Cystic Fibrosis Gene Therapy: Methods for the optimisation of CFTR gene delivery.

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Abbreviations

µg	microgram
µL	microlitre
AAV	Adeno-associated virus
AdV	Adenovirus
ARO	anaplastic thyroid cancer cell line
ASL	Airway Surface Liquid
ATP	Adenosine Triphosphate
Baf-A1	Bafilomycin A1
BAL	Bronchoalveolar lavage
BPB	Bromophenol Blue
Ca^{++}	Calcium ion
cAMP	Cyclic Adenosine Monophosphate
CF	Cystic Fibrosis
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
CHOK-1	Chinese Hamster Ovary cells
CIP	Calf Intestinal Phosphatase
Cl^{-}	Chloride Ion
CT	Computed Tomography
Ct	Cycle Threshold
DGGE	Denaturing gradient gel electrophoresis
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid

EF1α	Elongation factor alpha
ENaC	Epithelial sodium channel
EtOH	Ethanol
EYFP	Enhanced Yellow Fluorescent Protein
FABp	fatty acid binding protein
FDA	Food and Drug Administration
FEV1	Forced Expiratory Volume in one second
gDNA	Genomic Deoxyribonucleic acid
H & E	Haemotoxylin and Eosin
HCl	Hydrogen Chloride
HeBS	Hepes Buffered Saline
HIV-1	Human Immunodeficiency Virus type 1
I.P	Intraperitoneal
IFN-γ	Interferon Gamma
Ig	Immunoglobulin
IL-	Interleukin-
IMVS	Institute of Medical and Veterinary Science
IRT	Immunoreactive Trypsinogen
K^+	Potassium Ion
K18	Cytokeratin-18
kbp	kilobase pairs
kDa	kilodalton
LiCl	Lithium Chloride
LMB	Leptomycin B
LPC	Lysophosphatidylcholine

LTR	Long Terminal Repeat
LV	Lentivirus
MDS	Membrane Spanning Domains
MeOH	Methanol
MHC	Major Histocompatibility Complex
mL	Millilitre
mm	Millimetre
MRI	Magnetic Resonance Imaging
mRNA	Messenger Ribonucleic Acid
Na^+	Sodium ion
NaCl	Sodium Chloride
NaOAc	Sodium Acetate
NaOH	Sodium Hydroxide
NBD1	Nuclear Binding Domain 1
NBD2	Nuclear Binding Domain 2
NBF	Neutral Buffered Formalin
NFQ	Non Fluorescent Quencher
nM	nanomolar
NO	Nitric Oxide
OCT	Optimal Cutting Temperature
OGTR	Office of the Gene Technology Regulator
OLA	Oligonucleotide Ligation Assay
ORCC	Outwardly Rectifying Chloride Channel
PAMP	Pathogen-associated Molecular Patterns
PBS	Phosphate Buffered Saline

PCL	Periciliary Liquid
PCR	Polymerase Chain Reaction
PD	Potential Difference
PEG	Polyethylene glycol
PET	Positron Emission Tomography
PFT	Pulmonary Function Testing
Pgk	Phosphoglycerate kinase
PKC	Protein Kinase C
pM	picomolar
PMA	phorbol 12-myristate 12-acetate
PRR	Pathogen Recognition Receptors
RSV	Respiratory Syncytial Virus
RT-PCR	Real Time Polymerase Chain Reaction
Saf O	Safranin O
SDS	Sodium dodecyl sulphate
SELEX	Systematic Evolution of Ligands by Exponential Enrichment
SeV	Sendai Virus
SSC	Saline sodium citrate
SSCP	Single Strand Conformation Polymorphism
Th1	T-helper type 1
TLR	Toll Like Receptor
TNF-α	tumor necrosis factor-α
TPD	transepithelial potential difference
UNC	University of North Carolina
v/v	volume for volume

Vif	virion infectivity factor
Vpr	viral protein r
VSV-G	vesicular stomatitis virus G glycoprotein
WCH	Women's and Children's Hospital
w/v	Weight for weight
Z-LLL	Z-leu-leu-leu

Abstract

Gene therapy potentially holds the key for the treatment and cure of many genetic diseases, including cystic fibrosis. A number of delivery methods have been developed for the integration of a functional gene into the host genome, one of which is the use of a HIV-1 derived lentivirus, as is used in this thesis. However, a large number of issues need to be addressed before an effective gene therapy protocol can be developed, and some of these are described further in this thesis.

One such issue is that the response initiated by cells to the gene transfer vector need to be addressed, as organelles such as the proteasome and lysosome that break down foreign peptides and proteins may be involved in the degradation of our gene transfer vector, ultimately limiting the amount of gene transfer vector that is able to successfully integrate into the genome. Therefore, the potential use of proteasome and lysosome inhibitors for facilitating higher levels of gene transduction *in vivo* was investigated.

As this project uses a HIV-1 derived lentivirus for gene transfer, the use of an inhibitor of the IN1/PML innate antiretroviral response (Leptomycin B) was also assessed, again with the aim of increasing the efficiency, and hence level, of gene transfer obtained.

Using a robust animal model of disease is essential for testing lentivirus constructs containing the therapeutic gene and analysing phenotypic changes in disease. A mouse model of cystic fibrosis without gastrointestinal disease was bred to obtain a robust colony of mice that efficiently produce affected mice (CFTR knockout).

Visual analysis of therapeutic gene transfer in cystic fibrosis is often difficult due to the lack of antibodies available. Short DNA molecules that adopt a specific 3-D shape known as aptamers hold

much potential as agents that can be developed to bind to the CFTR gene product. These can then be labelled and used in the same way as antibodies to probe tissues excised from animals treated with the therapeutic CFTR gene.

Essential to gene therapy is the development of methods for the consistent determination of lentivirus titre. As the production of lentivirus becomes more sophisticated with the use of multiple transgenes in a single virus preparation, the need for multiple assays to determine the titres of each individual virus component are required. Real time PCR assays were developed for each individual transgene for titre determination. A real time PCR assay for use with CHOK-1 cells was also developed for comparison of real time PCR titre of a LacZ virus to the titre obtained using the traditional LacZ titre achieved *via* a staining assay in CHOK-1 cells. The use of a standard real time PCR assay for the determination of titre for all viruses- containing any transgene is essential to allow comparison by titre.

Determination of the level of gene expression required to achieve a therapeutic outcome in a cystic fibrosis mouse model is an important factor to consider, as high level expression in all cells may not achieve the best outcomes, and low level, ciliated-cell specific expression may in fact achieve a superior result. A range of different strength and cell targeted promoters (EF1α, pgk and K18) were tested for their effect on phenotypic correction of the cystic fibrosis knockout mouse model.

Lastly, targeting therapeutics to the disease affected areas is essential in achieving the best patient outcomes. As the main target organ in cystic fibrosis are the conducting airways of the lungs, delivery of our gene transfer vectors to the lungs as an aerosol is a necessary step towards moving this gene therapy protocol to the clinic. Aerosolisation of the treatment protocol was investigated in the rat lung for evidence of whether this is a viable means of lentiviral mediated gene delivery to the airways.

Declaration

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