Modelling neurodegeneration using a human genetically matched system: a next generation approach to study frontotemporal dementia and amyotrophic lateral sclerosis

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Abstract

Development of therapies to treat neurodegenerative diseases is hampered by the limited translatability (<10%) of existing preclinical animal models as well as the lack of reliable and consistent sources of in vitro models. Patient-derived human induced pluripotent stem cells (hiPSCs) enable generation of in vitro models that can recapitulate human disease phenotypes. However, conventional hiPSC differentiation protocols are often lengthy, complex, and difficult to scale. The lack of genetically matched controls for patient-derived models further complicates the investigation of disease phenotypes.

bit.bio has developed opti-ox*, a deterministic hiPSC programming technology that overcomes these limitations and enables generation of cell types and associated genetically matched disease models. Our objective was to generate disease models for frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) for use with genetically matched, wild type controls to improve screening specificity and accelerate drug discovery for these neurodegenerative disorders.

We used CRISPR/Cas9 gene editing to introduce the disease-relevant mutations in SOD1 and FUS in ioMotor Neurons*, in MAPT in ioGlutamatergic Neurons, and in TDP-43 (TARDBP) in both ioGlutamatergic Neurons and ioMotor Neurons.

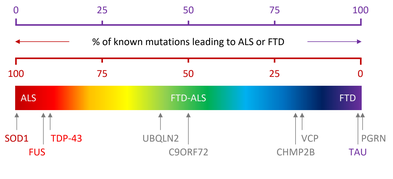
During the pathogenesis of FTD and ALS, mutant TDP-43, FUS, SOD1 and Tau proteins are prone to misfolding, aggregation, phosphorylation and/or mislocalisation, and have been reported to affect a range of neuronal subtypes, including cortical glutamatergic neurons and spinal lower motor neurons.

In this poster we showcase how opti-ox technology has been used to rapidly and deterministically program hiPSCs into motor neurons, termed ioMotor Neurons, which are a homogenous population of defined and functional cells (evaluated by MEA), that express key lower motor neuron marker genes MNX1(HB9), FOXP1, ISL2 and cholinergic markers ChAT and SLC18A3 (VAChT) by day 4.

Additional data demonstrates the utilisation of CRISPR/Cas9 gene editing in another neuronal cell type programmed with opti-ox (ioGlutamatergic Neurons) to infer a disease-related phenotype, demonstrating reduced neuronal activity in TDP-43 M337V/M337V neurons compared to TDP-43 M337V/WT and the genetically matched control.

1. Genetic and pathological overlap between ALS and FTD

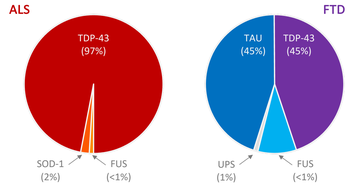
Genetics of ALS and FTD



Major known genetic causes for ALS and FTD are arranged based on their frequency in familial cases.

Image adapted from Ling et al., 2013

Pathological inclusions in ALS and FTD



Neuropathological protein inclusions in FTD and ALS are classified according to the main aggregating protein. TDP-43 and FUS inclusions depict the pathological overlap of these disorders.

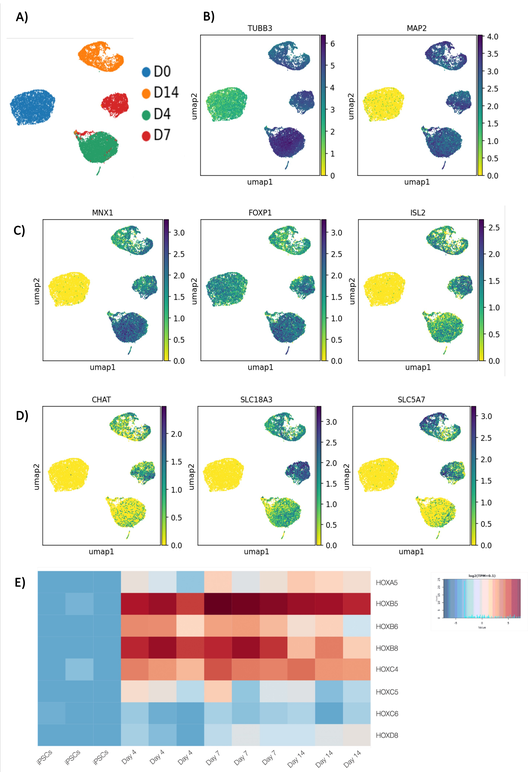
2. Generation of ioDisease Model Cells with ALS and FTD relevant point mutations

