Evidence of an expanded and dysregulated airway epithelial stem cell compartment in cystic fibrosis mice

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Introduction:

Recent studies have shown that FACS sorting and *in vitro* clonogenic assays are powerful tools for detection and quantitation of endogenous epithelial stem/progenitor cells (EpiSPC) in the adult mouse lung. We have used this approach together with *in situ* Ki-67 labeling to analyse the EpiSPC compartment in the tracheae of CF and normal mice.

In a separate series of experiments we employed a polidocanol detergent transient injury model to analyse the pattern of nasal airway EpiSPC regeneration following *in situ* lentviral (LacZ) reporter gene transduction of nasal airway epithelial cells in normal mice.

Methods:

EpiSPC isolation and colony-forming assay:

Tracheal cell suspensions from normal, CF (UNC) and CF(FABP) mice were prepared by mincing and collagenase digestion, and $CD45^{neg}CD31^{neg}EpCAM^{pos}CD24^{low}$ cells were isolated and co-cultured in our matrigel colony-forming assay together with supporting MLg cells to quantify epithelial colony-forming cells (Epi-CFU)[1,2]. The incidence of Epi-CFU in CF and normal tracheae was correlated with *in situ* measurement of epithelial cell proliferation by immunohistochemical detection of Ki-67 labeling.

Figure 1: Schematic description of the lung EpiSPC assay system.

LPC/LV-LacZ reporter gene transduction:

Mice were pre-treated with 0.3% lysophosphatidylcholine in PBS followed by instillation of a HIV-1-VSV-G pseudo typed lentiviral vector carrying the LacZ gene[3].

The Polidocanol transient injury model:

We employed a polidocanol detergent transient injury model to analyse the pattern of nasal airway epithelial regeneration in normal mice following *in situ* lentviral (LacZ) reporter gene transduction.

Following viral vector instillation, mice were randomly divided into three groups (n=11) with one group acting as a control. The second and third groups of mice received polidocanol at 4 weeks (single treatment) , or at 4 weeks and 8 weeks (double treatment) following LacZ reporter gene transduction to strip the epithelium according to the methods described by Borthwick *et al*[4].

Results:

Elevated incidence of Epi-CFU, and of epithelial hyperproliferation, in CF mouse trachea:

We detected a 5.2-fold and a 2.4-fold increase in the incidence of Epi-CFU in the $CD45^{neg}CD31^{neg}EpCAM^{pos}CD24^{low}$ tracheal cell fraction of CF(UNC) and CF(FABP) mice respectively, compared to normal wildtype mice (Fig 2A). The elevated incidence of tracheal Epi-CFU was correlated with a commensurate increase in the incidence of proliferating CF tracheal epithelial cells measured by in situ immunohistochemical Ki-67 labelling (Fig 2B).

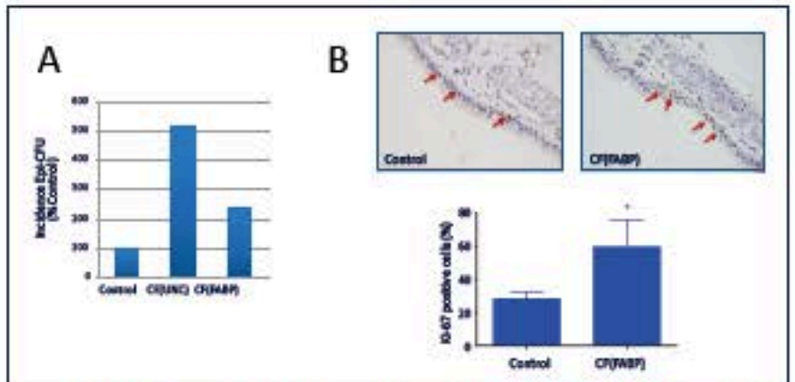

Figure 2: A) The incidence of Epi-CFU detected in sorted CF (UNC) (36/1000) & CF (FABP) (76/1000) cell suspensions is significantly higher than the equivalent fraction of normal tracheal epithelial cells (1/1000). B) Immunohistochemical staining of the proliferating nuclear antigen (Ki-67) in wildtype and CF(FABP) tracheal tissue sections.

Evidence of lasting nasal epithelial stem/progenitor cell transduction and regeneration in a polidocanol transient injury mouse model:

In a parallel series of experiments we analysed the pattern of *in situ* nasal epithelial stem/progenitor cell proliferation in the nasal septum of wildtype C57BL/6 mice which had been treated by instillation of a LacZ lentiviral vector as previously described[3].

Patchy expression of LacZ is evident 1 week following virus instillation, and was sustained in all animals for at least 14 weeks (Fig 3). By 14 weeks, the presence of large clusters of LacZ positive cells was consistent with the proliferation of transduced cells, and the analysis of LacZ expression in the polidocanol transient injury model provides evidence of transduction of long-lived nasal epithelial stem/progenitor cells.

Polidocanol denudes nasal airway surface epithelium and rapid re-epithelialisation occurs in the next week (Fig 3). Single and double polidocanol-ablated mice were characterised by the presence of large clusters of $LacZ^{pos}$ cells derived from polidocanol resistant nasal stem/progenitor cells with high regenerative capacity.

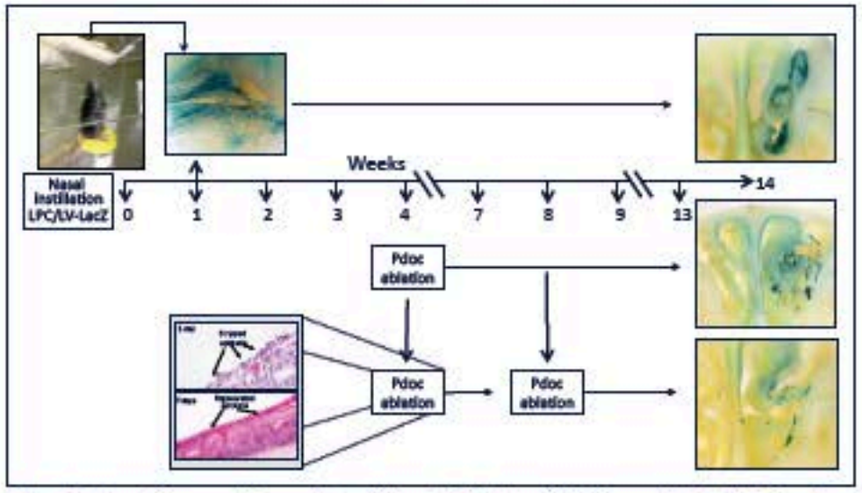

Figure 3: Schematic representation of the polidocanol (Pdoc) transient injury model for detection of long-lived LacZ-transduced nasal epithelial stem/progenitor cells. Images of *en face* mouse nasal airways displaying LacZ positive staining were captured by serial focusing. Images were then combined using Z-stacking to generate a single in-focus image of the section.

Summary:

Our findings are consistent with the notion that:

1) An expanded and dysregulated airway EpiSPC compartment contributes to airway remodelling and mucous cell hyperplasia in CF mice.
2) Flow cytometric analysis and sorting can be used to identify and prospectively isolate EpiSPC which could be targeted in CF cellular therapies.
3) Transient epithelial injury reveals the pattern of nasal airway epithelial stem/progenitor cell regeneration.

References:

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Acknowledgements:

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