FLUORESCENT CELLULAR MODEL FOR ALS/FTD DRUG SCREENING TARGETING TDP-25 AGGREGATES

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innoprot cell based solutions

Abstract

Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) share pathogenic mechanisms, including TDP-25 aggregation, which contributes to neuronal toxicity. We developed a fluorescent cellular model to visualize and quantify TDP-25 aggregates in real time, providing a powerful tool for drug screening. This system enables efficient evaluation of compounds targeting TDP-25 aggregation, offering a promising platform for identifying new ALS/FTD therapies.

Introduction

The accumulation of misfolded protein aggregates is a hallmark of several neurodegenerative diseases, including ALS and FTD. In particular, TDP-43 and its truncated fragment TDP-25 have been implicated in neuronal toxicity in these disorders. Due to the lack of effective treatments, it is crucial to develop cellular models that enable the evaluation of potential drugs aimed at reducing these aggregates.

In this study, we present a fluorescence-based cellular model for real-time detection of TDP-25 aggregates. By expressing a fluorescently tagged construct, we can monitor aggregate formation dynamics and assess the impact of different compounds on their reduction. This approach represents a significant advancement in drug screening, providing an efficient and quantifiable platform for identifying novel therapeutic strategies against ALS and FTD.

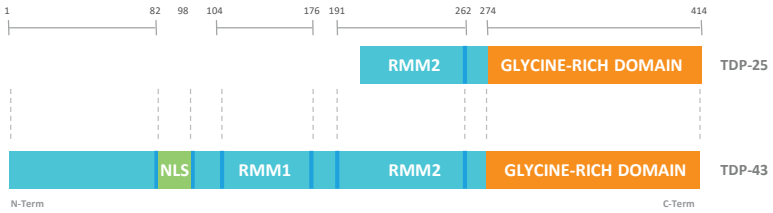

Figure 1. Schematic representation of the domains of TDP-43 protein and its truncation TDP-25.

Materials and Methods

Cell Line Development: U2OS cells were transfected with TDP25-tGFP cDNA.
Aggregation Assay: Cells were treated overnight with 50 µM Sodium Arsenite in OptiMEM.
HTS: Cells were incubated overnight with 10 µM Prestwick library compounds diluted in 50 µM Sodium Arsenite-OptiMEM.
Imaging: After treatments, PBS replaced the medium images were captured and analyzed using the Thermo Fisher Cell Insight CX7 (Fig. 5).

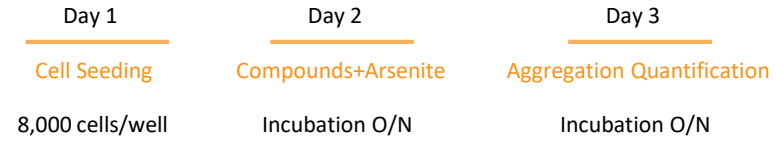

Figure 2. Assay Workflow: Cells were seeded at 8,000 cells per well in 96-well plates (Day 1) and treated with test compounds diluted in 50 µM Sodium Arsenite-OptiMEM overnight (Day 2). The following day (Day 3), the medium was replaced with PBS, and TDP-25 aggregates were quantified via high-content imaging system.

Results

Stress Induction

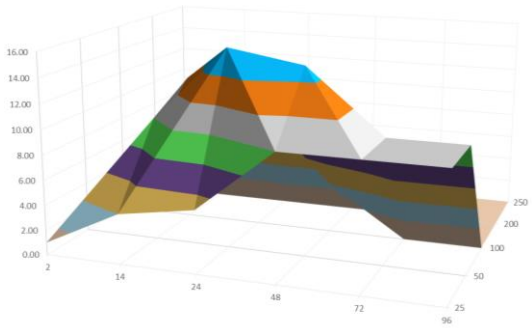

Figure 3. Stress granule formation over time. Cells were treated with increasing concentrations of sodium arsenite for up to 96 hours. The 3D graph represents the number of stress granule spots (Y-axis) as a function of compound concentration (Z-axis) and time (X-axis), illustrating the dynamic changes during granule formation.

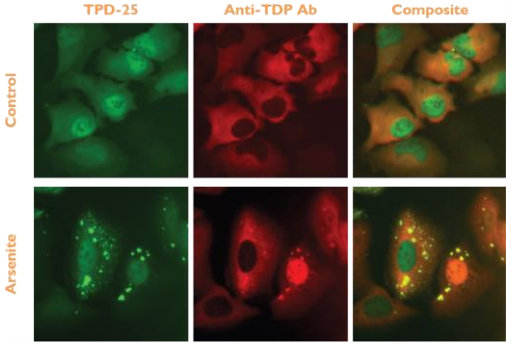

Figure 4. Immunofluorescence analysis of TDP-25 localization. The first column shows green fluorescent TDP-25 cells, the second column displays antibody labeling (TDP-43 Antibody), and the third column presents the merged images. Control cells (top row) are compared to arsenite-treated cells (bottom row) to assess changes in TDP25 distribution.

High-ThroughPut Assay

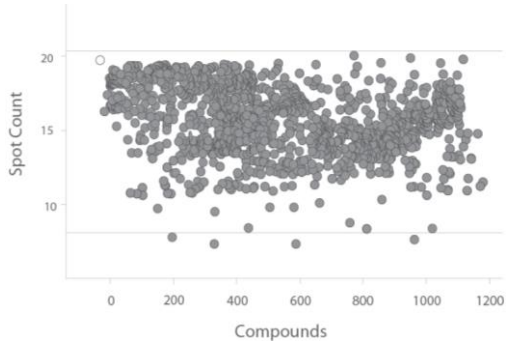

Figure 5. Assay development. A 1200-compound library was screened using TDP-25 cells (10 µM). Hits were defined as mean + 3SD. Toxic compounds (<75% viability) were excluded. Four hits were identified (Z' = 0.68 ± 0.12).

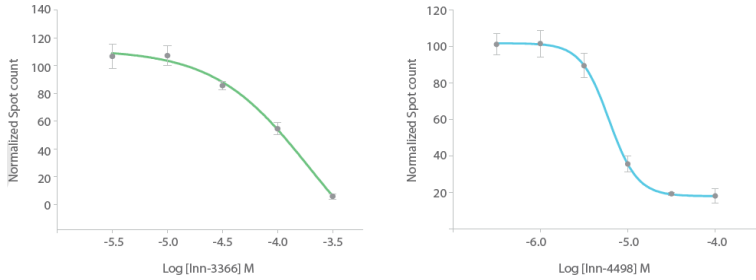

Figure 6. Dose response curves of two positive hits. The curves illustrate the protection rate of two of the four positive hits, Inn-3366 and Inn-4498, respectively.

Conclusions

Fluorescence-Based Model Validation: Our model effectively tracked TDP-25 aggregate formation and clearance, proving its value for drug screening in neurodegenerative disease research.

Advancement in Drug Discovery: This study supports the development of cellular models for high-throughput screening, advancing research for TDP-43-related disease therapies.

Compound Efficacy: Two compounds from the library reduced TDP-25 aggregation in a dose-dependent manner, highlighting their potential as therapeutic candidates for ALS and FTD.