STRONG CHILDREN'S RESEARCH CENTER

Name: Estee Wu
School: University of Rochester
Mentors: Ravi Misra, Ph.D & Gloria Pryhuber, M.D.

ABSTRACT

Title: Expression of Mast Cells in Bronchopulmonary Dysplasia

Background: Bronchopulmonary dysplasia (BPD) is a chronic inflammatory lung disease that primarily affects preterm infants less than 29 weeks to 32 weeks of gestation. In affected patients, lung development is noticeably impaired, with heterogeneous pathology throughout the lungs, including decreased alveolarization and emphysema. This pathology can lead to persistent airway and pulmonary vascular disease that can have lasting implications into adulthood[1]. Inflammation is a common pathway that is thought to contribute to the BPD phenotype. Genome-wide transcriptional profiling revealed an accumulation of mast cells, key players in the inflammatory cascade, in cases of BPD[2]. In efforts to better understand and validate the mast cell signature in BPD found in previous studies, the expression of mast cells was studied. To determine this, we targeted several genes and accessed their expression in human lung tissue to validate the hypothesis that there is an increase in mast cell expression in BPD than in non-BPD lungs. A greater understanding of the pathophysiology of BPD will provide a clearer understanding of disease mechanisms and enable the discovery of novel therapeutic targets.

Objective: To assess the expression of mast cells using specific mast cell markers in cases of BPD in preterm infants. Mast cell markers included c-KIT, KITLG, TPSAB1, CPA3, and CMA1.

Methods: Human lung samples were harvested within 6 hours of death and snap-frozen in liquid nitrogen. Twenty-four samples were selected for mast cell expression profiling, including 13 BPD and 11 non-BPD control cases, age matched for gestational age at birth and death. Frozen lung tissue was homogenized in Trizol reagent (Invitrogen, Carlsbad, CA) and total RNA was purified using a protocol including a DNase I treatment (MiniPrep kit, Agilent Technologies, Santa Clara, CA). The quality and concentration of purified RNA was assessed via a 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). cDNA was synthesized using purified RNA and qPCR was performed using primers for c-KIT, KITLG, TPSAB1, CPA3, and CMA1 to assess mast cell expression (Applied Biosystems, Carlsbad, CA).

Results: In the BPD donor tissue, the expression of mast cell markers was markedly increased compared to non-BPD control cases. In particular, it was found that the expression of c-KIT and KITLG was significantly increased in two cases of BPD donors, D039 and D141. Donor D141 was 11.18 months old with active and evolving BPD and donor D039 was 38.29 months with chronic BPD. Additionally, based on the results, the expression of mast cell markers KITLG and TPSAB1 correlates with age in BPD patients– increasing expression with increased age.

Conclusions: The results of our analysis match up with data collected through genome-wide transcriptional profiling done in previous studies. In future experiments, focus should be placed on the expression of c-KIT and KITLG in cases of BPD to elucidate their effects on pathology and potential as therapeutic targets of BPD.

References:

[1]Thébaud B, Goss KN, Laughon M, Whitsett JA, Abman SH, Steinhorn RH, Aschner JL, Davis PG, McGrath-Morrow SA, Soll RF, Jobe AH. Bronchopulmonary dysplasia. Nat Rev Dis Primers. 2019 Nov 14;5(1):78. doi: 10.1038/s41572-019-0127-7. PMID: 31727986; PMCID: PMC6986462.

[2]Bhattacharya S, Go D, Krenitsky DL, Huyck HL, Solleti SK, Lunger VA, Metlay L, Srisuma S, Wert SE, Mariani TJ, Pryhuber GS. Genome-wide transcriptional profiling reveals connective tissue mast cell accumulation in bronchopulmonary dysplasia. Am J Respir Crit Care Med. 2012 Aug 15;186(4):349-58. doi: 10.1164/rccm.201203-0406OC. Epub 2012 Jun 21. PMID: 22723293; PMCID: PMC3443810.