not in the Ror2-/- CRISPR cells) and 708 at 15 minutes. In keeping with the require-
ment of YAP for ROR2i-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 chon-
drogenesis, were involved including insulin, autophagy and focal adhesion. Many
phosphorylation events were previously poorly characterised.

Conclusion: ROR2 induces modulation of several signalling pathway in addi-
tion 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.

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POS0332
IDENTIFICATION OF DISTINCT CELLULAR POPULATIONS IN THE SYNOVIUM 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 synovial’s role in OA pathogenesis we sought to
determine 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 (KL1–2) and late (KL 3–4) 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 vali-
dated by immunohistochemistry. Pathway and gene ontology enrichment analy-
sis were 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
transcriptionally distinct macrophage subclusters (Figure 1). Cluster-based nuclear
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 3, 5 and 6 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 focussed on
selecting and targeting transcription factor(s) for both in vitro and in vivo anal-
yses by utilizing siRNAs in fibroblast culture and a cre-lox mouse system to iden-
tify the mechanisms associated with the synovial pathology during OA.

Conclusion: SnRNAseq 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 contri-
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POS0333
NLRP12 EXACERBATES OSTEOARTHRITIS BY PROMOTING DEGRADATION OF NOD2 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]. Activa-
tion of macrophages plays a crucial part in synovial inflammation in OA [2]. In
our previous researches, we have demonstrated NOD2 as an inhibitor of mac-
rophyage activation in vitro. Besides, bioinformatic analysis suggested a potential
link between NLRP12 and NOD2.

Objectives: To investigate the role of NOD2 in OA in vivo and explore the poten-
tial interaction between NOD2 and NLRP12.

Methods: We established CIOA (Collagenase-induced OA) model with 8-week-
old C57BL/6J mice, and injected NOD2 over-expression (oe-NOD2) lentiviral
vectors into the knee joint cavity as experimental group (CIOA + oe-NOD2), with
the empty vectors as control (CIOA + 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-immunoprecip-
itation (Co-IP), and ubiquitination assays to investigate the interaction between
NOD2 and NLRP12, as well as its mechanism of action in the regulation of mac-
rophyage activation.

Results: In vivo over-expression of NOD2 showed significant inhibition of patho-
logical changes (Figure 1A, 1B). Though NLRP12 had no impact on mRNA level
of NOD2 in RAW264.7 macrophages (Figure 1C), NOD2 expression at protein
level was negatively correlated with NLRP12 (Figure 1D), suggesting that
NLRP12 may influence the degradation of NOD2. Co-IP experiments also
confirmed the existence of interaction between NLRP12 and NOD2 at protein level,
which was influence by MG132, inhibitor of ubiquitin-proteasome pathway (Fig-
ure 1E). Besides, NLRP12 over-expression impaired inhibition of macrophage
inflammation by NOD2 (Figure 1F). Since HSP90 binds NOD2 to prevent its

Figure 1. a. UMAP of clustered cell types and their proportions in synovium from KL1
(EF08-E216) and KL3/4 (L44-L370) patients. b. UMAPs of fibroblasts and macrophages
from the subclustering analysis. c. Subclustering analyses of fibroblasts and macrophages
presented as UMAPs for each patient.