CYSTIC FIBROSIS: THE ROLE OF AIRWAY STEM CELLS IN SUSTAINED GENE EXPRESSION BY LENTIVIRAL DIRECTED GENE THERAPY

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Research conducted in the Department of Respiratory and Sleep Medicine at the Women’s and Children’s Hospital, Adelaide, South Australia.
Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Signed:

Date: 28 April 2015
This Thesis is dedicated to my daughter

Ella Farrow.

My very special little girl, your determination to enjoy life to its fullest despite having the insidious disease Cystic Fibrosis is a source of inspiration which drives me every day. Darling this is for you.
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Cmielewski, P, and Farrow, N, were equal contributing first authors.


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Ivan Bertoncello, **Nigel Farrow**, Jonathan McQualter, David Parsons.

2012


2011

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Robinson Research Institute, research travel award 2014

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Cystic Fibrosis Australia PhD scholarship 2011-2014

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Golden Key Honour Society 2009

Flinders University Chairperson’s Letter of Commendation 2008 and 2009
Synopsis

In this thesis transduction of airway stem cells (basal cells) in the nasal and tracheal airways was investigated to determine the causality of sustained transgene expression following a gene therapy protocol that utilised an LPC pre-treatment and a HIV-1 VSV-G pseudotyped lentivirus vector treatment, as previously published. To assess stem cell transduction and epithelial regrowth a forced-injury model was employed at a number of time points after the gene therapy protocol.

Epithelial remodelling in cystic fibrosis and normal airways of mice was also assessed. Airway stem cell hyperplasia and goblet cell hyperplasia and hypertrophy, and epithelial mucin content were assessed in the trachea and in some instance the nasopharynx in the nasal airways of CF and normal mice.

Additionally, the effectiveness of the LPC / lentiviral gene therapy protocol was assessed in lung airways of normal ferrets and the marmoset, a non-human primate, to determine if airway transduction of both differentiated ciliated cells and stem cells reflected observations noted in previously-published mouse-based studies. These ferret and marmoset animal studies have been published prior to thesis submission.

Airway stem cells transduction was confirmed in the trachea and nasal airways of mice following pre-treatment with LPC and subsequent treatment with a HIV-1 VSV-G pseudotyped lentiviral vector carrying the LacZ marker gene. A forced injury model was employed to force regeneration of the airway epithelium after vector treatment. Following the ablation and subsequent regeneration of the airway epithelium, clusters of LacZ positive were observed in both the trachea and nasal airways suggesting transduction of the airway stem cells and the passing of the
transgene to their progeny upon differentiation.

Airway epithelial remodelling was demonstrated in both airway stem cells and goblet cells in CF mice. Hyperplasia of airway stem cells and goblet cells in CF mice was observed. Hypertrophy and change in mucin acidity of goblet cells was also observed. Additionally, remodelling of the cartilage rings in the trachea was observed in CF mice. This is the first study to demonstrate the presence of goblet cell hyperplasia, hypertrophy and change in mucin acidity in the presence of airway stem cell hyperplasia. Importantly, the hyperplasia of airway stem cells in CF airways had previously been proposed however, this is the first study to directly quantitate the airway stem cell compartment using a novel flow cytometry and clonogenic assay approach.

Finally, the transduction of airway stem cells and ciliated cells is shown in normal ferrets and marmosets, a non-human primate. Validation of transducing relevant airway cell type in these animals adds to the gene therapy proof of principle foundation previously demonstrated in the airways of mice.
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