Mitophagy impairment in PBMCs of sporadic ALS patients

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Objectives. Functional defects, mislocalization, aggregation and altered mitochondrial morphology were found in soma and proximal axons of skeletal muscle and spinal motor neurons of ALS patients (1,2). An inefficient clearing of damaged mitochondria is an important issue in ALS pathogenesis. The present work aimed an investigation of mitochondrial characterization in PBMCs, evaluating several pathways in which mitochondria are involved, such as dynamism, apoptosis and mitophagy.

Methods. We analyzed by TEM the morphology of mitochondria in PBMCs of healthy controls and patients. Moreover, we studied by both WB and RT-qPCR protein level of several proteins involved in mitochondrial fusion and fission (OPA1, MFN1, DRP1), apoptosis (Cyt C, Bcl-2) and mitophagy (LC3, PINK1). By both immunofluorescence and MitoTracker™ Red FM we evaluated the accumulation of autophagosomes and mitochondria in cytoplasmic compartment of PBMCs of healthy controls and patients. Finally, we studied the relative number of total mitochondria using the mtDNA quantification by qPCR (3).

Results. TEM analysis revealed that in PBMCs of sALS patients mitochondria appear damaged, enriched in vacuoles (yellow arrows) inside of them and disrupted disorganized cristae (red arrows) (**Fig. 1**). We do not found any change in mitochondrial dynamism, without statistically significant difference expression of MFN1 and DRP1 between healthy controls and sALS patients (**Fig. 2**). Unchanged protein levels of OPA1, MFN1 and DRP1 were observed in PBMCs of sALS patients when compared to CTRL and between Cytoplasmic and Mitochondrial compartment (**Fig. 3**). No statistically significant change in protein levels of Cyt C release and anti-apoptotic protein Bcl-2 were observed in PBMCs of sALS patients when compared to CTRL and between Cytoplasmic and Mitochondrial compartment (**Fig. 4**). We found statistically significant increase in LC3-II/LC3-I ratio and in PINK1 protein level (*p<0.05) in PBMCs of sALS patients when compared to CTRL (**Fig. 5**). In sALS patients we observed co-localization of PINK1 and LC3, while we did not found it in healthy controls. In green: PINK1; in red: LC3; in blue: DAPI (nuclei). Using Mitotracker we did not see accumulation of mitochondria in PBMC of healthy controls, while we observe it in sALS patients (**Fig. 6**). We quantified relative mitochondria with quantification of mtDNA using qPCR. We used tRNA-LEU as mitochondrial DNA gene and β2-microglobulin as nuclear DNA gene. No difference was found between CTRL and sALS (**Fig. 7**). We proposed impairment mechanism: there is no change in mitochondrial dynamism and apoptosis in PBMCs of sALS patients, while we observe an increase in mitophagy pathway that lead to accumulation of damaged mitochondria in cytoplasmic compartment (**Fig. 8**).

Conclusions. These results led us to hypothesize that in patients' PBMCs mitochondria, damaged by the oxidative stress, enter in the mitophagy pathway. ALS is characterized by the inefficiency of mitophagy, thus, damaged mitochondria rounded by autophagosomes accumulates in the cytoplasmic compartment. The presence of dysfunctional mitochondria is not balanced by the replacement with normal mitochondria, so, it results in an additional stress for cell metabolism and survival.

