A 3D system to model human pancreas development and its reference single-cell transcriptome atlas identify signaling pathways required for progenitor expansion

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Method Article

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Abstract

Human organogenesis remains relatively unexplored for ethical and practical reasons. Here we report the establishment of a single cell transcriptome atlas of the human fetal pancreas between 7 and 10 post-conceptional weeks of development. To interrogate cell-cell interactions we developed InterCom, an R-Package for identifying receptors-ligand pairs and their downstream effects. We further report the establishment of a human pancreas culture system starting from fetal tissue or human pluripotent stem cells, enabling the long-term maintenance of pancreas progenitors in a minimal, defined medium in three-dimensions. Benchmarking the cells produced in 2D and those expanded in 3D to fetal tissue reveals that progenitors expanded in 3D are transcriptionally closer to the fetal pancreas. We further demonstrate the potential of this system as a screening platform and identify the importance of the EGF and FGF pathways controlling human pancreas progenitor expansion.

Introduction

Reagents

Equipment

Procedure

3-D long-term pancreatic progenitor spheroid (PP-spheroid) culture protocol

Preparation of PP-spheroid expansion medium and other reagents

1. PP-spheroid expansion medium (PEm)

DMEM/F12, Glutamax (ThermoFisher 31331028)

FGF2 (human bFGF), 64 ng/ml (Peprotech 100-18B)

B27, 1 X (Thermofisher 17504044)

ROCK inhibitor Y-27632, 10 µM (Calbiochem 616464)

2. Thawed growth factor reduced Matrigel (Fisher Scientific 354277). Keep on ice!

3. Dissociation reagents: TrypLE (Thermofisher 12604013) and 0.25% Trypsin+0.5 M EDTA

4. Inactivation reagent: Hi-FBS (heat inactivated FBS) or 10% FBS in DMEM/F12, Glutamax medium. Keep on ice.

5. PBS without Ca^{2+}, Mg^{2+} ($PBS^{-/-}$)

6. 5 ml tubes with 35 µm cell strainer snap cap (Fisher Scientific 10585801)

7. 4-well plate (VWR Nunc 176740)

8. Freezing medium: 80% FBS or KOSR + 20% DMSO

Culture protocol of hPSC-derived PP-spheroids

1. Differentiate hPSCs to pancreatic progenitors using Ameri protocol (Ameri et al., 2010) or Rezania protocol (Rezania et al., 2014) until the end of stage 4 (St4d8 in Ameri or St4d3 in Rezania).

2. Place 4-w plate in an incubator to warm it up.

3. Remove differentiation medium and wash cells with prewarmed $PBS^{-/-}$.

4. Dissociate cells using TrypLE and incubate for 3-5 min, until cells are rounded up and about to detach.

5. Add equal volume of Hi-FBS or 5X 10% FBS to the well and gently dissociate cells.

6. Transfer all the suspensions through cell strainer (5 ml tubes with 35 µm cell strainer snap cap) and centrifuge the dissociated cells at 500 g for 3 min.

7. Discard supernatant and resuspend cells in PEm.

8. Count cells, and adjust cell concentration in PEm to $4x10^6$/ml.

9. Take 40 µl of cells and place them on a pre-cold Eppendorf tube on ice for 4 well of 4-well plate (10 µl cells (40,000 cells)/well).

10. Using a pre-chilled pipette tip, add 3X volume of thawed Matrigel to the cold cells, and mix well by pipetting up and down at least 20X. Important, avoid making bubbles!

11. Take pre-warmed 4-w plate, and place 40 µl of cell-Matrigel mix on to each well by pipetting slowly to make a dome.

12. Place the plate in the incubator without adding medium for 10 min for the gel to solidify.

13. Add 500 µl of pre-warmed PEm to each well, and place in the incubator.

14. Change media every 3 days, and passage cells in every 10 days.

Passaging PP-spheroids

1. Pre-warm 4-well plate in the incubator.

2. Wash the wells once with 1 ml $PBS^{-/-}$ and add 500 µl/well of dissociating reagent. Break Matrigel by pipetting up and down a few times.

3. Incubate 7-8 minutes in the incubator.

4. In the meantime prepare a 15 ml falcon tubes per plate with equal vol of Hi-FBS or 5X 10% FBS per tube on ice.

5. Add 500 µl/well of inactivation reagent and pipette up and down a few times to help break down Matrigel and to dissociate the cells. Transfer cells in solution to the tube with inactivation reagent.

6. Spin down at 500 g for 3 minutes.

7. Remove supernatant from the tubes and add resuspend cells in 1 ml PEm.

8. Transfer all the suspensions through cell strainer (5 ml tubes with 35 µm cell strainer snap cap). Keep cells on ice.

9. Count the cells resuspended in PEm (keep notes of the cell count, total suspension volume and number of wells in suspension). Take required number of cells in suspension (40,000 cells/well) into a 1.5 ml Eppendorf tube.

10. Spin down at 500 g for 3 min, and remove excessive supernatant to make 4000 cells/µl concentration. This way, you are keeping 40,000 cells in 10 µl volume for 1 well.

11. Using pre-chilled pipette tip, add 3X volume Matrigel and resuspend and mix well by pipetting up and down ~20X to distribute cells homogeneously in Matrigel. Important, avoid making bubbles!

12. Take pre-warmed 4-w plate, and place 40 µl of cell-Matrigel mix on to each well by pipetting slowly to make a dome.

13. Place the plate in the incubator without adding medium for 10 min for the gel to solidify.

14. Add 500 µl of pre-warmed PEm to each well, and place in the incubator.

15. Change media every 3 days, and passage cells in every 10 days.

Freezing PP-spheroids for cryopreservation: whole-PP-spheroid freezing (1 cryovial/well of 4-well plate)

1. At day 10, wash the wells once with 1ml $PBS^{-/-}$ and add 500 µl/well of dissociating reagent. Break Matrigel by pipetting up and down a few times.

2. Incubate 1-2 min at 37°C just to free spheres from Matrigel.

3. Add equal volume of ice-cold Hi-FBS, and spin down at 500 g for 3 min.

4. Remove supernatant and add 200 µl PEm/well.

5. Transfer 200 µl cell suspension to cryovial, and add equal volume of ice-cold freezing medium.

6. Place the cryovials in Mr. Frosty and keep them at -80°C for overnight.

7. Place the vials in liquid nitrogen tank.

Defrosting cryopreserved PP-spheroids and culture

1. Pre-warm PEm, 10% FBS + 10 µM ROCK inhibitor Y-27632 in DMEM/F12, Glutamax (5X volume of cryopreserved cell solution), and 4-well plate.

2. Quickly thaw cryovials, and add 0.5 - 1 ml of prewarmed 10% FBS + 10 µM ROCK inhibitor Y-27632 in DMEM/F12, Glutamax drop by drop to the thawed cells.

3. Tranfer the PP-spheroid suspension into the falcon tube with 10% FBS + 10 µM ROCK inhibitor Y-27632 in DMEM/F12, Glutamax, and spin down at 500 g for 3 min.

4. Remove supernatant and resuspend PP-spheroids in 10 µl PEm/cryovial.

5. Add 3X volume Matrigel to the PP-spheroid suspension and mix well by pipetting up and down around 20 times.

6. Take pre-warmed 4-w plate, and place 40 µl of cell-Matrigel mix on to each well by pipetting slowly to make a dome.

7. Place the plate in the incubator without adding medium for 10 min for the gel to solidify.

8. Add 500 µl of pre-warmed PEm to each well, and place in the incubator.

9. Change media every 3 days, and passage cells when PP-spheroids become confluent at day 5-7.