Understanding Airways and Blood Vessels in the Lung

P234 SPUTUM CYTOKINES AND CLINICAL BIOMARKERS IN SEVERE ASTHMA

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Introduction Emerging treatments for type-2 high asthma such as anti-IL-5 (mepolizumab) and anti-IL-4 and IL-13 (dupilumab) target specific cytokine pathways resulting in type-2 inflammation. Whether patients with type 2 inflammation respond equally to both treatment or have distinct IL-13 and IL-5 profiles is currently unclear. We have tested the hypothesis that these pathways may function independently of each other and that simple biomarkers can help differentiate IL-13 and IL-5 high patients.
Methods Patients with well characterised, severe asthma were evaluated with the blood eosinophil count and fractional exhaled nitric oxide (FeNO). Patients also had paired measurements of type-2 cytokines in induced sputum samples. Sputum cytokines were measured using a Luminex assay.
Results We found that there was no relationship between the blood eosinophil count and FeNO. There was a positive correlation between FeNO and sputum IL-13 ($r = 0.51$, $p < 0.01$) and blood eosinophils and sputum IL-5 ($r = 0.47$, $p < 0.01$).
Conclusions These findings suggest that readily available, non-invasive biomarkers may be able to differentiate sub-phenotypes in type-2 high asthma. Post-hoc analysis of clinical trial data of anti-IL-5 and anti-IL-4 and IL-13 treatments based on the predominant clinical biomarker would be of interest to see if these predict response to treatment. Simple biomarkers may be of use in deciding which of the emerging biological treatments to use in severe, type-2 high asthma.

P235 EPIGENETIC LANDSCAPE OF THE ASTHMATIC AIRWAYS

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The airway epithelium of asthmatics exhibits distinct genomic and phenotypic characteristics. However the mechanisms underlying the establishment and chronicity of these characteristics remains unknown. We investigated if epigenetic changes underpin the genomic characteristics of the asthmatic airways by determining the chromatin landscape of bronchial epithelial cells (BECs) in healthy and asthmatic adults.

We employed ChIP-seq of histone H3 acetylation (H3K27ac) to determine the chromatin landscape in e*x vivo* cultured BECs from healthy and allergic-atopic asthmatics (n = 3 donors each). Regions of differential enrichment were identified (MEDIPS) and associated genes and pathways determined (GREAT). Gene expression profiles were investigated by microarray (Illumina) and differential analysis conducted (Partek Genome Suite). Super enhancers (SEs) were identified (ROSE) and enrichment of transcription factor motifs (MEME) and their tissue distribution (protienatlas.org) determined.

We identified 33,744 differentially enriched regions (DERs) of H3K27ac between asthma and healthy BECs. DERs were associated with genes (e.g. SERPINB2, TSLP) and pathways (e.g. leukotriene synthesis, antiviral response) previously implicated in asthma and had little overlap with known glucocorticoid receptor binding sites (1.7% of total). DERs occurred up to 100kb from gene promoters and gain or loss of H3K27ac was associated with increased and decreased gene expression in asthmatics respectively. Using a comparative approach, we identified SEs that were common (i.e., present across all donors) and distinct to health and asthma. In addition to established asthma genes (e.g. CLCA1) and transcription factors (e.g. TP63), asthma-SEs encompassed non-coding RNAs (up to 32% of genes) and epithelial-specific transcription factors (e.g. GCM2) previously unreported in asthma.

Our data indicates that asthma influences the chromatin landscape of BECs and suggests the genomic differences observed in the asthmatic airway epithelium are underpinned by established epigenetic mechanisms.

P236 THE ROLE OF HISTONE ARGININE METHYLATION IN GENE EXPRESSION OF AIRWAY SMOOTH MUSCLE CELLS IN ASTHMA

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Introduction and objectives Asthma is estimated to affect at least 300 million people globally. About 25% of the patients do not respond to therapy; therefore we need to develop novel treatments. ASM cells have a crucial role in asthma, contributing to airway remodelling, inflammation and airflow obstruction. We have previously shown that epigenetic histone modifications, particularly histone lysine acetylation and methylation regulate the secretion of inflammatory mediators from ASM cells. Here we tested the hypothesis that histone arginine changes are also involved. Protein arginine N-methyltransferases (PRMTs) are the enzymes which catalyse histone arginine methylation (HRme, the addition of a methyl group to arginine residues on the N-terminal tails of histones), and inhibiting them represents a strategy to reduce the secretion of inflammatory mediators from ASM cells.
Methods Studies were performed in cultured human ASM cells from asthmatic and non-asthmatic donors at passage 6. PRMT expression in human ASM cells was investigated by qPCR. Protein levels of four PRMTs in human ASM cells were investigated by western blotting. As PMRT1 has previously been suggested to play a role in mouse asthma models, we studied the association of PRMT1 with eotaxin, IL-6, IP-10 and CXCL8 promoters in healthy ASM cells, under basal conditions and following stimulation with TNF-α (1ng/ml), by chromatin immunoprecipitation (ChIP). IgG was used as a negative control, while acetylated histone H4 (AcH4) was used as a positive control.
Results We found that ASM cells express the PRMT1, PRMT2, PRMT3, CARM1, PRMT5, PRMT6, PRMT7 and FBX011 mRNA and PRMT1, CARM1, PRMT5, and PRMT6 protein. The analysis showed no difference in the levels of expression between cells isolated from asthmatic and non-asthmatic donors.

Under basal conditions, PRMT1 was associated with all of the promoters and association increased following 1 hour stimulation with TNF-α.