Ncardia

Development of a human iPSC-derived ALS model

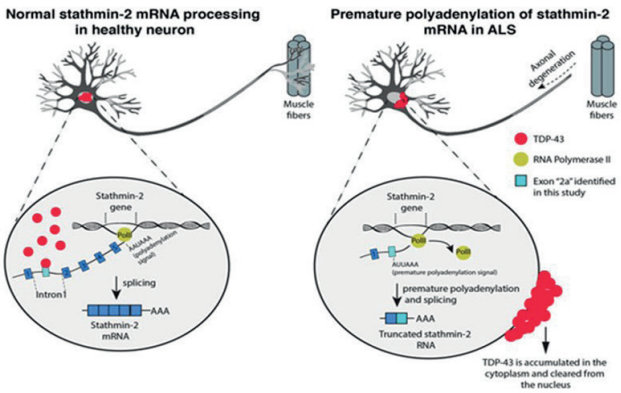

Figure 1: Loss of nuclear TDP-43 results in premature polyadenylation of Stathmin2 mRNA

- Production of a truncated Stathmin2 and premature stop
- Lowers levels of Stathmin2 and increased axonal degeneration
- Re-expression of Stathmin2 reduces axonal degeneration
- Considered a key aspect of the molecular etiology of ALS

Background

Amyotrophic lateral sclerosis (ALS) is a rare neurological disease that primarily affects the nerve cells (motor-neurons) responsible for controlling voluntary muscle movement. ALS is characterized by stiff muscles, muscle twitching, and gradually worsening weakness due to muscles decreasing in size. This results in difficulty speaking, swallowing, and eventually breathing.

~90% of ALS cases have no known etiology while the remaining 10% have a genetic cause. Mutations in over 20 genes have been associated with familial ALS with mutations in 4 genes accounting for most familial cases.

The majority of ALS cases and approximately 50% of FTD cases (both familial and sporadic) present with pathological aggregates of TDP-43. Familial mutations within TDP-43 are the most common genetic cause of ALS and mutation of TDP-43 has been reported to have multitude of effects.

Case study

For this case study, Ncardia used iPSC-derived motor neurons (MNs) with a CRISPR engineered TDP-43* point mutation to quantify disease relevant phenotypes:

- Mis-localization of TDP-43
- Aggregation of TDP-43
- Mis-splicing of STMN2
- Electrophysiological properties of wild type and mutant iPSC-derived motor neurons.

* iCell ® Motor Neurons, 01279 from FUJIFILM Cellular Dynamics, Inc.

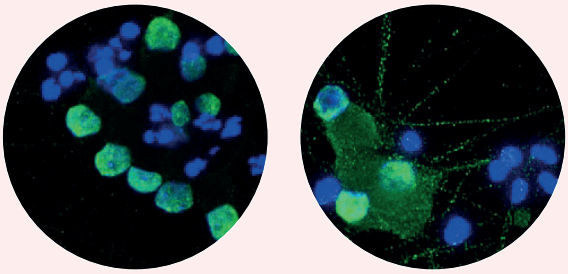

Figure 2: Ncardia's ALS model, using iPSC-derived motor neurons from TDP-43 mutant (right) and isogenic control (left).

Mis-localization of TDP-43

hiPSC derived MNs from TDP-43 mutant and isogenic control were matured and subsequently chronically treated with a stressor to induce TDP-43 mis-localization. MNs were fixed and immunocytochemistry performed to stain, TDP-43 or phospho-TDP-43 (green) and nuclei (blue).
Cytoplasmic localization of TDP-43 and phospho-TDP43 can be observed in the mutant neurons as well as in all neurons treated with the stressor.

Automated high content imaging with high throughput image analysis, segmented and quantified nuclear verses cytoplasmic TDP-43. Ratiometric quantification of nuclear and cytoplasmic TDP-43, confirmed mis-localization of TDP-43 from the nucleus to the cytoplasm both with and without the stressor. Data was normalized to the wild type MNs and a two-way ANOVA performed.

Untreated TDP43 M337V MNs and MNs chronically treated with a stressor show mis-localization of TDP-43 to the cytoplasm.

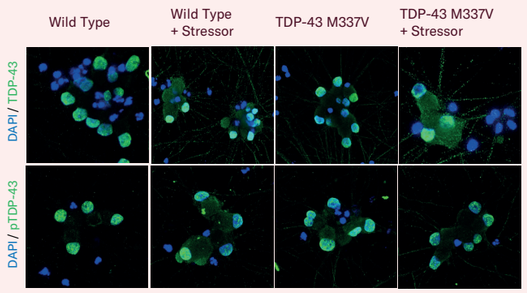

Figure 3

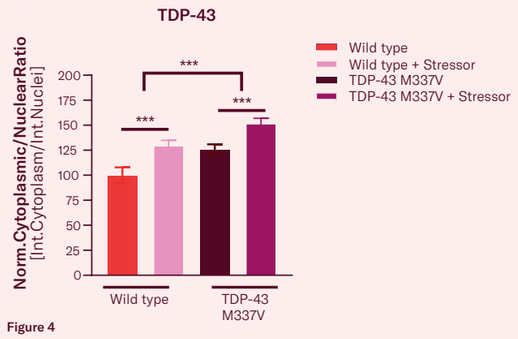

Figure 4

Aggregation of TDP-43

The majority of ALS cases and approximately 50% of FTD cases (both familial and sporadic) present with pathological aggregates of TDP-43.

