Development of an *in-vitro* testing platform for *AAV-UPF1* gene therapy to treat ALS

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Abstract

Aim: The purpose of the study is to examine the effect of AAV-based vectors expressing the *UPF1* gene on 2D motor neuron and myotube cultures derived from Amyotrophic Lateral Sclerosis (ALS) patients.

Methods: Motor neurons (MNs) and myotubes were derived from diseased and non-diseased iPSC lines. MNs and myotubes from each cell line were characterized by RT-qPCR, western blot, and immunohistochemistry. The *UPF1* gene was then expressed for each cell line in 2D cultures by transduction with AAV-based vectors employing different expression cassette designs.

Results: We show here that ALS patient-derived neurons show characteristic disease morphology and gene expression changes. Upon addition of the therapeutic vectors, we see a range of levels of rescue. These results will provide a basis for further translational research to develop gene therapy treatments for ALS.

Introduction

Amyotrophic Lateral Sclerosis (ALS)

ALS is a neurodegenerative disease that causes progressive loss of neurons in the motor cortex and corticospinal tracts. The disease causes muscle weakness, leading to the inability to move or breathe, and is usually fatal within 3-5 years of onset. There is no cure. The current treatments, Riluzole and Edaravone, are of limited efficacy and only prolong life by a few months. ALS affects around 4-5 per 100,000 people. While dozens of genes have been associated with ALS, expansion of the *C9orf72* hexanucleotide is a major contributor to both familial and sporadic ALS.

UPF1

UPF1 is an RNA helicase and ATPase that plays a critical role in the nonsense-mediated decay pathway. Mutations in *UPF1* have not been shown to cause ALS; however, work done in yeast, mammalian cell culture and rodent models of ALS driven by TDP-43, FUS and C9orf72, has indicated that overexpression of UPF1 reduces ALS-associated neurotoxicity.

Our original UPF1 promoter vector (“RK”) is too large to be packaged into the AAV capsid, so we are testing smaller constructs that vary in their promoters, 3’UTRs, polyadenylation sequences, and codon optimization.

The goal of our research is to develop a 2D motor neuron and myotube cultures with which we can assess the efficacy and potency of our UPF1 constructs.

Development of 2D cultures to model ALS

To create a 2D culture model for ALS, we started with iPSC cells from three separate patients with *C9orf72*-associated ALS, as well as one non-diseased control. These iPSCs underwent patterning and terminal differentiation to create both motor neurons (left) and myotubes (right). We found wide variability between cell lines in their morphology, expression of cell type-specific genes, and characteristic ALS changes.

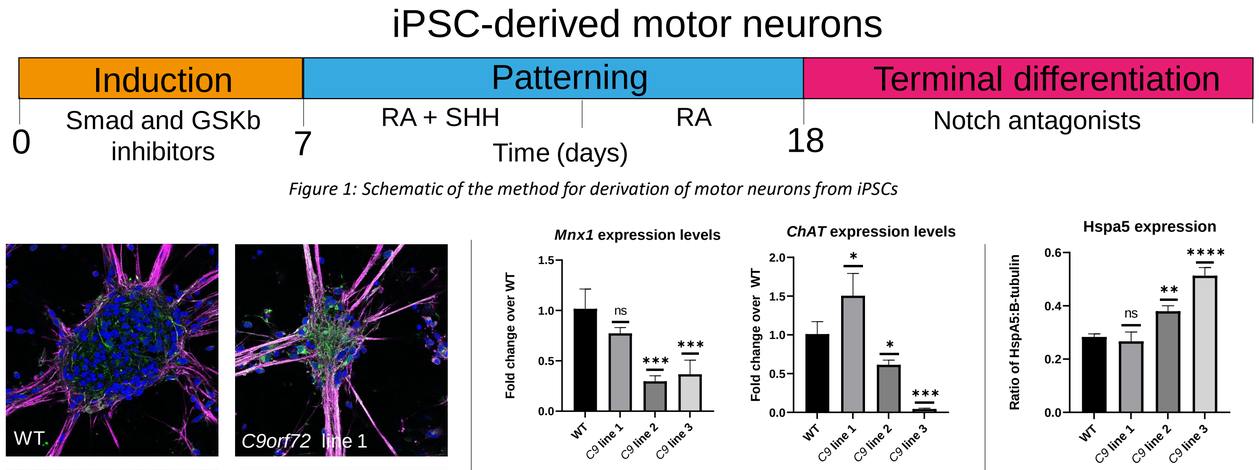

Figure 1: Schematic of the method for derivation of motor neurons from iPSCs

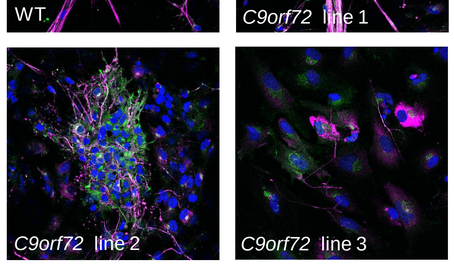

Figure 2: Immunohistochemistry images showing axonal outgrowth in each of the four cell lines. Blue: DAPI. Green: synaptophysin. Magenta: ChAT

