TRAM (Train and Retain Academic Musculoskeletal clinicians)
MB-PhD Project Summary

PhD project Title
Common translational mechanism's in musculoskeletal fibrosis

PhD supervisors (please provide name, affiliation and email) *[At least two supervisors]*	
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Background

Fibrosis is a complex process of aberrant tissue healing leading to loss of physiological tissue structure and function with inflammatory processes playing a critical role in disease chronicity across a spectrum of anatomical sites. Motivated by huge clinical burdens, continuous intense research on drug targeting fibrosis have been conducted, many of which have led to clinical trials with little success.

Importantly Dupuytren's disease (DD), Frozen shoulder (FS) and tendinopathy (TN) represent accessible diseases to interrogate common mechanisms of fibrosis in less accessible organs such as lung, liver and kidney. As with all fibrotic diseases the heterogeneity of human tissue samples and limited availability of non-diseased tissues for accurate interpretation of 'blue sky' biology remains difficult. The use of these soft tissue diseases as model systems for fibrosis is rather unique due to high availability (4-5 patient samples per week) yielding tissue samples across the spectrum of early versus intermediate versus late disease and thus has the potential to provide a unique stratification of the biology across the spectrum of fibrotic disease. This is extremely challenging in other fibrotic diseases in which tissues are more difficult to access and is undoubtedly a factor in the slow pace of development of novel antifibrotic medicines.

Single-cell multi-omics approaches are transforming our understanding of disease pathogenesis across medicine, making it possible to study cell populations in health and disease at unprecedented resolution. Indeed these technologies are vital to develop novel therapeutics developed for one fibrotic disorder may be applicable to a wide range of fibrotic diseases because of the shared pathways across organs that are uncovered by this work. Drug repositioning efforts may also be assisted by these studies. Our previous single cell work on tendon disease has helped identify key immune cell (T cell/macrophage) and fibroblast interactions that may contribute to disease.

Additionally one of the earliest signs of fibrosis is the increased deposition of COL-3, a triple helix protein comprised of 3 identical α1 chains, each one being encoded by the col3a1

gene in mice. This unbalanced overproduction, in detriment of other types of collagens (mainly collagen type I), is observed in different diseases, such as osteoarthritis, tendinopathy, hypertrophic scars and liver cirrhosis]. Little is known, however, about the cells that are expressing more col3a1 and, by doing so, increasing the levels of collagen type III protein. Knocking out the gene responsible for COL-3 is not possible, as studies have shown that absence of col3a1 expression is lethal in homozygous mice . Therefore, to examine col3a1-expressing cells and their potential as a biomarker/therapeutic target, we developed a mouse reporter that simultaneously expresses the col3a1 gene and a far-red fluorescent protein, mKate-2, under the control of the col3a1 promoter.

This project aims to utilise human datasets to investigate key fibrogenic pathways that will then be interrogated in the novel col3a1 reporter mice. We envisage that this system and generation of col3a reporting fibroblast cell lines, will lead to investigation of novel translational pathways in fibrosis.

Aims

1) Analysis of fibrogenic pathways in DD/FS/TN scRNAseq and overlay with available scRNAseq fibrosis databases from liver/renal fibrosis.

2) Validate the *col3a1*mKate-2 reporter mouse as an efficient model to detect col3a1 expression at different timepoints and identify *col3a1*-expressing cells by flow cytometry in a tendon injury fibrotic model.

3) Create a *col3a1*-reporting cell line from fibroblasts that would be used for evaluating treatments *in vitro*, based on the transcriptomics findings.

4) Utilise above datasets to explore the potential for small molecule (JAK/STAT) and anti-cytokine (IL-13R1, IL-17A, IL-11) therapies in fibrosis with both human and mouse *in vitro systems*.

Training and experience provided *[Include types of methodologies that will be employed]*

- Single cell RNA sequencing for transcriptional profiling
- Flow Cytometry:
- Mouse Tendon injury model:
- Cultivating col3a1 expressing tenocytes from mouse model
- In vitro translational studies (including siRNA)

Expected outcomes

Novel pathway discovery in human fibrotic diseases utilising overlay analysis between three distinct visceral and central fibrotic conditions.

Validation of novel col3a1 KO mice and first use of tendon injury


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References

1. Akbar, M., et al., *Attenuation of Dupuytren's fibrosis via targeting of the STAT1 modulated IL-13Rα1 response.* Sci Adv, 2020. **6**(28): p. eaaz8272.
2. Nielsen, M.J. and M.A. Karsdal, *Chapter 3 - Type III Collagen*, in *Biochemistry of Collagens, Laminins and Elastin*, M.A. Karsdal, Editor. 2016. p. 21-30.
3. Wynn, T.A., *Cellular and molecular mechanisms of fibrosis.* J Pathol, 2008. **214**(2): p. 199-210.
4. Baues, M., et al., *Fibrosis imaging: Current concepts and future directions.* Adv Drug Deliv Rev, 2017. **121**: p. 9-26.
5. Millar, N.L., G.A. Murrell, and I.B. McInnes, *Inflammatory mechanisms in tendinopathy - towards translation.* Nat Rev Rheumatol, 2017. **13**(2): p. 110-122.
6. Steenvoorden, M.M., et al., *Transition of healthy to diseased synovial tissue in rheumatoid arthritis is associated with gain of mesenchymal/fibrotic characteristics.* Arthritis Res Ther, 2006. **8**(6): p. R165.
7. Montesi, S.B., et al., *Molecular imaging of fibrosis: recent advances and future directions.* J Clin Invest, 2019. **129**(1): p. 24-33.
8. Poo, J.L., et al., *Benefits of prolonged-release pirfenidone plus standard of care treatment in patients with advanced liver fibrosis: PROMETEO study.* Hepatol Int, 2020.
9. Distler, O., et al., *Nintedanib for Systemic Sclerosis-Associated Interstitial Lung Disease.* N Engl J Med, 2019. **380**(26): p. 2518-2528.
10. Kuivaniemi, H. and G. Tromp, *Type III collagen (COL3A1): Gene and protein structure, tissue distribution, and associated diseases.* Gene, 2019. **707**: p. 151-171.
11. Millar, N.L., et al., *MicroRNA29a regulates IL-33-mediated tissue remodelling in tendon disease.* Nat Commun, 2015. **6**: p. 6774.
12. Liu, X., et al., *Type III collagen is crucial for collagen I fibrillogenesis and for normal cardiovascular development.* Proc Natl Acad Sci U S A, 1997. **94**(5): p. 1852-6.
13. Radhakrishnan, R., S.A. Moore, and K.A. Sluka, *Unilateral carrageenan injection into muscle or joint induces chronic bilateral hyperalgesia in rats.* Pain, 2003. **104**(3): p. 567-77.
14. Nickdel, M.B., et al., *Dissecting the contribution of innate and antigen-specific pathways to the breach of self-tolerance observed in a murine model of arthritis.* Ann Rheum Dis, 2009. **68**(6): p. 1059-66.
15. Maffia, P., et al., *Inducing experimental arthritis and breaking self-tolerance to joint-specific antigens with trackable, ovalbumin-specific T cells.* J Immunol, 2004. **173**(1): p. 151-6.
16. Benson, R.A., et al., *Cellular imaging in rheumatic diseases.* Nat Rev Rheumatol, 2015. **11**(6): p. 357-67.
17. Scales, H.E., et al., *Assessment of murine collagen-induced arthritis by longitudinal non-invasive duplexed molecular optical imaging.* Rheumatology (Oxford), 2016. **55**(3): p. 564-72.