TRAM MB-PhD Project Summary

(The completed form should not exceed 2 pages)

PhD project Title
Molecular regulation of stem and progenitor cell fate in the repair of skeletal joints.

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Background

Arthritis affects around 10 million people in the UK, with osteoarthritis (OA) being the most common form. Traumatic joint injury increases the risk of developing OA, which is characterised by the breakdown of joint tissues and aberrant repair processes, leading to pain and disability.

The synovium, a thin membrane surrounding the joint, contains stem and progenitor cells throughout life [1,2]. Using cell fate mapping in mice, we have shown that progeny of the *Gdf5*-expressing cells in the embryo that form the synovial joints (e.g., the knee) persist as stem and progenitor cells in the adult synovium and can repair damaged joints [1]. Notably, these cells also remodel joint tissues in OA, leading to the formation of osteophytes, bony outgrowths covered by cartilage at joint edges [3]. Our work has uncovered a progenitor cell subset within the *Gdf5*-lineage cell population that resides in the synovial lining. Following acute cartilage injury, these progenitors proliferate and differentiate into fibroblast-like synoviocytes (FLS), a specialised cell type unique to the synovium [4]. In a post-traumatic model of OA, they differentiate into chondrocytes that cap the cartilage of osteophytes [3]. This indicates bipotency of synovial lining progenitor cells towards FLS and chondrocytes.

Trajectory and gene regulatory network analyses based on single-cell transcriptomic data from mouse models of acute joint surface injury and ACL rupture leading to post-traumatic OA have identified candidate transcription factors (TFs) that regulate synovial cell differentiation [4,5]. Interestingly, several TFs associated with the FLS differentiation trajectory, such as Sox5 and Creb5, are also important TFs in articular cartilage [6,7], suggesting shared molecular mechanisms in the differentiation and maintenance of FLS and articular chondrocytes. This points to context-dependent roles for these TFs in regulating synovial stem and progenitor cell differentiation.

Aims

The overall aim of this project is to investigate how TFs control the fate of synovial stem and progenitor cells, driving their differentiation into FLS or chondrocytes. The specific aims are:

Aim 1: Identify key TFs regulating synovial cell differentiation. Single-cell transcriptomic analyses have revealed candidate TFs and putative downstream target genes that regulate synovial cell differentiation [4,5]. In this project, we will select TFs associated with FLS and chondrocyte differentiation for further investigation by analysing chromatin accessibility using single-cell ATAC-

seq data of purified *Gdf5*-lineage cells from injured mouse knees (data already available in our laboratory). We will identify accessible TF binding motifs enriched in FLS, chondrocytes, and their differentiation trajectories, and link these motifs to regulatory regions of key target genes. Literature screening and analysis of publicly available human datasets will be further used to prioritise TFs for experimental investigation.

Aim 2: Test TF function using organoid culture models. Three-dimensional *in vitro* culture models established in our lab will be used for functional investigation. These include a synovial organoid model in Matrigel for studying FLS differentiation, and a high-density pellet culture model for chondrogenesis [1]. Gene knockout/silencing or overexpression/activation of the TFs selected in Aim 1 will be employed in these models to assess their role in cell differentiation. Markers of differentiation will be analysed using fluorescent reporters, qRT-PCR or flow cytometry for screening, followed by single-cell RNA-seq for detailed phenotyping.

Aim 3: Investigate TF binding to regulatory regions. We will investigate the binding of TFs to regulatory regions of target genes to understand how they exert their context-dependent functions. Regions identified by scATAC-seq will be validated experimentally using advanced assays such as Cleavage Under Targets and Tagmentation (CUT&Tag). Functional relevance will be determined by inactivating or activating target regions using CRISPRi/CRISPRa in the organoid models described in Aim 2.

Training and experience provided

This PhD studentship will be based in the Rheumatology Research Group and will provide extensive training and experience in synovial joint biology and pathology, and cutting-edge molecular techniques. It will offer hands-on experience in advanced bioinformatics tools for analysing single-cell transcriptomic and chromatin accessibility data. Additionally, training will be provided in laboratory skills, including organoid culture systems, CRISPR-based genetic manipulation, qRT-PCR, and flow cytometry. The project also benefits from world-leading expertise in bioinformatics and molecular medicine at the Institute and will equip the candidate with highly relevant skills for both academia and industry.

Expected outcomes

This project will enhance our understanding of the transcriptional mechanisms controlling cell differentiation into FLS or chondrocytes. By identifying key TFs and regulatory regions involved in these processes, we aim to uncover molecular regulators that could be targeted for therapeutic intervention in joint repair and OA. Additionally, this project will enable development of *in vitro* models for drug screening, including pro-regenerative therapeutics.

References

[1] Roelofs AJ, Zupan J, Riemen AHK, Kania K, Ansboro S, White N, Clark SM, De Bari C. Joint morphogenetic cells in the adult mammalian synovium. Nature Communications 2017, 8:15040-15053.
[2] De Bari C , Dell'Accio F, Tylzanowski P, Luyten FP. Multipotent mesenchymal stem cells from adult human synovial membrane. Arthritis and Rheumatology 2001, 44(8):1928-42.
[3] Roelofs AJ, Kania K, Rafipay AJ, Sambale M, Kuwahara ST, Collins FL, Smeeton J, Serowoky MA, Rowley L, Wang H, Gronewold R, Kapeni C, Méndez-Ferrer S, Little CB, Bateman JF, Pap T, Mariani

FV, Sherwood J, Crump JG, De Bari C. Identification of the skeletal progenitor cells forming osteophytes in osteoarthritis. Annals of the Rheumatic Diseases 2020, 79(12):1625-1634.
[4] Collins FL, Roelofs AJ, Symons RA, Kania K, Campbell E, Collie-Duguid ESR, Riemen AHK, Clark SM, De Bari C. Taxonomy of fibroblasts and progenitors in the synovial joint at single-cell resolution. Annals of the Rheumatic Diseases 2023, 82(3):428-437.
[5] Knights AJ, Farrell EC, Ellis OM, Lammlin L, Junginger LM, Rzeczycki PM, Bergman RF, Pervez R, Cruz M, Knight E, Farmer D, Samani AA, Wu CL, Hankenson KD, Maerz T. Synovial fibroblasts assume distinct functional identities and secrete R-spondin 2 in osteoarthritis. Annals of the Rheumatic Diseases 2023, 82(2):272-282.
[6] Lefebvre V. Roles and regulation of SOX transcription factors in skeletogenesis. Curr Top Dev Biol. 2019;133:171-193.
[7] Zhang CH, Gao Y, Hung HH, Zhuo Z, Grodzinsky AJ, Lassar AB. Creb5 coordinates synovial joint formation with the genesis of articular cartilage. Nature Communications 2022, 13(1):7295.