Generation of stem/progenitor cultures from mouse lungs

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Method Article

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Abstract

Introduction

Cell renewal is essential to maintain tissue homeostasis and repair throughout the life of multicellular organisms. These functions rely on the presence of stem cells that have the potential to differentiate into specialized cell types as well as the capacity for long-term self-renewal. Adult organs therefore need long-life reservoirs of somatic stem cells to ensure the turnover of the tissue. These stem cells are localized in specific areas known as “niches”, which provide the right environment for the viability and function of the stem cell. The adult lung is an organ with slow turnover. There are different regions in the respiratory system, such as large airways and small distal bronchioles, which house different stem cell types that contribute to the maintenance and repair of lung tissue. Recently, a population of stem cells referred to as bronchioalveolar stem cells, has been identified at the bronchioalveolar duct junctions and has been shown to be important in maintaining bronchiolar Clara cells and alveolar cells of the distal lung. However, very little is known about the regulation of these lung stem/progenitor cells and the signals that induce their conversion into cancer cells. We have now used a modified version of previous protocols to isolate and maintain in culture lung stem/progenitor cells.

Reagents

DMEM media PBS Fetal Bovine Serum FibrOutTM \(CHI-SCIENTIFIC) FGF, EGF, Insulin Antibodies: Sca1, CD45, PECAM, SP-C, CC-10

Equipment

96 well, 6 well, 35 mm and 100 mm culture plates Flow Cytometer 40 and 70 microns strainers Tissue culture incubators at 37C, 5% CO_2

Procedure

1. Mechanical disruption of mice lungs passing the homogenates through a 70 µm strainer to obtain single cells. 2. Plate the cells in DMEM supplemented with 20% FBS and 30 µg/ml Insulin \(Sigma). Incubate at 37C in a 5% CO_2 atmosphere. 3. After 4 h to allow the cells to attach to the plate, FibrOutTM \(CHISCIENTIFIC) was added to eliminate fibroblasts. 4. Keep he cells under these conditions for 2 weeks, changing the media every 3 days and splitting them when needed. 5. After that time, the cells are changed to serum free DMEM supplemented with FGF \(10 ng/ml) and EGF \(20 ng/ml). 6. After 2 or 3 weeks the resulting cell population must be analyzed by flow cytometry. An aliquot of these cells fixed, permeabilized and labeled with SPC and CC-10 antibodies confirming the percentage of the double positive cells \(SPC+/CC10+). 7. The remaining cells are to be sorted for Sca-1+/CD45−/PECAM− using a FACS Aria cytometer \(BD Bioscience). 8. After re-plating, the sorted cells are maintained in serum free media \(plus FGF and EGF). An aliquot of these cells must be analyzed again by flow cytometry and

confirmed to be SPC+/CC10+. Thus, the final population in culture should be SP-C+/CC-10+/Sca1+/CD45–/PECAM–. 9. To isolate clones, serial dilutions of the cells were plated in 96 well plates to obtain wells with single cells. Six of these clones obtained can be expanded in serum-free media and then tested for their potential to differentiate. The stem/progenitor cells tend to aggregate when maintained in serum free media, but in the presence of 20% FBS the cells expand to form single cell layers and start differentiating.

Timing

5 to 7 weeks

Critical Steps

2. This is a critical step. A good quality population of cells is needed to success. If the cells have been damaged too much during isolation the culture could not progress. Try not to be too harsh during isolation and do not stress the cells. Allow them to recover from the stressing procedure before going on.

Troubleshooting

1. Avoid the proximal lung tissue \(trachea and bronchus) and use exclusively tissue from the distal lung \(alveolar epithelia), where the SPC+/CC10+ double positive stem cells are localized. 2. Allow the cells from the lung homogenates to set and attach to the plates properly before adding the FibOut. 4. Do not split the cells when they are at low confluency.

Anticipated Results

You should obtain a culture of cells forming clusters or aggregates when maintained in serum free media plus FGF and EGF. These cells should be able to slowly proliferate under these conditions.

References

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