TRAM MB-PhD Project Summary

(The completed form should not exceed 2 pages)

PhD project Title
Investigating the transcription factor FoxO1 in synovial fibroblasts

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Background

The synovium is a specialised tissue that encapsulates synovial joints and forms a boundary between the fluid-filled synovial cavity and surrounding tissues. Healthy synovium consists of two layers, the synovial lining, which is adjacent to the joint space, and the sublining (1). The synovial lining consists of a unique and specialised fibroblast known as a fibroblast-like synoviocyte (FLS). FLS are critical for the maintenance of joint homeostasis through secretion of lubricating factors such as lubricin and hyaluronic acid (2). However, following joint injury or during arthritis, FLS expand and become functionally deranged to sustain synovial hyperplasia and perpetuate synovial joint pathology (3). While studies have looked at changes to FLS in late-stage disease, very little is known about FLS differentiation and maintenance in the healthy synovium.

Using single-cell RNA sequencing (scRNA-seq), in conjunction with a triple-transgenic mouse model that allows us to determine fibroblast ontogenetic origin, we revealed that FLS express high levels of the transcription factor FoxO1 and its downstream target genes, which is not observed in other synovial fibroblast subpopulations (4). In addition, *in silico* knockout of FoxO1 was predicted to block FLS differentiation while *in vitro* overexpression of FoxO1 in primary synovial fibroblasts induced expression of mature FLS genes. These data suggest that FoxO1 plays an important role in modulating FLS differentiation and / or phenotype maintenance, though its exact mechanisms remain unknown. Furthermore, levels of FoxO1 expression in rheumatoid arthritis (RA) synovial tissue are negatively correlated with serum C-reactive protein, erythrocyte sedimentation rate and 28-joint disease activity score (DAS28) disease markers (5), while mouse models of FoxO1 loss in embryonic type II collagen-expressing cells or adult joint aggrecan-expressing cells resulted in development of osteoarthritis (OA)-like pathologies and synovial inflammation (6). These findings suggest that FoxO1 has an important protective role in the synovial joint.

This project will investigate the molecular regulation of synovial fibroblast FoxO1 expression and activity via interrogation of inter- and intra-cellular signalling pathways, with the potential to identify novel therapeutic targets for the treatment of arthritis.

Aims

Aim 1: Ligands, involved in FLS differentiation and identified through bioinformatic analysis of synovial scRNA-seq datasets, derived from healthy and perturbed joints, will be tested using a synovial fibroblast FoxO1-luciferase cell line to determine whether they induce FoxO1 expression.

Aim 2: Primary synovial fibroblasts will be treated with ligands identified in Aim1, known to induce FoxO1 expression, and bulk transcriptomic datasets generated. Analysis of these datasets will reveal whether the ligands, following the induction of FoxO1 expression, induce expression of FLS-specific markers.

Aim 3: Existing inhibitors targeting FoxO1 will be used in conjunction with ligands, identified in Aim 1, to treat primary synovial fibroblasts. In parallel, CRISPR/Cas9 or CRISPRa systems will be used to knockout / overexpress genes identified to be part of the FoxO1 gene regulatory network and bulk transcriptomic datasets generated. These experiments will determine whether FoxO1 is critical for the translation of the ligand signal to FLS-specific gene expression.

Training and experience provided

This research project will take place in the Rheumatology Research group as part of the Collins and De Bari laboratories and provide the Ph.D student with a deep understanding of the synovial joint and associated pathologies. Throughout the studentship they will be trained in *in vitro* cell culture and genetic manipulation, bioinformatic analyses and statistical analysis. In addition, students will learn how to successfully plan and perform experiments, write scientifically, critically interpret data and present it to their peers through attending and contributing to group lab meetings and journal clubs.

Expected outcomes

Identification of ligands involved in FLS differentiation will provide important insight into the intercellular pathways that govern FLS maintenance in the healthy and perturbed joint. Additionally, identifying the molecular regulation and downstream targets of synovial fibroblast FoxO1 activity will reveal potential novel therapeutic targets for the treatment of joint pathologies. Furthermore, the student will be encouraged to present their work at international and national scientific conferences as well as submit it to peer-reviewed high-impact journals.

References

1. Smith MD. The Normal Synovium. Vol. 5, The Open Rheumatology Journal. 2011.
2. Palmer DG, Selvendran Y, Allen C, Revell PA. Features of synovial membrane identified with monoclonal antibodies. Vol. 59, Clin. exp. Immunol. 1985.
3. Kurth TB, Dell'Accio F, Crouch V, Augello A, Sharpe PT, De Bari C. Functional mesenchymal stem cell niches in adult mouse knee joint synovium in vivo. Arthritis Rheum. 2011;63(5):1289–300.
4. Collins FL, Roelofs AJ, Symons RA, Kania K, Campbell E, Collie-Duguid ESR, et al. Taxonomy of fibroblasts and progenitors in the synovial joint at single-cell resolution. Ann Rheum Dis [Internet]. 2023 Nov 22;82(3):428–37. Available from: http://www.ncbi.nlm.nih.gov/pubmed/36414376
5. Grabiec AM, Angiolilli C, Hartkamp LM, Van Baarsen LGM, Tak PP, Reedquist KA. JNK-dependent downregulation of FoxO1 is required to promote the survival of fibroblast-like synoviocytes in rheumatoid arthritis. Ann Rheum Dis. 2015 Sep 1;74(9):1763–71.
6. Matsuzaki T, Alvarez-Garcia O, Mokuda S, Nagira K, Olmer M, Gamini R, et al. FoxO transcription factors modulate autophagy and proteoglycan 4 in cartilage homeostasis and osteoarthritis [Internet]. 2018. Available from: https://www.science.org