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

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Introduction

Corrective gene transfer to treat or cure the airway disease in CF remains achievable, although problems with efficiency, appropriate targeting and sufficient longevity have limited progress.

We have examined in-vivo gene transfer using mouse nose and mouse lung to enable rapid development; sheep lung to asses 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 pre-treatment 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 improves airway gene transfer) and followed by the lentiviral gene vector containing a reporter gene (producing blue stained cells (LacZ), or bio-luminecscence (Luc)). CFTR gene transfer was examined in mouse nasal airways. Acute effects were assessed after 7 days, while long-term effects were followed for up to two years.

Acknowledgements

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References

a) Stocker et al J Gene Med (online) in press 2009; b) Parsons et al, J Anatomy 2008; Donnelley et al, J Synch Radiation 2009.

Results

Mice: In nasal airway[a] a single brief dose can produce reporter-gene (LacZ) expression that lasts for a mouse lifetime (~2 years) (Fig 1a). Importantly, CFTR gene transfer into CF mouse nasal airway produced significant gene correction for at least 12 months (Fig 1b); an (incomplete) confirmatory study shows sustained ~40% correction towards normal levels for more than 9 months to date (not shown). In mouse lung we found that these gene transfer procedures also produce substantial reporter gene (LacZ) transfer in conducting airway (Fig 2), the site of CF pathophysiology. Recent LVLuc gene transfer studies suggest late recovery of gene expression in mouse lung (not shown).

Sheep: In lung airway we observed successful, but very 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 human-sized lung. Improved vector dose production is required and studies of improved techniques are planned.

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 lentivirus gene transfer in this small human-like primate lung. The gene deliveries were 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.

Transduced cell types: Mouse, sheep, and marmoset airway gene transfer was observed in the desired ciliated surface cells as well as basal cells (Fig 5). The latter cell type is thought to contain airway progenitor cells that have the potential to produce long lived, perhaps permanent gene transfer.

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

Delays: An unexpected major barrier was administrative, not biological. Despite full approval of the scientific, ethical & bio-safety requirements, our cross-institutional animal-model studies were severely hampered by legally-required but unproductive duplicative approvals and some studies were delayed for over 18 months.

Fig 1a: LacZ gene expression can persist for a mouse lifetime. 1b: corrective CFTR gene transfer persists for > 12 months.

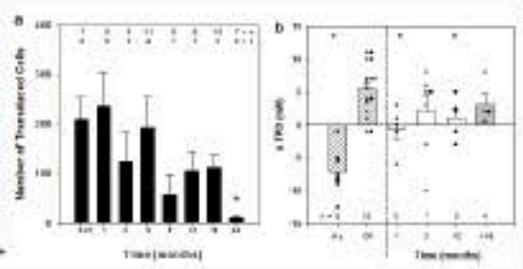

Fig 2: LacZ gene transfer is effective in mouse lung. Five levels shown, L1M is high mag of L1 (arrow).

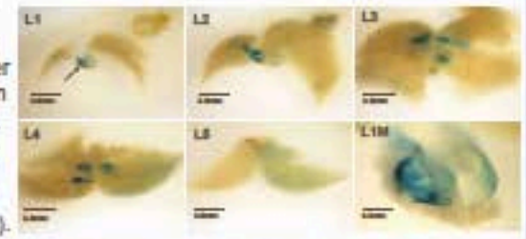

Fig 3: Sheep lung LacZ gene expression is present, but very low.

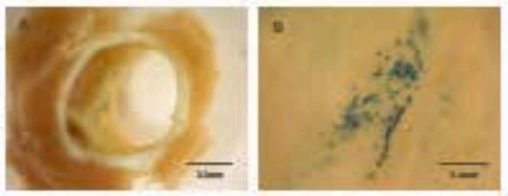

Fig 4: Marmoset lung shows patchy but strong gene expression

Fig 5: Cross sections show transduction of surface and basal airway cells.

Conclusions

Single dose lifetime airway gene transfer is possible in mice, indicating involvement of airway progenitor cells in the persistence of gene expression. Lung gene transfer developed in nasal airways translated well into mouse lung, but larger doses are needed to properly assess gene transfer in the large lungs of sheep. The first-ever trials of lentivirus gene transfer in the small marmoset monkey revealed satisfactory gene transfer into conducting airways. Taken together, these findings suggest LPC-enhanced lentivirus based gene transfer may be an effective method for delivering therapeutic genes into lung airways for cystic fibrosis. Finally, our new X-ray based imaging of airway mucociliary function has the potential to provide rapid non-invasive measurement of airway physiology associated with airway gene transfer in small animals.