

Characterization of regenerated cartilage after knee joint distraction, a human case-study

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INTRODUCTION: Knee joint distraction (KJD) is a joint-preserving surgical procedure in which cartilage-regenerating effects are observed. No human studies have been reported in which the proteomic landscape of the regenerated cartilage tissue have been dissected. In this case study a patient underwent bilateral KJD, which was followed by total knee arthroplasty (TKA) several years later. The objective of this study was to compare the proteome of KJD-regenerated cartilage to the proteome of corresponding contralateral native cartilage originating from the same patient, in order to acquire a unique insight into the proteomic nature of the KJD-regenerated cartilaginous tissue.

METHODS: This study was conducted under Medical Ethical Approval (METC: 2017-0183) and additional individual patient consent. KJD treatment was performed using an external fixation frame consisting of two monotubes, fixed to the femur and tibia on the lateral and medial side of the joint with four pairs of bone pins. The joint was distracted 2 mm at surgery, and gradually extended by 1 mm per day until 5 mm distraction was reached and radiographically confirmed. TKA was performed using the Attune posterior stabilized system with fixation using GentaPalacos cement. Before, during and after clinical interventions, X-rays were acquired for radiographic assessment of the knee joint, classifying the severity of the joint by Kellgren and Lawrence scale. After TKA, cartilage biopsies were collected from the femoral head for the medial KJD-regenerated cartilage and lateral native cartilage from the same knee and processed for proteomics analysis. After LC-MS/MS, proteins were identified using Mascot (Matrix Science) and quantified with Progenesis-QI (non-linear Dynamics). For data analysis and statistics, consistent distributions of the proteomics data, a median based normalization with log₂ transformation was applied. Thereafter a simple t-test was performed to use the raw unadjusted p values to rank the proteins and perform data-analysis. Thereafter pre-ranked Gene Set Enrichment Analysis was applied to test for enrichment at the top and bottom of the ranks.

RESULTS SECTION: Longitudinal Kellgren-Lawrence classification of both knees showed improvement between pre-KJD and post-KJD, which was still present pre-TKA. Optical examination of the TKA-explanted knee joint tissues showed that the KJD-regenerated medial femoral cartilage tissue was remarkably white and presented with a relatively irregular surface. This was in contrast to the native cartilage, which had a yellow appearance and smooth surface. Biochemically, Safranin-O staining of corresponding tissue sections showed a lower GAG content in the KJD-regenerated cartilage compared to the native cartilage. Proteomic analysis of cartilage biopsies from KJD-regenerated cartilage versus native cartilage from both knees did not reveal major differences for cartilage extracellular matrix proteins such as COL2A1, COMP, FMOD, FN1, DCN, and ACAN. Using pre-ranked KEGG Gene Set Enrichment Analysis (GSEA), we found enrichment of the leading-edge subsets of the associated gene sets including ribosomal proteins in the KJD-regenerated cartilage (core enrichment consisted of RPL23, RPL28, RPL19, RPS2, RPL11, RPS3, RPL26L1, RPL24, RPL18A, RPL9, RPL23A, RPL3L, RPL31, RPL13, RPS12, RPL18 and RPL7A). When using pre-ranked Reactome GSEA, we found highest normalized enrichment in the RNA Polymerase II Transcription Termination set (SRSF3, SNRPB, SRSF7 and SNRPD3) for the KJD-regenerated cartilage. Furthermore, proteins that were in general the highest upregulated in native cartilage include MYO1C, GDF10, PGK2 and ATP5F1A and proteins that were highest upregulated in KJD-regenerated cartilage include RPL19, RPS2, RPL23 and RPL11. Taken together, these results indicate that protein translation processes are more active in the KJD-regenerated cartilage.

DISCUSSION: This unique case study demonstrates striking proteomic similarities between KJD-regenerated cartilage compared to native cartilage. Levels of major extracellular matrix protein components are indistinguishably present in the KJD-regenerated cartilage. Remarkably, we found a strikingly higher expression of proteins that are part of the core ribosome or that act in protein translational processes. This indicates that protein translation processes are more active in KJD-regenerated cartilage, which would make sense from a molecular tissue regeneration perspective. While this is a case study with an intrinsic statistical power issue, this unique study for the first time describes the proteomic landscape of human KJD-regenerated cartilage by comparing KJD-regenerated cartilage with native cartilage from two knees of the same patient.

SIGNIFICANCE/CLINICAL RELEVANCE: This human case study is the first of its kind describing the proteomic landscape of KJD-regenerated cartilage and compare it to native cartilage from the same knee. Changes found at the protein extracellular matrix level in KJD-regenerated cartilage compared to native cartilage and the identification of a protein translation regenerative signature in the KJD-regenerated cartilage underpins the cartilage regenerative potential of KJD as a joint preserving treatment, while at the same time provides potential avenues to molecularly catalyze the KJD treatment outcome.