ELUCIDATING THE MECHANISM OF ACTION DOWNSTREAM OF ROR2 BLOCKADE: A PHOSPHOPROTEOMIC APPROACH

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Background: Osteoarthritis (OA) affects 12% of the population, and yet we still have no disease-modifying treatment. Cartilage breakdown is the hallmark of OA, and patients suffer from pain and loss of joint function/independence, severely affecting quality of life. Therefore, there is a huge unmet clinical need. Receptor tyrosine kinase-like orphan receptor 2 (ROR2) is a non-canonical WNT receptor that regulates the planar cell polarity pathway, controlling limb outgrowth. During skeletal development chondrocytes require ROR2 to undergo hypertrophy in the process of endochondral bone formation (1). Loss of function mutations in human causes Recessive Robinow Syndrome (limb shortening and brachydactyly (2,3)). Although absent from healthy adult articular cartilage, our initial studies identified high ROR2 expression in chondrocytes from patients with OA, suggesting a role in the disease process.

In a murine model of OA, blocking ROR2 in therapeutic regime using siRNA resulted in reduced cartilage destruction and rapid and sustained pain relief. Due to the limited expression pattern of ROR2 in adulthood, no systemic or local toxicity were expected, nor were any observed. We also found that blocking ROR2 supports cartilage formation in a human cartilage organoid model in nude mice using chondrocytes from patients with OA, and proposed ROR2 blockade as a potential disease-modifying OA treatment.

The mechanism of action of ROR2 blockade was independent of modulation of canonical WNT signaling. YAP inhibition was required, but not sufficient, for the chondrogenic effect (4). Therefore, additional, yet unknown mechanisms must be involved downstream of ROR2 blockade.

Objectives: To study the phosphorylation events downstream of ROR2, to further our understanding of the mechanism of action of ROR2 blockade.

Methods: Phosphoproteomics (label free liquid chromatography tandem-mass spectrometry with TiO2 based phospho-enrichment), in vitro studies in cells, CRISPR.

Results: WNT5A is the most well established ligand for ROR2. We confirmed that WNT5A stimulation leads to time-dependent phosphorylation of ERK in C3H10T1/2 cells. We then deleted ROR2 in C3H10T1/2 cells by CRISPR, and performed phosphoproteomics analysis on wildtype (control) C3H10T1/2 cells and ROR2-CRISPR C3H10T1/2 cells stimulated with 100ng/ml recombinant WNT5A for 5 or 15 minutes.

ROR2-dependant phosphorylation targets were defined as proteins differentially phosphorylated in control cells upon WNT5A stimulation, but that did not occur in ROR2-CRISPR cells.

YAP, and phosphorylated YAP by WNT5A in a ROR2-dependant manner. WNT5A/ ROR2 also modulated the YAP signaling pathway at other levels.

KEGG analysis revealed that other pathways with known roles in the pathogenesis of OA were enhanced, including NF-kB, mTOR signaling and cellular senescence.

With the current technology, ROR2 blockade requires intra-articular injections of siRNA conjugated to atelocollagen every 5 days. Preliminary efficacy data of our ongoing ROR2 siRNA clinical trial indicate a robust safety profile. Our approach involves a single treatment at the time of surgery, extending the duration of benefit to patients requiring surgery.

Conclusion: ROR2 blockade has potential as a disease-modifying treatment for OA, resulting in cartilage protection and rapid and sustained pain relief in a murine model. Here, we studied the mechanism downstream of ROR2 blockade using a phosphoproteomics approach. We confirmed that YAP signaling was modulated by ROR2, and identified novel pathways and cellular processes regulated by WNT5A/ROR2. Further studies are needed to clarify their role downstream of ROR2 blockade in OA.

Our current siRNA-atelocollagen based technology requires intra-articular injections too frequently to be acceptable for patients. We are developing ROR2 blockade which can be administered systemically or intra-articularly not more often than every 3 months.