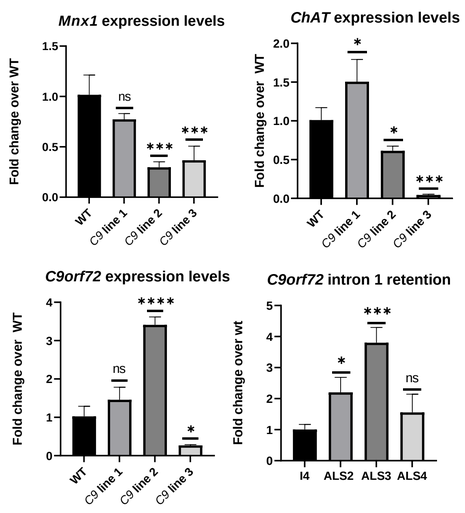

Figure 3: qPCR results showing gene expression changes in each cell line.

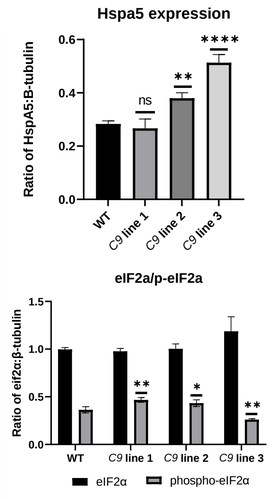

Figure 4: Western results showing changes in HSPA5 and eIF2α expression levels

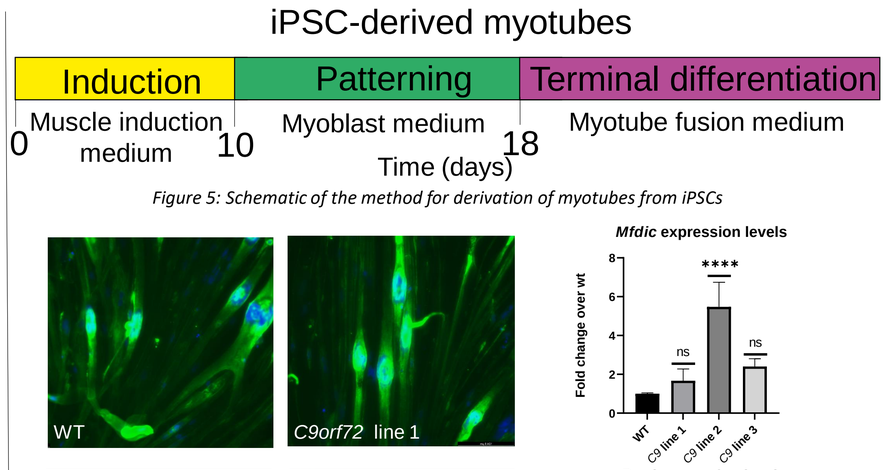

Figure 5: Immunohistochemistry images showing myotube growth. Blue: DAPI. Green: Titin

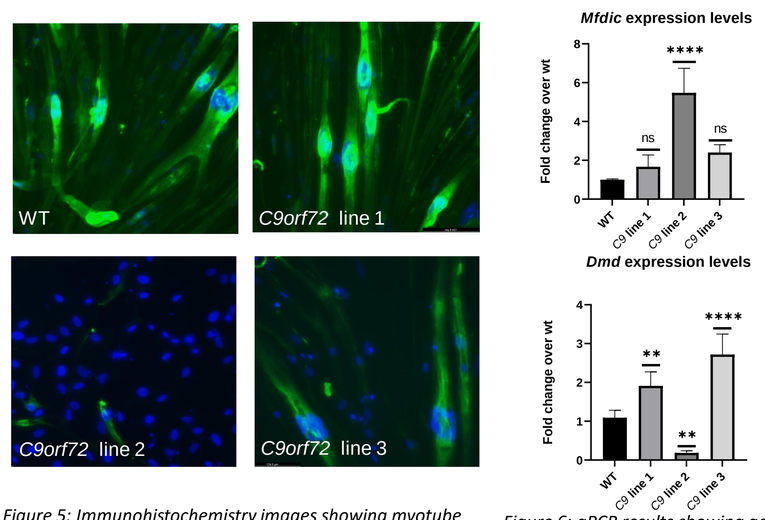

Figure 6: qPCR results showing gene expression changes in each line

Results

Serotype analysis shows AAV2-retro capsid has highest expression levels

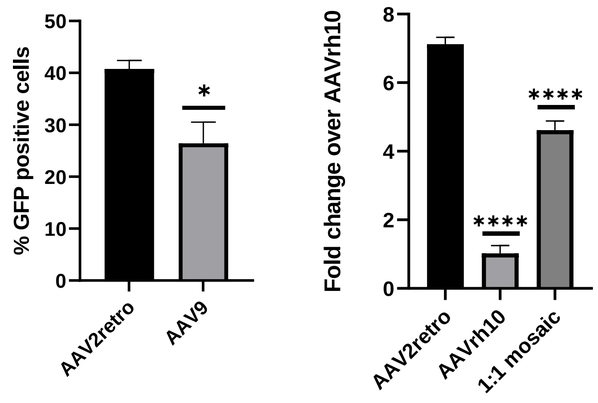

Figure 6: FACS analysis of AAV2-retro versus AAV9 capsids expressing CAG-eGFP in the C9orf72 *cell line 1.*

