An integrated platform for drug screening of neurological disease models

Hilaria Mollica[1], Paola Bonetti[1], Katharina Montag[1], Lucia Rutigliano[1], Silvia Cainarca[1], Paola Tarroni[1], Lia Scarabottolo[1], Stefan Lohmer[1] and Fernanda Ricci[1].

[1] Axxam S.p.A., Via Meucci 3, 20091 Bresso (Milan), Italy

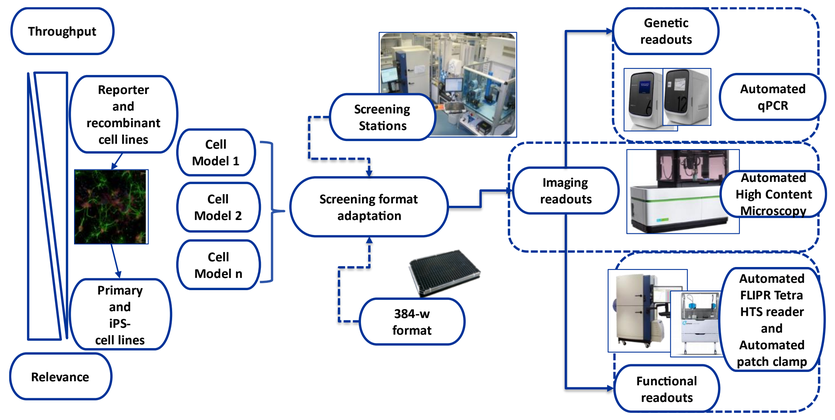
Introduction

Several human neurodegenerative diseases share a common pathological feature: the formation and accumulation of prion-like aberrant protein aggregates and insoluble inclusions, which lead to progressive neuronal dysfunction and toxicity. Here we present a multilayer platform to address TDP43 dysfunctions, a protein involved in RNA processing and the onset of pathological aggregates, using imaging, optogenetic assays, high-throughput miniaturized formats and relevant cell models, as a proof-of-concept to approach different neuro-related disorders and establishing the basis for therapeutic strategies .

TDP43

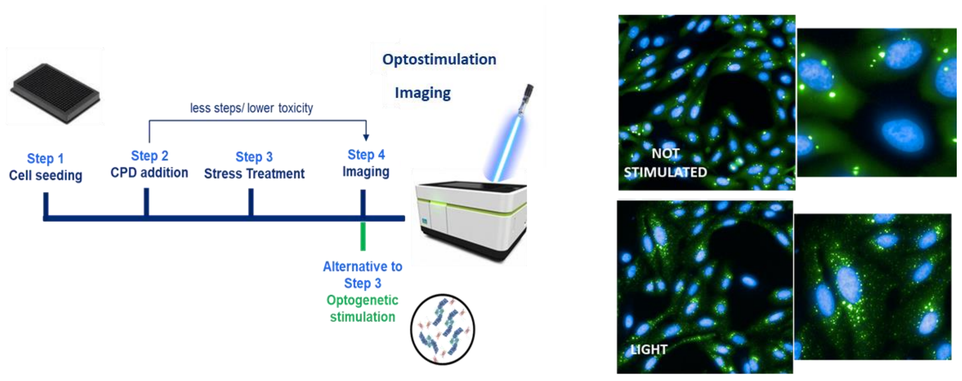
TDP-43 sequence with 2 RNA-recognition motifs (RRMs) and a prion-like, Low Complexity Domain (LCD). LCD is an intrinsically disordered region (IDR) domain, which renders TDP-43 aggregation prone.
LCDs are common in RNA-Binding Proteins (RBPs) and mediate protein-RNA interactions through a liquid-liquid phase separation (LLPS) process.
Mutations clustered within the LCD and the RRM domains may led to several proteinopathies.

Axxam multilayer framework platform for screening

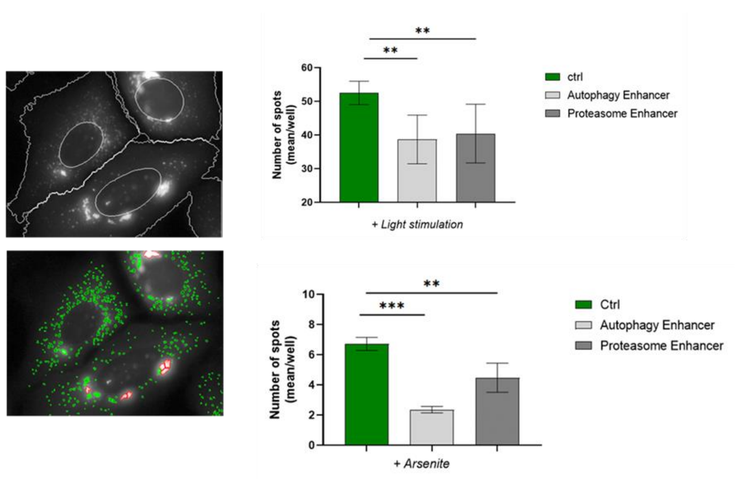


Several assays can be used sequentially and/or in parallel, combining and balancing throughput, relevant models and multiple readouts.