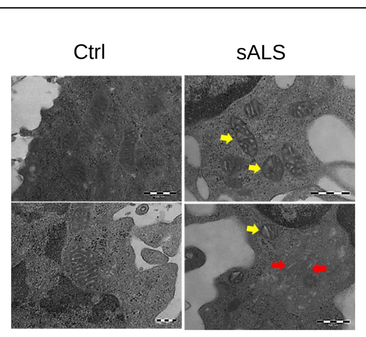

Fig. 1 – TEM analysis of mitochondria

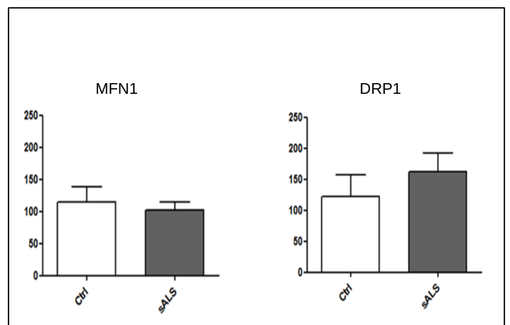

Fig. 2 – mRNA expression of MFN1 and DRP1

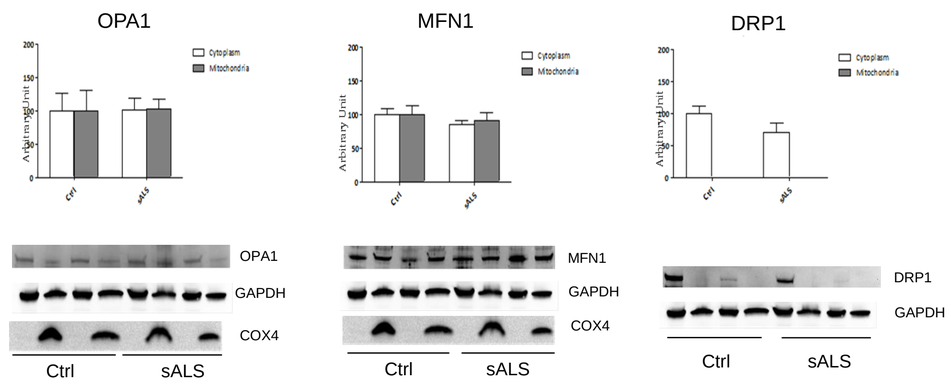

Fig. 3 – Protein expression of OPA1, MFN1 and DRP1

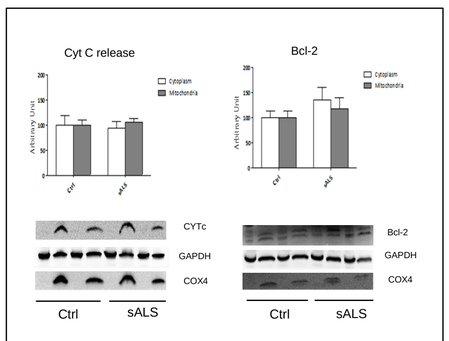

Fig. 4 – Evaluation of apoptotsis

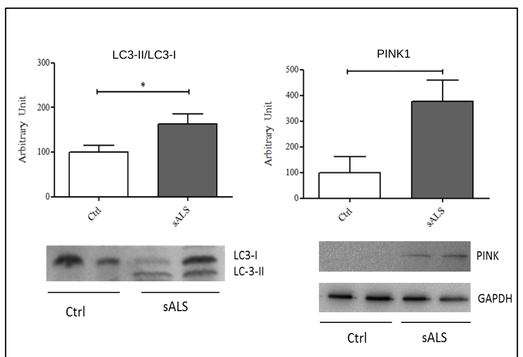

Fig. 5 – Investigation of mitophagy

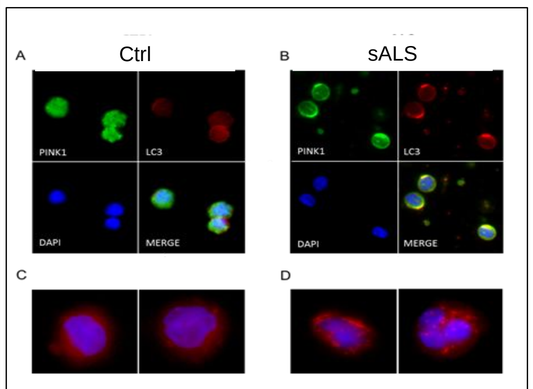

Fig. 6 – Co-localization of PINK1/LC3 and accumulation of mitochondria

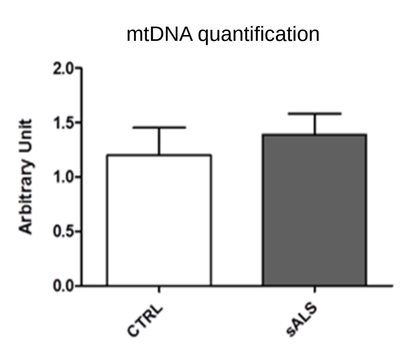

Fig. 7 – Quantification of mitochondria

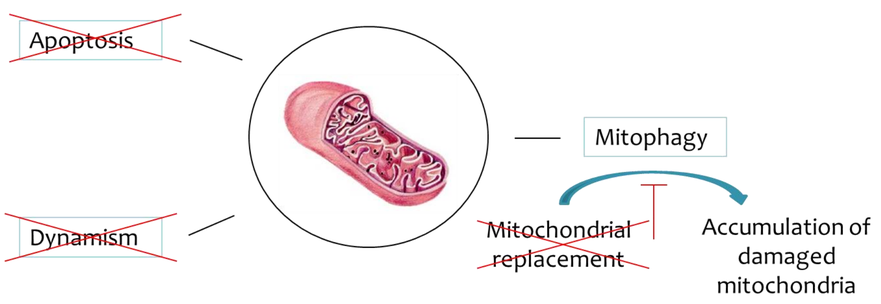

Fig. 8 – Proposed mechanism of mitophagy impairment

References

1) Chung M.J. et al., *Ultrastruct Pathol.* 2002
2) Sasaki S. et al., *J. Neuropathol. Exp. Neurol.* 2007
3) Venegas V. et al., *Curr Protoc Hum Genet.* 2011