The time course and impacts of TDP-43 loss on stathmin-2 gene expression, protein abundance, Golgi apparatus morphology, neurite outgrowth, and response to proteotoxic stress in human induced pluripotent derived motor neurons

Gray T1 , Hamwi Y 1 , Kamelgarn M 1 , Bussgang J 1 , Elbaum D 1 , Hinckley S 1
[1]QurAlis, Cambridge Massachusetts, United States; [2]Department of Biology

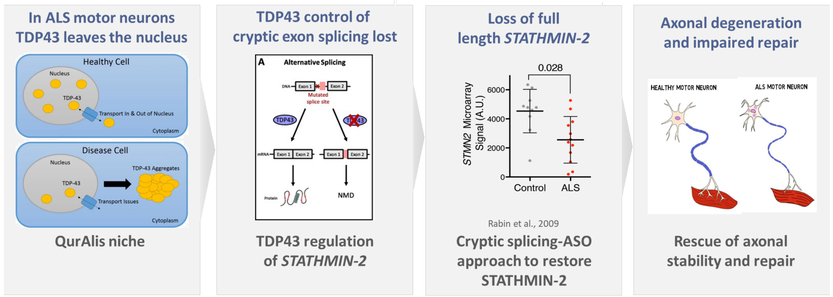
Abstract

- **Objective:** To understand the impact of TDP-43 loss on Stathmin-2 mRNA, protein, protein localization, and functional impact within motor neurons
- **Methods:** Human iPSC-derived motor neurons were transfected with a TDP-43 or Stathmin-2 gapmer ASO and were observed over 21 days. Over the time course cell lysates were collected for western blots and RNA isolation as well as fixed for ICC analysis. When assessing our splice-switching Stathmin-2 rescue ASOs, the splice switching ASO was added at the same time as the TDP-43 gapmer. To confirm our observations in a non-ASO induced TDP-43 loss of function model we utilized MG132 to invoke proteotoxic stress and force TDP-43 from the nucleus. To assess Stathmin-2 function within motor neurons we assessed ICC staining in established 2D human motor neuron cultures as well as in a 3D Xonachip system observing neurite outgrowth after injury.
- **Outcome:** The addition of TDP-43 gapmer ASO resulted in a loss of TDP-43 mRNA followed by loss of TDP-43 protein. The loss of Stathmin-2 mRNA then protein and induction of Stathmin-2 cryptic exon mRNA slowly followed loss of TDP-43, confirming the relation that TDP-43 is required for Stathmin-2 mRNA transcription. With knock down of TDP-43, Stathmin-2 is lost from established and new growth neurites, the Golgi Apparatus, Golgi outposts located in dendrites. Additionally, when TDP-43 exits the nucleus due to MG132 induced proteotoxic stress, Stathmin-2 protein is lost from neurites.

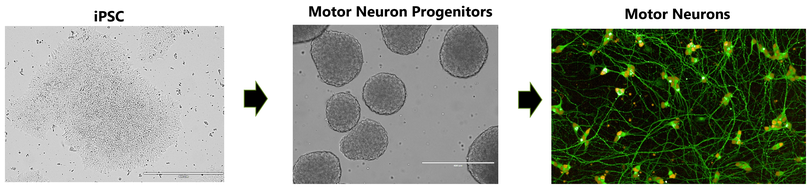
Background

- Approximately 97% of amyotrophic lateral sclerosis (ALS) cases have nuclear clearing or insoluble TAR DNA binding protein-43 (TDP-43) cytoplasmic aggregates present in the brain and/or spinal cord. TDP-43 is a nuclear RNA/DNA binding protein involved in RNA regulation and metabolism. The loss of nuclear TDP-43 results in the mis-splicing of several genes, including Stathmin-2. (Prasad et al., 2019, Klim et al., 2019, Melamed et al., 2019)
- Stathmin-2 is a tubulin stabilizing/destabilizing protein that associates with the Golgi apparatus and the growth cone during neurite outgrowth. Stathmin-2 normally co-localizes to the Golgi Apparatus organelle and Golgi outposts (in neuronal processes). Dysfunction in intracellular axonal trafficking has been implicated for the denervation seen in ALS in vitro and in vivo models. (Paolo et al. 1996, Horton & Ehlers, 2003)
- Golgi pathology has been described in ALS patient spinal and cortical motor neurons. (Sundaramoorthy et al. Fujita and Okamoto, 2005)
- Disruption of Golgi function is known to cause motor axonal degeneration.[8] (Schaefer et al. 2007)

Hypothesis



Patient derived induced pluripotent stem cell motor neurons



QurAlis utilizes a highly efficient culture driven protocol that produces 98% pure patient iPSC-derived motor neurons for all *in vitro* potency and efficacy studies

