

Early View

Research letter

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

Sangita Bhaker, Michael A. Portelli, Kamini Rakkar, Dominick Shaw, Simon Johnson, Christopher Brightling, Ian Sayers

Please cite this article as: Bhaker S, Portelli MA, Rakkar K, *et al.* Human bronchial epithelial cells from patients with asthma have an altered gene expression profile. *ERJ Open Res* 2021; in press (<https://doi.org/10.1183/23120541.00625-2021>).

This manuscript has recently been accepted for publication in the *ERJ Open Research*. It is published here in its accepted form prior to copyediting and typesetting by our production team. After these production processes are complete and the authors have approved the resulting proofs, the article will move to the latest issue of the ERJOR online.

Copyright ©The authors 2021. This version is distributed under the terms of the Creative Commons Attribution Non-Commercial Licence 4.0. For commercial reproduction rights and permissions contact permissions@ersnet.org

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

Sangita Bhaker^{1*}, Michael A. Portelli^{1*}, Kamini Rakkar^{1*}, Dominick Shaw¹, Simon Johnson¹, Christopher Brightling², Ian Sayers¹

¹*Translational Medical Sciences, NIHR Respiratory Biomedical Research Centre, School of Medicine, Biodiscovery Institute, University Park, University of Nottingham, Nottingham, UK.*

²*University of Leicester and Department of Respiratory Medicine University Hospitals of Leicester NHS Trust, Leicester, United Kingdom*

*contributed equally

Take Home Message: 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

To the Editor,

Asthma is a multi-factorial disease presenting with wheeze and shortness of breath and is known to be exacerbated by triggers such as pollen, house dust mite 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 that are features of asthma. Bronchial epithelial cells 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 bronchial epithelial cell 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 bronchial epithelial cells themselves? To answer this question, we completed transcriptomic analyses of bronchial epithelial cells (BEC) cultured in 2D 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 BTS guidelines (n=33) were compared to those from non-asthma control subjects (n=18). The asthma group was predominantly female (64.7%) with an average age of 54 (Range:19-64) years and mean percentage predicted FEV₁ =81.9±17.6, while the control group was predominantly male (75%), with an average age 39 (Range:20-64) and a mean percentage predicted FEV₁ of 101.5±9.55. Cells

were expanded in 2D culture to passage 3, as previously described[7], RNA 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 x 100bp) in pools of multiple samples. The resulting read depth ranged from 10,361,312-19,395,273 paired-end reads. Resultant data was then processed using Scythe/Sickle[®], Bowtie2[®] and TopHat[®] software packages. Differential gene counts were determined using 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 identify a total of 40 differentially expressed genes (5%FDR), of which 23 were upregulated in cells from asthma patients when compared to cells obtained from control subjects Fig 1A&B. 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*, *TJP1*) or lung function testing (*SNRPN*). Interestingly, other genes, i.e., *CXCL5*, *DCR1*, *IFITM1* & *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 <1.3E-04.

These initial analyses demonstrate that cultured bronchial epithelial cells 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 i) antiviral responses, specifically type-1 interferon responses (FDR=1.02E-10), ii) interferon alpha/beta signalling (FDR=1.34E-09), iii) Immune responses (FDR=5.64E-05) and iv) cellular division and proliferation (FDR=8.0E-09).

Importantly, we observe 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 <3.0E-04, based on comparisons to 150 published genes, we observe that *CEACAM5*, a cell adhesion protein that may regulate cell differentiation, apoptosis and polarity, and *IL1RL1*, the IL33 receptor that is involved in type-2 inflammation, achieve significance in both our and the published studies. Several other published genes (*MMP1*, *DAPK1*, *APOC1*, *ACKR3*, *AKAP12*, *PTGS1*, *SPOCK3*, *VNN1*)[5] (*FFR1*, *KYNU*, *FAM83D*, *MCAM*)[6] were observed using a nominal p-value cut-off of <0.05 in our study but did not achieve the adjusted $P < 3E-04$. 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 cultured asthma BECs cultured independently of the airway environment.

To our knowledge, we show for the first time that the transcriptomic profile of bronchial epithelial cells isolated from patients with asthma and cultured is crucially different from those from non-asthma subjects and that these changes 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.

This work was supported from grants from Asthma UK (08/017, 10/006), the MRC (G1100163), Nottingham Hospitals Charity Research Fund, the Nottingham NIHR Biomedical Research Centre and the Leicester NIHR Biomedical Research Centre.

