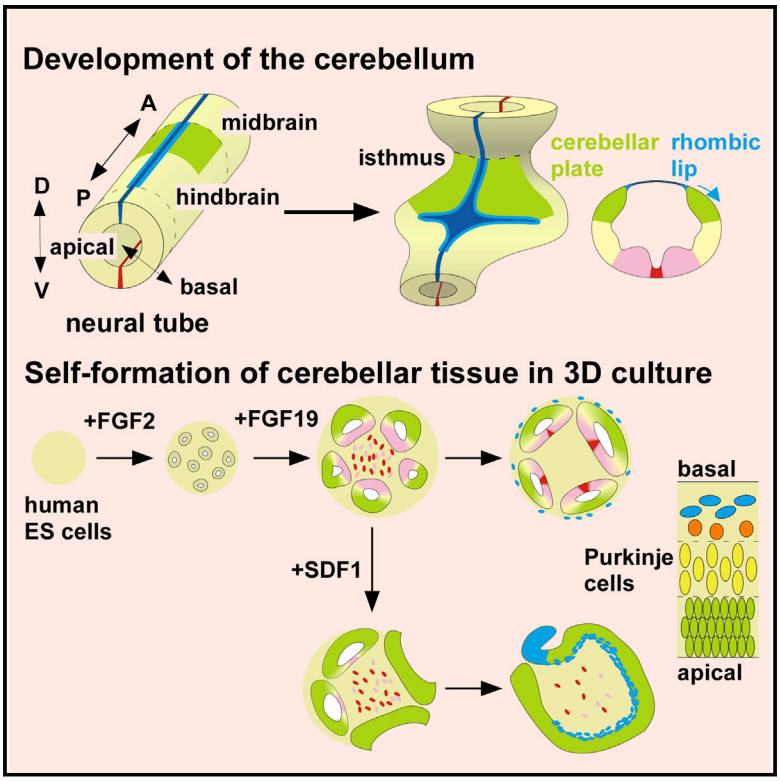
Article

Cell Reports

Self-Organization of Polarized Cerebellar Tissue in 3D Culture of Human Pluripotent Stem Cells

Graphical Abstract



Authors

Keiko Muguruma, Ayaka Nishiyama, ..., Kouichi Hashimoto, Yoshiki Sasai

Correspondence

muguruma@cdb.riken.jp

In Brief

Muguruma et al. developed a method to generate neurons of the cerebellum by 3D culture of human ESCs with sequential addition of growth factors. The induced cells self-organize into neural-tube-like structures with dorsoventral and apicobasal polarities. They eventually form layered structures that recapitulate cerebellar ontogenesis.

Highlights

- Neurons of the cerebellum are generated from FGF2-treated cultures of human ESCs
- The induced human Purkinje cells exhibit conserved and human-specific characteristics
- FGF19 and SDF1 promote the self-formation of polarized neural-tube-like structures
- The cerebellum is self-formed by ESC-derived cerebellar plate and rhombic lip neurons

Accession Numbers

GSE63015

Muguruma et al., 2015, Cell Reports *10*, 537–550
February 3, 2015
http://dx.doi.org/10.1016/j.celrep.2014.12.051

Cell Reports
Article

Self-Organization of Polarized Cerebellar Tissue in 3D Culture of Human Pluripotent Stem Cells

Keiko Muguruma,[1,*] Ayaka Nishiyama,[1] Hideshi Kawakami,[2] Kouichi Hashimoto,[3] and Yoshiki Sasai[1]
[1]Laboratory for Organogenesis and Neurogenesis, RIKEN Center for Developmental Biology, Kobe 650-0047, Japan
[2]Department of Epidemiology, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, 734-8551, Japan
[3]Department of Neurophysiology, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, 734-8551, Japan
*Correspondence: muguruma@cdb.riken.jp
http://dx.doi.org/10.1016/j.celrep.2014.12.051

