Title: Age-Dependent Rewiring of Ubiquitin Signaling: A Molecular Link Between Aging and Neurodegeneration

Abstract:

Aging is the major risk factor for many neurodegenerative diseases, yet the molecular mechanisms that link age-dependent loss of cellular homeostasis to neurodegeneration remain poorly understood. Protein homeostasis (proteostasis) is essential for maintaining cellular and neuronal function, and ubiquitin signaling plays a central role in controlling protein fate, including proteasomal degradation. Here, we investigate how ubiquitin signaling is remodeled during aging and how these changes contribute to longevity and neurodegenerative disease.

Using *Caenorhabditis elegans* as a model of aging, we found that aging induces profound changes in the ubiquitinated proteome, characterized by a global reduction in protein ubiquitination driven largely by increased deubiquitinase activity. These age-dependent changes impair the ubiquitin–proteasome pathway and lead to the accumulation of specific structural and regulatory proteins that influence lifespan. Among these, the RAC signaling regulator EPS-8 emerged as a critical mediator linking age-dependent proteostasis defects to neuronal dysfunction. Increased EPS-8 levels and hyperactivation of EPS-8/RAC signaling alter cytoskeletal organization in neurons and muscle and promote the pathological aggregation of proteins associated with neurodegenerative diseases, including Huntington's disease-related polyglutamine repeats and amyotrophic lateral sclerosis (ALS)-associated mutant FUS and TDP-43 variants. Conversely, reducing EPS-8/RAC signaling suppresses protein aggregation and protects neuronal function during aging.

We further identify the deubiquitinating enzyme USP4 as an evolutionarily conserved regulator of EPS8 ubiquitination and degradation in both *C. elegans* and human cells. Age-associated upregulation of USP4 contributes to EPS8 accumulation, whereas reducing USP4 activity prevents EPS8 dysregulation, extends lifespan and attenuates disease-associated phenotypes. Together, these findings reveal an age-dependent rewiring of ubiquitin signaling that connects impaired proteostasis to aberrant cytoskeletal signaling, pathological protein aggregation and neurodegeneration. Our work identifies the conserved USP4–EPS8/RAC axis as a potential molecular link between aging and neurodegenerative disease and suggests that restoring ubiquitin-dependent protein regulation may provide new therapeutic opportunities for age-related neurodegeneration.