Topical Delivery of mRNA to the Murine Lung and Nasal Epithelium

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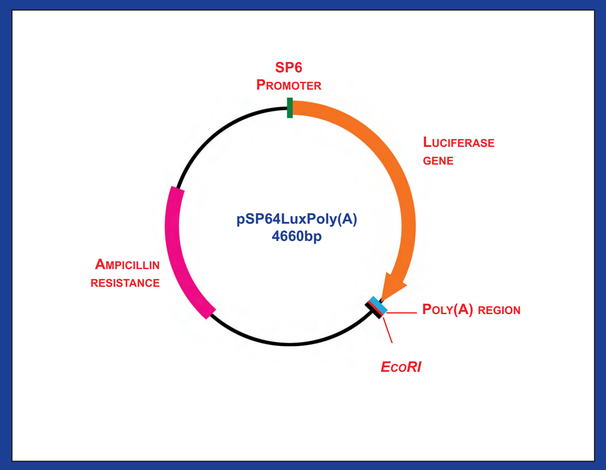
Introduction

- Gene replacement therapy is being developed for Cystic Fibrosis lung disease.
- Terminally differentiated cells in the respiratory epithelium lining the airways are the target.
- Non-viral vectors are being investigated to ensure successful repeat adminstration to these cells.
- Penetration of the nuclear membrane is a significant barrier to gene transfer in non-dividing cells *in vivo*.
- mRNA, which does not need to penetrate the nuclear membrane because it is translated in the cytoplasm, could be useful as a gene transfer vector.
- mRNA was investigated as a potential gene transfer agent in the murine nose and lung.

Figure 1: *In Vitro* Transcription

A plasmid expressing firefly luciferase was constructed by incorporating the luciferase gene into plasmid pSP64Poly(A) (Promega). The plasmid contains an SP6 promoter 5' to the luciferase gene, which is followed by a synthetic poly(A) tail. The plasmid was linearised with *Eco*RI and used as a template for *in vitro* transcription using the RiboMAX SP6 Large Scale RNA Production System (Promega) with a cap analogue (m7G(5')ppp(5')G):GTP ratio of 1:1. The transcribed mRNA was purified from transcription enzymes, unincorporated nucleotides and cap analogue before being electrophoresed on a denaturing 1% formaldehyde gel. The transcripts were of the expected size and did not show signs of degradation (data not shown).



The concentration of mRNA was measured by spectroscopy (average yield = 150-200μg per 100μl reaction). To test the translatability of the mRNA, HEK293T cells were transfected with 2μg of the luciferase-encoding mRNA complexed with 4μg DOTAP (Sigma). Luciferase expression (90,000 ± 13,000 RLU/mg) was observed 9 hours after transfection (data not shown).

Figure 2: mRNA Delivery to the Mouse Lung

Luciferase-expressing mRNA was complexed with Genzyme lipid GL67 and the complexes (80μg mRNA/60nmol GL67) were delivered to the lungs of female BALB/c mice (6-8 weeks) via intranasal instillation. Lungs were harvested at 3, 6, 12, and 24 hours after delivery (n = 5-6 per group). At 3 hours, mean luciferase expression was five-fold greater than that of naïve animals (ANOVA, p = 0.0001). Reporter gene expression decreased with time and was lower than expression from luciferase expressing plasmid DNA (pDNA) (80μg pCIKLux/60nmol GL67) (data not shown).

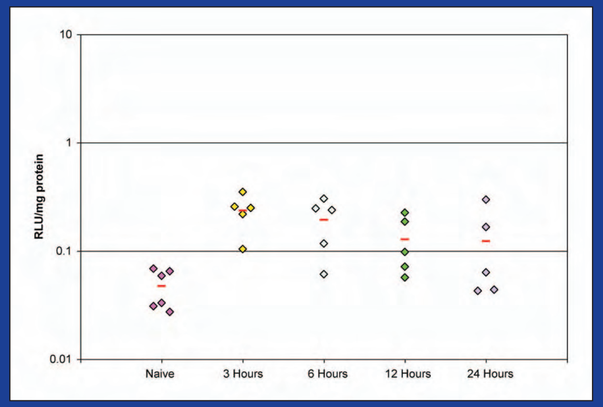


Figure 3: mRNA Delivery to the Mouse Nose

Luciferase-expressing mRNA was delivered to the mouse nose via nasal perfusion (100μl to the left nostril over 15 minutes). Three different formulations were tested: naked (i.e. uncomplexed) mRNA (150μg), mRNA/DOTAP (100μg) or mRNA/ Megafectin (DOTAP-Chol) (25μg). Nasal tissue was harvested at 4 hours post-dose and luciferase activity measured in the extracts. Luciferase expression 475-fold over naïve levels was seen in the naked mRNA group (ANOVA, p = 0.005), but mRNA/DOTAP and mRNA/Megafectin gave expression at the level of naïve animals (p = 0.60 and 0.23, respectively).

