AIRWAY CONDITIONING ENHANCES LONG-TERM LENTIVIRAL REPORTER GENE EXPRESSION IN MOUSE LUNG

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•Lentiviral (LV) gene vectors pseudotyped with the vesicular stomatitis virus G glycoprotein (VSV-G) have the potential to treat cystic fibrosis (CF) long-term. However physiological barriers of the airway prevent efficient vector access to the receptors on the basolateral surface of ciliated and basal (stem) cells residing on the basement membrane.

•[illegible] demonstrated the effectiveness of our lysophosphatidylcholine (LPC) conditioning pre-treatment and VSV-G LV vector dosing.

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

•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 oro-tracheal intubation, 1 hour after a pre-treatment with either 15µl of PBS (control), 0.1% LPC, or 0.3% LPC.

•Mice were humanely killed 3 months post 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).

•Few LacZ stained cells were noted in the peripheral airways from any treatment.

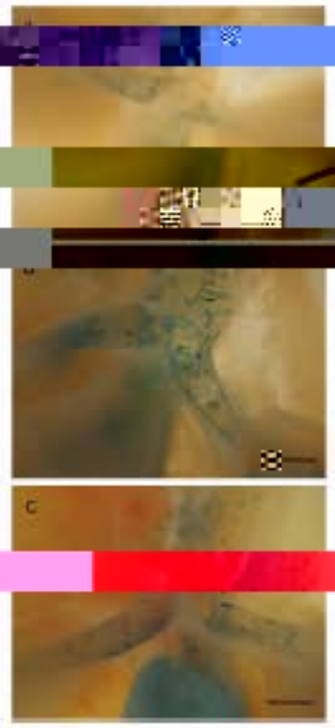

Figure 1. Example of lung LV-LacZ transduction of conducting airways at 3 months following a) control PBS, b) 0.1% LPC, and c) 0.3% LPC pre-treatment.

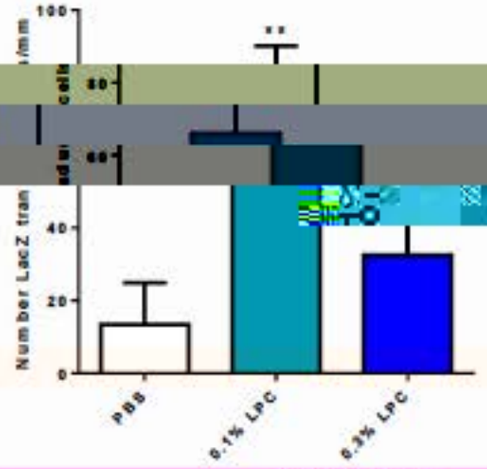

Figure 2. Significant increase in LV-LacZ transduction in lung conducting airways following 0.1% LPC and 0.3% LPC compared to PBS control pre-treatment (**$p<0.01$ and *$p<0.05$, ANOVA vs PBS)

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

CONCLUSION:

LPC conditioning of the airways prior to Vector delivery [illegible] used by other research groups is sufficient for producing extended lung expression [illegible] avoided. The LPC conditioning groups also showed basal cell transduction, consistent with the ability to target and transduce the normally inaccessible resident airway stem cells likely to be responsible for producing persistent gene expression.

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