Biomolecular Condensate/Aggregates Assay

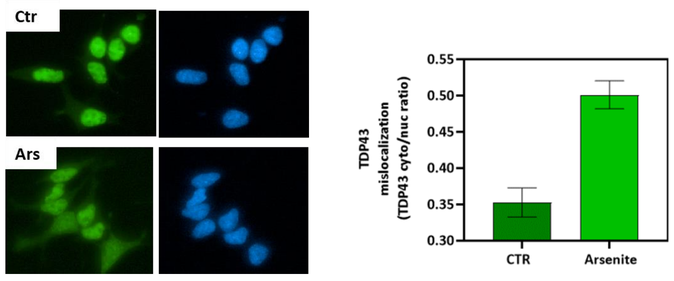


TDP43 biomolecular condensate/aggregation puncta formation by light stimulation (or chemical stress) in U2OS reporter cell lines. Left: Experimental protocol workflow in miniaturized format (384-w). Right: Images pre- and post- light stimulation were acquired by means of a **High Content Microscope (Operetta CLS, PerkinElmer)**; 40X magnification.

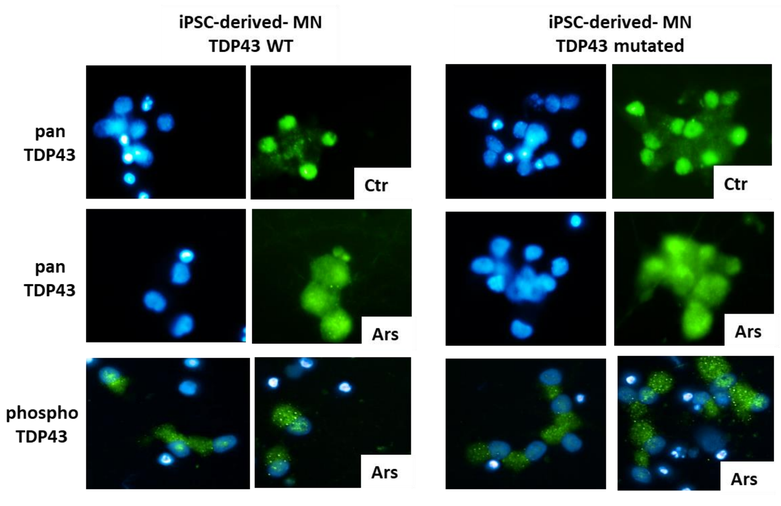


Cellular clearance pathways decrease the TDP43 puncta accumulation. Left: Green objects represent TDP43 positive small condensates, red objects large aggregates. Right: plots of number of spots detected with the two different stimulation protocols (light or chemical stressor, in presence of autophagy or proteasome enhancer drugs).

TDP43 mis-localization in relevant cell models

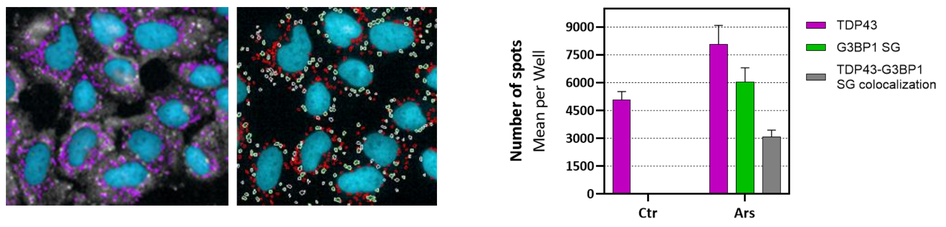


TDP43 mis-localization in human neuroblastoma cells. Endogenous level of TDP43 (green channel) upon stressor treatment (Arsenite, Ars) measured by immunostaining in human neuroblastoma cell lines, SH-SY5Y cells, counterstained with Dapi for nuclei (blue channel). Plot represents the ratio of TDP43 cytosol mis-localization.



