Developing High Efficiency Gene Transfer Techniques using Human (Air Liquid Interface) Epithelium

Nigel Farrow[1-3], Martin Donnelley[1-3], Eugene Roscioli[3,4], Patricia Cmielewski[1,2], Nathan Rout-Pitt[1-3], Chantelle McIntyre[1-3], Ivan Bertoncello[5] and David W Parsons[1-3]

[1] Department of Respiratory and Sleep Medicine, Women's and Children's Hospital, South Australia.
[2] Robinson Research Institute, and [3]Adelaide Medical School, University of Adelaide, South Australia.
[4]Chronic Inflammatory Lung Disease Research Laboratory, Department of Thoracic Medicine, Royal Adelaide Hospital, South Australia.
[5] Lung Health Research Centre, Department of Pharmacology and Therapeutics, University of Melbourne, Victoria

Introduction:

To develop an airway gene therapy for treating CF airway disease, rodent and other animal models may not predict treatment effectiveness in humans. Careful optimisation of the effectiveness and safety of our lentiviral CFTR gene vector is required for human use, so we have recently established human air-liquid-interface (ALI) cultures for this purpose. Our initial findings are presented here.

Methods:

Normal Human Bronchial Epithelial Cells obtained from LONZA (CC-2540S) were seeded (as per manufacturer's instructions) into 6 well plates at 2.5 x 10^5 cells per well (~70% confluence). Our lentiviral gene vector (HIV1-based, VSV-G pseudotype) containing the LacZ reporter gene (1.4 x 10^9TU/ml,) was applied to cells (2 hours after seeding) at MOI's of 100, 10 and 1 as well as untreated controls (n=3 per group). Immunohistochemistry staining using anti-keratin 5, a known airway basal stem cell marker was used to confirm the cell type used in the study. *ALI*: Normal Human Bronchial Epithelial Cells obtained from LONZA (CC-2540S) were seeded (as per manufacturer's instructions), expanded over 2 weeks, reseeded onto 24 well transwell plates and air lifted at confluency (~3 days). ALI cultures (n=6) were treated with our lentiviral vector carrying the LacZ marker gene alongside no treatment controls (n=6).

Results:

Anti-keratin 5 staining confirmed that the cells used in the study were human airway basal stem cells (Fig 1).

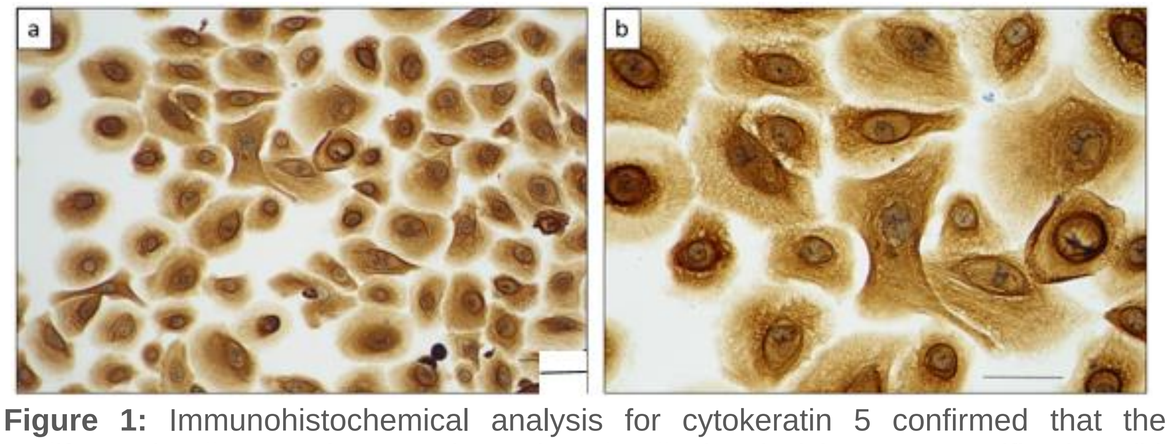

Figure 1: Immunohistochemical analysis for cytokeratin 5 confirmed that the proliferated cells were human airway basal cells (n=3). (a) 200x magnification, (b) 400x magnification. (Scale bar 20µm).

Gene transfer studies showed essentially complete LacZ transduction at MOI 100: 99.9% (0.3% (SD), similar levels at an MOI of 10: 97.7% (0.7%), and at MOI 1: 47.4% (3.7%) (Fig 2).

ALI: After 4 weeks the ALI cultures (n=24) contained active mucus producing cells and ciliated cells that together produced organized mucociliary transit activity, observed using fluorescent beads. Gene transfer observed via LacZ marker gene expression was present in all vector treated ALI cultures (Fig 3).

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Conclusion:

Mucociliary transit and histological data confirm the potential of our human ALI cultures. Our LV gene transfer data in the recently-seeded human airway cell cultures show highly efficient gene transfer and expression

High efficiency can be used either to boost the level of gene expression to ensure a therapeutic effect, or enable use of lower vector doses where only partial improvement in CFTR function is therapeutically effective (such as in CF lung disease) and so reduce the potential of inflammatory and immune function side effects. Gene transfer optimisation and testing studies using established ALI cultures are now in progress.

