Alterations of Aerocyte Capillary Phenotype During Postnatal Development and in Response to Injury

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Background: Angiogenesis is a key driver of alveolarization, and disrupted angiogenesis and alveolarization is observed in premature infants with bronchopulmonary dysplasia (BPD). Aerocytes have been recently identified as specialized lung capillary EC that highly express the enzyme carbonic anhydrase-4 (Car4), and extend along the alveolus, intimately associated with alveolar type 1 cells. However, the temporal progression of aerocyte emergence and stimuli inducing aerocyte differentiation are not known. Further, how aerocyte phenotype is altered by injuries that disrupt alveolarization has not been explored.Methods: Single lung cells were obtained from embryonic and neonatal mice at various timepoints under control conditions, and from mice exposed to chronic hyperoxia for 14 days and analyzed at P7 (acute injury) and P21(recovery). Flow cytometry to detect CAR4 and CD31 was utilized to analyze aerocyte abundance. Deep, plate-based single cell RNA sequencing (scRNA-Seq) was performed via SmartSeq2. Differentially expressed genes (DEGs) were subjected to pathway analysis using Metascape.Results: In the embryonic lung, aerocytes represented only 1% of total lung EC. At birth, aerocyte abundance markedly increased, comprising 18% of lung EC at P1, and 27% by P7. Exposure to chronic hyperoxia increased aerocyte abundance from 22.5 to 36.3 at P7 and 25.3 to 38.8 at P14 via FACS (p value less than 0.001, n =3-4). Upon recovery, aerocytes remained elevated at 29% in P21 mice exposed to hyperoxia vs. 11% in control mice (P=0.04, n=3). In normoxia, most aerocytes were distinguished from general capillary EC (gCAP) by unique markers. However, in hyperoxia recovery, cells exhibiting an intermediate phenotype (iCAP) characterized by a combination of aCAP (e.g. Car4, Ednrb, and Igfbp7) and gCAP marker genes (e.g. Kit and Plvap), increased from 6% to 10%. Pathway analysis of DEGs in hyperoxia-exposed aerocytes identified an enrichment of up-regulated genes related to ATP synthesis and aerobic metabolism and down-regulation of caveola assembly, semaphorin signaling, and migration at P7. Upon recovery, these same metabolic pathways were down-regulated, and up-regulated pathways included positive regulation of angiogenesis and cell migration.Conclusions: Aerocyte abundance markedly increases after birth coinciding with the transition from the hypoxic fetal environment to the normoxic prenatal environment. Postnatal hyperoxia increases aerocyte abundance, and alters genes important for metabolism, migration, and cell guidance. Taken together, these preliminary data suggest that physiologic stimuli at birth (e.g. hyperoxia, cyclic stretch, blood flow) may be important drivers of aerocyte speciation and highlight the sensitivity of aerocytes to injury.

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