Using *In Vitro* Lung Models for Personalized Approaches to Treat Rare Lung Diseases

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Lung diseases represent a large source of morbidity and mortality throughout the world, at all ages of life, but we have no specific therapies for lung diseases, just symptomatic management. One of the reasons why we have not yet developed effective cures for lung diseases is the lack of representative human models.

To address these issues, we have generated and extensively characterized an induced pluripotent stem cell (iPSC) based *in vitro* disease model that closely phenocopies IPF in a dish. These cells scar spontaneously and progressively when cultured over several days. Live cell imaging, transcriptome analysis, atomic force microscopy, immunostaining, apoptosis assays and cytokine arrays were used to characterize these scars. We found that the progressive scarring is driven by signals that include damage associated molecular patterns that drive a robust pro-inflammatory response, leading to the observed pathology.

Using the model, we developed a primary phenotypic high throughput drug screening assay to identify compounds that would target one or more of the phenotypic characteristics of our model, such as increasing apoptosis of hyper-proliferative fibroblasts, targeting extracellular matrix interactions, or targeting the stiffness of the cells.

We have also generated an induced pluripotent stem cell-based, 3D model of IPF in a dish. This was accomplished by engineering a cell-hydrogel bead composite that self-organized in a rotating bioreactor to generate an organoid that resembles the architecture of the interconnecting alveolar sacs of the human distal lung. We then used several inducers of fibrosis in the organoid cultures, such as TGF-β1, to generate fibrosis in the organoid. The lung organoid formation process is modular, allowing for the use of patient-specific cells and for the generation of lung organoids in 96 and 384 well plates, making this amenable to high throughput drug screening. Using a fluorescent reporter for alpha-smooth muscle actin, one of the hallmarks of the fibrosis seen in IPF, we developed a primary screen with the ImageExpress 3D confocal device to identify compounds that reverse TGF-β1 induced fibrosis.