REFERENCES:

THE MIR-320 FAMILY IS UPREGULATED IN FAST-PROGRESSING RADIOGRAPHIC KNEE OSTEOARTHRITIS: DATA FROM THE OSTEOARTHRITIS INITIATIVE

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Background: There is an outstanding need for prognostic biomarkers to reliably detect fast-progressing knee osteoarthritis (OA) such that preventive interventions can be targeted to this patient population. MicroRNA-sequencing is an unbiased approach for comprehensive profiling of circulating microRNAs in liquid biopsies to discover novel biomarkers of disease. As negative regulators of gene expression, microRNAs hold potential not only as biomarkers, but also as mechanistic drivers of knee OA.

Objectives: To apply microRNA-sequencing to identify unique circulating microRNAs as potential biomarkers that distinguish fast-progressing radiographic knee OA from both slow- and non-progressing radiographic knee OA.

Methods: Leveraging the Osteoarthritis Initiative (OAI) longitudinal cohort, we applied our customized microRNA-sequencing pipeline [1] to blood plasma samples collected at baseline and 4-year follow-up from 106 participants. The disease trajectory for each participant was constructed by plotting their Kellgren-Lawrence (KL) grades over an 8-year period. Based on these trajectories, we defined fast-progressors as an increase from KL 0/1 at baseline to KL 3/4 by 4-year follow-up, slow-progressors as an increase from KL 0/1 at baseline to KL 2/3 by 8-year follow-up, and non-progressors as no increase from KL 0/1 at baseline throughout the 8-year follow-up. Following differential expression analysis, we assessed predictive performance and identified putative gene targets for prioritized microRNAs.

Results: Comparing fast-progressors to both slow-progressors and non-progressors, we identified differentially expressed microRNAs within timepoints (i.e., 48 microRNAs at baseline and 2 microRNAs at 4-year follow-up) and across timepoints. Among these microRNAs were four members of the mir-320 family, with mir-320d showing an increase in fast-progressors at both timepoints, compared to both slow- and non-progressors. The predictive models we constructed included mir-320 family members and had good accuracy (area under the receiver operating characteristic curves ranging from 82.6 to 91.9) in distinguishing fast-progressors. Putative gene targets of the mir-320 family included members of the 14-3-3 gene family (Table 1), including YWHAE, whose downregulation in OA cartilage was reported to promote deterioration [2].

Table 1. Predicted gene targets of the mir-320 family include members of the 14-3-3 gene family.

<table>
<thead>
<tr>
<th>mir-320 family member</th>
<th>hsa-mir-320b</th>
<th>hsa-mir-320c</th>
<th>hsa-mir-320d</th>
<th>hsa-mir-320e</th>
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<tr>
<td>YWHAE</td>
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All mirDIP results with bold text indicating the prediction was among the top 1% for that microRNA/gene target pair. Letters denote the mirDIP score class with V=very high, H=high, M=medium, and L=low.

Conclusion: This microRNA-sequencing study is the first of its kind, profiling circulating microRNAs at two timepoints in 106 participants with data-driven construction of knee OA trajectories. We identify the mir-320 family of microRNAs to be associated with fast-progressing radiographic knee OA over time. While our data suggest this microRNA family could have applications as prognostic biomarkers for knee OA, and could be regulating gene targets to impact OA severity, validation of these findings in independent longitudinal cohorts is required.

REFERENCES:

Disclosure of Interests: None declared


POS0231

GENETIC BIOMARKERS, SNP GENES AND MTDNA HAPLOGROUPS, PREDICT OSTEOARTHRITIS STRUCTURAL PROGRESSORS THROUGH THE USE OF SUPERVISED MACHINE LEARNING


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Background: Knee osteoarthritis is the most prevalent chronic musculoskeletal debilitating disease. Current treatments are only symptomatic and to improve this, we need a robust prediction model to stratify patients at an early stage according to the risk of joint structure disease progression. Some genetic factors, including single nucleotide polymorphism (SNP) genes and mitochondrial (mt) DNA haplogroups/clusters, have been linked to this disease.

Objectives: For the first time, we aim to determine, by using machine learning, whether some SNP genes and mtDNA haplogroups/clusters alone or combined could form a new knee osteoarthritis structural predictors.

