Internship Proposal

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Project Title:
Determine the impact of impaired acylation on nervous tissue using a novel mutant mouse

Level:

Master Student

Project Summary:

Zdhhc14, a member of the ZDHHC family responsible for protein palmitoylation, has emerged as a significant factor in human disorders, particularly neurodevelopmental conditions. Its deficiency, often associated with microdeletion syndrome, leads to a spectrum of abnormalities including microcephaly, delayed development, and hearing deficiency. The multifactorial nature of these disorders suggests a complex interplay of genetic factors, with ZDHHC14 deficiency contributing to neurological dysfunction. In order to understand the in vivo functions of Zdhhc14 and the effects of its deficiency, we used CRISPR-Cas9 technology to generate a loss-of-function mouse mutant. Understanding the role of ZDHHC14 in these disorders will shed light on the molecular mechanisms underlying neurodevelopmental abnormalities and may offer potential avenues for therapeutic interventions.

Work to be developed by the student:

The aim is to determine the target proteins acylated by Zdhhc14 activity and characterize the Zdhhc14 knockout mice and its underlying neuropathology. Using the acyl biotinyl exchange (ABE) assay you will determine which proteins are acylated by Zdhhc14. To characterize Zdhhc14, you will analyze mRNA and protein expression and determine the expression pattern of Zdhhc14 in tissues and cells. Using several histological and microscopy approaches (confocal and electron microscopy), you will characterize the neuropathology in the brain of Zdhhc14 mice. Based on preliminary experiments, Zdhhc14 KO mice have myelination defects and neuron degeneration.

As such, you will acquire hands-on experience in several techniques including molecular biology (isolation mRNA, cDNA preparation, PCR, gel electrophoresis), biochemistry (acylation assays, western blot), cell biology (cell culture, light microscopy, electron

microscopy, histology) and training in laboratory mouse manipulations.

References:

-Wild AR, et al. Exploring the expression patterns of palmitoylating and de-palmitoylating enzymes in the mouse brain using the curated RNA-seq database BrainPalmSeq. Elife. 2022 Jul 12;11:e75804. doi: 10.7554/eLife.75804.

-Shimell JJ, et al. The X-Linked Intellectual Disability Gene Zdhhc9 Is Essential for Dendrite Outgrowth and Inhibitory Synapse Formation. Cell Rep. 2019 Nov 19;29(8):2422-2437.e8. doi: 10.1016/j.celrep.2019.10.065.

-Michelson M, et al. Delineation of the interstitial 6q25 microdeletion syndrome: refinement of the critical causative region. Am J Med Genet A. 2012 Jun;158A(6):1395-9. doi: 10.1002/ajmg.a.35361.

-Sanders SS, et al. The palmitoyl acyltransferase ZDHHC14 controls Kv1-family potassium channel clustering at the axon initial segment. Elife. 2020 Nov 13;9:e56058. doi: 10.7554/eLife.56058.

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