Small molecule TDP-43 oligomer/aggregation inhibitor, WTX-245, corrects transcriptional dysfunction across multiple mRNAs in a neuronal cell model for ALS

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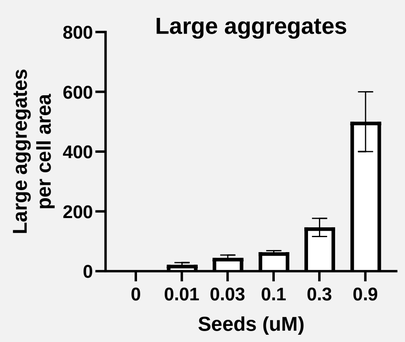
Background and Objective

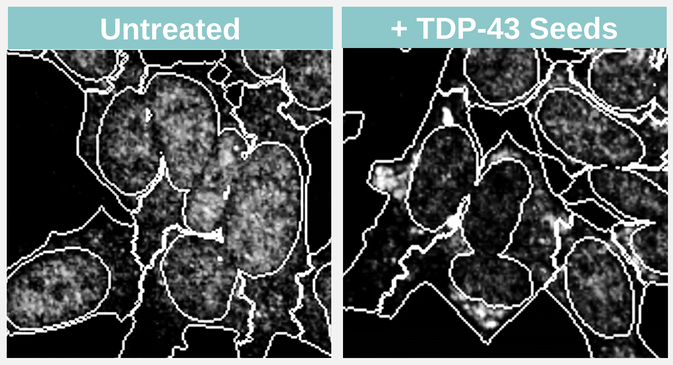
- Aggregation of TDP-43 is a central hallmark of neurodegeneration in ALS and FTD, which leads to nuclear depletion and the disruption of normal mRNA splicing.
- We have developed a cell model that emulates the full spectrum of aggregation pathology and disease biology and is suited for therapeutic translational development.
- Here we evaluate the efficacy of WTX-245, a small molecule TDP-43 nucleation inhibitor, in a neuronal cell assay that recapitulates the core neuropathological features of ALS: nuclear TDP-43 depletion, cytoplasmic aggregation, and mRNA transcriptional dysfunction.

WaveBreak's proprietary TDP-43 cell assay

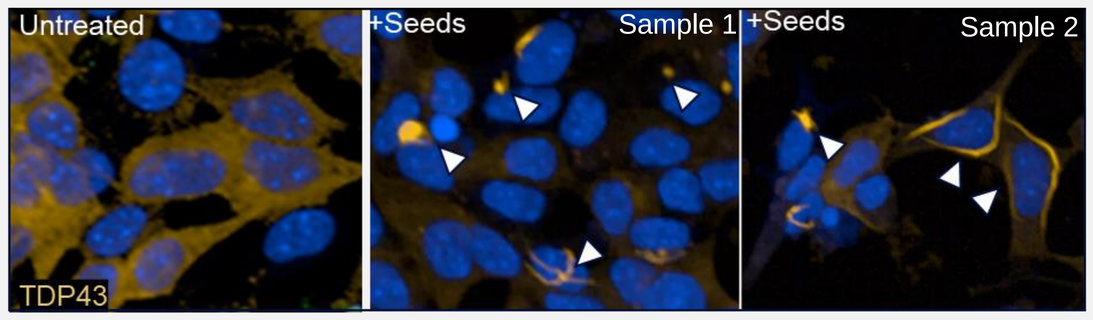
WaveBreak has pioneered the development of a neuronal model that uses proprietary, tag free TDP-43 amyloid fibrils to seed aggregation in our TDP-43 cytoplasmic aggregation neuroblastoma cell assay (T43CA).

I. The seeded cell assay induces TDP-43 aggregation in the cytoplasm and induces loss of nuclear TDP-43



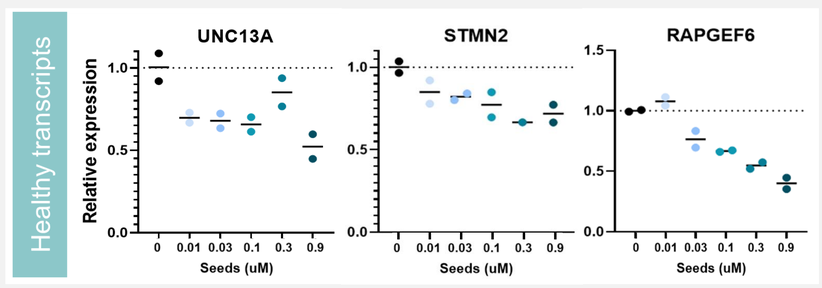


II. The seeded cells exhibit robust formation of TDP-43 aggregates that reproduce the morphology observed in patient samples



III. Neuronal mRNA transcription is disrupted with the onset of TDP-43 aggregation

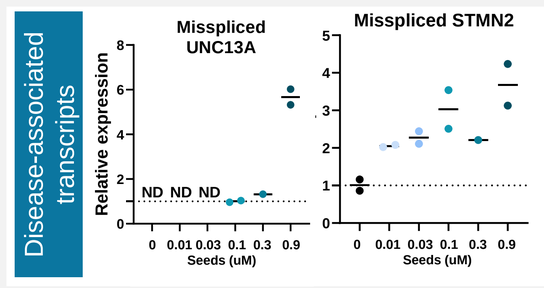
Aggregation-driven loss of correctly spliced mRNAs



