A combined TDP43-Tau cellular model for the understanding of LATE-NC Proteinopathy

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Abstract

TDP-43 protein is known as the major disease protein in amyotrophic lateral sclerosis (ALS) and in the most common variant of frontotemporal lobar degeneration (FTLD)[1]. Recently, TDP-43 proteinopathy has been related with a newly recognized type of dementia, limbic-predominant age-related TDP-43 encephalopathy (LATE-NC)[2]. Several studies have shown that almost 50% of patients with LATE-NC also present TDP-43 protein deposits in their brain cells. Although the true role of TDP-43 in LATE-NC and its relationship with pathological forms of Tau is currently unknown, in recent years it has been revealed that they may coexist in exacerbated forms of the disease[3].

The development of a cellular assay aimed to study the behavior of the TDP43-TAR protein in combination with hyperphosphorylated Tau protein may allow us to elucidate new pathways of molecular signaling in LATE disease, as well as to screen compounds that may intercede in the pathological synergy between these two targets. Using this HCS assay in 96 well format, we performed the screening of a small synthetic chemical library of 1,200 compounds. The Z′ factor of the assay was over 0.5 demonstrating the robust performance of the assay. After the screening campaign, the positive compounds were chosen for further testing, based on the strength of the initial response and the lack of cytotoxicity.

Methods

Assay development: U2OS cell line stably expressing IPTG-induced TDP43-tGFP protein was transfected with a TAU variant containing three mutations (TM): G272V, P301L and ΔK280 tagged with the far red FP650 fluorescent protein.

Cells were treated with 300 µM sodium arsenite during 90´, cells were dyed with 0.5 µg/ml hoechst the last 30´of the sodium arsenite treatment. 1 µM ISRIB and 30 mM LiCl were used as TDP-43 and Tau controls, respectively. Fluorescent images were acquired in the Cell insight CX7 high content equipment from Thermo Fisher (Fig 1). The activity of the proteins was quantified with the "spot detector" (TDP-43) and "bundle quantification" (Tau) applications from HCS Studio Cellomics software (Fig 2).

Day 1	Day 2	Day 3
Cell seeding ITPG treatment	**Treatments with test compounds**	**Treatments with Sodium Arsenite**
18,000 cells/well in 96 well-plates	Incubation O/N	Incubation 90 min

HCS Analysis: Cells were pre-treated overnight (O/N) with 1200 test compounds at 10 µM from the Prestwick library and then treated with sodium arsenite during 90´(Fig 3). Hit compounds were used to perform a dose-response curve (Fig 4).

Bibliography

1.- TAR DNA-binding protein 43 in neurodegenerative disease. Alice S. Chen-Plotkin (2010). doi:10.1038/nr-neurol.2010.18.

2.- Limbic-predominant age-related TDP-43 encephalopathy (LATE): consensus working group report. Peter t. Nelson (2019). doi:10.1093/brain/awz099.

3.-TDP-43 expression influences amyloidbeta plaque deposition and tau aggregation. Davis SA (2017). doi.org/10.1016/j.nbd.2017.04.012

Results

Assay development & analysis

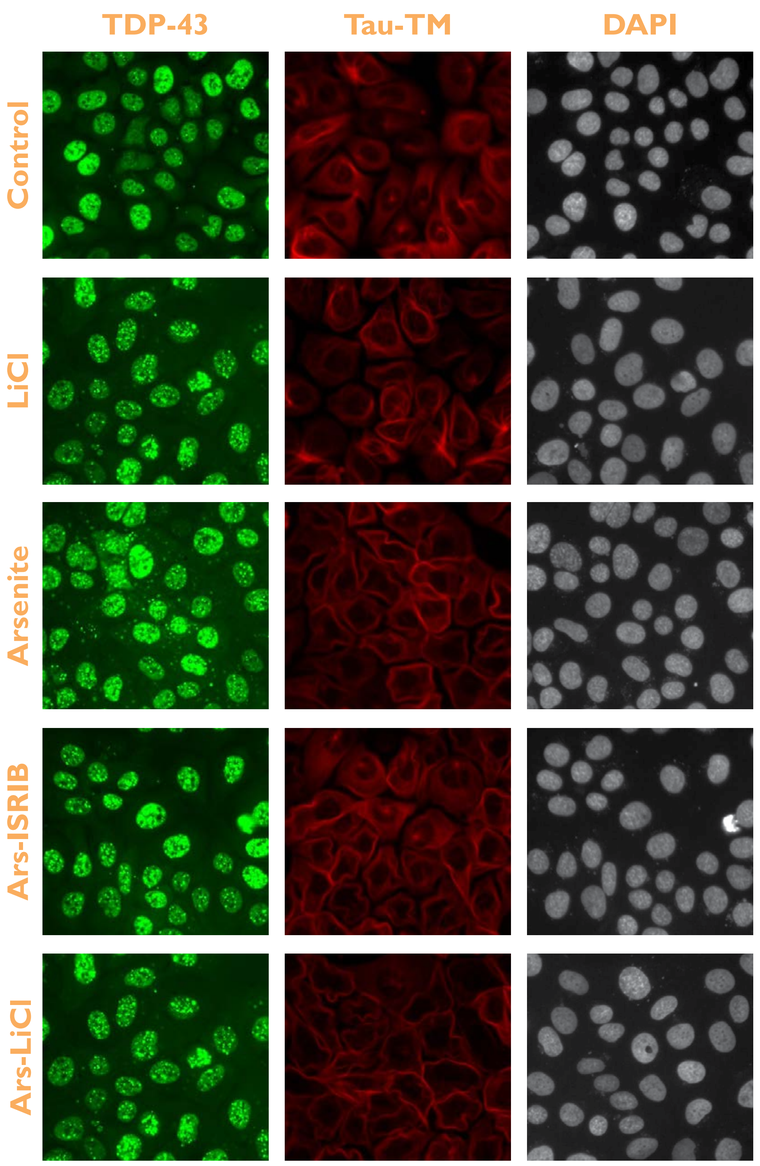

Fig 1. Assay development. Representative images of TDP-43 (left column),Tau-TM (central column) and nuclei (right column) in the different experimental conditions.

