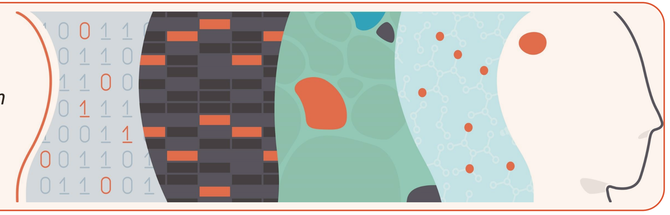
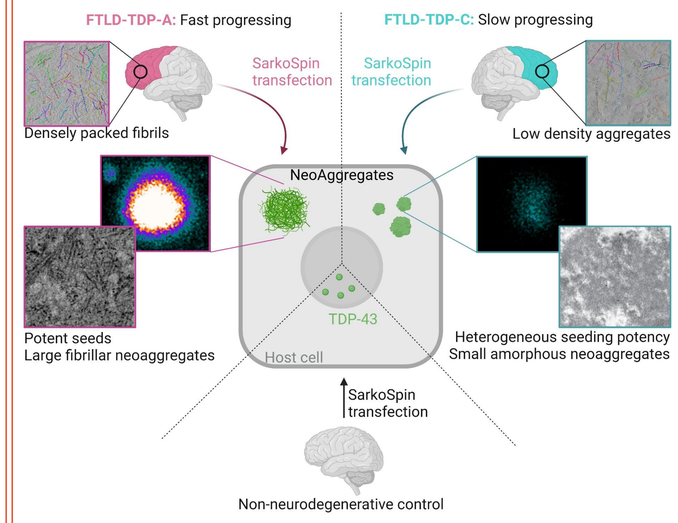
Template-dependent amplification of pathological TDP-43 and roles of phosphorylation

Pierre De Rossi[1], Amanda J Lewis[2], Johanna Furrer[1], Laura De Vos[1], Tomas Demeter[1], Aurélie Zbinden[1], Weijia Zhong[1], Vera I Wiersma[1], Carlo Scialo[1], Carolin Böing[3], Tammaryn Lashley[4,5], Henning Stahlberg[2] and Magdalini Polymenidou[1]

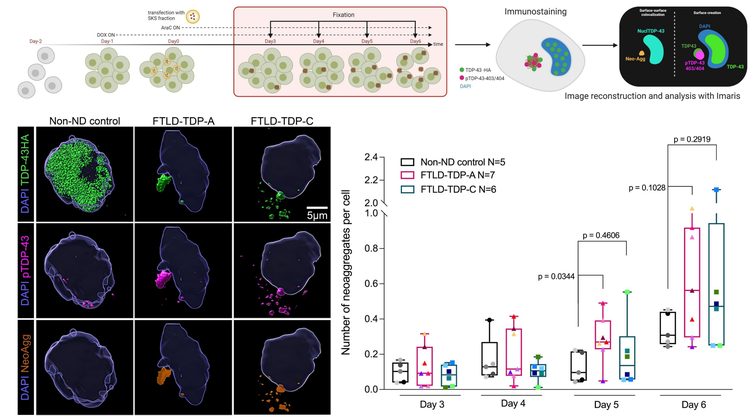
[1]Department of Quantitative Biomedicine, University of Zurich, Zurich, Switzerland, [2]Laboratory of Biological Electron Microscopy, Institute of Physics, SB, EPFL, Department of Fundamental Microbiology, Faculty of Biology and Medicine, UNIL, Lausanne, Switzerland, [3]C-CINA, Biozentrum, University of Basel, Basel, Switzerland, [4]Queen Square Brain Bank for Neurological diseases, Department of Movement Disorders, UCL Institute of Neurology, London, UK, [5]Department of Neurodegenerative Disease, UCL Institute of Neurology, London, UK



TDP-43 is an RNA binding protein found aggregated in several neurodegenerative diseases such as frontotemporal lobar degeneration (FTLD), amyotrophic lateral sclerosis (ALS) and Limbic-predominant age-related TDP-43 encephalopathy (LATE). The pathological hallmarks of these diseases are characterized by the presence of hyperphosphorylated TDP-43 within these pathological aggregates. It is assumed that TDP-43 proteinopathies follow the prion-like cascade, but the molecular mechanisms remained unknown. In our study, we demonstrated that isolated pathological seeds from post mortem tissue of patient with FTLD-TDP could trigger de novo aggregation in cells in a template-dependent manner. Our results also suggested that phosphorylation of these neoaggregates was sequential, N to C terminal, with subtype-specific timelines and aggregation profiles. We are currently investigating the role of TDP-43 phosphorylation in neuronal function, as well as disease pathogenesis and progression.

