Overview:

- The UK Cystic Fibrosis Gene Therapy Consortium is committed to the testing and development of gene therapy vectors for CF clinical trials.
- Successful CF gene therapy vectors will need to demonstrate not only adequate levels of transgene expression, but also appropriate distribution of expression in relevant lung cell types.
- Reporter genes such as enhanced green fluorescent protein (EGFP) are valuable tools for the localisation of gene expression.
- Cell type specific antibodies can be used to positively identify EGFP transfected cells in vivo.

We investigated the level and cellular distribution of transgene expression in the murine lung following nasal delivery of naked DNA and DNA conjugated to the cationic lipid GL67 (Genzyme Corporation, Framingham, USA).

For each animal treated with pEGFP-N1 or pEGFP-N1/GL67, the total number of GFP positive cells identified in 180 representative lung sections (see methods) was recorded. In a parallel study luciferase expression in lung homogenates from mice (n=6) dosed with control vectors (data not shown).

**Table 1: Expression profiles for pDNA and pDNA/GL67**

<table>
<thead>
<tr>
<th>细胞类型</th>
<th>pDNA</th>
<th>pDNA/GL67</th>
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<tbody>
<tr>
<td>Clara Cells</td>
<td>24.4%</td>
<td>1.3%</td>
</tr>
<tr>
<td>Type I Pneumocytes</td>
<td>12.8%</td>
<td>2.0%</td>
</tr>
<tr>
<td>Macrophages</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Ciliated Cells</td>
<td>61.6%</td>
<td>1.3%</td>
</tr>
<tr>
<td>Type II Pneumocytes</td>
<td>95.4%</td>
<td>2.0%</td>
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</tbody>
</table>

Figure 1: Visualisation of GFP positive cells in the mouse lung.

Female BALB/c mice (n=6) were dosed intranasally with 300µg of the plasmid pEGFP-N1 (Clontech, Oxford, UK) or 80µg of plasmid conjugated with GL67. 24 hours later, mice were sacrificed and 7µm cryosections examined for GFP expression. GFP positive cells were found in all treated mice with expression observed in several different cell types (Figure 1).

Figure 2: Comparison between GFP and luciferase reporter gene expression.

Methods:

Preparation of lung sections
Mice were killed by cervical dislocation and the lungs were inflated with 4% paraformaldehyde at a pressure of 20cm Hg for 10s. Lungs and trachea were then transected in 30% sucrose in PBS overnight at 4°C. The trachea, azygous, cardiac, diaphragmatic and left lung lobes were dissected away and individually embedded in OCT (Cellect, Newborn, UK) for cryosectioning. Sections were cut at 7µm and mounted onto glass slides. For each lung, 30 non-consecutive cryosections were taken from each lobe and the trachea. A total of 180 slides were prepared per mouse.

Cryosections containing GFP positive cells were stained with a range of cell specific antibodies (see methods) conjugated to the red fluorophore AlexaFluor 546. Co-localisation of GFP expression and antibody labelling was examined using confocal microscopy. Co-localisation of GFP expression with cell specific antibody labelling was observed for ciliated cells (Figures 3a and b), Clara cells (Figures 3c and d), type II pneumocytes (Figures 3e and f) and type I pneumocytes (Figures 3g and h) in animals treated with both pDNA and pDNA/GL67. However, the relative proportion of cells identified with each antibody was very different between the two vectors (Table 1).

Figure 3: Identification of GFP positive cells using cell type specific antibodies.

Cryosections from pEGFP-N1 and pEGFP-N1/GL67 treated animals were stained with a range of cell type specific antibodies. Following antibody staining the distribution of antibody labelled GFP positive cells was recorded for each vector. For animals treated with pDNA, the majority of positive cells (61.6%) were identified as ciliated epithelial cells with the remaining largely made up of Clara cells (24.4%) and type II pneumocytes (12.8%) (Table 1). In contrast, the vast majority of GFP positive cells in animals treated with pDNA/GL67 were identified as type I pneumocytes (95.4%) with relatively little expression in other cell types.

Conclusions:

- GFP expression from non-viral gene transfer vectors can be visualised in the murine lung.
- Different gene transfer vectors can target different cell populations within the lung.
- Identification of target cells should be considered an important parameter in the assessment of new gene therapy vectors.

Antibody staining:
Lung sections were initially blocked with 10% fetal calf serum (FCS) in phosphate buffered saline containing 0.5% Triton X-100 for 30min. Blocking solution was then replaced with primary antibody blocked in blocking solution and incubated at room temperature for 1 hour. Identification of Clara cells was performed using the rabbit anti-human Clara protein 1 antibody (DAKO, Ely, UK). Ciliated cells were identified using the mouse anti-a-defensin 2 antibody (Biocell, San Ramon, CA, USA). Type I pneumocytes were identified using the human anti-lung keratin antibody (Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA, USA). Type II pneumocytes were identified using the anti-endothelial surfactant protein B (Seph) (Chemicon, Temecula, CA, USA). Alveolar macrophages were identified using the rat anti-mouse F4/80 antigen antibody (Serotec, Oxford, UK). Detection of primary antibodies was performed using appropriate isotype-matched secondary antibodies and peroxidase-conjugated to AlexaFluor 546 (Molecular Probes, Leiden, Netherlands).