CRISPR/Cas9 gene editing was used to engineer genetically ALS and FTD disease-relevant mutations in the parental cell lines of human iPSC-derived ioGlutamatergic Neurons and ioMotor Neurons, two cell types primarily affected by ALS and FTD.

Gene	Mutation	ioCells background	Disease relevance
SOD-1	G93A	ioMotor Neurons	ALS
FUS	P525L	ioMotor Neurons	ALS
TDP-43	M337V	ioMotor Neurons	ALS/FTD
TDP-43	M337V	ioGlutamatergic Neurons	ALS/FTD
MAPT	P301S	ioGlutamatergic Neurons	FTD
MAPT	N279K	ioGlutamatergic Neurons	FTD

3. Single cell RNA-sequencing show ioMotor Neurons are a pure population of neurons with lower motor neuron identity

A) Single cell RNA-sequencing analysis was performed with ioMotor Neurons at four timepoints: day 0 (iPSCs), 4, 7, and 14. By day 14, the population has a distinct expression profile indicating a pure population (>99.9%) of post-mitotic neurons.
B) From day 4, ioMotor neurons show consistent expression of the pan-neuronal markers MAP2 and TUBB3, with >99.9% of the cells co-expressing both genes on day 14.
C) Starting from day 4, the expression of the key spinal motor neuron marker genes MNX1 (HB9), FOXP1, and ISL2 is detected in the culture, with close to 80% of cells expressing MNX1 and 50% of cells expressing ISL2 on day 14. These percentages are likely to be an underestimation due to limitation of single cell RNA-seq as immunocytochemistry for HB9 and ISL1 and ISL2 shows homogeneous expression of these markers in our cultures.
D) Within 7 days, the expression of the key cholinergic marker genes CHAT, SLC18A3 (VAChT), SLC5A7 is also detected in a high proportion of ioMotor Neurons.
E) Expression of HOX genes was evaluated using bulk RNA-seq data. Heatmap shows expression of genes from the B cluster and expression of HOXC4 and HOXC5, although at lower levels. This data, together with the marker expression from single cell RNA-seq, suggests that ioMotor Neurons have a posterior hindbrain or spinal cord (cervical region) identity.



Note, this data is from cells in continuous culture, so minor variations may exist between this data and data from cryopreserved cells.

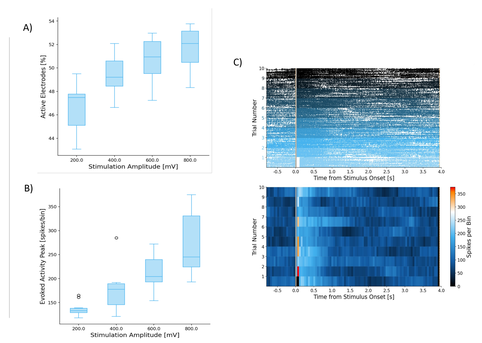
4. ioMotor Neurons respond to electrical stimulation

Electrical stimulation was performed at Day 42 for four different voltages (200mV, 400mV, 600mV, and 800mV) over 10 different trials. ioMotor Neurons were plated with astrocytes at a 2:1 ratio.

