Analysis of Human Cartilage Reveals Distinct Metabolomic Profiles Between Osteoarthritis and Healthy Patients

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INTRODUCTION: Osteoarthritis (OA) is a chronic joint disease that is characterized by the breakdown of articular cartilage (AC) and other joint tissues over time. The imbalanced activity between matrix anabolism and catabolism contributes to the observed changes in AC and other tissues affected by OA. Previous studies explored altered metabolism in various OA tissues, both *in vivo* and *in vitro*, to identify disease-associated metabolic activity. However, a significant limitation in many of these studies is the absence of healthy controls which hinders the comprehensive understanding of the role of metabolism in OA development. To fill this gap in knowledge, we compared metabolomic profiles from radiography-confirmed end-stage OA (KL grades III and IV) with healthy cartilage samples using liquid chromatography-mass spectrometry (LC-MS) metabolomic profiling. Global metabolomic profiling is advantageous because it detects thousands of metabolites, enabling the generation of biochemical signatures that represent the overall physiological state of the tissue. Thus, the objective of this study was to identify disease-associated profiles to shed light on the pathological mechanisms underlying OA. Furthermore, tandem LC-MS (LC-MS/MS) was used to identify potential biomarkers or drug targets that could slow, stop, or reverse OA progression. With this approach, we aim to uncover specific metabolic patterns and identified metabolites that may serve as valuable indicators of disease status or therapeutic targets.

METHODS: Under IRB approval, 35 femoral heads were obtained following total joint arthroplasty from local musculoskeletal clinics with patient information including age, sex, height, and weight. Additionally, healthy cartilage samples were obtained from Articular Engineering to serve as healthy controls for comparison. All cartilage samples (n=35 OA, n=10 healthy) were cut into shavings, submerged in 3:1 methanol:water, and homogenized using a tissue grinder. Metabolites were extracted using our established protocol for cartilage consisting of centrifugation, protein precipitation, and vacuum concentration. Metabolite extracts were subsequently analyzed by LC-MS and processed via XCMS. MetaboAnalyst was used to statistically analyze and visualize metabolic differences between healthy and OA cartilage using hierarchical cluster analysis, principal component analysis (PCA), partial least squares-discriminant analysis (PLS-DA), t-test, fold change, and volcano plot analysis. Populations of metabolite features identified as discriminatory by statistical analyses underwent metabolic pathway analysis to detect biologically relevant pathways. Significance was determined with a false discovery rate corrected $p < 0.05$. Pooled samples that were subjected to LC-MS/MS were analyzed using Progenesis QI. HMDB was used to compare acquired and theoretical fragmentation patterns for metabolite identification purposes.

RESULTS: To visualize metabolic differences between healthy and OA cartilage, HCA (unsupervised), PCA (unsupervised) and PLS-DA (supervised) were used. All clustering techniques displayed clear separation of healthy and OA cartilage demonstrating distinct metabolomic profiles (Fig. 1). T-test, fold change, and volcano plot analyses were performed to distinguish dysregulated populations of metabolite features between groups (Fig. 2A-C). Volcano plot analysis identified 987 metabolite features that were higher in abundance in OA cartilage compared to healthy (Fig. 2B). These metabolite features mapped to numerous lipid-related pathways (Omega 3 & 6 fatty acid metabolism, fatty acid activation & oxidation, polyunsaturated and saturated fatty acid beta-oxidation, glycosphingolipid metabolism). Conversely, 1,193 metabolite features were higher in abundance in healthy cartilage compared to OA cartilage. These metabolite features mapped to amino acid metabolism (methionine, cysteine, histidine, tryptophan, glycine, serine, alanine, threonine) and purine metabolism. Using LC-MS/MS data, histidinyl-histidine was identified and highest in abundance in healthy cartilage, whereas PC (14:0/0:0) was highest in OA cartilage (Fig. 2D) amongst other identified metabolites. Collectively, these findings unveil specific metabolites and metabolic pathways that show altered cellular mechanisms of OA and improve our understanding of OA pathophysiology.

