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Figure 1. Schematics illustrating intra-articular injection of TA-NM@Lip can effectively relieve pain, alleviate synovitis and protect cartilage in MIA rats and ACLT + pMMx rats.

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POSO330
SYNOVIAL M1 MACROPHAGES AND FIBROBLASTS DUAL-TARGETING LIPOSOMES ASSISTED DELIVERY OF TRIAMCINOLONE ACETONIDE IS EFFECTIVE AGAINST JOINT PAIN AND CARTILAGE DEGENERATION IN OSTEOARTHRITIS

Keywords: Treat to target, Pain, Osteoarthritis

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Background: The latest professional guidelines recommend intra-articular (IA) glucocorticoids (GCs) for pain relief in patients with knee osteoarthritis (OA). However, their effect is small-to-moderate and short-term only. There is also concern about the possible risk of cartilage deterioration caused by repeated IA GCs. M1 macrophages and activated synovial fibroblasts (SFs) mutually contribute to the propagation of joint pain and cartilage destruction in OA by constructing a positive feedback loop. To alleviate joint pain and potentially over a longer period without increasing the risk of cartilage deterioration, we developed nanometer-porous cellular membrane (NM@Lip) for targeted delivery of triamcinolone acetonide (TA), one of the IA GCs most commonly used for OA, to both M1 macrophages and activated SFs in the synovium of osteoarthritic joints.

Objectives: To investigate the phenotypic reprogramming effect of TA-loaded NM@Lip (TA-NM@Lip) in M1 macrophages and activated SFs and determine their ability in relieving pain and alleviating OA progression in rodent models.

Methods: TA-NM@Lip was fabricated using the thin-film hydration method. The mRNA and protein levels of pathogenic mediators secreted by M1 macrophages and activated SFs were measured after treatment with TA-NM@Lip or TA-NMx Lip for 24 hours to evaluate the ability of relieving pain and alleviating OA progression in rodent models.

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Results: TA-NM@Lip was the most efficient in downregulating the expression of M1-related genes and upregulating M2-related genes compared with other treatment groups. The mRNA and protein levels of pro-inflammatory cytokines, adhesion molecule and proteolytic enzyme secreted by activated SFs were significantly downregulated by TA-NM@Lip. NM@Lip was retained in the joint for up to 28 days and selectively distributed into both M1 macrophages and activated SFs in synovium with low distribution in cartilage. In MIA-induced model, a single IA injection of TA-NM@Lip attenuated synovitis and achieved complete pain relief without inducing adverse effects. In ACLT + pMMx-induced model, repeated TA-NM@Lip did not cause apoptosis of chondrocytes or damage cartilage and attenuated cartilage degeneration.

Conclusion: TA-NM@Lip exhibited extended joint-retention time and selectively mediated the phenotype of synovial M1 macrophages and activated SFs in OA joints. This dual-targeting scaffold effectively alleviated synovitis, decreased cartilage degeneration, and achieved complete relief of joint pain in two distinct rodent models of OA.

REFERENCE:

POSO331
MOLECULAR MECHANISMS OF CHONDROPROTECTION INDUCED BY ROR2 BLOCKADE

Keywords: Osteoarthritis, Cell biology

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Background: We previously reported that ROR2 blockade (ROR2i) induces chondrogenesis in vitro and in vivo, improving pain and cartilage integrity following joint destabilization in mice (Thorup et al., 2020). Downregulation of YAP signaling was required for chondrogenesis induced by ROR2i, but per se not sufficient. Overexpression of constitutively active YAP negated ROR2i-induced chondrogenesis, but the YAP inhibitor Verteporfin was unable to induce chondrogenesis. This means that something else must take place in addition to YAP inhibition for ROR2i to induce chondrogenesis.

Objectives: In this study, we used a phosphoproteomic approach to detail downstream ROR2 signaling in chondrogenic cells.

