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Differentiation of human pluripotent stem cells into Medial Ganglionic Eminence vs. Caudal Ganglionic Eminence cells

Sandra Ahn [a], Tae-Gon Kim [b], Kwang-Soo Kim [b,c], Sangmi Chung [a,c,*]

[a] Translational Stem Cell Neurobiology Laboratory, Department of Psychiatry and Program in Neuroscience, McLean Hospital/Harvard Medical School, Belmont, MA 02478, USA
[b] Molecular Neurobiology Laboratory, Department of Psychiatry and Program in Neuroscience, McLean Hospital/Harvard Medical School, Belmont, MA 02478, USA
[c] Harvard Stem Cell Institute, McLean Hospital/Harvard Medical School, Belmont, MA 02478, USA

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ABSTRACT

Human pluripotent stem cells (PSCs) represent an opportunity to study human development *in vitro*, to model diseases in a dish, to screen drugs as well as to provide an unlimited and ethically unimpeded source of therapeutic cells. Cortical GABAergic interneurons, which are generated from Medial Ganglionic Eminence (MGE) cells and Caudal Ganglionic Eminence (CGE) cells during embryonic development, regulate cortical neural networks by providing inhibitory inputs. Their malfunction, resulting in failure to intricately regulate neural circuit balance, has been implicated in brain diseases, such as schizophrenia, autism and epilepsy. In this study, using combinatorial and temporal modulation of developmentally relevant dorsoventral and rostrocaudal signaling pathways, we efficiently generated MGE cells vs. CGE cells from human PSCs, which predominantly generate Parvalbumin-expressing or Somatostatin-expressing interneurons vs. Calretinin-expressing interneurons, respectively. Efficient generation of specific differentiated progenies of hPSCs as shown in this study will be a pivotal step to realize the full potential of hPSCs for regenerative medicine, developmental studies, disease modeling, bioassay, and drug screening.

1. Introduction

During early neurodevelopment, cortical interneuron progenitors arise from the ventral telencephalic area, such as Medial Ganglionic Eminence (MGE) and Caudal Ganglionic Eminence (CGE) [1]. Secreted signaling molecules from nearby organizers, such as SHH [2,3], Wnt [4] and FGF8 [5,6], play a crucial role during this early phenotype specification of ventral telencephalon. These signals trigger the regulatory cascades that lead to cortical interneuron development by inducing key transcription factors, such as Nkx2.1 in MGE [7–9] and CoupTFII in CGE [8,9]. Once phenotypically specified in the ventral area, MGE- or CGE-derived GABAergic interneurons migrate tangentially to their target sites, where they form local synaptic connections and critically modulate cortical circuitry. MGE cells mostly mature to generate Parvalbumin$^+$ or Somatostatin$^+$ interneurons, which comprise more than half of the entire cortical interneuron population, while CGE cells mostly mature to generate Calretinin$^+$ interneurons [10].

Dysfunction of interneurons has been implicated in various brain diseases, such as epilepsy, schizophrenia and autism [11], for which more effective treatments are desperately needed. Human pluripotent stem cells (hPSCs), especially human induced pluripotent stem cells (hiPSCs), provide an unprecedented opportunity to study disease mechanisms and develop novel therapeutics for these brain diseases [12–17], as long as specific neuronal subtypes can be efficiently derived from hiPSCs. We and others have recently reported on the efficient generation of MGE-type interneurons from hPSCs [18–21] by employing developmentally relevant signaling pathway activation, and the derivation of CGE-type interneurons using various methods [21–23].

In this study, we provide a detailed description of the method for the stepwise and combinatorial treatment of developmentally relevant signaling molecules to efficiently generate MGE vs. CGE

Abbreviations: PSCs, pluripotent stem cells; hPSCs, human pluripotent stem cells; MGE, Medial Ganglionic Eminence; CGE, Caudal Ganglionic Eminence; hiPSCs, human induced pluripotent stem cells; iPSC, induced pluripotent stem cell; ESCs, embryonic stem cells; E8, Essential 8; KSR, knockout serum replacement; OMe, Boc-Asp; BAF, fluoromethyl ketone; PLO, Poly-L-ornithine; FN, fibronectin; BSA, bovine serum albumin; NDS, normal donkey serum; PV, Parvalbumin; SST, Somatostatin; ip, intraperitoneal injection; Calb, Calbindin; Calr, Calretinin; NPY, Neuropeptide Y; hNuc, human nuclei.

* Corresponding author at: Translational Stem Cell Neurobiology Laboratory, Department of Psychiatry and Program in Neuroscience, McLean Hospital/Harvard Medical School, Belmont, MA 02478, USA.
E-mail address: schung@mclean.harvard.edu (S. Chung).

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cells from hPSCs. In this protocol, we first ventralize early neuroectoderm using SHH activation and Wnt inhibition, followed by further rostralization and caudalization of ventralized neuroectoderm using FGF8 vs. FGF19 signaling activation to induce MGE vs. CGE phenotype. This highly efficient method, which recapitulates early developmental processes, will help accelerate the realization of the full potential of induced pluripotent stem cell (iPSC) technology in the study of disease mechanisms and the development of novel therapeutics.

