RAPID GENERATION OF MATURE CORTICAL AND SPINAL ASTROCYTES FROM HUMAN iPSCs

Michael Hendrickson <mhendrickson@brainxell.com>, Ben Dungar, Zhong-Wei Du. BrainXell, Inc., Madison, WI, USA

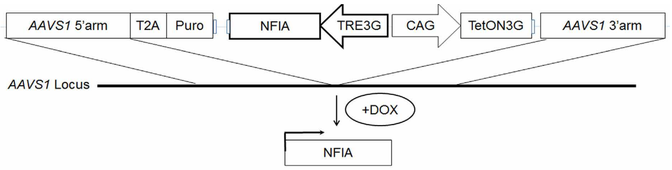
Introduction

Growing evidence implicates glia, particularly astrocytes, in neurological and psychiatric diseases. Astrocytes perform a variety of essential functions including glutamate regulation, axonal guidance, trophic support, inflammatory response, wound healing, formation of the blood-brain barrier, and neuronal synapse formation. Human cortical astrocytes are larger, structurally more complex and diverse, and respond differently to extracellular glutamate compared to their rodent counterparts. Given the unique biology of human astrocytes, it is critical that improved human-specific cell-based systems be established to enable the study of human astrocytes in health and disease. Because of the limited availability of primary human astrocytes, human iPSCs are currently used as a source of astrocytes. However, existing methods for astrocyte generation are slow (up to 6 months) or require additional selection to reduce heterogeneity. To rapidly generate mature astrocytes for disease modeling, we have developed a novel protocol that uses inducible expression of astrocyte differentiation master transcription factors NFIA or SOX9 and an optimized astrocyte differentiation medium. Human cortical or spinal astrocytes can be generated from normal or disease iPSCs in only two months. They express key astrocyte markers GFAP and S100β at >90% and exhibit mature process-bearing morphologies. These astrocytes can promote neuron synapse formation and functional activity in electrophysiology and calcium imaging applications, This protocol represents an important tool for modeling neurological diseases using a human iPSC-based astrocyte-neuron co-culture platform, allowing the role of diseased astrocytes in neuronal degeneration to be investigated.

Materials and Methods

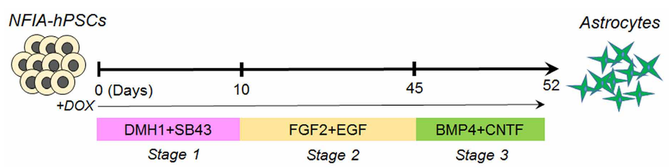
Establishment of inducible TRE3G-NFIA or SOX9 iPSC Line

The human iPSC line WC-30 (normal wildtype) was used in this study. We applied TALEN technology to integrate the TRE3G-NFIA or TRE3G-SOX9 into the AAVS1 safe harbor site.



Rapid Generation of mature astrocytes

TRE3G-NFIA or TRE3G-SOX9 iPSCs were first induced to neuroepithelia by the EB method or monolayer method in the presence of DMH1 (2μM) plus SB431542 (2μM) for 10 days. The cells were treated with DOX (1μg/ml) to induce astrocyte differentiation. The astrocyte progenitors were expanded with FGF2 (20ng/ml) and EGF (20ng/ml) for about 30 days. After 40 days of induction, the cells were dissociated with accutase (Chemicon) and attached to Matrigel coated plates at the density of 5,000-10,000/cm2 in the presence of BMP4 (10ng/ml) and CNTF (10ng/ml) for another 7 days for maturation.



Mature Cortical and Spinal Astrocytes from Human iPSCs

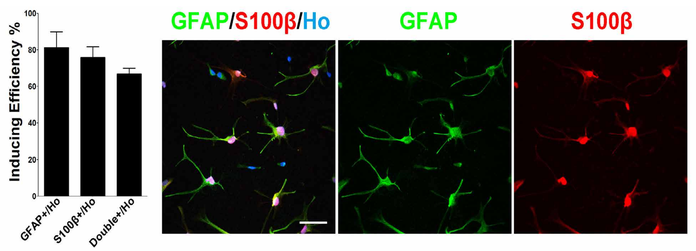


Figure 1. Rapid generation of mature astrocytes. Representative images and quantification of induced GFAP+S100β+ astrocytes after 52 days of differentiation. >90% cells were GFAP+ or S100β+ astrocytes. Scale bar: 100 μm.

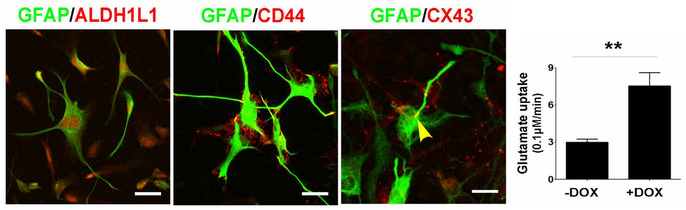
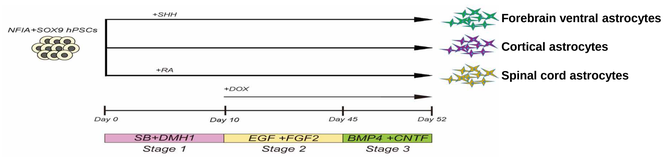


Figure 2. Astrocytes express typical markers and uptake glutamate. Representative images of induced GFAP+ astrocytes co-express ALDH1L1, CD44, and CX43. Scale bar: 50 μm. Kinetics of cellular uptake of glutamate from medium. The "+/-Dox" indicates the cells induced with/without Dox. The data are presented as the mean +/− SEM. **P<0.01(Student's t-test).



