Development of *in vitro* ALS discovery and translational assays with patient-derived cells

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INTRODUCTION

The low success rate observed in ALS clinical trials for developing new therapies emphasizes the urgent need to develop additional models that enhance our ability to accurately predict disease progression and to assess human response to a given therapy. With the advent of iPSC technology, a number of new molecules have recently emerged into the clinics, proving that not only do iPSC-derived cells help us to better understand disease mechanisms, but also provide a tool for assessing new compounds in cellular assays. Patient-derived cells can be used to generate disease-relevant cells including motor neurons (MNs) and glial cells, to model the non-cell autonomous mechanism underlying ALS, and to investigate the glial-driven MNs toxicity. Additionally combining these cells in a spheroid model holds tremendous potential for unraveling the mechanisms and progression of neurodegenerative diseases, as their ability to faithfully recreate the three-dimensional neuronal structure and cell-to-cell interactions offers a more physiologically relevant platform compared to traditional 2D models.

OBJECTIVES

Our objective is to use iPSC-derived cells from patients with sporadic ALS or with ALS-associated mutations, to develop disease-relevant assays to screen for small molecules, and to develop disease-relevant models in 2D and 3D to study ALS and its progression.

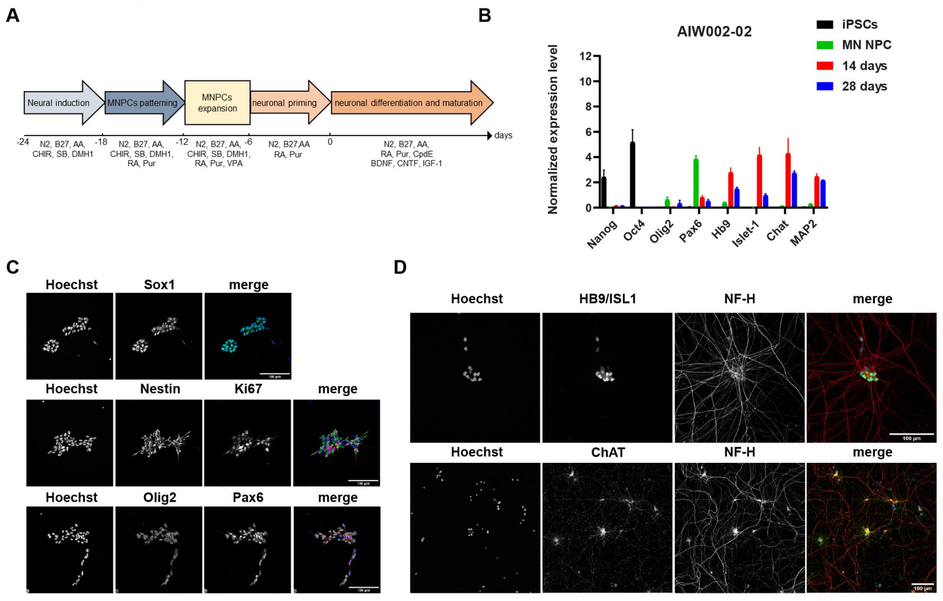

Figure 1: Generation of iPSC-derived MNs. (A) Overview of the process for differentiating iPSCs into MNPCs and subsequently into MNs. **(B)** qPCR depicting normalized expression levels of pluripotency markers NANOG and OCT4, MNPCs markers OLIG2 and Pax6, MNs markers HB9, ISL1 and CHAT, and the neuronal marker MAP2, in iPSCs, MNPCs and D14 and D28 iPSC-derived MNs. **(C)** Representative images of iPSC-derived MNPCs visualized by immunocytochemistry. Cells were stained with the neural progenitor markers Sox1 and Nestin, and the proliferative marker Ki67 as well as with the MNPCs markers OLIG2 and Pax6. Nuclei were counterstained with Hoechst 33342. **(D)** Representative images of iPSC-derived MNs visualized by immunocytochemistry with the combined HB9 and Islet1 MN markers (HB9/ISL1), the choline acetyltransferase protein (ChAT), and the neuronal marker neurofilament heavy chain (NF-H) after 28 days of final differentiation. Nuclei were counterstained with Hoechst 33342. (modified from Deneault et al., 2021).