TDP43 mis-localization and puncta formation in iPSC-derived motor neurons. Analysis of TDP-43 (green channel) localization, upon stressor treatment (Arsenite, Ars), was performed in human iPSC-derived motor neurons (MN**; iCell Motor Neurons FCDI**) possessing either non-mutant (WT, left panel) or mutant TDP-43 (Mutated, right panel). TDP-43 was measured by means of both total TDP-43 (pan TDP43) and phospho-TDP-43 (phospho TDP43) immunofluorescence and counterstained with DAPI for nuclei (blue channel).

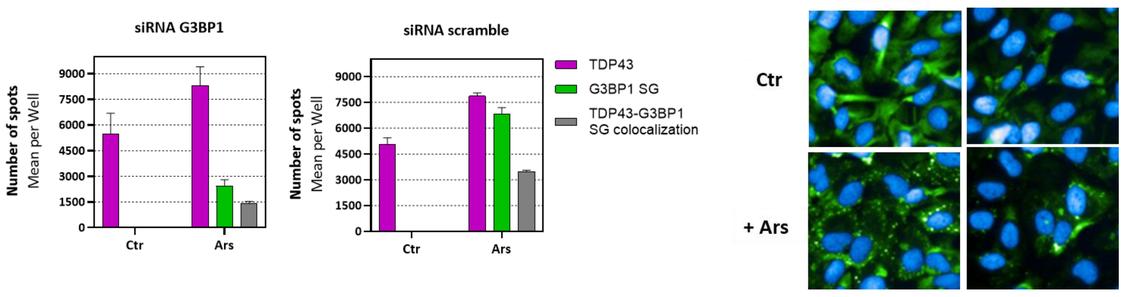
Stress Granules (G3BP1 positive) Assay



TDP43 and Stress Granules (SG) -G3BP1 positive- co-localization. G3BP1 SG measurement in presence/absence of a stressor treatment (Arsenite, Ars) using multiplexing staining with G3BP antibody (green channel), Dapi for nuclei (blue channel) and TDP43 reporter (pink channel). Several features can be analyzed: white spots represent the TDP43 spot co-localized with the G3BP1 positive SG over the discarded red one. Plot: number of puncta in presence and absence of arsenite stressor.

RNAi automated genomic screening

Stress granule marker G3BP1 efficiently silenced through automated reverse RNAi. Top: High-throughput RT-qPCR workflow in miniaturized (384-well) format and semi-automated conditions. Bottom: G3BP1 mRNA expression levels upon transfection with siRNA G3BP1 pool and siRNA non targeting control (scramble) pool in two different cell lines. Data presented in Relative Expression Units (REU).



G3BP1 silencing resulted in TDP43 re-localization. Silencing of the SG marker G3BP1 following by arsenite stress (left plots), resulted in both a ~ 60% reduction of the number of G3BP1 spots and a 40% decrease of TDP43-G3BP1 co-localized puncta, compared to the scramble controls, respectively. A slight increase of cytosolic TDP43 compared to control was also observed after G3BP1 knockdown. Right: Representative images of cells upon silencing in presence/absence of arsenite. Blue channel for Dapi, green channel for G3BP1 staining.

Conclusion

Here we have shown the use of an integrated platform with several assays structured at different levels of complexity as a proof-of-concept to evaluate the dysfunctions of RNA-binding proteins (RBPs), such as TDP43. The wide-angle approach together with the automated miniaturized formats of the assays can be relevant for the study of neurodegenerative diseases.

References: M. J. et al. Neuron, 2019; F. Y. M. et al. Neuron 2019; A. S. et al. Nature Reviews Molecular Cell Biology, 2021; H. M. et al. Cell, 2021

Contacts

Director, Discovery Services
Lia Scarabottolo
email: lia.Scarabottolo.ls@axxam.com

Director, Business Development
Sabrina Corazza
email: sabrina.corazza.sc@axxam.com

Unit Manager, High Content Screening
Fernanda Ricci
email: fernanda.ricci.fr@axxam.com

Corporate Offices & Laboratories

Axxam S.p.A.

OpenZone Campus: Via Meucci 3
Kunzi Campus: Via Ariosto 19
20091 Bresso (Milan) / Italy

Novartis Campus
Via Provinciale Schito 131
80058 Torre Annunziata (Naples) / Italy

info@axxam.com

Axxam GmbH

Building 7, The Plant Konstanz
Byk-Gulden-Strasse 2
78467 Konstanz / Germany

info.de@axxam.com

Business Offices

Cambridge Innovation Center
1 Broadway, Cambridge, MA 02142 / USA

Copenhagen Bio Science Park - COBIS A/S
Ole Maaløes Vej 3, 2200 Copenhagen / Denmark

Switzerland Innovation Park Basel Area AG
Hegenheimermattweg 167A,
4123 Allschwil / Switzerland