Figure 1: The loss of TDP-43 is correlated with the loss of Stathmin-2 over time

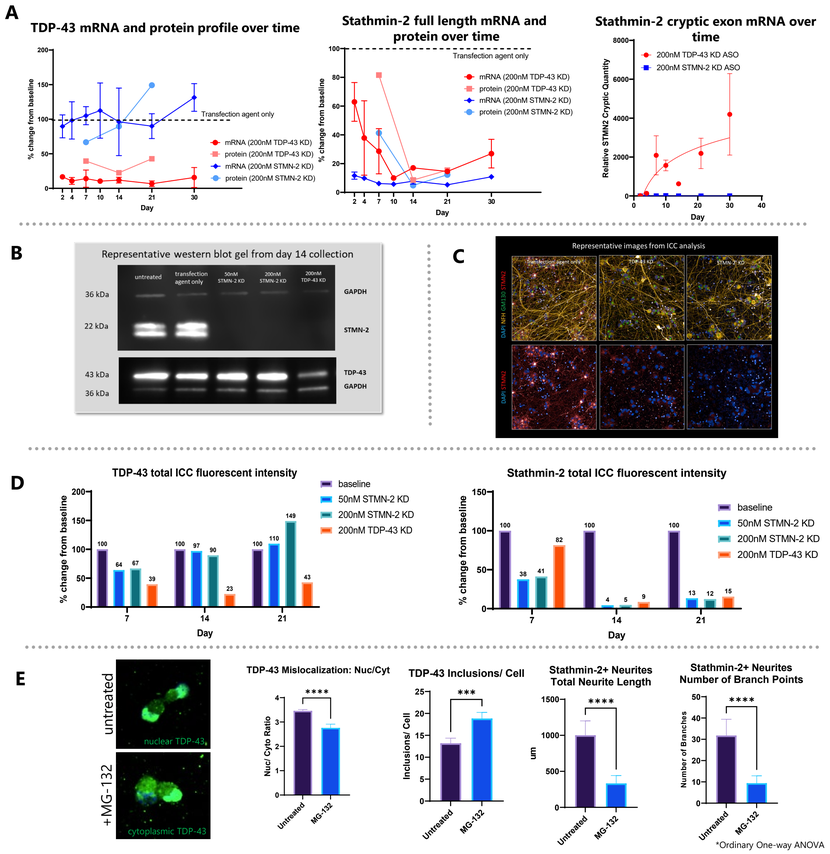


Figure 1: A) STMN2-FL mRNA and protein levels are reduced when TDP-43 is KD while STMN-2 cryptic exon increases over time B) Representative western blot membrane from the time course. C) Representative images of stained motor neurons used for the time course ICC quantification. D) Quantitation of total TDP-43 and STMN2 staining over the time course. Total TDP43 and STMN2 staining has a sustained reduction compared to baseline over time. E) Representative images from motor neurons treated with MG-132 for 48h. Quantitation of the ratio of TDP-43 nuclear mis-localization and inclusions per cell with matching ICC analysis of STMN2 neurite properties showcasing the impact of TDP-43 mis-localization on STMN2

Figure 2: Loss of stathmin-2 impacts motor neuron neurite outgrowth after injury

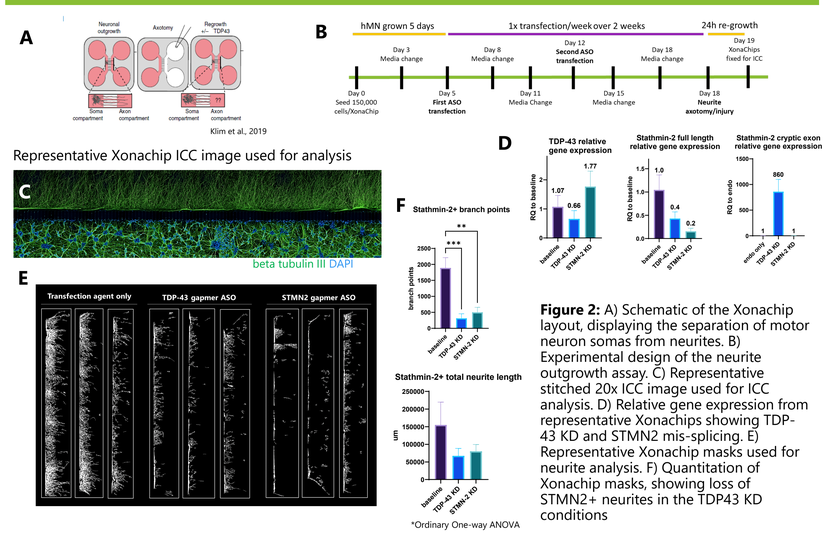


Figure 2: A) Schematic of the Xonachip layout, displaying the separation of motor neuron somas from neurites. B) Experimental design of the neurite outgrowth assay. C) Representative stitched 20x ICC image used for ICC analysis. D) Relative gene expression from representative Xonachips showing TDP-43 KD and STMN2 mis-splicing. E) Representative Xonachip masks used for neurite analysis. F) Quantitation of Xonachip masks, showing loss of STMN2+ neurites in the TDP43 KD conditions

