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PROTEOMIC OF THE HUMAN NORMAL ARTICULAR CHONDROCYTES

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Introduction: The chondrocyte is the only cell type present in mature cartilage, and it is important in the control of matrix turnover. There is currently a great lack of knowledge about the chondrocyte proteome. 2-dimensional electrophoresis (2-DE) is a powerful technique for resolving complex mixtures of proteins into individual polypeptides for analysis by mass spectrometry.
Aim: To obtain a 2-DE proteome map of the human normal articular chondrocyte.
Material and Methods: Cells were isolated from five cartilages collected from autopsies without history of joint disease. Cultured cells were used to obtain protein extracts which were resolved by 2-DE and visualized by silver nitrate or Coomassie blue staining. Spots were excised from the gels and analyzed using matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) or MALDI-TOF/TOF mass spectrometry. Proteome of chondrocytes was compared with proteome of other mesenchymal cells (Jurkat and fybroblastic sinovial cells).
Results: A total of 124 proteins from 180 excised spots (69%) were identified by means of peptide mass fingerprinting (PMF). Further 12 samples that could not be recognized with the information available in the PMF were identified by tandem mass spectrometry using MALDI-TOF/TOF technology. All together, our analysis leads to the identification of 136 spots that represent 93 different proteins. A significant proportion of proteins are involved in cell organization (26%), energy (16%), protein fate (14%), metabolism (12%), and cell stress (12%). From all identified proteins, cathepsin D, Hsp47, mitochondrial superoxide dismutase (SODM), cytoskeleton-related proteins (vimentin, transgelin and destrin), and members of the annexin family were more abundant in chondrocytes than in other types of mesenchymal cells.
Conclusion: As the metabolic program is altered in osteoarthric chondrocyte, this proteome map is an important tool for future pathogenic studies on this pathology.

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NITRIC OXIDE EFFECTS ON THE MITOCHONDRIA OF HUMAN OSTEOARTHRITIC SYNOVIOCYTES

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Aim of study: To investigate the effect of NO on fibroblastic synovial cells on the mitochondrial activity as well as its relation with the apoptosis.
Methods: Human OA synovium was obtained from 6 patients who were undergoing hip joint replacement. The level of cell death was evaluated by microscopy (DAPI), MTT and LDH assay. Mitochondrial function was evaluated analyzing mitochondrial membrane potential (MMP) and ATP production as well as respiratory chain enzyme complexes and citrate synthase (CS) activities. Variations in MMP were measured by DePsipher dye using flow cytometry. Intracellular ATP was quantified by a luminescence detection assay. The activities of the mitochondrial respiratory chain (MRC) complexes (complex I: NADH CoQ_1 reductase, complex II: succinate dehydrogenase, complex III: ubiquinol-cytochrome *c* reductase, complex IV: cytochrome *c* oxidase) and CS were measured spectrophotometrically. Protein synthesis analysis was performed by Western-blot. SNP (Sodium NitroPrusside) was used as a NO compound donor (Alexis).
Results: SNP at 0.5 mM for 24, 48 and 72 hours induced cellular death (percentage of survival: 86.11±4.39, 85.51±5.91 and 77.85±6.46 respectively vs basal 100%; $p<0.05$) and loss of the LDH enzyme at 12 and 24 hours (4.51±0.39 and 4.74±0.48 respectively, vs positive control 100%; $p<0.01$). Furthermore, SNP 0.5 mM induced depolarization of the mitochondrial membrane in 62% of cells at 12 hours. In addition to this, the time course analyses of treatment with SNP at 0.5 mM for 6, 24, 48 and 72 hours demonstrated that treatment with SNP reliably and significantly reduced the intracellular ATP synthesis (at 48 hours 75±12.94 vs basal 100%; $p< 0.05$). However, the analysis of the MRC showed that, at 48 hours, the NO donor at 0.5 mM increased the activity of complex I and III (64.32±13.41 vs 40.36±5.84 and 145.05±30.96 vs 62.4±9.74 nmol/min/mg protein respectively). Finally, SNP downregulated bcl-2, mcl-1 and the caspase 3 proenzime.
Conclusions: This study suggests that the NO reduces the OA synoviocytes survival. Its effect can be mediated by its activity on mitochondrial function.

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IDENTIFICATION OF TRANSCRIPTIONAL CHANGES DURING INITIATION OF OSTEOARTHRITIS IN A CANINE MODEL

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Aim of Study: The aim of this study was to identify transcriptional changes that occur in osteoarthritis pathogenesis by utilizing a canine model of OA.
Methods: *Canine OA model:* Dogs were hound crosses ranging in age from 10 months to 4 years at the time of surgery. Transection of the anterior cruciate ligament (ACLT) was performed under general anesthesia in an IACUC-approved protocol. Sham surgery (opening of the joint without anterior cruciate transection) was performed on the contralateral knee of a subset of dogs. Articular cartilage was harvested post-surgery at time points ranging from 1 to 39 months.
RNA isolation and Microarray analysis: RNA was extracted from frozen pulverized articular cartilage tissue from individual joints and subjected to gene expression profiling analysis using the Affymetrix whole genome canine array.
Statistical Analysis: A mixed model analysis was employed to allow multiple known sources of variability to be identified. An initial data filter was instituted, requiring a gene to be called "present" in at least one sample, and at least one sample must have a signal intensity of greater than 100 Signal Units. All P values were subjected to false discovery rate (FDR) correction and the data was filtered for an FDR $<$ 1% (0.01) in any test. Cluster analysis using Ward's Method in Spotfire determined the ability of significantly regulated genes to segregate the cartilage samples as a result of the treatment or time post surgery.
Results: Surgically-induced knee instability in dogs formed the basis for an experimental model of OA resulting in progressive destructive changes in the articular cartilage over a period of 39 months. Thus, the cartilage samples obtained from the knees of these dogs from 1 month to 39 months post surgery allowed study of gene regulation throughout initiation and progression of disease. Cluster analysis showed good segregation between surgery and sham cartilage samples isolated from the early time points. Analysis of later time points showed segregation more by animal, indicative of a strong donor effect. Significantly dysregulated genes were binned into networks by Ingenuity to uncover