Wnt signaling underpins fibro-adipogenic dynamics of intra-articular fat during post-traumatic osteoarthritis

Alexander Knights[1], Seth Kahan[1], Danny Nguyen[1], Aanya Mohan[1], Rida Pervez[1], Stephen Redding[1], Easton Farrell[1], Michael Newton[1], Tristan Maerz[1]
[1]University of Michigan, Ann Arbor, MI
aknights@umich.edu

Disclosures: None.

INTRODUCTION: The pathophysiological roles of intra-articular adipose tissue in osteoarthritis (OA) are not well understood. Some clinical studies have indicated that intra-articular fat may have pro-regenerative functions in joint homeostasis[1] and confer protection from OA[2], but dysfunctional fat in OA can assume deleterious functions[3]. Fibrosis of intra-articular fat, along with co-located synovial tissue, is a clinical manifestation in OA. However, whether this fibrosis is a pathological driver of pain and disease progression remains to be elucidated. Using a non-invasive mouse model of post-traumatic OA (PTOA) and various *in vitro* models, we characterized the spatiotemporal dynamics of intra-articular adipose tissue after joint injury. We hypothesized that in OA, intra-articular fat would undergo a shift from adipogenic to a pathological, fibrotic phenotype, resulting in aberrant function and diminution of the fat depot.

METHODS: We induced PTOA in mice by ACL rupture (ACLR)[4], with Shams (anesthesia/analgesia only) as controls. Mice were harvested 7d or 28d post-ACLR. All animal work was approved by IACUC. We assessed intra-articular adipose tissue using 2D histomorphometry of SafO-stained sagittal knee sections to compare anterior fat pad area to total anterior synovial tissue area. We then compared healthy mouse knee fat pad to classical white adipose tissue from the inguinal subcutaneous depot (IWAT) by RNA-seq, and used gene expression and oil red O staining of lipid droplets to assess adipogenic capacity of primary progenitors derived from IWAT, healthy fat pad, or PTOA fat pad (7d ACLR). To test the effect of mechanical loading on fat pad-derived progenitor differentiation, we subjected cells to 0.5 Hz of biaxial strain (10% stretch) for 1hr on day 0, 3, 6 of adipogenesis, using the FlexCell system.

RESULTS: There was a marked reduction in 2D relative fat pad area in late stage PTOA joints (28d ACLR) compared to Sham joints, with no appreciable differences in the early stage of disease (7d ACLR) (Fig 1A-B). 5,456 differentially expressed genes were detected between the intra-articular fat pad and classical adipose tissue, IWAT (Fig 1C). Compared to IWAT, which was enriched for immune and hematopoietic functions, fat pad was enriched for terms like osteogenesis, chondrogenesis, angiogenesis and matrix regulation, supporting a dynamic, pro-regenerative role in the joint (Fig 1D). Cultured stromal/progenitor cells from IWAT and healthy fat pad had comparable adipogenic differentiation capacity, as measured by oil red staining of lipid droplets and induction of adiponectin (*Adipoq*) – however, adipogenic capacity was greatly blunted in cells from the fat pad of PTOA mice (Fig 1E-F). Cells from injured fat pad expressed higher levels of the fibrotic marker *αSMA* before and after differentiation (Fig 1G), suggesting that these progenitors may be predisposed towards a fibrotic state instead of an adipogenic-primed state. The Wnt signaling agonist R-spondin 2 (*Rspo2*), which is inhibitory for adipogenesis[5] and which we have previously shown to drive pro-fibrotic and pro-inflammatory functions in synovial fibroblasts[6], was more highly expressed at baseline and after differentiation in cells derived from PTOA fat pad compared to healthy cells (Fig 1H). Progenitors differentiated in the presence of rRSPO2 (200ng/mL), to activate Wnt signaling, had impaired adipogenesis (Fig 1I). Finally, given the link between joint destabilization, abnormal mechanical forces, and fibrosis[7], we subjected fat pad-derived progenitors to mechanical strain during adipogenic differentiation. Injurious mechanical loading reduced adiponectin (*Adipoq*) gene induction and upregulated expression of the fibrotic marker *αSMA* (Fig 1J).

DISCUSSION: Dysfunctional intra-articular fat is associated with OA pain and inflammation, however, mechanisms by which fat acts as a driver of disease are not well explored. In addition to diminished fat pads in mice with PTOA, we found that fat pad-derived progenitor cells have an impaired adipogenic capacity and are instead predisposed to a fibrotic phenotype. This may explain both the reduced fat pad volume and provide a mechanism for the dysfunctional, pathological role of intra-articular fat in OA. Wnt signaling is recognized as overactive in OA/PTOA joints, especially synovium, and can elicit both pain and inflammation[6] by mechanisms yet undescribed. Here we propose that chronically overactive Wnt signaling blunts adipogenesis in fat pad progenitors, instead promoting a pro-fibrotic phenotype. *In vivo* lineage tracing and gain-of-function experiments are now required to examine this phenotypic shift during OA and then to test interventions that may reverse fibrosis and concomitant inflammation and pain.

