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Multiplexed and volumetric imaging of human organs 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 an 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 (3-6). 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 (7). 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 (6).

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 multiplexed imaging (so-called 4i imaging, 6, 8) and 3D light sheet fluorescent microscopy (LSFM, 9-10)**.

The student will apply advanced imaging techniques, such as two-dimensional (2D) multiplexed imaging and 3D volumetric LSFM, to map cell state changes of ECs in time and to reveal the complex 3D evolution of the ectopic vessel network during lung fibrogenesis. In detail, the student will start to perform 1) multiple rounds of IF staining on 2D FFPE sections after antibody elution in each round in health and fibrotic human lung tissues; 2) whole-mount staining of 3D lung sections followed with tissue clearing and LSFM imaging (Figure 1). The 3D lung tissues will be cut into a size of ca. 0.5-1 cm^3 and become optically transparent after tissue clearing. Pan-endothelial and disease-specific protein markers identified with single-cell omics will be used to visualize and quantify the trajectory of cellular dynamics during cytokine-induced ex vivo lung fibrogenesis. To analyze the 2D/3D imaging datasets, the student will also learn to use advanced image analysis software like Imaris for machine-learning based 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.

1. *Workflow*

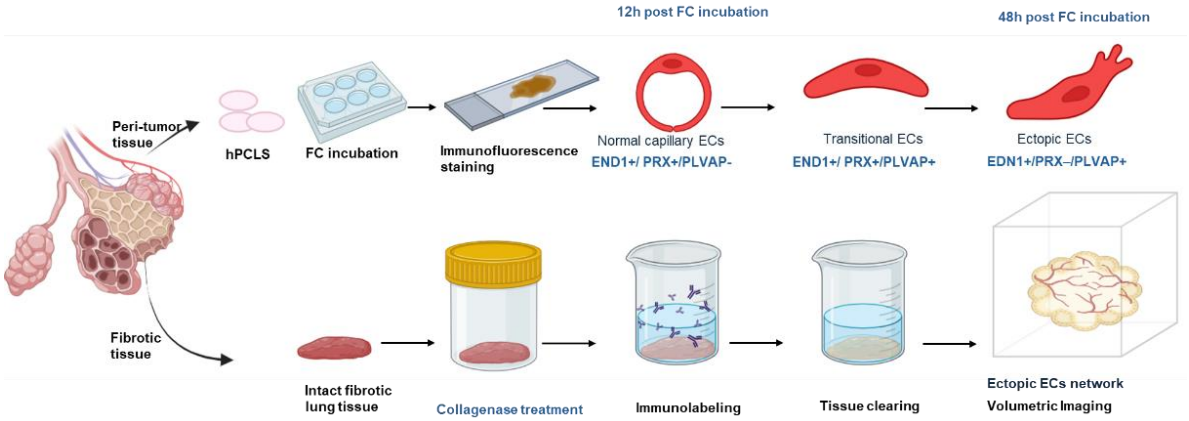

Figure 1: Schematic workflow of multiplexed imaging and tissue-cleared LSFM for 2D and 3D characterization of endothelial cell states in health and diseased human lungs. FC: Fibrotic Cocktail of cytokines.

2. *Preliminary data*

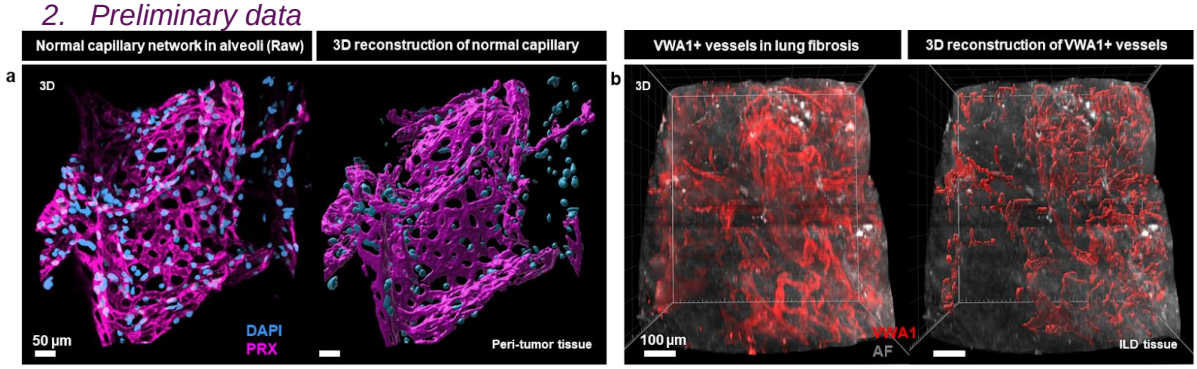

Figure 2: The physiological organization of abundant capillary bed in peri-tumor tissues (a) and the aberrant 3D structure of interconnected VWA1+ vessels appeared in human IPF lung tissue (b) scanned with laser scanning confocal microscopy and tissue clearing and light sheet fluorescence microscopy, respectively. AF: tissue autofluorescence.

Tasks for the project will include:

- Performing 4i staining on 2D FFPE sections from in vivo and ex vivo human lung tissues.
- 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.
- Extracting biological information from 2D/3D images with computational analysis using advanced analytical tools like Imaris, Python.
- 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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