The Role of IL-13 in Chronic Obstructive Pulmonary Disease in Distal Airway Stem Cells

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ABSTRACT

Chronic Obstructive Pulmonary Disease (COPD) is a type of lung disease that involves the rapid destruction of the respiratory tract where oxygen exchange takes place and is widely known as the leading cause of death. COPD is caused due to reduced air flow in and out of the airways due to the destruction of the walls of the air sacs which results in massive inflammation[1]. Pathological changes in COPD are commonly detected in the airways and alveoli. A common symptom of COPD is mucus hypersecretion caused by elevated numbers of goblet cells (goblet cell metaplasia or GCM) in patients' tissues[2]. This mucus overproduction is usually caused by airborne pollutants, microscopic organisms, and regulators of inflammation[2]. A common regulator of GCM is Interleukin 13 or IL-13. Interleukins are a group of natural proteins that regulate communication between cells. They regulate cell growth, and trigger inflammation when secreted in response to a stimulus. IL-13 is known to be a central regulator of GCM[2]. Our objective is to prove is the presence of IL-13 truly leads to overproduction of goblet cells. By comparing both normal and advanced COPD tissues, we are able to detect visible phenotypic differences which will help us link IL-13 to GCM.

METHODS

Identification of phenotypic diversity- Cells were harvested using the Air-Liquid Interface cell culture method where stem cells are grown in their respective media while the top of the cellular layer is exposed to air. We grew cells in a controlled environment and one treated with IL-13. The purpose is to study the goblet cells in the lining of the respiratory epithelium. Then they were embedded in paraffin wax and cut into sections with Thermo Scientific Microm HM 355 S Microtome, and stained accordingly. Two main methods of staining were used in the process:

- **Hematoxylin & Eosin**- The slides were deparaffinized by Xylene (StatLab) and rehydrated through a series of ethanol. They were then stained in Hematoxylin (Poly Scientific) and Eosin (Poly Scientific) and identified by microscopy (Nikon Eclipse Ti) using Elements imaging software (Nikon) for a detailed structure of the cells.

- **Immunofluorescence Antibody Staining**- The slides that were stained with various antibody markers to differentiate the distal airway ALIs include Alpha Tubulin (Santa Cruz), Mucin 5AC (Abcam), and Mucin 5B (Abcam). The cells were identified by microscopy and DAPI was utilized for nuclear counterstaining.

RESULTS

Figure 1

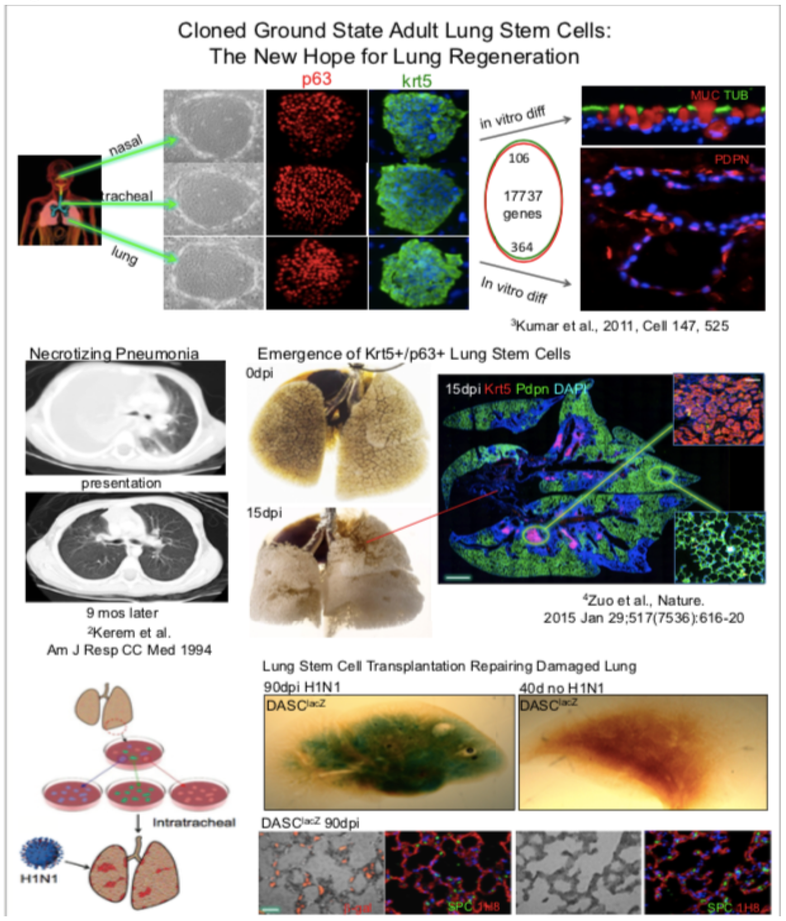
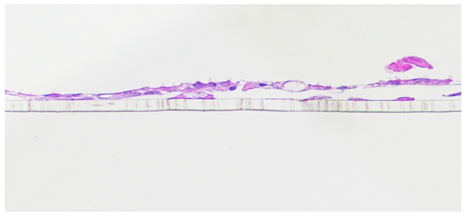
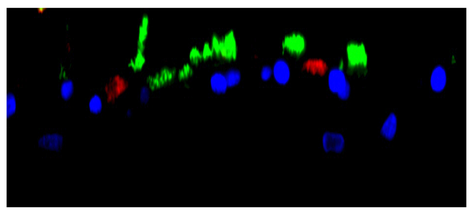


