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Development of Gonadotropin-Releasing Hormone-Secreting Neurons from Human Pluripotent Stem Cells

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SUMMARY

Gonadotropin-releasing hormone (GnRH) neurons regulate human puberty and reproduction. Modeling their development and function in vitro would be of interest for both basic research and clinical translation. Here, we report a three-step protocol to differentiate human pluripotent stem cells (hPSCs) into GnRH-secreting neurons. Firstly, hPSCs were differentiated to *FOXG1*, *EMX2*, and *PAX6* expressing anterior neural progenitor cells (NPCs) by dual SMAD inhibition. Secondly, NPCs were treated for 10 days with FGF8, which is a key ligand implicated in GnRH neuron ontogeny, and finally, the cells were matured with Notch inhibitor to bipolar TUJ1-positive neurons that robustly expressed *GNRH1* and secreted GnRH decapeptide into the culture medium. The protocol was reproducible both in human embryonic stem cells and induced pluripotent stem cells, and thus provides a translational tool for investigating the mechanisms of human puberty and its disorders.

INTRODUCTION

The onset of puberty is regulated by a small population of hypothalamic gonadotropin-releasing hormone (GnRH) neurons, which secrete GnRH decapeptide to the hypophyseal portal system. Unlike other neuroendocrine cell types that reside in the hypothalamus, GnRH neurons are born outside the CNS in the frontonasal area. The olfactory pit, which is formed by the olfactory placodes (OP) and surrounding mesenchyme, provides a niche for GnRH neuron specification. Prenatally, GnRH neurons migrate from the frontonasal mesenchyme along with olfactory axons to the forebrain and into the hypothalamus, where their final maturation occurs (Wray, 2010). The early events that lead to GnRH neuron specification and the origin of their progenitors in the olfactory pit are currently poorly understood. Many existing data support OP as the source of GnRH neurons (Kim et al., 1999; Schwanzel-Fukuda and Pfaff, 1989; Wray et al., 1989a, 1989b), whereas others have proposed that at least some GnRH neurons arise from multiple sources, such as the neural crest, and adenohypophyseal or CNS progenitor cells (Forni et al., 2011; Markakis et al., 2004; Salvi et al., 2009; Whitlock et al., 2003).

GnRH neuron specification occurs under explicit spatiotemporal conditions, involving fibroblast growth factor 8 (FGF8) signaling and bone morphogenetic protein/transforming growth factor β (BMP/TGF-β) pathway inhibition (Forni et al., 2013; Kawauchi et al., 2005; Rawson et al., 2010). The indispensable role of FGF8 signaling through fibroblast growth factor receptor 1 (FGFR1) in this process has been established in various animal models (Chung and Tsai, 2010; Sabado et al., 2012). These findings are mirrored by reports in humans: mutations in *FGF8* and *FGFR1* are found in patients with congenital hypogonadotropic hypogonadism, a rare genetic disease that causes GnRH deficiency (Dode et al., 2003; Falardeau et al., 2008).

Human pluripotent stem cells (hPSCs), including embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs), allow in vitro differentiation of specialized cell types, including neurons (Chambers et al., 2009; Davis et al., 2012; Hay et al., 2008). Here we report a protocol for the generation of GnRH-expressing neurons from hPSCs.

RESULTS

A schematic of the protocol is presented in Figure 1A. In the first step, we employed dual SMAD inhibition on hPSCs by blocking BMP and TGF-β/activin signaling pathways with dorsomorphin (DM) and SB431542 (SB), respectively, for 10 days to produce neural progenitor cells (NPCs) (Chambers et al., 2009). This was followed by 10-day treatment with FGF8, a key ligand in GnRH neuron development, and 4–8 days of treatment with both FGF8 and

