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CIRCULATING MIR-126-3P IS ELEVATED IN LATE-STAGE RADIOGRAPHIC KNEE OSTEOARTHRITIS

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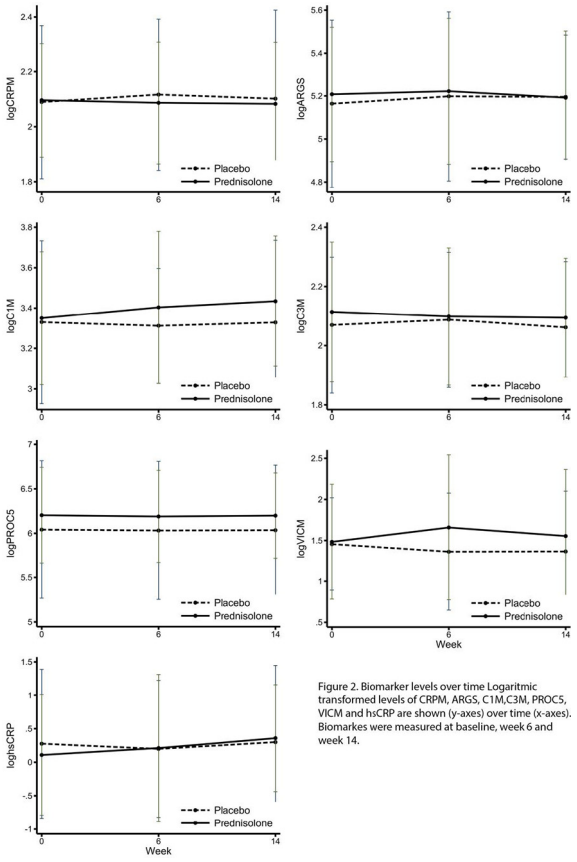

Figure 2. Biomarker levels over time Logaritmic transformed levels of CRPM, ARGS, C1M,C3M, PROC5, VICM and hsCRP are shown (y-axes) over time (x-axes). Biomarkes were measured at baseline, week 6 and week 14.

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BLOOD MICRO-RNAS DELINEATING SENESCENCE PHENOTYPES IN OSTEOARTHRITIS

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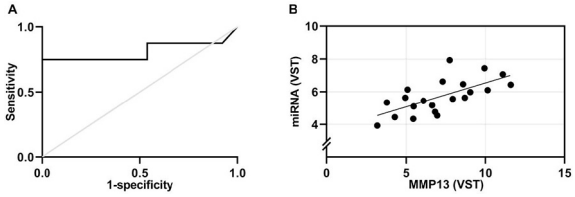
Purpose: The rapid increase of aged individuals is associated with a concomitant increase in chronic diseases, such as osteoarthritis (OA). A hallmark of such age-related degenerative diseases is the heterogeneous accumulation of senescent cells (SCs) affecting tissues by virtue of a well-defined senescence-associated secretory phenotype (SASP). In this regard, cellular senescence was shown to be a bona fide driver of OA in all stages in mice. Identification of non-invasive reliable biomarkers that sensitively reflect cellular senescence in human articular cartilage is essential to allow efficient monitoring and to evaluate therapy efficacy with e.g. senolytics. In the current study, we set out to characterize heterogeneity of cellular senescence within preserved osteoarthritis (OA) cartilage while identifying blood-biomarkers in the form of microRNAs (miRNAs).

Methods: By applying a literature search, 162 senescence genes were selected for in silico analyses in a previously assessed RNA sequencing dataset of 57 macroscopically preserved and 44 lesioned cartilage samples of the RAAK study. To reduce dimension of the transcriptomics senescence data, principal components analysis (PCA) was performed (genes were retained with a correlation |>0.4| to each component). Afterwards, Least Absolute Shrinkage Selector Operator (LASSO) analysis was applied with 5-fold validation to identify predicting genes per component. 399 circulating miRNA's of previously assessed miRNA-seq dataset of blood plasma (n=21 preserved OA samples overlapping with mRNA-seq cartilage dataset) were correlated to the components. After pre-selection, a panel of predicting circulating miRNAs was selected per component using LASSO analysis. Receiving operating curves (ROC) of circulating miRNAs panels was performed to estimate their predictive value on the components. After which, individual correlation between predicting genes and miRNAs per component was performed.

