Modelling Alzheimer's disease: Development of a scalable, high throughput-compatible assay to detect tau aggregates using iPSC-derived cortical neurons maintained in a 3D-culture format

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HIGHLIGHTS

Here we describe a robust, scalable and disease relevant model of tau aggregation using iPSC-derived cortical neurons that can be applied to drug discovery programs in neurodegeneration. The resulting assay is highly reproducible across users and works in different commercially available iPSC-lines, providing a reliable tool for better understanding tau pathophysiology and for the identification of novel treatments against Alzheimer's Disease (AD).

ABSTRACT

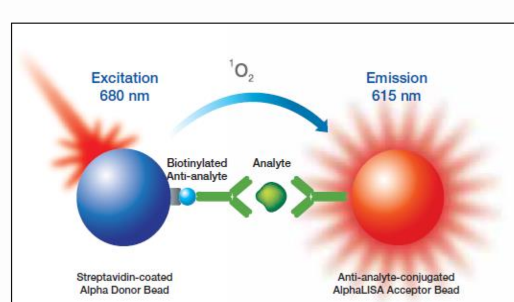
Tau aggregation is the pathological hallmark that best correlates with the progression of Alzheimer's Disease (AD). The presence of neurofibrillary tangles (NFTs), formed of hyperphosphorylated tau, leads to neuronal dysfunction and loss, and is directly associated with the cognitive decline observed in AD patients. The limited success in targeting β-amyloid pathologies has reinforced the hypothesis of blocking tau phosphorylation, aggregation and/or spreading as alternative therapeutic entry points to treat AD. Identification of novel therapies requires disease-relevant and scalable assays capable of reproducing key features of the pathology in an in vitro setting. Here we use induced pluripotent stem cells (iPSC) as a virtually unlimited source of human cortical neurons to develop a robust and scalable tau aggregation model compatible with high throughput screening (HTS). We downscaled cell culture conditions to 384-well plate format and used diluted matrigel to introduce an extra physical barrier that protects against cell detachment and reduces stress linked to plate handling. We complemented the assay with AlphaLISA technology for the detection of tau aggregates in a high throughput-compatible format. The assay is reproducible across users and works with different commercially available iPSC-lines, representing a highly translational tool for the identification of novel treatments against tauopathies including AD.

METHODS

iPSC and neural differentiation: Two commercially available cell lines generated from clinically unaffected donors were used: IPSC0028 (Sigma) and ChiPSC6b_m1 (Cellectis). iPSCs were differentiated into Neuronal Progenitor Cells (NPCs) by Axol Biosciences by adapting already published protocols (Shi et al., 2012).

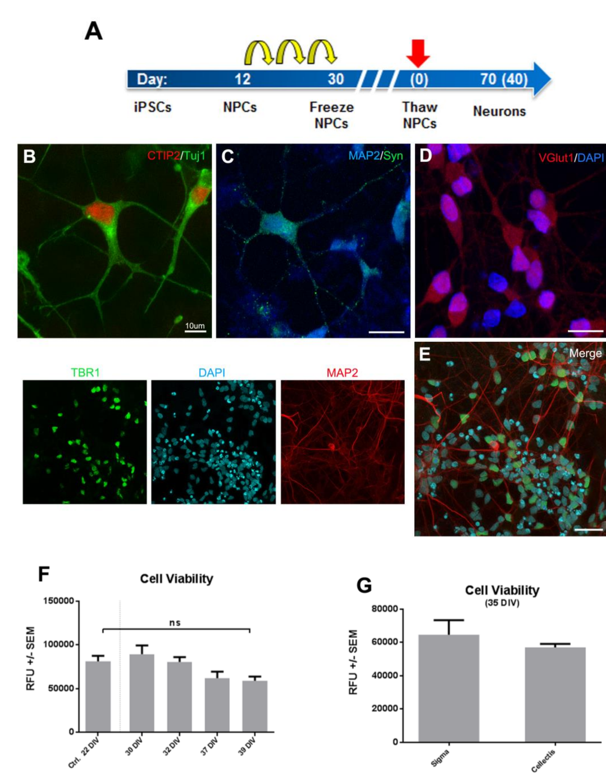
Induction of tau aggregation: Tau aggregation was carried out following previously described protocol (Guo et al., 2011) with slight modifications. NPCs were transduced with adenovirus driving expression of human P301L mutant tau form under the human synapsin promoter. Seeding with fibrils was performed by adding recombinant truncated tau containing four microtubule-binding repeats with P301L mutation (k18) at a concentration of 50nM per 96-well/12-wells, and 133nM per 384 well.

