

Air–Liquid Interface Models of Human Airway Epithelium for Enhanced Drug Discovery and Safety Assessment

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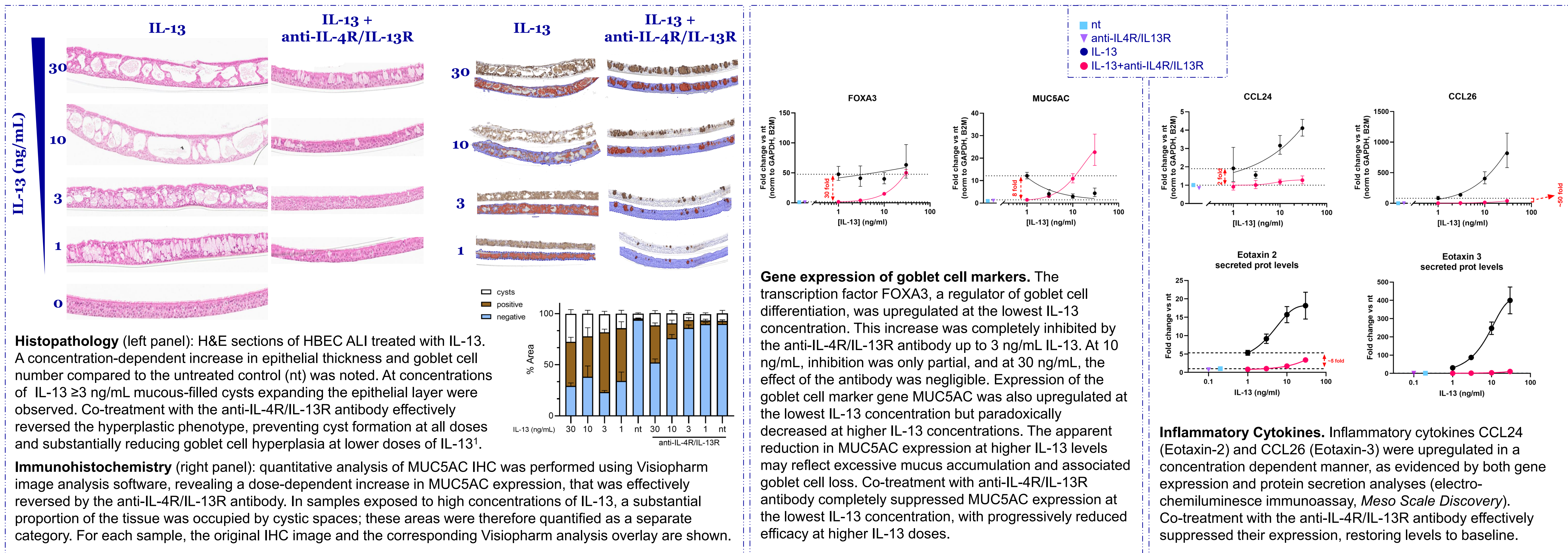
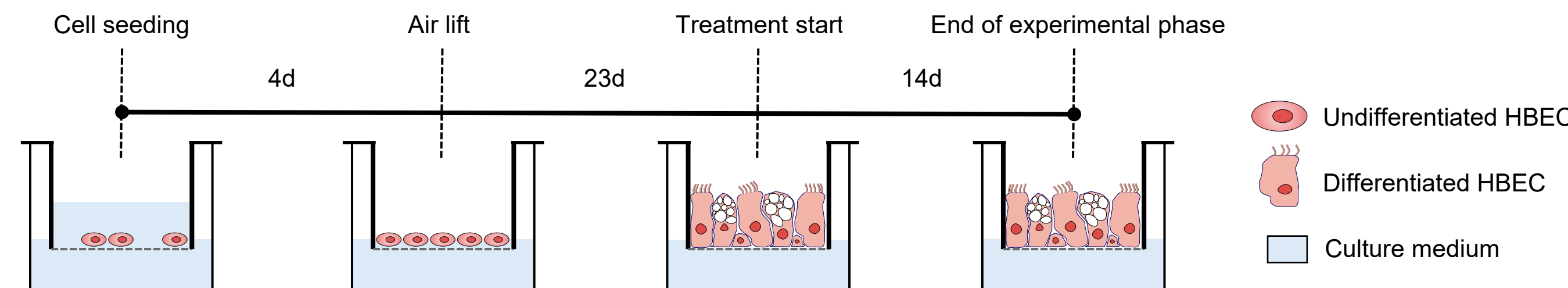
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INTRODUCTION