Results: To characterize the expression level and pattern of selected senescence genes (162 senescence genes), we performed *in silico* look up of a previously assessed RNA sequencing dataset of preserved (n=57) and lesioned (n=44) OA. From the total dataset of 162 genes (n=101), 137 genes were expressed in OA preserved and lesioned cartilage and were considered for further analysis. To investigate different senescence processes active in preserved OA cartilage, we performed a PCA analysis on the 137 senescence genes in cartilage (n=57) which identified 4 unique component, retaining 71% of the total variation, reflecting different networks of correlating senescence genes or subtypes. Pathway analysis with DAVID revealed NF-kappa Beta signalling pathway as the most significant (FDR 6.95E-07) and unique pathway of component 1 (Senescence subtype 1). In component 2 (senescence subtype 2), angiogenesis/VEGF pathway revealed to be important (FDR 1.21E-02 and 2.17E-02 respectively) with the involvement of *VEGFA*. These results suggest that different senescence processes are active in preserved OA cartilage such as NF-kappa beta in senescence subtype 1 and angiogenesis/VEGF pathways in senescence subtype 2. To identify gene expression patterns efficiently marking the active senescence subtypes we performed LASSO analyses. In total 23 and 28 genes were retained for subtype 1 and 2 respectively. In subtype 1, *PLAUR* had the highest positive effect with LASSO coefficient (beta, β) of 0.13. Moreover, other notable genes that marked senescent subtype 1 were *CDKN1A* and *MMP13* representing, respectively, both general and specific senescence processes. To identify non-invasive blood biomarkers for identified senescence subtypes, an overlapping plasma miRNA RNA sequencing dataset (n=21) was explored. LASSO analyses identified a panel of 4 circulating miRNAs marking subtype 1 specific active senescence processes. As can be seen in Figure 1A, these circulating miRNAs had a predicting value (area under the curve) of >0.80 reflecting the capability of the model to correctly classify cases in senescence subtype 1 with >80% chance, indicating clinical relevant predictive capabilities. Notably, individual miRNA's from this panel showed strong positive correlation to e.g. *MMP13* (r=0.68, Figure 1B).

Conclusions: By exploring a mRNA dataset of cartilage, we identified 4 different components reflecting heterogeneous senescence processes in preserved cartilage of OA patients. By subsequently integrating a miRNA dataset of blood plasma, potential new blood biomarkers in the form of miRNAs were identified marking senescence component 1 with high reliability. To be able to translate this further into the clinic, validation of the results is necessary. Together, these data contribute to a better understanding of the heterogeneity of the OA pathophysiology with the further development of diagnostic biomarkers to further advance the OA diagnostics.



(A) ROC curve of baseline characteristic with a panel of 4 miRNAs (black) and a reference line (grey), showing an area under the curve of >0.8. (B) Scatterplot of normalized expression counts of a representative individual cartilage gene (MMP13) and circulating miRNA of senescence subtype 1, showing correlation of 0.68.

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CIRCULATING MIR-126-3P IS ELEVATED IN LATE-STAGE RADIOGRAPHIC KNEE OSTEOARTHRITIS

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Purpose: There is an outstanding need to identify minimally invasive biomarkers for reliable detection of knee osteoarthritis (OA). Current clinical diagnostic methods are limited since OA symptoms do not always correlate with structural degeneration in the joint. Soluble biochemical markers provide a better readout of disease activity, and a variety of blood, synovial fluid, and urine biomarkers have been explored in OA, including microRNAs. As small, non-coding RNAs, microRNAs are promising biomarker candidates since they are easy to detect in biofluids, are relatively stable (i.e. resistant to enzymatic degradation), and can be reliably quantified such that levels can be linked to disease. Furthermore, microRNAs are known drivers of OA pathology, and their expression may precede joint degeneration, when opportunities for intervention still exist. Based on this, circulating microRNAs have strong potential to serve as biomarkers for knee OA, but a major limitation is lack of reproducibility across studies profiling circulating microRNAs in OA. While sequencing is the gold standard method for unbiased profiling of microRNAs, there are critical experimental design and analysis parameters that can impact the results. The objectives of this study are to identify circulating microRNAs in late-stage radiographic knee OA compared to non-OA controls using existing microRNA-sequencing data, and to validate the findings using our recently established Henry Ford Health System (HFHS) Osteoarthritis cohort.

Methods: We searched the literature for microRNA-sequencing studies profiling circulating microRNAs in OA versus non-OA participants and identified two studies, one conducted in Norway (Aae et al., 2020) and the other in France (Rousseau et al., 2020). We obtained raw sequencing data from the authors and re-analyzed the data by applying our recently reported method for microRNA-sequencing analysis. Among other changes (e.g. normalization method), we re-defined the cohorts to include participants with only Kellgren-Lawrence (KL) grades 3 and 4 in the OA group (compared to KL 0 to 4 and total knee arthroplasty in the original Norway study and KL 2 and 3 in the original France study) and with KL grade 0 in the non-OA group (consistent with the original Norway study and compared to KL 0 and 1 in the original France study). Following differential expression analysis using a multivariate model adjusted for age, sex, and body mass index, we prioritized microRNAs that were common to the OA groups in both cohorts. We next performed validation by real-time PCR in the HFHS Osteoarthritis cohort utilizing plasma samples from participants with unilateral and/or bilateral knee and/or hip OA and non-OA controls.

