Airway gene-addition therapy for cystic fibrosis: Comparative efficiency of HA and VSV-G pseudotyped lentiviral vectors

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Introduction:

- Lentiviral (LV) vectors are the lead vector for treating cystic fibrosis (CF) airway disease by delivering functional copies of the CFTR gene into airway epithelial cells.
- The choice of LV-vector pseudotype is important to ensure that the correct cell types and locations are effectively targeted. The VSV-G and HA envelope proteins target airway receptors on the basolateral and apical surfaces, respectively.
- Conditioning the airway surface with the compound lysophosphatidylcholine (LPC) prior to LV-vector delivery may increase transduction efficiency in the lungs by transiently breaking epithelial tight-junctions, exposing the basolateral surface to the LV vector.
- A HIV-1 pseudotyped LV-vector carrying either the *LacZ* or Luciferase (*Luc*) reporter genes was used to determine which pseudotype is more effective, and whether LPC conditioning resulted in higher transduction levels from either pseudotype.

Methods:

- Normal C57Bl/6 female mice were anesthetised and intubated.
- The trachea and conducting airways were conditioned with 10 µl of PBS (control, n=7-12) or LPC (n=7-12), followed one hour later by two 15 µl aliquots of VSV-G (n=7-12) or HA (n=7-12) pseudotyped LV-vector containing either the *LacZ* or *Luc* reporter genes.
- One week after LV-vector instillation, mice that received *LacZ* were humanely killed via CO_2 asphyxiation and their lungs inflation fixed. LacZ transduction was assessed *en face* after histochemical (X-gal) analysis, while cell types were determined by histological methods.
- Bioluminescence imaging (BLI; Xenogen, IVIS) was performed at 1 week, and then monthly for 12 months (currently) after LV-vector instillation to assess Luc expression levels in the lungs over time.

Results: Short-term *LacZ* study

- *En face* LacZ staining assessment of mouse lungs one week after dosing indicated that airway conditioning with LPC resulted in stronger initial LacZ transduction levels than PBS, independent of the pseudotype (Fig 1).

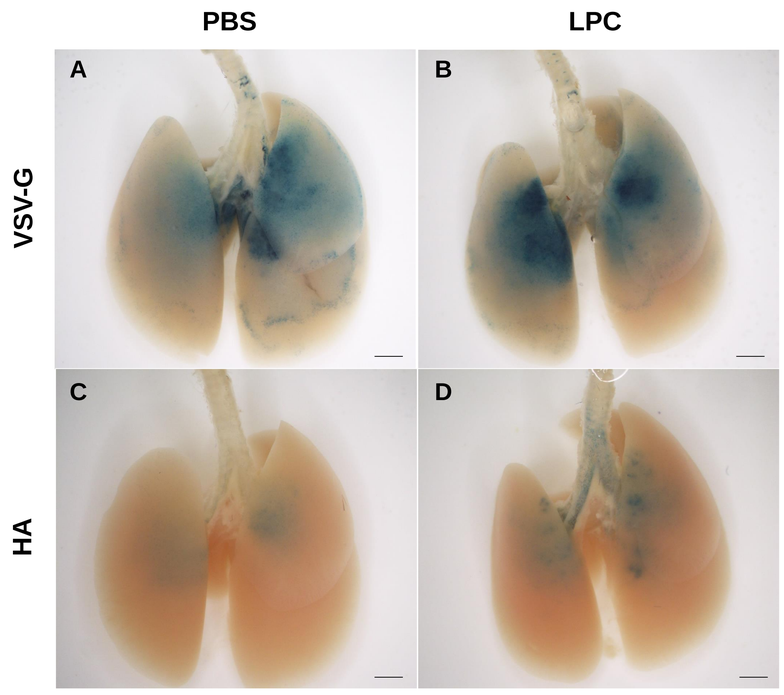

Figure 1: Examples of LV-LacZ transduction of mice lungs at one week following LV-vector instillation with (A) PBS VSV-G, (B) LPC VSV-G, (C) PBS HA and (D) LPC HA. (x7.5 mag, Scale bar 2mm).

- In the VSV-G treated mice, LacZ transduction was typically more pronounced in the trachea and upper bronchioles, regardless of the conditioning treatment (Fig 1A & B).
- In the HA treated mice, LPC produced higher levels of LacZ transduction in the trachea and bronchioles (Fig 1D), compared to PBS (Fig 1C).

- Preliminary histological analysis indicated that for both pseudotypes, ciliated cells were the predominant LacZ expressing cell type in the upper and middle conducting airways (Fig 2).
- Qualitative assessment of the HA pseudotype suggested that conditioning with LPC produced higher numbers of LacZ expressing cells than PBS.
- For both pseudotypes, a small number of Type I and II alveolar cells were transduced, regardless of the conditioning treatment.

