Results: Of 535 subjects with CE MRI, 453 subjects had complete data on synovitis and WOMAC pain. Mean age was 59.2±7.2 years, mean BMI 29.7±5.0, and 48% were women. Moderate/severe synovitis was uncommon (15% of knees) but of knees with at least moderate pain, 49% had moderate/severe synovitis with 4.8 times increased odds of knee pain compared to those without synovitis. Other WOMAC definitions of pain severity yielded similar results. Among knees without ROA (n = 297), while few had moderate/severe synovitis, synovitis itself was still associated with increased prevalence of pain.
Conclusions: Moderate/severe synovitis has a strong relation with knee pain severity, an association detected more clearly with CE MRI than suggested by prior studies using nonCE MRI measures of synovitis.

Proteomics & Metabolomics

473 DIFFERENTIAL PROTEOME OF ARTICULAR CHONDROCYTES FROM PATIENTS WITH OSTEOARTHRITIS

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Purpose: Cartilage damage is a major problem in osteoarthritis (OA). To gain insight into the pathogenesis of OA, we have analyzed the differential proteome of articular chondrocytes from these patients.
Methods: Protein extracts were prepared from cultured chondrocytes from 6 patients with end-stage OA and 6 normal donors without special radiographic signs of OA. Samples were then analyzed by 2D gels using the DIGE approach. Gel image analysis was performed using the DeCyder Differential Analysis Software Release 6.5 and statistical module EDA 1.0 (GE Healthcare). Protein spots corresponding to statistically significant expression differences were excised from the gels and submitted to tryptic digestion, and the resulting peptides were mass analyzed using an Ultraflex MALDI-TOF/TOF mass spectrometer (Bruker-Daltonics). Protein identification was achieved through database searching with MALDI MS and MS/MS data.
Results: Significant differential expression was observed for 27 proteins, with 14 underexpressed and 13 overexpressed chondrocyte OA proteins. Of special interest was the identification of destrin, cofilins, gelsolin, annexin A2, glycolytic enzymes, chaperones, cathepsin D, proteasome beta 9 subunit isoform 2 proprotein and proteasome activator hPA28.
Conclusions: The altered expression of these proteins is consistent with events such as cytoskeleton binding protein disruption, apoptosis, and glycolysis, demonstrating the ability of the 2D-DIGE/MS platform to identify proteins with altered expression in chondrocytes from patients with end-stage OA. The identification of these proteins may open new lines of research for this disease.

474 A ROBUST METHOD FOR PROTEOMIC CHARACTERIZATION OF MOUSE CARTILAGE USING SOLUBILITY-BASED FRACTIONATION AND TWO-DIMENSIONAL ELECTROPHORESIS

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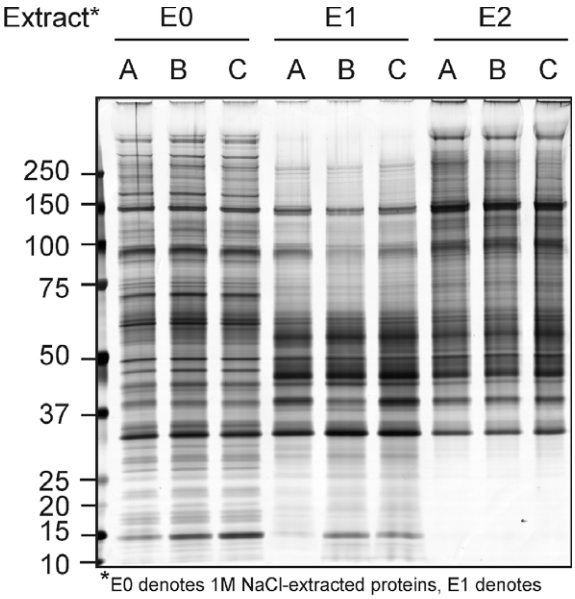
Purpose: There is increasing interest in proteomic techniques for global profiling of normal and pathological cartilage samples, to elucidate underlying disease mechanisms identify novel biomarkers. Identification of protein expression differences using two-dimensional electrophoresis (2-DE) and liquid chromatographic (LC)-based proteomics depends critically on reproducibility throughout sample preparation and analysis. This applies particularly where sample fractionation is used to remove high abundance or interfering components to facilitate deeper mining of the proteome. Here we have developed and validated a procedure for solubility-based cartilage sample fractionation and reproducible resolution by 2-DE.
Methods: Triplicate independent *sequential* extractions were performed on mouse femoral head cartilage. Pulverized explants were digested with chondroitinase ABC then extracted in Tris acetate containing 1 M NaCl for 24 hrs (E0 fraction). The NaCl-insoluble fraction was then further extracted for 18 hrs in sodium acetate containing 4 M GuHCl (E1 fraction). Finally, to prepare samples for 2-DE, high molecular weight components that prohibit isoelectric focusing (hyaluronan and aggrecan) were removed by 100 kDa cut-off centrifugal filtration (E2 fraction). Triplicate E0, E1 and E2 fractions were first profiled by SDS-PAGE. E0 and E1 were then resolved by 2-DE in triplicate to compensate for variation in 2-DE and silver staining and the 18 gel images were analyzed in ImageMaster. The E0 and E1 extracts were characterized by identification of protein spots by tandem mass spectrometry (MS).
Results: The 1-D profiles of E0, E1 and E2 fractions were highly consistent between extractions (see figure). Centrifugal filtration caused partitioning of some proteins <100 kDa into the E2 fraction but this was also consistent between samples. E0 and E1 fractions produced distinct protein 2-DE spot patterns, with greater complexity in E0. Automated spot analysis reported 70% spot matching in E0 gel triplicates and 75% matching in E1 gel triplicates, representing approximately 600 and 500 matched spots, respectively. E0-specific spots were mostly cellular proteins, e.g. BiP, triosephosphate isomerase and gelsolin, whereas E1-specific spots were abundant matrix proteins, e.g. collagen VI, matrilins 1 and 3, and lactadherin. Interestingly, some specific proteins such as link protein and beta-actin partitioned almost equally between E0 and E1 extracts. MS results were validated by immunoblotting.



*E0 denotes 1M NaCl-extracted proteins, E1 denotes 4M GuHCl-extracted proteins and E2 denotes proteins removed by 100 kDa cut-off centrifugal filtration

Conclusions: This study has, using the minimal amounts of tissue available from mouse tissue, established a new approach to 2-DE based analysis of cartilage extracts. This method can be used to enrich one or both protein fractions for deeper mining of the cartilage proteome and to investigate cellular mechanisms and matrix components involved in developmental and degenerative cartilage disease.

475 PROTEOMIC CHARACTERIZATION OF MOUSE CARTILAGE DEGRADATION *IN VITRO*

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Purpose: To develop proteomics to analyze mouse cartilage degradation and correlate transcriptional and translational responses to catabolic stimuli.
Methods: Proteomic techniques were used to analyze catabolism in mouse femoral head cartilage. Using specific methods to prepare cartilage extracts and conditioned media for two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) and subsequent tandem mass spectrometry (MS), we identified novel proteins and fragments released into the media of control, interleukin-1α (Il-1) and all-*trans*-retinoic acid (RetA)-treated explants. Fluorescent difference gel electrophoresis (2-D DIGE) was used to quantify protein expression changes. We also measured changes in mRNA expression to distinguish transcriptional and post-translational regulation of released proteins.
Results: We identified 20 differentially-abundant proteins in media from control and treated explants, including fragments of thrombospondin-1