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Specific AIMs

Alpers-Huttenlocher Syndrome is a severe neurological disorder that also affects other parts of the body. The disease is known for having episodes of seizures that can often lead to a series of them, and are untreatable. The disease affects 1 in 100,000 to 250,000 and 80% of individuals present symptoms within the first two years of their lives. The result of this disease is fatality and usually by the means of liver failure.The gene POLG (aka POLG1) is a major contributor to a multitude of genetic diseases and the most severe is Alpers-Huttenlocher Syndrome. The POLG gene encodes the α (alpha) subunit of the protein polymerase γ (gamma). This protein is necessary for mitochondria to replicate mitochondrial DNA (mtDNA), and cell survival. POLG mutations cause non functionality in the α subunit and a decreased amount of mtDNA replication which leads to a drop in the amount of energy the cell receives. Although we know the phenotype caused by the POLG mutations, we do not know the affect the mutations have on the alpha subunit protein and insight into this could lead to treatments of this disease as well as many other diseases associated with this gene.

My goal is to obtain a better and more complete understanding of the effects of the POLG gene on the mitochondria and the human body overall. We will test our hypothesis that certain POLG mutations change the conformation of the α subunit so it becomes non functioning and unsuccessful in participating in the DNA polymerase complex in the mitochondria.

1. Identifying mutations on the POLG gene is critical to understanding how the cell is affected. We will use **RNA sequencing** to compare the wild-type to an array of mutants in order to identify the different RNA modifications in each mutant. We can use this information to identify the critical areas that cause modifications to the α subunit directly.

2. Identifying modifications to our protein is extremely useful in determining the reasons we have our disease phenotype. One such way we can test this is through **Small Molecule assays**. By determining what small molecules interact with our α subunit we may better understand the cause of its ineffectiveness within the mitochondria.

3. We will study the protein interactions within the protein polymerase γ and how they will affect the function of mtDNA replication. Specifically we will look at the α subunit by using **Pull-Down assays** (Immunoprecipitation), Pull-down assays are useful for confirming the existence of a protein-protein interactions.

4. We want to measure the overall expression of our α subunit protein in both our wild-type and in our mutants. **Mass Spectrometry** can be paired with our Pull-down assays in order to identify the amount of our α subunit. This will be useful to determine if we notice a difference in the overall amount of our protein in functioning and nonfunctioning DNA polymerase.

This project will help us better understand Alpers-Huttenlocher syndrome and possibly lead us to more effective treatments and a better understand of the mitochondria as a whole. Along with this disease we will also be able to better understand other disorders associated with the POLG1 gene.

References

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- *"Correlative Light- and Electron Microscopy with Chemical Tags." Sciencedirect.com. Journal of Structural Biology, May 2014. Web. 26 Feb. 2015*
- *"Pull-Down Assays." Pull-Down Assays. N.p., n.d. Web. 26 Feb. 2015.*