Unraveling the Synovial Contribution to Ankle Osteoarthritis and Pain

Shotaro Kamijo[1,2], Ruiling Xu[1,3], MaCalus V. Hogan[1,4], Hang Lin1[1,4]
[1]University of Pittsburgh, Pittsburgh, PA, [2]Showa Medical University, Japan,
[3]Central South University, China, [4]Orland Bethel Family Musculoskeletal Research Center, Pittsburgh, PA.
KAMIJOS@pitt.edu

Disclosures: Shotaro Kamijo (N), Ruiling Xu (N), MaCalus V. Hogan (N), Hang Lin (N)

INTRODUCTION: Post-traumatic osteoarthritis (PTOA) of the ankle is a joint disorder that leads to chronic pain and significantly diminishes the quality of life, similar to the impact of knee and hip osteoarthritis. The ankle joint is the most commonly injured joint during sports activities, with approximately 78% of ankle osteoarthritis cases linked to trauma, such as ankle sprains (Valderrabano et al., Clin Orthop Relat Res, 2009). As a result, ankle osteoarthritis is a significant concern in the field of sports medicine. In knee osteoarthritis, pathological changes in synovial tissues have been shown to precede cartilage damage, correlate with pain, and contribute to the progression of the disease. However, the changes in synovial tissues associated with ankle osteoarthritis and their effects on other tissues, such as cartilage, are not well understood. In previous research, we developed a rat model of ankle osteoarthritis by resecting the anterior talofibular ligament (ATFL) and the calcaneofibular ligament (CFL) (ORS 2024 Annual Meeting). In this study, we evaluated the synovial changes in this animal model and investigated the underlying molecular and cellular mechanisms contributing to cartilage degradation and the generation of pain.

METHODS: *(1) Surgical resection of the anterior talofibular ligament (ATFL) and calcaneofibular ligament (CFL).* With the IACUC approval, adult male Wistar rats were used. All animals were assigned randomly to one of two groups: the CFL and ATFL resection group (Resection) and the Control group (Cont). All rats had surgery on the left leg. CFL and ATFL on the left side were dissected in the surgery group, and skin and capsule were cut in the Cont group. *(2) Pain-related behavior assessment.* Ankle mechanical-hyperalgesia (AMH) and Static weight-bearing + String pulling (SWBSP) were performed at 10 weeks after surgery. AMH was performed by pressing on the lateral side of the ankles with a Pressure Application Measurement (PAM) device (Ugo Basile). The force was applied until pain-related behaviors such as leg movement, muscle twitching, or vocalization were observed. Each rat underwent four tests per session, with the middle two results averaged. SWBSP was performed with a sensor device (BIOSEB Static Weight Bearing Touch device). The results were calculated by subtracting the left legs from the right legs: Right – Left, and taking the average of the three. *(3) Histopathology.* After fixation in 10% neutral buffered formalin and decalcification, HE staining was performed to evaluate synovitis on the front side of the ankle joint (Krenn, et al., Histopathology 2006). Three images per joint were taken and averaged for the joint. *(4) bulk RNA sequencing (RNASeq) with DRG.* The left side of L5 Dorsal Root Ganglion (DRG)was collected at 10 weeks. The total RNA was analyzed for RNA sequencing by the University of Pittsburgh Health Sciences Sequencing Core. *(5) Single-cell RNA sequencing.* Ankle joint synovium was collected from the front side of the joint and put together in each group. Synovium was digested by DNase, Liberase, and Collagenase IV. Cells from 3 rats were pooled to get a sufficient cell number for sequencing. *(6) Statistical analysis.* Pain-related behavior assessments, Synovitis score, and relative gene expression in qRT-PCR and bulk RNA sequence were analyzed using a two-tailed Student's t-test, with significance set at 5%.

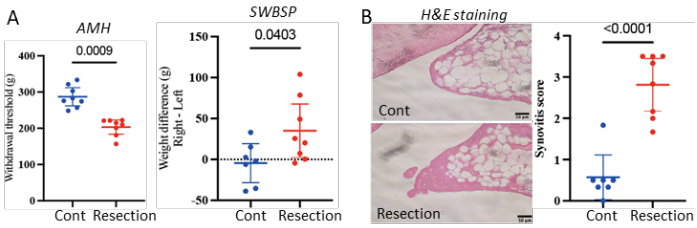

Fig. 1 Pain and pathological changes of synovial tissues in ankle OA. (A) AMH and SWBSP tests were conducted 10 weeks after surgery to assess pain levels in the Cont and Resection groups. SWBSP measured the difference in right and left load and calculated as right-left. (n=8). (B): HE staining of the representative synovial tissues and synovitis score. Scale bars, 50 µm. (n=8).