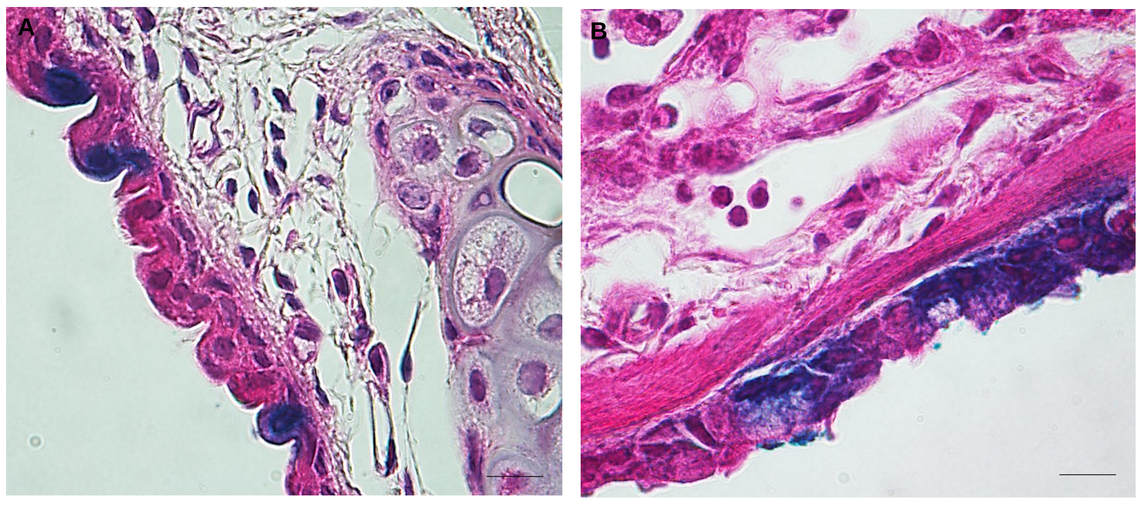

Figure 2: Histology examples of upper and middle lung sections of LacZ transduced ciliated cells one week post LV-vector instillation with (A) LPC and HA, and (B) LPC and VSV-G. (x100 mag). Scale bar 10µm.

Results: Long-term *Luc* study

- Lung luminescence was detected by BLI at all imaging time points in the PBS and LPC conditioned mice that received either pseudotyped LV-vector (Fig 3).
- At one week, the VSV-G group had significantly higher expression levels than HA, independent of the conditioning treatment.
- Longer term (1-12 months) there was no quantifiable difference in lung luminescence between PBS or LPC and VSV-G or HA.

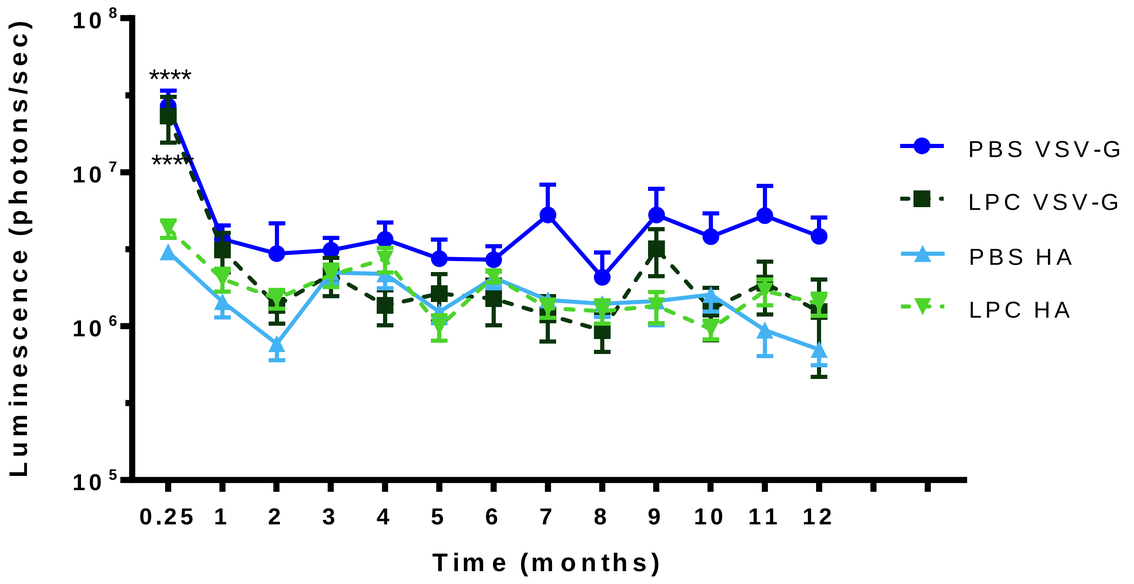

Figure 3: *Luc* gene expression in the lung airways of mice conditioned with either LPC or PBS followed one later by VSV-G or HA LV-vector instillation. (**** $p < 0.0001$, RM two-way ANOVA, Tukey's at 1 week, n=7-8 per group, mean ± SEM).

Conclusion:

- At one week the LacZ and Luc data both suggest that the VSV-G pseudotype is more effective at transducing airway cells than the HA pseudotype.
- Both pseudotypes transduced the correct cell types in the upper conducting airways for the treatment of CF airway disease.
- The long term Luc results suggest that conditioning the airways with LPC prior to LV vector delivery does not increase the total long-term lung transduction level for either pseudotype, suggesting that LPC may not be required for efficient long-term gene expression.
- BLI will be continued to observe total lung gene expression levels over time, while further LacZ analysis will be performed to quantify the number of LacZ expressing cells produced by either pseudotyped LV-vector.

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