SUMMARY

During cerebellar development, the main portion of the cerebellar plate neuroepithelium gives birth to Purkinje cells and interneurons, whereas the rhombic lip, the germinal zone at its dorsal edge, generates granule cells and cerebellar nuclei neurons. However, it remains elusive how these components cooperate to form the intricate cerebellar structure. Here, we found that a polarized cerebellar structure self-organizes in 3D human embryonic stem cell (ESC) culture. The self-organized neuroepithelium differentiates into electrophysiologically functional Purkinje cells. The addition of fibroblast growth factor 19 (FGF19) promotes spontaneous generation of dorsoventrally polarized neural-tube-like structures at the level of the cerebellum. Furthermore, addition of SDF1 and FGF19 promotes the generation of a continuous cerebellar plate neuroepithelium with rhombic-lip-like structure at one end and a three-layer cytoarchitecture similar to the embryonic cerebellum. Thus, human-ESC-derived cerebellar progenitors exhibit substantial self-organizing potential for generating a polarized structure reminiscent of the early human cerebellum at the first trimester.

INTRODUCTION

The cerebellum is a highly ordered brain structure related to motor functions with several distinct types of cells. Its early development is conserved among amniotes. Initially, the isthmic organizer, formed at the midbrain-hindbrain boundary (MHB), induces the cerebellar plate (CP) in the dorsal region (alar plate) of rhombomere 1 (r1) (Wingate and Hatten, 1999; Joyner et al., 2000). Cerebellar cells are generated in two distinct germinal zones in r1 (Figure S1A). The ventricular zone (VZ) of the CP expresses the basic helix-loop-helix (bHLH) transcription factor Ptf1a. Ptf1a$^+$ progenitors produce GABAergic neurons of the cerebellar cortex (Purkinje cells and interneurons) and of the deep cerebellar nuclei (DCN). The rhombic lip (RL) at the dorsal margin of r1 expresses another bHLH factor, Atoh1 (also known as Math1). Atoh1$^+$ progenitors generate glutamatergic neurons, including granule cells (GCs), unipolar brush cells, and large DCN projection neurons (Ben-Arie et al., 1997; Hoshino et al., 2005; Machold and Fishell, 2005; Wang et al., 2005; Fink et al., 2006). Recent knowledge on the mechanism of cerebellar differentiation has promoted technical advancement for in vitro generation of cerebellar neurons from pluripotent stem cells (Su et al., 2006; Salero and Hatten, 2007; Muguruma et al., 2010; Tao et al., 2010; Erceg et al., 2010). However, it remains unknown how the several cellular components are assembled to form the intricate structure of the cerebellum.

We previously reported that cerebellar neurons could be efficiently generated from mouse embryonic stem cells (mESCs) by recapitulating the self-inductive signaling microenvironment in 3D culture (Muguruma et al., 2010). mESCs formed isthmic organizer-like tissue in response to fibroblast growth factor 2 (FGF2) and insulin. Further inhibition of Shh signaling triggered the differentiation of the CP neuroepithelium (NE) (CPNE, hereafter) that expresses the Purkinje cell-progenitor marker Kirrel2 (also known as Neph3). On the other hand, the addition of bone morphogenetic protein (BMP) signals promoted differentiation into Atoh1$^+$ GCs and Tbr1$^+$ DCN neurons at the expense of Purkinje cells. Although we succeeded in induction of cerebellar neuronal components, 3D construction of cerebellar structures has not been so far recapitulated.

In the present study, we applied the self-formation principle to human ESC (hESC) culture for the generation of human cerebellar tissues in vitro. We found in vitro production of major cerebellar cell types. Of note, we demonstrated a set of electrophysiological analyses of human Purkinje cells. Moreover, we identified two factors, FGF19 and SDF1, that promote self-formation of ordered CP-like tissues in distinct manners. Here, we demonstrate that the addition of FGF19 promotes spontaneous formation of hindbrain neural-tube-like NE structures with dorsal-ventral (D-V) polarity. Sequential addition of FGF19 and SDF1 induces the generation of continuous CPNE that differentiates into a multilayered structure as seen in the cerebellar ontogenesis. NE margins form distinct RL-like germinal zones. We discuss the self-organizing nature of hESC-derived cerebellar tissues with regard to spontaneous polarity formation in 3D stem cell culture.