Stem Cell Reports
Article

ISSCR

OPEN ACCESS

Derivation of Diverse Hormone-Releasing Pituitary Cells from Human Pluripotent Stem Cells

Bastian Zimmer,[1,2] Jinghua Piao,[1,3] Kiran Ramnarine,[1,4] Mark J. Tomishima,[1,4] Viviane Tabar,[1,3] and Lorenz Studer[1,2,3,*]

[1]The Center for Stem Cell Biology
[2]Developmental Biology Program
Sloan Kettering Institute for Cancer Research, 1275 York Avenue, New York, NY 10065, USA
[3]Department of Neurosurgery, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, USA
[4]SKI Stem Cell Research Facility, 1275 York Avenue, New York, NY 10065, USA
*Correspondence: studerl@mskcc.org
http://dx.doi.org/10.1016/j.stemcr.2016.05.005

SUMMARY

Human pluripotent stem cells (hPSCs) provide an unlimited cell source for regenerative medicine. Hormone-producing cells are particularly suitable for cell therapy, and hypopituitarism, a defect in pituitary gland function, represents a promising therapeutic target. Previous studies have derived pituitary lineages from mouse and human ESCs using 3D organoid cultures that mimic the complex events underlying pituitary gland development in vivo. Instead of relying on unknown cellular signals, we present a simple and efficient strategy to derive human pituitary lineages from hPSCs using monolayer culture conditions suitable for cell manufacturing. We demonstrate that purified placode cells can be directed into pituitary fates using defined signals. hPSC-derived pituitary cells show basal and stimulus-induced hormone release in vitro and engraftment and hormone release in vivo after transplantation into a murine model of hypopituitarism. This work lays the foundation for future cell therapy applications in patients with hypopituitarism.

INTRODUCTION

Human pluripotent stem cells (hPSCs) provide a unique resource for basic as well as translational research. Both human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) are widely used to study early human development (Zhu and Huangfu, 2013), assess the toxic effects of chemicals (Dreser et al., 2015; Zimmer et al., 2012), model human diseases or cancer (Bellin et al., 2012; Funato et al., 2014; Merkle and Eggan, 2013), and discover novel potential drugs (Lee et al., 2012). Furthermore, access to greatly improved protocols for lineage-specific differentiation has led to the first experimental applications of hPSC-derived lineages in regenerative medicine such as in patients with macular degeneration (Schwartz et al., 2015). Other hPSC-based applications that are being pursued intensely include the replacement of hormone-producing cells such as in type 1 diabetes (Pagliuca et al., 2014; Rezania et al., 2014). Replacing hormone-producing cells is a particularly attractive approach for cell therapy, especially if restoration of feedback mechanisms with subsequent dynamic release of hormones can be achieved by the grafted cells.

The pituitary gland is considered the master gland of hormone function. Hypopituitarism is a disease condition with insufficient or absent function of the pituitary gland. Pituitary tumors are the most common cause but many other triggers can induce pituitary dysfunction including inborn genetic defects, brain trauma, immune and infectious diseases, or radiation therapy. The prevalence of hypopituitarism has been estimated at 46 per 100,000 (Regal et al., 2001), but this is likely an underestimation. The consequences of pituitary dysfunction are particularly serious in children where they can lead to severe learning disabilities, growth and skeletal problems, as well as effects on puberty and sexual function (Chemaitilly and Sklar, 2010). Chronic hypopituitarism requires lifelong complex hormone replacement therapies that are very costly and compromise quality of life. Furthermore, static delivery of hormones can only poorly mimic the dynamic secretion of the intact pituitary gland, which reacts to feedback mechanisms such as the hypothalamic-pituitary-adrenal (HPA) axis or the circadian clock. Therefore, there is a considerable clinical need to direct current treatment paradigms toward a more physiological and complete hormone replacement therapy (Smith, 2004).