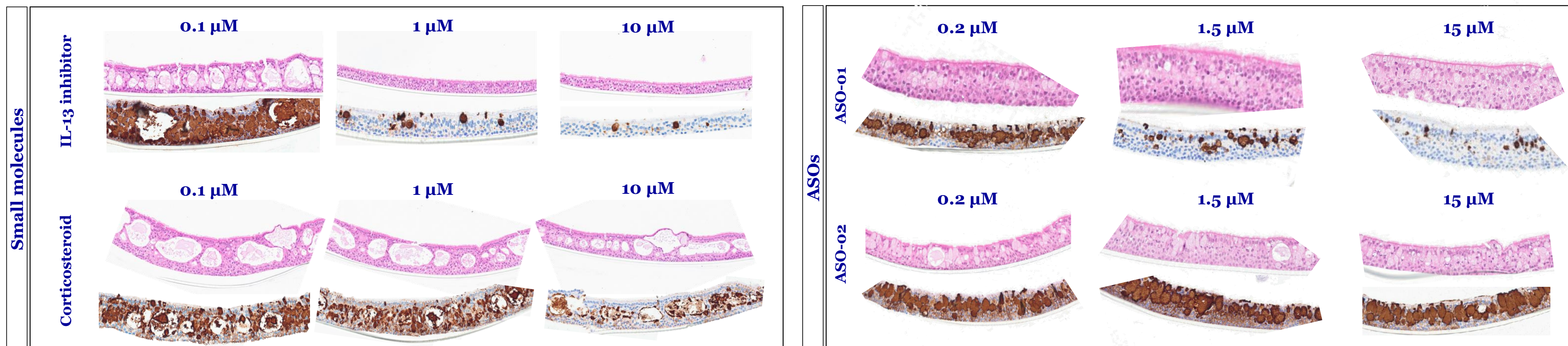
In vitro models and new approach methodologies (NAMs) provide ethical, cost-effective, and disease-relevant complements to animal testing for drug discovery and toxicological screening. Air–liquid interface (ALI) cultures of primary human bronchial epithelial cells (HBECs) recapitulate key structural and functional features of the airway epithelium, including epithelial remodeling under pathological conditions, such as IL-13 driven goblet cell hyperplasia. Here, we describe the development, validation, and application of advanced ALI systems, including monoculture and multicellular co-culture models, under basal and stimulated conditions, highlighting the usefulness of pathology endpoints in guiding model development and defining translationally relevant phenotypic changes.

AIR-LIQUID INTERFACE MODEL DEVELOPMENT AND VALIDATION

HBECs were seeded onto 24-well plate-fitting transwell inserts (n=84) and initially cultured with medium added to both the apical and basal compartments. Upon reaching confluence (4 days post-seeding), culture medium was supplied only to the basal chamber (air-lift), driving differentiation towards a mucociliary phenotype and mimicking the anatomical conditions of the airway epithelium. To induce goblet cell hyperplasia, a hallmark of asthma and other chronic airway disorders, cells were treated with IL-13 at scalar concentrations (30, 10, 3, 1 ng/mL) every other day. Untreated cells (nt) served as negative controls, while co-treatment with an anti-IL-4R/IL-13R antibody (100nM) was used as positive control to inhibit IL-13 effects.



