TDP-43 AND STRESS GRANULES IN ALS AND FTD: ARE MULTIPLE CELLULAR MODELS REQUIRED?

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ABSTRACT

We characterised two TDP-43 proteinopathy cellular models that were based on different aetiologies of disease. These were a sodium arsenite-induced chronic oxidative stress model and a disease-relevant TDP-43 mutant model (M337V). These two models were exposed to small molecule chemical probes and different effects were observed. For example, one compound decreased cytoplasmic TDP-43 and increased soluble levels of stress granule marker TIA-1 in the cellular stress model without impacting these levels in the mutant cell line. This study highlights the challenges of using cellular models in lead development during drug discovery for ALS and FTD and reinforces the need to perform assessments across a variety of cell lines and aetiological models.

BACKGROUND

- Proteinopathy relating to TDP-43 is a pathological hallmark in almost all ALS and 50% of FTD patients
- Mislocation of TDP-43 from nucleus to cytoplasm: loss-of-function and gain-of-toxicity
- Stress granule formation implicated in TDP-43 proteinopathy
- ↓ stress granule-associated proteins can rescue degenerative phenotypes in ALS mouse models
- Mislocation and stress granules potential targets for disease-modifying treatments
- To date, there is no consensus about which *in vitro* assay or TDP-43 model best replicates the disease pathology

AIMS

Conduct an in-depth characterisation of two common cellular models of ALS and FTD.

1. HEK-293 cells overexpressed WT TPD-43, treated with a chronic dose of sodium arsenite (SA).
2. SH-SY5Y cells expressing disease-relevant mutant form of TDP-43 (TDP-43 M337V)

Oxidative Stress Model

- HEK-293 cells transfected with tetracycline (Tet)-inducible WT TDP-43
- Tet treated cells (2 µg/mL, 48 h): overexpression of TDP-43 (red), especially in the nucleus, low levels TIA-1
- Tet + SA (15 µM, 18 h): mislocalisation of TDP-43 which colocalised with upregulated TIA-1 (green) in cytoplasm
- Tet + SA mirror many aspects seen in postmortem FTD/ALS tissue

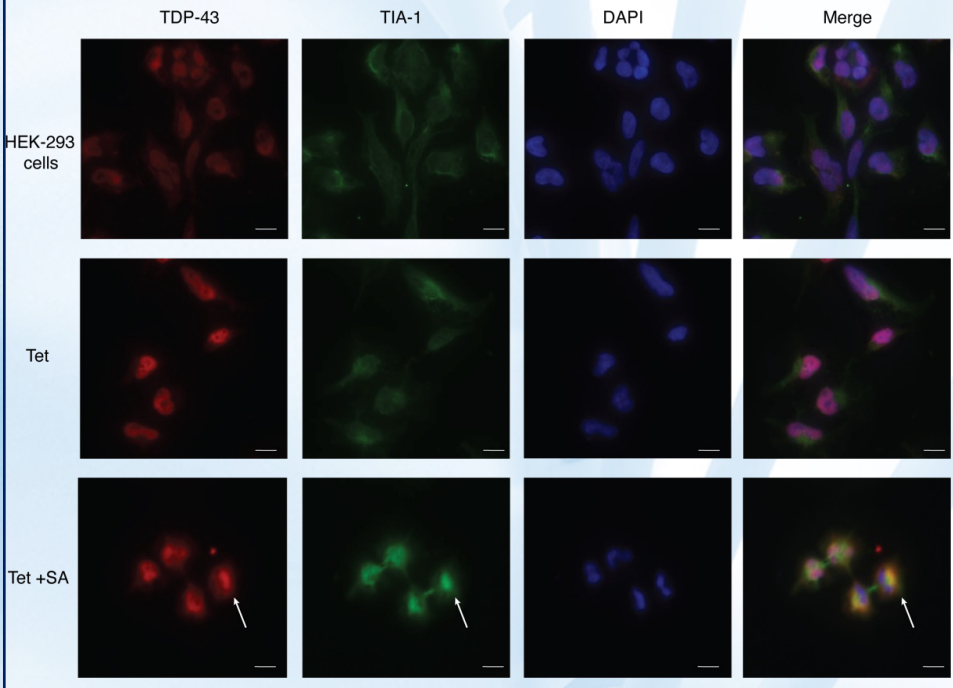


Figure 1. Immunofluoresence imaging of TDP-43 and TIA-1 in stably transfected HEK-293 cells. DAPI (blue) used to indicate nucleus. Scale bar = 10 µM

