Mutations in VCP induce lysosomal alterations and autophagy activation in ALS neuronal models

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Valosin Containing Protein (VCP) is an ATPase protein member of the AAA+ protein family. VCP is involved in various pathways that concur in maintaining cellular homeostasis. VCP mutations have been correlated different proteinopathies including neurodegenerative diseases as ALS. VCP-mutants are associated associated with the presence of alteration of the Protein Quality Control System: ubiquitin inclusions, TDP-43 mis-localization and aggregation, and abnormal vacuoles. To date, the mechanisms correlated to VCP-mutants that lead to cell toxicity and death are still not defined.
In this study, we identify VCP-mutants pathological mechanisms in an ALS-model. We overexpress VCP WT, VCP R155H and VCP R191Q in NSC-34, a motor neuron mouse immortalized cell line. In first instance, we found that both VCP mutants from insoluble aggregates and induce lysosomal alteration in size, morphology, activity and membrane breakage.
Lysosomal damage is known to lead to cell toxicity and death, so cells activate different mechanisms to remove damaged lysosome as the activation of autophagy. Therefore, we studied variance in the autophagic flux in presence of VCP-mutants by analysing LC3 conversion and p62 accumulation. We could determine that VCP-mutants are correlated with an activation of the autophagic flux. Moreover, by analysing transcription factors that regulate autophagy we determined that VCP-mutants positively regulates autophagic flux by specifically activating the transcription factor TFE3. Results also determined that TFE3 activation triggered by VCP-mutants presence is mediated by calcineurin, a Ca2+ dependent phosphatase. In parallel, we excluded the involvement of TFEB in this pathway. Overall, these data suggest that lysosomal damage and leakage induced by VCP-mutants activate calcineurin which in turn mediates TFE3 dephosphorylation and nuclear translocation inducing autophagy. In support to this we found that VCP mutants enhances insoluble protein-aggregates with a specific dependency from the autophagic pathway.