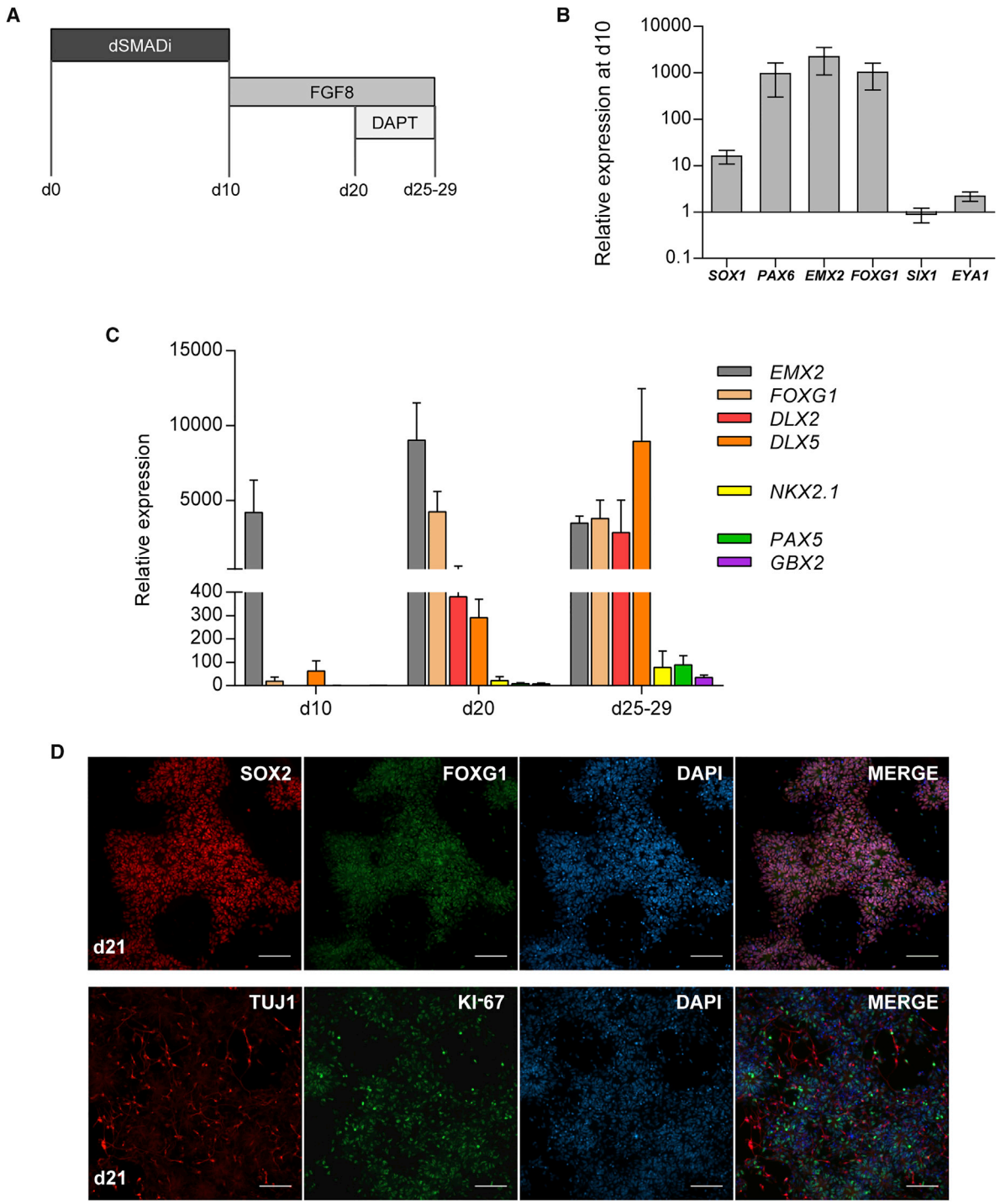

Figure 1. Differentiation Protocol Schematic, and Expression of Anterior Neural Progenitor Markers after Neural Induction

(A) Schematic representation of the protocol. For the first 10 days the cells were treated with dual SMAD inhibition (dSMADi) using dorsomorphin and SB431542. FGF8 was added at d11, and Notch inhibitor DAPT was added at d21.

(B) Real-time qPCR results at d10 showing an increased expression of pan-neural marker *SOX1* and forebrain- and olfactory placode-associated genes *EMX2* and *FOXG1*. Preplacodal markers *SIX1* and *EYA1* remained low. Expression levels are relative to d0 hPSCs (HEL11.4 and H9 representative experiments, n = 6, mean ± SEM).

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