2. Materials

2.1. Reagents

2.1.1. Cells

- Human embryonic stem cells (ESCs): H9 cells, WA09 (WiCell, Madison, WI, passage 45–55)
- Human ESCs: H7 cells, WA07 (WiCell, Madison, WI, passage 41–51)
- Human iPSC2497 (a kind gift from Dr. Fred Gage, [15], passage 30–40)

2.1.2. Growth media and supplements

- DMEM (Life Technologies, Carlsbad, CA, cat. no. 11965–118)
- Essential 8 medium kit (E8 medium; Life Technologies, Carlsbad, CA, cat. no. A1517001)
- ROCK inhibitor Y-27632 (Selleck Chemicals, Houston, TX, cat. no. S1049)
- Knockout serum replacement (KSR; Life Technologies, Carlsbad, CA, cat. no. 10828–028)
- Penicillin–streptomycin (Life Technologies, Carlsbad, CA, cat. no. 15140–163)
- 2-Mercaptoethanol (Sigma–Aldrich, St. Louis, MO, cat. no. M3148)
- L-Glutamine, 200 mM (Life Technologies, Carlsbad, CA, cat. no. 25030–164)
- LDN193189 (Stemgent, Cambridge, MA, cat. no. 04–0074)
- SB431542 (Selleck Chemicals, Houston, TX, cat. no. S1067)
- IWP-2 (Selleck Chemicals, Houston, TX, cat. no. S7085)
- SAG (Cayman Chemical, Ann Arbor, MI, 11914)
- Recombinant human FGF-8 (PeproTech, Rocky Hill, NJ, cat. no. 100–25)
- Recombinant human FGF-19 (PeproTech, Rocky Hill, NJ, cat. no. 100–32)
- N-2 supplement (Life Technologies, Carlsbad, CA, cat. no. 17502–048)
- DMEM-F12 (Life Technologies, Carlsbad, CA, cat. no. 11320–082)
- Recombinant human GDNF (PeproTech, Rocky Hill, NJ, cat. no. 450–10)
- Recombinant human BDNF (PeproTech, Rocky Hill, NJ, cat. no. 450–02)
- HBSS, calcium, magnesium, no phenol red (Life Technologies, Carlsbad, CA, cat. no. 14025–134)
- Boc-Asp(OMe) fluoromethyl ketone (BAF; Sigma–Aldrich, St. Louis, MO, cat. no. B2682)

2.1.3. Enzymes and other reagents

- Matrigel, hESC-qualified matrix (BD Biosciences, San Jose, CA, cat. no. 354277)
- DPBS, without calcium and magnesium (Life Technologies, Carlsbad, CA, cat. no. 14190–250)
- EDTA (Sigma–Aldrich, St. Louis, MO, cat. no. E6758)
- Poly-L-ornithine solution (PLO; Sigma–Aldrich, St. Louis, MO, cat. no. P4957)
- Fibronectin (FN; Sigma–Aldrich, St. Louis, MO, cat. no. F0895)
- Trypsin–EDTA solution, 0.5% (Life Technologies, Carlsbad, CA, cat. no. 15400–054)
- Fixation/permeabilization solution kit (BD Biosciences, San Jose, CA, cat. no. 554714)
- Bovine serum albumin (BSA; Sigma–Aldrich, St. Louis, MO, cat. no. A9418)
- Saponin (Sigma–Aldrich, St. Louis, MO, cat. no. 47036)
- TRIzol reagent (Life Technologies, Carlsbad, CA, cat. no. 15596–026)
- PureLink RNA mini kit (Life Technologies, Carlsbad, CA, cat. no. 12183018A)
- SuperScript II reverse transcriptase (Life Technologies, Carlsbad, CA, cat. no. 18064–014)
- Oligo d(T)12–18 (Gene Link, Hawthorne, NY, cat. no. 26–4000-05)
- SYBR green I nucleic acid gel stain (Sigma–Aldrich, St. Louis, MO, cat. no. S9430)
- Formaldehyde, 37% (Electron Microscopy Sciences, Hatfield, PA, cat. no. 15686)
- Normal donkey serum (NDS; Sigma–Aldrich, St. Louis, MO, cat. no. D9663)
- Triton X-100 (Sigma–Aldrich, St. Louis, MO, cat. no. X100)
- Hoechst 33342 (Life Technologies, Carlsbad, CA, cat. no. H3570)
- Fluoromount-G (SouthernBiotech, Birmingham, AL, cat. no. 0100–01)
- Isoflurane (Sigma–Aldrich, St. Louis, MO, cat. no. 792632)
- Buprenorphine hydrochloride (Sigma–Aldrich, St. Louis, MO, cat. no. B9275)
- Triple antibiotic ointment (Fisher Scientific, Pittsburgh, PA, cat. no. 19–075-383)
- Pentobarbital (Sigma–Aldrich, St. Louis, MO, cat. no. P0500000)
- Heparin sodium (Sigma–Aldrich, St. Louis, MO, cat. no. 1304005)
- Sodium chloride standard, aqueous solution (Fisher Scientific, Pittsburgh, PA, cat. no. 7213095)
- Sucrose (Fisher Scientific, Pittsburgh, PA, cat. no. S6–12)

2.1.4. Antibodies

Table 1.

2.1.5. Primers

Table 2.

2.2. Equipment and software

- Laminar flow hood
- CO_2 incubator (Sanyo, Osaka, Japan)
- Refrigerator, 4 °C (Sanyo, Osaka, Japan)
- Inverted microscope with phase contrast (Olympus, Center Valley, PA, CK40)
- Centrifuge with swing-bucket rotor (Eppendorf, Hauppauge, NY, 5810)
- Pipet-aid (Drummond Scientific, Broomall, PA, cat. no. 4-000-101)
- Serological pipettes (25, 10 and 5 ml; BD Biosciences, San Jose, CA, cat. nos. 357535, 357551 and 357543, respectively)
- Pipetman (P1000, P200, P20 and P10; Gilson, Middleton, WI)
- Sterile pipette tips (1 ml, 200 and 10 μl; Fisher Scientific, Pittsburgh, PA, cat. nos. 02-681-165, 02-681-457 and 02-681-440, respectively)
- Sterile filter units, 500 ml (Thermo Scientific, Waltham, MA, cat. no. 566-0020)

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