Controlled astrogliogenesis enables automated, high-throughput generation of astrocytes from human pluripotent stem cells

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Introduction

Astrocytes play important roles in normal brain development, synaptic function, neurodegenerative diseases, and various pathological conditions (e.g. opioid addiction). Derivation of human astrocytes from a scalable source such as induced pluripotent stem cells (iPSCs) is an attractive approach for disease modeling and drug discovery; however, currently available protocols are variable, inefficient, and lengthy (lasting up to several months). Here, we developed a highly efficient and controlled astrocyte differentiation protocol that overcomes the limitations of previously published methods. By identifying and simultaneously manipulating several critical pathways, we achieved direct astrogliogenesis from iPSCs with over 95% efficiency in less than 30 days, thereby largely bypassing neurogenesis. The differentiation protocol was automated using a robotic cell culture system, which now enables standardized production of large quantities of astrocytes for high-throughput screening and other translational applications.

Differentiation protocol

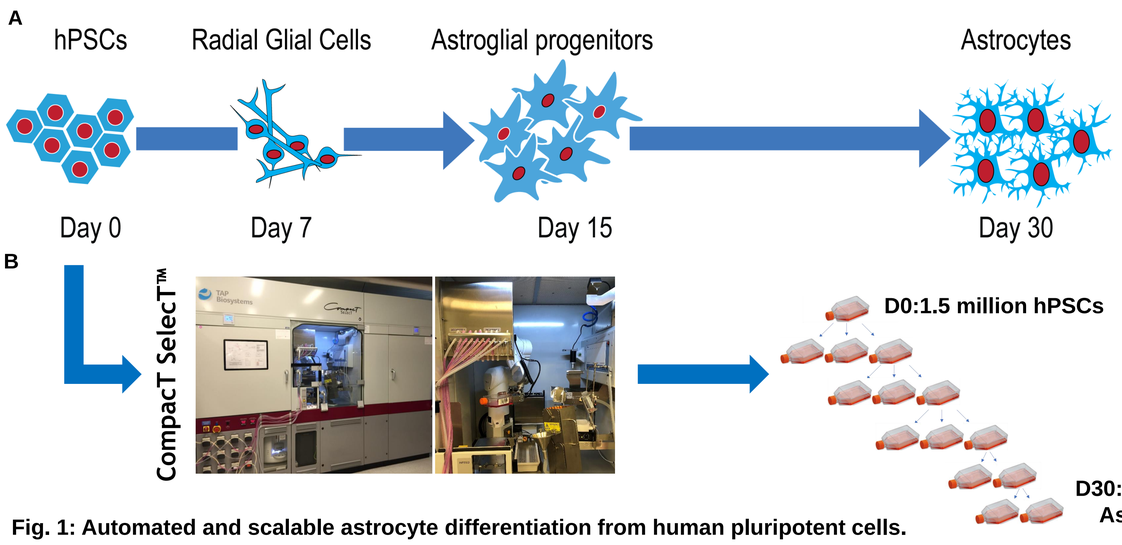


Fig. 1: Automated and scalable astrocyte differentiation from human pluripotent cells.

A. Novel approach for stepwise and controlled differentiation of hPSCs into astrocytes **B.** Differentiation protocol is amenable for automated cell culture and production of large numbers of human astrocytes for cryopreservation and on-demand use.

