EPITHELIX

In vitro respiratory solutions

Characterisation of IL-13 induced Airway Goblet Cell Metaplasia

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1 Introduction

Goblet cell metaplasia can be induced in a fully differentiated human airway epithelium following an IL-13 two-weeks treatment. Epithelix' previous data suggested that IL-13-induced epithelial remodeling was dependent on epithelium anatomical origin (Figure2) as bronchial tissue (MucilAir™-Bronchial) responded better to IL-13 than bronchiolar (SmallAir™) and nasal (MucilAir™-Nasal) tissues. To investigate this further, pooled versions of these epithelium models representing various anatomical regions were reconstituted using primary cell mix from several donors and were directly compared for their ability to acquire hallmark features of metaplasia following Il-13 exposure. Using both a newly developed platform based on a combination of immunofluorescence (IF) whole tissue staining and 3D confocal microscopy pictures analysis, and an IHC traditional approach, we demonstrated that MUC5AC increases in a dose-response manner in response to Il-13 concentrations ranging from 10 to 30 ng/ml in all 3 types of epithelia. Specifically, bronchial tissue gave the best response to IL-13 and nasal epithelium showed a less pronounced effect.

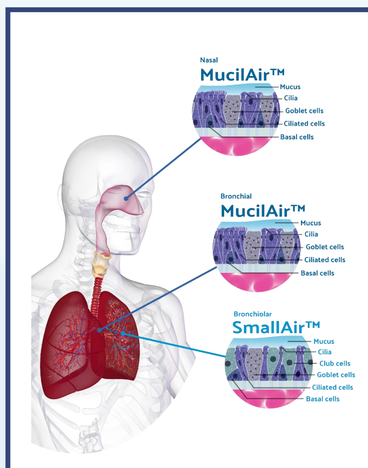


Figure 1_ Epithelix Human Airway in Vitro Models cultured at the air-liquid interface (ALI) and reconstituted with primary cells isolated from different anatomical regions.

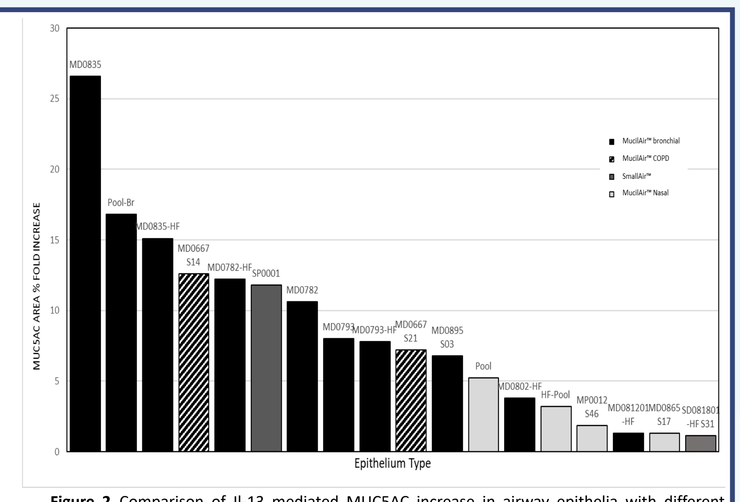


Figure 2_ Comparison of Il-13 mediated MUC5AC increase in airway epithelia with different anatomical origins.

