

# The hippo pathway component AMOTL2 impacts lung epithelial cell reprogramming in pulmonary fibrosis.

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**Background:** Idiopathic pulmonary fibrosis (IPF) is a severe and usually fatal lung disease characterized by increased ECM deposition, stiffening of lung tissue, and destruction of alveoli. IPF is thought to be in part, driven by the failure of appropriate differentiation of alveolar stem cells (primarily alveolar type 2 – AT2) into the gas-exchanging alveolar type 1 (AT1). Single-cell RNA sequencing has identified an aberrant AT2 cell phenotype in IPF, referred to as basaloid cells. A similar aberrant cell type has been found in murine models of pulmonary fibrosis, alveolar differentiation intermediate (ADI) cells. We and others show that the Hippo effector Yes-associated protein 1 (YAP) is upregulated in AT2 cells in IPF and promotes ADI formation; here we examine its regulator AMOTL2. We hypothesize that AMOTL2 downregulation in AT2 cells leads to increased nuclearization of YAP where it upregulates genes related to reprogramming of AT2 cells.

**Materials and Methods:** We examined localization of AMOTL2 in human lung tissue from healthy and IPF donors (n=10/group -IPF vs control) via single-cell RNA Seq and immunofluorescent (IF) staining of AMOTL2 alongside specific AT2 cell-markers. We knocked out AMOTL2 in cultured primary mouse AT2 cells from *Amotl2* fl/fl mice using Adeno-cre ex vivo and analyzed up and downregulated genes and pathways in addition to their ADI phenotype using bulk RNA Seq (n=4). AT2 specific AMOTL2 knockout mice were treated with bleomycin to induce lung fibrosis and were compared to controls in bodyweight, lung function and histologically.

**Results:** We found that AMOTL2 is downregulated in AT2 cells in IPF, and its loss in primary mouse AT2 cells ex vivo, induces transcription of pro-fibrotic and altered epithelial identity pathways. The AMOTL2 knockout cells also show upregulation of ADI-associated genes such as Keratin 8 and Claudin 4, indicating loss of normal AT2 cell reprogramming. Our in vivo experiment with bleomycin-induced lung injury in mice with AT2 cell-specific loss of AMOTL2 showed that our knockout mice lose less bodyweight following bleomycin, but they exhibit increased lung stiffness compared to control. These data indicate worsened fibrotic mechanical remodeling despite a potential attenuated initial immune-related systemic effect from the bleomycin treatment.

**Conclusion:** Loss of AMOTL2 contributes to AT2 cell reprogramming and thus might contribute to the development and progression of pulmonary fibrosis.