Synovium and joint fat pad share common mesenchymal progenitors and undergo coordinated changes in osteoarthritis

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INTRODUCTION: Osteoarthritis (OA) affects all tissues in the knee joint. Previous OA research has focused extensively on articular cartilage and subchondral bone, but often overlooked surrounding soft tissues, such as synovium and intra-articular adipose tissues (IAATs) that are attached to each other. During OA, both synovium and IAATs undergo pathological fibrosis, thickening, stiffening, and inflammation. However, whether these two tissues share the same progenitor cells and hence function as a single unit in joint homeostasis and diseases is largely unknown. This study aims to investigate the cellular heterogeneity of this compartment and analyze its responses to OA.

METHODS: *Animals*- All animal work was approved by the IACUC at the University of Pennsylvania. *Dpp4-CreER Rosa-tdTomato* (*Dpp4ER/Td*), and *Prg4-CreER Rosa-tdTomato* (*Prg4ER/Td*) mice were generated. For lineage tracing, mice at 2 months of age received tamoxifen (Tam) injections (75 mg/kg/day) for 5 days followed by destabilization of medial meniscus (DMM) at their right knees. *Single cell RNA-sequencing (scRNA-seq)*- Synovium and infrapatellar fat pad (syn/IFP) from 3 groups of male *WT C57BL/6* mice (control/uninjured, n=20; 1 week post DMM, n=8; 2 months post DMM, n=12) were harvested, enzymatically digested, and sorted for live cells to be loaded into Chromium controller for scRNA-seq. *Histology*- Knee joints were processed either for paraffin sections followed by H&E staining or for frozen sections followed by Prg4, αSMA, and Perilipin immunofluorescent staining. *Cell culture*- Syn/IFP cells were cultured in 10% DMEM for CFU-F assay. For differentiation assays, sorted Td+ and Td- cells were cultured in either osteogenic medium (10% DMEM, 10 nM dexamethasone, 10 mM β-glycerophosphate, and 50 μg/mL ascorbic acid) for 2 weeks followed by Alizarin red staining or adipogenic medium (10% DMEM, 0.5 mM isobutylmethylxanthine, 10 mM indomethacin, 1 μM dexamethasone, and 10 μg/mL insulin) for 1 week followed by Oil Red O staining. *Statistics*- Data are expressed as means±SD and analyzed by one way ANOVA and unpaired, two-tailed Student's t-test.

RESULTS: The extrasynovial space of uninjured mouse knee joints were filled with IAATs, including the anterior IFP and posterior fat pad (PFP), which were continuously and seamlessly attached to the synovium membrane (Fig. 1). After DMM, both IFP and PFP were quickly replaced by densely packed fibroblastic cells. The fat pads re-appeared 1 month later and expanded, but were still smaller than control fat pads 3 months later. Single-cell transcriptomic profiling of synovium and IFPfrom control and DMM mice (1 week and 2 months post-surgery) revealed five mesenchymal clusters (total 17,097 cells, Fig. 2A). Our analysis identified presumptive multipotent mesenchymal progenitor cells (MPCs, marked by *Cd34*, *Pi16*, and *Dpp4*) for synovial lining fibroblasts (SLFs, marked by *Prg4* and *Has1*), myofibroblasts (MFs, marked by *Acta2*, *Tagln*, *Myl9*), preadipocytes 1 (preAD1, marked by *Icam1*) and preadipocytes 2 (preAD2, marked by *F3*, Fig. 2B). To validate these computational predictions, we examined knee joints of *Dpp4ER/Td* and *Prg4ER/Td* mice that label MPCs and SLFs, respectively, after Tam injections. As shown in Fig. 3A (top panel), Dpp4+ MPCs resided in the synovial sublining layer of control mice and remained there as Prg4- cells after 3 months of tracing (Fig. 3B). DMM induced the rapid expansion of Td+ cells (Fig. 3A bottom panel) and their differentiation into αSMA+ MFs (Fig. 3C). Three months later, we detected many Prg4+;Td+ SLFs and Perilipin+;Td+ adipocytes (Fig. 3A, B). While Td+ cells only represented a small percentage of Cd45- cells (1.7%) in Syn/IFP from control knees, they possessed mesenchymal progenitor properties, including high CFU-F activity (Fig. 4A) and the capacity for osteogenic and adipogenic differentiation (Fig. 4B). In *Prg4ER/Td* mouse joints, Td+ cells stayed in the synovium and did not become adipocytes (Fig. 5A). Similar to Dpp4+ MPCs, Prg4+ SLFs rapidly expanded and gave rise to αSMA+ MFs shortly after DMM (Fig. 5B). Sorted Td+ cells from Syn/IFP had much less CFU-F forming ability compared to Td- cells (Fig. 5C).

