Neonatal Hyperoxia Causes Vascular Smooth Muscle Specific Transcriptomic Changes and Pulmonary Hypertension

D. T. Dinh[1], E. A. Barnes[2], R. Ito[3], C. Knutsen[4], X. Che[1], F. Zanini[5], C. M. Alvira[6], D. N. Cornfield[2]; [1]Division of Pediatric Pulmonology, Stanford University School of Medicine, Palo Alto, CA, United States, [2]Pediatrics, Stanford University, Stanford, CA, United States, [3]Pediatrics, Jikei Medical University, Tokyo, Japan, [4]Division of Critical Care, Stanford University School of Medicine, Stanford, CA, United States, [5]Prince of Wales Clinical School, University of New South Wales, Sydney, Australia, [6]Stanford University, Palo Alto, CA, United States.

Corresponding author's email: dtdinh@stanford.edu

Background: Premature infants can develop bronchopulmonary dysplasia (BPD), a chronic lung disease of infancy characterized by an arrest of secondary septation, compromised angiogenesis, and, often, pulmonary hypertension (PH). Though pulmonary vascular smooth muscle cells (PVSMC) play a central role in neonatal PH, insight into the cell-specific transcriptomic alterations that might drive proliferation, migration, and contractility remains limited.Objective: To test the hypothesis that single cell RNA-sequencing would identify a PVSMC-specific transcriptomic signature in a murine model of hyperoxia-induced neonatal lung injury. Methods: Pulmonary mesenchymal cells (MC; 5493 cells) were isolated from mice at early saccular (E18.5), late saccular (P1), early alveolar (P7) and late alveolar (P21) stages of development, and from P21 mice exposed to chronic hyperoxia (H;FiO_2=0.8 x 7d), a preclinical model of bronchopulmonary dysplasia (BPD). Single MC were sequenced on Illumina NovaSeq at a depth of ~10^7 reads per cell. MC populations and genes of interest were validated with in situ hybridization. In separate experiments, lung histology, right ventricular systolic pressures (RVSP) and vascular reactivity were measured at 6 weeks (echocardiogram) and 12 weeks (catheterization)Results: Unsupervised clustering identified 14 transcriptionally distinct MC clusters, with dynamic changes in diversity across development. PVSMC were identified by coexpression of smooth muscle (Tagln, Acta2) and mural (Pdgfrb) genes. In most lung MC, differential gene expression (DEG) from P21 H compared to normoxic (N) mice was relatively conserved, including upregulation of known anti-proliferative genes Cdkn1a, Junb, and Btg2, a signature was absent in H PVSMC. The most DEG in H (n=73 cells) compared to N (n=81 cells) PVSMC were relatively unique with decreases in Btg2 (16-fold), Egr1 (8-fold), and increase in Cnn1 (8-fold), and Col18a1 (8-fold) molecules encoding proteins that are anti-proliferative, involved in injury repair, procontractile, and anti-angiogenic, respectively. Medial wall thickness, RVSP, and hypoxic pulmonary vasoconstriction (HPV) were greater in H, compared to N at 6 ($p<0.01$; n=9 N; n=10 H) mice and 12 ($p<0.01$; n=9 N; n=10 H) weeks Conclusions: In the lung, neonatal MC transcriptome demonstrates progressive increases in cellular diversity and dynamic changes in gene expression. Neonatal hyperoxia caused vascular remodeling, pulmonary hypertension and accentuated HPV. Hyperoxic PVSMC demonstrated a unique transcriptomic signature with dynamic changes in genes that play roles in cell proliferation, injury response, contraction, and angiogenesis. These results provide molecular insights and potential therapeutic targets to address the vascular remodeling that underlies neonatal PH.

This abstract is funded by: None

Am J Respir Crit Care Med 2023;207:A4657
Internet address: www.atsjournals.org Online Abstracts Issue