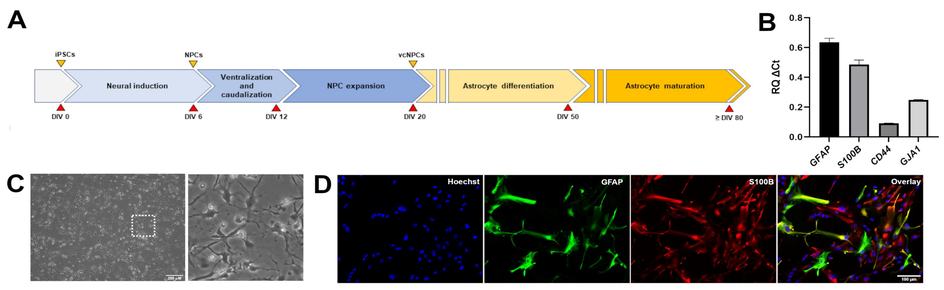

Figure 2: Characterization of human iPSC-derived caudalized astrocytes. (A) Overview of the differentiation protocol. **(B)** Real-time PCR analysis of the expression of GFAP, S100B, CD44, GJA1. **(C)** Phase contrast image of astrocytes derived from ventralized and caudalized NPCs, showing that most cells have stellate-like morphologies with long and thin extensions. (D) Representative double-labeling immunofluorescence analysis of GFAP (green) and S100B (red) expression; Hoechst counterstaining (blue) is shown (modified from Soubannier et al., 2022).

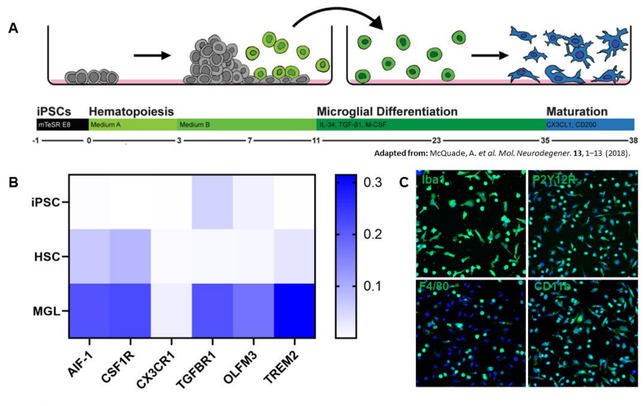

Figure 3: Generation of human iPSC-derived microglia. **(A)** Overview of the differentiation protocol. **(B)** Graphic representation of the expression of the main microglial genes during the three main phases of the protocol (iPSC, hematopoietic stem cells, mature microglia). **(C)** Immunofluorescence analysis of mature iPSC-derived microglia showing the expression of the main microglia marker Iba-1, P2Y1R, F4/80 and CD11b in green. Hoechst counterstaining is shown in blue.

