Human Alveolar Epithelial Type 2 Cells in 3-D Culture

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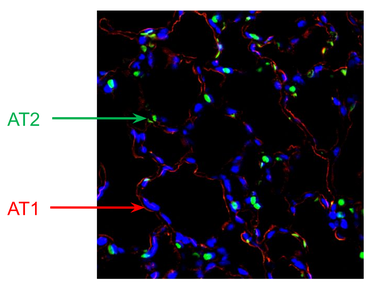
Abstract

The lung alveoli are vulnerable to damage and injured alveoli leading to respiratory failure in chronic lung diseases such as idiopathic pulmonary fibrosis (IPF). Alveolar epithelial type 2 (AT2) cells have been discovered to act as progenitor cells for the alveolar epithelium repair after lung damage. Understanding the regenerative potential of AT2 cells is crucial for studying and developing treatments on alveolar cells dysfunction. Our study focus on investigating the effects of different growth conditions on the proliferation and differentiation of AT2 cells, in order to establish a standard model for AT2 cell culturing. Here, we use isolated human AT2 cells and grow them in 3-D culture under different culture conditions, and we investigate the formed spheroids through high-resolution imaging. To eliminate confounding effects, we excluded MRC5, a fibroblast cell line typically used for supporting AT2 cell growth, in our cell culture. Our results show AT2 cell form spheroid and express the AT1 marker, AQP5, in the differentiation condition.

Introduction

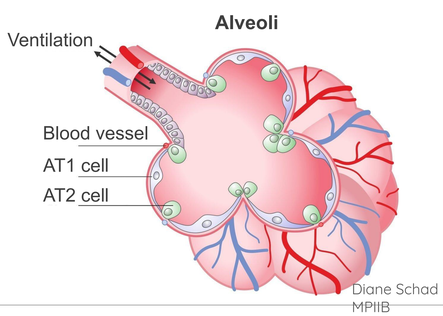
The lung alveolar epithelium contains two types of epithelial cells. Alveolar type 1 (AT1) cells are thin squamous cells that function for gas exchange, and express a transmembrane protein called aquaporin 5 (AQP5). Alveolar type 2 (AT2) cells are cuboidal shaped and produce surfactant proteins to prevent alveolar collapse. Thus, AT2 cells are able to be detected with surfactant protein C (SPC) staining, which is expressed exclusively in type 2 cells. AT2 cells also act as progenitor cells that self renew and give rise to AT1 cells during lung repair.



Fluorescence microscopy of AEC1 and AEC2 in rat lung

Nkx2.1 staining: nucleus, green

AQP5 staining: apical membrane, red

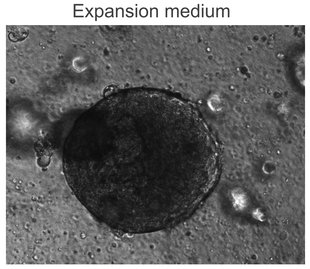


Methods

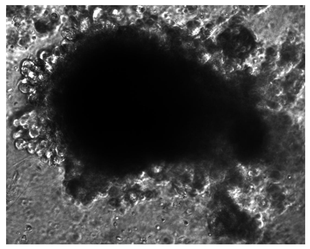
3-D culture: AT2 cells were suspended in a solution of 1:1 growth factor reduced (GFR) Matrigel and expansion medium (STEMCELL). All 6 wells were initially treated with expansion medium, and half of the wells were changed to treat with differentiation medium starting at Day 11. The cells were cultured for a total of 20 days.

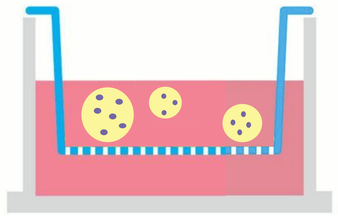
10x Microscope Image at Day 20

Expansion medium



Differentiation medium



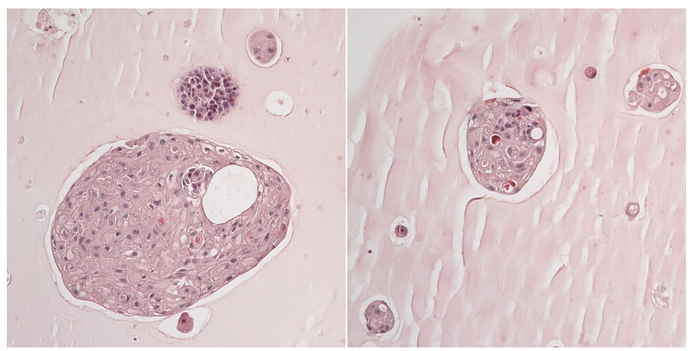


Results

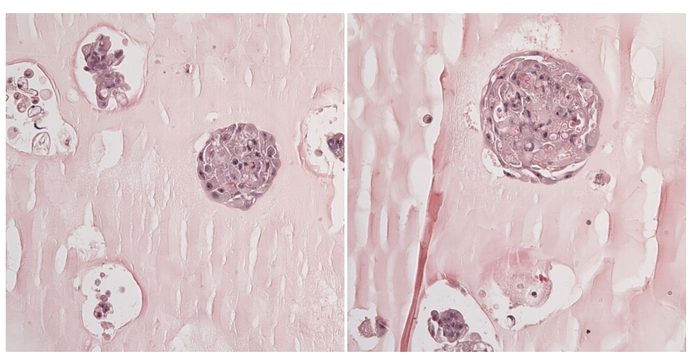
Hematoxylin and Eosin (H&E) Staining is used in histology and helps give a detailed view of tissue structures.

- Purple staining (Hematoxylin) – nucleus
- Pink staining (Eosin) – cytoplasm

Expansion medium 20x



Differentiation medium 20x

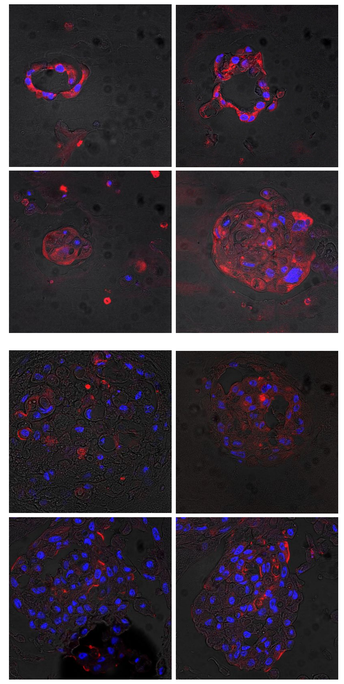


Results

Immunofluorescence (IF) staining: Antibodies against AT1 cells (AQP5) and against AT2 cells (SPC) were used. Results were imaged by Confocal microscopy, which provides high resolution images on detected areas and eliminate out-of-focus parts.

Expansion medium
Red – SPC (AT2 marker)
Blue – nucleus

Differentiation medium
Red – AQP5 (AT1 marker)
Blue – nucleus



Summary and Future Directions

- Overall, variations in the development of AT2 cell colonies in different culture conditions can be seen through the microscope images. The edges of the colonies in expansion medium had a round and smooth edge, while the colonies in the differentiation medium had bubble-like aggregates around the edge.
- The different morphologies are also shown through the H&E images, where in the expansion medium, colonies appear solid throughout the cross-section, while ones in differentiation medium have less nucleus, which may be caused by increased cell death as a result of differentiation.
- The Immunostaining show some expression of AQP5 in the differentiation group, suggesting differentiation of AT2 cells into AT1 cells occurred.
- Additional information from immunostaining using antibodies against more AT2 and AT1 markers is still needed to have a comprehensive view on AT2 cell differentiation.

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