Project title: Fibrosis Unmasked: A Metabolic Cell Odyssey in Liver Fibroblasts

Supervision: Oxford: Profs. Chris Buckley, Mark Coles, Calliope Dendrou; GSK: Steve Atkinson (no supervisor agreed yet)

Summary: 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 tissue 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 liver, and is a major contributor to increasing global morbidity and mortality (Thannickal et al. 2014). For chronic liver fibrosis, 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 the liver to other organs? Does inflammation contribute to fibrosis? We propose that the reason these questions remain is that the role of metabolic changes in driving pathogenesis liver fibrosis has not been adequately addressed so far and how this relates to fibrosis in other tissues. 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 resulting driven by metabolic products.

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 chronic liver fibrotic disease.

DPhil project Aims: Our overarching goal is to identify the breadth of metabolic pathways that drive aberrant fibroblast activation in human liver disease with an emphasis on advanced liver disease using a computational approach building on human datasets.

Our specific aims of this project are to: 1) Integrate single-cell transcriptomic data from fibroblasts normal and fibrotic liver disease and use this integrated dataset for comparative metabolic pathway and metabolic flux inference analyses. **2)** To assess the spatial relevance of fibroblast metabolic pathways in livers across the disease spectrum through analysing spatial transcriptomic data (Xenium 5K panel) from selected human tissues (from patients and controls) and to validate spatially resolved cellular and extracellular matrix signatures at the protein level using high dimensional Phenocycler Fusion on these samples.

Alignment with therapeutic area and key scientific theme(s): This project will align with the theme of Liver Fibrosis using primary human tissues across a disease spectrum. It will combine single cell RNAseq datasets with spatial transcriptomics using computational metabolomics to predict the mechanisitic basis for fibrosis formation to identify pathways regulating fibrosis progression and regression in response to therapeutic intervention.

Project delivery:

Aim 1: We have access to unique in-house single-cell transcriptomic datasets derived from patients displaying inflammation-associated fibroblast activation and fibrosis (e.g. longitudinal inflammatory bowel disease gut data and arthritis synovial data), as well as access to publicly available cross-tissue/cross-disease datasets. Collectively, we estimate that we have collated single-cell transcriptomics data from >2 million fibroblasts derived from ~20 different human tissues (including gut, **liver**, synovium, lung, heart and vasculature, kidney, and skin, amongst others) and at least 30 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 and use this to explore the liver comparing to fibrosis in other tissues. 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 in the liver and how it differs from other 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).

Aim2: To better delineate how shared or distinct aberrant fibroblast metabolic states arise and are maintained in different liver diseases, we will investigate how these states map to inflammatory, pro-fibrotic niches in the liver. For these niche analyses we will utilise unique in-house and available spatial transcriptomic datasets generated on the 10x Genomics Xenium 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 Phenocycler Fusion platform.

Research environment:

The Buckley – Coles – Dendrou group has world leading experience in fibroblast biology and the development of giga single cell atlases containing the depth of information required to generate a mechanistic understanding of human cell disease leveraging the human cell atlas. The group is based in the Kennedy Institute of Rheumatology with access to BMRC and compute capacity. The group has pipelines to undertake metabolic pathway analysis using single cell data sets and single cell transcriptomics. The fellow will have no expectation of additional clinical commitments.

References:

- Buckley CD & Midwoord KS. Targeting the origins of lung fibrosis. (2024)
- Nature Immunol. 25, 1517-1519; Bull, JA et al. MuSpAn: A Toolbox for Multiscale Spatial Analysis. (2024) bioRxiv 2024.12.06.627195; doi:10.1101/2024.12.06.627195
- Croft A et al. Distinct fibroblast subsets drive inflammation and damage in arthritis. (2019) Nature 570, 246-251
- Curion F et al. Panpipes: a pipeline for multiomic single-cell and spatial transcriptomic data analysis. (2024) Genome Biol 25, 181
- Dakin SG et al. Pathogenic stromal cells as therapeutic targets in joint inflammation. (2018) Nature Rev Rheumatol 14, 714-726
- Davidson S et al. Fibroblasts as immune regulators in infection, inflammation and cancer. (2021) Nature Rev Immunol 21, 704-717
- Drucker DJ & Holst JJ. The expanding incretin universe: from basic biology to clinical translation. (2023) Diabetologia 66,1765-1779
- Faust HJ et al. Adipocyte associated glucocortcoid signaling regulates normal fibroblast function wich is lost in inflammatory arthritis. Nature Commun (2024), 15(1):9859. doi: 10.1038/s41467-024-52586-x.
- Korsunsky I et al. Cross-tissue, single-cell stromal atlas identifies shared pathological fibroblast phenotypes in four chronic inflammatory diseases. (2022) Med 3, 481-518
- Liu C et al. GLP-1R activation attenuates the progression of pulmonary fibrosis via disrupting NLRP3 inflammasome/PFKFB3-driven glycolysis interaction and histone lactylation. (2024) J Transl Med 22, 954
- Pucino V et al. Metabolic checkpoints in rheumatoid arthritis. (2020) Front Physiol 11.
- Pucino V et al. Differential effect of lactate on synovial fibroblast and macrophage effector functions. (2023) Front Immunol 14;
- Thannickal VJ et al. Fibrosis: ultimate and proximate causes. (2014) J Clin Invest 124, 4673-4677
- Zhao M et al. Targeting fibrosis, mechanisms and clinical trials. (2022) Signal Transduct Target Ther 7, 206.