Figure 3: Control ALI *in vitro* characterization

Hematoxylin & Eosin



Muc5AC & Alpha Tubulin



Muc5B

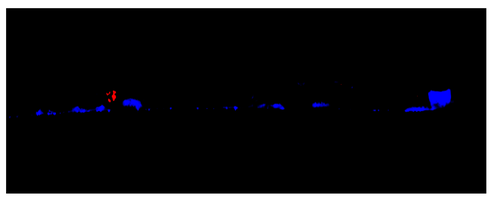
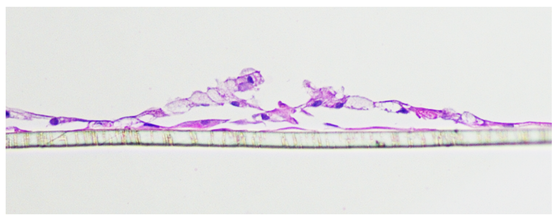
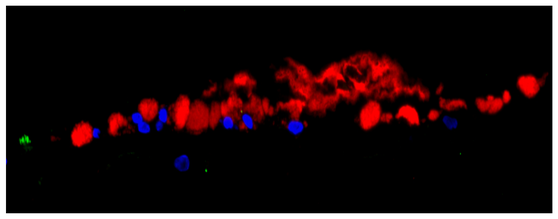


Figure 4: Advanced COPD ALI *in vitro* characterization

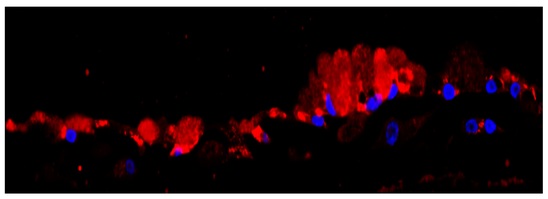
Hematoxylin & Eosin



Muc5AC & Alpha Tubulin



Muc5B



RESULTS

- The H&E staining shows a minimal goblet cells count in the control ALI while the ALI with advanced COPD reveals overproduction of goblet cells due to IL-13.
- In the IF staining of Muc5AC & Alpha Tubulin markers, the COPD ALI expose a large number of goblet cells that secrete mucus, which is a sign of goblet cell metaplasia possibly due to the presence of Interleukin 13.
- The Muc5B staining also reveals inflammation of mucus secretory cells in the COPD ALI compared to the minimal staining in the control ALI.

CONCLUSIONS

- When comparing the distal airway stem cells of patients with and without COPD, the ALIs show numerous pathogenic phenotypes in the ALI treated with IL-13 like goblet cell metaplasia. These features could contribute to the advancement of chronic obstructive pulmonary disease
- Future research must be geared towards understanding the epigenetic structures of the pathogenic stem cells to eliminate lung diseases.

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