Advanced Modeling of Amyotrophic Lateral Sclerosis Using a Human iPSC-Derived Motor Neuron, Astrocyte, and Microglia Tri-culture System

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Malika Bsibsi[1], Narendra Chunduri[1], Yull Bakridi[1], Larisa Turtureanu[1], Lianne van Beek[1], Cindy J.M. Loomans[1], Ross Wheatcroft[2], Anne-Marie Zuurmond[1]

[1] IQVIA Laboratories, Leiden, NL
[2] BrainXell, Madison, US

Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease driven by motor neuron degeneration and contributions from astrocytes and microglia. Conventional models often fail to capture these complex multicellular interactions, limiting their translational relevance. Physiologically relevant multicellular models are therefore needed to better recapitulate ALS pathology, improve therapeutic translation, and reduce reliance on animal models.

Methods

We established a multicellular ALS co-culture model comprising iPSC-derived motor neurons, astrocytes, and microglia from WT and TDP-43 G298S backgrounds. TDP-43 pathology was induced with puromycin and MG132 and assessed by high-content imaging of TDP-43 mis-localization and aggregation. Loss of TDP-43 function was measured by qPCR of truncated STMN2 transcripts.



Figure 1. Experimental design and timeline. WT and TDP-43 G298S motor neuron monocultures and tri-cultures (MN:AC:MG, 2:1:3) were treated with puromycin (5 or 10 µg/mL) or MG132 (0.2 or 0.6 µg/mL) for 48 or 72 h.

Results

Immunostaining of cell-specific markers in tricultures

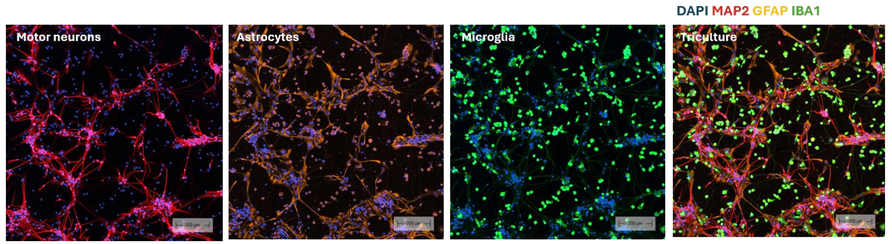


Figure 2: Images of Motor neurons, Astrocytes and Microglia in triculture. Cells stained for nuclei (DAPI in blue), MNs (MAP2 in red), astrocytes (GFAP in orange), microglia (IBA1 in green)

Puromycin induces TDP-43 cytoplasmic translocation and puncta formation

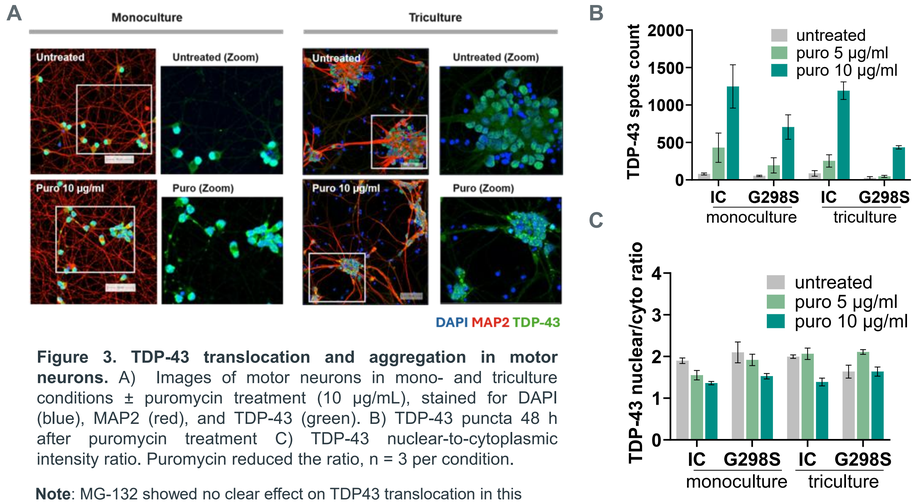


Figure 3. TDP-43 translocation and aggregation in motor neurons. A) Images of motor neurons in mono- and triculture conditions ± puromycin treatment (10 µg/mL), stained for DAPI (blue), MAP2 (red), and TDP-43 (green). B) TDP-43 puncta 48 h after puromycin treatment C) TDP-43 nuclear-to-cytoplasmic intensity ratio. Puromycin reduced the ratio, n = 3 per condition.

Note: MG-132 showed no clear effect on TDP43 translocation in this model (Data not shown)

MG132, but not puromycin, induces STMN2 mis-splicing

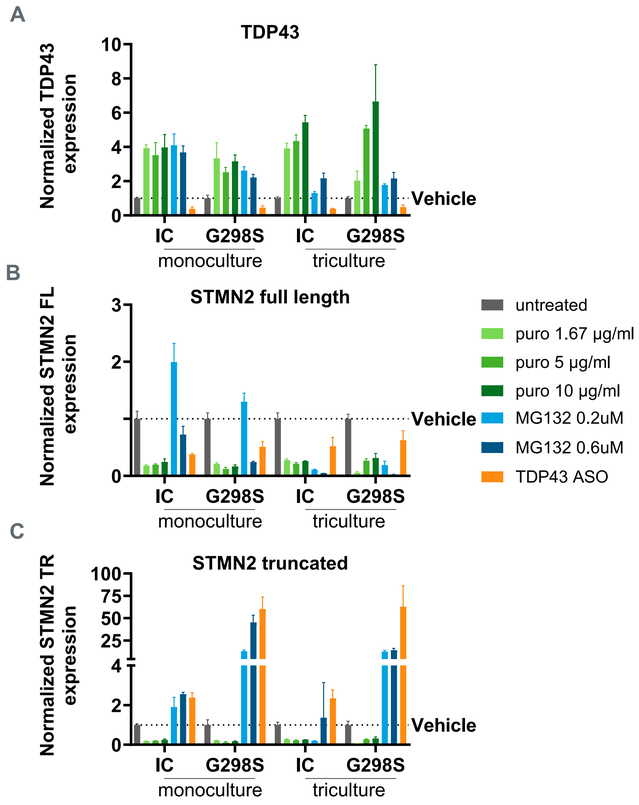


Figure 4. Effects of puromycin and MG132 on TDP-43 function assessed by STMN2 mRNA processing. Normalized expression of A) TDP-43, B) full-length STMN2, and C) truncated STMN2 measured by qPCR. TDP-43 ASO used as positive control. n = 3 for treatments; n = 6 for vehicle.

Conclusions

We offer iPSC-derived motor neuron monocultures and multicellular co-culture models that recapitulate key features of ALS, providing a physiologically relevant platform for more predictive therapeutic evaluation and reduced attrition in CNS drug discovery.

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