SIGNIFICANCE/CLINICAL RELEVANCE: Intra-articular fat is a key site of pain, inflammation, and fibrosis in OA patients. Restoring normal function of the fat pad by reprogramming progenitors from a fibrotic to an adipogenic predisposition is an attractive therapeutic avenue for treating OA.

REFERENCES:[1]Eymard *ARD* 2017 [2]Pan *ARD* 2015 [3]Zhou *JOR* 2022 [4]Christiansen *OAC* 2012 [5]Dong *Nat Metab* 2022 [6]Knights *ARD* 2023[7]Sono *Path* 2020

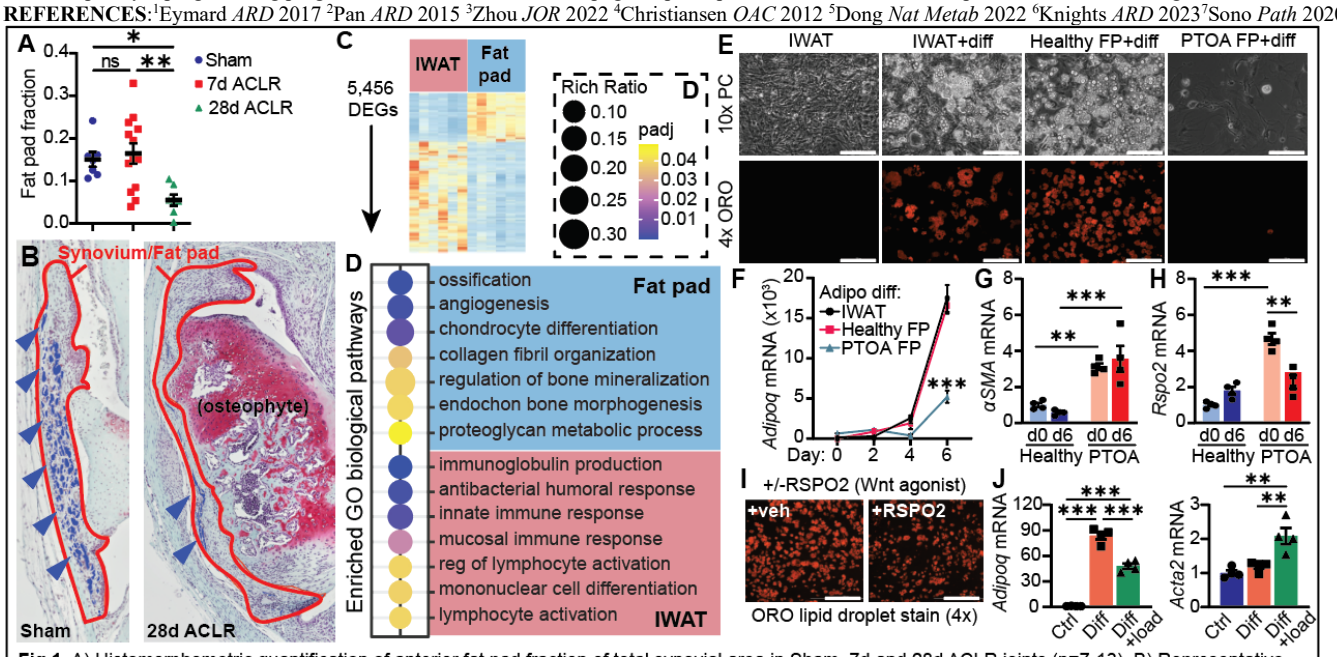

Fig 1. A) Histomorphometric quantification of anterior fat pad fraction of total synovial area in Sham, 7d and 28d ACLR joints (n=7-13). B) Representative sagittal images of Sham, 28d ACLR synovium (red outline) and fat (blue mask) - blue arrows point to fat areas. C) Heatmap of differentially-expressed genes/DEGs (padj<0.05; log2FC>|1|) between healthy fat pad and IWAT (n=6). D) Biological pathways enriched in fat pad or IWAT based on DEGs. E) Adipogenic differentiation of progenitor cells from IWAT, healthy, or PTOA fat pad (FP); includes IWAT cells grown in only growth medium (left). Top: 10x phase contrast; Bottom: oil red O lipid droplet staining. F) Adiponectin (*Adipoq*) expression during adipogenic differentiation of IWAT, healthy and PTOA fat pad-derived progenitors. G) α-smooth muscle actin (*αSMA*) and H) R-spondin 2 (*Rspo2*) expression in healthy or PTOA fat pad progenitors at day 0 and 6 of adipogenesis. I) Oil red O after differentiation + or - RSPO2 (200ng/mL). J) Fat pad progenitors were subjected to growth medium, adipogenic differentiation, or adipogenic differentiation with FlexCell mechanical loading at day 0, 3, 6 (10% biaxial strain, 1hr, 0.5 Hz). *Adipoq* and *αSMA* expression was assessed (n=4). One-way ANOVA was done for A, F, G, H, J where *p<0.05, **p<0.01, ***p<0.001. Errors bars are mean ± SEM. Scale bars: 200μm.