Non-biased proteomics discovery of pulmonary artery hypertension biomarkers periostin and matrix metalloproteinase 9
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**Background:** Identification of a pulmonary hypertension (PH) biomarker would aid in risk stratification, non-invasive monitoring of therapeutic efficacy and even as a potential therapeutic target. Our aim was to identify PH biomarkers via proteomic analysis of PH and control lung, followed by complementary techniques to demonstrate alteration in PH lung and plasma.

**Methods:** Homogenized lung extract from the Pulmonary Hypertension Breakout Initiative (PHBI) biorepository from end stage PH patients (idiopathic PAH=5, PAH-associated with congenital heart disease=5, control= 5) was analyzed by non-biased, high resolution mass spectrometry (Orbitrap Elite). Selection of lead biomarkers was by biological feasibility and spectral counting with > 2 or <0.5 fold change between PH and control. Lung western blot, IHC and ELISA were performed on PHBI homogenized lung, paraffin embedded lung and plasma, respectively, from adult PAH patients and controls. Data was analyzed by one way ANOVA and Kruskal Wallis.

**Results:** 860 non-redundant lung proteins were identified (APAH= 620, IPAH= 688, control=688; figure 1); 38 proteins were > 2 fold up or < 0.5 fold down in PH versus control lung. Periostin (IPAH 7.5 fold, APAH 9.5 fold) and matrix metalloproteinase 9 (MMP9) (IPAH and APAH <<0.5 fold) were identified as promising lead biomarkers. Western blot revealed elevated periostin while MMP9 decreased in PH versus control lung (figure 1a and 1b). Periostin IHC stained alveolar endothelial cells (figure 2). ELISA assay showed increased periostin in PH versus control plasma (n= 17, PH median 9738 ng/mL, control median 1433 ng/mL, p= 0.0502) (figure 3).

**Conclusions:** Periostin and MMP9 have reciprocal expression in PH lung. Periostin is predominately endothelial expressed, with circulating periostin a promising new PH biomarker. MMPs play a significant role in PH pathogenesis. Future studies will include larger sample sets, including pediatric patients, and evaluate additional potential PH biomarkers generated from proteomic lung analysis.