LYMPHOBLASTOID CELL LINES: AN INTERESTING MODEL TO STUDY ALS DISEASE MECHANISM

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Objectives. New evidences switch the ALS hypothesis from a neurocentric to a multisystemic or non-neurocentric point of view. From 2006, we focused on the study of non-neural cells, in particular patient PMBCs and lymphoblastoid cell lines (LCLs) (1,2). We first characterized LCLs of sALS and patients carrying SOD1, TARDBP, FUS mutations to identify biologically relevant signature associated with ALS. Moreover, we highlighted whether and how mutations differentially affect ALS-linked pathways, i.e. protein mis-localization/aggregation and mitochondrial dysfunction.

Methods. PBMCs were immortalized into LCLs via Epstein-Barr Virus (EBV) infection (3). Protein aggregates and mis-localization were investigated by WB and immunofluorescence. Mitochondrial dynamics (OPA1, MNF1 and Drp1) and morphology were investigated by WB and Transmission Electron Microscopy (TEM). The Seahorse XF-24 Bioanalyzer was used to simultaneously measure oxygen consumption rate (OCR) and glycolytic flux by determining the extracellular acidification rate (ECAR).

Results. In fractionated nucleus and cytoplasm, LCLs from *sALS patients* showed a decrease in SOD1, TDP-43 and FUS protein expression compared to Ctrl. No protein re-localization or aggregation were reported. Mitochondria maintained a normal longitudinal shape but appeared smaller, probably related to Drp1 increased expression. In *SOD1 mutated patients*, we observed SOD1 cytoplasm/perinuclear aggregates. No changes were reported in fusion and fission protein expression. Smaller and round-shaped mitochondria account for favoring fission pathways. An increased mitochondrial respiration and a decrease Spare Respiratory Capacity (SRC) referred to mitochondrial difficulties to cope with high energy demand. In *TARDBP mutated patients*, SOD1 and FUS maintained their normal localization; instead, TDP-43 mis-localized and accumulated in the cytoplasm, where round-shaped toxic aggregates appeared. Mitochondria showed elevated levels of MFN1 suggesting an increased fusion, further confirmed by giant degenerated mitochondria. No significant alterations in LCLs of *FUS mutated patients* were reported. Megamitochondria pointed at increased mitochondria damage.

Conclusions. Different gene mutations affect the various cellular mechanisms: TARDBP and FUS mutations mainly affect fusion, while sALS and SOD1 mutation mainly affect fission. Aggregates formation is similar in TARDBP and SOD1 mutated patients. Independently from mutation, LCLs are not able to work under excessive energy request, suggesting a significant metabolic defect.. LCLs are an intriguing tool to study ALS specific pathological pathways and/or to identify new ones, because they share features typical of degenerating MNs.

