Evaluation of KB407, an HSV-1-Based Gene Therapy Vector for the Treatment of Cystic Fibrosis, in Healthy and Patient-Derived Airway Cells Including an Apical-Out Diseased Airway Organoid Model

ATS 2024
Annual Meeting
POSTER P122

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Background

- Cystic fibrosis (CF) is caused by mutations in the CF transmembrane conductance regulator (*CFTR*) gene and characterized by recurrent chest infections, increased airway secretions, and progressive pulmonary impairment.
- To address the need for gene-corrective therapies, particularly for patients that can not take approved modulator therapies, we have developed KB407, a herpes simplex virus type 1 (HSV-1)-based gene therapy vector encoding full-length human CFTR.
- The vector backbone underlying KB407 is non-replicating, accommodates the large size of the *CFTR* gene, harbors a natural tropism for epithelial cells, and has an established record of clinical safety after repeated administration in other conditions.
- Here, KB407-derived CFTR was evaluated for proper glycosylation upon transduction of healthy and CF primary human bronchial epithelial cells (HBECs) and small airway epithelial cells (SAECs) in two-dimensional cell culture.
- The ability of KB407 to deliver its genetic cargo to the polarized airway epithelium via the apical membrane was then established using a three-dimensional, apical-out airway organoid (AOAO) model differentiated from human primary healthy and CF bronchial cells.

Glycosylation of KB407-Derived CFTR in Clinically Relevant Primary Human Airway Cells Reveals Appropriate (Mature) Protein Processing

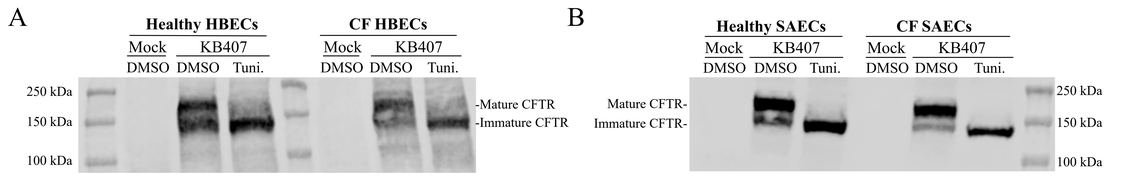

Figure 1. CFTR glycosylation, a modification necessary for trafficking of the protein to its site of action at the plasma membrane, was visualized in KB407-transduced healthy and CF patient-derived (**A**) HBECs and (**B**) SAECs via western blotting (WB) with and without treatment using a glycosylation inhibitor (tunicamycin, 'Tuni.' at 2.5 µg/mL). KB407 transduced both primary healthy and CF patient-derived HBECs and SAECs, resulting in robust expression of full-length, fully glycosylated CFTR, while treatment with tunicamycin revealed only the immature CFTR band. Mock-treated cells and DMSO (vehicle) treatment served as controls.

A Novel AOAO Model System from CF Patient-Derived Cells Effectively Transduced by an HSV-1-Based Fluorescent Reporter Virus (SAR)