AlphaLISA: AlphaLISA® technology, the non-wash ELISA alternative, is a bead-based sensitive method used to study biomolecular interactions. Beads are coated with highly specific antibodies (hTAU10, HT7) that mediate binding to the target analyte (TAU). To detect aggregated Tau, both biotin-and acceptor beads are conjugated to the same antibody (hTAU10). Only when the distance between Donor and Acceptor bead is ≤200nm, energy transfer occurs producing a luminescent signal that can be quantified (Calafate et al., 2015).



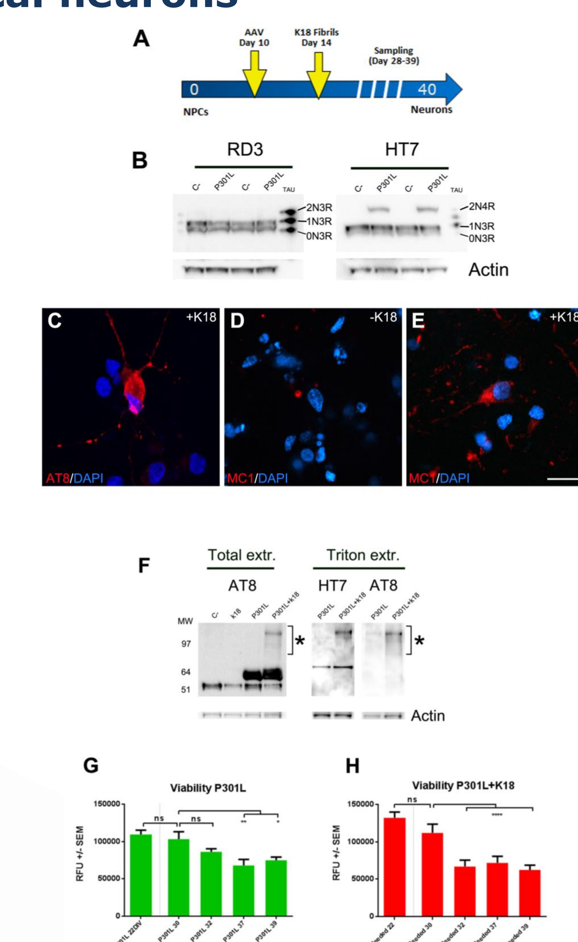
RESULTS

Healthy cortical neurons can be differentiated in 384-well plates for at least 5 weeks



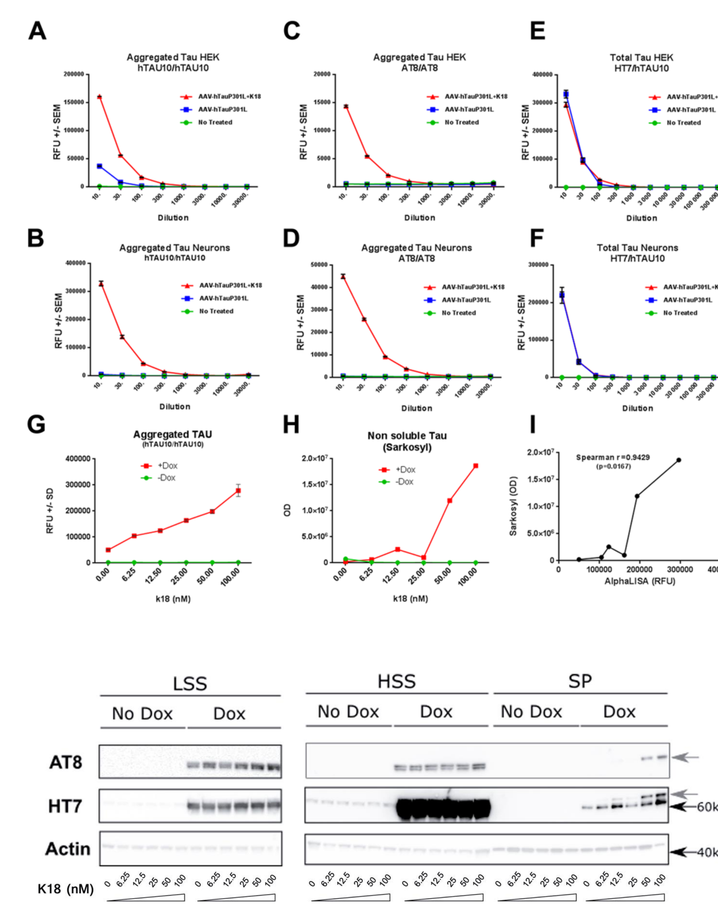
Scheme in A depicts the protocol used for the amplification (first 30 days) and final differentiation of NPCs. After neuronal induction and expansion, NPCs are frozen. After thawing NPCs are subplated in 384/96 well plates using diluted matrigel. Numbers represent days in culture (in brackets the number of days after thawing.) B, cortical identity (CTIP2) and early maturation markers (Tuj1) are expressed since day 7 of culture. Typical synaptic markers as synapsin (C) and VGlut1 (D) are present at 18 and 30 days respectively. MAP2/TBR1 staining shows complex connectivity network of glutamatergic cortical neurons after 30 days of differentiation (E). F, Cells maintained in culture for 39 days show similar metabolic activity as cells cultured for 22 days (measured by quantifying ATP, CelltiterGlo), indicating no alteration in cell viability (n=4 independent experiments). G, no significant difference was observed in cell viability in the two cell lines tested in this study after 35 days in culture. Scale bars: 10um in B, 20um in C -D, and 40nm in E. DIV: days in vitro. n=3 independent experiments, One-way Anova. ns, no significant ,*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 (Dunnett's multiple comparison test versus non seeded control).

