PROGRESS AND BARRIERS TOWARD LENTIVIRAL AIRWAY GENE TRANSFER DEVELOPMENT WITHIN THREE ANIMAL MODELS

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Research for the future health of our children

Introduction

[illegible] gene transfer to treat or cure the airway disease in CF remain[illegible]vable, although problems with efficiency, appropriate targ[illegible] sufficient longevity have limited progress.

We have examined in vivo gene transfer using [illegible] mouse nose and [illegible] development; s[illegible] to assess effects in a large lung, and marmoset lung to assess suitability in a primate lung.

Using a lentiviral gene vector coupled with a brief airway [illegible] treatments designed to access airway stem/progenitor cells, we sought successful gene transfer techniques with potential to produce permanent or transient CFTR gene expression to reverse CF airway disease.

Methods

Airways were dosed in two steps, starting with LPC (lysophosphatidylcholine, a component of lung surfactant that [illegible] followed [illegible] was examined in mouse nasal airways. Acute effects were assessed after 7 days, while long-term effects were followed for up to two years.

Results

Mice: In nasal airways a single brief dose can produce reporter ge[illegible] LacZ) expression that lasts for a mous[illegible] transfer into CF mouse nasal airway produced significant gene correction for at least 12 months (Fig 1b); an (incomplete) confirmatory study shows sustained ~40% [illegible] correction towards normal levels for more than 9 months so to date (not shown). [illegible] pathophysiology. Recent LVLuc gene transfer studies s[illegible] late recovery of gene expression in mouse lung (not sh[illegible]

Fig 1a: [illegible] can persist for a mouse lifetime. 1b: [illegible] corrective [illegible] persists for > 12 months.

Sheep: In lung airway we observed successful h[illegible] low level reporter gene transfer (Fig 3). In part, these were due to the small dose volumes we were able to generate for studies in this harnessed lung. Improved vector dose procedures is required and studies of improved techniques are planned.

Fig 2: LacZ gene transfer is effective in mouse lung. Five levels shown, L1M is high mag of L1 (arrow).

Marmosets: In the two marmoset monkeys studied to date LacZ reporter gene expression was observed in the conducting airways (Fig 4), showing for the first time well tolerated, but we noted some evidence of (recovering) epithelial cell disturbance, this is to be investigated in further studies. A transient (day 2) serum antibody response to the virus-vector surface protein (VSV-G) was lost by day 3.

Fig 3: Sheep lung LacZ gene expression is present, but [illegible] lung shows patchy but strong gene expression.

Transduced cell types: Mouse, sheep and marmoset airway gene transfer was observed in the desired ciliated surface cells as well as basal cells. [illegible] Produce [illegible]

New measurements of airway [illegible]tion: The novel non-invasive airway-surface imaging technique using synchrotron X-rays that we recently described is providing the first insights into mucociliary transport behaviour of individual particles in live mice. This method may be able to track therapeutic improvements in airway mucociliary function in mice, with potential to monitor airway function in CF mice.

Fig 5: Cross [illegible] of surface and basal

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Conclusions

Single dose lifetime airway gene transfer is possible in mice, indicating involvement of airway progenitor cells in the persistence of gene expression. Lung ge[illegible] [illegible]se lung, but larger doses are needed to prope[illegible] [illegible] method for delivering therapeutic genes into lung airways for cystic fibrosis. Finally, our new X-ray b[illegible] has the potential to provide rapid non-invasive measurement of airway physiology associated with a[illegible]

References

a) Stocker et al J Gene M[illegible] 2009; b) Parsons [illegible] [illegible]atomy 2009; Donnelley et al, J Synch Radiation 2009.