LENTIVIRAL AIRWAY GENE TRANSFER IN NORMAL FERRETS

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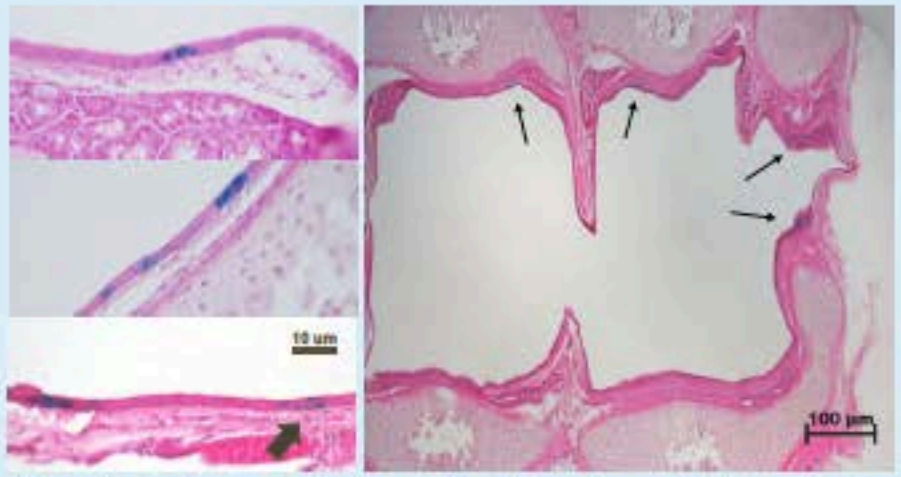
BACKGROUND: With the recent availability of the cystic fibrosis (CF) ferret model, we wished to assess the ability of our HIV-1 based Lentiviral (LV) vector to produce initial transduction in ferret airways.

Our airway gene delivery protocol employs a lysophosphatidylcholine (LPC) pre-treatment, which enables robust expression in marmoset lung, and long lasting gene expression in mouse nasal airways.
This pilot study asked whether this protocol was effective in normal ferret lung airway, prior to consideration of studies in CF ferrets.

METHODS: Six recently-weaned ferrets (2 M, 4 F, 335 to 460 gram BW at 7 weeks of age) were anaesthetised and orally intubated. Our HIV-1 based LV vector containing a nuclear-localised LacZ gene (500 µl, ~ 5 x 108 TU/ml) was delivered 1 hour after treatment of the airway with LPC (150 µl of 0.1% w/v in PBS). Both LPC and vector were delivered via a PE cannula projecting 2 mm from the ET tube into the lower ¼ of the trachea. Blood was taken at baseline and on alternate days. One week later animals were humanely killed (Lethabarb i.p.). The upper right lobe was removed prior to inflation fixation for molecular biological analyses, along with other non-airway tissues, and the trachea and lung tissues were inflation-fixated and processed to reveal LacZ protein expression (standard X-Gal procedures). The airway tissues were first examined *en face* to identify transduced regions. Subsequently, selected regions of transduced tissue were sectioned and counter-stained (eosin) to identify transduced cells.

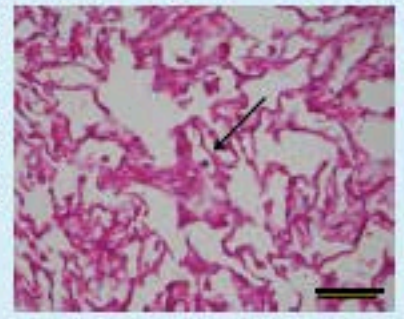
RESULTS: Clear but low-level LacZ gene expression was present in the 6 ferrets, evident primarily as blue-stained cells in the trachea (below).

Low levels of LacZ transduction in the trachea of the 6 ferrets studied. *En face* view. x20 magnification except lower left is x30



(Above) Together with clusters of ciliated and non-ciliated cells (fine arrows) and basal cells (thick arrow on low power section) were transduced in the tracheal epithelium.

In the lung only rare LacZ-stained alveolar macrophages (arrow) were detected in one or two lobes, in some animals (right). Scale bar 10 µm



Vector presence in serum was sought via p24 assay; p24 was not detected above baseline levels on any day (pre, 1, 3, 5 & 7 days) in any animal.

Using qPCR analysis of lung, spleen, liver and gonads harvested at day 7 the LacZ gene was not detected in these tissues in any animal.

CONCLUSION:
Our combined LPC/LV delivery protocol can produce airway gene transfer in normal ferret lung airways. Compared to the strong and extensive LacZ gene expression we have reported in airways of mice and marmosets, the extent and efficiency of gene expression was (qualitatively) low and did not warrant quantification.
Factors influencing this reduced LacZ gene expression may be LV vector spreading and dilution after delivery within the very long trachea; a sub-optimal LPC pre-treatment dose provided by single 150 µl volume used; and it is possible that setting a vector dose volume by scaling on body weight is not appropriate. In the lung the rarity of alveolar macrophages is consistent with an inadequate dose volume. The absence of both vector p24 protein and LacZ transgene in blood and harvested organs, respectively, could also be due to an inadequate vector dose. Alternatively there may be species-related factors that reduce transduction efficiency in ferret lung compared to other animal models. Future dose-response studies would be informative.

Nevertheless, the conducting airway cell-types that were transduced were similar to those we have found in mice, sheep and marmosets, and relevant to CF airway gene therapy. These findings supporting the utility of our airway gene transfer method by extending it to another animal species.

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