Title: Targeting miRNA-based medicines to cystic fibrosis airway epithelial cells using nanotechnology

Body: Cystic Fibrosis (CF) is characterised by chronic pulmonary inflammation. microRNAs (miRs) are regulatory RNAs which inhibit gene expression. miR-126, a regulator of TOM1, is decreased in CF bronchial brushings; TOM1 is reciprocally increased. Polymeric nanoparticles of Polyethylenimine (PEI) and Chitosan-TPP can be used to deliver nucleic acids into bronchial epithelial cells. Here a proof-of-concept study was performed testing their efficacy at delivery of premiR-126 into non-CF and CF bronchial epithelial cells lines. PremiR-126-nanoparticles were prepared and characterized using a Zetasizer and used to transfect CFBE41o- and 16HBE14o- cell lines. RNA extraction was carried out 24h post transfection, cDNA was prepared and miR-126 and TOM1 expression was assessed using qRT-PCR. Toxicity was measured by high content analysis (HCA). Over-expression of miR-126 resulted in down-regulation of TOM1 in both cell types. The use of polymeric, cationic nanoparticles was shown to efficiently deliver premiR-126 into cells in order to achieve this knockdown. PremiR-126 encapsulated in PEI at a nanoparticle/premiR (N/P) ratio of 1:1 resulted in knockdown of TOM1 in CFBE41o- cells, with a reduction of ∼47% in TOM1 expression in comparison to a scrambled negative control complexed with commercial transfection reagent (p≤0.05). HCA showed no significant difference in cell counts between untreated cells and cells treated with PEI- and chitosan-TPP-premiR-126 nanoparticles, suggesting they are relatively non-toxic. miRs are potential novel targets for respiratory gene therapy and could be targeted to CF bronchial epithelial cells in the lungs using PEI nanoparticles. Funding:SFI and HRB PhD/2007/11.