AIRWAY PRE-TREATMENT ENHANCES MOUSE LUNG LENTIVIRAL REPORTER GENE EXPRESSION

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BACKGROUND:

•Cystic fibrosis (CF) is the most common, fatal genetic disease among the Caucasian population. VSV-G pseudotyped lentiviral (LV) gene vectors have the potential to treat CF long-term, however physiological barriers prevent efficient vector access to ciliated and basal (stem) cells residing on the basement membrane.

•We have demonstrated the effectiveness of our lysophosphatidylcholine (LPC) pre-treatment and LV vector dosing technique for producing ciliated and basal cell transduction in mouse nasal airways.

•The aim of this experiment was to determine if LPC pre-treatment enhances LV reporter gene transduction in mouse lung conducting airways, the primary target of a CF gene therapy.

METHODS:

•A 20 µl bolus of a HIV-1 LV vector carrying the LacZ transgene was instilled directly into the trachea of C57Bl/6 mice (n=10 per group) via orotracheal intubation, 1 hour after a pre-treatment of either 15µl of PBS (control), 0.1% LPC, or 0.3% LPC.

•LacZ expression was assessed by X-gal staining of the lungs after mice were humanely killed 3 months after dosing.

RESULTS:

•Dosing was well tolerated, however there was a mild transient respiratory depression at the time of LV delivery.

•There was little to no LacZ transduction in the control group that received the PBS pre-treatment, (Fig. 1a) however there was a consistent pattern of strong LacZ transduction of the conducting airways in both LPC treatment groups (Fig. 1b &c).

•Histological staining of lung sections revealed a significant difference in the number of LacZ transduced cells/mm of cartilaginous-associated upper airways from both LPC treatment groups compared to PBS ($p<0.01$ and $p<0.05$, ANOVA , 0.1% LPC and 0.3% LPC respectively, Fig. 2).

•The majority of cell transduction occurred in ciliated epithelial cells, with some basal cell transduction also present (Fig. 3).

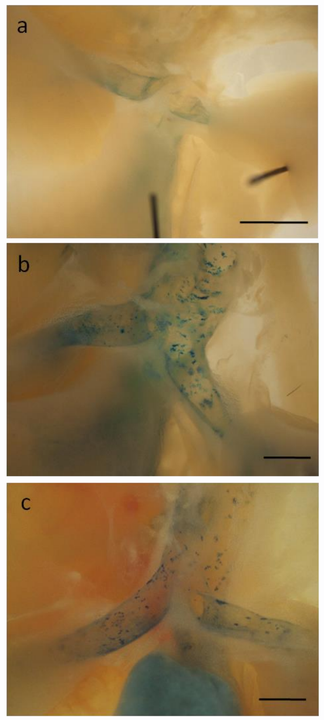

Figure 1. Example of lung LV-LacZ transduction of conducting airways at 3 months following a) control PBS, b) 0.1% LPC and c) 0.3% LPC. Scale bar 1 mm

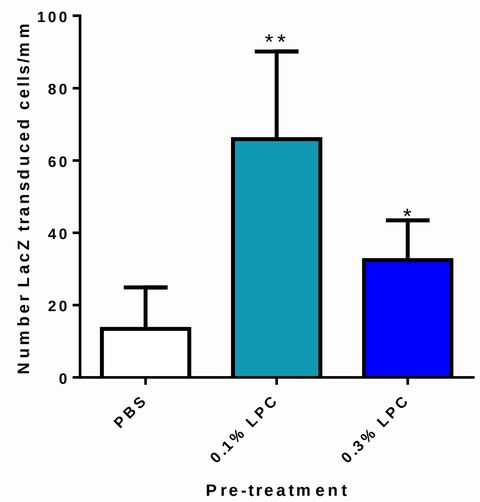

Figure 2. Significant increase in LV-LacZ transduction in lung conducting airways following 0.1% LPC and 0.3% LPC compared to PBS control pre-treatments. (**p<0.01 and *p<0.05, ANOVA vs PBS)

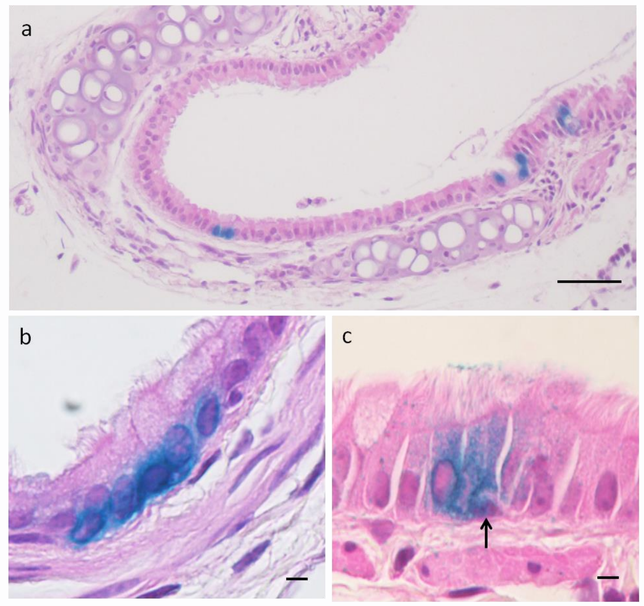

Figure 3. LV-LacZ transduced cells (blue staining) of a) the cartilaginous-associated upper conducting airways in the lung of b) ciliated cells and c) basal cells designated by arrow. Haematoxylin and Eosin staining. Scale bar a) 10 µm and b, c) 1 µm.

CONCLUSION:

LPC pre-treatment prior to LV vector delivery into mouse lungs substantially improves the level of transduction achieved. The LPC pre-treatment groups also showed basal cell transduction that may indicate effective targeting of resident airway stem cells. Doses of LPC lower than the 0.1% level used here should now be trialled to determine the minimally effective conditioning treatment for enhanced lung gene transduction *in vivo.*

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