Methods: Cartilage differentiation was assessed in C3H10T1/2 cells cultured in micromass using Alcian Blue staining and the expression of mRNA encoding chondrogenic markers. Osteogenesis was assessed by molecular marker analysis, alkaline phosphatase activity and alizarin red staining. Phospho-proteomics screening was performed by precipitation of phosphoproteins on TiO2 columns followed by mass spectrometry. Par2-/- C3H10T1/2 cells were generated in CRISPR/Cas9. Results: In keeping with the delayed endochondral ossification and chondrocyte hypertrophy observed in Par2-/- mice (DeChiara et al., 2000), Par2-/- C3H10T1/2 cells failed to undergo osteogenesis when exposed to osteogenic medium as assessed by alkaline phosphatase activity, extracellular matrix calcification and expression of hypertrophy/osteogenic markers. We established suitable time points for ROR2-mediated phosphorylation events based on ERK (early) and JNK (late) phosphorylation following stimulation with WNT5A. Phosphoproteomics analysis using a label-free approach, revealed 1109 early (57%) phosphorylation events that were ROR2 dependent (taking place in the wild type but not in Par2-/- cells) and 304 late (50%) phosphorylation events that were ROR2 dependent (taking place in the wild type but not in Par2-/- cells).

REFERENCE:
not in the Ror2-/- CRISPR cells) and 708 at 15 minutes. In keeping with the requirement of YAP for ROR2-dependent chondrogenesis (Thorup et al., 2020), several phosphorylation events of molecules within the YAP pathway occurred including YAP and LATS1/2. In keeping with this, YAP dependent transcription was modulated by WNT5A in a ROR2-dependent manner in a reporter assay. Several phosphorylation sites were non-conventional, suggesting a complex, non-canonical, regulation of YAP by ROR2. Other signalling pathways, previously known to be involved in chondrogenesis, were involved including insulin, autophagy and focal adhesion. Many phosphorylation events were previously poorly characterised.

Conclusion: ROR2 induces modulation of several signalling pathway in addition to YAP. The hierarchy of these phosphorylation events needs to be resolved to identify the detailed molecular mechanism underpinning the efficacy of ROR2i in osteoarthritis.

**REFERENCES:**


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**POS0332**

**IDENTIFICATION OF DISTINCT CELLULAR POPULATIONS IN THE SYNOVIIUM OF EARLY- AND LATE-STAGE RADIOGRAPHIC KNEE OA USING SINGLE NUCLEUS RNA SEQUENCING**

**Keywords:** Osteoarthritis

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**Background:** Osteoarthritis (OA) is a complex, multifactorial and heterogeneous joint disease of unknown etiology. OA research has largely focused on articular cartilage degeneration with little attention given to other joint tissues, including the synovium. The synovium lines the joint capsule, produces synovial fluid for lubrication, and is emerging as a contributor to OA pathogenesis. In OA, the synovium exhibits increased vascularization, inflammation, hyperplasia and fibrosis. Synovial cells that contribute to these pathological events during early and advanced stages of OA are not well characterized. The emergence of RNA sequencing (RNAseq) at the resolution of a single cell or nucleus allows for the identification of distinct cells that may contribute to OA pathogenesis.

**Objectives:** To delineate the synovium’s role in OA pathogenesis we sought to identify if distinct cell subtypes exist in the synovium of early (KL1) versus late stages (KL3/4) of radiographic knee OA using single nucleus RNA sequencing.

**Methods:** Synovia from patients with early (KL1; n=5) and late (KL III/IV; n=4) stage radiographic knee OA were subjected to single nucleus (sn)RNAseq and to bioinformatics analyses. Canonical cell-specific markers were used to identify cell types from the unsupervised clustering analysis and prominent cell types were re-clustered. Differentially expressed gene (DEG) lists between the subclusters were determined based on top gene expression within a cell type between early and late OA synovium. Cell surface markers identified from the DEGs were validated by immunohistochemistry. Pathway and gene ontology enrichment analysis was performed on fibroblast subclusters to identify prominent pathways and transcription factors that were upstream regulators. Ongoing in vivo and in vitro methods are being used to assess these transcription factors in both fibroblast cell culture and an OA mouse model.