Figure 7: RT-qPCR analysis of AAV2-retro, AAVrh10, and a mosaic of the two expressing CAG-mCherry in the C9orf72 *cell line 1*

C9orf72 cell line 1 motor neurons were transduced with AAV2-retro containing either the original large ‘RK’ construct or one of five other smaller constructs that vary in their promoter, 3’UTR, polyadenylation sequence, and codon optimization. Seven days after transduction, cells were harvested for analysis.

Construct	Size (bp)
RK	6451
Meira A	5179
Meira B	5179
Construct 11	4906
Construct 6	4800
Construct 11i	4906

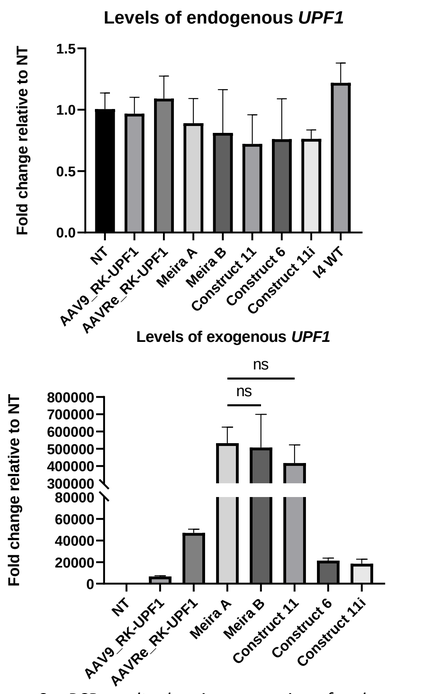

Figure 8: qPCR results showing expression of endogenous (top) or exogenous (bottom) UPF1 *upon transduction with the UPF1 constructs.*

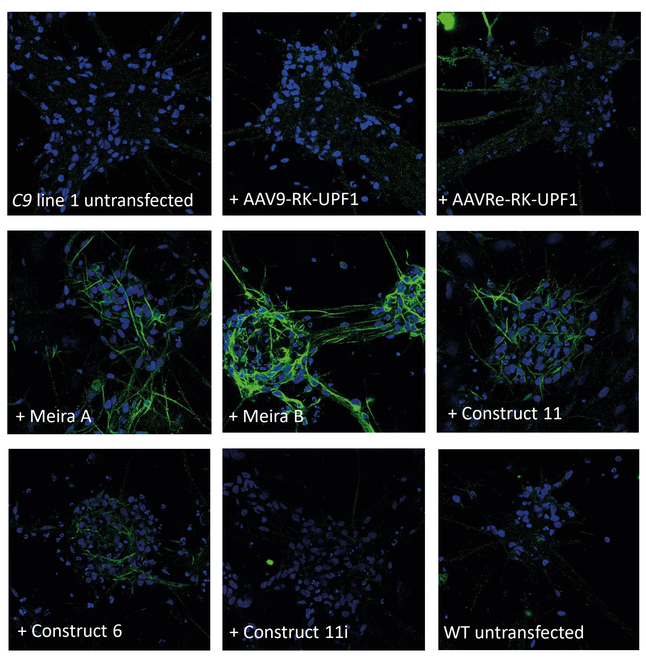

Figure 8: immunohistochemistry results showing expression of UPF1. Blue: DAPI. Green: UPF1

Conclusions

- Patient-derived ALS cell lines show considerable variability in their growth, terminal differentiation, and characteristics
- AAV2-retro shows higher expression levels in our patient derived iPSC motor neuron cultures than either AAV9 or AAVrh10
- qPCR results indicate that construct ‘Meira A’, ‘Meira B’, and ‘Construct 11’ show the highest expression levels in ALS motor neurons, while immunohistochemistry shows that ‘Meira A’ and ‘Meira B’ have the highest levels

Future work

- Construct analysis will be repeated in multiple *C9orf72* cell lines, as well as cell lines with other ALS-causing mutations
- Motor neurons and myotubes will be cultured together in microfluidic devices to examine neuromuscular junctions
- Transduction of motor neurons will be tested for ability to rescue ALS pathology in each cell type

References

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2. Barmada, S. J. *et al.* Amelioration of toxicity in neuronal models of amyotrophic lateral sclerosis by hUPF1. *Proc Natl Acad Sci U S A* **112**, 7821–7826 (2015).
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4. Hall, C. E. *et al.* Progressive Motor Neuron Pathology and the Role of Astrocytes in a Human Stem Cell Model of VCP-Related ALS. *Cell Rep* **19**, 1739–1749 (2017).