Human bronchial epithelial cells from patients with asthma have an altered gene expression profile

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To the Editor:

Asthma is a multifactorial disease presenting with wheeze and shortness of breath, and is known to be exacerbated by triggers such as pollen, house dust mites and viral infection. In the lung, the bronchial epithelium is recognised as a central driver of airway structural changes, including epithelial goblet cell hyperplasia and metaplasia, which are features of asthma. Bronchial epithelial cells (BECs) isolated from patients with asthma and cultured *in vitro* have altered barrier properties [1], elevated expression of remodelling factors [2] and defective repair [3]. Interestingly, genome-wide association studies (GWAS) of asthma have implicated a number of genes that are known to be expressed and functional in the airway epithelium, including *IL33*, *IL1RL1*, *TSLP* and *MUC5AC* [4]. To identify the molecular mechanisms underlying altered BECs phenotype in asthma patients, several studies have completed transcriptomic analyses using bronchial brush samples. Two recent meta-analyses [5, 6] suggested that alterations in chemical stimulus, extracellular region, pathways in cancer and arachidonic acid metabolism were features of the bronchial epithelium in the lungs of patients with asthma, and included 78 up- and 75 down-regulated genes [5]. While useful, a key question is how much the airway environment of a patient is driving this differential gene expression profile (GEP) and how much is intrinsic to the BECs themselves? To answer this question, we completed transcriptomic analyses of BECs cultured two-dimensionally through multiple passages in the laboratory that had originally been isolated from control subjects without disease or patients with asthma. An attrition rate (for successful culture) of 54% and 42% was observed in the asthma and control populations respectively.

BECs derived from bronchoscopic bronchial brushes of moderate to severe asthma subjects, as defined by British Thoracic Society guidelines, (n=33) were compared to those from non-asthma control subjects (n=18). The asthma group was predominantly female (64.7%) with a mean average age of 54 years (range 19–64 years) and mean forced expiratory volume in 1s (FEV_1) of 81.9±17.6% of predicted, while the control group was predominantly male (75%), with an average age of 39 years (range 20–64 years) and a mean FEV_1 of 101.5±9.55% predicted. Cells were expanded in two-dimensional culture to passage 3, as previously described [7]. RNA was collected from this cellular monolayer and transcriptomic changes determined through RNA sequencing. Briefly, sequence libraries were generated using TruSeq RNA sample preparation kits (Illumina) *via* the Sciclone NGS Liquid Handler (Perkin Elmer). These libraries were sequenced on an Illumina HiSeq2500 (Paired End 2×100 bp) in pools of multiple samples. The resulting read depth ranged from 10 361 312 to 19 395 273 paired-end reads. Resultant data were then processed using the Scythe/Sickle, Bowtie2 and TopHat software packages. Differential gene counts were determined using the Cufflinks software package (CuffDiff). Pathway analysis was carried out using DAVID 6.8, analysing all genes returning with a p-value of <0.05.

We identified a total of 40 differentially expressed genes (5% false discovery rate (FDR)), of which 23 were upregulated in cells from asthma patients when compared to cells obtained from control subjects (figure 1). Mining of human gene–disease associations (GDAs) using the online DisGeNET platform [8] confirmed that a number of these differentially expressed genes have previous GDAs to asthma (*CEACAM5/6*, *COL5A1*, *CXCL5*, *DDR1*, *DISP2*, *GAS5*, *NOS1*, *THBD* and *TJP1*) or lung function testing

Shareable abstract (@ERSpublications)
Gene changes observed in asthma bronchial epithelial cells are maintained following repeated culture, presenting with an exaggerated response to viral infection and immune responses as well as having differences in the rate of cell division and replication https://bit.ly/3Cq2xKf