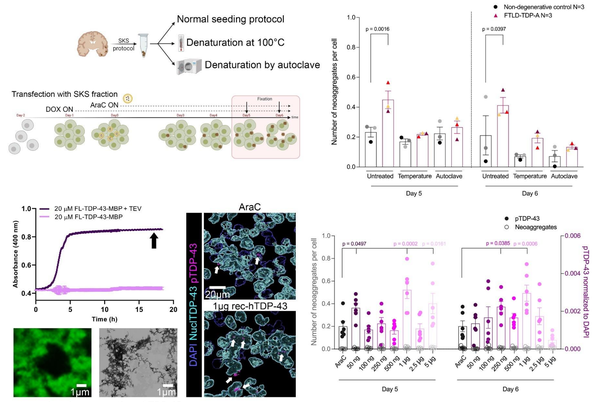


1 FTLD patient-derived TDP-43 aggregates seed neoaggregates in cells with subtype-specific potency



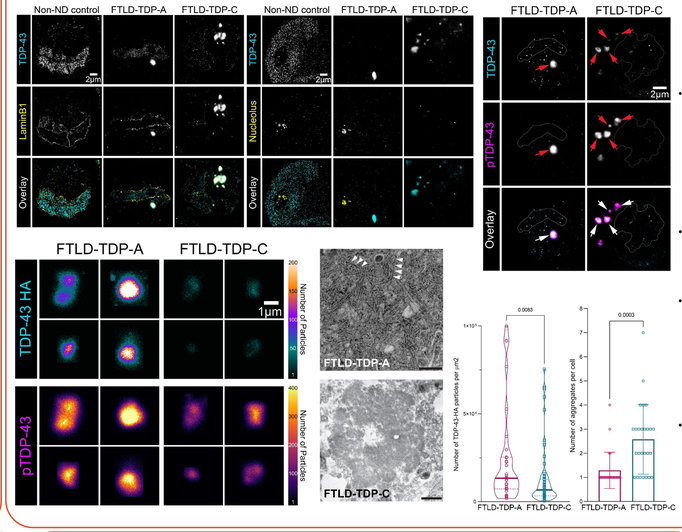
- Workflow of the seeding experiments and analyses.
- High magnification imaging of cells seeded for 6 days revealed presence of large cytoplasmic TDP-43-HA aggregates in cells seeded with FTLD-TDP-A and multiple cytoplasmic TDP-43-HA aggregates in cells seeded with FTLD-TDP-C. Surface representation generated with Imaris showing DAPI, TDP-43-HA (green), phosphorylated TDP-43 (pTDP-43, magenta) and neoaggregates (NeoAgg, orange).
- Quantification of the number of neoaggregates over time in (n = 3) independent experiments, with multiple biological replicates: non-ND control (n = 5), FTLD-TDP-A (n = 7) and FTLD-TDP-C (n = 6). Whiskers in the box plot represent median and minimum/maximum values. 2-way ANOVA repeated measure, with post-hoc Fisher's LSD, $F(3,15) = 1.3$, $P = 0.3110$, NS difference for Day 3 and 4. Day 5: Non-ND control vs FTLD-TDP-A $P = 0.0344$, non-ND control vs FTLD-TDP-C $P = 0.4606$. Day 6: non-ND control vs FTLD-TDP-A $P = 0.1028$, non-ND control vs FTLD-TDP-C $P = 0.2919$.