2 Methods & Experimental Strategy

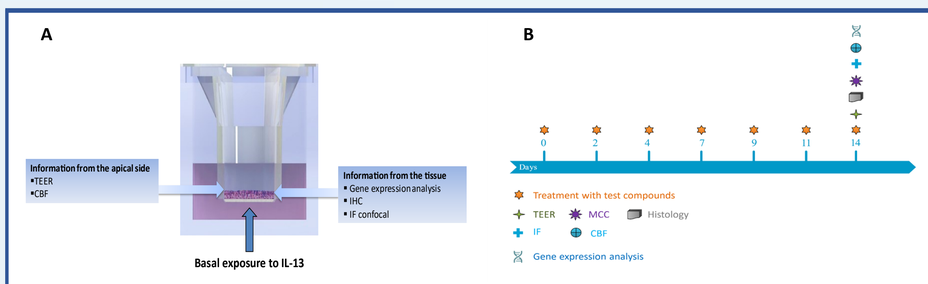


Figure 3_A. Schematic representation of *In Vitro* 3D epithelium cultured at the ALI interface in a Transwell insert. Basal exposure of IL-13 is indicated (vertical arrow). Experimental outcomes of this study include transepithelial electrical resistance (TEER) and Cilia Beating Frequency (CBF), Immunofluorescence (IF), Immunohistochemistry (IHC) and tissue lysis for MUC5AC gene expression analysis.
B. Testing sequences description. MucilAir™ epithelia were exposed during 14 days to IL-13. Monitoring of TEER and CBF were performed at D14. Epithelia (N=3) for MUC5AC gene expression analysis were lysed at the end of the experiment (D14) and epithelia for IF analysis (N=3), and IHC procedures were fixed at D14.

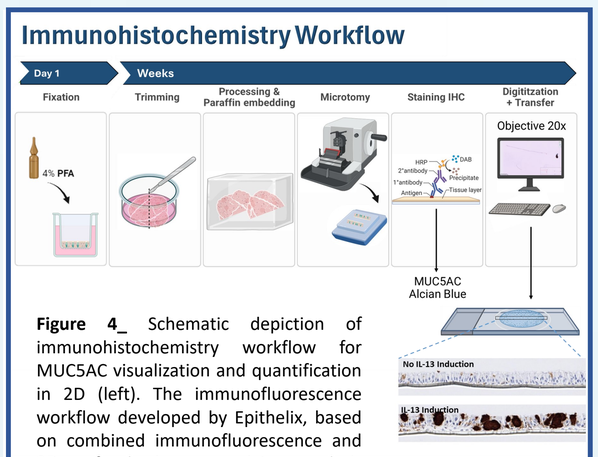
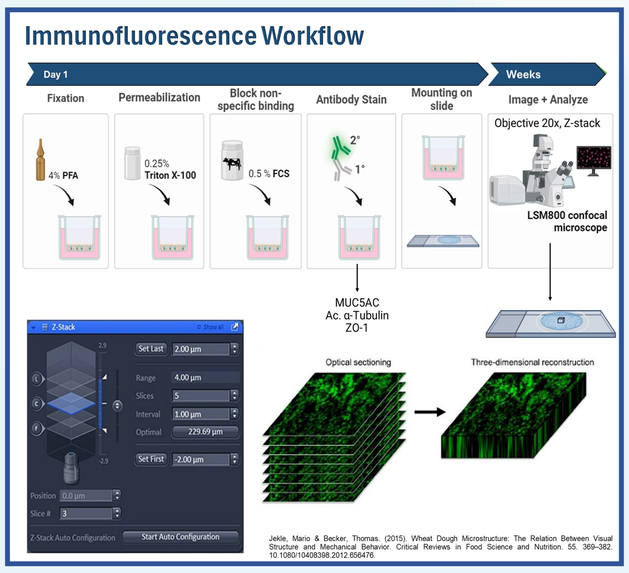


Figure 4_ Schematic depiction of immunohistochemistry workflow for MUC5AC visualization and quantification in 2D (left). The immunofluorescence workflow developed by Epithelix, based on combined immunofluorescence and 3D confocal microscopy picture analysis is represented on the right.