MNs from wild type and mutant iPSC lines were matured, lysed and a HTRF assay performed to quantify TDP-43 aggregation.

The mutant MNs show a suggestive increase in TDP-43 aggregation but the addition of a stressor, consistently and robustly increases TDP-43 aggregation

TDP43 M337V MNs and MNs chronically treated with a stressor show aggregation of TDP-43.

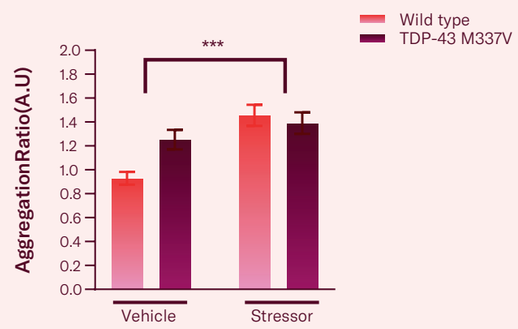

Figure 5: HTRF quantification of TDP-43 aggregation in wild type and mutant MNs. Two-way ANOVA were performed to determine statistical significance.

Downregulation and mis-splicing of STMN2

iPSC derived MNs from TDP-43 mutant and isogenic control were matured and subsequently chronically treated with a stressor to induce STMN2 dysregulation.

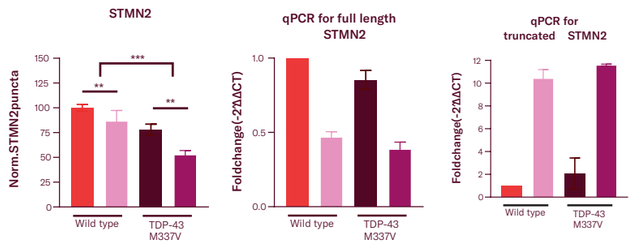

Figure 6: MNs were fixed and immunocytochemistry performed to visualize STMN2 and nuclei. Reduction in of STMN2 can be significantly and robustly observed in mutant neurons treated with the stressor. Data was normalized to the wild type neurons and a two-way ANOVA performed.

MNs chronically treated with a stressor show a reduction of STMN2 and the appearance of the truncated variant.

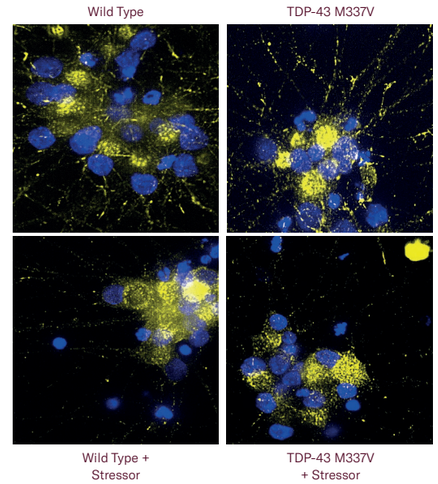

Figure 7: In parallel, MNs were lysed and qPCR performed to quantify RNA levels of STMN2 and truncated STMN2, Upon treatment with the stressor, STMN2 levels significantly decreased while there was a significant increase in truncated STMN2 levels. Data was normalized to the wild type neurons.

Electrophysiological dysfunction in iPSC MNs

- Representative raster plots (180 seconds) of culture conditions in 48-well MEA plate;
- Neuronal firing in complex and structured patterns (detection of bursts and network bursts) was observed for wild type and mutant MNs;
- A network burst event (pink lines) assumes the participation of at least 4 active electrodes.
- MNs with the TDP43 mutation show deficits in activity and network burst frequency.
- Electro-physiological deficits observed at multiple time points during neuronal maturation.

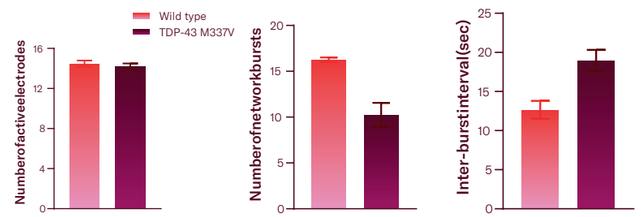

Figure 9

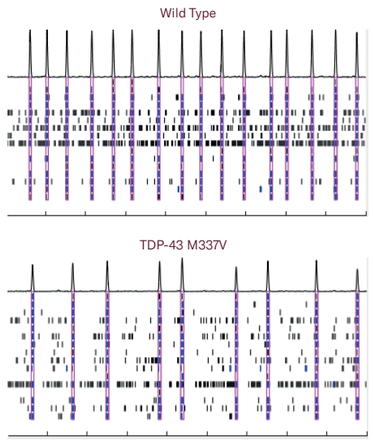

Figure 8

Ncardia's solutions

At Ncardia, we have more than a decade of iPSC experience, building the know-how and capabilities to makethis technology accessible for therapeutic developers to advance their science consistently and efficiently.

We combine a deep iPSC knowledge with broad assay capabilities and a demonstrated ability to integrate thebiology of human diseases into in vitro testing stages, to help our customers make critical decisions earlier and with more confidence.

Modeling and large-scale manufacturing

Our human iPSC-based in vitro models are physiologically relevant and fully functional, customized to your specifications and manufacture to scale.

Assay development and screening

We provide high-quality and reproducible assays amenable to evaluate a wide range of phenotypes and screenany therapeutic modality at high throughput, with highly translational results.

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Wondering if your original application could be a good fit for Ncardia's iPSC platform? Let's find out together!

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Know now, to win then