2 TDP-43 seeding depends on specific aggregate conformation(s)



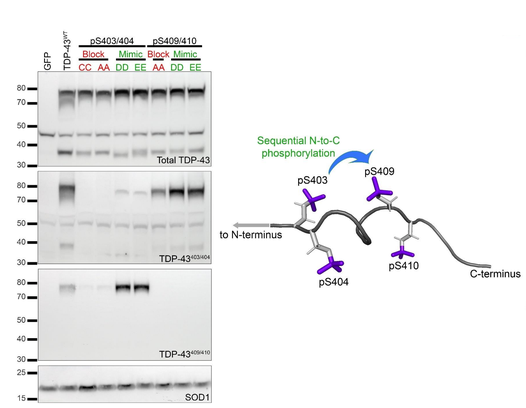
- Strategy to explore the prion-like properties of TDP-43 seeds.
- Bar graph showing the seeding effect at day 5 and day 6 post-seeding in untreated and denaturating conditions. One-way ANOVA, with post-hoc Fisher's LSD test, Day 5: $F(5,12) = 6.713$. (N = 3 biological replicates for non-ND control, FTLD-TDP-A and FTLD-TDP-C, bar graph showing mean ± SEM).
- Aggregation kinetics of full length (fl) recombinant human TDP-43 (rec-hTDP-43) measured as an increase in absorbance. Rec-hTDP-43 aggregates used for seeding were collected 18 h after the addition of TEV protease.
- Rec-hTDP-43 aggregates stained by Thioflavin T (ThT). Transmission Electron Microscopy (TEM) image of the rec-hTDP-43 aggregates.
- Rec-hTDP-43 aggregates were transfected on mitotically arrested cells and fixed after 5 or 6 days post-transfection. Surface masks generated with Imaris depict DAPI (blue), NuclTDP-43 (cyan) and pTDP-43 (magenta).
- Quantification of neoaggregates (left y-axis) and pTDP-43 (right y-axis) at day 5 and day 6 post-transfection for increasing concentrations of rec-hTDP-43. 2-way ANOVA repeated measure, with post-hoc Fisher's LSD, $F(7,80) = 5.886$, $P < 0.0001$. N = 6 biological replicates for each concentration; bar graph showing mean ± SEM.

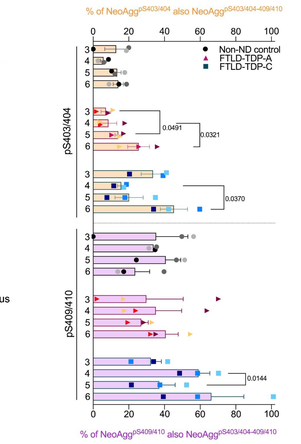
3 Distinct features of cellular neoaggregates resemble subtype-specific characteristics in FTLD brains



- dSTORM images of cells seeded for 6 days with non-neurodegenerative control (non-ND control), FTLD-TDP-A and FTLD-TDP-C seeds extracted from post-mortem brain tissue. Staining shows localization of TDP-43 (HA tag, cyan) along with LaminB1 (nucleus) (yellow)
- dSTORM images of cells containing aggregates after 6 days of seeding with SarkoSpin fractions of postmortem brain from patients with FTLD-TDP-A or FTLD-TDP-C. Red arrows point to cytoplasmic aggregates. Cells were labeled with TDP-43 (HA-tag, cyan) and phosphorylated TDP-43 pS403/404 (pTDP-43, magenta).
- Heatmap representation of the density of TDP-43-HA and pTDP-43 particles for four representative neoaggregates of FTLD-TDP-A and FTLD-TDP-C seeded cells.
- Violin plots showing the density of particles TDP-43 per µm2. Non-parametric unpaired t test FTLD-TDP-A vs FTLD-TDP-C, $P = 0.0083$. (Continuous line represent median, dashed lines represent quartiles, n = 3 independent experiments, six biological replicates for each condition).
- Bar graph showing the number of neoaggregates per cell. Non-parametric unpaired t test FTLD-TDP-A vs FTLD-TDP-C, $P = 0.0003$. Error bars represent mean ± SEM, n = 3 independent experiments, six biological replicates for each condition.

