Co-culture of human type I and type II pneumocyte cell lines as a model of alveolar epithelium

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The epithelial tissues of the distal lung are continuously exposed to inhaled air, and are thus of research interest in studying respiratory exposure to both therapeutic and hazardous materials. There is a need therefore to develop sophisticated models of the human alveolar epithelium, which better represent the different cell types present in the native lung and interactions between them. Our aim was to develop an air-liquid interface (ALI) model of the alveolar epithelium by incorporating human cell lines which bear features of type I (hAELVi) and type II (NCI-H441) epithelial cells.
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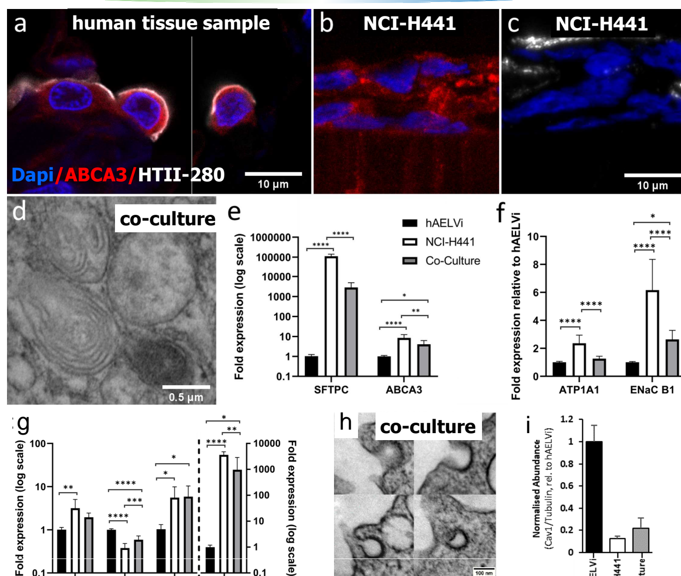
Materials and methods

Single cultures of NCI-H441 (ATCC) and hAELVi (Inscreenex) , and co-cultures consisting of equal proportions of both were seeded at $1*10^5$ cells/cm^2 on permeable supports (Transwell 0.4 µm pore inserts) coated with collagen I in advanced DMEM supplemented with 1% glutamax, 200 nM dexamethasone, 1% Fetal bovine serum and antibiotics. After reaching confluence (typically within 1 week) the cells were cultured at ALI. TEER was measured every 2-3 days and cells were fixed after 14 days at ALI for immunolabelling and TEM or lysed for mRNA (RT-qPCR) and protein analysis (WB). *See Brookes et al 2021 for details.*

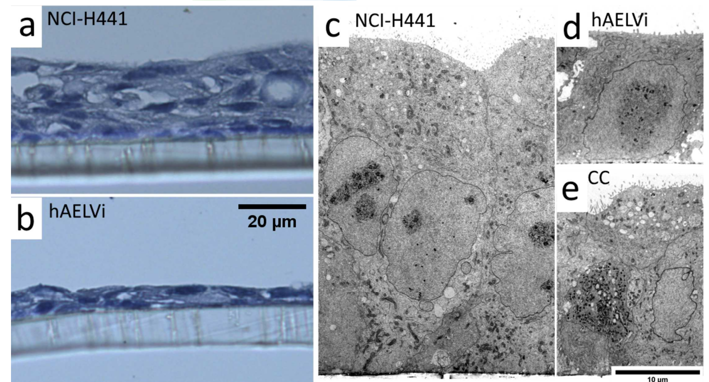
Key functional protein and gene expressions



Immunofluorescent labeling of the lamellar body component ABCA3 (red) and type-II specific antigen HTII-280 (white) in normal human tissue samples (a) and in NCI-H441 ALI cultures (b and c). TEM image of lamellar bodies (d) in co-cultures. RTqPCR of surfactant protein SFTPC (e), lamellar body protein ABCA3 (e), ion pumps ATP1A1 (f) ENaC B1 (f) and water channels aquaporins AQP1, AQP3, AQP5 and AQP4 (g) (fold expression relative to hAELVi after normalization to reference genes RPL13, RPL19 and HPRT). TEM image of caveolae in co-cultures (h) and quantification of caveoline 1 protein expression by western blot (i) (abundance relative to hAELVi after normalization to tubulin). n = 3 replicates within 3 independent cultures (4 days at ALI).

NCI-H411 mono-cultures expressed type II specific markers (SFTPC, ABCA3) and higher levels of AQP1, AQP5, AQP4, ATP1A1 and ENaC B1 compared to hAELVi. In contrast, levels of type I specific proteins (Caveoline) and AQP3 were higher in hAELVi mono-cultures. Co-cultures showed intermediate expressions and structural markers of type I (caveoli) and type II cells (lamellar bodies).

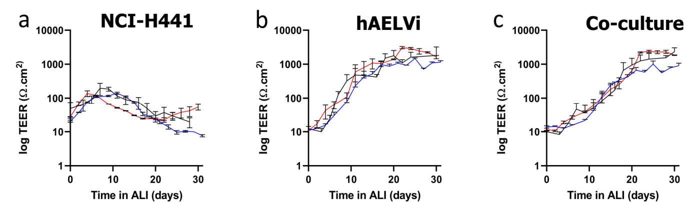
Supra-cellular structures of ALI cultures



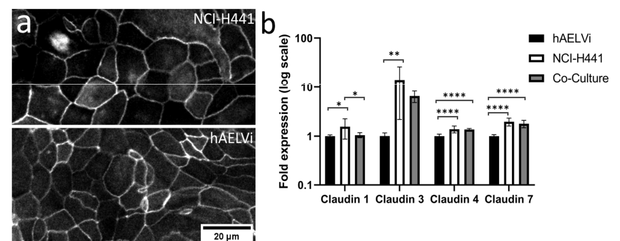
Ultrathin sections (a, b) and TEM images (c, d, e) of NCI-H441 (a, c), hAELVi (b, d) or the coculture (e) after 14 days at ALI.

NCI-H441 cultures exhibit a loose stratified cell layer, while hAELVi typically form a flatter monolayer. Co-cultures do exhibit stratification but form a more compact culture than NCI-H441 in mono-cultures.

Barrier properties of ALI cultures



TEER measurements of NCI-H441 (a), hAELVi (b) and an equal co-culture of the two (c) over a period of thirty days following the establishment of ALI conditions. n = 3 transwells within 3 independent experiments.



Immunolabeling of ZO1 in the two parent lines (a) and RTqPCR of tight junction proteins claudin 1, 2, 3 and 4 (b) (fold expression relative to hAELVi after normalization to reference genes RPL13, RPL19 and HPRT). n = 3 replicates within 3 independent cultures (14 days at ALI).

Both single cell lines establish functional tight junctions in ALI conditions. TEER are greater and persistent in hAELVi but claudin expressions are higher in NCI-H411. The profile of claudin expression of co-cultures is similar to NCI-H411 whereas the TEER is close to hAELVi but with a slower kinetic.

The co-culture model was observed to form a stable barrier akin to that seen in hAELVi and barrier properties can be maintained for 30 days. Co-cultures express markers of type II pneumocytes (surfactant protein C, lamellar bodies, Ag HTII-280) and type I pneumocytes (caveoli) and having a profile of expression of ion pumps, claudins and aquaporins appropriate for the distal lung.
In summary, our results support the co-culture of these two cell lines to produce a model which better represents the breadth of functions seen in native alveolar epithelium.

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