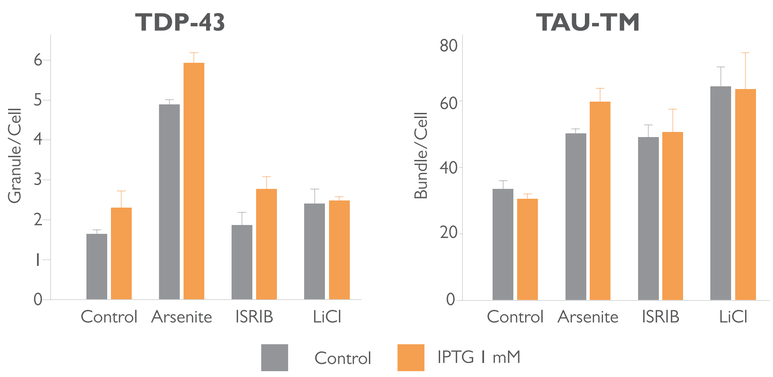

Fig 2. Image analysis. The image analysis provides the number of TDP-43 granules (left) and Tau bundles (right) per cell. The addition of 300 µM sodium arsenite during 90´ increased the TDP-43 aggregates number 2.9-fold. The treatment with 30 mM LiCl increased the number of bundles 2.08-fold. The treatment with 1 µM ISRIB significantly reduced the number of TDP-43 granules.

HCS Analysis

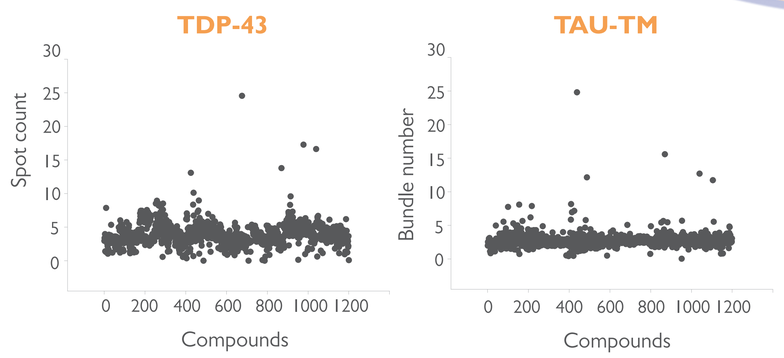

Fig 3. Assay development. A screening of 1200-compound library was performed with the TDP43_Tau-TM cell line. Cells were treated with each compound at 10 µM. The cuts off set to find the hits were fixed at mean -1.5*SD for TDP43 protein and mean + 1.5*SD for Tau protein. Compounds with a viability lower than 75 % compared to the control were considered toxic and were discarded for next steps. Under these conditions 16 positive compounds were detected for TDP43 and 8 for TAU. Z' = 0.67 ± 0.11.

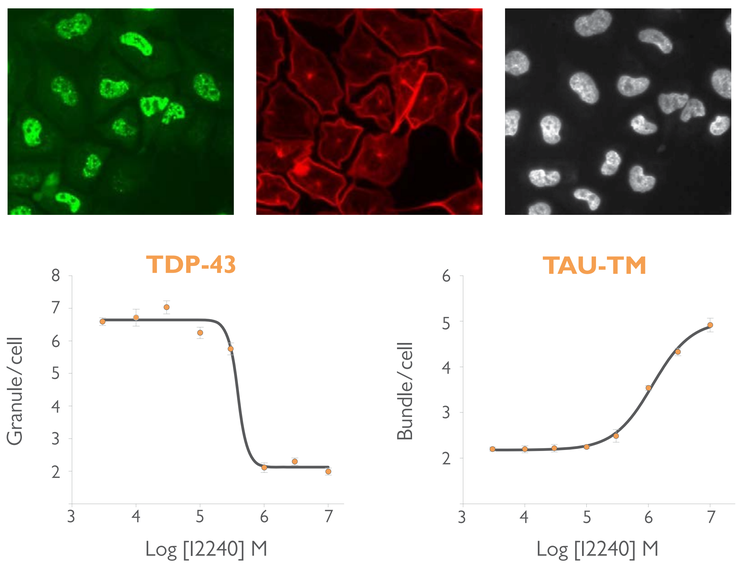

Fig 4. Dose-response Assay. 3 compounds were found to be positive for both proteins but only compound I2240 performed a dose response curve. Cells were treated with 8 decreasing concentrations starting with 10 µM. This compound showed a dose response effect with an EC_{50} of 0.39 µM for TDP43 and 1.16 µM for TAU. Data points represent the mean ± SD at each condition for a single experiment performed in triplicate.

Conclusions

-An increase in the expression of TDP-43 does not affect the behavior of the Tau protein.

- This cellular model allows the evaluation of compounds that may present synergies in neuroprotection through their effect on both cellular targets.

-In the search for synergistic compounds for the TDP-43 and Tau targets, 3 possible candidates have been found. Only compound I2240 shows a neuroprotective effect against both targets in a dose-dependent manner, decreasing the number of TDP-43 stress granules and increasing the number of Tau bundles.