1. Xiao C, Puddicombe SM, Field S, Haywood J, Broughton-Head V, Puxeddu I, Haitchi HM, Vernon-Wilson E, Sammut D, Bedke N, Cremin C, Sones J, Djukanovic R, Howarth PH, Collins JE, Holgate ST, Monk P, Davies DE. Defective epithelial barrier function in asthma. *J Allergy Clin Immunol* 2011; 128(3): 549-556 e541-512.
2. Lopez-Guisa JM, Powers C, File D, Cochrane E, Jimenez N, Debley JS. Airway epithelial cells from asthmatic children differentially express proremodeling factors. *J Allergy Clin Immunol* 2012; 129(4): 990-997 e996.
3. Kicic A, Hallstrand TS, Sutanto EN, Stevens PT, Kobor MS, Taplin C, Pare PD, Beyer RP, Stick SM, Knight DA. Decreased fibronectin production significantly contributes to dysregulated repair of asthmatic epithelium. *Am J Respir Crit Care Med* 2010; 181(9): 889-898.
4. Shrine N, Portelli MA, John C, Soler Artigas M, Bennett N, Hall R, Lewis J, Henry AP, Billington CK, Ahmad A, Packer RJ, Shaw D, Pogson ZEK, Fogarty A, McKeever TM, Singapuri A, Heaney LG, Mansur AH, Chaudhuri R, Thomson NC, Holloway JW, Lockett GA, Howarth PH, Djukanovic R, Hankinson J, Niven R, Simpson A, Chung KF, Sterk PJ, Blakey JD, Adcock IM, Hu S, Guo Y, Obeidat M, Sin DD, van den Berge M, Nickle DC, Bosse Y, Tobin MD, Hall IP, Brightling CE, Wain LV, Sayers I. Moderate-to-severe asthma in individuals of European ancestry: a genome-wide association study. *Lancet Respir Med* 2019; 7(1): 20-34.
5. Nie X, Wei J, Hao Y, Tao J, Li Y, Liu M, Xu B, Li B. Consistent Biomarkers and Related Pathogenesis Underlying Asthma Revealed by Systems Biology Approach. *Int J Mol Sci* 2019; 20(16).
6. Loffredo LF, Abdala-Valencia H, Anekalla KR, Cuervo-Pardo L, Gottardi CJ, Berdnikovs S. Beyond epithelial-to-mesenchymal transition: Common suppression of differentiation programs underlies epithelial barrier dysfunction in mild, moderate, and severe asthma. *Allergy* 2017; 72(12): 1988-2004.
7. Slater M, Torr E, Harrison T, Forrester D, Knox A, Shaw D, Sayers I. The differential effects of azithromycin on the airway epithelium in vitro and in vivo. *Physiol Rep* 2016; 4(18).
8. Pinero J, Ramirez-Anguila JM, Sauch-Pitarch J, Ronzano F, Centeno E, Sanz F, Furlong LI. The DisGeNET knowledge platform for disease genomics: 2019 update. *Nucleic Acids Res* 2020; 48(D1): D845-D855.
9. Beale J, Jayaraman A, Jackson DJ, Macintyre JDR, Edwards MR, Walton RP, Zhu J, Man Ching Y, Shamji B, Edwards M, Westwick J, Cousins DJ, Yi Hwang Y, McKenzie A, Johnston SL, Bartlett NW. Rhinovirus-induced IL-25 in asthma exacerbation drives type 2 immunity and allergic pulmonary inflammation. *Sci Transl Med* 2014; 6(256): 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(2): 343-351.

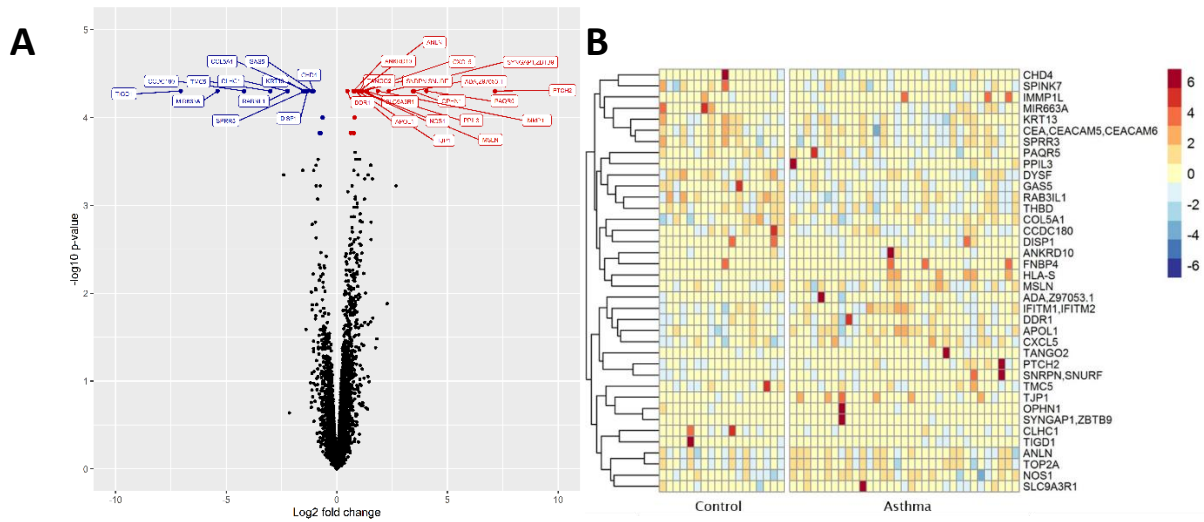


Figure 1: RNA-sequencing of primary bronchial epithelial cells taken from 33 moderate-severe asthma patients and 18 controls subjects and cultured in vitro identifies modest differences in gene expression between cases and controls (Panel A), that may be in part due to the high heterogeneity observed in our sample population (Panel B). Of the transcriptomic signature of 40 differentially expressed genes meeting 5% FDR (17 upregulated, 23 downregulated) identified as unique to asthma cells.