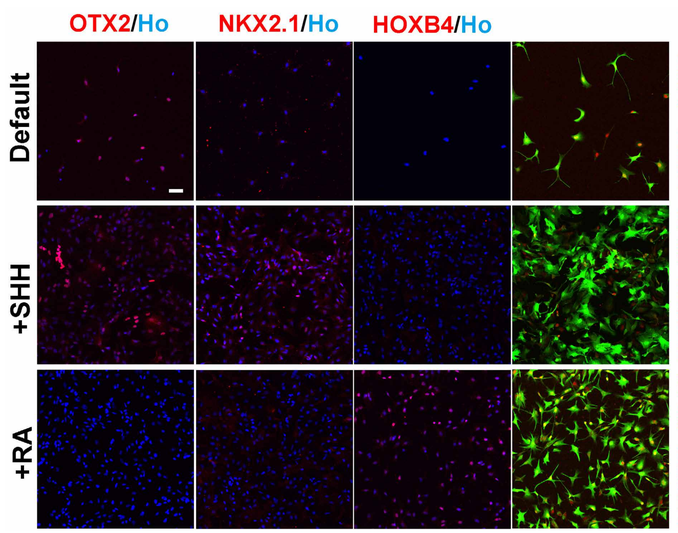


Figure 3. Generation of regional subtype astrocytes with the new method. Astrocytes co-expressing regional-specific markers: OTX2 (forebrain), NKX2.1 (ventral), and HOXB4 (spinal). Scale bar: 100 μm.

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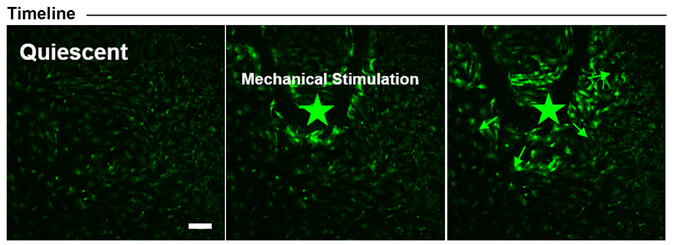

Figure 4. Astrocytes propagate calcium wave. Images of propagating distances for the calcium wave after mechanical stimulation. Asterisk indicates the site of stimulation. Scale bar: 50 μm.

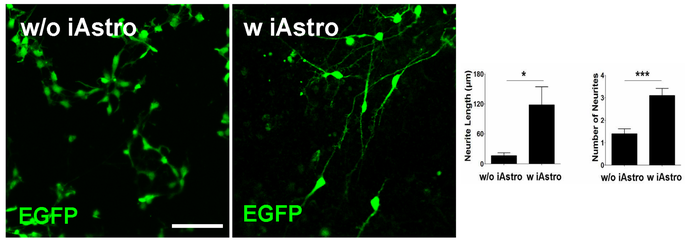

Figure 5. Astrocytes promote neurite outgrowth. Images of neurite outgrowth for GFP neurons co-cultured with or without induced astrocytes (iAstro). Scale bar: 100 μm. The data are presented as the mean +/− SEM. *P<0.05; ***P<0.001 (Student's t-test).

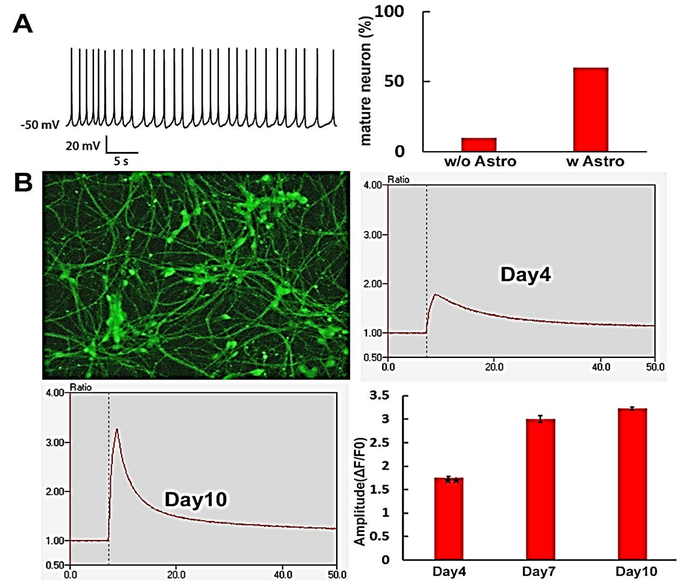

Figure 6. Astrocytes promote mature neuron activity. (A) >60% motor neurons showed spontaneous action potentials when co-cultured with astrocytes for 14 days, but <10% without astrocytes. (B) Motor neurons showed maximal calcium change when co-cultured with astrocytes for 10 days.

Conclusions

- We developed a rapid method to generate pure mature astrocytes from human iPSCs in 2 months.
- Induced astrocytes exhibit similar characteristic cellular and functional properties as primary astrocytes.
- This method can be applied to generate regional-specific astrocytes from patient iPSCs in the same time frame.