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Human RA-gastruloid induction from pluripotent stem cells

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We use this protocol and it's working

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Disclaimer

Abstract

Gastruloids are a powerful in vitro model of early human development. However, although elongated and composed of all three germ layers, human gastruloids do not morphologically resemble post-implantation human embryos. Here we show an early pulse of retinoic acid (RA), together with Matrigel, robustly induces human gastruloids with posterior embryo-like morphological structures, including a neural tube flanked by segmented somites, and diverse cell types including neural crest, neural progenitors, renal progenitors, and myocytes. Through in silico staging based on single-cell RNA-seq (scRNA-seq), we find human RA-gastruloids to be more advanced than other embryo models, and comparable to E9.5 mouse and CS11 cynomolgus monkey embryos. We leverage chemical and genetic perturbations of RA-gastruloids to confirm that WNT and BMP signalling regulate somite formation and neural tube length in the human context, while transcription factors TBX6 and PAX3 underpin presomitic mesoderm and neural crest, respectively. Looking forward, RA-gastruloids are a robust, scalable model for decoding early human embryogenesis.

Materials

- StemPro Accutase (Thermo, A1110501)
- Wash media

Note

DMEM/F-12 (Thermo, 11320033) + 0.1% BSA (Thermo, 15260037)

- 10 µM Y-27632 (Sellek, S1049)
- Nutristem hPSC XF medium (Biological Industries, 05-100-1A)
- 30mM CHIR99021 (CHIR, Millipore, SML1046)

Note

(Make 6µL aliquots and stored in -20C, and test the batch before the use)

- 100mM Retinoic Acid (RA) stock (Millipore Sigma, R2625)

Note

Make 10µL aliquots and stored in -20C)

- Essential 6 medium (Thermo, A1516401)
- Matrigel (Corning, 354230)
- Non-adhesive 96-well plate, such as Nunclon™ Sphera™ 96-Well, Nunclon Sphera-Treated,U-Shaped-Bottom Microplate and PrimeSurface® 3D culture: Ultra-low Attachment Plates: 96 well,U bottom, Clear plates
- Vitronectin (Gibco, A14700)

Note

Make 200 µl aliquots and store them in -20C and dilute them x100 in PBS before the use and keep remaining in 4C and use it within 2 weeks

Troubleshooting

Day0, Passage human PSCs onto vitronectin-coated NutriStem

1 Coat wells with Vitronectin and incubate them in 37C for at least 15 min

2 Dissociate pre-treated ESCs

3 Aspirate media from wells and wash wells with PBS(-) and aspirate PBS (-)

4 Add 500 µL Accutase to each well and incubate them at 37C for 4 min

5 Quench the reaction by adding 2 mL of Wash media containing 10 µM Y-27632 media and pipette up and down ~10 times in a well to dissociate cells

6 Transfer them to a new 15mL tube and centrifuge for 3 min at 300 g

7 Suspend them in NutriStem containing 10 µM Y-27632 and spread 2 × 10^4 cells for 1/12 well onto each vitronectin-coated well

Note

The number of seeding cells on the pre-treatment plate is important for later gastruloid elongation and needs to be optimized for each lab and each cell line.

Day 1-3, Pre-treatment of ESCs (CHIR stimulation)

8 **On day 1**, Change medium to fresh NutriStem medium containing 5 µM Y-27632

9 **On day 2**, Change the medium to fresh NutriStem medium containing 4 µM CHIR

Note

- CHIR concentrations and duration of treatment should be optimized for each CHIR batch and/or each cell line.
- CHIR should be used within three months from the aliquoting.

10 **On day 3**, Change medium to fresh NutriStem medium containing 4 µM CHIR + 500 nM RA

Note

- The concentrations of CHIR and RA should be optimized for each cell line.

Day 4-9, Human RA-gastruloid induction

11 **On day 4**, prepare the following reagents

Note

- Accutase = 500µL for 1/12 well
- Wash media + 10 µM Y-27632 = 2 mL for 1/12 well
- Essential 6 medium + 1 µM CHIR + 5 µM Y-27632 = 50µL / well + 10%

12 Aspirate media from wells and wash wells with PBS(-) and aspirate PBS (-)

13 Add 500µL Accutase to each well and incubate them in 37C for 4 min

14 Quench the reaction by adding 2 mL of Wash+Y media to each well and pipette up and down ~10 times in a well to dissociate cells

15 Transfer them to a new 15 mL tube and centrifuge for 3 min at 300 g

16 Wash again with 2 mL Essential6 + 1µM CHIR + Y and centrifuge it again

17 - Resuspend them in 1-2 mL Essential 6 + Y medium and count cells by Countess

18 Transfer the necessary number of cells (4000 cells/well) into a reservoir containing a medium (50 µl/well)

19 Spread cells with a multi-channel pipette

20 Incubate them in 37C incubator

21 - **On day 5 (+24h from RA-gastruloid induction)**, add 150 µL Essential 6 media to each well.

22 - **On day 6 (+48h from RA-gastruloid induction)**, carefully remove 150 µL medium and add 150 µL of fresh Essential 6 medium, containing 100 nM RA and 5% Matrigel

> Note
>
> At this moment, you would see some elongation of RA-gastruloids

23 **On day 9 (+120h from RA-gastruloid induction)**, you should see the fully elongated RA-gastruloid with neural tube and segmented somites