ENDOGENOUS STEM/PROGENITOR CELL COMPARTMENTS OF CONDUCTING AIRWAYS DIFFER IN CYSTIC FIBROSIS AND NORMAL MICE

Nigel Farrow[1, 2], Jonathan L McQualter[3], David Parsons[1,2], Ivan Bertoncello[3].

[1]Department of Respiratory and Sleep Medicine, Women's and Children's Hospital; [2]Center for Stem Cell Research, and Children's Research Center, University of Adelaide, South Australia.

[3] The Lung Health Research Centre, Department of Pharmacology, University of Melbourne, Victoria.

Introduction:

Recently McQualter *et al* (PNAS 107:1414) prospectively isolated and characterized rare EpCAM^{pos}α6-integrin^{pos}β4-integrin^{pos}CD24^{low} multipotent adult lung epithelial stem/progenitor cells (EpiSPC) in mice. These cells can self-renew and give rise to lineage-restricted airway and alveolar progenitor cells when co-cultured with lung stromal cells and cytokines. We have used this assay to compare the incidence and proliferative potential of EpiSPC in the tracheae of CF and normal mice. In situ Ki-67 labelling has been used to compare differences in the proliferative activity of tracheal epithelium.

Methods:

The conducting airways from heterozygous (normal), CF (UNC), and CF(FABP) mice were excised and disaggregated. FACS sorted EpCAM^{pos}Sca-1^{low}α6-integrin^{pos}β4-integrin^{pos}CD24^{low} cells from each group of mice were cultured in our matrigel-based clonogenic assay to quantify EpiSPC. Parallel studies analysed site-specific zones of cell proliferation revealed by immunohistochemical staining of tracheae for Ki-67.

Background and Results:

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-1^{pos}EpCAM^{neg} mesenchymal cells and mesenchyme-derived growth factors (Fig 1 & 2).

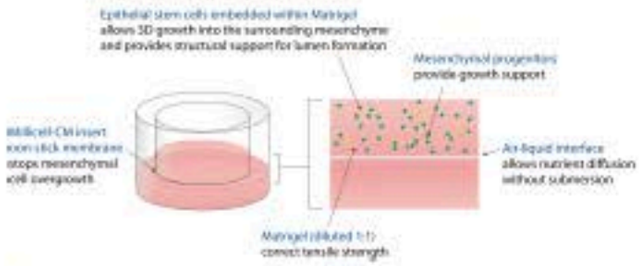

Figure 1: Schematic description of the lung EpiSPC assay system.

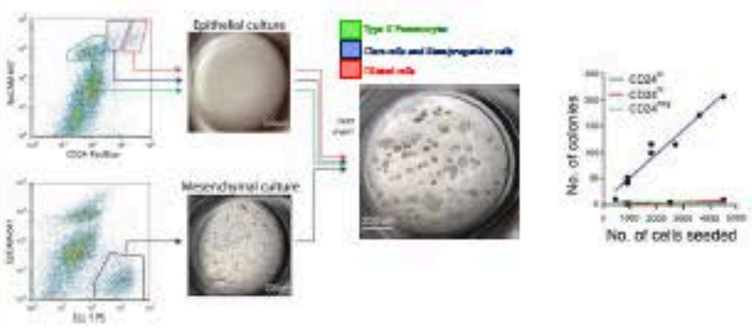

Figure 2: The clonal growth of CD45^{neg}CD31^{neg}EpCAM^{hi} CD24^{low} EpiSPC reveals an obligatory requirement for mesenchymal support. There is a linear relationship between CFU incidence and cells plated. Colony-forming potential is regulated by mesenchyme-derived stimulatory and inhibitory factors.

Upper airway epithelial stem/progenitor cells in mouse models of cystic fibrosis :

Using our assay we have observed an increase in airway progenitor cells in the trachea of cystic fibrosis mice. We detected a 5.2-fold and a 2.4-fold increase in the incidence of EpiSPC in the tracheal epithelia of CF (UNC) and CF (FABP) mice respectively, compared to normal heterozygous mice (Fig 3).

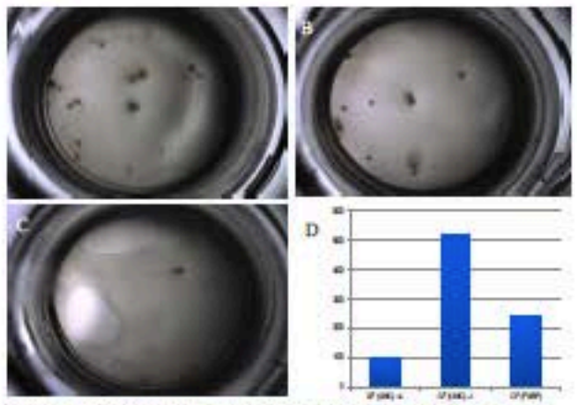

Figure 3: The incidence of EpiSPC colonies in isolated tracheal epithelial cells of A) CF (UNC -/-) (36/1000 cells) (B) CF (FABP) (76/1000 cells) is increased compared to C) heterozygous (UNC +/-) mice (1/1000 cells).

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)

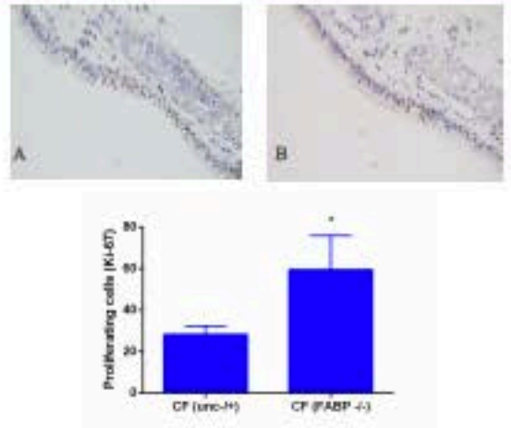

Figure 4: : Histology and immunohistochemistry for KI-67 in control and cystic fibrosis (CF) upper airways sections. CF (A) and control (B) tissue sections (5µm) stained for proliferating nuclear antigen KI-67 and is depicted graphically (bottom graph)

Summary:

These findings are consistent with the notion that expanded and dysregulated airway EpiSPC could contribute to CF airway remodelling and mucous cell hyperplasia in progression of dysfunction in CF lungs, and may have application in identifying target airway EpiSPC to sustain gene expression in a gene therapy setting.

Acknowledgements :

This work was supported by grants from the Australian National Health and Medical Research Council and the Cure4CF Foundation. Nigel Farrow is supported by the M8 McLeod Fellowship.

References:

1. McQualter JL, Brouard N, Williams B, Baird BN, Sims-Lucas S, Yuen K, Nilsson SK, Simmons PJ, Bertoncello I. (2009) Endogenous fibroblastic progenitor cells in the adult mouse lung are highly enriched in the Sca-1 positive cell fraction. Stem Cells 27: 623-633.
2. McQualter JL, Yuen K, Williams B, Bertoncello I. (2010) Evidence of an epithelial stem/progenitor cell hierarchy in the adult mouse lung. Proc Natl Acad Sci USA 107:1414-1419
3. Bertoncello I, McQualter JL. (2011) Isolation and clonal assay of adult lung epithelial stem/progenitor cells. Curr Protoc Stem Cell Biol. Chapter 2: Unit 2G.1.