Survival assay : glutamate-induced toxicity

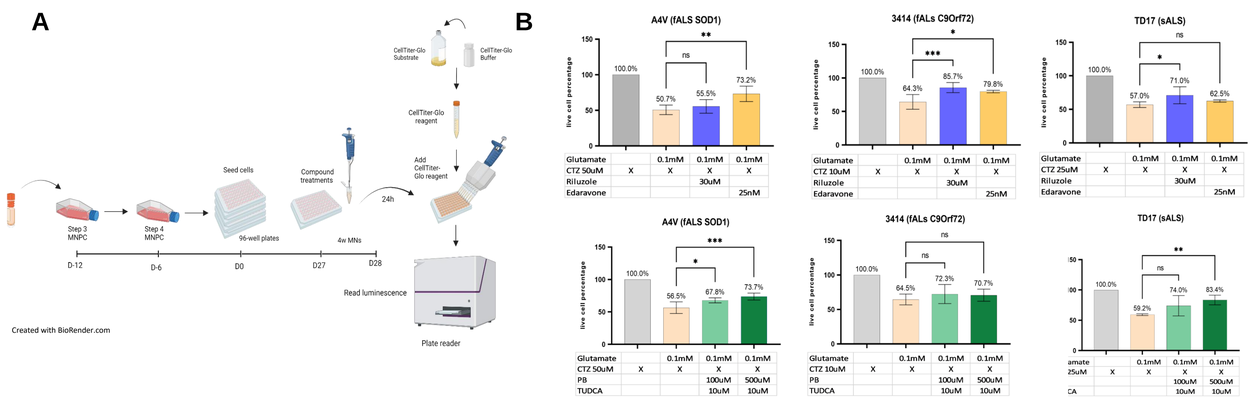

Figure 4: Survival of ALS patient lines after glutamate treatment. (A) Schematic representation of the survival assay using the CellTiter-Glo Luminescent Cell Viability Assay from Promega. **(B)** Survival of fALS SOD1-A4V, fALS C9Orf72 and sALS iPSC-derived MNs after glutamate insult and addition of standard of care treatment for ALS Riluzole 30µM, Edaravone 25nM, or a combination of PB and TUDCA. Bar graphs show mean +/-SEM, n= 6 technical replicates. The statistical significance for each treatment was analysed using one-way ANOVA tests *p<0.05, **p<0.01; ***p<0.001.

Apoptosis and autophagy assays

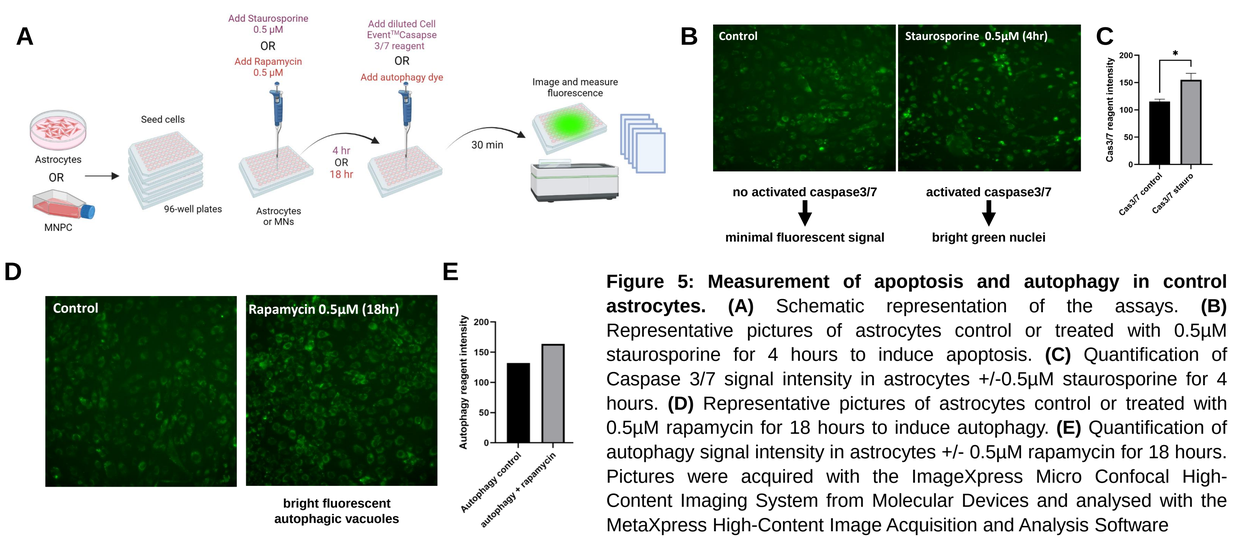

Figure 5: Measurement of apoptosis and autophagy in control astrocytes. (A) Schematic representation of the assays. **(B)** Representative pictures of astrocytes control or treated with 0.5µM staurosporine for 4 hours to induce apoptosis. **(C)** Quantification of Caspase 3/7 signal intensity in astrocytes +/-0.5µM staurosporine for 4 hours. **(D)** Representative pictures of astrocytes control or treated with 0.5µM rapamycin for 18 hours to induce autophagy. **(E)** Quantification of autophagy signal intensity in astrocytes +/- 0.5µM rapamycin for 18 hours. Pictures were acquired with the ImageXpress Micro Confocal High-Content Imaging System from Molecular Devices and analysed with the MetaXpress High-Content Image Acquisition and Analysis Software

