Use of Ciliated Cell Specific Promoter FoxJ1 in Gene Transfer Vectors for the Airway Epithelium

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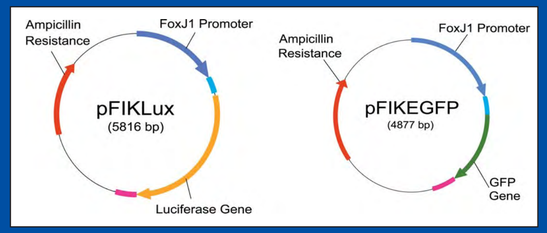
Overview of Project:

- Gene replacement therapy is currently being developed for the treatment of Cystic Fibrosis (CF) lung disease
- Non-viral vectors are being developed to ensure successful repeat administration to maintain therapeutic gene expression
- The majority of studies use viral promoters such as CMV which become attenuated *in vivo,* particularly in the lung (1)
- Human promoters such as the polyubiquitin C (UbC) promoter can overcome this problem, allowing persistent transgene expression (1)
- We used the ciliated cell-specific promoter FoxJ1 (2) to investigate transgene expression in the mouse lung and nose

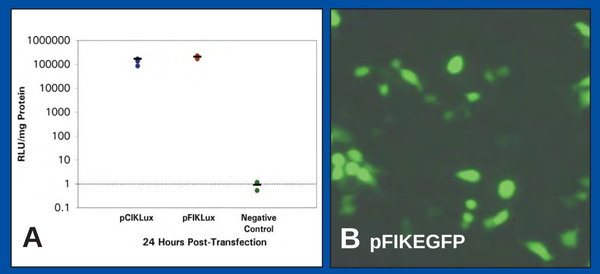
Results:

Figure 1: FoxJ1 Reporter Plasmids.



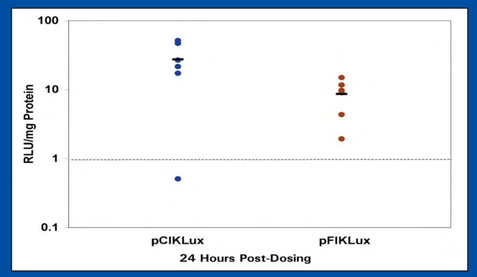
A 1 kb DNA fragment containing the FoxJ1 promoter sequence was amplified from plasmid pTG1 (kind gift of W. O'Neal, University of North Carolina) using PCR. The fragment was used to replace the CMV promoter in pCIKLux and pCIKEGFP in order to generate pFIKLux and pFIKEGFP.

Figure 2: FoxJ1-Driven Reporter Gene Expression in HEK 293T Cells.



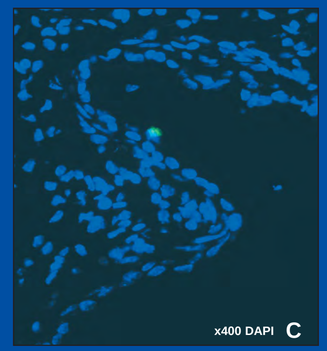
HEK 293T cells were transiently transfected with pCIKLux, pCIKEGFP, pFIKLux and pFIKEGFP complexed with polyethylenimine (PEI). Reporter gene expression was assayed 24 hours post-transfection. (A) Luciferase expression from pFIKLux was not significantly different from pCIKLux (P=0.0628, ANOVA), and both treatments gave expression 100,000-fold greater than background levels (dashed line). Luciferase expression from the GFP vectors gave expression at or below background. (B) Transfection efficiency with both pCIKEGFP and pFIKEGFP was around 75%, with both vectors giving equivalent intensity of expression.

Figure 3: FoxJ1-Driven Luciferase Expression in the Mouse Lung.



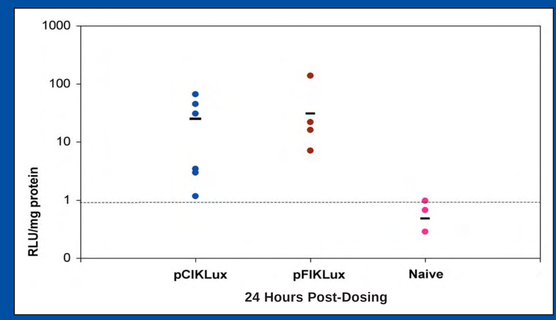
100*μ*g of plasmids pFIKLux and pCIKLux were delivered to the lungs of female BALB/c mice (6-8 wk) via intranasal instillation and reporter activity measured 24 hours post-administration. Luciferase activity was normalised to protein content. Reporter gene expression from pCIKLux was on average 40-fold greater than background (0.04 ±0.015) levels (dashed line). Expression from pFIKLux was on average 10-fold greater than background levels. Expression from pFIKLux was not significantly different from pCIKLux (P=0.3427, ANOVA).

Figure 4: FoxJ1-Driven GFP Expression in the Mouse Lung.



