Generation and Culture of CRC Fibroblasts

Contact address: Henner F. Farin (Georg-Speyer-Haus, Institute for Tumor Biology and Experimental Therapy, Frankfurt/Germany; email: h.farin@georg-speyer-haus.de)

Version 1.0 Version Date: 11 April 2023, generated by Benardina Ndreshkjana

Introduction

Cancer-associated fibroblasts (CAFs) are a key component of the tumor microenvironment and comprise a heterogeneous and plastic population. Through different processes, such as paracrine signaling or metabolic changes, CAFs can modulate the cancer phenotype. For establishment and culture of patient derived CAFs different protocols have been described.

Purpose

This standard operating procedure (SOP) provides a complete procedure for the establishment, expansion, freezing and thawing of fibroblast cultures from CRC tissue as well as the composition of the culture media.

1. Equipment, materials and chemicals

1.1 Equipment

- Cell culture incubator adjusted at 37 °C and 5 % CO2
- Centrifuge (Rotanta 460R, Hettich)
- Evos Fl microscope with 2X, 4X, 10X objectives
- Pipettes with sterile tips
- Pump + Aspiration pipettes
- Water bath or heating block
- Pipette boy

1.2 Materials

- 15- and 50-ml Falcons
- 5 ml Eppendorf tubes
- Sterile cell culture dishes
- Cryovials
- 5-, 10- and 25-ml pipets
- Freezing box

1.3 Chemicals

- Primocin (InvivoGen, #ant-pm-1)
- Recovery™ Cell Culture Freezing Medium (Thermo Fisher, #12648010)
- PBS (Thermo Fisher, #10010015)
- 0.1 % gelatin in PBS (Sigma-Aldrich, #G2500-100G)
- Trypsin (Thermo Fisher, #12605-010)

2. Medium for tumor fibroblast expansion

Component	Concentration (stock)	Amount
DMEM (Thermo Fisher, #31966-047)	1x	500 ml
Fetal Bovine Serum (Sigma-Aldrich, #F7524)	10x	60 ml
Pent/Strep (Thermo Fisher, #15140-122)	100x	5ml

3. Fibroblast isolation

Colorectal cancer tissue collection and processing has been described in detail in SOP 1 and for fibroblast isolation we use remaining tissues from step *5.1.3*.

- Collect the remaining pieces from step *5.1.3* in a 50 ml falcon with 10 ml fibroblast medium and pipette vigorously using a 10 ml pipette to remove any remaining epithelial cells.
- Let the pieces to sediment and discard the supernatant.
- Add 20 ml of medium to wash the tissue pieces and remove blood cells.
- Allow to settle down and remove the supernatant. Repeat the previous step 2-3 times.

- Collect the pieces in 10 ml medium supplemented with 1:500 Primocin, transfer to a regular 10 cm culture dish and place the plate in a cell culture incubator for one day.
- Next day, coat a 10 cm culture dish with 3 ml of 0.1 % gelatin in PBS and incubate for 20-30 min in incubator (take the gelatin up before seeding the pieces).
- Collect the seeded pieces from the previous day in a 50 ml falcon tube and repeat the washing steps similar as above.
- Collect the clean pieces in 10 ml pre-warmed medium supplemented with 1:500 Primocin, transfer it in the gelatin-coated culture dish and place it in the incubator.
- Leave it in a fixed position for at least one week.
- Carefully change medium weekly and do not disturb the pieces to allow the attachment to the plastic and the migration of the fibroblasts.
- Generally, the pieces take 2-3 weeks to attach and to see fibroblasts migrating out and 1-2 more weeks to fill the plate.
- Remove the pieces once the plate is confluent with fibroblasts.

4. Expanding and splitting fibroblasts

- Prepare 10 cm culture dishes with 3 ml gelatin and place them in the incubator for 20-30 min.
- Once the fibroblast dish is confluent, remove the medium and wash with 3 ml of PBS.
- Add 1 ml of Trypsin and place it back in the incubator for 4-5 min.
- After 4 min check under the microscope if the cells started to detach and collect with 4 ml medium in a 5 ml Eppendorf tube.
- Centrifuge at 1500 rpm for 5 min at 4 °C.
- Remove the supernatant and collect the pellet in 1 ml fibroblast medium.
- Take the gelatin-coated cell culture dishes from the incubator and remove the remaining gelatin.
- Add 9.5 ml of pre-warmed medium and 1:500 Primocin for the first 3 passages.
- To the prepared dishes add 0.5 ml of the fibroblast suspension.
- Usually, a full 10 cm dish is split 1:2 and should take maximum 2 weeks to grow again a full dish.

5. Freezing and thawing of fibroblasts

- To freeze the fibroblast, remove the medium and wash with 3 ml PBS.
- Add 1 ml of Trypsin and place it back in the incubator for 4-5 min.
- After 4 min check under the microscope if the cells started to detach and collect with 4 ml of fibroblast medium expansion in a 5 ml Eppendorf tube.
- Centrifuge at 1500 rpm for 5 min at 4 °C.
- Remove the supernatant and resuspend the cell pellet in 500 µl Recovery™ Cell Culture Freezing medium/dish in one cryovial.
- Place the cryovials in a freezing box at -80 °C.
- After one day, transfer the vials from -80 °C to a liquid nitrogen tank.
- When thawing frozen fibroblasts, thaw the vial on ice and add 1 ml of medium.
- Add the cells in a 5 ml Eppendorf tube with 4 ml of fibroblast medium.
- Spin down at 1500 rpm for 5 min at 4 °C.
- Remove the supernatant and add 1 ml medium.
- Seed the fibroblasts in a gelatin-coated 10 cm culture dish containing 9 ml of pre-warmed medium and place it in the incubator.