Title of Proposal: INTERROGATING LIPOFUSCIN TO IMPROVE BRAIN AGING

Abstract (500 words)
The accumulation of lipofuscin, an autofluorescent storage material comprised of oxidized proteins, lipids, and metals, is a natural phenomenon in the aging brain. While common neurodegenerative diseases such as Alzheimer's disease (AD) exhibit accelerated lipofuscin storage, it is most pronounced in Neuronal Ceroid Lipofuscinoses (NCL), a group of pediatric neurodegenerative diseases where lipofuscin serves as the defining pathology. NCLs falls under the lysosomal storage diseases (LSDs) and are characterized by lysosomal deficiency, indicating compromised clearance mechanisms in the brain. Initial insights into lipofuscin nature stemmed from the NCL gene, PPT1/CLN1, encoding a depalmitoylating enzyme implicated in protein degradation. To unravel lipofuscin's composition and role, we developed a method to purify lipofuscin from aged wildtype (WT) and PPT1 knockout (KO) mice brains, the latter serving as a CLN1 model. Subsequently, we developed a mass spectrometry-based quantitative spatial multiOMICs platform to study protein-lipid organization directly from native membranes. Surprisingly, we observed similarities between age-related and NCL-related lipofuscin, with both being enriched in S-acylated proteins (>95%) and containing the majority of PPT1 substrates. Notably, mitochondrial proteins constituted the largest protein class in lipofuscin, corroborated by lipidomics data exhibiting key mitochondrial markers. We also serendipitously made a critical discovery that the long-chain polyunsaturated fatty acids are enriched (lc-PUFA) in lipofuscin lipidome, indicating clear lipotoxic stress. Based on these findings, our proposal centers around the hypothesis that accumulation of protein lipidation through S-acylation, triggered by organellar stress, drives lipofuscin biogenesis. Aim 1 focuses on molecularly characterizing lipofuscin and delineating the role of protein S-acylation/de-acylation dynamics in its formation. Model membrane studies will elucidate how an overdrive of S-acyl lipidation of the mitochondrial proteome leads to lipofuscin formation, connecting organellar dysfunction and lipid homeostasis. In Aim 2, we plan to test the impact of decreasing S-acylation on lipofuscin clearance in iNeurons. Using human iPSC-derived iNeurons, two strategies will be employed to reduce S-acylation: inhibiting palmitoyl acyltransferases and increasing de-acylation with small molecules. The efficacy of these strategies in rescuing lipofuscin formation will be assessed, alongside monitoring protein turnover and iNeuron health markers. Successful implementation of these aims will shed light on whether resolving lipofuscin accumulation can enhance health-span, paving the way for novel gerotherapeutics.