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Large-Scale Production of Mature Neurons from Human Pluripotent Stem Cells in a Three-Dimensional Suspension Culture System

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SUMMARY

Human pluripotent stem cells (hPSCs) offer a renewable source of cells that can be expanded indefinitely and differentiated into virtually any type of cell in the human body, including neurons. This opens up unprecedented possibilities to study neuronal cell and developmental biology and cellular pathology of the nervous system, provides a platform for the screening of chemical libraries that affect these processes, and offers a potential source of transplantable cells for regenerative approaches to neurological disease. However, defining protocols that permit a large number and high yield of neurons has proved difficult. We present differentiation protocols for the generation of distinct subtypes of neurons in a highly reproducible manner, with minimal experiment-to-experiment variation. These neurons form synapses with neighboring cells, exhibit spontaneous electrical activity, and respond appropriately to depolarization. hPSC-derived neurons exhibit a high degree of maturation and survive in culture for up to 4–5 months, even without astrocyte feeder layers.

INTRODUCTION

With the seminal discovery of human pluripotent stem cells (hPSCs) (Thomson et al., 1998; Takahashi et al., 2007), human cells that would be difficult or impossible to obtain can be produced using in vitro cell-culture techniques. This in turn has raised hopes that hPSCs can be used to study and treat different forms of disease, including neurological and neuropsychiatric disorders (Dolmetsch and Geschwind, 2011; Fox et al., 2014; Han et al., 2011; Imaizumi and Okano, 2014; Kanning et al., 2010; Liu and Zhang, 2010; Mariani et al., 2015). However, a key step in the utilization of hPSCs for these purposes is the ability to obtain cell types of interest. This has often proved to be challenging for several reasons including neural diversity, culture-to-culture and line-to-line variability, and limitations on large-scale cell production.

Several methods have been described to obtain neurons of specific subtypes through differentiation of hPSCs, either via formation of three-dimensional (3D) embryoid bodies (EBs) or using monolayers as starting material (Amoroso et al., 2013; Boissart et al., 2013; Boulting et al., 2011; Eiraku and Sasai, 2012; Eiraku et al., 2008; Espuny-Camacho et al., 2013; Hu and Zhang, 2009; Kim et al., 2014; Li et al., 2009; Qu et al., 2014; Shi et al., 2012; Zeng et al., 2010). An alternative approach is transcriptional programming, whereby the forced overexpression of a cocktail of transcription factors instructs PSCs, fibroblasts, or other cell populations to adopt a specific neuronal fate (Hester et al., 2011; Vierbuchen et al., 2010). These methods have provided important insights into human neurogenesis and the pathogenesis of neurodevelopmental disorders, but they have limitations. For instance, EB-based protocols generally have comparatively low efficiencies (10%–40%) and require a relatively long time in culture to generate functional motor neurons. In addition, the neurons generated often require cellular feeder layers to survive for longer times in culture (Hu and Zhang, 2009; Boulting et al., 2011; Amoroso et al., 2013). Moreover, EB methods typically result in the formation of spheres of cells varying in size and shape, leading to differences in the kinetics and efficiency of differentiation within individual plates and from experiment to experiment. Monolayer-based protocols for the generation of both cortical and motor neurons have also been published, with recent work describing improved efficiencies (Qu et al., 2014). However, a disadvantage of this adherent monolayer-based protocol is that the neurons need to be passaged, and successful long-term culture after replating has not been described. Another common theme in the field has been the problem of obtaining mature cells from hPSCs. It has been shown that maintaining differentiated cells in culture can be challenging, thereby precluding experiments studying aspects of cellular functions that take longer times to manifest (Bellin et al., 2012; Grskovic et al., 2011).

