BACKGROUND

The problem: Inefficient lung cytosol delivery

Lung epithelial cells provide structural integrity, allow gas exchange and enhance ion and fluid transport. Damaged epithelium leads to the development of respiratory diseases, such as chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis (IPF), cystic fibrosis, and, lately, the 2019 coronavirus disease (COVID-19).

Effective use of macromolecular therapeutics targeting intracellular components of the lung is severely restricted due to poor cytosolic delivery to the respiratory epithelium (Moschos et al., 2017). Outer membrane vesicles (OMVs) are spherical structures (20-250nm) produced by pathogenic and commensal bacteria. They carry small portions of bacterial membranes bulge away from the cell, pinch off, and release into the surrounding environment (Momen-Heravi et al., 2018).

OMVs allow bacteria to interact with their environment and, as a result, exert diverse functions such as host cell interaction and the initiation of pathogenesis, survival during stress conditions by toxin sequestration, and regulation of microbial interactions. These functions are afforded through biologically active proteins and nucleic acids naturally encapsulated in these liposome or virus-like structures. Their utility to man is well-established through their scaled bioproduction and application in several commercially successful vaccine preparations.

Recent production of OMVs from BL21 (DE3)-derived, LPS-deficient Escherichia coli (Valentine et al., 2016), commercially available as ClearColi® (Mamat et al., 2015), introduced the possibility of pathogen associated molecular pattern-free OMVs, at least in terms of TLR4 activation.

Our solution: bioengineered microbial vesicles

Overcome cytosolic lung delivery bottlenecks by bioengineering LPS-free, recombinant, biocompatible OMVs biotherapeutics.

PROJECT OBJECTIVES

- Produce ClearColi® OMVs expressing a proprietary transmembrane protein (TMP) which facilitates active internalisation pathways across respiratory epithelia specifically.
- Produce bioactive OMVs featuring a series of imaging reporter functions.
- Demonstrate TMP-enhanced OMV targeting to respiratory epithelial cells and cytosolic reporter protein delivery.
- Demonstrate functional biotherapeutic compound delivery within respiratory epithelial cells in vitro and air-liquid interface culture.

STUDY DESIGN

• Stage 1: Clone production
  - Clone proprietary TMP, far-red fluorescence protein (tdTomato), and N- or C-terminally-tagged TMP in BL21 (DE3) and ClearColi® E. coli.
  - Optimise recombinant protein expression.

• Stage 2: Scalable production of ClearColi® OMVs
  - Develop scalable OMV production methods.
  - Characterise clone impact on OMV proteome, transcriptome.

• Stage 3: Evaluate cytosolic access in cells
  - OMV cell trafficking, RNA-Seq, and immunomodulation:
    - In submerged cell culture.
    - In Air Liquid Interface (ALI) primary cell culture.

• Stage 4: Demonstrate efficient cytosolic biotherapeutic delivery
  - Generate therapeutic RNA and ribonucleoprotein-containing, TMP-labelled OMVs.
  - Quantify pharmacological activity in translationally relevant cell culture systems.

REFERENCES


CONCLUSIONS & FUTURE PLANS

- Eukaryotic receptor ligand TMs can be ectopically expressed in TLR4-evading ClearColi® bacteria.
- N-terminal tagging enhances TMP loading onto OMV membranes but substantially alters OMV proteome profile.
- Validation of TMP® ClearColi® OMV loading and internalization by lung epithelial cells may unlock oligonucleotide, ribonucleoprotein or recombinant/mRNA delivery for chronic, hereditary, and infectious lung disease such as IPF, COPD, cystic fibrosis, and COVID-19.

Bioengineering bacterial outer membrane vesicles as delivery systems for RNA therapeutics targeted to lung epithelial cytosols.

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