CYSTIC FIBROSIS MOUSE MODELS DISPLAY AGE DEPENDENT AIRWAY BASAL STEM CELL HYPERPLASIA

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Introduction:
We have shown that rare multipotent airway basal stem cells in adult mouse airways can be identified and isolated via FACS ($EpCAM^{pos}\alpha6\text{-integrin}^{pos}\beta4\text{-integrin}^{pos}CD24^{low}$) and subsequent clonogenic assay. These basal stem cells can self-renew and produce lineage-restricted airway and alveolar progenitor cells when co-cultured with lung stromal cells and cytokines. Using this assay system we have previously reported stem cell hyperplasia in the airways of 7 month old CF mice compared to age-matched normal mice. The aim of this pilot study was to assess whether basal stem cell hyperplasia was also present in CF mice earlier in life.

Methods:
The tracheal airways from 4.5 month and 7 month old normal CF littermates (Het UNC), CFTR knockout (CF UNC), and CFTR gut-corrected (CF FABp) mice were excised and cells disaggregated. FACS-sorted cells ($CD45^{neg}\,CD31^{neg}\,EpCAM^{pos}\,\alpha6\text{-integrin}^{pos}\,\beta4\text{-integrin}^{pos}\,CD24^{low}$) from the six groups of mice were then cultured within a matrigel-based clonogenic assay system to quantify the number of basal stem cells present as a percentage of total airway basal cells isolated per trachea.

The lung epithelial colony-forming cell assay:
$CD45^{neg}CD31^{neg}EpCAM^{hi}\,CD24^{low}$ lung cells generate colonies (CFU) comprising cells of both airway and alveolar epithelial lineages when co-cultured in matrigel with $Sca\text{-}1^{pos}EpCAM^{neg}$ mesenchymal cells and mesenchyme-derived growth factors (Fig 1 & 2).

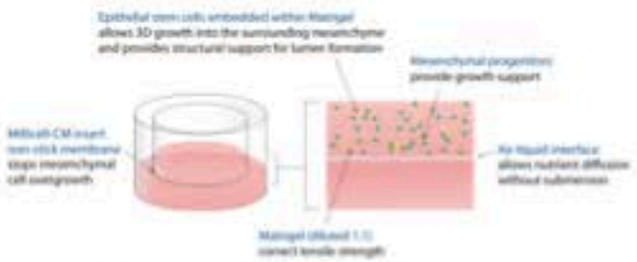

Figure 1: Schematic description of the airway stem cell assay system.

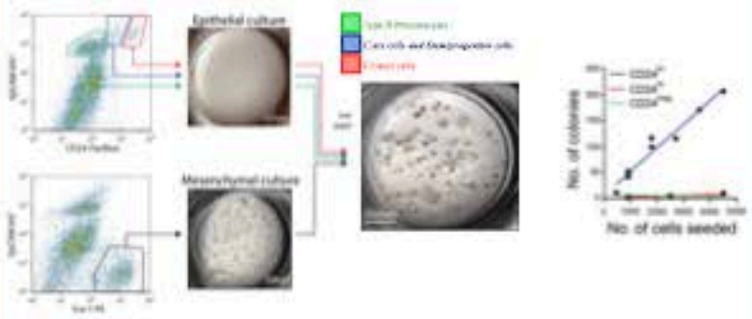

Figure 2: The clonal growth of $CD45^{neg}CD31^{neg}EpCAM^{hi}\,CD24^{low}$ basal stem cells reveals an obligatory requirement for mesenchymal support. There is a linear relationship between CFU incidence and cells plated. Colony-forming potential is regulated by mesenchyme-derived stimulatory and inhibitory factors.

Upper airway epithelial stem/progenitor cells in mouse models of cystic fibrosis :
The 4.5 month age group displayed a 1.4- fold increase in the number of basal stem cells in the tracheal epithelia of CF UNC mice, compared to normal heterozygous (Het UNC) mice. However, the number of basal stem cells in the CF FABp and Het CF mice were the same at 4.5 months. In contrast, in the 7 month age groups we observed 4.9-fold and 1.9-fold increases in basal stem cells in CF UNC and CF FABp mice respectively, compared to Het CF mice (Fig 3).