Recently, a 3D culture system that yields brain tissue from hPSCs in the form of neural organoids has been described (Bershteyn and Kriegstein, 2013; Lancaster

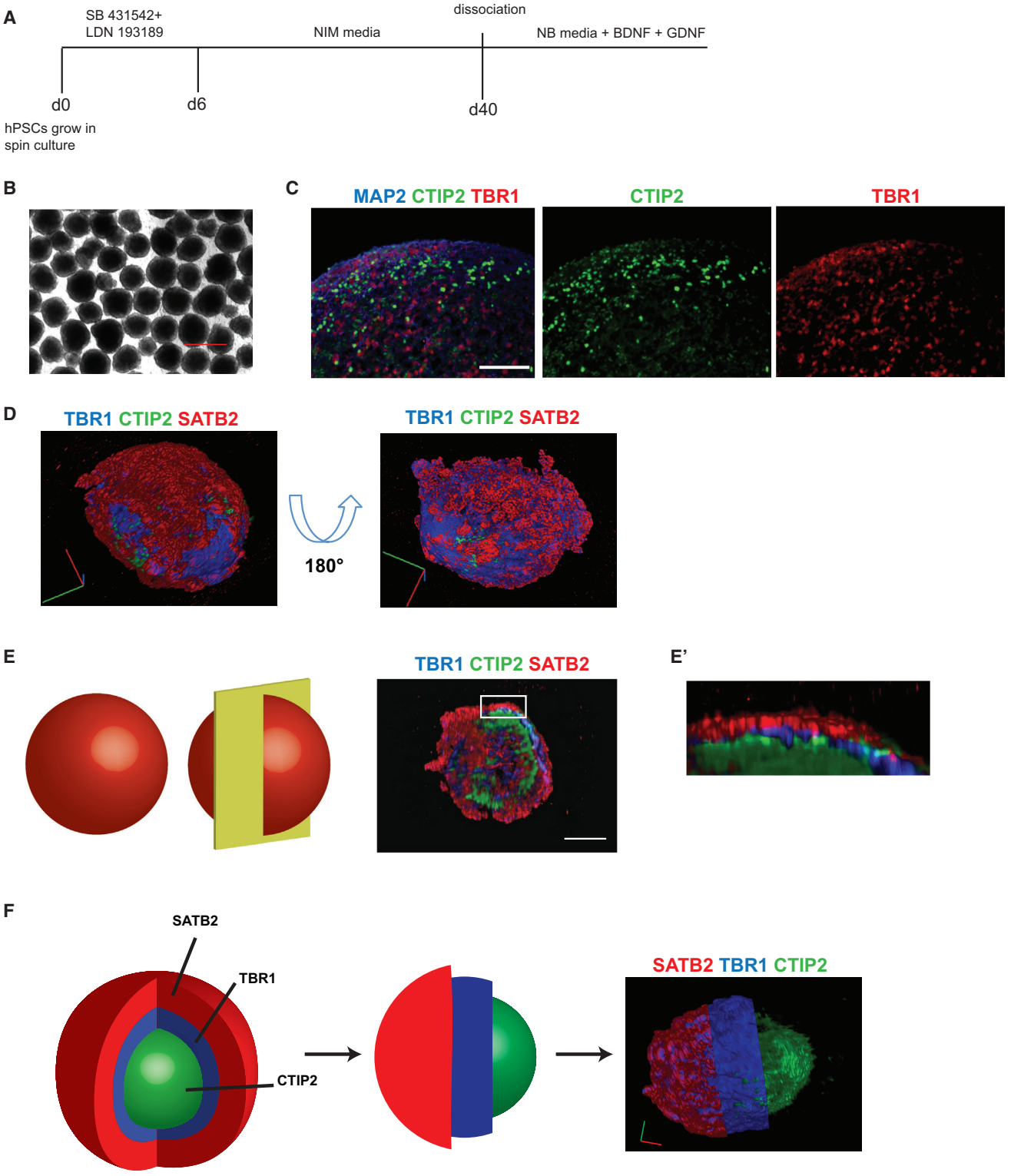

Figure 1. 3D Neuronal Spheres from hPSC Lines Adopt a Predominantly Cortical Identity by Default

(A) Schematic illustration of the differentiation strategy used to obtain cortical neurons from 3D spheres. d, day; BDNF, brain-derived neurotrophic factor; GDNF, glial cell-derived neurotrophic factor.

(B) Representative bright-field image of HUES8-derived cortical spheres at day 15 of differentiation.

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