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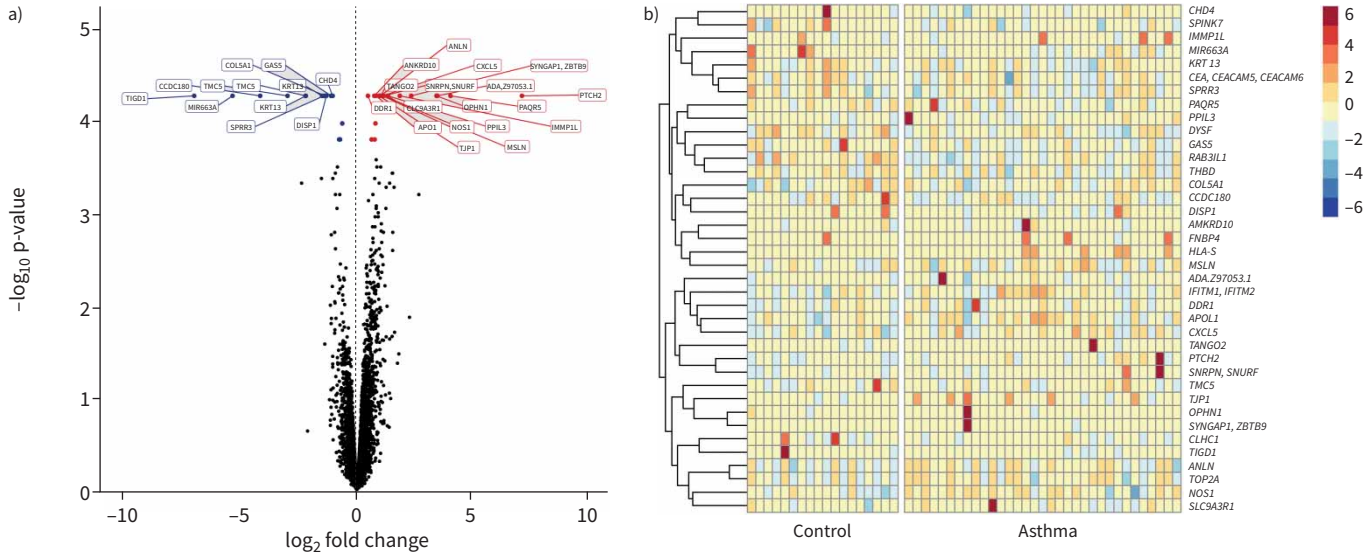

FIGURE 1 RNA sequencing of primary bronchial epithelial cells taken from 33 moderate–severe asthma patients and 18 control subjects, and cultured *in vitro*, identifies a) modest differences in gene expression between cases and controls, that may be in part due to b) the high heterogeneity observed in our sample population. Of the transcriptomic signature of 40 differentially expressed genes meeting a 5% false discovery rate, 17 upregulated and 23 downregulated genes were identified as unique to asthma cells.

(*SNRPN*). Interestingly, other genes, *i.e. CXCL5*, *DCR1*, *IFITM1* and *SNRPN*, were also associated to viral infections, which are recognised drivers of asthma exacerbation [9]. We also cross referenced 382 asthma GWAS genes, as defined by the search "Asthma" in GWAS Catalog (https://www.ebi.ac.uk/gwas/) and identified that *COL15A1* is differentially expressed based on a Bonferroni corrected p-value of $<1.3\times10^{-4}$.

These initial analyses demonstrate that cultured BECs originally isolated from asthma patients have an altered GEP *in vitro*. Pathway analysis using DAVID 6.8 identified enrichment of multiple pathways in cells from asthma patients, that are broadly grouped into: 1) antiviral responses, specifically type-1 interferon responses (FDR 1.02×10^{-10}); 2) interferon-α/β signalling (FDR 1.34×10^{-9}); 3) immune responses (FDR 5.64×10^{-5}); and 4) cellular division and proliferation (FDR 8.0×10^{-9}).