Methods: Participants (901) were first classified for the probability of being structural progressors. Genotyping included SNP genes TP63, FTO, GNL3, DUS4L, GDF5, SUPT3H, MCF2L, TGFA, mtDNA haplogroups H, J, T, Uk others, and clusters HV, TJ, KU.-Others. They were considered for prediction with major risk factors of osteoarthritis, namely, age, and body mass index (BMI). Seven supervised machine learning methodologies were evaluated. The support vector machine was used to generate gender-based models. The best input combination was assessed using sensitivity and synergy analyses. Validation was performed using 10-fold cross-validation as well as an external cohort (TASOAC).

Results: From 277 models, two were defined. Both used age and BMI in addition for the first one of the SNP genes TP63, DUS4L, GDF5, FTO with an accuracy of 85%; the second profits from the association of mtDNA haplogroups and SNP genes FTO and SUPT3H with 82.5% accuracy. The highest impact was associated with the haplogroup H, the presence of CT alleles for rs8044769 at mtDNA group F, the presence of CT alleles for rs8044769 at mtDNA group H, the presence of CT alleles for rs8044769 at mtDNA group H, and the absence of AA

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POS0232

IDENTIFICATION OF THE CORE REGULATORY GENE NETWORKS REGULATING SUBCHONDRAL BONE AND MARROW ADIPOSE TISSUE REMODELING IN HUMAN KNEE OA

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Background: Subchondral bone and the marrow adipose tissue (BMAT) contained therein undergo elevated turnover and remodeling during progression of osteoarthritis (OA). BMAT changes, detected as bone marrow lesions (BML) on MRI images, are considered important diagnostic and prognostic imaging biomarkers for OA. However, a comprehensive understanding of the regulatory mechanisms in individual BMAT-resident cell populations that mediate marrow fibrosis and subchondral bone sclerosis is still lacking. Recently generated single-cell transcriptomic atlas of human adipose tissue has opened new avenues for computational bulk tissue cell type deconvolution and dissecting gene regulatory networks.

Objectives: To infer core gene regulatory networks underlying subchondral bone sclerosis and BMAT remodeling in human knee OA.

Methods: We conducted comparative transcriptomics on bulk transcriptomic profiles of human subchondral bone tissue from three independent studies comprising tibial plateaus from non-OA controls (n=20) and BMLs (n=14), non-sclerotic (n=45) and sclerotic (n=45) regions from patients with medial knee OA. Cell type deconvolution (adipocyte/pre-adipocyte/fibroblast/endothelial/macrophage) of differentially expressed genes (DEGs) was performed using single-cell atlases of human brown and white adipose tissue. Cell type annotated DEGs were functionally annotated using Gene Ontology enrichment. To infer transcription factors (TFs) regulating gene networks we performed ChIP-Seq enrichment analyses and assessed the presence of TF binding sites in the proximal promoter (+500 to +100) sequence of DEGs. Tissue explants were stained en bloc with Oil red O and Hoechst to evaluate distribution of neutral lipids and nuclei in BMAT from non-sclerotic and sclerotic regions.

Results: 534 upregulated and 363 downregulated DEGs were shared between at least two independent datasets. Cell deconvolution revealed the majority of upregulated DEGs were expressed in fibroblasts (38%), pre-adipocytes (27%) and endothelial cells (22%). Downregulated DEGs were predominantly expressed by endothelial (31%), adipocyte (25%) and fibroblast (16%) cell populations. We inferred major TF regulatory networks driving upregulated DEGs in pre-adipocytes (PRRX1/SMAD4/TWIST1) and fibroblasts (SP7/SMAD4/RUNX2/ILX5) and loss of PPARG/SOX17/SNAI1-driven gene expression in adipocytes and endothelial cells. Pre-adipocyte genes were functionally enriched for collagen fibroin organization, ossification and cell migration. Fibroblast genes associated with biomineral tissue development and negative regulation of angiogenesis. Downregulated DEGs were enriched for triglyceride catabolism and sequestration (adipocytes) and regulation of vascular permeability and endothelial cell differentiation (endothelial). Whole mount staining displayed homogenous distribution of neutral lipids in adipocytes and low cell abundance in BMAT from non-sclerotic tissue. In contrast, BMAT from sclerotic tissue showed heterogeneously distributed and lower neutral lipid content as well as high cell abundance (Figure 1).

Figure 1.