Sox5 as a transcriptional mediator of synovial disease in PTOA

Adrienne A. Giannone[1], Lindsey Lammlin[1], Scarlet Howser[2], Isabelle Smith[3], Alexander Knights[3], Tristan Maerz[1]
[1]ETH Zürich, Zürich, Switzerland ; [2]University of Michigan, Ann Arbor, MI, [3]Washington University St. Louis, St. Louis, MO
agiannone@ethz.ch

Disclosures: T. Maerz is a paid consultant for Relation Rx.

INTRODUCTION: Synovial lining hyperplasia is clinically correlated to worse disease outcomes in osteoarthritis (OA) and post-traumatic OA (PTOA). Fibroblast-like synoviocytes (FLS) of the lining layer produce vital synovial fluid lubricants and nutrients; however, in OA/PTOA they undergo rampant proliferation (hyperplasia) and drive fibrosis, inflammation[1], osteophyte formation[2], and patient-reported pain[3]. Critically, the molecular mechanisms governing their maintenance, pathological expansion, and dysregulation remain poorly understood. Herein we identify SOX5 as a transcriptional mediator of FLS emergence, function, and pathological activation, providing critical insights into synovial physiology and chronic synovitis in PTOA.

METHODS: Single-cell RNA sequencing (scRNA-seq): Previously published scRNAseq datasets[4,5] were mined to assess SOX5 cell type-specific expression patterns in mouse and human OA synovial tissue. Immunofluorescence: C57 Bl/6 mice at 14 weeks of age were subjected to unilateral anterior cruciate ligament rupture (ACLR) as a non-invasive model of PTOA. At 28 days post-injury, ACLR and contralateral (control) limbs were stained for SOX5 and DAPI, then imaged using fluorescence microcopy. Bulk RNA sequencing: Primary murine hindpaw FLS were harvested from C57 Bl/6 mice (n=3M and 3F pooled) then transfected with either control siRNA (siCtrl) or siSox5 (25nM each) for gene knockdown (KD). FLS were then dosed with a vehicle (PBS) or IL1β (10ng/mL) and collected at 24 hrs post-treatment (n=3/condition). RNA was extracted and bulk RNA sequencing was performed (40M reads/sample). Differentially expressed genes (DEGs) were identified using pairwise comparisons (DESeq2) and gene ontology was conducted using PantherDB. ELISA: Conditioned media from the treated FLS (n=3/condition) were collected for cytokine quantification via ELISA. Inducible SOX5 knockout (KO) mice: 12 week-old $Sox5^{fl/fl}$- $RosaCre^{ERT2(+)}$ (SOX5 KO) and WT controls ($Sox5^{fl/fl}$-$RosaCre^{ERT2(-)}$) were generated and received tamoxifen (75 mg/kg IP) for five consecutive days before joint injury (ACLR). Synovial lining histology: Hindlimbs from WT and SOX5 KO mice were harvested at 28 days post-ACLR and stained with SafO/Fast Green. Histological scoring was performed in a blinded fashion using previously established criteria to assess synovial lining hyperplasia[6]. μCT analysis: Fixed hindlimbs were imaged using micro-computed tomography (μCT; SkyScan: 9 um voxel). Osteophyte size and maturity were quantified using Dragonfly software. Normalized total osteophyte volume was calculated by diving the total osteophyte volume by the femoral length. All animal work was IACUC-approved. Only male mice are currently represented in this study. While female littermates have also been collected and analyzed, their sample size is currently too small for inclusion. Contralateral limbs were used as controls to reduce the number of mice needed for this study (3R).

RESULTS: In both human OA and murine PTOA, SOX5 expression is highly enriched in the synovial lining (**Fig.1A**), particularly in cells undergoing hyperplastic expansion after ACLR (**Fig.1B**). In our ORS abstract last year, we showed that SOX5 expression in FLS is upregulated in response to inflammatory cytokine stimulation (via IL1β and TNFα). To next interrogate the role of SOX5 in the FLS inflammatory response, we knocked down SOX5 prior to treatment with IL1β *in vitro* and performed bulk RNA sequencing. SOX5 knockdown significantly attenuated the expression of genes involved in matrix remodeling (*Mmp3, Mmp9, Mmp13, Tgfb1*), inflammation (*Il6, Cxcl5, Il1a, Ccl2, Ccl6, Nlrp3, Nod2*), and neurotrophic signaling (*Ngf, Nrp1, Sema3a/c/e, Ntn4, Snca*) compared to control (siCtrl + IL1β) conditions (**Fig. 1C**). Gene ontology analyses revealed corresponding downregulation of migratory, pro-fibrotic, inflammatory and neurotrophic pathways (**Fig. 1D**). Additionally, secreted protein analysis showed that SOX5 silencing reduced secretion of key inflammatory (IL6, CCL2, GM-CSF, CXCL1/2/5) and pro-angiogenic (VEGF) proteins associated with pathogenic FLS activation (**Fig. 1E**). To examine SOX5's role *in vivo*, we globally knocked out SOX5 in mice prior to joint injury and assessed outcomes at 28 days post-ACLR, a timepoint representing established PTOA. SOX5-deficient mice exhibited a marked reduction in synovial lining hyperplasia scores (**Fig. 1F**) and an ~50% decrease in normalized osteophyte volume (**Fig. 1G**), indicating an overall decrease in PTOA severity when SOX5 is ablated.

