

Synovial fluid-based endotyping reveals two major knee osteoarthritis subgroups

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INTRODUCTION: Osteoarthritis (OA) has a complex etiology and discovery of clinically relevant molecular endotypes that could be treated in a joint preserving fashion is key to find disease-modifying solutions. Until now, identification of a single biomarker in body fluids of OA patients that correlate to OA disease status or predict disease progression has largely failed. We hypothesized that the cellular response to synovial fluid (SF) of knee OA patients molecularly integrates all biomolecular cues in the synovial fluid (growth factors, cytokines, metabolites etc.), providing a novel agnostic opportunity to endotype OA. Previously, we have established that Nanoluciferase is the optimal reporter gene to report on signaling pathway activity responses in combination with OA synovial fluid (1), and we described the overall response of human chondrocytes to non-OA and OA synovial fluid (2). In this work we used our Nanoluciferase reporters to screen a large OA-SF collection for its capacity to differentially modulate signaling pathways and resulting transcription factor activity, providing a unique basis for knee OA endotyping.

METHODS: Lentiviral Nanoluciferase reporter constructs were generated for 13 distinct signaling pathways. SW1353 chondrocytic cells were transfected and selected with puromycin to generate stable reporter cell lines. The reporter assays were miniaturized to a 384 wells plate format to obtain a high-throughput assay. OA synovial fluid from end-stage knee OA patients (N=160) was obtained from total knee replacement surgeries (METC 2017-0183). Reporter cells were stimulated for 6 hours with 0.01-33% OA-SF (v/v) to determine the ideal OA-SF concentration for measuring cellular responses. Cluster analysis of reporter assay data was done with Gaussian mixture modelling and was used to identify OA subgroups. The protein composition of OA-SF subgroups was evaluated with a cytokine array (L-507, RayBiotech) in a pooled design using 4 distinct pools consisting of 4 donors (N=12) per group. Cut-off for the cytokine array was p. value < 0.05. The cellular response to the OA-SF subgroups was determined by RNA-seq using the same pooled design. Cut-offs for RNA-seq data analysis were log₂ fold change > 1.25 and FDR adjusted p. value < 0.01. KEGG and Reactome pathway analysis was used.

RESULTS SECTION: Six reporter cell lines were selected for a large-scale screening (NFκB-RE, CRE, AP1-RE, SRE, SRF-RE and SIE) using 0.5% OA-SF. This OA-SF concentration fell within the 20-80% response-window of the reporters to prevent saturation of the Nanoluciferase signal. Two groups were identified based on the integrated cellular response of all six reporters (Figure 1A). Group-1 (N=121 or 77% of total) was characterized by higher NFκB-RE (1.5x), CRE (1.9x), SRE (1.1) and AP1-RE (1.1x) activity, while Group-2 (N=37, 23% of total) was characterized by higher STAT3 signaling (1.1x). These two sub groups did not correlate with clinical parameters (K&L score, BMI, gender, diabetes, hypertension, etc.). Additional cytokine arrays were performed to further characterize these subgroups and 426 proteins were detected of which 14 were significantly different between both groups. RNA-sequencing was performed at 2 hours and 72 hours post-stimulation of SW1353 cells with the same OA-SF pools. A large number of 4030 genes were differentially expressed (2200 up, 1830 down) between Group-1 and Group-2 OA-SF pools after 72 hours of stimulation. No genes were differentially expressed between groups at 2 hours of stimulation. Pathway analysis revealed that KEGG's "ribosome", Reactome's "eukaryotic translation elongation" and "translation" pathways were the most differentiating pathways between both OA-SF subgroups. Upon closer examination, 70 from 86 core ribosomal protein genes were higher expressed in cells exposed to OA-SF from Group-2.

DISCUSSION: The cellular response to OA synovial fluids from 160 knee OA patients allowed us to identify two distinct OA subgroups. This cell-integrated response provides more biological information than analytical SF content analyses. A large number of gene expression changes supported differential activation of pathways by the two identified OA-SF subgroups at 72 hours post-stimulation. The ribosome and regulatory elements of protein translation were the top differentially regulated pathways and this finding supports our overarching hypothesis that OA constitutes an acquired ribosomopathy (3). Future work will focus on validation of cytokine array data and identification of the proteomic consequences for cells exposed to these two different OA-SF subgroups and how this relates to OA. In addition, our OA-SF collection consists of end-stage OA patients. It would be valuable to analyze earlier stages of OA with our cell reporter assays and establish changes for detection of early OA.

SIGNIFICANCE/CLINICAL RELEVANCE: Successful stratification of osteoarthritis patients in biologically relevant molecular endotypes is expected to enable disease-modifying intervention in a joint preserving treatment strategy.

REFERENCES: 1. Housmans & Neeffjes, et al. SciRep 2021. 2. Housmans, et al. OA&C 2022. 3. Van den Akker, et al. Curr Opin Rheumatol 2022.

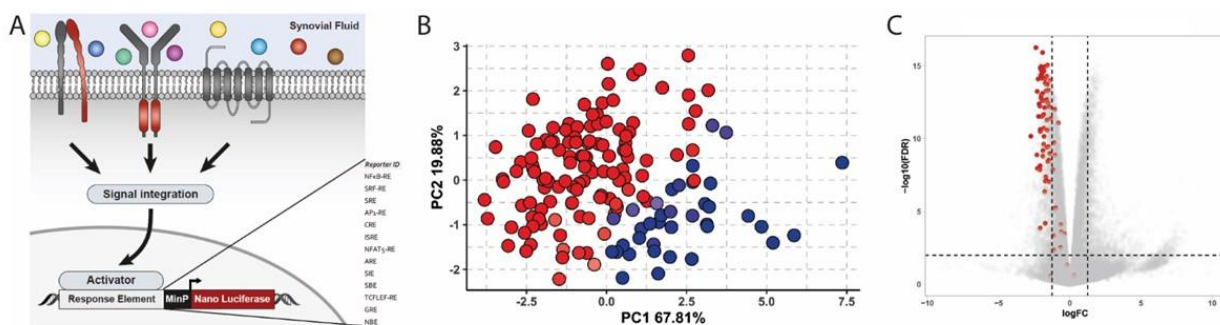


Figure 1: A) Synovial fluid was applied to reporter cells carrying specific transcription factor response elements for signal integration. B) PCA plot showing separation between group 1 (red) and group 2 (blue). C) Volcano plot showing the 2log FC (group1/group2) from RNA-seq. In red 86 core ribosomal proteins, dashed lines are cut-off value's.