Redistribution of EC-SOD alters interstitial macrophage populations in hypoxia-induced pulmonary hypertension

Caitlin Lewis, Ayed Allawzi, Christina Sul, Laura Hernandez, Claudia Mickael, Eva Nozik

Pediatric Critical Care, Cardiovascular Pulmonary Research Labs, Department of Pediatrics, University of Colorado Anschutz Medical Campus

Hypoxia leads to reduced circulating pro-inflammatory Ly6C\text{hi} and increased lung nonclassical Ly6C\text{low} monocytess in both WT and R213G mice

**Background**

- Extracellular superoxide dismutase (EC-SOD) expression and activity is reduced in pulmonary hypertension (PH)\(^1\).
- The R213G EC-SOD SNP leads to redistribution of EC-SOD from tissue into extracellular fluid and increased risk of vascular disease in humans\(^2\).
- Mice with the R213G polymorphism have exacerbated vascular remodeling and PH\(^3\).
- Interstitial macrophages (IM) are increased in PH and can be subdivided into IM1, IM2 and IM3 populations\(^1,4\).
- We hypothesize that IM populations will be altered in R213G mice under hypoxia, contributing to worsened PH.

**Methods**

**Mice:** Male and female C57BL/6 (WT) and R213G mice were exposed to hypobaric hypoxia in chambers simulating 18,000 ft altitude. Normoxic (NMX) controls were maintained at Denver altitude.

**Flow Cytometry:** Retro-orbital (RO) injection of CD45 antibody was used to identify intravascular cells. Lungs were digested to form single cell suspensions and stained with antibodies to identify intravascular and lung monocytes. Absolute cell counts for 1 ml blood or whole lung digest are shown.

**Statistics:** 2-way ANOVA was used to assess effects of hypoxia and/or genotype on variance with Tukey’s post hoc testing, n=6-13, square= male, circle= female, *P<0.05.

**Results:**

- Despite reduced lung IMs at D4, R213G mice exhibit worsened hypoxia-induced PH, suggesting potential protective actions of these cells.

**Conclusions**

- Pro-inflammatory monocytes exit systemic circulation and both monocyte subpopulations exit lung vasculature four days after induction of hypoxia.
- Increased nonclassical monocytes are observed in lung and may give rise to IMs.
- All three IM subpopulations are increased with hypoxia.
- The redistribution of EC-SOD in R213G mice has no effect on monocyte populations but prevents increases in IM1 and IM2 populations.

**Future directions**

- Characterize ROS levels in WT vs R213G lung compartments
- Examine IM transcriptomic profiles and the potential effect of the R213G SNP on hypoxic reprogramming
- Investigate localization of IM subsets within the lung
- Explore potential roles of different IM subsets in the development of PH using mice deficient in selected subsets

**Disclosures**

- This work was funded by NIH/NHLBI R35HL139572 (ESN)