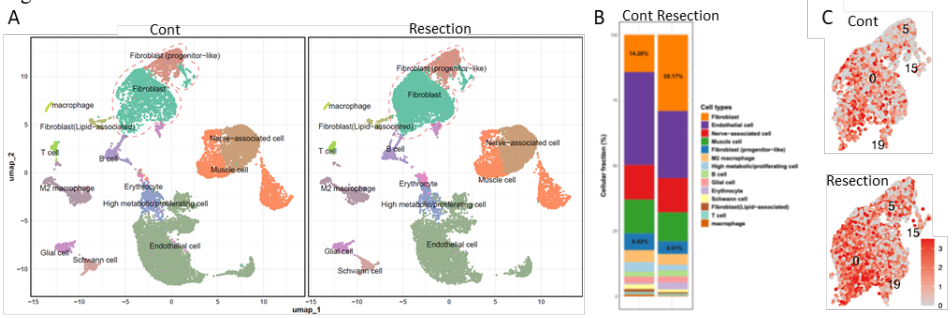

Fig. 2 Single-cell RNASeq for ankle synovial tissues. (A) All cell populations in ankle synovial tissues from the Cont and Resection group. (n=3). (B) Percentage of each cell types. (C) *Ctgf* gene expression in fibroblasts from two groups.

RESULTS SECTION: Compared to the sham surgery control group (Cont), the Resection group showed a significantly lower threshold in the AMH test (**Fig. 1A**), as well as displayed remarkably higher scores in SWBSP, which indicated less load on the left paw (with CFL and ATFL resection) compared to the right (without surgery). These results together suggested the generation of ankle pain in the Resection group. Interestingly, we also observed a significantly higher synovitis score in the Resection group when compared to the Cont group (**Fig. 1B**). Single-cell RNASeq for synovial tissues revealed an increased population of fibroblasts in the Resection group (**Fig. 2A&B**). Interestingly, fibroblasts from the synovial tissues also displayed higher expression of inflammation and fibrosis-associated genes, such as connective tissue growth factor (*Ctgf*) (**Fig. 2C**). In bulk RNASeq for DRG, expression levels of *P2rx3, Trpv1, Scn11a, Scn9a, Prdm12*, *Scn10a,* and *Cacna1b*, which are genes associated with neural activation, were significantly upregulated in the Resection group (**Fig. 3**).

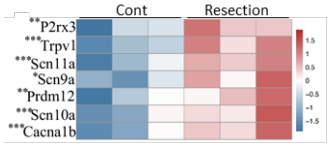

Fig. 3 Bulk RNASeq for DRG. Expression levels of selected pain associated genes in the Cont and Resection groups. P2rx3: Purinergic Receptor P2X 3; Trpv1: Transient Receptor Potential Vanilloid 1; Scn 9a, 10a, 11a: Nav 1.7, 1.8, 1.9; Prdm12: PR domain zinc finger protein 12; Cacna1b: alpha-1 subunit of the N-type voltage-gated calcium channel. *,p<0.05; **,p<0.01, ***, p<0.001.

DISCUSSION: Synovial tissues in the Resection group were thickened, as shown in HE staining. Single-cell RNA sequence further revealed an increased fibroblast population and upregulated *Ctgf* expression in ankle OA. Previously, CTGF was shown to promote fibroblast numbers and induce inflammation in the synovium (Liu, et al., PLoS One. 2012). Furthermore, increased expression of pain-related genes was observed in DRG from the Resection group. Future work will continue to analyze the RNASeq data, validate the findings from RNASeq, and identify potential druggable targets. We will also examine the crosstalk between fibroblasts and other cells, such as macrophages and chondrocytes.

SIGNIFICANCE/CLINICAL RELEVANCE: Our study for the first time explored the critical roles of pathological changes of synovial tissues in ankle OA progression. In particular, we identified the critical roles of fibroblasts. These findings may provide new therapeutic strategies for treating ankle OA.

ACKNOWLEDGEMENTS: The project is partially supported by the Pittsburgh Foundation. We would like to thank NIH/NIAMS P30AR079206 and acknowledge the Chicago Center on Musculoskeletal Pain Research Core Center for support in developing pain-assessing methods. Dr. Tristan Maerz from the University of Michigan provides technical support in processing ankle synovial tissues for single-cell RNASeq.