

Cell culture models of non-viral transgene expression are not indicative of *in vivo* lung outcome

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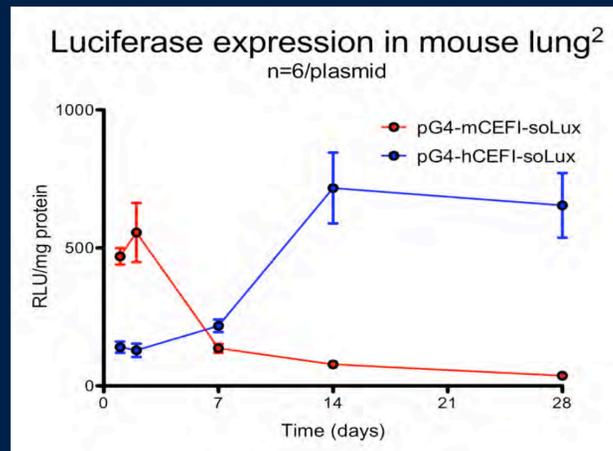
To develop a cell culture model that recapitulates non-viral gene expression observed in the mouse lung.

Two cell culture models have been investigated:

1. Human lung carcinoma cell line A549
2. Primary human air-liquid interface (ALI) cultures of respiratory epithelial cells

INTRODUCTION

- ▶ CpG-free plasmids¹ have been developed which can direct persistent expression in the mouse lung after aerosol delivery².



- ▶ Luciferase expression from the hCEFI promoter (pG4-hCEFI-soLux) persists for >6 months.
- ▶ Luciferase expression from the mCEFI promoter (pG4-mCEFI-soLux) lasts only a few days.
- ▶ The plasmids differ only in the presence of the human or equivalent murine CMV enhancer (hC or mC).

MATERIALS AND METHODS

A549 (ATCC) transient transfection: 5×10^4 cells/well, 3 μ g pDNA, 0.9 μ l 0.1M PEI in 2ml OptiMEM, 18h.

▶ Luciferase activity normalised against protein content (Relative Light Units/mg protein).

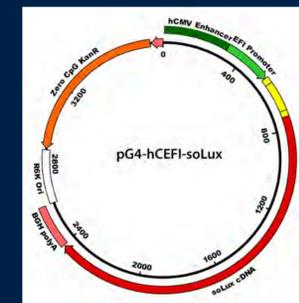
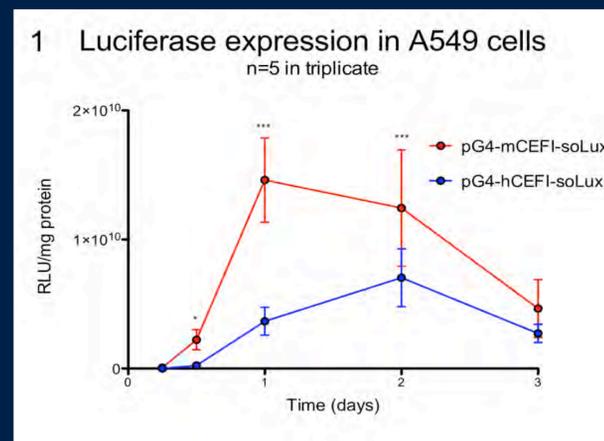
ALI (Epithelix Sarl, Switzerland) transient transfection: 5×10^5 cells/insert, 10 μ g pDNA, 25 μ l Lipofectamine in 100 μ l OptiMEM apically for 6h.

▶ EGTA treatment: where indicated, 10mM EGTA added apically for 30min prior to transfection (pre-treatment) or during transfection (co-treatment).

▶ Luciferase imaging: D-Luciferin added apically at 1 μ g/ml 15min before imaging using the IVIS100 bioluminescent system (Caliper Life Sciences). Values corrected for background (untransfected ALI).

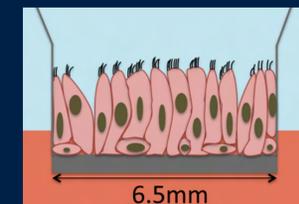
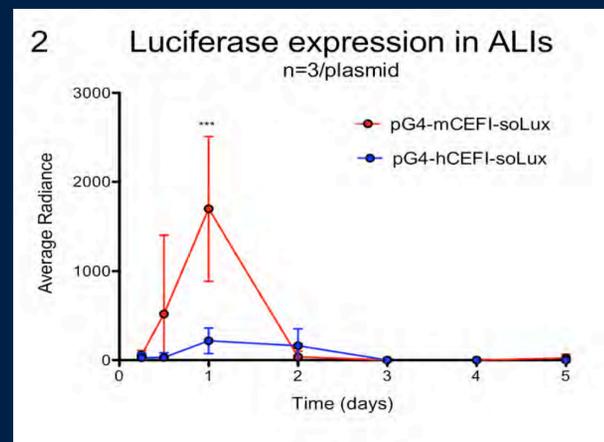
Statistics: 2-way ANOVA with Bonferroni posttests