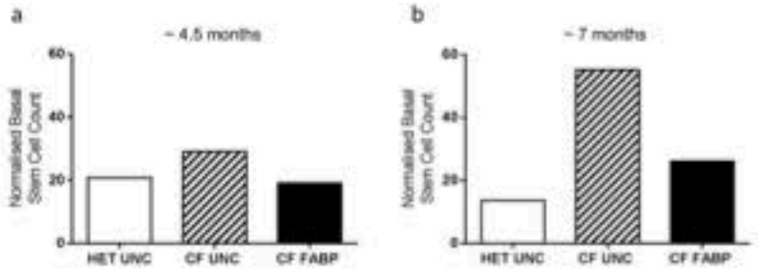

Figure 3: The relative incidence of tracheal airway basal stem cells in Het UNC, CF UNC, and CF FABp mice. A) At 4.5 month age CF UNC mice displayed a 1.4 fold increase in the number of epithelial stem cells compared to the Het UNC mice. B) At 7 month age the CF UNC and CF FABp mice displayed 4.0 and 1.9 fold higher numbers of respiratory epithelial stem cells respectively when compared to the Het UNC controls.

Upper airway epithelial stem/progenitor cell proliferation index:
Sections containing excised tracheae were evaluated for stem/progenitor cell proliferation via immunohistochemistry for a nuclear proliferation antigen, Ki-67. A 2.1 fold increase in proliferating cells was observed in homozygous CF (FABP) mice compared to heterozygous CF (UNC) mice. (Fig 4)

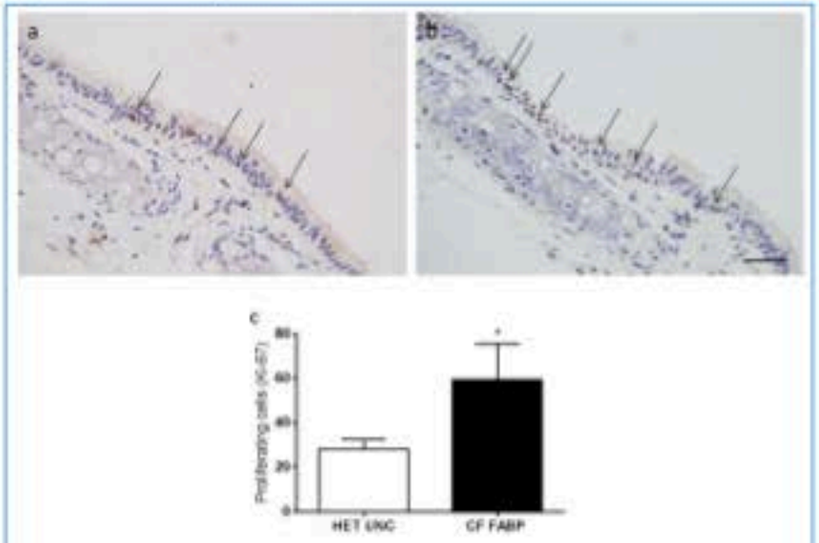

Figure 4: Ki-67 immunohistochemistry of the tracheal airway epithelium from (a) Het UNC and (b) CF FABp mice. (c) There was a significantly greater number of proliferating cells positive for Ki-67 (black arrows) in the CF FABp mice than the controls (*$p < 0.05$, t-test), (scale bar 50 µm)

Summary:
These findings suggest that basal stem cell hyperplasia in the airways of CF mice is not present initially, but may develop as mice age. The increased incidence of basal stem cells in older CF mice suggests there is a progressive increase in the activity of the stem cell compartment, which may contribute to the progressive remodelling of CF airways with age. These findings suggest that early gene therapy correction of CF mouse airways may prevent abnormal hyperplasia of CF airway basal stem cells. If so, hyperplasia of descendant lineages, such as mucin-containing goblet cells, might similarly be beneficially reduced.

Acknowledgements :
Cure4CF Foundation, Centre for Stem Cell Research, Robinson Research Institute, N Farrow was supported by a MS McLeod PhD Scholarship.