M337V Mutant Model

- SH-SY5Y cells transfected with disease-relevant M337V TDP-43 (V5-tagged).
- V5 tag allows differentiation between the effect of drugs on mutant and endogenous TDP-43.
- SH-SY5Y cells and M337V TDP-43 SH-SY5Y cells showed similar expression levels and distribution of TDP-43 (red), stress granule marker, TIA-1 (green).
- Arrows shows colocalisation of TDP-43 and stress granule aggregates

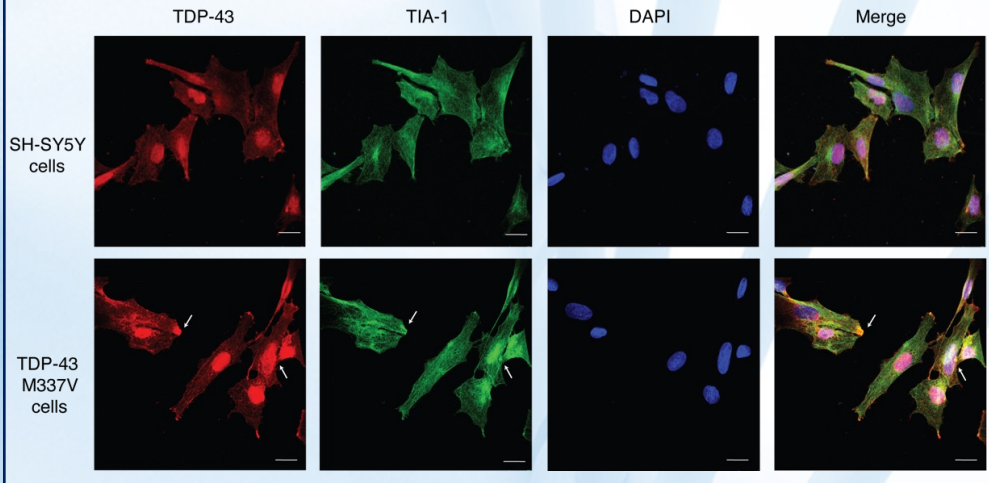
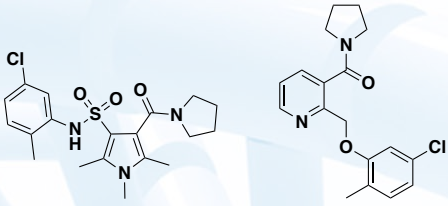


Figure 2. Immunofluoresence imaging of WT human neuroblastoma SH-SY5Y cells compared to SH-SY5Y cells expressing ALS-linked mutation TDP-43 M337V. DAPI (blue) was used to indicate the nucleus. Scale bar = 10 μM

TDP-43 Expression Profile

Screened reported compound (**1**) and novel bioisoteric analogue (**2**) at 300 nM



1 **2**

- **1** reduced cytoplasmic TDP-43 levels in oxidative stress model, without altering nuclear levels or levels in soluble and insoluble fractions.
- Lack of impact of **1** on insoluble fraction conflicts previous reports[1] with GFP-tagged TDP-43 and high conc. acute sodium arsenite dose
- **1** and **2** no effect in mutant model

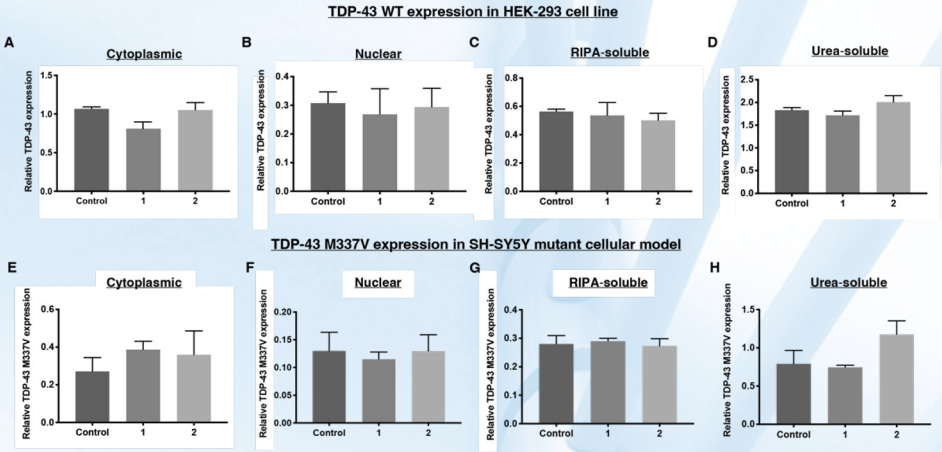


Figure 3. Relative TDP-43 WT expression levels after treatment

Stress Granule Solubility

Oxidative Stress Model

- **1** no effect on TIA-1 distribution
- **2** significantly decreased nuclear TIA-1 expression, no effect on cytoplasmic
- **1** and **2** increased RIPA-soluble and decreased urea-soluble TIA-1