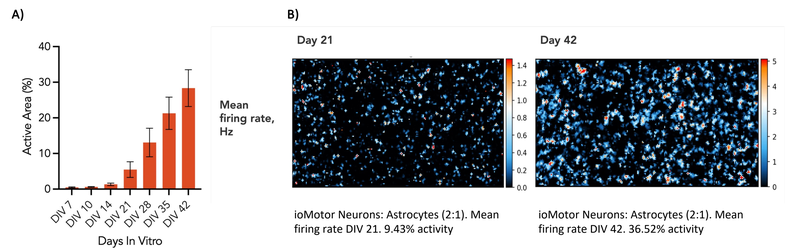
A) ioMotor Neurons become more active as the intensity of the stimulus increases.

B) ioMotor Neurons evoke a higher peak activity as the stimulus increases.

C) Plots showing immediate response to the stimulus before returning to baseline.



5. ioMotor Neurons rapidly gain functional activity

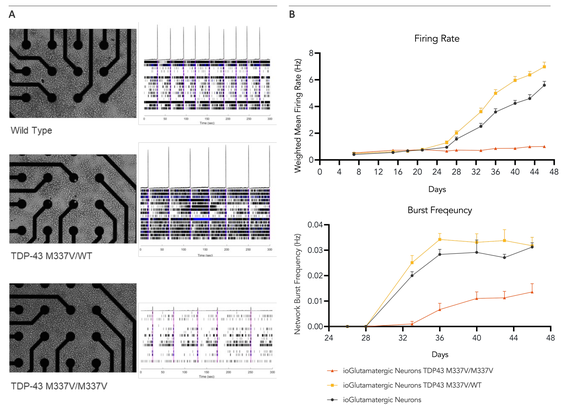


A) ioMotor Neurons were plated with astrocytes at a 2:1 ratio to perform Multi-Electrode Array (MEA) analysis over a period of 42 days. ioMotor Neurons show electrical activity as early as 14 days post-thaw, with the percentage of active area increasing throughout the length of the experiment. DATA NOT SHOWN – additional experiments were carried out at different ratios and culture conditions. When ioMotor Neurons were in monoculture, baseline electrical activity was detected, but significantly lower than ioMotor Neurons: Astrocyte co-cultures, indicating that ioMotor Neurons are of high purity and unable to form synapses without the presence of other cell types.

B) A heatmap showing an increase in mean firing rates from day 21 (9.43%) to day 42 (36.52%) in culture.

6. ALS disease model in ioGlutamatergic Neurons shows reduced neuronal activity

Reduced activity measured in TDP-43 M337V/M337V neurons compared to TDP-43 M337V/WT and isogenic control.
A) 100K ioGlutamatergic Neurons (Wild Type), TDP-43 M337V/WT, and TDP-43 M337V/M337V co-cultured with 20K human iPSC-derived astrocytes spotted to MEA plates. Brightfield at 26 DIV (left). Cells show good coverage of electrodes and produce clear burst and network burst activity as seen in the raster plot of activity (right).
B) Quantification of raster plots over the course of culture shows that TDP-43 M337V/M337V neurons have a lower weighted mean firing rate, and network burst frequency than Wild Type and TDP-43 M337V/WT neurons. No clear difference is noted between Wild Type and TDP-43 M337V/WT neurons.



Error bars indicate SEM, n=14 technical repeats.
Data courtesy of Charles River Laboratories, D. Magnani, M. Iovino.

Summary

- opti-ox technology reliably produces hiPSC-derived motor neurons and glutamatergic neurons
- Genetically matched disease models were engineered to carry ALS- and FTD-relevant mutations
- Our panel of disease-relevant mutations in ioGlutamatergic and ioMotor Neurons will help to understand the similarities and differences between the molecular mechanisms causing ALS and FTD
- Our motor neurons are a homogenous population of defined and functional cells
- MEA analysis revealed that the TDP-43 M337V homozygous mutation affected glutamatergic neuronal activity

July 2024