Results

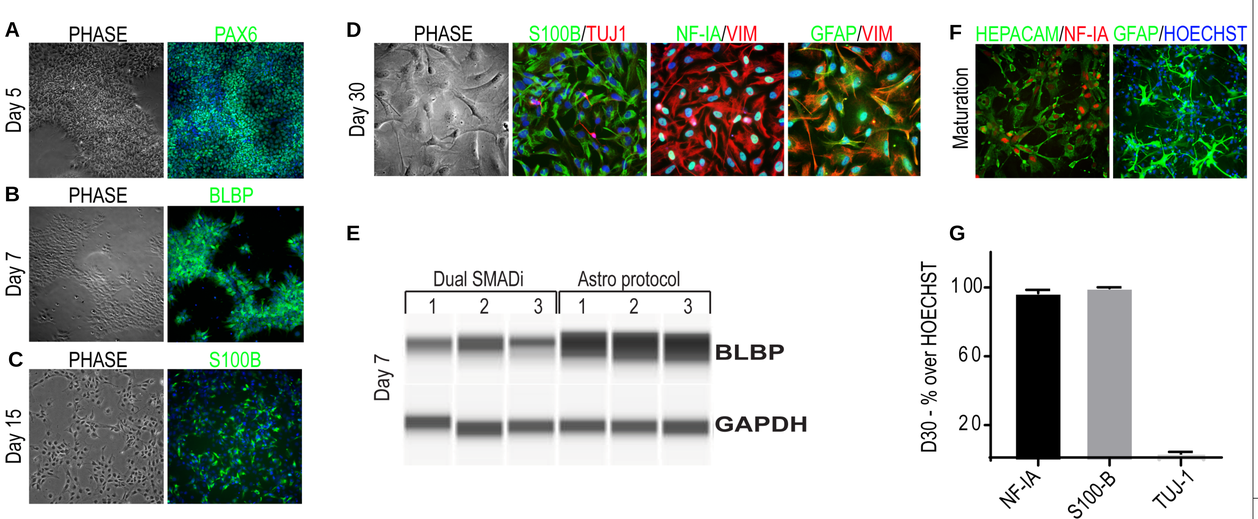


Fig. 2: Time-course of differentiation process and characterization of hPSCs-derived astrocytes

A,B. Phase-contrast images and immunostainings showing the specification of PAX6$^+$ neuroepithelium into BLBP$^+$ radial glia-like cells. **C.** Emergence of S100B$^+$ astroglia progenitors at day 15. **D**. Expression of various astrocyte markers at day 30. Note that only a few TUJ1$^+$ neurons are generated. **E**. At day 7, radial glia marker BLBP is expressed at much higher levels using new protocol versus the dual-SMAD inhibition method (SMADi). **F.** Prolonged culture of day-30 astrocytes for 4 passages results in marker expression indicative of more mature astrocytes. **G.** Quantification of astrocyte (NF-IA, S100-B) and neuronal (TUJ1) markers at day 30 showing the purity of glial cultures. Note the very low percentage of neuronal cells.

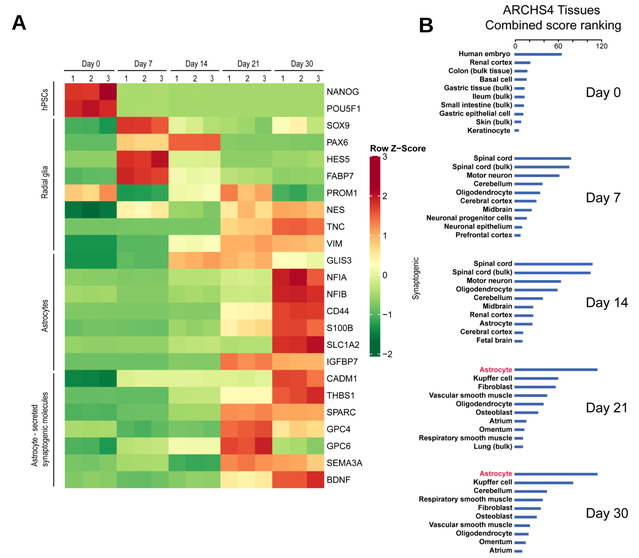


Fig. 3: Gene expression profiling of differentiating astrocytes (RNA-seq).

A. Heat-map illustrating directed differentiation and time-course (day 0-30) of important genes expressed by pluripotent cells, radial glia, and astrocytes. Note also the expression of neurotrophic and synaptogenesis-promoting factors expressed by astrocytes (e.g. BDNF, SEMA3A).

B. EnrichR analysis comparing the transcriptome of differentiating iPSCs at different timepoints (day 0-30). The dataset of differentiating iPSCs was compared to 65,429 human tissue samples in the ARCHS4 database. Note that the gene expression signature of iPSC-derived astrocytes is congruent with their *in vivo* counterpart.

Fig. 4: Single-cell analysis (scRNA-seq) of differentiated astrocytes (Day 30).

A. Heat-map comparing the gene expression signatures of iPSC-derived astrocytes to various other cell types or differentiation stages (pluripotency, neuroectoderm, neuronal, oligodendrocyte, microglia, and endothelial cells). Note the strongly expressed astroglia-specific genes further validating the efficiency of the cell differentiation protocol.