Importantly, we observed minimal overlap between the gene signatures observed in this study using cultured cells with reported differential gene expression in the bronchial epithelium in the lungs of patients with asthma [5, 6]. Considering an adjusted p-value of $<3.0\times10^{-4}$, based on comparisons to 150 published genes, we observed that *CEACAM5*, encoding a cell adhesion protein that may regulate cell differentiation, apoptosis and polarity, and *IL1RL1*, encoding the interleukin-33 receptor that is involved in type-2 inflammation, achieved significance in both our and the published studies. Several other published genes (*MMP1*, *DAPK1*, *APOC1*, *ACKR3*, *AKAP12*, *PTGS1*, *SPOCK3* and *VNN1*) [5], and *FDFR1*, *KYNU*, *FAM83D* and *MCAM* [6]), were observed using a nominal p-value cut-off of <0.05 in our study but did not achieve the adjusted $p<3.0\times10^{-4}$. These data suggest that while differential GEPs observed *in vivo* are at least part driven by the airway environment, certain transcriptomic changes are intrinsic, as highlighted by the presence of an altered GEPs in asthma BECs cultured independently of the airway environment.

To our knowledge, we show for the first time that the transcriptomic profile of cultured BECs isolated from patients with asthma is crucially different from those from nonasthma subjects, and that these changes not only confirm a subset of differences identified *in vivo*, but also identify additional new findings. These observed differences suggest that epithelial cells originating from asthma patients present with an exaggerated response to viral infection and immune responses, as well as having differences in the rate of cell division and replication. Although in general, *in vitro* epithelial responses are different from those observed *in vivo*, the few that do replicate relate well to both known morphological changes that occur in the bronchial epithelium in asthma, where epithelial cell hyperplasia is a known process occurring during airway remodelling and to altered response to infection by respiratory viruses [10]. These novel data provide greater insight into altered epithelial mechanisms in asthma and provide new understanding and potential targets for future work.

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References

1 Xiao C, Puddicombe SM, Field S, *et al.* Defective epithelial barrier function in asthma. *J Allergy Clin Immunol* 2011; 128: 549–556.
2 Lopez-Guisa JM, Powers C, File D, *et al.* Airway epithelial cells from asthmatic children differentially express proremodeling factors. *J Allergy Clin Immunol* 2012; 129: 990–997.
3 Kicic A, Hallstrand TS, Sutanto EN, *et al.* Decreased fibronectin production significantly contributes to dysregulated repair of asthmatic epithelium. *Am J Respir Crit Care Med* 2010; 181: 889–898.
4 Shrine N, Portelli MA, John C, *et al.* Moderate-to-severe asthma in individuals of European ancestry: a genome-wide association study. *Lancet Respir Med* 2019; 7: 20–34.
5 Nie X, Wei J, Hao Y, *et al.* Consistent biomarkers and related pathogenesis underlying asthma revealed by systems biology approach. *Int J Mol Sci* 2019; 20: 4037.
6 Loffredo LF, Abdala-Valencia H, Anekalla KR, *et al.* Beyond epithelial-to-mesenchymal transition: common suppression of differentiation programs underlies epithelial barrier dysfunction in mild, moderate, and severe asthma. *Allergy* 2017; 72: 1988–2004.
7 Slater M, Torr E, Harrison T, *et al.* The differential effects of azithromycin on the airway epithelium *in vitro* and *in vivo*. *Physiol Rep* 2016; 4: e12960.
8 Pinero J, Ramirez-Anguita JM, Sauch-Pitarch J, *et al.* The DisGeNET knowledge platform for disease genomics: 2019 update. *Nucleic Acids Res* 2020; 48: D845–D855.
9 Beale J, Jayaraman A, Jackson DJ, *et al.* Rhinovirus-induced IL-25 in asthma exacerbation drives type 2 immunity and allergic pulmonary inflammation. *Sci Transl Med* 2014; 6: 256ra134.
10 James KM, Peebles RS Jr, Hartert TV. Response to infections in patients with asthma and atopic disease: an epiphenomenon or reflection of host susceptibility? *J Allergy Clin Immunol* 2012; 130: 343–351.