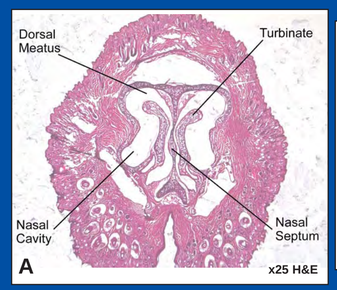
300*μ*g of plasmids pFIKEGFP and pCIKEGFP (positive control) were delivered to the lungs of female BALB/c mice (6-8 wk) via intranasal instillation. The animals were sacrificed 24 hours post-dosing and the lungs harvested. The lungs were inflated and fixed, and each lobe dissected away and embedded in Embedding Matrix. 7*μ*m cryosections were prepared and mounted using fluorescent mounting medium with DAPI stain. GFP expression was characterised using fluroescence microscopy. (A + B) GFP-positive cells following transfection with pCIKEGFP. Approximately 72% of the GFP-positive epithelial cells were ciliated (B) as determined by co-localisation of cell-specific antibodies (3). The remainder were non-ciliated. (C) Fluorescence microscopy following transfection with pFIKEGFP revealed very few GFP-positive cells in the lung epithelium. No GFP-positive cells were found in the parenchyma.

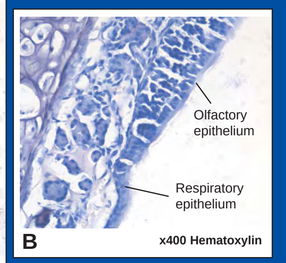
Figure 5: FoxJ1-Driven Luciferase Activity in the Mouse Nose.



The epithelium lining the mouse nose contains all the cell types of interest in CF gene therapy, and is also more easily accessible than the lung. This makes it a useful surrogate for measuring correction of the CF defect *in vivo*. 200*μ*g of plasmids pFIKLux and pCIKLux were delivered to the noses of female BALB/c mice (6-8 wk) via 15-minute nasal perfusion under general anaesthetic. Animals were sacrificed 24 hours post-dosing and whole nose lysates assayed for Luciferase activity. Expression from both pCIKLux and pFIKLux was on average 50-fold greater than background levels (dashed line). There was no significant difference between pCIKLux and pFIKLux, but both were significantly greater than naive expression levels (P=0.018, ANOVA).

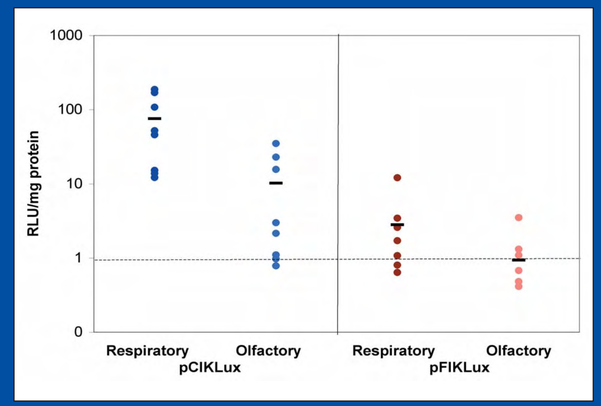
Figure 6: Cell Specificity in the Mouse Nose.





Although GFP-positive cells were detected in the nasal epithelium following delivery of AdCMV-GFP (data not shown), no GFP-positive cells were observed following delivery of plasmid vectors. A. The type of nasal epithelium varies depending on its depth in the nasal cavity. B. Respiratory cells predominate up to 2.5mm and transition to olfactory cells appearing from 3 - 3.5mm, A useful technique for identifying cell types transfected is to separate these areas of epithelium from each other and assay each for reporter gene activity (see Fig 7).

Figure 7: Determining the Specificity of pFIKLux in the Mouse Nose.



200*μ*g of plasmids pFIKLux and pCIKLux were delivered to the noses of female BALB/c mice (6-8 wk) via 15-minute nasal perfusion under general anaesthetic. The animals were sacrificed 24 hours post-dosing, the nasal tissues dissected out and the respiratory and olfactory areas separated. No statistical significance was observed between respiratory and olfactory epithelia with either vector but the trend indicates that the majority of expression is found in the respiratory epithelium.

Conclusions:

- Luciferase and EGFP expression from the FoxJ1 promoter *in vitro* was similar to that observed from CMV plasmids
- Levels of Luciferase expression from FoxJ1 plasmids were similar to CMV plasmids in the murine lung and nose
- The majority of Luciferase expression from FoxJ1 plasmids was localised to the respiratory epithelium
- More sensitive assays are required to identify specific cell types transfected with FoxJ1 plasmids *in vivo*
- Cell specific expression may help restrict transgene expression to the airway epithelium

References:

1. Gill, D. R. *et al* (2001). Increased persistence of lung gene expression using plasmids containing the ubiquitin C or elongation factor 1 alpha promoter. ***Gene Therapy.*** **8, 1539-1546.**

2. Ostrowski, L. E. *et al* (2003). Targeting expression of a transgene to the airway surface epithelium using a ciliated cell-specific promoter. ***Molecular Therapy*****. 8 : 637-645**

3. Davies, L. A. *et al* (2003). Transgene expression in the mouse lung following administration of gene transfer vectors expressing EGFP. ***Molecular Therapy*****. 7 : 74-75**

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