**Results:** Fibroblasts and macrophages constituted 75% of the cells from early and late-stage synovium and re-clustering analysis resolved 6 fibroblast and 6 macrophage subclusters (Figure 1). Cluster-based nucleotide proportion differences identified fibroblast clusters 1, 2, 4 and 6 and macrophage clusters 1, 2 and 5 to contribute to early-stage samples while fibroblast clusters 0, 3 and 5 and macrophage clusters 0, 3 and 4 to late-stage. Downstream analyses focused on fibroblasts and putative cell surface markers from fibroblast subclusters were identified from DEGs and confirmed by immunohistochemistry. The fibroblast subclusters were subjected to pathway analyses which identified clusters 0 and 1 to be the most prominent clusters which both shared common ECM related pathways. Upstream transcription factors that regulate ECM related genes were identified for both subclusters 0 and 1. Current efforts are focused on selecting and targeting transcription factor(s) for both in vitro and in vivo analyses by utilizing siRNAs in fibroblast culture and a cre-lox mouse system to identify the mechanisms associated with the synovial pathology during OA.

**Conclusion:** Single nucleus RNAseq analysis identified distinct subclusters of fibroblasts and macrophages to exist in human OA knee synovia. Certain subclusters were more representative of the early stage while others were more representative of the late stage of the disease. Further validation studies are being performed to assess the functional roles of these subclusters and whether targeting them would attenuate disease progression. KT and EG share equal first author contribution. DE and MK share equal senior author contribution.

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**POS0333**

**NLRP12 EXACERBATES OSTEOARTHRITIS BY PROMOTING DEGRADATION OF NOOD2 IN SYNOVIAL MACROPHAGES**

**Keywords:** Synovium, Innate immunity, Osteoarthritis

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**Background:** Osteoarthritis is the most common degenerative bone and joint disease. Hypothesis has been widely accepted that osteoarthritis (OA) starts from cartilage injury and loss, while emerging evidences suggest that synovial inflammation precedes cartilage loss during the progression of OA [1]. Activation of macrophages plays a crucial part in synovial inflammation in OA [2]. In our previous researches, we have demonstrated NOOD2 as an inhibitor of macrophage activation in vitro. Besides, bioinformatic analysis suggested a potential link between NLRP12 and NOOD2.

**Objectives:** To investigate the role of NOOD2 in OA in vivo and explore the potential interaction between NOOD2 and NLRP12.

**Methods:** We established CIAO (Collagenase-induced OA) model with 8-week-old C57BL/6J mice, and injected NOOD2 over-expression (oe-NOOD2) lentiviral vectors into the knee joint cavity as experimental group (CIAO + oe-NOOD2), with the empty vectors as control (CIAO + Mock). 8 weeks later, the knee joints were harvested and stained with Safranin O/fast green. Besides, three-dimensional reconstruction of Micro-CT images were employed to evaluate the pathological changes of OA. In addition, we applied lentiviral transfection, co-immunoprecipitation (Co-IP), and ubiquitination assays to investigate the interaction between NOOD2 and NLRP12, as well as its mechanism of action in the regulation of macrophage activation.

**Results:** In vivo over-expression of NOOD2 showed significant inhibition of pathological changes (Figure 1A, 1B). Though NLRP12 had no impact on mRNA level of NOOD2 in RAW264.7 macrophages (Figure 1C), NLRP2 expression at protein level was negatively correlated with NLRP12 (Figure 1D), suggesting that NLRP12 may influence the degradation of NOOD2. Co-IP experiments also confirmed the existence of interaction between NLRP12 and NOOD2 at protein level, which was influence by MG132, inhibitor of ubiquitin-proteasome pathway (Figure 1E). Besides, NLRP12 over-expression impaired inhibition of macrophage inflammation by NOOD2 (Figure 1F). Since HSP90 binds NOOD2 to prevent its interaction with NLRP12, it is possible to develop a new therapeutic strategy of NLRP12 for OA treatment.

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