Supplement E-Appendix 1:

Nasal Brushing and Cell Isolation

Subject's nasal epithelium was anesthetized with 1% tetracaine (Pontocaine; Sanofi Winthrope, New York, NY) by spraying 2 ml in each nostril with a mucosal atomization device (MAD) spray tip (Wolfe Tory Medical Inc., UT). In order to confine the tetracaine to the nasal passages, each subject took slow deep breaths through the mouth during application of the topical anesthetic. After waiting ten minutes for the tetracaine to exert its effect, each nasal passage was washed twice with 10 ml of warm sterile saline, a total of 40 ml. To collect cells, the nasal epithelium was brushed with a combined back and forth and rotating motion. The brush was a 115 cm disposable cytology brush, bristles extending 10 mm with a 5 mm diameter (Model BC-202D-5010, Olympus Medical Systems, Tokyo). The brush handle was cut to result in a working length of about 20 cm. The first brushing occurred along the lower medial margin of the inferior turbinate, and the second and third brushings each moved up the medial wall of the inferior turbinate. At the end of each brushing, the tissue samples were removed from the brush by manually flicking and shaking the brush in a centrifuge tube containing 10 ml of phosphate buffered saline (PBS) supplemented with penicillin (10^5 U/l), streptomycin (100 mg/l), gentamicin (100 mg/l) and amphotericin B (2.5 mg/l); hereafter referred to as PBS with antibiotics. Both sides of the nose were brushed in this manner for a total of six samples.

The nasal tissue consisting primarily of small aggregates of nasal mucosa and mucus was rinsed three times in PBS with antibiotics containing 5 mM dithiothreitol to reduce mucus contamination. After a final rinse in PBS with antibiotics, the tissue was incubated at 4^0C overnight in PBS with antibiotics containing 0.025% protease. The following day, the enzyme solution was neutralized with a 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F-12 (DF12) containing 5% fetal bovine serum (FBS) and antibiotics as described above. Any clumps of nasal airway epithelial cells remaining were dispersed by the addition of 0.05% trypsin, 0.02% EDTA in 0.9% Na^+Cl^- wt/v (saline-trypsin-versene; STV) and repeated agitation in a 10 ml pipette. After centrifugation of the cell suspension (100 x *g*), cell number and viability were determined using trypan blue and a hemacytometer.

Conditional Reprogramming of Nasal Epithelial Cells and Production of Differentiated Cell Cultures

Isolated nasal cells are then conditionally reprogrammed according to published methods with some modifications.[1; 2] 3T3-L1 fibroblasts (obtained from the Cell Culture Facility, University of California, San Francisco) were suspended in F-medium (3:1 Ham's F-12:DMEM, supplemented with 5% FBS, 5µg/ml bovine insulin (Sigma-Aldrich), 8.4 ng/ml cholera toxin (Sigma-Aldrich), 10 ng/ml recombinant human EGF (Atlanta Biologicals,), 250 ng/ml hydrocortisone (Sigma-Aldrich), 10µM Y27632-ROCK Inhibitor (Enzo Life Sciences), penicillin/streptomycin (as above), 250 µg/ml amphotericin B and 10 µg/ml gentamicin) and irradiated in the Gammacell 3000 Elan (Best Theratronics, Springfield, VA) at 30 Gy. Irradiated cells were then plated (3 x 10^6 per 10 cm tissue

culture dish; Corning) and allowed to attach for 2 hours at 37°C. 1.5 x 10^6 nasal cells were added to each dish and allowed to proliferate for 4 to 7 days (37°C, 5% CO_2). The nasal cells were harvested when approximately 80% confluent by differential trypsinization. The plate was rinsed twice in PBS with antibiotics, and then treated with 2 ml 0.05% STV for 4 minutes at 37°C to remove irradiated 3T3 fibroblasts. Following two PBS rinses, an additional 4 ml STV was added for 8 minutes at 37°C. The STV was neutralized in F medium, and cell number and viability were determined as above. Next, the conditionally reprogrammed cells were plated at 2.5 x 10^5 viable cells/cm^2 onto tissue culture inserts (Costar Transwell) coated with human placental collagen (20 μg/cm^2; Sigma) in DF12 containing 5% FBS and antibiotics. After 24h, the inserts were rinsed with PBS with antibiotics and the fed from the basal side only with UNC-ALI media, serum-free media that supports growth and functional differentiation of tracheo-bronchial respiratory epithelial cell cultures.[3] Medium was changed three times a week by addition of approximately 1 ml to basal side of the insert. Cultures were grown for 28 days to ensure full differentiation.

Determination of CFTR-mediated Cl^- currents in Ussing chambers

For measurements of transepithelial Cl^- current (I_{Cl}), confluent nasal cultures were mounted into water-jacketed (37°C) EasyMount Ussing chambers (Physiologic Instruments, San Diego, CA), and used for electrophysiological studies, as described previously.[4] Transepithelial voltage (V_T) was clamped to 0 mV. Short-circuit current (I_{Cl} under the conditions of these experiments) were measured using a four-electrode voltage clamp (VCC MC6 multichannel voltage clamp, Physiologic Instruments), with

Ag-AgCl electrodes (World Precision Instruments, Sarasota, FL) connected to the solutions through agar bridges containing 1 M KCl. V_T was briefly clamped from 0 to 1 mV and R_T was calculated from the corresponding current deflection using Ohm's law. Positive currents were defined as anion movements from serosa to mucosa. In the Ussing chambers, bicarbonate-buffered solutions (5 ml) recirculated on the apical and basolateral sides separately. A serosal-to-mucosal Cl^- gradient was used to increase the electrochemical driving force for Cl^- secretion. The basolateral solution contained (in mM) 120 NaCl, 25 $NaHCO_3$, 5 KCl, 1.2 NaH_2PO_4, 5.6 glucose, 2.5 $CaCl_2$, and 1.2 $MgCl_2$. The mucosal Cl^--free solution contained (in mM) 120 Na^+-gluconate, 20 $NaHCO_3$ 5 $KHCO_3$, 1.2 NaH_2PO_4, 5.6 glucose, 2.5 $Ca^{++}(gluconate)_2$, and 1.2 $MgSO_4$. All solutions were gassed with 5% CO_2-95% air, resulting in pH 7.4. Amiloride (100 uM; apical) was added mucosally to block Na^+ absorption and forskolin (20 uM; serosal) was added with acute exposure of ivacaftor (1 uM; apical) to activate cAMP-dependent CFTR Cl^- transport. Patient-specific CFTR Cl^- currents were quantified with 50 uM CFTRinh172 (CF Foundation Therapeutics, Inc.). Ivacaftor was purchased from Selleckchem.com

Supplement References

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