DISCUSSION: SOX5 expression is enriched in the synovial lining of both human and murine joints, identifying it as a conserved, tissue-specific transcriptional regulator. *In vitro* and *in vivo* experiments implicate SOX5 as a central hub controlling the FLS inflammatory program and injury response: SOX5 silencing blunts inflammatory, neurotrophic, and pro-fibrotic gene expression in FLS, and SOX5-deficient mice are considerably protected from disease. Collectively, these findings establish SOX5 as a key driver of synovial lining-associated pathology in PTOA, including osteophyte maturation, hyperplastic expansion, and nociceptive signaling.

SIGNIFICANCE/CLINICAL RELEVANCE: Our findings demonstrate SOX5 as a critical regulator of FLS-mediated pathology and the joint's *in vivo* response to injury. Targeting SOX5 to attenuate disease onset in the early post-injury period represents a potentially viable, novel therapeutic strategy for PTOA.

REFERENCES:[1]Culemann+, Nature, 2019; [2]Roelofs+, Ann Rheum Dis, 2020; [3]Bai+, Sci Trans Med, 2024; [4]Knights+, Ann Rheum Dis, 2023; [5]Tang+, Sci Transl Med, 2024; [6]Bergman+, Osteoarthr Cartilage, 2024.

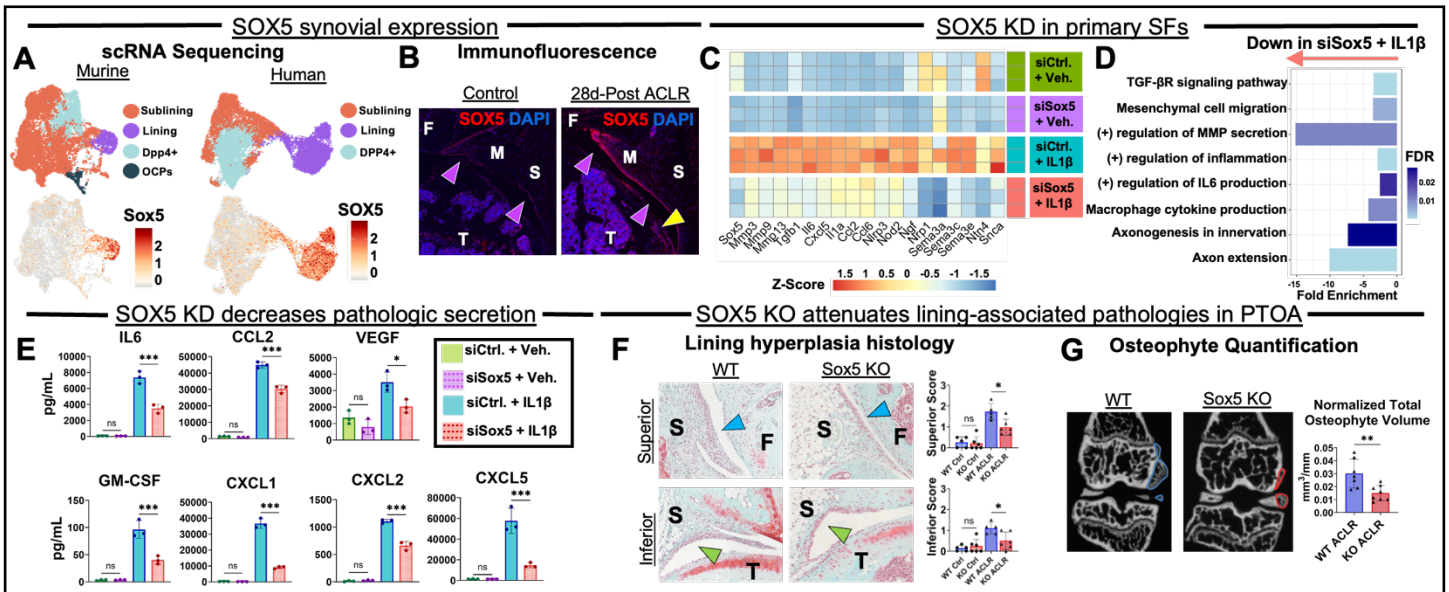

Figure 1. (A) scRNA-seq of murine (left) and human (right) synovial fibroblasts, showing normalized SOX5 expression. **(B)** Immunofluorescence imaging showing expression of SOX5 in a paired contralateral control limb (left) and 28d ACLR limb (right). Purple arrows indicate the synovial lining. Yellow arrow indicates osteophytes. S = synovium, M = meniscus, F = femur, T = tibia. **(C)** Heatmap of representative DEGs identified via DESeq2 computed between siSOX5 + IL1β and siCtrl + IL1β (log2FC > 0.585, p-adjusted > 10^{-14}; n=3/condition). **(D)** PantherDB gene ontology results using DEGs computed between siSOX5 + IL1β and siCtrl + IL1β. FDR = false discovery rate. **(E)** Cytokine ELISAs of conditioned media from the bulk RNA sequencing samples (n=3/condition). **(F)** Representative histology images of male WT and SOX5 KO mice and lining hyperplasia quantification (right; n=5-6/condition). Blue arrows indicate the superior anterior synovial lining, while green arrows indicate the inferior anterior lining. Ctrl indicates the uninjured contralateral limb. S = synovium, F = femur, T= tibia. **(G)** Representative images of osteophyte outlining and normalized total osteophyte volume (right; n=7-8/condition). All error bars are mean ± SD. *P<0.05, **P<0.01, ***P<0.001.