Tau aggregation model using human cortical neurons



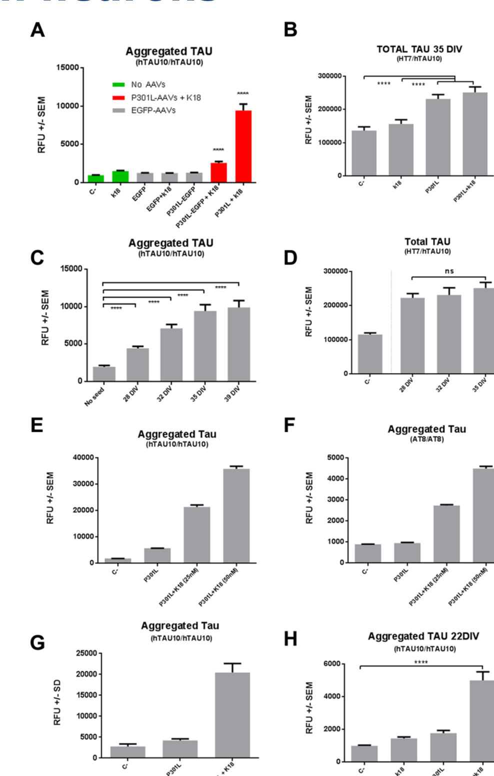
Scheme in A shows the protocol followed. 3R endogenous (c-) and 4R overexpressed (P301L) forms of tau detected with 3R-specific (RD3) and total (HT7) tau antibodies respectively. Insoluble aggregated tau is detected by IF after fixation with 4%PFA-1%Triton and staining with AT8 (C) and MC1 (E) only in seeded (+k18) samples. WB in F shows hyperphosphorylated forms of tau in total cell extracts detected by AT8. Note the presence of typical high molecular weight band (>90 kDa) and smear corresponding to aggregated tau detected both with AT8 and HT7 antibodies after triton extraction (*). A 50% decrease in cell viability is observed in transduced and seeded cells (H) but not in cells only overexpressing the transgene (G). RFU, relative fluorescence units . Scale bar: 20um. AAV: adenovirus, n=3 independent experiments, One-way Anova. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 (Dunnett's multiple comparison test versus non seeded control).

