TDP43/FUS Fluorescence cell-based assay to screen synergistic drugs against ALS disease

Rosa M. Mella, Meritxell Roura-Ferrer, Saray López-Benito, Clarisa Salado, Miguel Peco and Patricia Villacé

Innoprot (Innovative Technologies in Biological Systems), Parque Tecnológico de Bizkaia, Edificio 502, Planta 1, 48160, Derio, Bizkaia, Spain

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Speeding up drug discovery

Abstract

Amyotrophic Lateral Sclerosis disease (ALS) is characterized by the death of both upper and lower motor neurons in the motor cortex of the brain, the brain stem, and the spinal cord. Prior to their destruction, motor neurons develop intracellular protein inclusions in their cell bodies and axons[1]. These inclusions often contain ubiquitin, and generally incorporate one of the ALS-associated proteins: SOD1, TAR DNA binding protein (TDP-43, or TARDBP), or FUS[2].

Innoprot has developed a novel fluorescence cell-based assay in U2OS cells that expresses constitutively the FUS protein in a TPD43-induced model. This cellular model allows the study of the relationship between FUS and TDP-43 proteins aggregation pathways in the development of the disease. This novel ALS fluorescence cell-based assay has been designed for High Content Screening applications to find compounds able to inhibit or modulate TDP43/FUS aggregation after severe cytotoxic damage induction by Arsenite. In this work, this model was used to screen a library of 880 compounds. ISRIB and LiCl compounds were used as positive protection controls in the fluorescent TDP43/FUS aggregation model. After the screening campaign, positive compounds were chosen for further testing, based on the strength of the initial response and the lack of cytotoxicity. Our results indicated that the pharmacological inhibition or modulation of TDP43 and FUS aggregation implicated in ALS is a valid strategy for drug screening.

Methods

Assay development: U2OS cell line stably expressing IPTG-induced TDP43-tGFP protein was transfected with FUS protein tagged with the red fluorescent FP602 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 was used as TDP-43 and FUS aggregation inhibitor control. Fluorescent images were acquired in the Cell insight CX7 high content equipment from Thermo Fisher (Fig 1). The proteins aggregation was quantified with the "spot detector" application from the 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 880 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/nrneurol.2010.18.

2.- The role of TDP-43 mislocalization in amyotrophic lateral sclerosis. Terry S. Suk (2020). https://doi.org/10.1186/s13024-020-00397-1

Results

Assay development & analysis

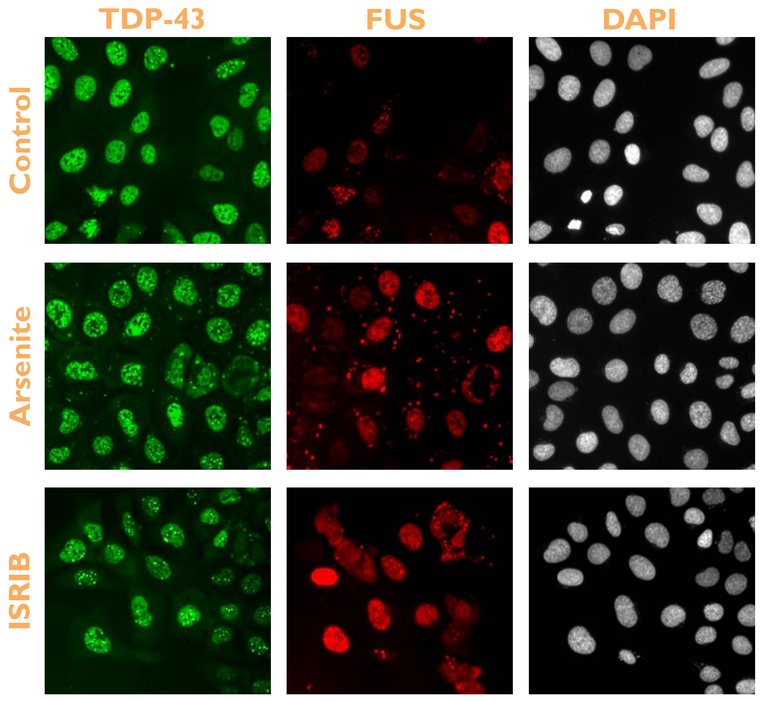


Fig 1. Assay development. Representative images of TDP-43 (left column), FUS (central column) and nuclei (right column) in the different experimental conditions.

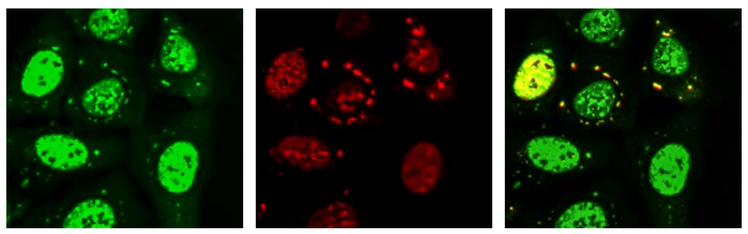


Fig 2. Representative images of the treatment with sodium arsenite, TDP-43 (left column), FUS (central column) and merge (right column).

