Time: 2024.08.17-2024.08.20

1. **Experiment:** Human hepatoma organoids culture and passage
2. **Time:** 2024.08.17-2024.08.20
3. **Member:** Xiaoyuan Chen, Hanyue Liu
4. **Material:**

Name	Supplier or Formulation
DMEM basic	Invitrogen
Stopping culture medium	DMEM basic, Penicillin-streptomycin (100 μg/mL), FBS (10%)
Complete culture medium	Advanced DMEM/F12, Penicillin-streptomycin (100 μg/mL), Double-antibody (1×), HEPES (10 mM), GlutaMAX-I (1×), A83-01 (500 nM), Y-27632 (10 μM), N-Acetylcysteine (1.56 mM), Nicotinamide (10 mM), FGF10 (10 ng/mL), B27 supplement (1×), Forskolin (10 μM), Wnt3A conditioned medium (30%), R-spondin conditioned medium (2%), Noggin conditioned medium (4%)
Ordinary culture medium	Advanced DMEM/F12, Penicillin-streptomycin (100 μg/mL), Double-antibody (1×), HEPES (10 mM), GlutaMAX-I (1×), A83-01 (500 nM), Y-27632 (10 μM), N-Acetylcysteine (1.56 mM), Nicotinamide (10mM), FGF10 (10 ng/mL), B27 supplement (1×), Forskolin (10 μM), EGF (50 ng/mL), Wnt3A conditioned medium (30%), R-spondin conditioned medium (2%), Noggin conditioned medium (4%)
Basal culture medium	Advanced DMEM/F12, Penicillin-streptomycin (100 μg/mL), Double-antibody (1×), HEPES (10 mM), GlutaMAX-I (1×)
Penicilin-streptomycin	Invitrogen
TrypLE	Invitrogen
Collagenase, Type 2	Invitrogen
Y-27632	Selleck
Wash medium	DMEM basic, Penicillin-streptomycin (100 ug/mL)
Digestion solution	Collagenase, Type 2 2.5 mg/mL Y-27632 10 uM
PBS 10X (1000 mL)	Na_2HPO_4 1.44 g KH_2PO_4 0.24 g Add distilled water to 800 mL
Matrigel	Corning (356231)

Time: 2024.08.17-2024.08.20

5. Method:

(1) Culture of human Hepatoma organoids

① For tumor tissue samples, approximately 1 cm^3 tissue blocks are placed in 10 cm culture dishes and washed twice with clean culture medium.
② Scissors and forceps were used to mince the tissue samples and transferred into 15 mL centrifuge tubes containing digestion solution (basal culture medium containing 2.5 mg/mL Type II collagenase and 10 μM Y-27632).
③ The tubes are placed on a tube rotator in a 37 °C cell culture incubator for thorough mixing and digestion for approximately one hour. Digestion can be stopped when no large tissue particles are visible in the centrifuge tubes.
④ The digestion product is filtered through a 70 μM filter and washed for several times.
⑤ The filtrate is centrifuged at 1700 rpm for 5 minutes. The supernatant was discarded.
⑥ 3-5 mL stopping culture medium is added. The centrifugation step is repeated at 1700 rpm for 5 minutes.
⑦ This process is repeated once more with 2 mL complete culture medium (Hepatoma tissue) or ordinary culture medium (normal liver tissue).
⑧ Based on the amount of cell precipitation obtained, 150 μL of the respective culture medium and 100 μL of Matrigel (Store on ice during the experiment) are seeded in suspension 6-well plates, or 30 μL of the respective culture medium and 20 μl of Matrigel are seeded in suspension 24-well plates.
⑨ After seeding, the plates are left undisturbed in a cell culture incubator (37 °C, 5% CO_2, and humidity maintained).
⑩ After 1 hour incubation, the seeded plates are supplemented with liquid medium (3 mL per well for 6-well plates; 1 mL per well for 24-well plates).

(2) Passage of human Hepatoma organoids

① Monitor the organoid culture via microscopy and passage the organoids when density reaches approximately 70% in suspension.
② Disperse the organoids using a 1 mL pipette gun into plate wells, then transfer into a 15 mL centrifuge tube.
③ Spin at 1500 rpm for 5 min (if the matrix gel-containing precipitate is suspended after centrifugation, the centrifuge can be slowed down)
④ Discard the supernatant to 1 mL, and blow the precipitate 80-100 times with a 1 mL pipette gun.
⑤ Under microscope observation, the organoid should form a mass of 30-50 uM.
⑥ Wash the solid organoids with pre-cooled phosphate-buffered saline (PBS) until no visible matrix gel remains following centrifugation. In cases where solid organoids are resistant to dissociation, the supernatant may be removed post-centrifugation, followed by the addition of 1-2 mL of TrypLE for enzymatic digestion at 37 °C within a cell culture incubator for approximately 8-10 minutes.
⑦ Add 5 mL of termination medium and centrifuge at 1000 rpm for 3 min to obtain the cell precipitate.
⑧ Resuspend the cells with the appropriate amount of corresponding medium and matrix gel, inoculate the cells on the suspension plate, and control the substitution ratio at 1:2~1:3. The pipette gun should not be sticked to the wall of the centrifuge tube to prevent cross contamination.