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Tissue clearing and 3D light sheet microscopy of human lungs to characterize capillary cell plasticity in lung injury and fibrogenesis

Supervisors

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Project background

Fibrogenesis after tissue injury is one of the most prevalent clinical complications and causes of death. One of the archetypal examples of a organ fibrosis is the lethal disease Idiopathic Pulmonary Fibrosis (IPF), a relentless fibrotic lung disease characterized by the progressive scarring of alveolar tissues with dramatic changes in epithelial, endothelial and fibroblast cell states during disease progression (1-2). The main aim of our research is to understand how these aberrant cell states are wired together into circuits and thereby influencing each other throughout disease evolution (3-5). To better understand the altered cell-cell communication inducing these disease-specific circuits, we use state-of-the art single cell -omics technologies, innovative imaging tools and organotypic ex vivo models.

Fibrogenesis in ARDS as seen in COVID-19 has been shown to feature several shared cell states with the more progressive and irreversible pulmonary fibrosis in IPF patients. For instance, recent single-cell transcriptomic analysis has revealed the appearance of similar ectopic endothelial cell states in both IPF and COVID-19 (6). In this work we investigate the cellular origin of the VWA1+/PLVAP+ injury induced endothelial cell state.

Using multiplexed immunofluorescence imaging in micro-CT staged IPF tissues we identified a substantial loss of capillaries and a gradual increment of newly-generated ectopic vessels (VWA1+/PLVAP+) with increased fibrotic remodeling. Larger VWA1+/PLVAP+ vessels were observed around airways, likely representing the systemic circulation around bronchi. However, we also identified de novo expression of VWA1+/PLVAP+ in the thickened alveolar septum of early stage IPF, indicating that these cells might emerge from capillary EC in the pulmonary circulation.

Using a mix of profibrogenic cytokines we experimentally induced human lung fibrogenesis *ex vivo* in human precision-cut-lung slices (hPCLS) and performed both whole mount and FFPE based immunostainings as well as scRNASeq. VWA1+/PLVAP+ ectopic EC specifically emerged in the alveolar septum only after treatment with profibrogenic cytokines and scRNAseq based trajectory inference suggests emergence of this population from capillary EC.

Taken together, our current data highlights so far underappreciated and not well studied capillary EC plasticity upon human lung injury, which gives rise to a VWA1+/PLVAP+ EC state in both acute lung injury during infectious disease as well as chronic interstitial lung disease.

Project description

In this project, the student will further investigate the nature of capillary changes in early-stage pulmonary fibrosis in patient tissues using 3D light sheet imaging.

The student will apply tissue-clearing light sheet fluorescence microscopy (LSFM, 7-8) to characterize the 3D spatial organization of the pulmonary and systemic vascular networks in human lung fibrosis. In detail, the student will start to perform whole-mount staining of 3D lung sections using the pan-endothelial and disease-specific protein markers. The student will then perform tissue clearing techniques to make the human lungs optically transparent and take 3D imaging of entire 3D lung sections (the sample size ca. 0.5-1 cm^3) using light sheet fluorescence microscopy. To analyze the 3D imaging datasets, the student will also learn to use advanced image analysis software like Imaris for 3D reconstruction and computational analysis.

Expert training will be received on whole-mount immunostaining techniques, tissue clearing protocols, state-of-the-art light sheet fluorescence microscopy, and bioinformatic analysis.

Preliminary data:

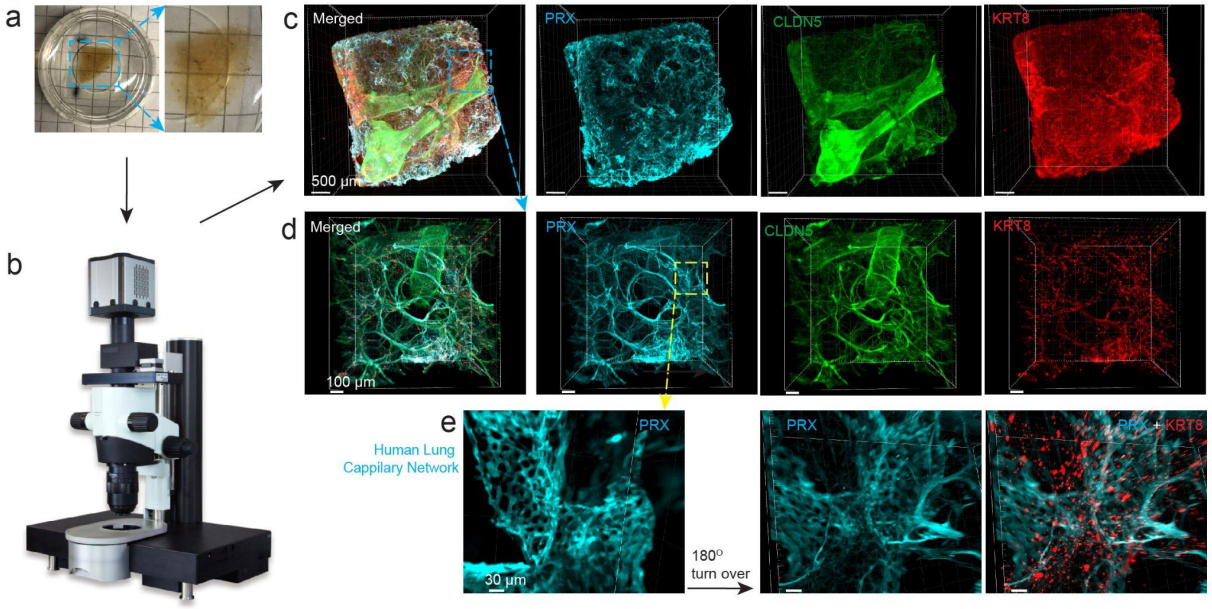

Figure 1: Schematic workflow of tissue-cleared LSFM for 3D imaging of pulmonary architecture in whole-mount immunostained human lungs. a: a tissue cleared lung with excellent optical transparency. **b**: the optical cleared lung was imaged by LSFM. **c**, **d**,and **e**: 3D architectures of a piece of human lung obtained from peritumor regions (in different magnifications) that has been stained by the pan-endothelial marker CLDN5, capillary marker PRX, and epithelial marker Keratin 8 (KRT8). The lung capillary network around a single alveolus can be observed using LSFM (e).

Tasks for the project will include:

- Optimization of new protein markers (antibodies) for use in whole-mount immunofluorescence.
- Learning the tissue-clearing light sheet fluorescence microscopy and applying it to understand ectopic endothelial systems in human IPF tissue with different disease severity states.
- Performing the 3D imaging analysis by Imaris or Fiji like 3D reconstruction of lung geometry and quantitative analysis.

- Understanding and defining the ectopic vascular networks and cell circuits from 3D imaging datasets.

Project requirements

This project will suit a highly motivated student with a background in biology or similar field with an interest in imaging and computational data analysis. Familiarity with computational data analysis is desirable but not required. Experience with staining techniques and optical tissue clearing is not required but will be developed during the project. There will be regular contact with supervisors but you should also be comfortable working independently. By completing this project the student will be exposed to a cutting edge experimental systems biology research group, develop a range of research and problem-solving skills and become familiar with the challenges of method development, data analysis, and biological interpretation of volumetric imaging datasets.

Diversity

Women and people from other underrepresented groups are strongly encouraged to apply and we will seek to provide any support you require to complete the project.

References

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