Results: As reported by the two original microRNA-sequencing studies, there were no significant differences in the Norway cohort and 3 differentially expressed microRNAs in OA (miR-139-5p, miR-1299, miR-200a-3p) in the France cohort, though none achieved validation in real-time PCR experiments. Following our re-analysis, we identified 23 and 82 differentially expressed microRNAs ($p<0.1$) in the Norway and France cohorts, respectively, with 3 microRNAs in common between the OA groups: miR-126-3p, miR-30c-2-3p, and miR-144-5p. Of these, miR-126-3p had the highest counts-per-million in both cohorts, showed an increased fold change in OA in both cohorts ($p<0.05$; Figure 1A and **1B**), and was found in 100% and 91% of OA samples and 0% and 35% of non-OA samples in the Norway and France cohorts, respectively. Furthermore, a report in 2014 by Borgonio Cuadra et al. identified circulating miR-126 to be elevated in OA (KL 2 and 3) compared to non-OA (KL 0) by both real-time PCR array and real-time PCR validation experiments (Figure 1C). This led us to explore miR-126-3p expression in plasma samples from the HFHS Osteoarthritis cohort where we found a consistent increase in knee OA (symptomatic, KL 3 or 4), irrespective of unilateral or bilateral, compared to non-OA controls (asymptomatic, KL 0), yet no significant increase in hip OA (Figure 1D).

Conclusions: Through application of our microRNA-sequencing analysis method, we identified circulating miR-126-3p to be increased in late-stage radiographic knee OA compared to non-OA controls in two studies originally reporting no validated differences. This finding is supported by previous literature identifying circulating miR-126 to be elevated in knee OA compared to non-OA controls and is extended by our data showing that the increase may be unique to knee OA and not hip OA. Taken together, there are now data from four independent cohorts demonstrating an increase in circulating miR-126-3p in knee OA, suggesting that this microRNA may have utility as a biomarker for OA.

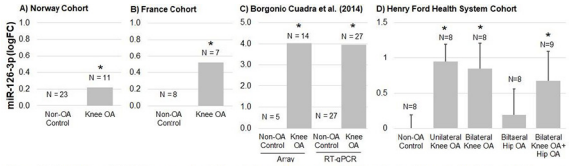

Figure 1. Circulating miR-126-3p expression in four independent osteoarthritis cohorts. All panels depict log fold change (logFC) in miR-126-3p expressed relative to non-OA controls and asterisks denote $p \leq 0.05$. A) Data re-analyzed from Aae et al. (2020; Norway Cohort) using counts-per-million. B) Data re-analyzed from Rousseau et al. (2020; France Cohort) using counts-per-million. C) Data presented as reported in Borgonio Cuadra et al. (2014). D) Data generated from the Henry Ford Health System Osteoarthritis Cohort using $\Delta\Delta C_T$. Bars = 95% confidence intervals.

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PROTEOMIC ANALYSIS OF INCIDENT AND PREVALENT HAND AND KNEE OSTEOARTHRITIS IN THE FRAMINGHAM HEART STUDY

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Purpose: Osteoarthritis (OA) at the knees and hands may not share the same etiology. For example, pathogenesis of large joint OA like knee OA may be determined in large part by mechanopathology, while hand OA has been long considered the consequence of metabolic or systemic influences. Identifying shared aspects of disease across different joint sites may provide insights into relevant pathways that may be commonly targeted across different OA joint sites. We aimed to determine whether there are proteins associated with both knee and hand OA and whether these proteins are associated with disease incidence and prevalence.

Methods: We analyzed data from the Framingham Heart Offspring study, a longitudinal community-based study that began in 1971 with the recruitment of 5,124 individuals who were children of the Original Study cohort and their spouses. Of the 3,236 participants who had a visit in 1991-1995 and had available plasma samples, 1,913 individuals had proteomic profiling using the SOMAscan platform (version 1.1, which contained 1,129 aptamers or version 1.3, which contained 1,305 aptamers). In each batch, age and sex-adjusted protein values were log transformed and standardized to a mean = 0 and standard deviation (SD) = 1. The median intra-assay CV was < 4% and median inter-assay CV was < 7% across batches. A subset of 625-636 Offspring study participants had x-ray imaging of the hands and knees, respectively, in 1991-1995 (mean age = 55 years, range = 32-79 years, 57% women) and again in 2002-2005 (~9.5 years later). We defined prevalent OA when a person had radiographic OA of KL grade >=2 in at least one joint in knee or hand at the initial exam in 1991-1995. We then excluded persons with prevalent OA in the target joints and defined incident disease as the new development of radiographic OA grade >=2 in 2002-2005. We conducted logistic regression adjusted for age, sex, and technical covariates to identify proteins associated with the odds of prevalent hand and/or knee OA in 1991-1995 (P-value < 0.05). We then assessed whether the identified proteins were also associated with the odds of incident hand and/or knee OA in 2002-2005 (P-value < 0.05).

Results: We identified 103 plasma proteins associated with knee OA (57 positive and 46 negative) and 52 plasma proteins associated with hand OA (32 positive and 20 negative) at the baseline visit in 1991-1995. Of these proteins, 10 plasma proteins were also associated with incident knee OA and 8 plasma proteins were also associated with incident hand OA at the follow-up visit in 2002-2005 (P-value < 0.05) (Table 1). We identified two proteins that were significantly associated with both prevalent and incident hand and knee OA, showing consistency of effects in both the knees and hands: Factor I, which regulates complement activation, and IGFBP-1, an insulin-like growth factor-binding protein, known to influence cartilage metabolism. Given the exploratory nature of our study and small sample, we report here nominal findings. One protein achieved an FDR < 0.05, IL-17D, which was