European Respiratory Society
Annual Congress 2012

Abstract Number: 3044
Publication Number: P3725

Abstract Group: 3.2. Airway Cell Biology and Immunopathology
Keyword 1: Cell biology **Keyword 2:** Epithelial cell **Keyword 3:** Epigenetics

Title: microRNA profiling of murine alveolar epithelial type II cells

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Body: Rationale Alveolar epithelial type II cells (ATII) have key roles in innate immune response, surfactant production and as precursors for ATI cells (Mason RJ, Respirology, 2006). To obtain insight in gene regulatory networks specific for ATII we established a method for negative isolation of ATII cells and investigated their microRNA (miR) profiles. Methods Single cell suspensions were prepared from whole lungs of female C57BL/6 mice (6-12w) using dispase and mechanical dissociation. ATII were obtained by sorting (BD FACS Aria II) CD45-/CD31-/autofluorescence-high cells (sATII). Sorted cells were characterized by flow cytometry, immunocytochemistry (ICC) and RT-qPCR. MiR profiling was performed using TaqMan® Array Cards (ABI). MiR profiles of sATII were compared to cells obtained by panning (pATII) according to a standard method (Koenigshoff M et al., JCI, 2009) using negative selection in antibody-coated (CD45, CD16/32) petri dishes. Results Up to 99% of sATII cells were CD45-/CD31-/CD74+. sATII expressed higher mRNA levels for SP-C while mRNAs for CD31, CD45, ZO-1 and α-SMA were expressed at lower levels compared to pATII. mRNA levels for CD74, AQP5 and SP-A were similar in both populations. ICC confirmed proSP-C expression on sATII. MiR profiling revealed 117 miRs expressed at equal abundance (-1.5x to 1.5x) in sATII and pATII. 128 miRs were up- (>1.5x) and 64 miRs were down-regulated (<-1.5x) in sATII compared to pATII. In addition, 3 miRs were only detectable in sATII while 14 miRs were unique to pATII. Conclusion This study presents novel data on microRNAs expressed in an important pulmonary cell type. Further analysis by in silico enrichment analysis may reveal ATII-specific mRNA targets and signalling networks.