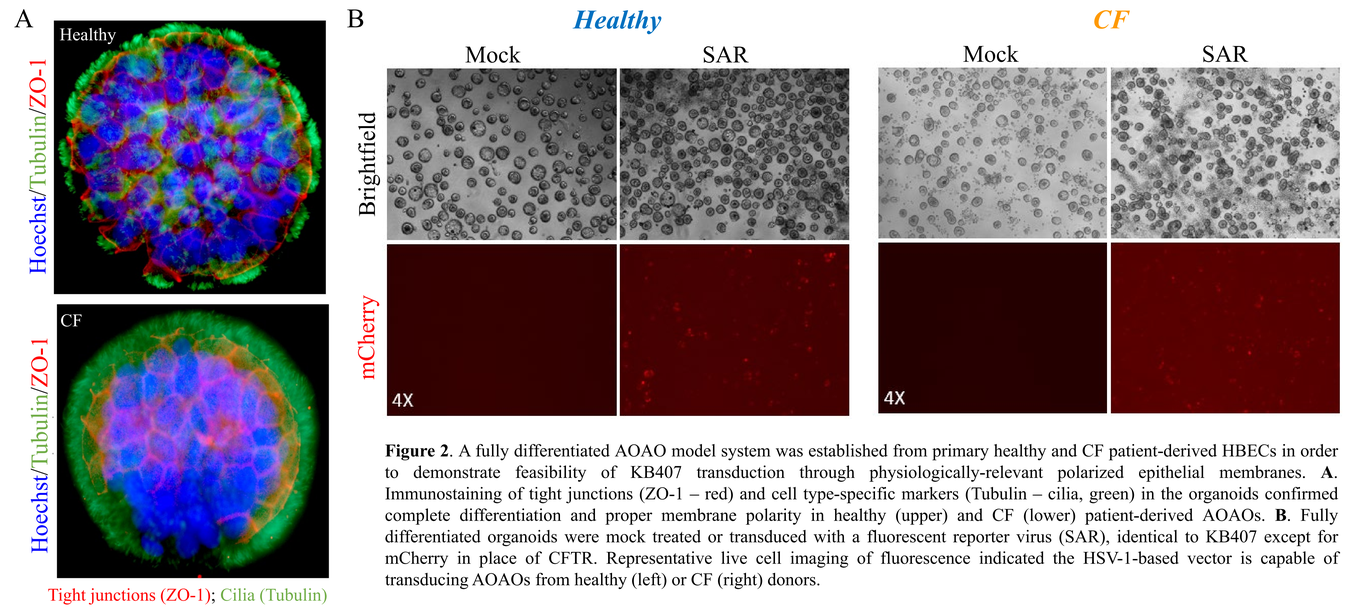

Figure 2. A fully differentiated AOAO model system was established from primary healthy and CF patient-derived HBECs in order to demonstrate feasibility of KB407 transduction through physiologically-relevant polarized epithelial membranes. **A**. Immunostaining of tight junctions (ZO-1 – red) and cell type-specific markers (Tubulin – cilia, green) in the organoids confirmed complete differentiation and proper membrane polarity in healthy (upper) and CF (lower) patient-derived AOAOs. **B**. Fully differentiated organoids were mock treated or transduced with a fluorescent reporter virus (SAR), identical to KB407 except for mCherry in place of CFTR. Representative live cell imaging of fluorescence indicated the HSV-1-based vector is capable of transducing AOAOs from healthy (left) or CF (right) donors.

