Fibrosis Unmasked: A Metabolic Cell Odyssey Across Tissues and Diseases

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Project outline

Background: Improved treatment of fibrotic diseases is a critical unmet clinical need (Dakin et al. 2018; Zhao et al. 2022). Fibrogenesis is a physiological mechanism required for wound healing and repair, however, upon chronic or repetitive injury, such as in the context of infection or autoimmunity, this process becomes dysregulated (Davidson et al. 2021). The aberrant fibroblast activation, perpetuated by inflammatory and epigenetic processes, drives pathogenic tissue scarring and remodelling, leading to fibrotic disease, impairment of organ function and eventually organ failure. Maladaptive fibrogenesis can affect multiple organ systems, including the musculoskeletal, gastrointestinal, respiratory, and cardiometabolic systems, and is a major contributor to increasing global morbidity and mortality (Thannickal et al. 2014). For many of these conditions, organ transplantation is the only final resort leading to high unmet care needs and health burdens. Two critical questions in the field remain: Is the route to fibrosis the same in all organs? Does inflammation contribute to fibrosis? We propose that the reason these questions remain is that the role of metabolic changes in driving pathogenesis across organs and diseases has not been adequately addressed so far.

Emerging evidence indicates that metabolism is central to fibrogenesis, and that perturbation of specific metabolic processes, such as inflammasome-dependent glycolysis and lactylation in fibroblasts, may attenuate fibrosis (Pucino et al. 2020; Drucker & Holst 2023; Faust et al., 2023; Liu et al. 2024). However, the full breadth of metabolic pathways that promote fibrosis have not been characterised and it is unknown to what extent these are shared between or distinct to different diseases – especially given our growing appreciation of the heterogeneity of fibroblast cell subsets and states associated with pathology across different tissue types (Croft et al. 2019; Korsunsky et al. 2022).

We have wide-ranging expertise in fibroblast biology that spans cross-tissue and cross-disease fibroblast single-cell and spatial transcriptomic data analysis, to novel fibroblast culturing and precision genome editing, and through to the investigation of fibroblast metabolic activity and its implications for cellular function (e.g. Korsunsky et al. 2022; Buckley & Midwood 2024; Pucino et al. 2023). For this proposal we will leverage our access to human data and tissues, and our fibroblasts culturing and functional assaying approaches to dissect the single-cell and spatial biology of the metabolic drivers of disease-associated fibrogenesis.

Hypothesis: We propose that a combination of metabolic pathways, triggered by inflammatory and other stimuli, through fibroblast-macrophage crosstalk promote the maladaptive fibrogenesis associated with fibrotic disease across different tissues.

Aims: Our overarching goal is to identify the breadth of metabolic pathways that drive aberrant fibroblast activation. Our specific aims are to:

1. To integrate single-cell transcriptomic data from >1 million fibroblasts derived from metabolically relevant human tissues across >15 diseases from >200 individuals, and to use

this integrated dataset for comparative metabolic pathway and metabolic flux inference analyses.

2. To assess the spatial relevance of fibroblast metabolic pathways by analysing spatial transcriptomic data from selected human tissues (from patients and controls) and to validate spatially resolved cellular and extracellular matrix signatures at the protein level through multiplexed ion beam imaging (MIBIscope) of tissue biopsies and through high-plex spectral cytometry.

3. To perform CRISPR-based metabolic pathway combined with high-throughput transcriptomics to screen primary human tissue fibroblasts to identify key regulators of metabolic activity upon cell activation.

WP1: We have access to unique in-house single-cell transcriptomic datasets derived from patients displaying inflammation-associated fibroblast activation and fibrosis, as well as access to publicly available cross-tissue/cross-disease datasets. Collectively, we estimate that we have collated single-cell transcriptomics data from >1 million fibroblasts derived from ~10 different metabolically active human tissue sites (including pancreas, adipose tissue, gut, liver, kidney, heart, vasculature, CNS) and at least 15 different diseases. Leveraging our single-cell and spatial multi-omics data processing and analysis pipeline, Panpipes (Curion et al. 2024), we have the capacity to perform high-speed and high-throughput integration of these datasets to create a comprehensive fibroblast mega-atlas. Using this mega-atlas, we will assess the presence of metabolic pathways through gene set and pathway enrichment analyses (e.g. scGSEA, cNMF) and we will infer metabolic flux balances (e.g. COMPASS, scFEA) to better characterise fibroblast metabolic single-cell states and their association with pathogenesis across tissues. We will also dissect how genetic determinants contribute to maladaptive fibrogenesis by further integrating our single-cell data with relevant genome-wide association study statistics (using tools such as fGWAS and SNP2cell).

WP2: To better delineate how shared or distinct aberrant fibroblast metabolic states arise and are maintained across different tissues and diseases, we will investigate how these states map to inflammatory, pro-fibrotic niches. For these niche analyses we will utilise unique in-house and available spatial transcriptomic datasets generated on the 10x Genomics Visium, Xenium and the Bruker CosMx platforms, including longitudinal data. These datasets will enable spatial niche microenvironment characterisation, assessing interactions between fibroblast cell states, tissue macrophages in the context of endothelial, innate/adaptive immune, and epithelial cells in their vicinity. These analyses will leverage our Panpipes pipelines (particularly for single-cell and spatial data integration) and our experience with downstream analysis packages such as MuSpAn (Bull et al., 2024). Transcriptomic findings will be validated at the protein level through targeted metabolic imaging using the MIBIscope platform and high-plex spectral cytometry (e.g. SCENITH).

WP3: We will functionally validate the most promising findings from WP1 and WP2 using a human fibroblast culture system with tandem CRISPR metabolic pathway screening, to investigate the impact of perturbing genes individually and/or in combination using high-throughput transcriptomics (Novo). We have access to cells from multiple donors, experience with fibroblast culture and co-culture systems in conjunction with metabolic pathway assaying, and we also have expertise in CRISPR screen design (e.g. via HDR and base editing approaches), implementation and off-target evaluation using primary human cells. Novo has expertise in high-throughput transcriptomics to deeply phenotype cell responses to genetic perturbations and assess their translatability to disease-associated cell behaviours.

Risk Mitigations: In this project will apply multiple different systems techniques to identify and validate metabolic pathways and cross talk driving fibrogenesis. We have access to multiple imaging technologies in addition to MIBIscope e.g. CellDive, Phenocyler Fusion (CODEX) and Hyperion.

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