The protective effect of naringenin in REEP1 drosophila model of hereditary spastic paraplegia is mediated by induction of ER-phagy

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Defects in endoplasmic reticulum (ER) membrane shaping and interactions to other organelles seem to be one of the crucial mechanisms underlying Hereditary Spastic Paraplegia (HSP), a complex genetic disorder characterized by the axonal degeneration of corticospinal tracts. The structural organization of this complex organelle is created and maintained thanks to a continuous process of membrane remodeling, governed by homotypic fusion events, tubulation and curvature rearrangements as well as cytoskeletal transport and autophagy (Goyal and Blackstone, 2013; Chen et al., 2013). In parallel to the ER remodeling process, lipid metabolism is another important emerging cellular aspect of HSP mechanism. The role of lipid droplets (LDs) in HSP mechanism has been highlighted by recent evidences that proteins such as seipin/spg17, erlin2/spg18, atlastin/spg3a, spartan/spg20, REEP1/spg31 and spastin/spg4 localize or affect cellular LDs turnover (Belzil and Rouleau, 2012; Papadopoulos et al., 2015; Tan et al., 2014). In spite of these findings, the relationship between ER and the lipid pathway with the related implications in neuronal disorder in HSP still remains unknown.

Here we report the analysis of a Drosophila melanogaster model for an autosomal dominant form of HSP caused by mutations in the SPG31 gene. SPG31 codifies for REEP1, a transmembrane protein belonging to the TB2/Dp1/HVA22 family. REEP1 interaction with atlastin-1 (SPG3A) and spastin (SPG4), the other two major HSP linked proteins, has been demonstrated to modify ER architecture in vitro. Indeed, REEP1 is required to confer stress resistance against the accumulation of unfolded proteins (UPR) induced by tunicamycin, thapsigargin and 1,4 dithiothreitol (DTT) (Appocher et al. 2014).

We manipulated the expression of Drosophila REEP1 by using loss of function or gain of function alleles and then we analyzed ER morphology and stress response, mitochondria defects, LDs biogenesis and autophagic pathway activation. D-REEP1 absence caused ER and mitochondria elongation, decreased LDs biogenesis and attenuated UPR response. The UPR response attenuation, sustained by ATF4 activation together with the decline XBP1 unspliced and spliced levels is a condition reported in prolonged ER stress. Treatments with DTT and thapsigargin determined UPR response of REEP1 loss of function model comparable to control flies. However, autophagic flux resulted only activated in control flies upon ER stress induction but not in REEP1-lacking flies. Conversely, REEP1 gain of function caused ER and mitochondria fragmentation together with the autophagic pathway activation, suggesting REEP1 involvement in controlling ER function and morphology by the autophagic flux.

Finally, treatment with naringenin, a compound demonstrated to induce LDs biogenesis in Drosophila nervous system and muscle tissues, activated the autophagic flux in control flies as well as in flies lacking REEP1, rescuing ER, LDs and mitochondria defects of REEP1 loss function alleles.

The finding of novel compounds such as naringenin in modulating the ER homeostasis by autophagic degradation is of particular relevance for the maintenance and the function of neuronal cells as well as for the development of novel therapeutic strategies for neurodegenerative diseases.

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