Expression of CD55 and ACTA2 in Bronchopulmonary Dysplasia

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Introduction: Bronchopulmonary dysplasia (BPD) is a term used to describe chronic lung disease developed as a result of inadequate lung development in premature infants (<37 weeks gestational age) and postnatal injury to developing lungs[1]. BPD disrupts alveolarization and microvascular development, leading to abnormal gas exchange and lung mechanics. Proteomics data generated by the research team at the Pacific Northwest National Laboratory (PNNL) suggests differential expression of certain lung proteins in BPD lungs, recovered lungs, and control lungs. In an effort to validate some of the proteomic data from PNNL, we chose to study the spatial location of smooth muscle alpha actin (ACTA2) to substantiate the proteomic findings that ACTA2 is more highly expressed in BPD lungs, using immunofluorescence microscopy. CD55—an inhibitor of complement activation, previously noted to be expressed in alveolar epithelial cells—was also assessed, because its role in lung development is not well described.

Methods: Formalin-fixed, paraffin embedded sections from the proximal lung and alveolar region for each donor were deparaffinized, rehydrated, and subjected to optimal antigen retrieval conditions for CD55 and ACTA2 multiplex. Each slide was blocked for 60 minutes, incubated overnight with antibodies for ACTA2 and CD55, then incubated with fluorophore conjugated secondary antibodies. A slide with no antibodies added and a second slide with secondary antibodies only were kept as negative controls. All slides were imaged with a Keyence BZ-X810 inverted epifluorescence microscope.

Results: Epithelial marker CD55 outlines structural differences in alveolar regions. Alveolarization is much less prominent in BPD tissue versus control, with fewer secondary crests and less alveolar complexity. There is increased cellularity and edema in alveolar septal walls in BPD tissue, making them appear thickened. The cells of the alveolar septal walls appear to be more cuboidal, indicating either more Type II cells than in control samples or misshaped Type I cells. Recovered BPD patients have thinner alveolar walls, showed reduced interstitial edema, and reduced intraseptal cellularity, making them more similar to control samples; however, there was still alveolar simplification and fewer secondary crests, consistent with effects of previous BPD.

Using the proteomics data, we expected ACTA2 to be differentially expressed in BPD lungs. Immunofluorescent imaging showed smooth muscle hyperplasia around distal airways and around vessel walls, with thicker more continuous lining around airways in BPD tissue. There seemingly was an increase of myofibroblasts in intra-alveolar septal space, instead of at the end of secondary crests (as seen in controls). Control tissue showed lower expression of smooth muscle actin, a less continuous smooth muscle lining around airways, and thinner walled vessels. BPD recovered tissue also lacked secondary crests and the associated myofibroblasts, but surprisingly had a more similar overall distribution of ACTA2 as compared to the control samples.

Conclusions: Immunostaining of ACTA2 corroborates PNNL's proteomics data by visualizing the placement and intensity of the ACTA2 signal and demonstrating excess expression of ACTA2 in BPD tissue. CD55 staining gave insight on the differences in alveolarization in BPD diseased lung tissue, and showed that recovered tissue has a similar intensity of CD55 as control tissue, but a different spatial configuration. Future direction should focus on quantifying ACTA2 expression through stereology and further characterization of CD55 expression.