M337V Mutant Model

- **1** and **2** no effect on TIA-1 distribution
- **1** significantly decreased urea soluble TIA-1, RIPA-soluble no effect
- **2** reduced both RIPA-soluble and urea-soluble TIA-1

Outcome

- Altered stress granule solubility profile from insoluble to soluble

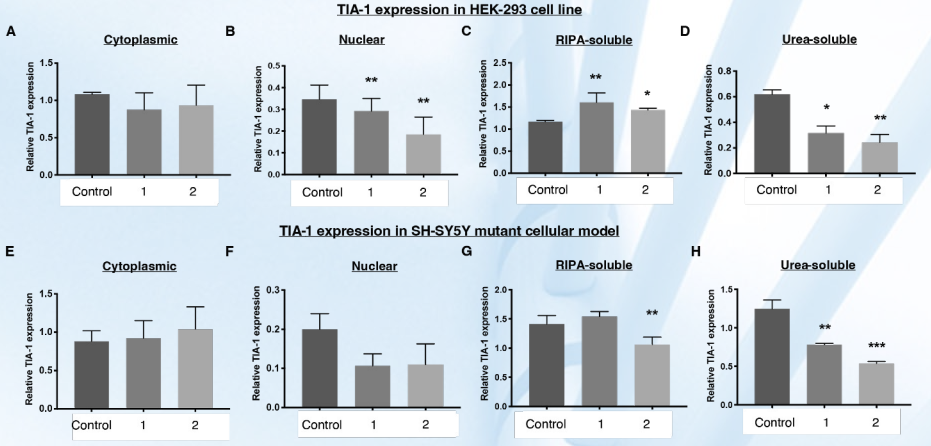


Figure 4. Relative distribution of TIA-1, a stress granule marker

Ubiquitination & Cell Viability

- Investigated whether stress granule solubility was associated with increased ubiquitin-proteasome system

Oxidative Stress Model

- **1** no effect on cellular ubiquitin levels
- **2** reduction of cellular ubiquitin levels

M337V Mutant Model

- **1** and **2** reduction of ubiquitination levels.

Outcome

- Neither stimulated the recruitment of the ubiquitin-proteasome system
- Inhibition did not correlate with an increase in stress granule formation

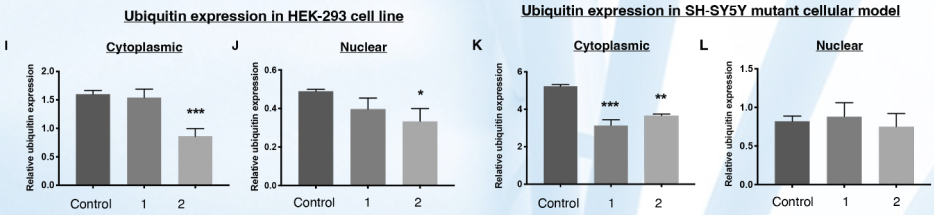


Figure 5. Relative distribution of ubiquitin

Cell Viability

- Effects of **1** and **2** on cell viability
- Neither protected or induced cell death
- May not confer cell survival benefits

CONCLUSION

- Characterised two ALS and FTD cellular models commonly used in literature
- Compounds **1** and **2** reduce insoluble stress granule levels and ubiquitin expression.
- Do not reduce stress-induced cell death, suggesting increased stress granule solubility may not confer cell survival benefits in TDP-43 models.
- Identified differing pathomechanisms in the stress and mutant cellular models
- Conflicting effect of **1** in WT HEK-293 oxidative stress model compared to reported study in PC-12 cells.
- Highlights challenges of using cellular models in lead development for ALS and FTD

REFERENCES

1. Boyd, J. D. *et al*, A high-content screen identifies novel compounds that inhibit stress-induced TDP-43 cellular aggregation and associated cytotoxicity. *J. Biomol. Screen* **2014**, 19, 44-56.

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