4 Phosphorylation of TDP-43 occurs sequentially from N- to C-terminal sites with a subtype-specific timeline

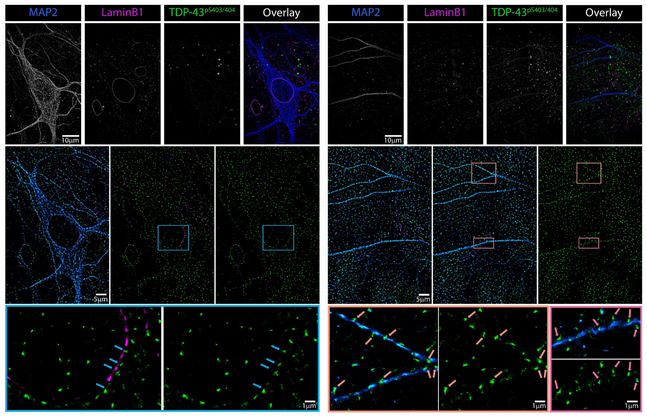




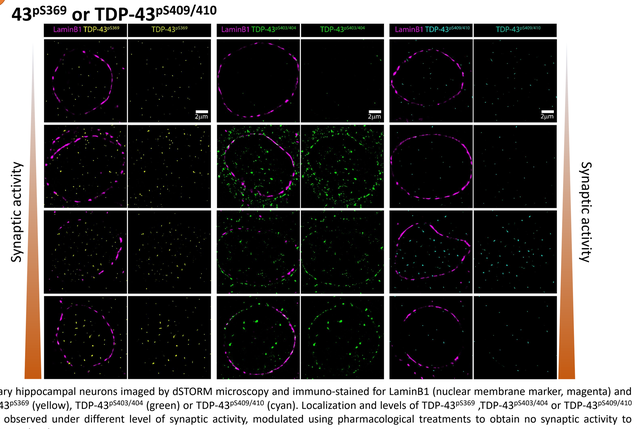
- Overexpression of GFP-TDP-43 variants with different mutations that either block (CC and AA mutation, red labels) or mimic phosphorylation (DD and EE, green label) at S403/404 or S409/410 in motor neuron-like NSC-34 cells. Western blot showed a specific signal for total TDP-43 (top panel), TDP-43$^{pS403/404}$ (middle panel) and TDP-43$^{pS409/410}$ (lower panel) and load control SOD1 (bottom panel).
- Schematic of the extreme C-terminal serine phosphorylation found in pathological TDP-43.
- Bar graph representing the percentage of NeoAggpS403/404 bearing double phosphorylation (left side, bars filled in light orange) and percentage of NeoAggpS409 bearing double phosphorylation (right side, bars filled in light purple). Each individual patient is labeled in different colored dots. N = 3 biological replicates per condition, graph bar showing mean ± SEM. Two-way ANOVA repeated measures, with post-hoc Fisher's LSD test, pS403/404 also double positive: $F(3,6) = 6.567$, $P = 0.0253$:

5 Physiological phosphorylation of TDP-43 in neurons uncovered by superresolution microscopy

Primary hippocampal neurons imaged by both confocal (top row) and dSTORM microscopy (middle and bottom rows) and immuno-stained for MAP2 (dendritic marker, blue), LaminB1 (nuclear membrane marker, magenta) and TDP-43$^{pS403/404}$. Bottom row shows enlargement areas indicated by the boxes on the middle row. Blue arrows point at the TDP-43$^{pS403/404}$ localized near the nuclear membrane. Orange and pink arrows point at synaptic-like structures positive for TDP-43$^{pS403/404}$ along dendrites.