B. tSNE plot shows the heterogeneity of iPSC-derived astrocytes (day 30) at the single-cell level consistent with published literature.

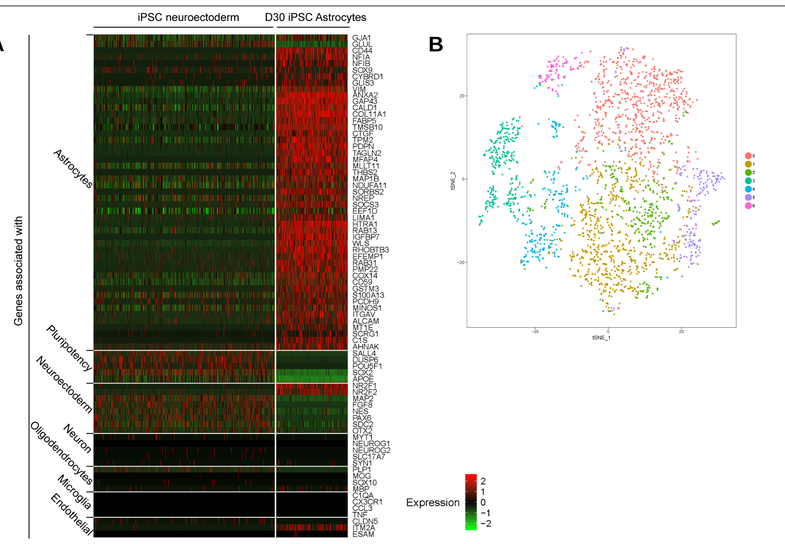
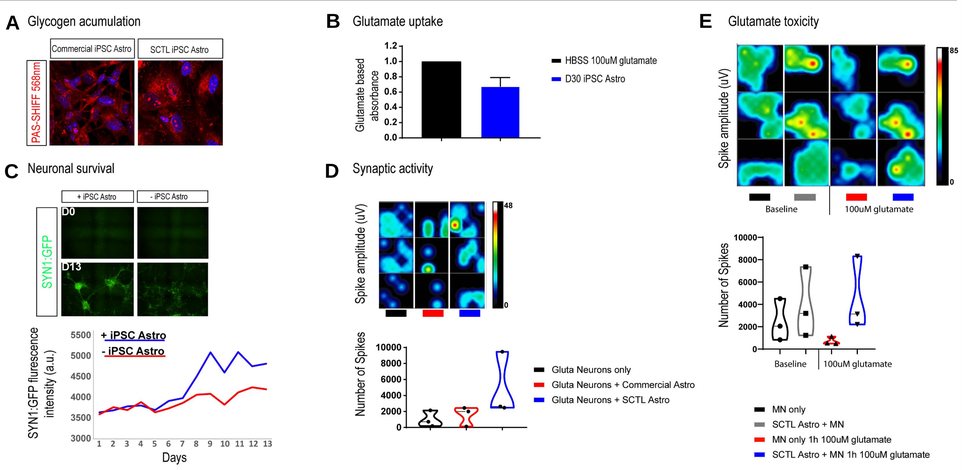


Fig. 5: Functional analysis of iPSC-derived astrocytes.

A. PAS-Schiff staining to visualize and compare glycogen accumulation in astrocytes from different sources.
B. Reduction in glutamate based absorbance after 3h incubation with astrocytes.
C. Neurons derived from hESC SYN1:GFP show stronger GFP signal when co-cultured with astrocytes.
D. Glutamatergic neurons show increased number of spikes when co-cultured with astrocytes for 72 h.
E. Motor neurons co-cultured with astrocytes maintain synaptic activity after exposure to 100 μM glutamate for 1 h.



Summary

1. Novel approach for stepwise and controlled astrogliogenesis. Conversion of hPSC into radial glial and further differentiation into astrocytes is possible without genetic manipulation. Remarkably, this process bypasses neurogenesis and produces functional astrocytes at high purity at day 30 (<95%).
2. Human PSCs-derived astrocytes show typical morphologies, express astrocyte-specific and neurotrophic genes, promote neuronal survival, increase synaptic activity, and display cytoprotective effects when co-cultured with neurons.
3. The protocol was adapted to an automatic cell culture system to standardize and scale-up the production of astrocytes.

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