Exogenous CFTR Co-Localizes with a Viral Marker in Transduced AOAOs

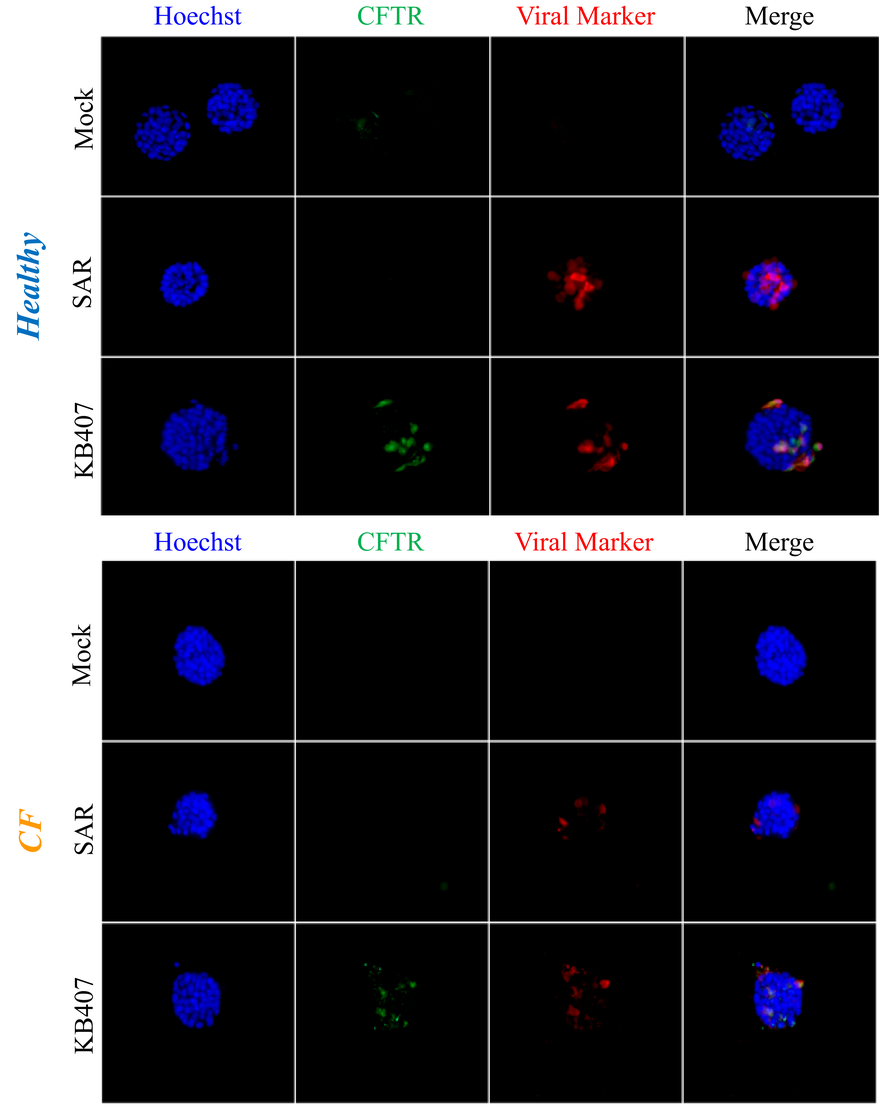

Figure 4. Immunofluorescence (IF) was used to demonstrate KB407 transduction of healthy (upper) and CF patient (lower) bronchial-derived AOAOs. Following KB407 transduction, CFTR protein (green) co-localized with an HSV-1 viral marker protein (red), expressed from the vector backbone of both SAR and KB407. In CF patient-derived samples, detection of CFTR protein was limited to cells that were HSV-1 marker protein-positive. Low levels of endogenous CFTR were detected in mock-infected healthy patient-derived samples. Hoechst (blue) was used to visualize nuclei.

KB407 Transduces Healthy and CF Bronchial-Derived AOAOs Resulting in *CFTR* Transcripts and Mature CFTR Protein

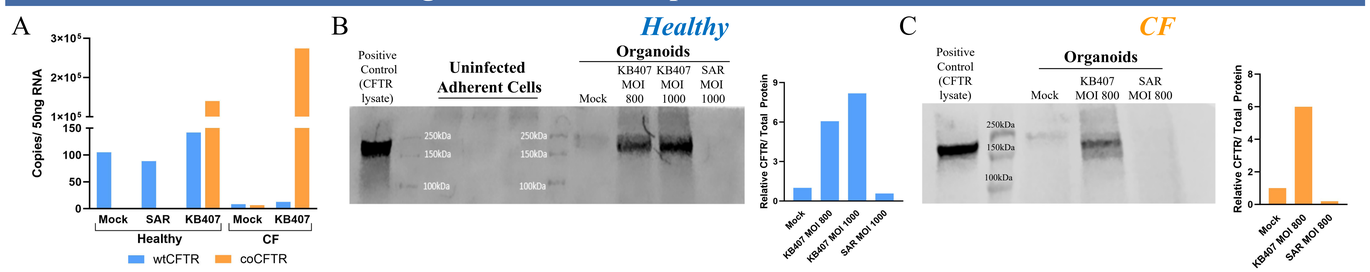

Figure 3. Following KB407 transduction, (**A**) codon-optimized (co) exogenous *CFTR* transcript levels were similar in healthy and CF patient-derived AOAOs as assessed by qRT-PCR. WB demonstrated exogenous CFTR was both full-length and fully glycosylated for both (**B**) healthy and (**C**) CF-patient derived AOAOs. The relative amount of CFTR to total protein is quantified (**B and C**, right) with the mock control set to 1. MOI = multiplicity of infection; wt = wild type.

Conclusions

KB407 is able to transduce clinically relevant airway epithelial cell populations *in vitro*, including via the apical membrane of a polarized, three-dimensional model, and deliver full-length, mature CFTR, supporting future clinical development of KB407 as an inhaled therapeutic for CF patients.

Disclosures

These studies were funded by Krystal Biotech, Inc. All authors are current employees of Krystal Biotech, Inc.