The use of Lentiviral vectors to treat airway disease

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F/HN Lentivirus mediates lifelong gene expression

F/HN Lentivirus expressing an EGFP-Luciferase fusion was delivered to the nasal and lung respiratory epithelium. Luciferase expression can be detected by bioluminescence imaging (BLI) in the mouse nose and lungs for up to 18 months post-delivery. EGFP expression (white punctate signal) was observed throughout the respiratory epithelium.

Conclusions and future work

Large-scale Lentiviral production

Large-scale lentiviral production is rate-limiting for use in the clinic. Therefore we have developed scalable, animal-free lentiviral production and purification methods using suspension cell culture, which have been approved by the MHRA for the production of virus for non-clinical regulatory toxicology studies.

We utilise a 25L Wave Bioreactor that controls temperature, pH, CO2 and O2 levels throughout production to maximise virus yield. Production of F/HN pseudotyped Lentivus expressing EGFP is easily monitored by a characteristic green colour change – even under normal lighting conditions.

Following production the virus is concentrated and purified using a combination of ultrafiltration and anion-exchange chromatography techniques.

Expression elements can be manipulated to enhance or knock-down gene expression as required. This new Lentivirus vector can be used to efficiently transduce the airway epithelium for lung gene therapy, including cystic fibrosis, alpha-1-antitrypsin deficiency and COPD.

F/HN Lentivirus expressing EGFP was delivered to the nasal respiratory epithelium. Nasal transgene expression was observed for the lifetime of treated mice (up to 449 days post delivery).

Modular dual-function Lentivirus for gene therapy

We have developed a modular Lentiviral vector that allows the insertion of various promoters/enhancers, RNA interference (RNAi) elements, and transgenes.

Expression elements can be manipulated to enhance or knock-down gene expression as required. This new Lentivirus vector can simultaneously knock down ENaC and deliver CFTR in human airway cells in culture.

Future studies will determine whether this dual-function vector can correct the functional defect in primary CF airways.

Dual-therapy Lentivirus to treat Cystic Fibrosis lung disease

CF is caused by mutations in the CFTR chloride channel. In the lung this leads to dysregulation of epithelial ion transport resulting in chronic bacterial infection, lung inflammation, and premature death. In CF chloride channel activity is reduced, whereas sodium channel (ENaC) activity is increased. We are using a dual-function Lentivirus to increase CFTR chloride expression and knock-down ENaC sodium transport to re-balance airway ion transport.

F/HN pseudotyping

The lentivirus Env coat protein has a limited range of receptors, therefore most lentiviral vectors are produced as pseudotypes expressing coat proteins from other viruses with a broader cell tropism. Lentivirus is often pseudotyped with the Vesicular Stomatitis Virus G protein (VSV-G), but this pseudotype does not efficiently transduce the lung. Lentivirus can be much more efficiently targeted to airway epithelial cells by pseudotyping with the respiratory Sendai Virus fusion (F) and hemagglutinin/neuraminidase (HN) coat proteins.

We can produce large quantities of F/HN-pseudotyped Lentivirus suitable for efficacy and toxicology studies. Initial work shows life-long duration in mouse with no significant toxicity.

Our dual-function lentiviral vector can simultaneously knock down ENaC and deliver CFTR in human airway cells in culture.

Future studies will determine whether this dual-function vector can correct the functional defect in primary CF airways.

This F/HN Lentivirus lung gene delivery system was awarded The Translational Research Innovation Award in the Medical Futures Innovation Awards 2011.

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