Investigating Genome wide DNA methylation in Bronchial and Lung Fibroblasts from healthy individuals and individuals with COPD

Rachel L. Clifford[1,3], Nick Fishbane[2], Poojitha Rajasekar[1], Andrew J. Fisher[4], Michael S. Kobor[2], Alan J. Knox[1], Tillie-Louise Hackett[2].

[1] Division of Respiratory Medicine and Nottingham Respiratory Research Unit, University of Nottingham, Clinical Sciences Building, City Hospital, Nottingham, United Kingdom

[2] University of British Columbia, Centre for Heart Lung Innovation, Dept of Anesthesiology, Pharmacology and Therapeutics, Vancouver, BC, Canada.

[3]Centre for Molecular Medicine and Therapeutics, Child and Family Research Institute, Department of Medical Genetics, University of British Columbia, Vancouver, British Columbia, Canada.

[4] Department of Respiratory Medicine, Newcastle Upon Tyne Hospitals NHS Foundation Trust, Newcastle Upon Tyne, UK Institute for Ageing, Newcastle University, Newcastle Upon Tyne, UK.

Rationale: Lung fibroblasts are implicated in respiratory disease pathology including chronic obstructive pulmonary disease (COPD). Phenotypic differences between fibroblasts isolated from the bronchi versus the lung parenchyma have been described but no studies have compared the cell types on a genome wide scale. DNA methylation is a reversible modification of DNA structure with the ability to affect cell function via the alteration of gene expression. Here we compared genome wide DNA methylation profiles from bronchial and lung fibroblasts and assessed modification to these profiles in cells isolated from individuals with COPD.

Methods: DNA was isolated from lung (LgF) and bronchial fibroblasts (BrF) at passage 4 and bisulphite treated. Site specific, quantitative genome wide methylation was determined using the Illumina 450K Infinium Methylation BeadChip array. Linear modelling and DMRcate functions identified differentially methylated sites and regions respectively between BrF and LgF and from cells isolated from healthy individuals versus those with COPD.

Results: 3980 CpG (methylation) sites significantly differed (Bonferroni correction) between BrF and LgF isolated from healthy individuals. These sites had a broad distribution of effect size, with 240 CpG sites displaying a difference in methylation of >50%. 78 of these sites validated in a second cohort of matched BrF and LgF isolated from the same individual. There was genomic proximity to these sites and DMRcate was used to refine the individual CpG sites to 5 regions of interest associated with 5 genes; HLX, TWIST1, CREB5, SKAP2 and PRDM16. Differences in methylation were less pronounced when comparing cells isolated from healthy individuals to those with COPD. In BrF 47 DMRcate regions were identified with a maximum difference in methylation of at least 20%. In LgF 3 DMRcate regions were identified with a maximum difference in methylation of at least 20%.

Conclusions: DNA methylation profiles are significantly different between BrF and LgF but only small modifications are associated with COPD. Future work will focus on validating a methylation based marker of lung versus bronchial fibroblasts and associating our differential observations with gene/protein expression.