The role of oxidative stress and dysregulation of bronchiolar epithelial cells in idiopathic pulmonary fibrosis

**Objectives:** To investigate the effects of Atp8b1 deficiency on behavior of Club-Clara cells in hyperoxia-induced acute lung injury

**Methods:** WT and Atp8b1 G308V homozygous mutant mice at 7-9 weeks of age were exposed to 100% O2. Immediately after hyperoxia, bronchoalveolar lavage (BAL) fluid and lung tissues were collected. Microscopic evaluation was performed on BAL cells. Histopathological evaluation was performed on H&E-stained lung tissues sections. To determine cell apoptosis, TUNEL staining was performed on lung sections. Immunohistochemical labeling for Club-Clara cell secretory protein (CCSP) (a Club-Clara cell marker), Cluaudin-10 (a second Club-Clara cell marker) and Ki-67 (a proliferation marker) was performed on lung sections.

**Results:** TUNEL staining on lung tissue sections revealed that Atp8b1 mutant lungs under hyperoxia exhibit enhanced cell death in alveoli. Meanwhile, the number of TUNEL-positive cell death was not changed in Atp8b1 mutant lungs. H&E-stained lung sections revealed a patchy thickening of bronchiolar epithelium. Immunohistochemical labeling revealed that a large portion of the cells in the thickened bronchiolar epithelium in Atp8b1 mutant lungs under hyperoxia are CCSP-positive, Claudin-10-positive and Ki-67-positive.
Microscopic evaluation of BAL fluid cells from hyperoxic mice revealed that Atb8b1 mutant mice under hyperoxic conditions showed a robust increase in the number of cells in airspace compared to WT mice. In BAL fluid from hyperoxic Atp8b1 mutant mice, a particular cell type reminiscent of Club-Clara cells was occasionally encountered.

**Conclusions:** Atp8b1 deficient Club-Clara cells are resistant to oxidative stress and proliferate under hyperoxic conditions.