Quantitative Mapping of Alveolar Epithelial Cell Renewal *in vivo*

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Abstract

The gas exchange region of the mature lung is lined by squamous type-1 (AT1) and cuboidal type-2 (AT2) alveolar epithelial cells. We employed a genetic nuclear protein label retention assay to 1) quantitatively map AT1 & AT2 cell activity and behavior *in vivo*, and to 2) determine whether or not AT1 cells have mitotic potential.

We pulse labeled AT1 & AT2 cells and mapped label dilution with progressive aging over a chase period, employing the well-established rtTA-TetO system. By measuring the quantal dilution of nuclear GFP in individual cells over time, we can estimate the frequency of AT1 & AT2 cellular division, and infer whether all or some AT2 cells function as progenitors.

Pulse-labeled AT1 & AT2 cells presented a normal distribution of nuclear GFP quantity. Following a 12-month chase, AT2 cells displayed significant GFP signal intensity loss; AT1 cells displayed significant GFP signal intensity loss following a 3-month chase. Our working model suggests that both AT1 and AT2 cells divide.

Introduction

Lung cancer is by far the leading cause of death in both males and females, with over 27% of cancer deaths being attributed to the disease; over 220,000 new cases of lung cancer will arise during 2014. Unlike other organ systems, many questions remain unanswered in the field of lung biology - where does proliferation of cells begin? Where does cell lineage begin? How do alveoli maintain themselves? Answers to these questions will allow us to develop pharmacological treatments for lung cancer, emphysema, and fibrosis, among others.

Introduction (cont.)

Currently, there are no stem cell transplant therapies for lung diseases, and very little is known about the location and function of progenitor cells in the lungs. Research suggests that rare AT2 cells can intermittently give rise to AT2 and AT1 cells in localized foci; however, the frequency and cellular mechanisms of renewal remain unknown. Understanding these lung biology fundamentals will allow us to discover the cellular mechanisms behind AT2 and AT1 cell differentiation.

Methods & Materials

- Pancreas and lung cells were marked with the histone-2B Green Fluorescent Protein (H2B-GFP) genetic nuclear label, paired with the Rat Insulin Promoter (RIP) in the pancreas and Surfactant protein C (SpC) & Aquaporin 5 (Aqp5) in the lungs.

Figure 1. Gene expression mechanisms. Cre recombinase (Cre) is produced from the Aqp5 promoter; Cre deletes the polyadenylation (pA) stop sequence, allowing for expression of the reverse tetracycline transactivator (rtTA). rtTA is activated by doxycycline (Dox), inducing GFP expression.

- rtTA/TetO-GFP system was used for nuclear labeling

Methods & Materials (cont.)

- Pancreas model was utilized to identify any endogenous turnover or degradation of the nuclear GFP label
- GFP expression is doxycycline (Dox) inducible, specimens were pulsed with Dox
- Mouse pancreas and lung specimens were collected at pulse and chase periods from mice that expressed desired characteristics
Pancreas: RIP-rtTA⁺/TetO-GFP(β -cell)
Lung: SpC-ER-rtTA⁺/TetO-GFP (AT2)
Aqp5-Cre⁺/LSL-rtTA/TetO-GFP (AT1)
- Tissues were fixed in PFA, then stained overnight with DAPI
- Pancreatic samples were viewed under a stereoscope to visualize pancreatic islets

Figure 2. Pancreatic β -cell islets. Groups of fluorescent β -cells (RIP).

- Samples were visualized with a Leica TCS SP8 Inverted Confocal Microscope
- Microscope images were analyzed with the Leica Advanced Fluorescence Application Suite and CellProfiler

Results

Figure 3. β -cell GFP retention. The pancreas model confirmed that there was no significant loss of GFP signal due to protein degradation between the pulse & chase experiments

Results (cont.)

Figure 4. Pancreatic β -cell fluorescent signal with 3 mg Dox dosage (RIP). Frequency of fluorescent signal levels remained constant and did not change significantly ($p > 0.05$) over the 23 day period during which pancreatic cells divide infrequently. *Insets: GFP-marked β -cells in respective chase samples.*

Figure 5. Quantification of Nuclear GFP in AT1 cells (Aqp). A 95 day gap between pulse & chase experiments yielded a 72% decrease in signal intensity ($p < 0.05$). *Visualized alveolar tissue - Image 1: 2 month, 6 day old mouse Image 2: 5 month, 15 day old mouse.*

Figure 6. Quantification of Nuclear GFP in AT2 Cells (SpC). A 12 month gap between chase experiments yielded a 58% decrease in signal intensity ($p < 0.05$). *Visualized alveolar tissue - Image 1: 4 month, 9 day old mouse Image 2: 16 month, 9 day old mouse.*

Conclusions

Both AT1 & AT2 cells demonstrated a loss of nuclear label with progressive specimen aging, presumptively due to cell division. AT1 cell labeling had a calculated half-life of 1 month 22 days; AT2 cell labeling had a calculated half-life of 9 months 22 days. As the endogenous turnover of the H2B-GFP nuclear marker is insignificant, the results suggest that the decrease in signal in AT1 and AT2 nuclei is due to a single cell division in this time period.

Future Research

Intermittent repeated cellular ablation will be used to distinguish distinct populations of AT2 cells. Analysis of multiple chase images after the inferred AT1 & AT2 cell division will allow us to determine the precise cell cycle duration of these cells. Multi-colored labeling and fate-mapping of potential stem cells can be used for clonal analysis of lung cells. Further understanding of AT1 & AT2 cell behavior and mechanisms will allow for therapies to be developed for disease treatment.

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