AlphaLISA technology to quantify Tau aggregation



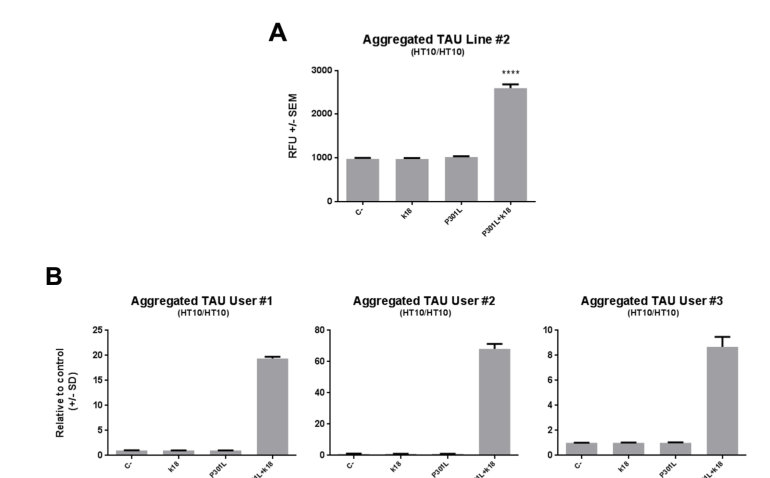
Antibody validation to quantify aggregated and total Tau using HEK cells (A, C, and D) and primary neurons (B,D and F). hTAU10/hTAU10 and AT8/AT8 was chosen for aggregate quantification based on the specific detection of aggregates in transduced and seeded cells (A-D, red). HT7/hTAU10 detected Tau overexpression but no difference between seeded and non-seeded (green) cells, making it more suitable for quantification of total Tau (E-F). G-H show the dose-response curves obtained from the same sample by AlphaLISA of total extracts and western blot of the sarkosyl insoluble fraction respectively. The computation between both signals is plotted in I showing a significant correlation (Spearman r=0.09429, p=0.0167). Total Tau levels are not affected by the increase of K18 concentration as detected by western blot with AT8 and HT7 in total (LSS) and sarkosyl-soluble fraction (HSS) (B). Only the sarkosyl-insoluble fraction (SP) increases (grey arrow). Dox: doxycicline. OD, optic density. n=3 technical replicates, Two-way Anova. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 (Dunnett's multiple comparison test versus non seeded control).

Quantification of Tau aggregates in human neurons



A shows baseline values of the assay defined by the counts obtained in all negative control used (i.e. non transduced cells, EGFP transduced cells, cells transduced with P301L but not seeded with K18 and cells only seeded with K18). In B , HT7/hTAU10 AlphaLISA confirming overexpression of Tau in transduced cells. Optimal sampling time was defined at 35 days as hTAU10/hTAU10 signal gives the highest value in the shortest time after reaching a pleateau (C). The levels of total tau do not increase after 28 days of differentiation (D). Tau aggregates are detected with hTAU10/hTAU10 (E) and AT8/AT8 (F) in a dose dependent manner. The signal-to-basal ratio values obtained from 384-well assay plates after 35 days provide a Z' factor of 0'52 (G). A shorter version of the protocol also provides significant values for Tau aggregation as soon as 22 days post-thawing (H). C-, untreated cells; k18 cells seeded with P301L-mutant fibrils; EGFP, transduced cells overexpressing EGFP; EGFP+k18, cells transduced with EGFP and seeded with k18; P301L-EGFP+k18, cells iverexpressing P301L and EGFP; P301L+k18, cells overexpressing P301L and seeded with K18 fibrils. n=3 biological replicates in A-D and H; n=6 technical replicates in E-F. One-way Anova. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 (Dunnett's multiple comparison test versus non seeded control).

Tau aggregation assay is reproducible in different lines and across users



A shows positive Tau aggregation values when the protocol is applied to the ChiPSC6b iPSC line. In B, hTAU10/hTAU10 normalized data obtained from different users using 96-well format and 20.000 cells per well. n=3 biological replicates in A; each plot in **B** represents a minimum of 3 technical replicates. A, One-way Anova. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 (Tukey's multiple comparison test).

CONCLUSIONS

- **We have developed a SOP for quantifying tau aggregation in human iPSC-derived cortical neurons differentiated in matrigel.**
- **The assay works across users and in different cell lines, overcoming variability issues typically related to iPSC technology.**
- **Its compatibility with biochemistry and imaging platforms makes it suitable for High Throughput and High Content Screenings.**

Future directions

- **Test tau aggregation capacity of neurons differentiated from iPSCs derived from patients carrying mutations associated to tauopathies (e.g MAPT IVS10+3 G>A, and others).**
- **Optimize protocol in order to accelerate maturation and to improve electrophysiological properties of the neurons.**
- **Test alternative scaffolds (e.g. chemically defined) aiming to reduce potential sources of variability and costs.**

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