determined at the mRNA and protein levels. Finally, expression of LOXL2 was determined in an Aspergillus fumigatus model of asthma. Asthmatic HASM cells activated 3-fold higher levels of TGFβ basally than non-asthmatic cells (p<0.01). Both diseased and control HASM cells increased TGFβ activation in response to methacholine confirming our previous data (Tatler et al 2011). A collagen gel contraction assay demonstrated that asthmatic HASM cells were hypercontractile compared with non-asthmatic cells under basal conditions (p<0.05) and that contractility weakly correlated with amount of TGFβ activated (p<0.05). Importantly, culturing non-asthmatic HASM cells on asthmatic ECM led to increased TGFβ activation (p<0.05) and culturing asthmatic HASM cells on non-asthmatic ECM decreased TGFβ activation (p<0.05). mRNA Expression of the ECM crosslinking enzymes LOXL2 and LOXL3 was significantly increased in asthmatic HASMs (p<0.05). Finally, LOXL2 protein was increased in asthmatic HASMs cells, and increased in the airway smooth muscle layer of animals challenged repeatedly with Asp. f compared with control challenged animals. In conclusion HASM cells derived from asthmatic patients exhibit enhanced activation of TGFβ compared with non-asthmatic HASM cells. This may be driven by the diseased ECM since asthmatic HASMs cells exhibit aberrant expression of ECM crosslinking enzymes.

Severe asthma represents a significant unmet clinical need and the molecular basis for disease persistence remains inadequately understood. Bronchial epithelial cells, at the interface of environment/tissue, are central to asthma pathogenesis. There is thus a need to evaluate genome-wide changes between health and asthma to better understand the molecular mechanisms underlying disease. The vast majority of genome-wide measurements have focused on determining changes at the DNA or mRNA levels, with little attention paid to how and which mRNAs are actually translated into protein. This may not disclose changes happening at the protein level, since mRNA and protein expression correlate poorly. To determine translation and its regulation in bronchial epithelial cells in severe asthma patients we analysed paired genome-wise expression of transcriptional (cytoplasmic) and translational (polysome-bound) mRNA levels employing Frac-seq (sub-cellular fractionation and RNA-sequencing) in primary bronchoepithelium in health and severe asthma patients. We also integrated those data with genome-wise profiling of microRNAs to understand their role in gene expression and impact on the pathophysiology of severe asthma bronchial epithelium. We found both genes (=all isoforms of a gene) and mRNA isoforms differentially expressed in severe asthma airways cells, with dysregulated transcriptional mRNA levels (194 genes) showing little overlap with dysregulated translational mRNA (243 genes) expression. We determined novel inflammatory and remodelling pathophysiological mechanisms disclosed solely by polysome-bound mRNAs, centred in epithelium remodelling and repair pathways. We also reveal six dysregulated microRNAs accounting for ~90% of cellular microRNA targeting, displaying preferential targeting of ~50% of mRNAs undergoing translation in severe asthma airways cells. Thus, microRNAs in human severe asthma are major regulators of translation in airways epithelium and offer potential as future therapeutic targets.