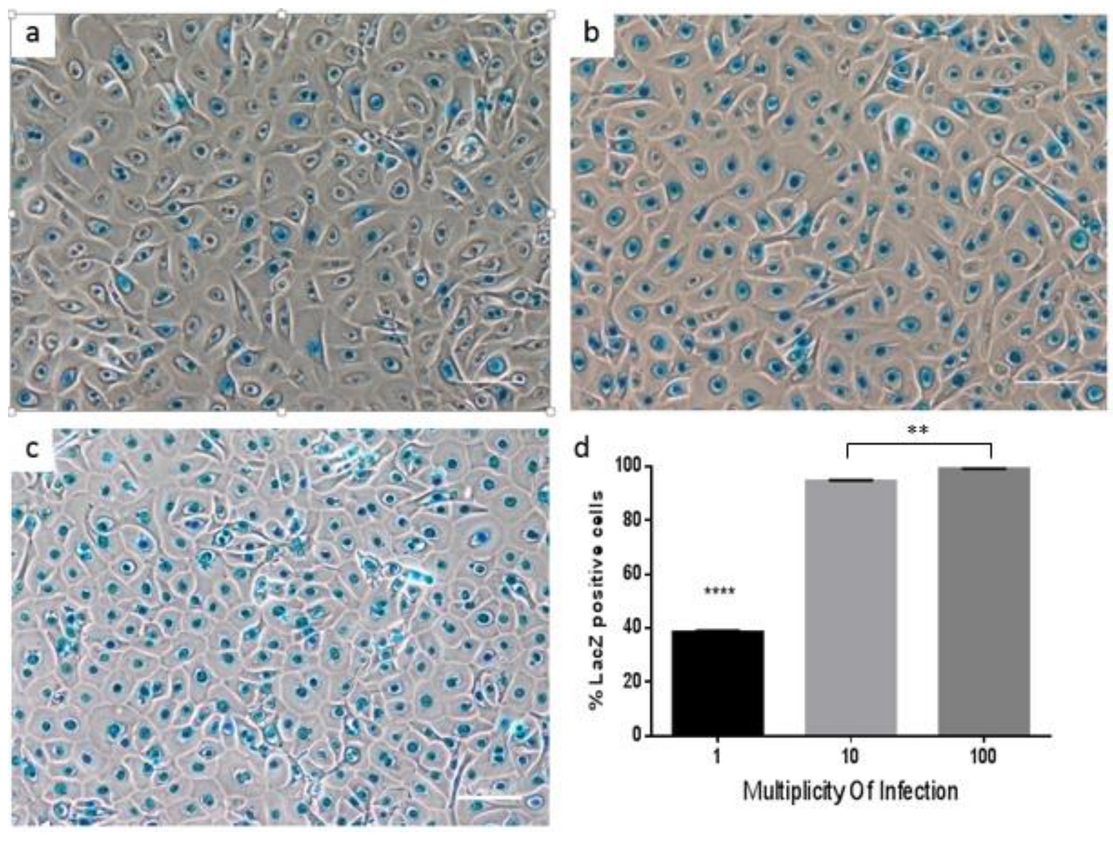

Figure 2: Examples of LacZ expression in human airway basal cells (a) MOI 1, (b) MOI 10, (c) MOI 100 (Scale bar 200µm). (d) At MOIs of 10 and 100 the proportion of transduced cells approached 100% (p****<0.0001, MOI 1 v MOI 10 and MOI 100, p**<0.01, MOI 10 v MOI 100 ANOVA, n=3).

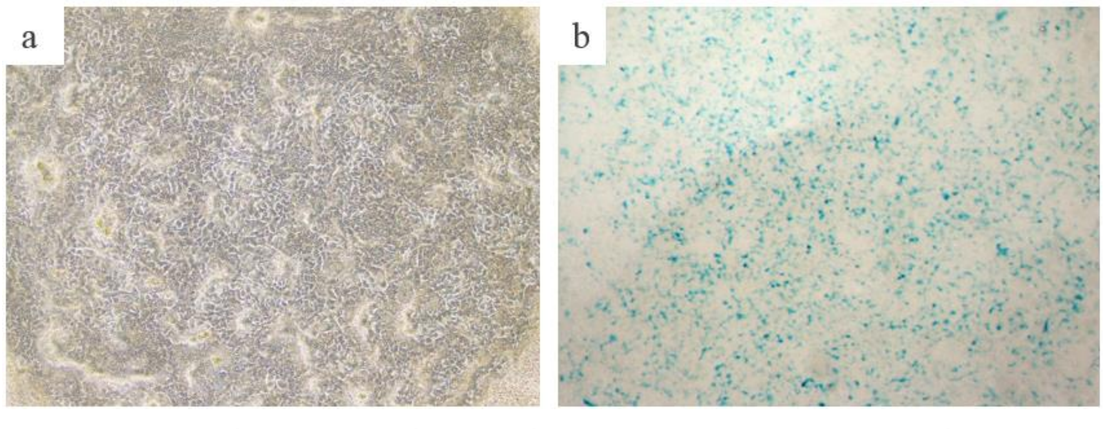

Figure 3: Examples of typical ALI cultures at ~ 4weeks post air lift showing (a) confluent layer and mucus production prior to gene transfer procedures and (b) LacZ staining of positively transduced cells. Both at 40x magnification, (n=6 wells per group).

Acknowledgments

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