ADMINISTRATION OF NARINGENIN AMELIORATES DSPASTIN LOSS OF FUNCTION PHENOTYPES IN *DROSOPHILA MELANOGASTER*

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Introduction: SPAST gene, known as SPG4, encodes the microtubule-severing protein called spastin. Mutations in SPAST are the most common cause of autosomal dominant hereditary spastic paraplegia (HSP). HSP is characterized by weakness and spasticity of the lower limbs due to the axonal degeneration of corticospinal tracts. Spastin is an AAA ATPase protein that regulates microtubules stability, lysosomal morphology, endosomal tubule fission and Lipid Droplets (LDs) biogenesis/turnover. Lysosomes and LDs biogenesis are strictly linked to authophagosomes formation and maturation but the role of Spastin in the autophagic pathway and the related implications in neurodegeneration mechanism of HSP still remain unknown. In Drosophila melanogaster, the depletion of Dspastin (Drosophila spastin) caused an excessive stabilization of micro-tubules at the neuromuscular junction synapse and muscles. Cytoskeleton defects produced by lack of Dspastin seem to be responsible of neuromuscular junction synaptic area reduction, the decrease of LDs biogenesis/turnover, the partial lethality of flies at pupal stage (low eclosion rates) and the reduction of locomotor activity.

Materials and methods: In this work, we analyzed the effects of loss of Dspastin on autophagy by using the RNAi (RNA interference) approach. We investigated the formation of early and late autophagosomes, the lysosomes and the autolysosomes using different fluorescent markers: the transgenic lines carrying the monomeric tandem construct GFP–mCherry-Atg8a to measure autophagosomes maturation, the fluorescent lysosomes markers GFP-Lamp1to quantify lysosomes defects and the marker mCherry-Atg8a to monitor the acidic compartments. Moreover, we used the refractory to Sigma P (Ref(2)P) antibody (fly homolog of human p62) to evaluate the autophagic flux. All fluorescent structures were analyzed by confocal microscopy and quantified by the ImageJ software.

Results: For this study, we quantified the differences of these fluorescent markers in neuronal and muscle tissues in control and RNAi spastin flies. Our data showed that loss of Dspastin impaired the autophagic flux, increasing the number of early/immature and late autophagosomes, and decreasing the number of autolysosomes. Moreover, we showed that in Drosophila the lack of Dspastin produces larger lysosomes, unable to fuse with the autophagosomes, leading to the accumulation of Ref(2)P.

Discussion and conclusions: Finally, we treated control and DSpastin loss of function flies with the flavonoid naringenin, complexed with hydroxylpropyl-β-cyclodextrin to ameliorate its bioavailability. Administration of naringenin at 0.5mM partially rescues the lethality phenotype of Dspastin loss of function, the locomotor behavior defects and the autophagy impairment, thus representing a promising strategy for the management of HSP symptoms.