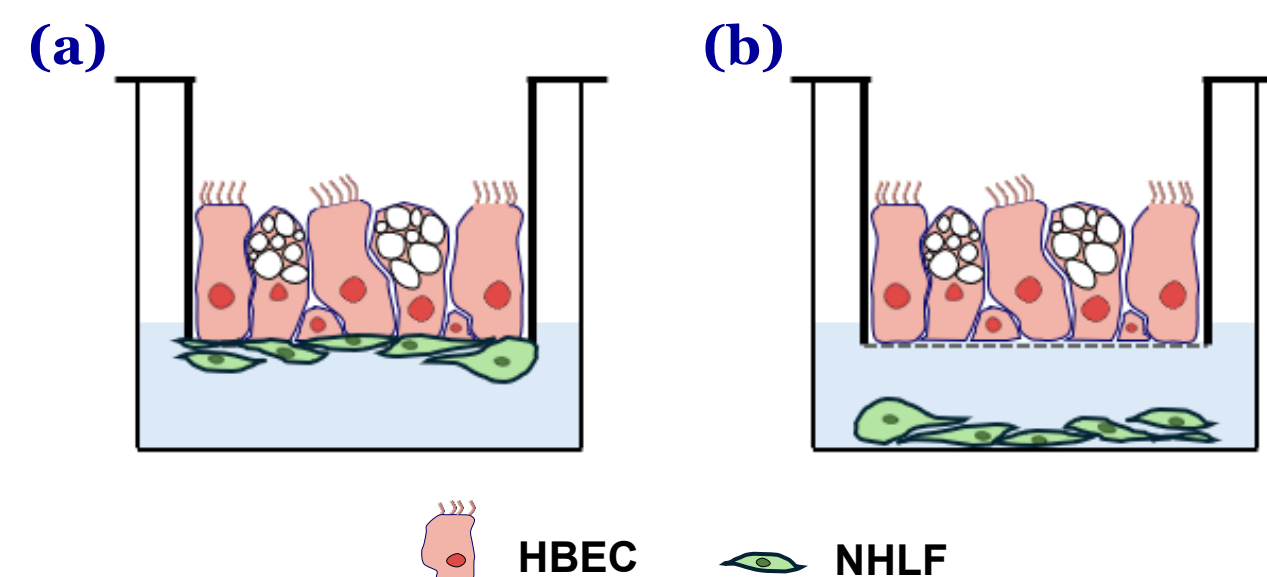
Further pharmacological validation was performed by evaluating the efficacy of two small molecules (an IL-13 pathway inhibitor and a corticosteroid) and two antisense oligonucleotides (ASOs; targets not disclosed), at different concentrations, to assess their ability to reverse disease-related phenotypic changes, specifically goblet cell hyperplasia. The hyperplastic phenotype was induced by treatment with IL-13 at 30 ng/mL for small-molecule testing and 1 ng/mL for ASO testing. Co-treatment with IL-13 and the test compounds was initiated on Day 23 post-air lift and continued every other day for 14 days.



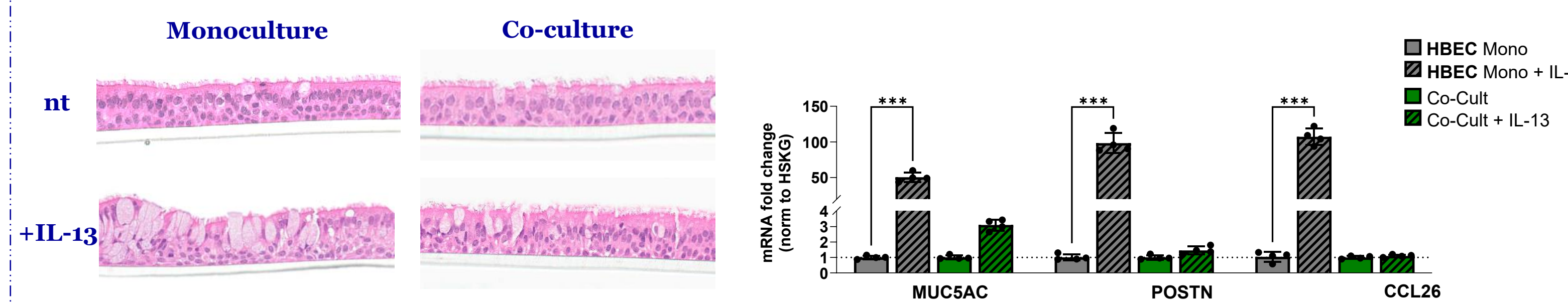
Histopathology. H&E and MUC5AC immunohistochemistry sections of HBEC ALI model treated with two small molecules (left panel) and two ASOs (right panel) at different concentrations. Histopathological assessment demonstrated consistent modulation of the IL-13-induced hyperplastic phenotype across the tested conditions. The IL-13 pathway inhibitor effectively reduced goblet cell hyperplasia at concentrations ≥ 1 μ M, as evidenced by a reduction in MUC5AC-positive cells and restoration of the epithelial morphology. In contrast, the corticosteroid did not result in a meaningful reversal of the hyperplastic phenotype, consistent with previously reported findings in the literature^{2,3}. ASO-01 produced a dose-dependent reduction in goblet cell hyperplasia, whereas ASO-02 did not show a detectable effect. Overall, these findings demonstrate the responsiveness of the model to pharmacological modulation of the IL-13 pathway and support its utility for evaluating novel therapeutic approaches targeting airway goblet cell hyperplasia.

