EXPERIMENTAL PROTOCOL: CO-CULTURE BETWEEN FIBROBLAST AND TUMORSPHERES

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Introduction

As a major component of tumor microenvironment, cancer-associated fibroblasts (CAFs) play an important role in cancer progression and drug resistance. 3D culture and interaction with stromal components are considered essential elements in the establishment of a 'more clinically relevant' tumor model. Tumor cells and fibroblasts migrate together with tumor cells in forming a center surrounded by fibroblasts, maximizing the contact between cells.

Reagents

- ATCC-PCS-201-030 Fibroblast Basal Medium (Ref. ATCC-PCS-201-030)
- ATCC-PCS-201-041 Fibroblast Growth Kit-Low serum (Ref. ATCC-PCS-201-042)
- RPMI 1640 / DMEM F12 10% FBS
- Spheroids medium (RPMI 1640, 7.5% BSA, 50 µg/ml EGF, 20 µg/ml bFGF and 20 µg/ml ITS)
- Trypan Blue Solution, 0.4%
- Phosphate Buffered Saline w/o Calcium w/o Magnesium (PBS)
- Fetal Bovine Serum (FBS)
- Penicillin-Streptomycin
- Trypsin 5% EDTA, 10X

Instruments and tools

- Laminar flow cabinet
- 75 cm^2 Cell Culture Flask
- 6 well-plate
- Neubauer chamber
- Centrifuge with swing-bucket rotors and acceleration and deceleration (braking) ramps
- Sterile 15 ml conical tubes

Before start

Here we describe the procedure for co-culture between a fibroblast cell line and tumor cells in 3D condition. 5 days before, tumorspheres should be induced from adherent cells with spheroids medium supplemented with 7.5% BSA, con EGF, FGF, ITS.

PROCEDURE

1. Culture the fibroblast cell line in a flask 75cm^2.
2. When fibroblast cells are in a confluence of 80%, detach the cells.
3. Wash the cells with PBS.
4. Trypsinize with 2ml of Trypsin for 5 min at 37ºC.
5. Neutralize with medium with FBS.
6. Centrifuge cells at 1500 rpm for 5 min.
7. Proceed with cell counting.
8. Plate in a 6 well-plate 300.000 fibroblast cells/well. After 2 hours, fibroblast will be attached.
9. Then, collect tumorspheres in a 15ml conical tube.
10. Trypsinize with 2ml of Trypsin for 5 min at 37ºC.
11. Neutralize with medium with FBS
12. Centrifuge cells 1500 rpm for 5 min.
13. Proceed with cell counting.
14. Plate 100.000 tumorspheres above the layer of fibroblast cells.
15. After 48h of co-culture procedure, you will have the co-culture stablished to perform different experiments.