Endogenous lung epithelial stem/progenitor cell compartments differ in cystic fibrosis and normal mice

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Introduction:

We have recently described a FACS-based cell separative strategy to identify, prospectively isolate and characterise endogenous mesenchymal stromal cells and epithelial stem/progenitor cells (EpiSPC) in the adult lung of normal mice (Figs.1 & 2)[1,2]. These studies show that rare EpCAM^{pos}α6-integrin^{pos}β4-integrin^{pos}CD24^{low} lung EpiSPC self-renew and give rise to colonies of mixed, and lineage-restricted airway and alveolar epithelial cells when co-cultured with lung stromal cells and cytokines in a 3-dimensional organotypic clonogenic assay providing evidence for the existence of an EpiSPC hierarchy in the lung (Figs.2 - 4)[2,3]. We have utilised these assays in this study to analyse the comparative incidence and proliferative potential of EpiSPC in the trachea of CF mice and their wildtype littermates.

Methods:

We excised and disaggregated the lungs and conducting airways from CF (UNC) colony mice that were heterozygous (normal) or homozygous CF (-/-) and from CF(FABP) colony mice. The sorted CD45^{neg} CD31^{neg} EpCAM^{pos} Sca-1^{low} α6-integrin^{pos} β4-integrin^{pos} CD24^{low} cells isolated from these three groups of mice were then cultured in our matrigel-based clonogenic assay to quantify EpiSPC.

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).

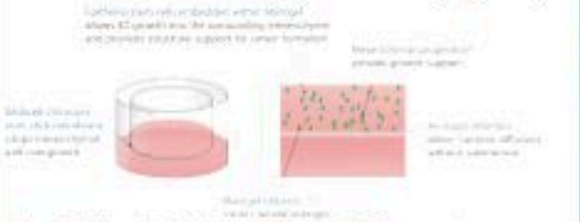

Figure 1: Schematic description of the lung EpiSPC assay system.

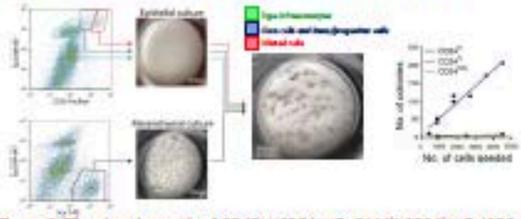

Figure 2: The clonal growth of CD45^{neg}CD31^{neg}EpCAM^{hi} CD24^{low} EpiSPC reveals an obligatory requirement for mesenchymal support. There is a liner relationship between CFU incidence and cells plated. Colony-forming potential is regulated by mesenchyme-derived stimulatory and inhibitory factors.

Multi-lineage potential of lung stem cells:

The morphological, immunohistochemical and gene expression profiling of epithelial colonies, and colony recloning experiments reveal the existence of distinct colonies with differing developmental potential. This has led us to propose the existence of an epithelial stem/progenitor cell hierarchy in the lung in which multilineage stem cells give rise to lineage restricted airway and alveolar progenitor cells that differentiate into all the epithelial cells in the adult lung (Fig 3).

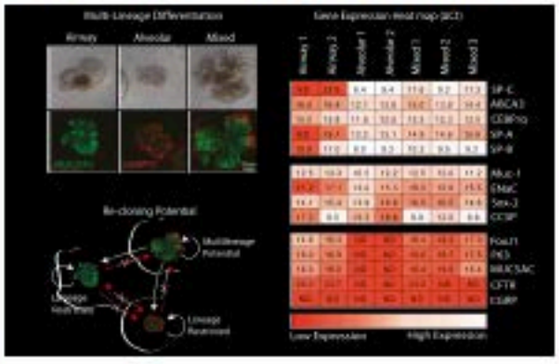

Figure 3: The differentiation potential of each epithelial colony type was assessed by immunohistochemistry and real-time PCR (expressed as ΔCt relative to 18s control). Mixed colonies demonstrate multi-lineage potential.

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 4).

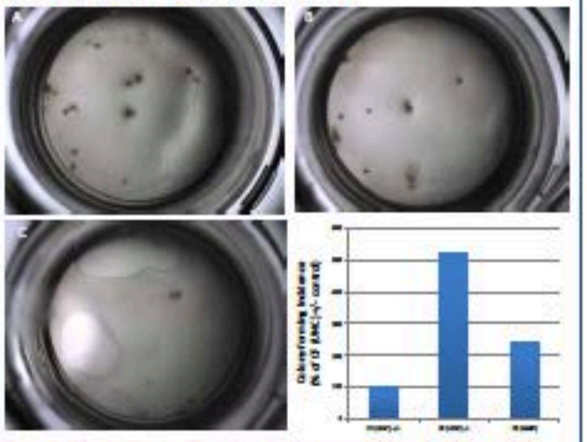

Figure 4: The incidence of EpiSPC colonies in isolated tracheal epithelial cells of A) CF (UNC -/-) (36/1000 cells) (B) CF (FABP) (76/1000 cells) is increased compared to C) heterozygous (UNC +/-) mice (1/1000 cells).

Summary:

These preliminary findings are consistent with the notion that an expanded and dysregulated airway EpiSPC compartment of CF mice could contribute to airway remodelling and mucous cell hyperplasia in progression of disease in the CF lung. The methods we describe may have application to CF pig and CF ferret lung tissues to help understand CF lung pathogenesis and disease in models more similar to humans and that are able to recapitulate CF airway disease development more closely than in mice. Similarly, these techniques may also be applicable to analysis of the regulation of EpiSPC in the human lung.

References:

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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.

Acknowledgements :

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