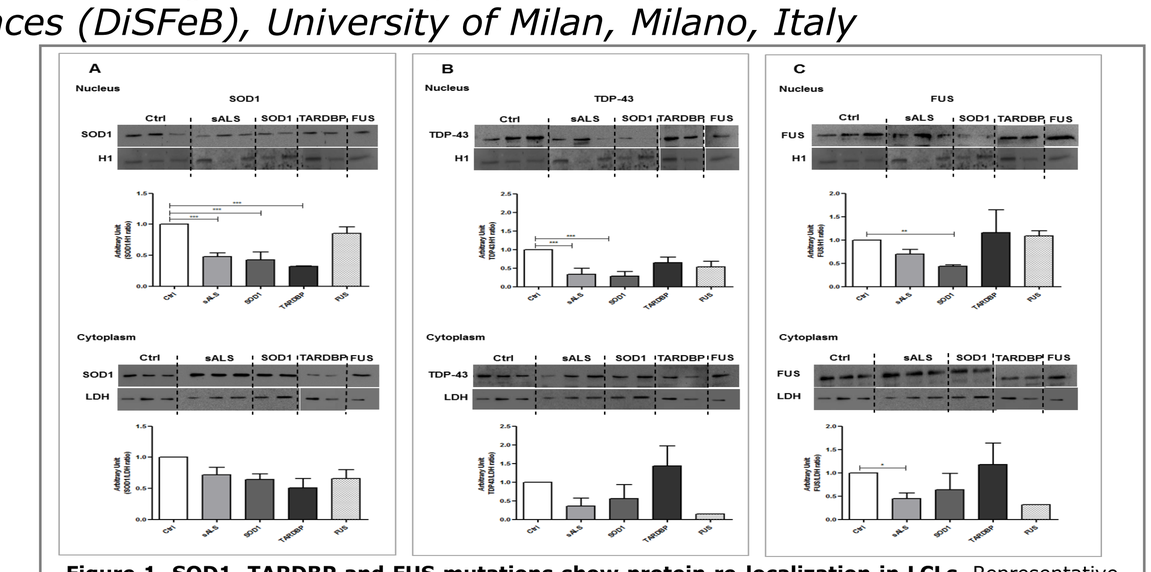

Figure 1. SOD1, TARDBP and FUS mutations show protein re-localization in LCLs. Representative immunoblots for nuclear and cytoplasm SOD1 (**A**), TDP-43 (**B**) and FUS (**C**) protein expression levels in Ctrl, sALS, SOD1,TARDBP and FUS LCLs. Histone H1 was used as nuclear sample normalization; LDH was used as cytoplasm sample normalization. Data are means ± S.E.M. ANOVA followed by Dunnet Multiple Comparison Test is used. *p<0.05 in C (Cytoplasm), **p<0.01 in C (Nucleus) and ***p<0.001 in A and B (Cytoplasm), all relative to Ctrl.

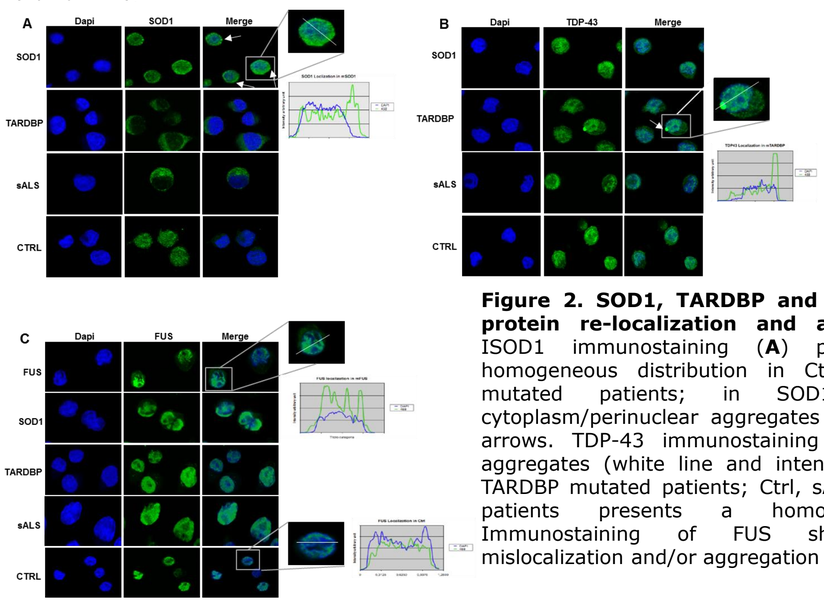

Figure 2. SOD1, TARDBP and FUS mutations show protein re-localization and aggregation in LCLs. ISOD1 immunostaining (**A**) presents a cytoplasm homogeneous distribution in Ctrl, sALS and TARDBP mutated patients; in SOD1 mutated patients cytoplasm/perinuclear aggregates are indicated by white arrows. TDP-43 immunostaining (**B**) shows cytoplasm aggregates (white line and intensity profile analysis) in TARDBP mutated patients; Ctrl, sALS and SOD1 mutated patients presents a homogeneous distribution. Immunostaining of FUS shows no cytoplasm mislocalization and/or aggregation of FUS protein (**C**).

