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SOLUBLE ADAM33 AUGMENTS THE PULMONARY IMMUNE RESPONSE PROMOTING ALLERGIC AIRWAY SENSITIVITY

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Rational A *disintegrin and metalloproteinase 33* (ADAM33) was discovered in 2002 as an asthma susceptibility gene. Genetic associations have been made between ADAM33 polymorphisms and asthma disease severity, bronchial hyperresponsiveness (BHR) and rate of lung function decline in both adults and children. A soluble, catalytically active form of the protein (sADAM33) has been identified in the bronchoalveolar lavage fluid of patients, levels of which correlate with disease severity (Lee JY et al, AJRCCM 2006). To study the role sADAM33, a lung specific, doxycycline (Dox) inducible, transgenic mouse, expressing the human pro and metalloproteinase domains of the full-length protein was generated (Ccsp-rtTA/Otet-ADAM33-Pro-MP). Induction of sADAM33, followed by house dust mite (HDM) sensitisation and challenge, resulted in increased BHR and airway inflammation (Davies ER et al, JCI-Insight 2016). The mechanisms by which ADAM33 promotes this susceptibility are unclear. The aim of this work is to identify pathways that are augmented by the induction of sADAM33, which promote increased sensitivity to allergen.

Methods RNA samples from whole lung of adult mice, where sADAM33 had been induced for 4 or 8 weeks, were analysed by next generation RNA sequencing. Identified genes were confirmed across experimental time points (72 hour, 7 day, 4 and 8 weeks on Dox) in wider sample cohorts of Ccsp-rtTA/Otet-ADAM33-Pro-MP and control mice through RTqPCR.

Results The predominant signal from the RNAseq output was for modulation of immune response genes at 4 weeks of sADAM33 expression (GO:0006955 Immune response: 31 genes, 58.49% coverage, FDR p value=7.09 E-22). Genes associated with an immune activation signature (*Ccl5*, *Irgm1*, *Gm12250*, *Gzmb*, *Ncr1*) were validated at least one time point. By 8 weeks of sADAM33 expression genes associated with negative regulation of the response were also validated (*Ido1*, *Cd274*).

Conclusion Induction of sADAM33 in murine lungs, without allergic sensitisation, augmented underlying immune processes in this transgenic mouse model, which may contribute to increased susceptibility to allergic airway inflammation and BHR when challenged with allergen. Further work is required to delineate how sADAM33 affects immune cell populations and their behaviour in the lung.

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THE EFFECT OF LONG ACTING BETA-AGONISTS ON GLUCOCORTICOID RECEPTOR AND IMPORTIN-7 NUCLEAR TRANSLOCATION IN AIRWAY SMOOTH MUSCLE CELLS

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Introduction and Objectives 5%–10% of asthmatic patients display relative insensitivity to the therapeutic benefits corticosteroids and are termed severe asthmatics. This has been attributed, in severe asthma (SA) airway smooth muscle cells (ASMCs), to defective corticosteroid-induced nuclear translocation of the glucocorticoid receptor (GR). Importins, including importin-7, mediate GR nuclear translocation. Studies report increased importin-7-GR interaction in response to corticosteroids in ASMCs. Long-acting beta agonists (LABAs) augment corticosteroid-induced GR nuclear translocation in COPD macrophages. The primary aim of this project was to determine whether the LABA salmeterol could reverse impaired corticosteroid (dexamethasone)-induced GR nuclear translocation in severe asthmatic ASMCs through an importin-7-dependent mechanism.

Methods ASMCs derived from bronchial biopsies from patients with non-severe asthma (NSA) (n=4) and SA (n=4) were cultured and treated with dexamethasone (10–8 M) in the absence or presence of salmeterol (10–10 M or 10–8 M). 30 min post-stimulation, nuclear and cytoplasmic protein fractions underwent western blot analysis to determine levels of importin-7 and GR. RNA extraction was conducted at 4 hours post-stimulation followed by RT-qPCR to investigate the levels of the GR-activated genes, mitogen activated protein kinase phosphatase-1 (MKP-1) and glucocorticoid leucine zipper mRNA levels (GILZ).

Results The Results show a reduced dexamethasone-induced GR nuclear translocation in SA when compared to NSA ASMCs, which was increased with co-treatment with dexamethasone and salmeterol. Co-treatment with dexamethasone and salmeterol also increased GR-activated gene expression leading to an increase in the expression of MKP-1 in SA ASMCs compared to dexamethasone alone. Nuclear importin-7 levels in NSA and SA ASMCs were not increased by co-treatment compared to dexamethasone alone.

Conclusion We have demonstrated impaired dexamethasone-induced GR nuclear translocation in SA ASMCs, which can be reversed by the addition of the LABA salmeterol in combination with dexamethasone. No differences were observed in importin-7 nuclear localisation after co-treatment compared to dexamethasone alone in SA ASMCs. This could suggest that importin-7 is either rapidly recycled back into the cytoplasm after entry into the nucleus, or that the effect of LABAs are not mediated through Importin-7 in severe asthmatic patients. Further real-time experiments would elucidate the role of importin-7 in LABA mediated GR nuclear translocation.

Cystic fibrosis: disease trajectory and evolving therapies

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EARLY GROWTH TRAJECTORIES IN CYSTIC FIBROSIS

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Introduction Within CF-EpiNet; Harnessing Data to Improve Lives (a CF Trust funded Strategic Research Centre) we will