3 Results

Epithelia barrier and ciliary properties upon Il-13 induction

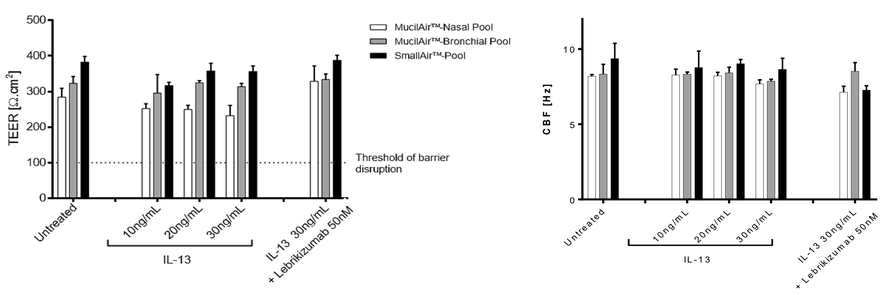


Figure 5. A. Effect of IL-13 repeated exposure on tissue integrity. MucilAir™-Nasal pool, Bronchial Pool and SmallAir ™- Pool epithelia models were exposed to IL-13 for 14 Days (by renewing compound at D2, D4, D7, D9 and D11). TEER was monitored at D14. Error bars indicate SEM (N=4). Dotted line (100 Ω.cm²) indicates the threshold of epithelium disruption.
B. Effect of IL-13 repeated exposure on Cilia Beating Frequency (CBF) of different epithelium types. MucilAir™ epithelia were exposed during 14 days to IL-13. Monitoring of CBF was performed at D14.

Figure 7. MUC5AC relative increase in response to IL-13 in various tissues. MucilAir™-Nasal pool, Bronchial Pool and SmallAirPool epithelia were compared for their ability to answer to IL-13. Results were represented in fold change of MUC5AC volume or % area.

Epithelium Type		Nasal	Bronchial	Bronchiolar
MUC5AC FOLD INCREASE	IHC	1,3	3	5,3
	IF	2,4	3	4,2

Measurements of TEER and CBF (Figure 5) indicated that epithelium barrier and ciliary functions remained unchanged compared to the untreated control for the three types of epithelia tested in this study.
Altogether, microscopy data evidenced an IL-13 dose-dependent increase of MUC5AC for all three types of epithelia, MucilAir™-Pool nasal, MucilAir™-Pool bronchial and SmallAir™-Pool using both analysis methods. Co-addition of Lebrikizumab_ a commercially available IL-13 antagonist_ to IL-13 efficiently prevented MUC5AC increase. Notably, MucilAir™-Pool Bronchial epithelium gives the best response to IL-13 exposure in term of MUC5AC production with a maximum value of 16 % MUC5AC area measured by IHC and 37255 μm^3 measured in 3D IF. Considering the fold increase of MUC5AC % area and volume, the bronchial epithelium was behind the bronchiolar tissue (Figure 7).
MUC5AC gene expression analysis also indicated a dose-dependent increase although the effect was moderate for MucilAir™-Pool Nasal and SmallAir™-Pool. These data are in line with microscopy results as bronchial epithelium displays the highest MUC5AC expression levels compared to the other tissue types.
Based on all these data, we concluded that MucilAir™-Pool Bronchial and SmallAir™-Pool were the tissues of choice to study metaplasia. MucilAir™-Pool nasal also responded to IL-13 but the effect was moderate (Figure 6 and 7).