ALS 3D model

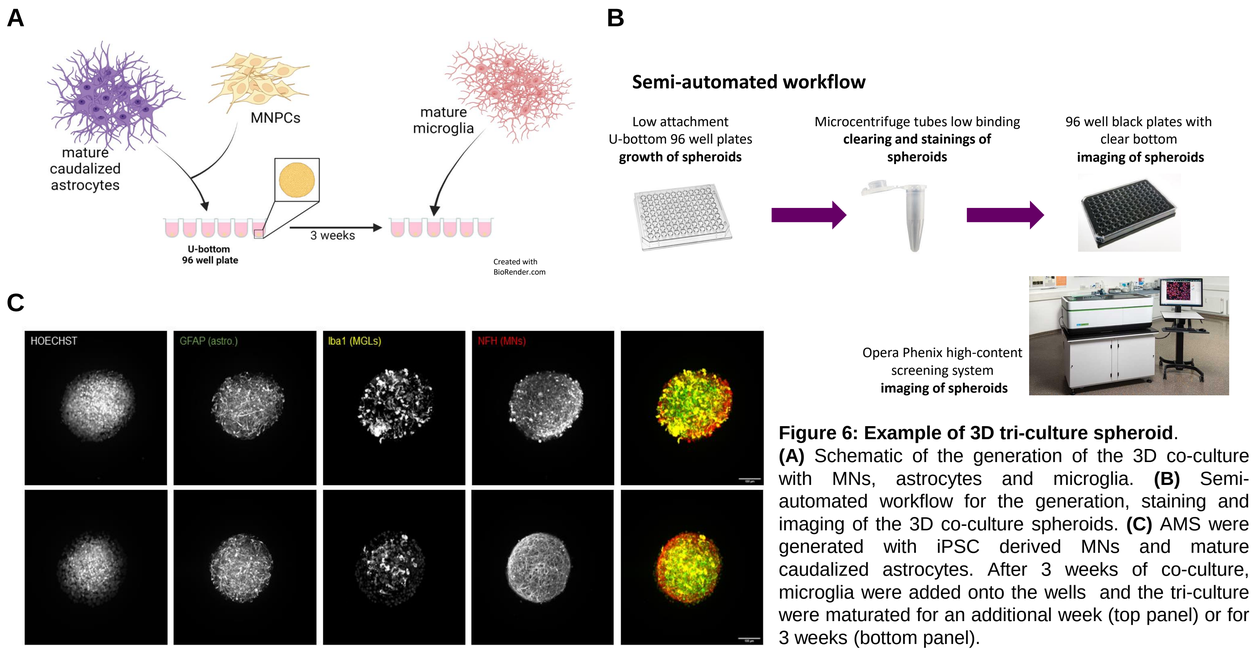

Figure 6: Example of 3D tri-culture spheroid. **(A)** Schematic of the generation of the 3D co-culture with MNs, astrocytes and microglia. **(B)** Semi-automated workflow for the generation, staining and imaging of the 3D co-culture spheroids. **(C)** AMS were generated with iPSC derived MNs and mature caudalized astrocytes. After 3 weeks of co-culture, microglia were added onto the wells and the tri-culture were maturated for an additional week (top panel) or for 3 weeks (bottom panel).

CONCLUSION

Taken together, we developed a panel of ALS-relevant in vitro assays that can be applied towards the screening of small molecules and assessing compound efficacy in addressing specific phenotypes associated with ALS. Additionally, we have successfully developed a 3D co-culture model combining iPSC-derived MNs, astrocytes and microglia providing us a unique opportunity to uncover disease-associated phenotypes in spheroids generated with cells from patients with fALS and sALS.

Acknowledgments

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