MULTICELLULAR AIRWAY ALI MODEL

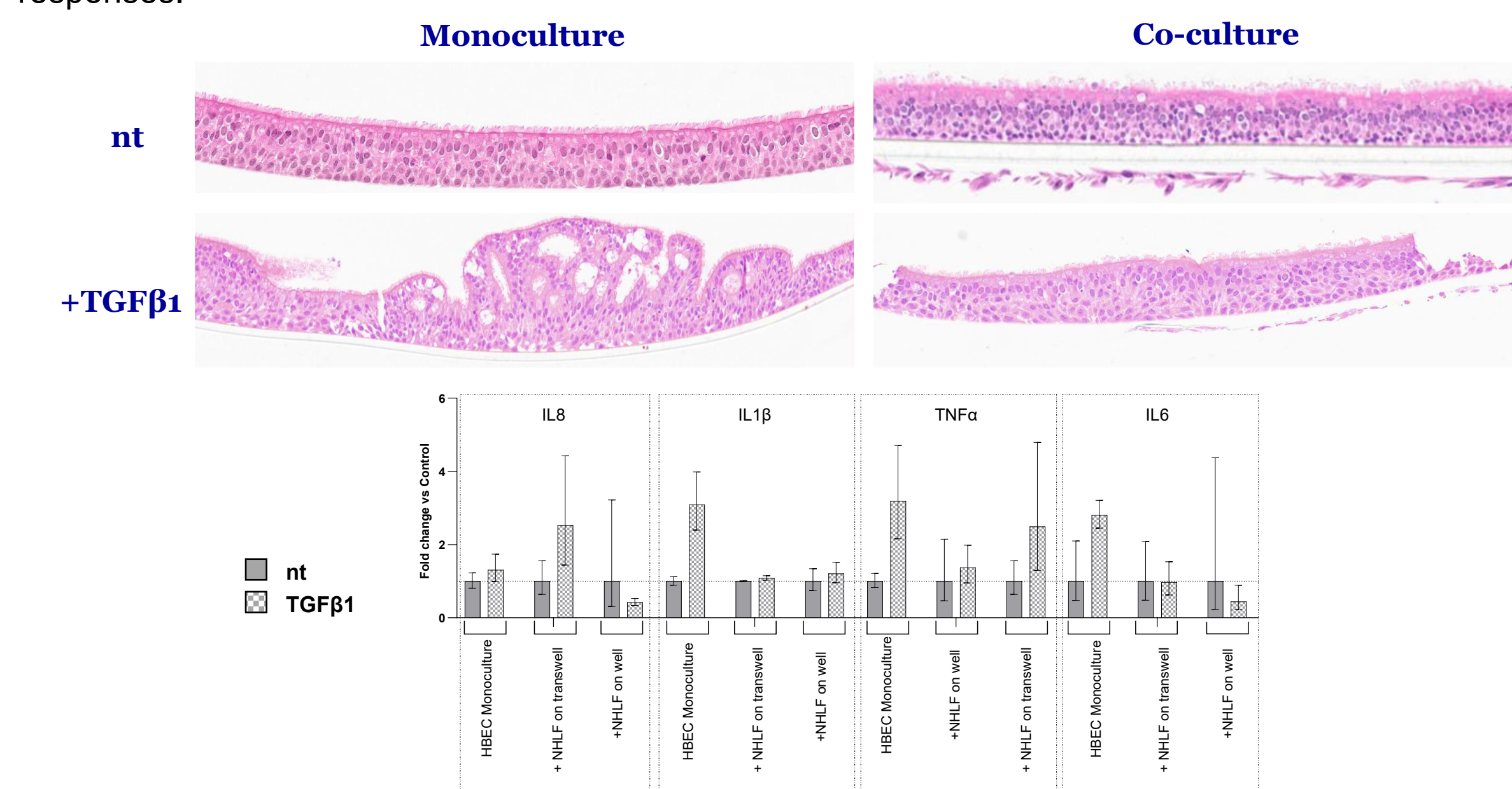
To enhance the translational relevance of the goblet cell hyperplasia model and investigate epithelial–stromal crosstalk, primary normal human lung fibroblasts (NHLFs) were incorporated into the HBEC ALI model, either at the bottom of the transwell membrane (a) or the 24-well plate (b). The resulting co-cultures were evaluated in two complementary experimental settings:



IL-13-induced goblet cell hyperplasia. IL-13 (1 ng/mL, 14 days) induced goblet cell hyperplasia in HBEC monocultures, whereas this response was markedly reduced in the presence of NHLFs, as demonstrated by histopathological evaluation (only condition b is shown in the images). Consistently, IL-13-induced upregulation of the goblet cell marker MUC5AC and inflammatory/remodeling markers CCL26 (Eotaxin-3) and POSTN (Periostin) in HBECs, was blunted by co-culture. *** p < 0.001.



TGF- β 1-induced pro-fibrotic response. Following exposure to TGF- β 1 (1 ng/mL, 7 days), HBEC monocultures showed altered epithelial growth, including increased epithelial thickness, surface irregularities and cyst formation. In contrast, both co-culture configurations (only condition a is shown in the images) maintained a normal pseudostratified respiratory phenotype. Consistent with these morphological findings, TGF- β 1 induced an approximately 3-fold increase in IL-1 β , TNF- α and IL-6 expression in HBEC monocultures, whereas no significant changes were observed in co-cultures. These findings suggest that epithelial–stromal crosstalk can attenuate both TGF- β 1-induced epithelial remodelling and inflammatory responses.



CONCLUSION

Together, these complementary ALI-based models recapitulate key features of airway remodeling and epithelial–stromal crosstalk. The incorporation of multiple human cell types adds biological complexity to the in vitro system, enhancing its physiological relevance and providing scalable, reproducible, and mechanistically informative platforms for therapeutic screening and safety assessment. Their versatility was demonstrated across different drug modalities, including ASOs, small molecules, and monoclonal antibodies. As a human-relevant New Approach Methodology (NAM), the ALI platform can also support personalized approaches using patient-derived cells and contribute to reducing animal use in preclinical research.

References: (1) Boomer J, et al. Increased Muc5AC and Decreased Ciliated Cells in Severe Asthma Partially Restored by Inhibition of IL-4Ra Receptor. *Am J Respir Crit Care Med*. 2024 Dec 15;210(12):1409-1420. doi: 10.1164/rccm.202307-1266OC. (2) Kanoh S et al. IL-13-induced MUC5AC production and goblet cell differentiation is steroid resistant in human airway cells. *Clin Exp Allergy*. 2011 Dec;41(12):1747-56. doi: 10.1111/j.1365-2222.2011.03852.x. (3) Groneberg, D.A. et al. Expression of respiratory mucins in fatal status asthmaticus and mild asthma. *Histopathology*, 2002; 40: 367-373. <https://doi.org/10.1046/j.1365-2559.2002.01378.x>