Efficient gene delivery to pig airways using helper-dependent adenoviral vectors

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Gene therapy is currently being developed for Cystic Fibrosis. While various gene therapy vectors have been successfully delivered to mouse lungs, gene transfer to the airway epithelium of humans has proven to be inefficient. Pigs share many features of lung biology with humans, including anatomy, electrolyte transport, and immune responses. Recently, a porcine CFTR−/− model has been created and the neonatal CF pigs displayed a high degree of similarity in electrophysiological and pathological changes to human neonates with CF (Rogers et al, Science, 2008). These results suggest that highly efficient delivery of HD-Ad vectors to the airway of large animals can be achieved and will provide important insights into the design of clinical studies for CF lung gene therapy.

Reference(s)

Retargeting human papillomavirus-mediated gene transfer to human airway epithelial cells

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Papillomavirus (HPV) pseudovirions are artificial vectors constituted of HPV major capsid protein (L1) and minor capsid protein (L2) packaged with plasmid coding for a gene of interest. These safe therapeutic gene delivery systems are able to transduce a wide range of cells. Nevertheless, they were shown to poorly transduce airway epithelium cells. In order to circumvent this problem, we inserted 3 different airway cell ligands in the HPV 16 capsid. Several motifs have been described to confer lung targeting like P1: THALWH, P2: RDFSLKLV and P3: GHPRQMSSHVY. These peptides were inserted in the DE, EF or FG loop of HPV16 L1. These recombinant L1 proteins were co-expressed with the HPV16 L2 protein in insect cells using the corresponding recombinant baculoviruses. The capacity of the virus-like particles obtained to transfer the luciferase gene was investigated in pulmonary cell (S9 and IB3−1). Five mutant VLPs were selected and tested for their gene transfer capacity. An increase in gene transfer by 400 to 500-fold was observed in IB3−1 and S9 cells lines compared to Hela cells, with P1DE and P2DE mutants. In addition, we identified by phage display two other motifs (VDRLOQK and PHPNRAQ). Each motif was inserted in the DE loops of HPV16 L1 and expressed in insect cells. The ability of the VLPs obtained to transfer gene in airway cells is currently investigated.

In conclusion, the results indicate that cell tropism of viral vectors can be effectively reengineered by insertion of short peptide ligands in order to produce improved vectors for application of gene transfer in human airway epithelial cells.

Re-emergence of luciferase expression in lung following a single nasal instillation of a lentiviral vector in normal and cystic fibrosis mice

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Non-invasive bioluminescence imaging permits repeated in-vivo quantification of long-lasting gene transfer in individual experimental animals. A single nasal delivery of our lentiviral (LV) gene transfer vector revealed unusual dynamic changes in mouse lung gene expression.

Aim: To monitor the location and persistence of LV luciferase gene transfer in airways in normal and CF mice.

Methods: Normal (C57Bl/6) and CF (CFTR<sup>−/−</sup>) mice received a nasal bolus instillation of lysophosphatidylcholine (LPC) or PBS control one hour prior to dosing with a Luciferase LV vector (10−3.10<sup>10</sup>tu/ml). Bioluminescence was measured 10−15 minutes after intranasal D-luciferin delivery (50μl, 15 mg/ml), at 1 week, 1, 3, 6, 9, 12, 15 and 18 months after vector dosing.

Results: CF transgenic mice: LPC pre-treatment also produced significantly higher luciferase activity than PBS controls in the nasal airways at all time points (p<0.05, RM ANOVA). Luciferase activity was also present in the lung in both pre-treatment groups. CF transgenic mice: LPC pre-treatment also produced significantly higher levels of luminescence in the nasal airways compared to PBS controls (p<0.05, RM ANOVA). In contrast to normal mice, lung luminescence was lost at 6 and 9 months in PBS pre-treated CF mice. Surprisingly, significant levels of luminescence in the lung returned at the 12 month time point.

Conclusions: Luciferase gene expression was significantly improved in mouse nasal airways using LPC pre-treatment in both strains. The re-emergence of gene expression in lung many months after a single transfection event suggests involvement of in situ airway stem/progenitor cell transduction in the murine CF knockout lung.

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High-resolution synchrotron X-ray imaging of live mouse airways: overcoming challenges in physiological assessment

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Introduction: While small animal models are useful for studying respiratory diseases such as CF the complexity of physiological studies is increased dramatically when imaging live animal airways using synchrotron X-rays. Over the last 5 years we have developed techniques at the Japanese SPring-8 synchrotron to facilitate accurate and reliable visualisation of mouse airways using high-resolution 2D imaging. We will present our effective approaches and discuss continuing challenges.

Methods: Images of airway surfaces (1.8 mm × 1.2 mm, pixel resolution 0.45 μm), were captured from live, Nembutal anaesthetised, intubated and ventilated (SCIREQ flexiVent) mice using a phase-contrast X-ray imaging (PCXI) setup. Animals were secured on an imaging board for precise orientation in the X-ray beam. Images were captured at set intervals or gated to ventilator end-expiratory trigger signals.

Results: Two special challenges were the fixed location and orientation of the horizontal X-ray beam and minimising the effects of small respiratory, cardiac and muscle movements that can blur images at such high levels of magnification. Rapid tracheal intubation facilitated long term study stability, and additional body/muscle movements that can blur images at such high levels of magnification.

Conclusions: Synchrotron PCXI of mouse respiratory systems has special challenges but continues to evolve. Attention to animal-handling and imaging techniques will permit continued development of novel, high-resolution, live animal airway physiology imaging for use in respiratory research.