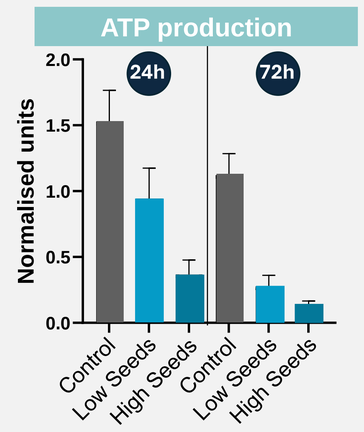
These mRNA's play key roles in Motor Neurons

Gene	Function
UNC13A	Synaptic transmission
STMN2	Axonal projection
RAPGEF6	Axon maintenance

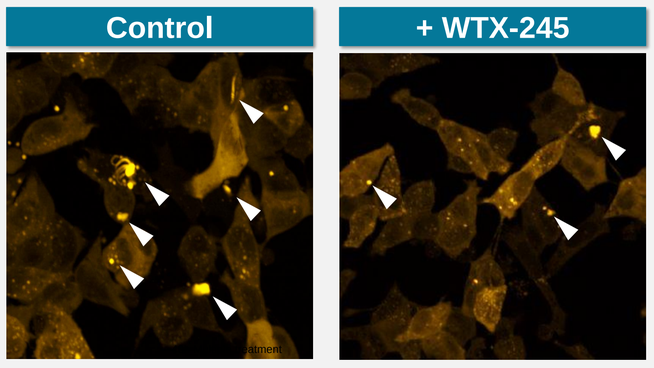
Aggregation-driven enrichment of mis-spliced mRNAs

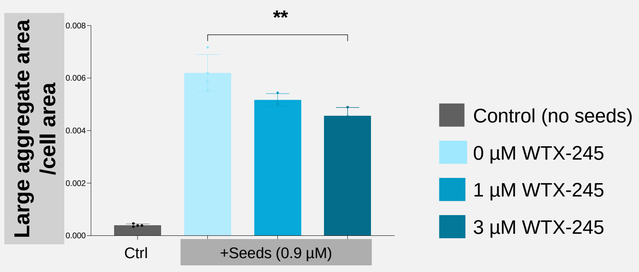


IV. Mitochondrial function is rapidly compromised in the seeded cells

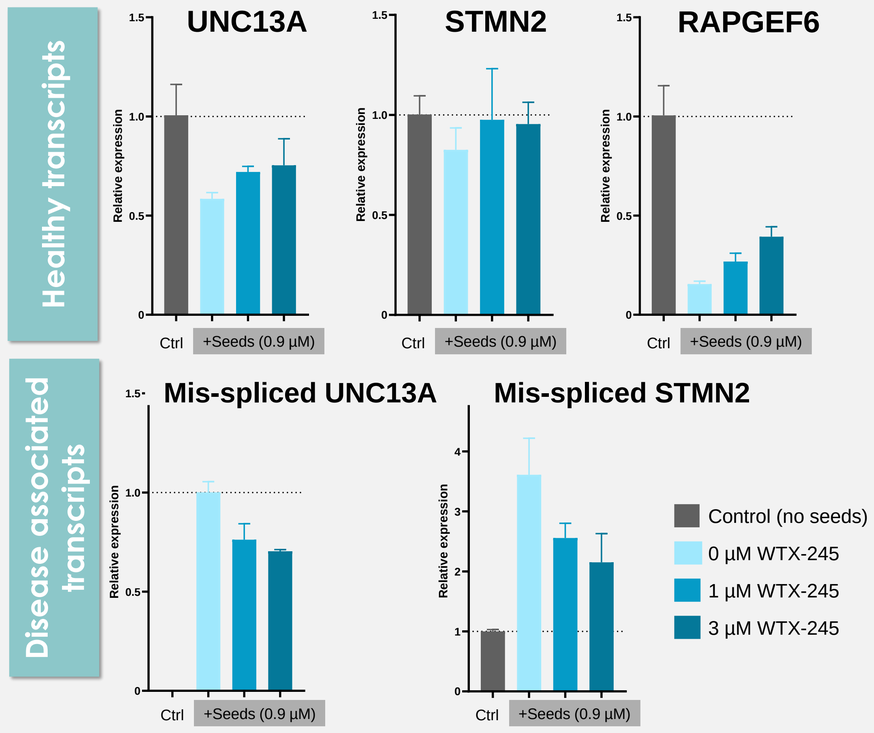


WTX-245 inhibits TDP-43 aggregation, restores healthy mRNA transcription and reduces mis-splicing





Methods: SH-SY5Y cell line treated with 0.9 uM TDP43 seeds +/- WTX-245. Data represent the mean ±SD. * P<0.05 ** P<0.01 versus vehicle-treated. Ordinary one-way ANOVA, Šídák's multiple comparisons test. High content imaging was performed at 40 h time point using Opera Phenix microscope. Images were analyzed using Opera Harmony software. Aggregates were selected using a pre-trained algorithm.



Methods: qPCR performed 72 hours after seeding. Data represent the mean ±SD. Normalised to seed treated control. Reduced mis-spliced transcripts, coincident with a significant reduction of TDP-43 aggregates. Dose-dependent trend for rescuing healthy mRNA transcripts.

Conclusions

- To support the development of a new class of TDP-43 therapeutics, we have developed a proprietary seeded neuronal cell assay that recapitulates TDP-43 pathology: aggregation, neuronal depletion, mRNA mis-splicing, and metabolic dysregulation.
- WTX-245 is a small molecule designed to directly inhibit the TDP-43 nucleation mechanisms that are disease-specific and initiate the cascade of molecular events leading to the formation of protein aggregates.
- WTX-245 inhibits TDP-43 aggregation, restores healthy mRNA transcription and reduces mis-splicing in this TDP-43 seeded neuronal cell assay.