DISCUSSION: While altered metabolism is increasingly recognized as a crucial factor in OA development, further investigation is needed to understand the role of aberrant metabolism in OA pathophysiology. The results of this study demonstrate that human cartilage has distinct metabolomic profiles between healthy and end-stage OA patients. By taking a comprehensive approach to assess differences between the metabolomes of healthy and OA cartilage, several metabolic pathways with distinct regulation patterns were detected. Specifically, metabolites associated with lipid metabolism were upregulated in OA cartilage compared to healthy, aligning with previous studies on OA synovial fluid affected[1,2]. This dysregulation may signify that the lipid profile associated with OA is both diverse and distinct from healthy controls. Additionally, upregulation of lipid-related pathways may indicate a bioenergetic shift to generate ATP and reflect mitochondrial alterations in OA. Conversely, amino acid metabolism was significantly downregulated in OA cartilage compared to healthy controls. The magnified downregulation of amino acid-related pathways, like glycine and histidine, supports previous studies that have found decreasing trends in these amino acids as disease progresses[3]. This observed dysregulation of amino acids may indicate their potential role in responding to disease and reflect the degree of joint damage. Taken together, the detected alterations of amino acids and lipids highlight key differences in bioenergetic resources, structural degradation, and mitochondrial dysfunction in OA cartilage compared to healthy. Considering these metabolic signatures, a greater understanding of altered cartilage metabolism in OA may lead to the identification of candidate biomarkers and drug targets to slow, halt, or reverse cartilage damage in end-stage OA.

SIGNIFICANCE/CLINICAL RELEVANCE: The results of this study provide clear evidence of OA-induced metabolic perturbations in human articular cartilage. With this approach, we uncovered specific metabolic patterns and identified metabolites that may serve as valuable indicators of disease status or therapeutic targets.

REFERENCES: [1]Bastiaansen-Jenniskens+ (PMID: 26069676); [2]Van De Vyer+ (PMID: 30203669); [3]Zhang+ (PMID: 25861152)
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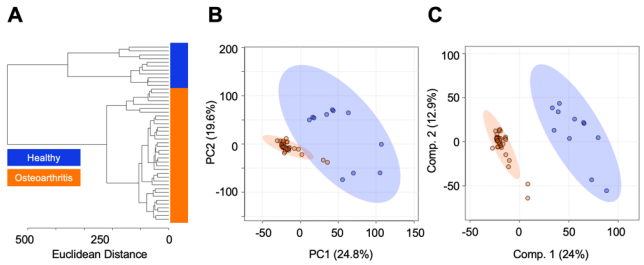

Figure 1. Metabolomic profiles of human articular cartilage from healthy and OA patients are metabolically distinct. (A) HCA. (B) PCA. (C) PLS-DA. Orange = osteoarthritis. Blue = healthy.

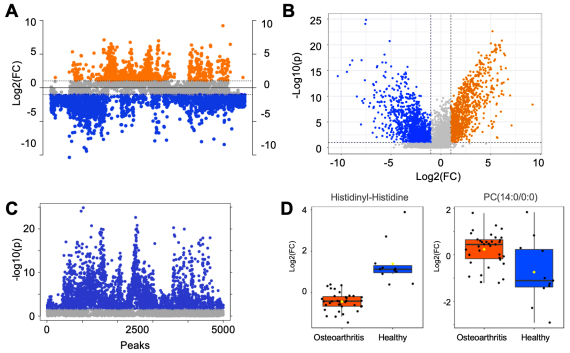

Figure 2. (A-C) Patterns of metabolites and (D) identified metabolites statistically differ in abundance between healthy and osteoarthritic articular cartilage. (A) T-test. (B) Fold Change. (C) Volcano Plot. (D) Identified metabolites: Histidinyl-Histidine and PC (14:0/0:0). Orange = osteoarthritis. Blue = healthy.