It is conceivable that replacing the damaged cells via cell transplantation can restore pituitary function and permanently cure chronic hypopituitarism. Previous work in mouse ESCs has shown that anterior pituitary cells, capable of hormone secretion, can be generated in 3D cultures by recapitulating some of the complex morphogenetic interaction between the developing hypothalamic and oral ectoderm tissues in vitro (Suga et al., 2011). Our laboratory has recently reported a first attempt at generating functional adenohypophyseal cells from human PSCs (Dincer et al., 2013), and very recently pituitary cells have been generated from hPSCs using a 3D organoid approach (Ozone et al., 2016). While these studies represent a promising proof of concept, current protocols remain

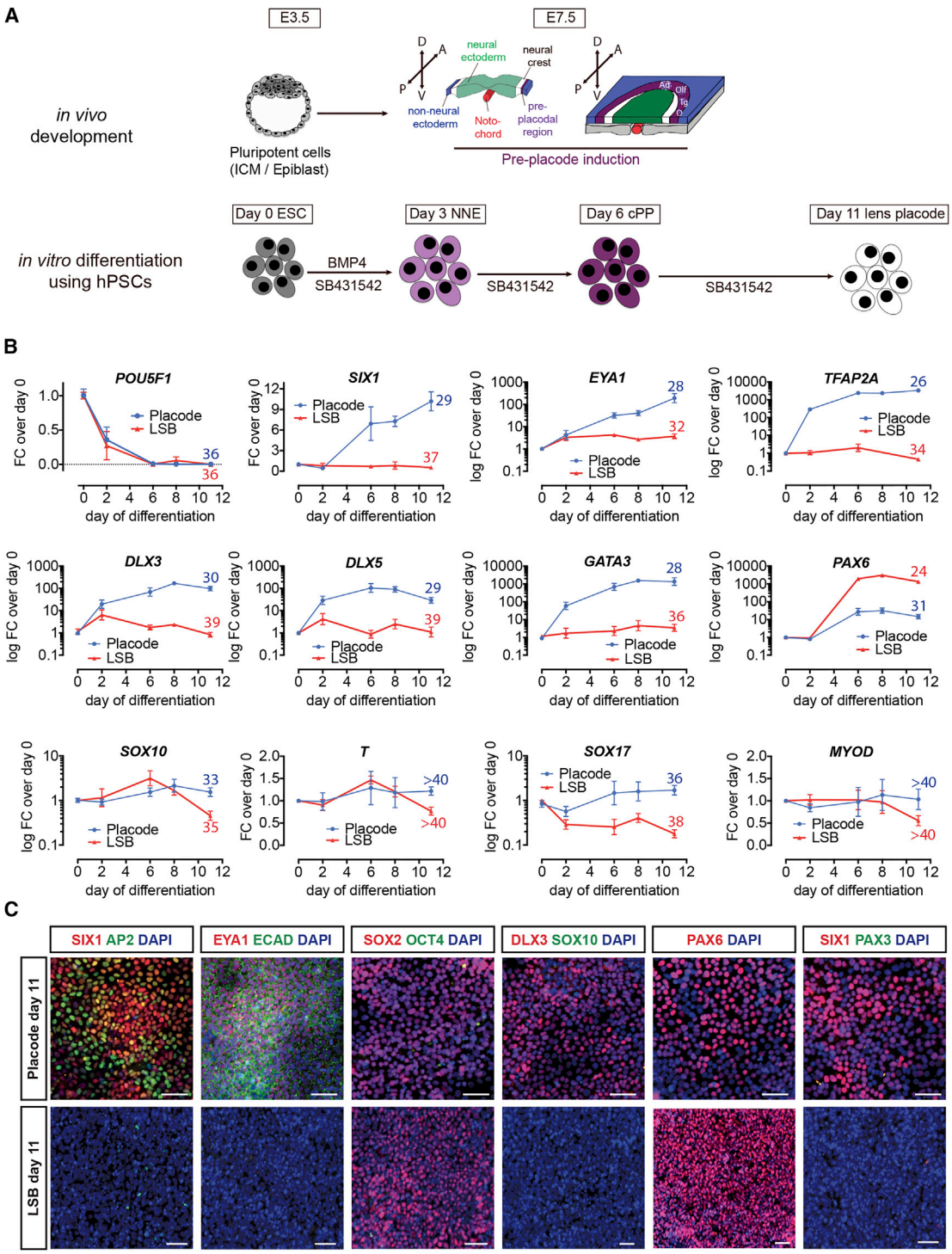

Figure 1. Differentiation of hPSCs into Cranial Placode Using Chemically Defined Conditions

(A) Schematic representation of cranial placode in vivo development and protocol for directed differentiation of hPSCs. ICM, inner cell mass.

(B) Real-time PCR gene expression time course of key cranial placode (*SIX1*, *EYA1*) and non-neural ectoderm (*TFAP2A*, *DLX3/5*, *GATA3*) genes as well as genes probing for potential contaminates (*SOX10*, *T*, *SOX17*, *MYOD*). Values are normalized to *GAPDH* and expression on

(legend continued on next page)