Supplementary Materials

In Vitro α-SMA Flow Cytometry Assay

Samples

- 2 × T25 flasks with MRC-5 cells for each sample. Seeded day 0.

Treatments

- TGF-β3 treatment: TGF-β3 solution added to cell medium at concentrations of 0.01 ng/mL, 0.5 ng/mL or 50 ng/mL on day 1 (24 hours after seeding)
- Controls: Sham irradiated on day 2.
- Irradiated: Irradiated on day 2 (48 hours after seeding)

Harvesting, staining and flow cytometry was performed 96 hours after seeding.

Protocol

1. Aspirate medium and wash with 2 mL PBS
2. Add 0.5 mL trypsin and incubate 1–2 min (37°C)
3. Tap cells gently against counter to ensure detachment, add 3 mL cell medium, and use a 2 mL pipette to resuspend to single cells
4. Transfer **two cell flasks to one centrifuge tube**, to ensure sufficient number of cells for analysis
5. Centrifuge at 200 G for 5 min, and remove supernatant
6. Resuspend in 8 mL PBS and split each sample in two: **sample** and **secondary ab control**
7. Add 2 mL PBS to all tubes, centrifuge at 200 G for 5 min, and remove supernatant
8.
    a. To **samples**: Resuspend in 150 µL primary ab solution
    b. To **secondary ab controls**: Resuspend in 150 µL PBS with 1% BSA
9. Incubate 30 min (on ice)
10. Wash twice in 5 mL **ice cold** PBS (centrifuge 200 G, 5 min, 4°C) and remove supernatant
11. Resuspend all samples in 150 µL secondary ab solution and incubate 30 min in the dark (on ice)
12. Wash twice with 5 mL **ice cold** PBS (centrifuge 200 G, 5 min, 4°C) and remove supernatant.
13. Resuspend in 150 µL PBS
14. Keep samples on ice in the dark until flow cytometry analysis
15. Immediately before analysis, add 0.5 µL PI solution for live/dead staining
16. Filter and run flow

Solutions

- **TGF-β3 solution**
    - 50 µg/mL in 4 mM HCl
- **PBS**
- **Trypsin (37°C)**
- **PBS with 1% BSA**
- **Primary ab solution**
    - Anti-α-SMA primary antibody diluted 1:600 in PBS with 1% BSA
- **Secondary ab solution**
    - Alexa Fluor 647 goat anti-rabbit IgG diluted 1:400 in PBS with 1% BSA

- **PI solution**
    - 1.0 mg/mL in PBS

Data Analysis

α-SMA expression intensity $\bar{I}$ is calculated using the following formula:

$$\bar{I} = \frac{(\overline{S-S_2})-(\overline{C-C_2})}{\overline{C_2}};$$

where S is the median α-SMA fluorescence intensity of a given sample, S_2 the median intensity of its secondary control, C the median intensity of the control sample, C_2 the median intensity of the secondary control to the control sample, and $\overline{C_2}$ is the mean of the median intensities of secondary controls to the control samples in the experiment.