Assessing the Impact of NOTCH Signaling on RAS Cell Progenitor Function: Implications for Pathophysiology

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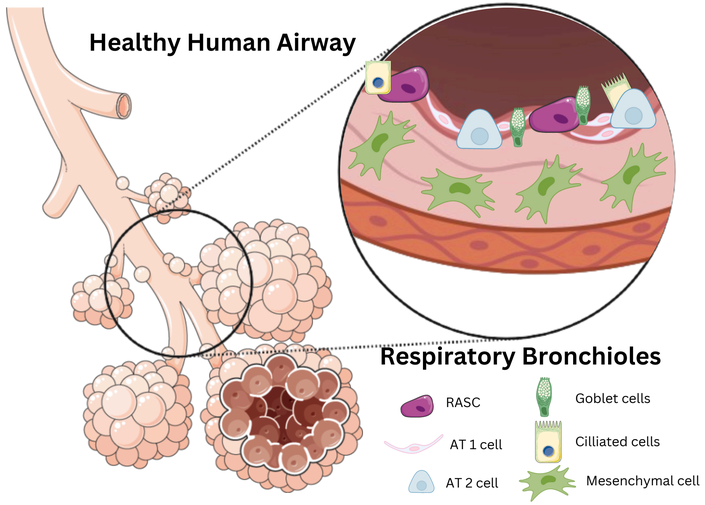
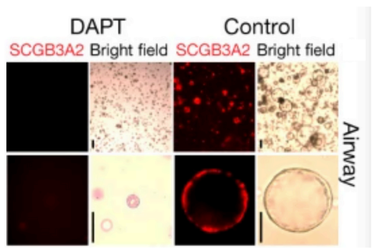
Introduction

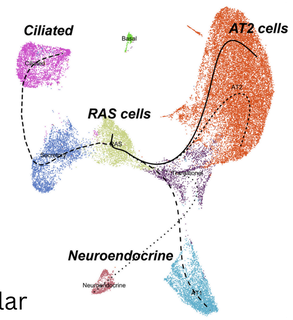
- Respiratory Airway Secratory (RAS) Cells are a newly identified progenitor cell located in the human respiratory bronchiole, which are the smallest airway in human lungs
- RAS cells have been shown to be progenitors of alveolar epithelium type 2 (AT2) cells
- Our evidence suggests they may also have a progenitor function for the airway epithelium
- These cells are suspected to play an important role in the pathogensis of chronic obstructive pulmonary disease (COPD) and other diseases
- In COPD the transcriptional profile of RAS cells is altered, leading to associated atypical alveolar type 2 cell states, a phenomenon seen in smoking exposure in both humans and ferrets.
- These cells are only found in the distal airways which are known to be an injury site for COPD
- NOTCH and WNT have been shown to play a role in RAS cell identity



Background Data

Notch inhibition using DAPT of iRASC, revealed loss of RAS cell fate





Pseudo time analysis of cells from distal human lung reveal putative relationships between RAS cells and other epithelial cells

Designing a Human Distal Airway ALI interface

- iRAS cells in 24-well ALI plate. 25k cells per well
- Culture was collected after 21 days

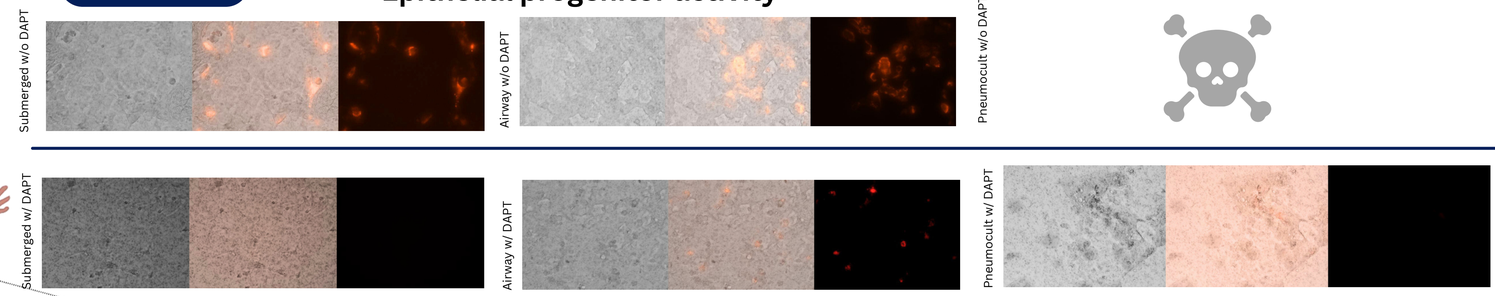
CONDITIONS:

- Airway media submerged: w/o DAPT & w/ DAPT
- Airway media airlifted: w/o DAPT & w/ DAPT
- Pneumacult media airlifted: w/o DAPT & w/ DAPT

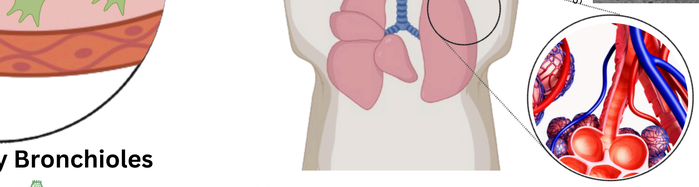
Hypothesis

The NOTCH signaling pathway modulates RAS cell Airway Epithelial progenitor activity

Results

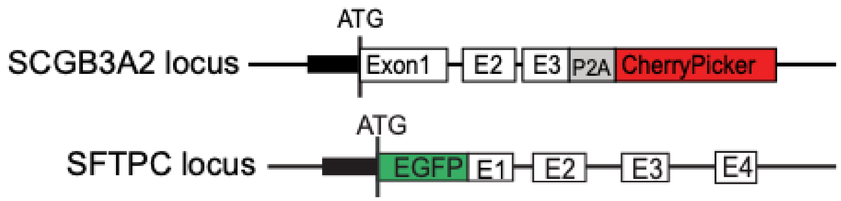


Immunofluoresence of ALI after 21 days of culturing

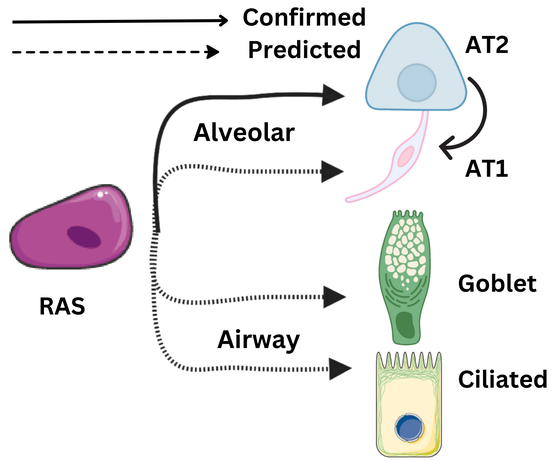


Conclusion:

RAS cell fate was significantly reduced with the addition of DAPT



RAS cell progenitor ability:



References

1. Basil, Maria C, et al. "Lung Repair and Regeneration: Advanced Models and Insights into Human Disease." Cell Stem Cell, vol. 31, no. 4, 1 Apr. 2024, pp. 439–454, https://doi.org/10.1016/j.stem.2024.02.009. Accessed 21 Aug. 2024.
2. Basil, M.C., Cardenas-Diaz, F.L., Kathiriya, J.J. et al. Human distal airways contain a multipotent secretory cell that can regenerate alveoli. Nature 604, 120–126 (2022). https://doi.org/10.1038/s41586-022-04552-0

Next steps

1. Confirm model and determine if change in airlift duration impacts system efficiency
2. Determine Epithelial cell types in each condition and quantify differentiation with imaging and RT-PCR
3. Test NOTCH over expression using lentiviral NOTCH-3-ICD
4. Test how differentiation is impacted when RAS cells are exposed to a hypoxic environment and cigarette smoke