Figure 3: Loss of stathmin-2 impacts on motor neuron Golgi Apparatus

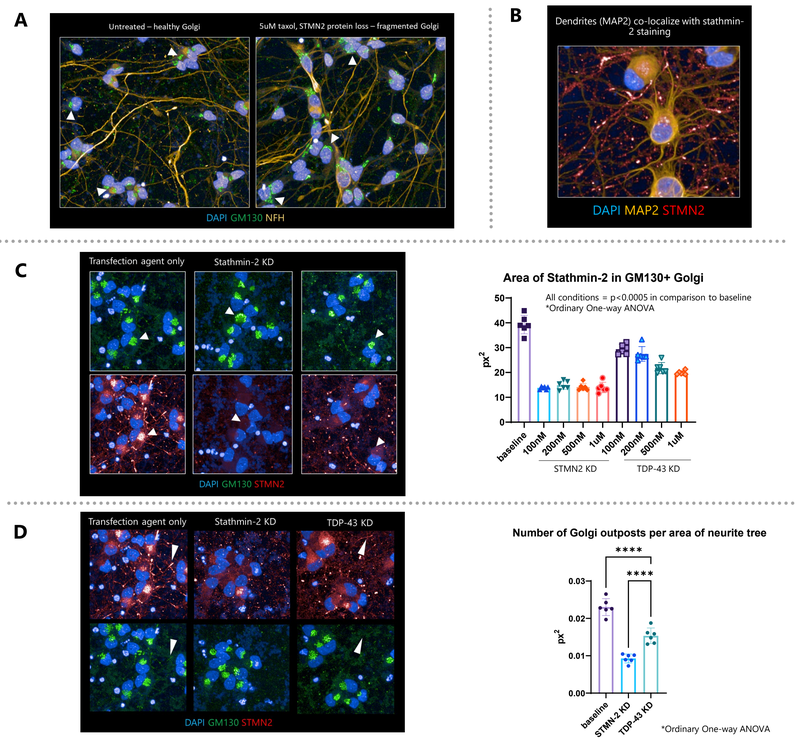


Figure 3 : A) The addition of taxol, which reduces Stathmin-2 protein levels, results in Golgi fragmentation showcasing Stathmin-2's crucial role in golgi apparatus homeostasis. B) In human motor neurons Stathmin-2+ neurites co-localize with MAP2, showing it to be present in dendrites. C) TDP-43 KD significantly reduces Stathmin-2 co-localization to GM130+ (cis-) golgi compared to baseline. D) Stathmin-2 neurite golgi outposts stained by GM130 are significantly reduced in the TDP-43 KD compared to baseline

Figure 4: Stathmin-2 ASO rescues stathmin-2 mis-splicing caused by TDP-43 loss

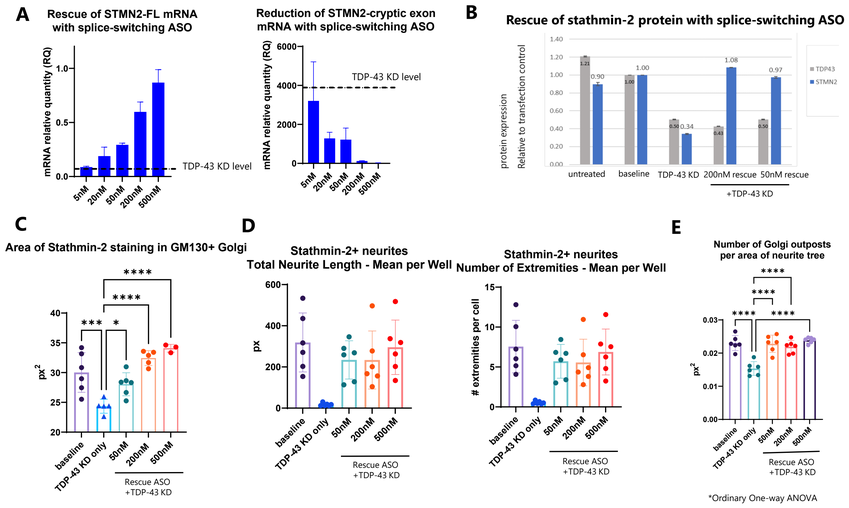


Figure 4: A) Despite the addition of TDP-43 gapmer ASO (loss of TDP-43), at 20nM our Sathmin-2 splice switching ASO can recover *STMN-2* full length mRNA and reduce *STMN-2* cryptic exon above the TDP-43 KD condition. B) At 50nM the Stathmin-2 splice switching ASO can recover Stathmin-2 protein levels despite TDP-43 KD. C) In a dose dependent manner, the splice switching Stathmin-2 ASO returns Stathmin-2 to the Golgi apparatus in comparison to the TDP-43 KD condition despite TDP-43 loss. D) Stathmin-2 is returned to the neurites despite TDP-43 loss when the Stathmin-2 rescue ASO is added at all dose points. E) GM130+ golgi is returned to neurites with the Stathmin-2 rescue is added at all dose points