Of the genes responsible for NCLs, Cln1, Cln2, Cln3, Cln5, Cln6 and Cln8 have been cloned and characterized to some extent. We are working with a mouse model of NCLs with a mutation in the Cln1 gene that exhibits degeneration of various nervous system cells. Previously, we were able to quantitate storage body build-up as a function of autofluorescence. However, this technique does not allow us to visualize the lysosomes with enough quality or quantitate them accurately. Therefore, my project has involved testing LysoTracker, an organelle-selective, fluorescent probe which labels and tracks acidic organelles (lysosomes) in live cells at nanomolar concentrations. Before we will be able to use LysoTracker on brain slices of deficient mice, we first need to confirm its ability to label lysosomes in simple attached cell cultures. We are testing LysoTracker on several different types of plated cells, which include Cln1 deficient fibroblasts, neuralized B5 embryonic stem cells, neuralized B5 embroid bodies, and bone marrow derived cells. After the cells are plated and exposed to LysoTracker for a given amount of time, we view the cells on a confocal microscope in order to quanitate the accumulation of storage bodies. If we are able to successfully label the lysosomes of the different cells types with the LysoTracker, then we will be able to investigate the application of LysoTracker in different neuronal cell types. In the future, we would like to use LysoTracker to label lysosomal storage bodies in live brain slices of Cln1 deficient mice that have undergone stem cell therapies. The ability to quantitate a reduction of autofluorescence correlates to a reduction in disease phenotype and possibly symptoms.