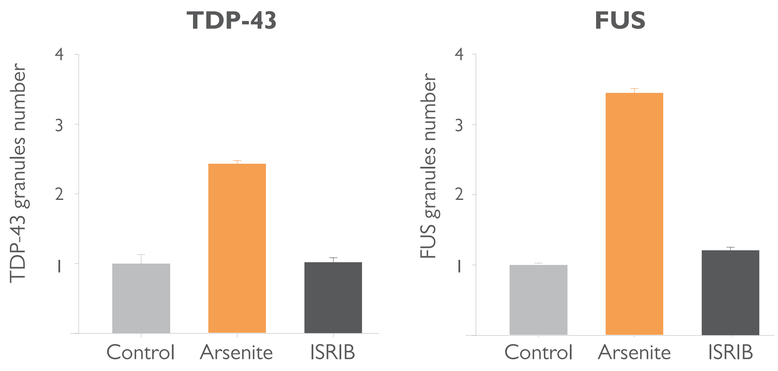


Fig 3. Image analysis. The image analysis provides the number of TDP-43 (left) and FUS (right) granules per cell. The addition of 300 µM sodium arsenite during 90´ increased the number of TDP-43 and FUS aggregates 2.42 and 3.44-fold, respectively. The treatment with 1 µM ISRIB reduced the aggregation levels to values close to zero.

HCS Analysis

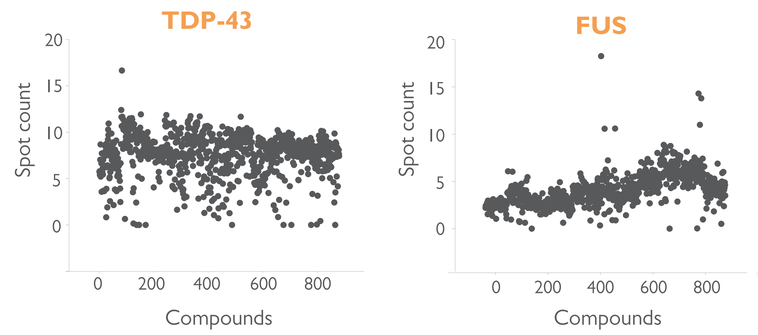
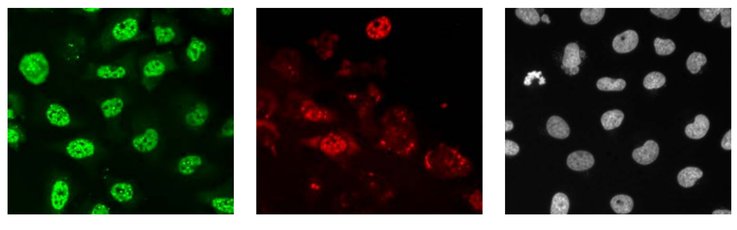


Fig 4. Assay development. A screening of 880-compound library was performed with the TDP43_FUS cell line. Cells were treated with each compound at 10 µM. The cuts off set to find the hits were fixed at mean -1.5*SD for TDP43 protein and mean + 1.5*SD for Tau protein. Compounds with a viability lower than 75 % compared to the control were considered toxic and were discarded for next steps. Under this conditions 8 positive compounds were detected for TDP43 and 9 for FUS. Z' = 0.67 ± 0.11.



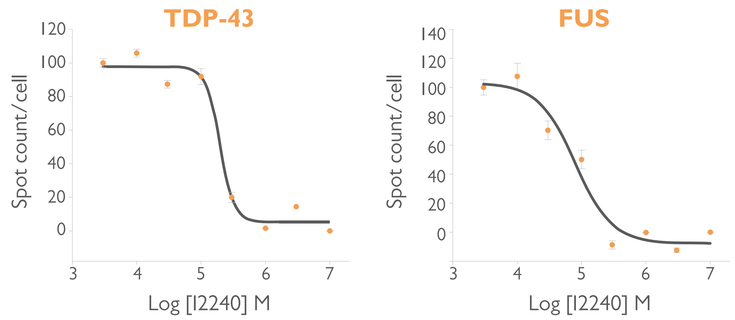


Fig 5. Dose-response Assay. 3 compounds were found to be positive for both proteins but only compound I2240 performed a dose response curve. Cells were treated with 8 decreasing concentrations starting with 10 µM. This compound showed a dose response effect with an EC_{50} of 0.20 µM for TDP43 and 0.08 µM for FUS. Data points represent the mean ± SD at each condition for a single experiment performed in triplicate.

Conclusions

- The treatment of the TDP-43_FUS cell line with 300 µM sodium arsenite induces the cytosolic granule formation of both proteins. The preincubation with 1 µM ISRIB prevents the formation of cytosolic granules of TDP-43 and FUS.
- This cellular model allows the evaluation of compounds that may present synergies in neuroprotection through their effect on both cellular targets.
- In the HCS analysis one possible candidate for further studies was found. Compound I2240 showed a neuroprotective effect against sodium arsenite intoxication decreasing the number of TDP-43 and FUS stress granules.