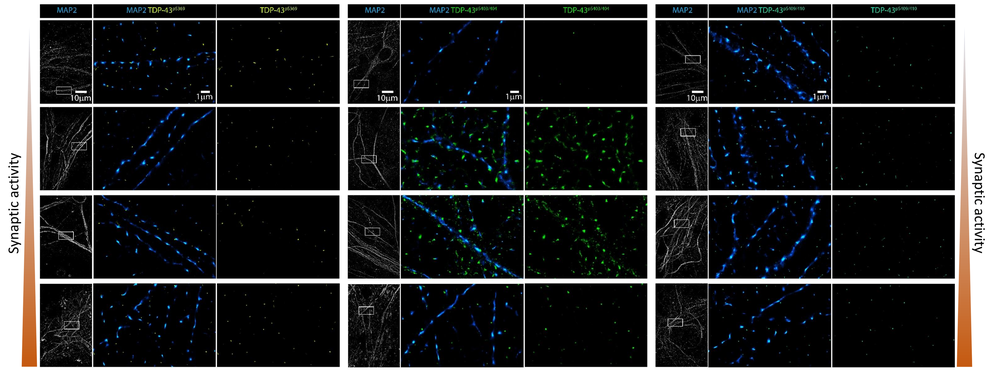


6 Synaptic activity modulates nuclear level of TDP-43$^{pS403/404}$ but not TDP-43^{pS369} or TDP-43$^{pS409/410}$



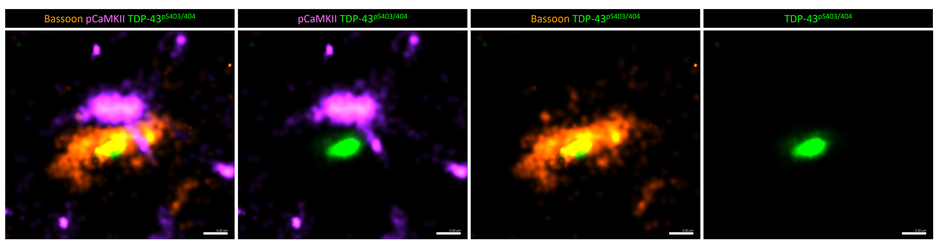
Primary hippocampal neurons imaged by dSTORM microscopy and immuno-stained for LaminB1 (nuclear membrane marker, magenta) and TDP-43^{pS369} (yellow), TDP-43$^{pS403/404}$ (green) or TDP-43$^{pS409/410}$ (cyan). Localization and levels of TDP-43^{pS369} ,TDP-43$^{pS403/404}$ or TDP-43$^{pS409/410}$ were observed under different level of synaptic activity, modulated using pharmacological treatments to obtain no synaptic activity to excitotoxic levels.

7 Synaptic activity modulates dendritic level of TDP-43$^{pS403/404}$ but not TDP-43^{pS369} or TDP-43$^{pS409/410}$



Primary hippocampal neurons imaged by dSTORM microscopy and immuno-stained for MAP2 (dendritic marker, blue) and TDP-43^{pS369} (yellow), TDP-43$^{pS403/404}$ (green) or TDP-43$^{pS409/410}$ (cyan). Localization and levels of TDP-43^{pS369} ,TDP-43$^{pS403/404}$ or TDP-43$^{pS409/410}$ were observed under different level of synaptic activity, modulated using pharmacological treatments to obtain no synaptic activity to excitotoxic levels.

8 Synaptic localization of TDP-43$^{pS403/404}$



Primary hippocampal neurons imaged by dSTORM microscopy and immuno-stained for pCaMKII (active synapses marker, purple), Bassoon (presynaptic active zone, orange) and TDP-43$^{pS403/404}$ (green). We detected the presence of TDP-43$^{pS403/404}$ at the presynaptic site, in colocalization with Bassoon.

9 Conclusions

1. Pathological TDP-43 extracted from post mortem tissue of patients with FTLD is able to trigger neoaggregation after transfection
2. Neoaggregation is dependent on the seed structure
3. Seeds extracted from different subtypes trigger structurally different neoaggregation
4. Phosphorylation timeline of the neoaggregates is different between subtypes
5. TDP-43$^{pS403/404}$ is present under physiological conditions in different compartments of the neurons
6. TDP-43$^{pS403/404}$ levels are modulated by synaptic activity

We are now working at understanding what drives these changes and what are the functional roles of TDP-43 phosphorylation

This project was/is supported by

DEMENTIA RESEARCH
SYNAPSIS FOUNDATION SWITZERLAND
erc
AFTD
FNSNF

CV Pierre

Polymenidou Lab

1 2 3 4

Adapted from

De Rossi, P., Lewis, A. J., Furrer, J., De Vos, L., Demeter, T., … Polymenidou, M. FTLD-TDP assemblies seed neoaggregates with subtype-specific features via a prion-like cascade. *EMBO Reports (2021)*