RESULTS



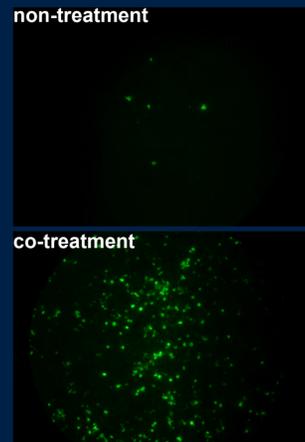
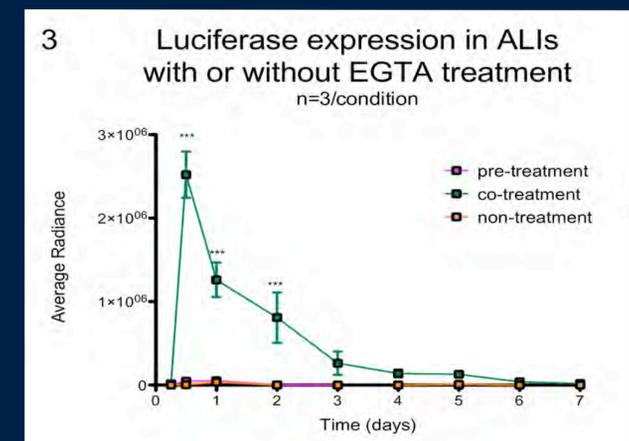
- ▶ Luciferase activity from pG4-mCEFI-soLux differed from pG4-hCEFI-soLux at 12h ($p < 0.01$), 24h ($p < 0.001$) and 48h ($p < 0.001$).

- ▶ Neither plasmid directed persistent transgene expression.



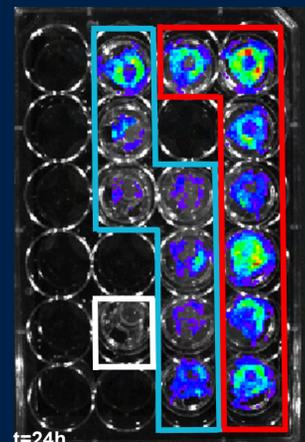
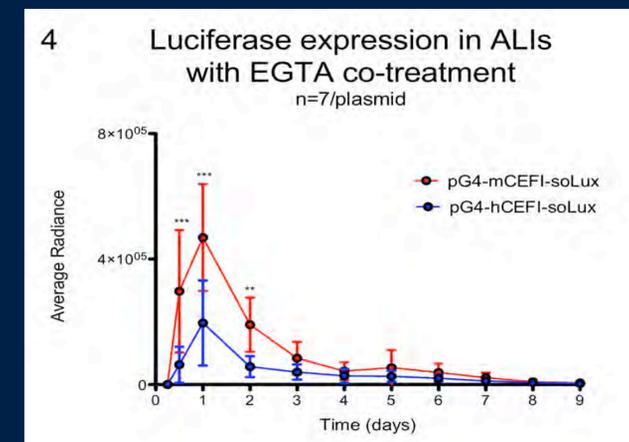
- ▶ Luciferase activity was significantly higher with pG4-mCEFI-soLux than pG4-hCEFI-soLux at 24h ($p < 0.001$).

- ▶ Average radiance was very low with both plasmids at all time-points.



- ▶ To increase transgene expression, ALIs were treated with EGTA to transiently disrupt tight junctions.

- ▶ Co-treatment with pG4-mCEFI-soLux and EGTA resulted in a 63-fold increase in luciferase activity at 12h ($p < 0.001$) over pre-treated inserts. This was confirmed with pEGFP-N1 +/- EGTA.



- ▶ Luciferase activity directed by the two plasmids was different at 12h ($p < 0.001$), 24h ($p < 0.001$) and 48h ($p < 0.01$).

- ▶ Neither plasmid directed persistent transgene expression.

CONCLUSIONS

- ▶ There was no persistence of non-viral gene expression in human A549 cells.
- ▶ EGTA co-treatment of primary ALI cultures results in 63-fold increased transgene expression.
- ▶ Non-viral transgene expression in primary ALI cultures did not mimic *in vivo* results.
- ▶ Currently the reasons for this are unknown, but may include species and cell type differences.

1. Utilising CpG-free plasmid technology developed by Cayla Invivogen (Toulouse, France)
2. Hyde et al., 2008, Nature Biotechnology, 26, 549-551