A33

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Development of an In Vitro Model For the Study of The Cross-talk Between Alveolar type II cells and Pulmonary Fibroblasts

Alveolar type II cells (T2C) specialize in the synthesis and secretion of pulmonary surfactant. Previous research in our lab showed that knockdown of low density lipoprotein receptor related protein 1 (LRP1 KD) in human A549 T2C-derived results in a decrease of surfactant lipid in vitro, with decreased mRNA expression of lipid metabolic markers. In vivo, our inducible T2C-specific LRP1 knockout mice (SPC-LRP1-/-) exhibits decreased lung compliance and surfactant lipid. Fibroblasts are the main cell in the lung producing and secreting all components of the extracellular matrix. We developed culture systems to study the cross-talk between control or LRP1 KD T2C and fibroblasts.

For the non-direct, T2C cells were cultured on transwell inserts coated with an equimolar mix of collagen 1 and 3, with fibroblasts plated on the bottom of the plate. For the direct contact, both sides of the insert were coated and T2C cells were cultured as above on transwells, and fibroblasts on the basal side of the insert. In both conditions, the cells were placed at the air liquid interface (ALI), removing the media from the upper chamber, to allow the cells to differentiate into polarized T2C. We performed qPCRs for detection of metabolic and inflammation markers.

The non-direct culture condition showed severely higher gene expression for the metabolic genes for both control and LRP1 KD cells, and most of the baseline differences were maintained. The direct contact culture increased the gene expression of the metabolic genes, more dramatically in LRP1 KD than in control cells. In both co-cultures, the inflammation markers increased in the T2C, except CXCL8 in the non-direct culture, and the fibroblast cell gene expression were unaffected by the LRP1 KD in the T2C.

These co-culture models allow us model the alveolar environment, reflecting epithelial cells' apical and basal polarity to study cell to cell communication through cell signaling.