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 ... and quantitation of endogenous epithelial stem/progenitor cells (EpiSPC) ... approach together with *in situ* ... to analyse the EpiSPC compartment in the trachea ... CF and normal mice.

In a separate series of experiments we employed a polidocanol transient injury model ... to analyse ... regeneration following *in situ* lentiviral (LacZ) ... 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 ... CD24^{low} cells were isolated and co-cultured in ... together with supporting MLg cells to qua... (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:

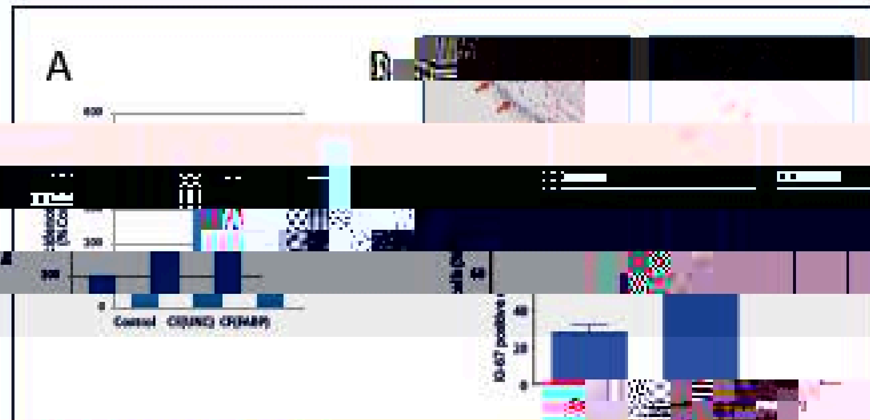
We employed a polidocanol detergent transient injury model to analyse the pattern of nasal airway epithelial ... lentiviral (LacZ) reporter ge...

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

Results:

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

... CD45^{neg}CD31^{neg}EpCAM^{pos}CD24^{low} tracheal cell fraction of CF ... mice respectively, compared to normal wildtype mice ... the incidence of proliferating CF tracheal epithelial cells measured *in situ* ... immunohistochemical Ki-67 labeling (Fig 2B).



... cell suspensions is significantly higher than the equivalent fraction of normal tracheal epithelial (1/1000). B) Immunohistochemical staining of the proliferation nuclear antigen (Ki-67) in ... and CF(FABP) tr... tissue sections.

... and regeneration in a polidocanol transient injury mouse model:

In a parallel series of experiments we analysed the pattern of *in situ* ... epithelial stem/progenitor cell proliferation in the nasal septum of ... sustained ... 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 ... stem/progenitor cells.

Polidocanol denudes nasal airway surface epithelium ... occurs in the next week (Fig 3). Single and double p... characterised by the presence ...

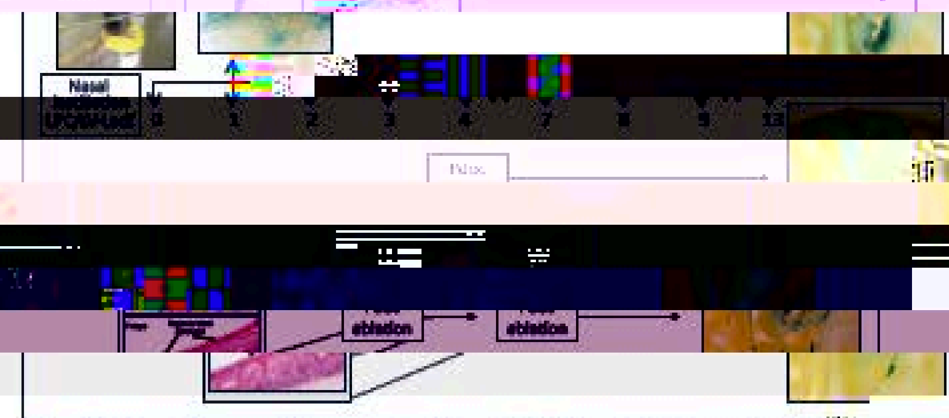


Figure 3: Schematic representation ... of polidocanol (PdOC) trea... long-lived LacZ-transduced nasal ... stem/progenitor cell...

1) An expanded and dysregulated airway EpiSPC compartment contributes to airway remodelling and mucous cell hyperplasia in CF mice.

... isolate EpiSPC which could be targeted in CF cellular therapies.

3) Transient epithelial injury reveals the pattern of nasal airway ... regeneration.

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3. ... Stocker AG, Kremer KL, Koldej R ... lifetime airway gene expression." J Gene Med 11: 861-867.
4. Borthwick DW, Shahbazian M, Krantz QT, Dorin JR, Randell SH. (2001). Evidence for stem-cell niches ...

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