Characterization of CI-huAEC airway epithelial cells for air-liquid exposure experiments

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Objective

Cell-based lung models of human origin are needed for toxicity and efficacy studies as well as for assessing the toxic potential of environmentally and occupationally relevant airborne substances. Immortalized cell lines often do not adequately reflect the in vivo situation in lung tissue, while primary cells, on the other hand, are of limited availability. The CI-SCREEN technology aims to overcome this problem by implementation of expansion genes into freshly isolated primary cells. As a result primary cells can thereby be expanded to cell numbers enabling reliable and reproducible testing protocols while retaining functions of primary cells. In this study, CI-huAEC cells were characterized in a first step to barrier formation, CYP expression and inducibility, expression of surface markers and ALI-exposure.

Methods

Figure 1: Scheme of the experimental setup for exposing CI-huAEC cells to airborne test substances (formaldehyde) using air-liquid interface conditions. The system consists of three parts: A: Generation and transport of the test substance atmosphere (1-4) or the clean air (5) control respectively. B: The exposure unit for the target cells, grown under air-liquid interphase conditions (P.R.I.T.® ExpoCube® ;6). C: Analysing unit for test atmosphere (FT-IR Monitor). Position 4 represents the exhaust of excess test atmosphere.

Cell culture: CI-huAEC Cells (INS-CI-1011) were purchased by InSCREENex GmbH, Braunschweig and cultivated with serum free medium according to the manufacturer’s instructions. Cells were routinely split twice a week. For air-liquid interface culture cells were grown on PET-membranes (BD Falcon) with 1cm² in diameter and 0.4µm pore size. The transepithelial electrical resistance (TEER) was measured to monitor the barrier properties of the cell monolayers with chipstick electrodes. Viability measurements were performed by using the WST-Assay. Detection of surface markers was performed by FACS whereas expression of xenobiotic metabolizing enzyme genes was shown by RTq-PCR.

Results

Figure 2: Population doubling time depending on the number of passages (left) and development of TEER-values under air/liquid interface conditions depending on seeding density, cultivation time and passage; (early passage ≤ 12, late passages ≥ 20) (right).

Figure 3: Dose effect curve of CI-huAEC cells exposed to formaldehyde for 1hr followed by a 24hrs post incubation period. Confidence interval of 95% is stained grey. EC50 value = 2086 ppm

Figure 4: Analysis of Ep-CAM (A) Podoplanin (B) and Caveolin-1 (C) staining. Nearly all cells expressed Ep-CAM in contrast to podoplanin and caveolin-1.

Conclusion

The CI-huAEC cell line may represent a very promising model for inhalation toxicology studies.

COI: Tobias May is shareholder of InSCREENex GmbH which commercializes the CI-huAEC cell line

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