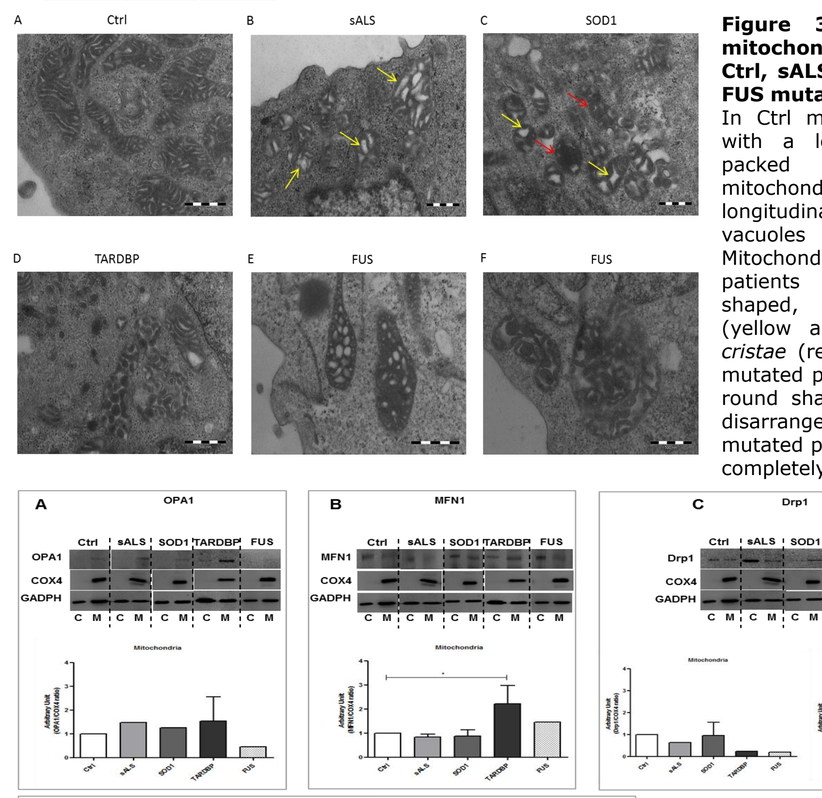

Figure 3. TEM analysis of mitochondria morphology in Ctrl, sALS, SOD1, TARDBP and FUS mutated LCLs. In Ctrl mitochondria are normal with a longitudinal shape and packed *cristae* (**A**). sALS mitochondria are smaller, longitudinal with abundant vacuoles (yellow arrows) (**B**). Mitochondria of SOD1 mutated patients are smaller, round shaped, with several vacuoles (yellow arrows) and paucity of *cristae* (red arrows) (**C**). TARDBP mutated patients show bigger and round shaped mitochondria, with disarranged *cristae* (**D**). In FUS mutated patients mitochondria are completely degenerated (**E**, **F**).

Figure 4. SOD1, TARDBP and FUS mutations show differences in mitochondrial dynamic in LCLs. Representative immunoblots for mitochondrial fusion proteins, OPA1 and MFN1 (**A, B**) and for mitochondrial and cytoplasm fission proteins, Drp1 (**C**) protein expression levels in Ctrl, sALS, SOD1, TARDBP and FUS LCLs. In D, representative immunoblot for cytochrome c release; Cyt-C shows increased cytoplasmic levels, in FUS and TARDBP mutated patients, while no changes are reported in Ctrl, sALS and SOD1 mutated samples. COX4 was used for mitochondrial sample normalization; GADPH was used for cytoplasm sample normalization. was reported in panel D. Data are means ± S.E.M. ANOVA followed by Dunnet Multiple Comparison Test is used. *p<0.05 in B and C (Cytoplasm), all relative to Ctrl.

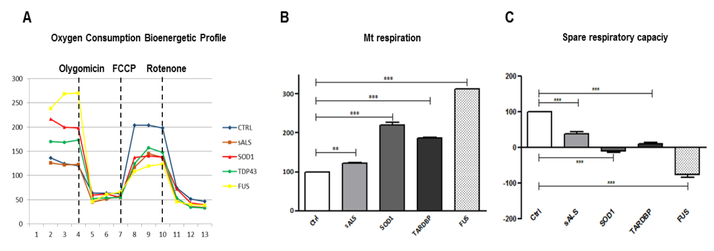

Figure 6. SOD1, TARDBP and FUS mutations show differences in mitochondrial respiration in LCLs. Mitochondrial respiration increases significantly in mutated patients (p<***p<0.01) and sALS (**p<0.01) compared to Ctrl (**B**). The spare respiratory capacity (SRC) is reduced in all patients compared to Ctrl (***p<0.001) (**C**). Data are means ± S.E.M. ANOVA followed by Dunnet Multiple Comparison Test is used.

References.

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