DISCUSSION: In this work, we examined the cellular heterogeneity of the integrated IAATs and synovium at a single cell level. Using single cell transcriptomic analysis, lineage tracing, a mouse OA model, and cell culture experiments, we demonstrate that Dpp4+ MPCs residing in synovial sublining layer serve as a multipotent mesenchymal progenitor population for Prg4+ SLFs and Perilipin+ adipocytes during joint development (data not shown) and OA progression. After DMM injury, both MPCs and SLFs expand quickly to become MFs, which contributes to hyperplastic synovial thickening at late OA stage.

SIGNIFICANCE: Our novel finding advances the knowledge of previously understudied joint tissues and provides new directions for drug discovery to treat joint disorders.

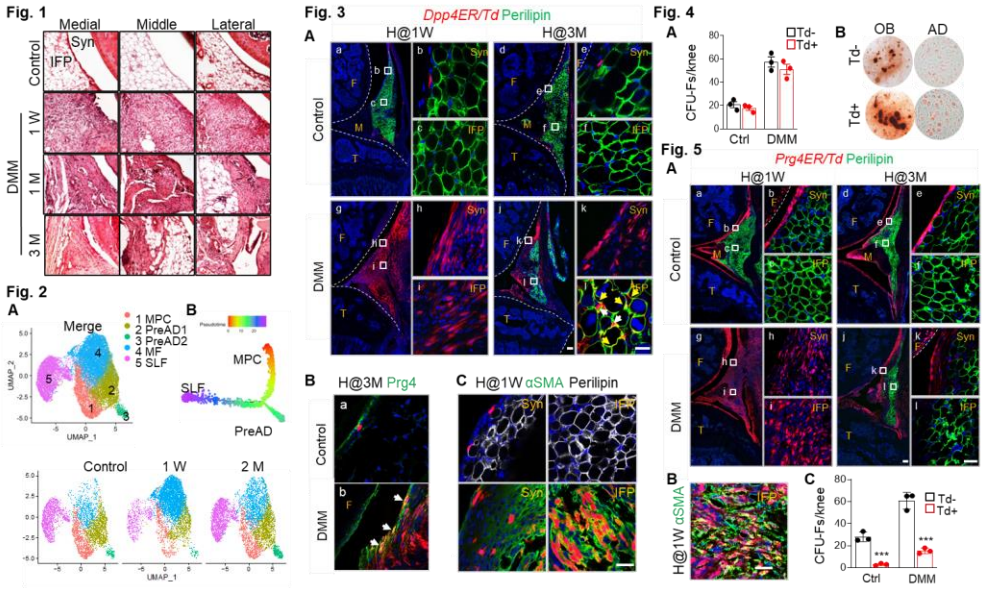

Fig. 1. Synovium and IAATs are an integrated joint tissue. H&E staining of medial, middle, lateral sections from control and DMM knees at 1 week, 1 month, or 3 months post-surgery to show Syn/IFP. **Fig. 2. Subclustering fibroblasts predicts a common progenitor for Syn/IFP.** (A) UMAP plots of fibroblasts in control, DMM 1-week and 2-month datasets are shown as merge and split views. (B) Monocle pseudotime trajectory. **Fig. 3. Dpp4+ MPCs are progenitors for Syn/IFP in OA**. (A) Fluorescent images of *Dpp4ER/Td* knee joints with Perilipin staining. Mice received Tam injections for 5 days at 2 weeks before DMM and euthanized at 1 week and 3 months post surgery. White boxes on the left are magnified at the right. Arrows point to Td+ Perilipin- (white) and Td+Perilipin+ (yellow) cells. (B) Prg4 staining shows that Td+ cells overlap with Prg4+ SLFs in 3-month DMM knees (arrows). (C) αSMA staining shows Td+ cells overlap with αSMA+ MFs in 1-week DMM knees. **Fig. 4. Dpp4+ MPCs possess mesenchymal progenitor properties in vitro.** (A) CFU-F assay of Td- and Td+ cells from Syn/IFP of control and 1-week DMM *Dpp4ER/Td* mice. (B) Alizarin red and Oil Red O staining of sorted Td- and Td+ cells that undergo osteogenic (OB) and adipogenic (AD) differentiation, respectively. **Fig. 5. Prg4+ SLFs are not progenitors for Syn/IFP**. Fluorescent images of *Prg4ER/Td* knee joints with Perilipin staining. Mice received daily Tam injections for 5 days at 2 weeks before DMM surgery and euthanized at 1 week and 3 months post surgery. White boxes on the left are magnified at the right. (B) αSMA staining shows Td+ cells overlap with αSMA+ MFs in 1-week DMM knees. (C) CFU-F assay of Td- and Td+ cells from Syn/IFP of control and 1-week DMM mice. ***: p<0.001.