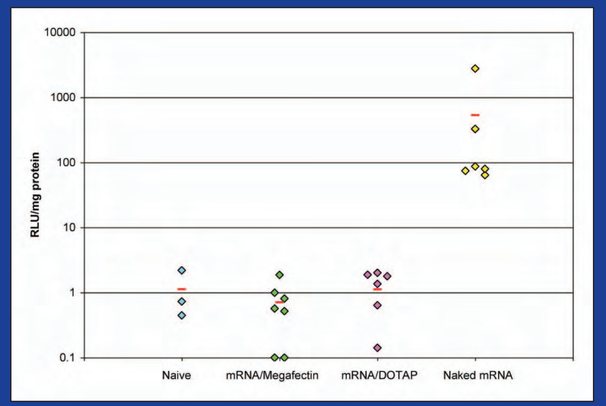


Figure 4: mRNA & pDNA Dose Response in Mouse Nose

Increasing concentrations of naked mRNA or plasmid pCIKLux were delivered to the mouse nose (details as fig 3). A total of 12.5, 25, 50, 100 and 150μg mRNA or pDNA was delivered. Nasal tissue was harvested at 4 or 24 hours post-dosing for mRNA or pDNA respectively. All doses of mRNA and pDNA resulted in expression over background levels (ANOVA, p < 0.05) with no statistically significant differences between the different doses (ANOVA, p > 0.05). Also, the same doses of mRNA and pDNA gave equivalent expression levels (ANOVA, p > 0.05).

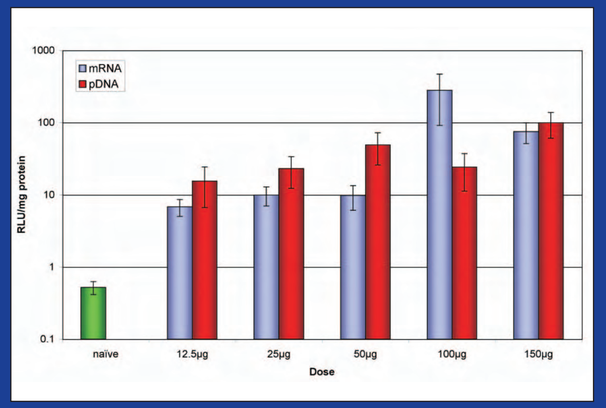
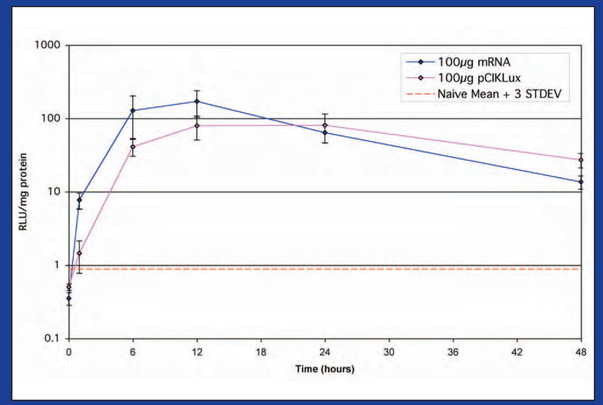


Figure 5: mRNA & pDNA Time Course in Mouse Nose

To find the peak time and duration of expression, 100μg naked mRNA or pCIKLux was dosed (details as fig 3). Nasal tissue was harvested at 1, 6, 12, 24, 48 and 168 hours (n = 6-7 per group). Overall pDNA and mRNA showed very similar time course profiles, except for the slightly shorter duration and earlier peak of mRNA expression compared to pDNA. Expression from mRNA was seen as early as 1 hour, reaching a peak at 12 hours (mean = 170 ± 67 RLU/mg), and falling back to naïve values by 7 days (data not shown). Expression from pDNA was not seen until 6 hours post-dose, with peak levels at 24 hours (mean = 80 ± 34 RLU/mg protein). Modest expression from pDNA was detectable at 7 days (4.0 ± 1.3 RLU/mg protein, p = 0.04) (data not shown).



Conclusions

- mRNA complexed with GL67 gives modest luciferase expression in the mouse lung.
- Naked mRNA delivery results in luciferase expression in the mouse nose, whereas mRNA complexed with DOTAP or DOTAP-Chol does not.
- As little as 12.5μg mRNA delivered to the mouse nose results in readily detectable luciferase expression.
- mRNA derived luciferase expression in the mouse nose can be seen as early as 1 hour post dose and lasts for over 48 hours.
- mRNA is equivalent to pDNA in terms of duration and expression levels.
- Re-administration studies will investigate the potential for giving multiple doses of mRNA to the same animal.
- Studies using GFP constructs to investigate the number and type of cells transfected *in vivo* are underway.

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