

# Single-cell RNA Sequencing Analysis Of Human Synovial And Adipose Mesenchymal Stem Cells For Knee Osteoarthritis Treatment

Yuki Hidaka, Kentaro Endo, Ichiro Sekiya

Center for Stem Cell and Regenerative Medicine, Tokyo Medical and Dental University

**Disclosures:** Y. Hidaka: None. K. Endo: None. I. Sekiya: None.

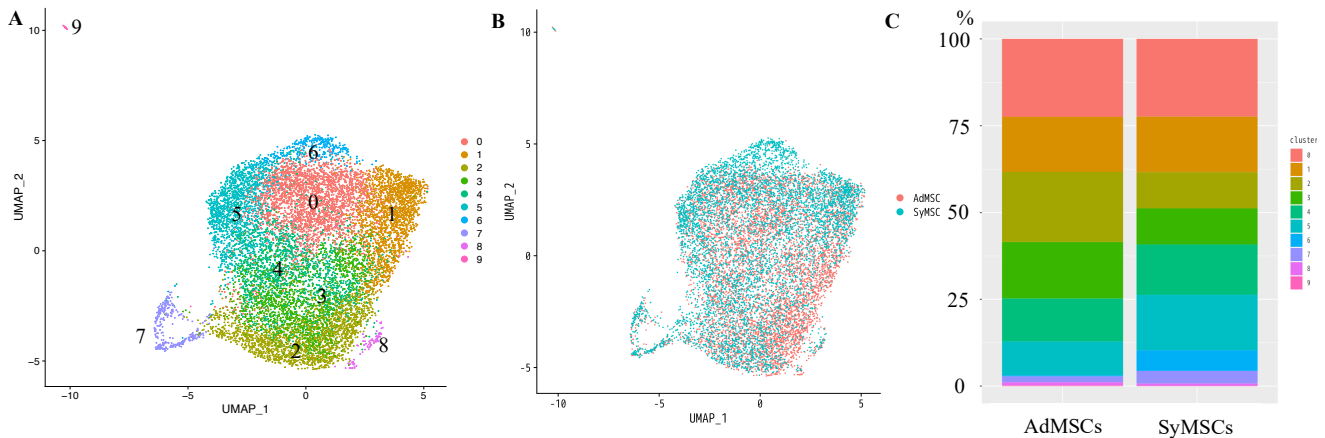
**INTRODUCTION:** Mesenchymal stem cells (MSCs) offer a promising treatment for knee osteoarthritis (OA). These cells can be isolated from various tissues and their potentials differ from their origins. We have previously reported that the intra-articular injections of synovial MSCs (SyMSCs), which have a higher chondrogenic capacity than MSCs derived from other tissues, increased the amount of cartilage in patients with knee OA. However, adipose MSCs (AdMSCs) have been commonly used for treating OA. Although recent studies have suggested that the heterogeneity of MSCs can influence therapeutic outcomes, the differences between SyMSC and AdMSC subpopulations are not fully understood. In this study, we performed single cell RNA-sequencing (scRNA-seq) of human SyMSCs and AdMSCs to compare the composition and function of their respective subpopulations.

**METHODS:** The study was approved by the ethics committee of the Tokyo Medical and Dental University, and informed consent was obtained from the patients. Synovial tissue and subcutaneous fat were harvested from 3 patients with OA who underwent total knee arthroplasty. The tissues were digested with Liberase and the isolated cells were cultured at 500 cells/cm<sup>2</sup> in  $\alpha$ -MEM supplemented with 10% fetal bovine serum (FBS). After 2 weeks of culture, the cells from 3 patients were pooled and subjected to single-cell RNA sequencing analysis. The scRNA-seq was processed by the Cell Ranger 6.1.2 pipeline with default parameters. Further analysis was performed using R with Seurat package. Gene enrichment analysis was performed using the Gene Ontology Resource database (<http://geneontology.org>). For exploring surface markers, we used the in silico human surfaceome database (Bausch-Fluck et al. PNAS. 2018).

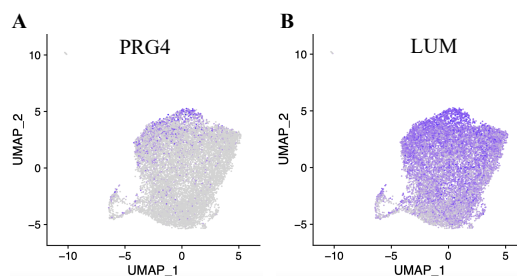
**RESULTS:** Clustering analysis identified 10 distinct subsets in SyMSCs and AdMSCs (Fig. 1A, B). The cluster 6 was specific to SyMSCs (Figure 1C). The proportion of cluster 2 and 3 were abundant in AdMSCs compared with SyMSCs. The top 2 upregulated genes of differentially expressed genes (DEGs) for cluster 6 were PRG4 and LUM, known as the proteoglycan family (Fig. 2A, B). Gene enrichment analysis of DEGs for cluster 6 identified extracellular matrix and growth factor-related terms (Table 1). Surfaceome analysis demonstrated that the cluster 6 had higher expression of SLC2A12, SLCA5, STEAP4, and lower expression of THY1 than other clusters.

**DISCUSSION:** This study found the difference in subpopulation of human SyMSCs and AdMSCs. Notably, SyMSCs had a specific subpopulation (cluster 6) which highly expressed PRG4, highlighting that the cluster 6 potentially characterized the unique functionalities of SyMSCs. PRG4 codes lubricin which is a glycoprotein to reduce a friction force in the knee joint. As lubricin has been shown to inhibit inflammation and OA progression, the cluster 6 may possess greater ability to protect joints and reduce pains. This study identified specific surface marker candidates for the cluster 6, therefore, our next step is to sort this cluster and evaluate its biological functions. One of the limitations in this study was that we used the cells pooled from three patients for scRNA-seq. Samples should be prepared for each patient to clarify individual differences in subpopulations and their proportion.

**SIGNIFICANCE/CLINICAL RELEVANCE:** Analyzing the differences in subpopulation of SyMSCs and AdMSCs will contribute to a better understanding of the differences in biological function and OA therapeutic effect between them.



**Fig. 1** UMAP display of scRNA-seq data and proportion of each cluster.



**Fig. 2** Feature plot of PRG4 and LUM.

GO molecular function complete	fold Enrichment	FDR
syndecan binding	> 100	2.63E-03
platelet-derived growth factor binding	79.31	1.16E-05
extracellular matrix structural constituent conferring compression resistance	31.72	3.13E-03
BMP binding	27.55	4.19E-02
extracellular matrix structural constituent conferring tensile strength	27.14	9.92E-06
collagen binding	26.04	2.47E-08
fibronectin binding	23.26	7.92E-03
extracellular matrix binding	19.39	4.24E-04
antioxidant activity	12.32	3.26E-03
heparin binding	11.83	7.29E-07

**Table 1.** Top 10 enriched terms for the cluster 6.