Transcriptomic response to osteoarthritis using single-cell RNA sequencing in an equine model

Dylan T. Ammons[1], Lyndah Chow[1], Blaine Larson[1], Luke Bass[1], Laurie Goodrich[1], and Steven Dow[1,2] and Lynn M. Pezzanite[1]
[1]Department of Clinical Sciences, [2]Department of Microbiology, Immunology, Pathology, Colorado State University, Fort Collins, CO
Email of Presenting Author: lynn.pezzanite@colostate.edu

Disclosures: Lynn M. Pezzanite (EqCell), Steven Dow (EqCell, Validus Cellular Therapeutics).

INTRODUCTION: Understanding of the pathogenesis is still evolving, but osteoarthritis (OA) is increasingly thought to be a multifactorial disease. Although many cell types have been identified as contributing to disease progression, there is compelling evidence which suggests the innate immune system, particularly myeloid cells, play an important role in regulating and perpetuating low-grade inflammation. The chronic inflammation results in continued articular cartilage breakdown for several years following initial joint trauma. Synovial macrophages are the most common myeloid cell type and one of the most metabolically active cells in the joint; however, their phenotype and functional properties have not been clearly defined in OA joints. This understudied but vital aspect of the interaction between macrophages and joint tissues has major implications towards understanding OA pathogenesis. The objective of this study was to use single-cell RNA sequencing (scRNAseq) to describe the transcriptomic responses of synovial cells of OA and healthy joints. The equine preclinical model of joint disease was used due to similarity in joint volume, cartilage thickness and articular cartilage loading forces to humans. We hypothesized that scRNA-seq would enable identification and characterization of synovial cell types in OA that were previously unrecognized.

METHODS: ScRNAseq was completed on cells isolated from the synovial fluid (SF) of three healthy and three OA joints. Horses (n=3 geldings, aged 3-20 years) used in the study presented with carpal OA based on physical examination, musculoskeletal examination, lameness evaluation, diagnostic imaging. Healthy tibiotarsal joints of horses were used for healthy synovial fluid collection. Cells (5,000/sample) were targeted for analysis with sequencing depth of 50,000 reads/cell. Raw sequencing data was demultiplexed, adaptors trimmed, and aligned to equine genome (EquCab3.0) using Cell Ranger (10x Genomics). Count matrices were imported into Seurat to complete spatial reconstruction and downstream analysis of single cell gene expression data.

RESULTS: Computational analysis revealed the presence of 8 major cell populations (**Figure 1**). Major cell identities were classified using algorithmic and manual approaches, with canonical features used to inform manual classification. Cell abundance analysis was then completed to determine how healthy and OA samples contributed to the composition of each major cell type. Macrophage clusters were noted to come largely from OA samples, while T cells primarily were obtained from normal SF samples. These data indicate a shift in the immune composition of SF following onset of OA, suggesting synovial macrophages may migrate into joint fluid in the diseased state. Following classification of major cell types, data were subset to focus on macrophage populations then unsupervised clustering analysis was repeated which revealed 12 transcriptionally distinct macrophage clusters (**Figure 2**). To investigate how the overall macrophage transcriptome was altered in OA, differential gene expression analysis was performed which revealed 47 upregulated genes and 66 downregulated genes in macrophages obtained from OA SF. Notable expression changes included the upregulation of APOE/FABP5 and the downregulation of MARCO in macrophages isolated from OA synovial fluid. To determine which clusters were driving the differential expression, we visualized select features using a split UMAP plot. The visualization revealed that the reduction in MARCO expression in SF macrophages was largely confined to macrophages. Overall, the preliminary findings reported here suggest macrophage play a role in responses to OA within equine synovial fluid.

DISCUSSION: To develop effective therapeutic strategies for early pharmacologic and surgical interventions, it is critical to understand the changes in joint tissues at the cellular and molecular level in the acute post-traumatic phase and asymptomatic period of the chronic phase of post-traumatic osteoarthritis. These studies begin to address a critical gap in our understanding of the pathogenesis of the common and debilitating condition of OA, with multiple genes identified as potential therapeutic targets for future studies. Study limitations include small sample size and heterogenous population enrolled that were diagnosed with degenerative joint disease.

SIGNIFICANCE/CLINICAL RELEVANCE: The preliminary studies described provided the computational and theoretical foundation to pursue further studies using scRNA-seq to further our understanding of changes within the joint in OA which may lead to improved therapies.

IMAGES AND TABLES:

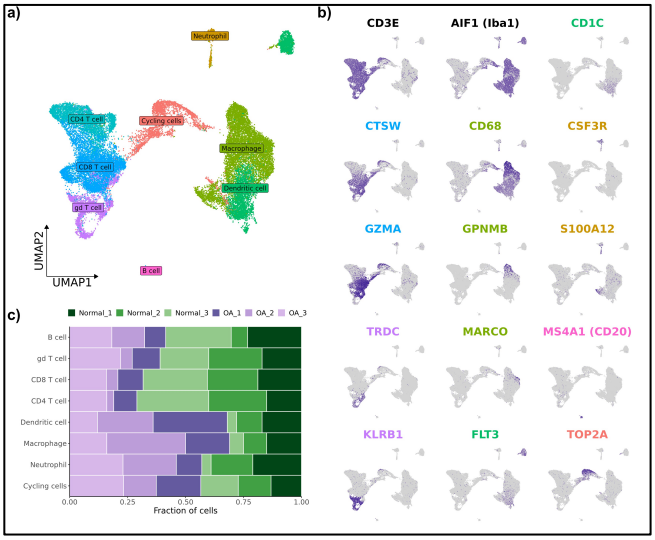

Figure 1: Single cell transcriptomics reveals 8 major cell populations in equine synovial fluid. A) UMAP plot depicting the results of unsupervised clustering colorized by the assigned cell identity. B) Depiction of key gene expression used in cell classification. C) Stacked bar graph depicting the composition of each cluster. Purple bars indicate cells came from OA synovial fluid while green bars indicate cells came from normal synovial fluid.

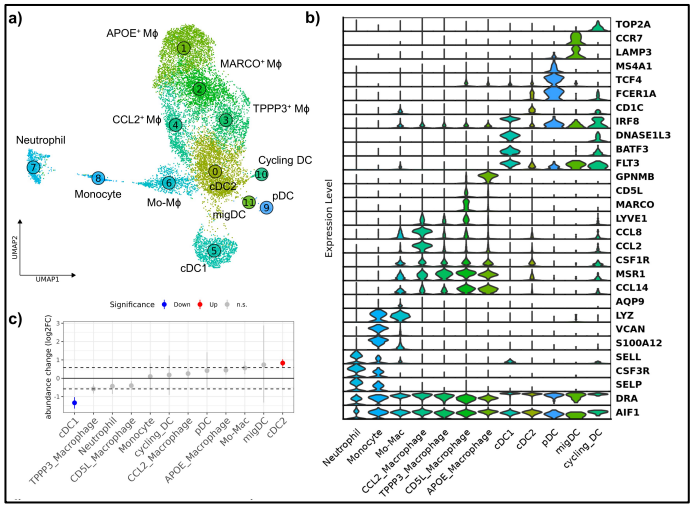

Figure 2: Unsupervised clustering reveals 12 distinct macrophage clusters in equine synovial fluid. A) UMAP plot depicting results of unsupervised clustering colorizing unique cell clusters identified. B) Violin plots depicting the expression of features that define the macrophage subpopulations. C) Differential analysis of macrophage subpopulations in OA relative to normal synovial fluid (y-axis indicating the log-fold change, significance determined using Monte Carlo permutation test with threshold set to 0.05).