Internship Proposal

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Project Title:
Unraveling how very-long-chain fatty acids impact the neuronal cytoskeleton

Level:

Master

Project Summary:

The project's primary goal is to determine how the accumulation of very-long-chain fatty acids impacts neuronal health and the dynamics of the neuronal cytoskeleton. To achieve our goal, we use a mutant mouse with a defect in VLCFA degradation that causes the build-up of fatty acids, and serves as a disease model for a rare disorder. Using neuron cultures from WT and Knockout (KO) mice, we will characterize tubulin and actin dynamics and organelle transport (e.g., mitochondria and synaptic vesicles) along axons. In addition, because VLCFA are solely derived from endogenous synthesis (through the process of fatty acid elongation), we will also induce VLCFA accumulation in neuron cultures by adding precursor fatty acids. Based on our preliminary data, it is expected that the work reveals how defects in the cytoskeleton and/or organelle transport mediate the large axon swellings and organelle accumulations observed in the brain and spinal cord of mutant mice.

Work to be developed by the student:

The student will carry out the isolation and culture of primary cortical neurons from WT and KO mice, and the different assays to evaluate the neuronal cytoskeleton. To characterize microtubule dynamics, neurons will be transfected with Eb3-GFP to detect the dynamic growth and shrinkage of microtubules. Using specialized software, the live cell time-lapse recordings will allow the measurement of microtubule growth speed, shrinkage speed, catastrophe rate, and rescue rate. Similarly, the F-actin organization and dynamics will be assed in cultured neurons transfected with Lifeact-GFP. The evaluation of organelle transport in neurons from WT and KO mice will be carried out using in vivo imagining of mitochondria (labeled with MitoTracker) and synaptic vesicles (labeled with Synaptophysin-RFP). The work plan will also involve manipulating VLCFA using shorter fatty acids and the rescue of neuronal defects using a gene therapy plasmid.

References:

1- Ferdinandusse S, et al (2017) ACBD5 deficiency causes a defect in peroxisomal very long-chain fatty acid metabolism. J Med Genet. 54(5):330-337.

2- Costello JL, et al. (2017) ACBD5 and VAPB mediate membrane associations between peroxisomes and the ER. J Cell Biol.216(2):331-342.

3- Teixeira CA, et al (2014) Early axonal loss accompanied by impaired endocytosis, abnormal axonal transport, and decreased microtubule stability occur in the model of Krabbe's disease. Neurobiol Dis. 66:92-103.

4- Pinto-Costa R, et al (2020) Profilin 1 delivery tunes cytoskeletal dynamics toward CNS axon regeneration. J Clin Invest. 130(4):2024-2040.

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