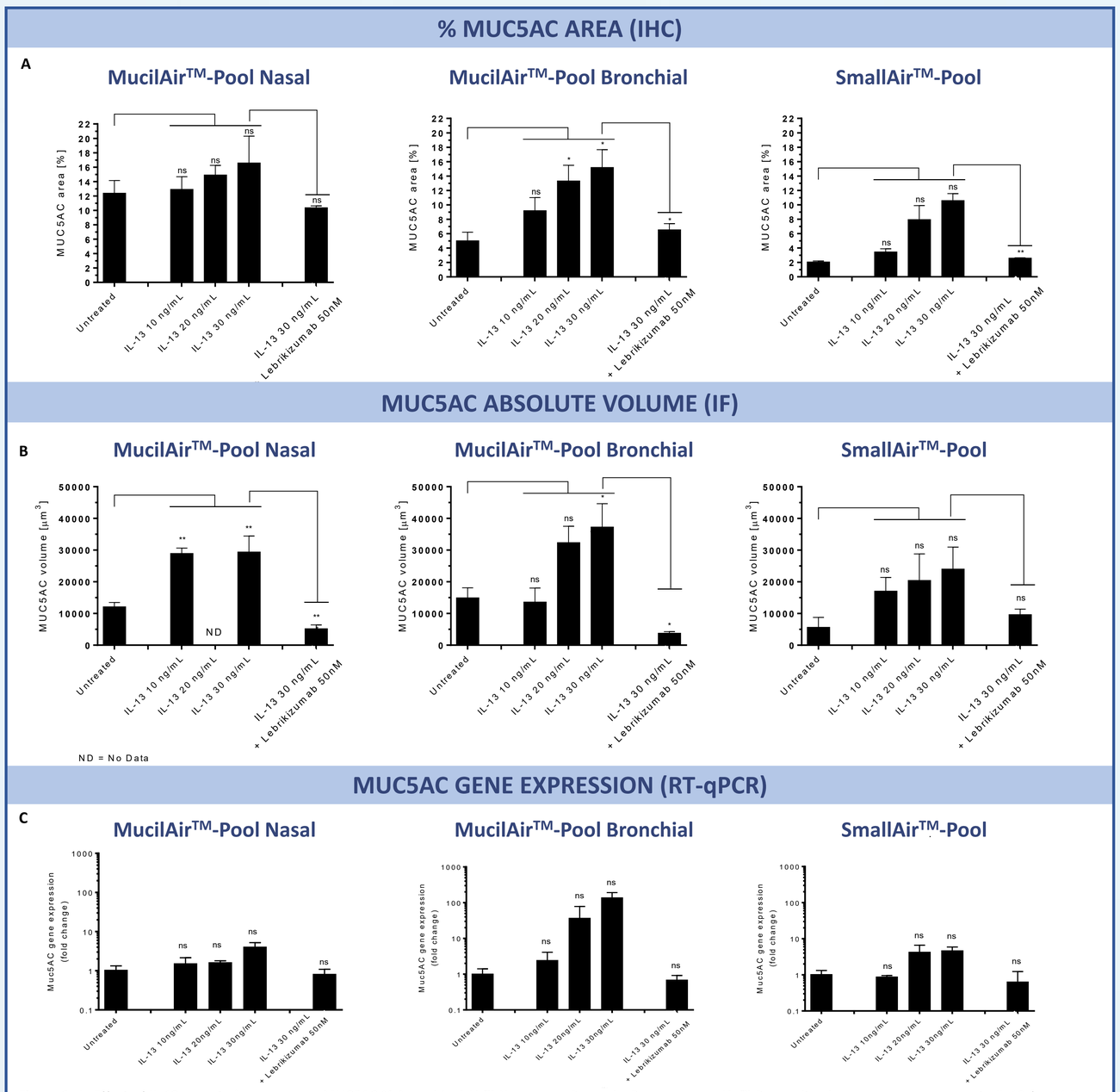


Figure 6. A. Effect of IL-13 repeated exposure on MUC5AC % Area. MucilAir™-Nasal pool, Bronchial Pool and smallAir ™-Pool epithelia models were exposed to IL-13 for 14 Days. Immunohistochemistry-labelled tissue section pictures (n=3) were quantitatively analysed using Epithelix image analysis software. **B. Effect of IL-13 repeated exposure on MUC5AC volume.** After fixation and staining, 3D stacks were taken using confocal microscope and analysed using Epithelix algorithm. **C. Effect of IL-13 repeated exposure on MUC5AC gene expression.** Cultures were lysed on D14 and transcripts for MUC5AC were quantified by RT-PCR (N=3, mean ± SEM). Data were normalized by TBP (TATA-Box Binding protein) and are expressed relative to the Vehicle Control as fold change. Statistical analysis was performed using two-way ANOVA or one-way with Dunnett's post-tests (Prism 6.0 GraphPad, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

4 Conclusions

- The new approach developed by Epithelix to quantify MUC5AC, based on combined immunofluorescence and 3D confocal microscopy picture analysis (Figure 6B) is operational as it led to results that were aligned with immunohistochemistry traditional method (See Figure 6A).
- MucilAir™-Pool Bronchial epithelium is the tissue of choice for studying metaplasia as it gave the highest response in term of MUC5AC production. SmallAir™-Pool is also a valuable epithelium for studying metaplasia in terms of MUC5AC fold change.