from the cartilage samples. Focusing on the healthy and OA cartilage samples, the identified transcripts included known nuclear and mitochondrial genes, as well as previously unannotated chromosomic regions. The most abundant transcripts in both sets of samples included DCN, GPX3, CLU or VIM. Selection of differentially expressed genes produced a list of 85 and 91 genes overrepresented in HNC and OA, respectively. Some of them coded for known markers of cartilage integrity (EGR1) or cartilage damage, (COMP, CRTAC1, CTGF…). Among genes overexpressed in HC we found DPP10, CSDA and the ribosomal proteins RPL11 and RPL41. To identify genes negatively or positively associated to the OA process, we performed RT-PCR quantitation of selected genes. We confirmed overexpression of NFKBIA, OSMR and CSDA in healthy cartilage. In the case of CSDA, western blot analysis also suggested a distinctive protein pattern between healthy and OA cartilage. Interestingly, this gene is a known positive regulator of CDKN1A and EGR1, genes that were also found overrepresented in the healthy cartilage samples.
Conclusions: Data mining of sequences stored in public databases can be a useful tool to identify genes involved in biological process. We have been able to identify a set of genes differentially expressed in normal and OA cartilage. Confirmation of the results on clinical samples has revealed CSDA as a potential mediator of cartilage integrity.

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FRZB IS A CRITICAL MODULATOR OF CANONICAL WNT SIGNALLING IN CARTILAGE BIOLOGY

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Purpose: Polymorphisms in Frizzled-related protein (FRZB), a WNT antagonist, have been associated with osteoarthritis (OA). However, a recent meta-analysis failed to find a consistent effect of FRZB genetic variants on OA susceptibility. Our transcriptomics analysis in Frzb-/- mice provided evidence for a tight regulation of WNT signalling and highlighted the complex role for FRZB in joint homeostasis. We previously demonstrated that Frzb-/- mice have increased damage when dramatically challenged by papain, collagenase or severe inflammation. As these models are acute and short-term, we aimed to further investigate the effect of Frzb loss in a true translational model of OA and to study molecular interactions in the ATDC5 micromass in vitro model using RT-PCR and Western blot analysis.
Methods: Surgical destabilization of the medial meniscus (DMM) was performed on the right knee of eight-week-old male Frzb-/- and wild-type mice. Eight weeks after surgery, mice were sacrificed and histological scores were determined for the femoral and tibial articular surfaces following the OARSI histopathology initiative guidelines.
Results: Overexpression of Frzb in ATDC5 micro-masses boosted chondrogenesis with up-regulation of Col2a1 and Aggrecan transcription, whereas downregulation of Frzb lead to a decreased expression of Col2a1 and Aggrecan. These results corresponded with a reduction or increase in the activation of canonical WNT signalling pathway, respectively. Fluctuating levels of Frzb did not influence the Wnt/CamKII signalling pathway. The semi-quantitative OARSI score showed a significant increase in cartilage erosion in DMM-operated Frzb-/- mice compared to wild-type.
Conclusions: Our data show that, in addition to the higher susceptibility to OA in acute induced models, Frzb-/- mice are more prone to OA in a full translational model of the disease characterized by slowly progressive joint damage. Overexpression of Frzb stimulates chondrogenesis by its inhibitory role on WNT/β-catenin signalling.

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STUDY OF DEAMIDATION OF CARTILAGE EXTRACELLULAR MATRIX PROTEINS TO INVESTIGATE CARTILAGE TURNOVER

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Purpose: Under physiological conditions proteins are susceptible to various non-enzymatic covalent modifications such as racemization, isomerization, and deamidation. These modifications may affect the structure, function, charge, and stability of proteins. One of these modifications, deamidation (the hydrolysis of the amide group on the side chain of Asn or Gln to form Asp and Glu) has no enzymatic repair system and may accumulate in a time-dependent manner. This phenomenon could function as a molecular clock since deamidation occurs spontaneously at a regular rate. However, deamidation can only be observed when it occurs within the lifespan of a protein because newly synthesized proteins eventually replace deamidated proteins. Under these circumstances, deamidation could be utilized to determine the half-lives of in vivo proteins and could provide protein tags to monitor the level of turnover and repair of a tissue. To prove this concept, we have investigated deamidation of two cartilage matrix proteins, aggrecan core protein and cartilage oligomeric matrix protein (COMP) and identified deamidation residues within both these proteins by mass spectrometric evaluation of cartilage extracts. The result of this study will serve as the pilot data for a quantitative approach to describe in vivo protein turnover.
Methods: To achieve the goals of this research, we have optimized the procedure of extracting proteins from cartilage extracellular matrix (ECM) without confounding by intracellular proteins of chondrocytes. The optimized procedure relies on depletion of chondrocytes from frozen tissue sections based on their sensitivity to change in osmotic pressure and ice crystal formation; we use serial rapid freeze/thaw cycles (between -20°C degrees and 25°C) in hypotonic solution (deionized H2O). Any few remaining chondrocytes after treatment with hypotonic solution are depleted by laser capture microscopy (LCM). To overcome the limitation of very small quantities of protein from frozen sections, we perform in situ hyaluronidase and trypsin treatment in ammonium bicarbonate buffer to efficiently release the peptide fragments into a buffer compatible with mass spectrometry. We subsequently performed qualitative screening by 1D reverse phase LC coupled with tandem mass spectrometry in a DDA (Data Dependent Acquisition) mode of acquistion. To obtain the broadest coverage of cartilage proteins, we subsequently performed 2D 5-fraction LC/LC-MS/MS analysis operated in DDA mode. Deamidated residues were identified by their monoisotopic mass shift of 0.984 Da from the native non-deamidated peptide.
Results: The efficiency of chondrocyte depletion and effects on ECM recovery were monitored by quantification of dsDNA, glycosaminoglycans (GAGs), and protein within the hypotonic solution. Results indicated that most of the chondrocytes were depleted in the first freeze/thaw cycle. To date, by mass spectrometry of cartilage ECM, we have identified a total of 16 deamidated epitopes in 21 trypsin-generated peptides in the aggrecan core protein; some peptides contained doubly deamidated epitopes. We have identified a further 15 deamidated epitopes in 13 trypsin-generated peptides in COMP.
Conclusions: We have optimized the handling and processing of cartilage frozen tissue sections to enable mass spectrometric analyses of ECM proteins with minimal loss of protein and confounding by intracellular proteins. Deamidated residues within cartilage matrix proteins have been identified and will serve as the targets for a quantitative approach to determining protein half-lives in vivo.
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AGRIN EXPRESSION IS DOWNREGULATED IN OA AND IS REQUIRED FOR CHONDROCYTE DIFFERENTIATION

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Purpose: Background: Agrin is a large basement membrane heparan sulphate proteoglycan best known for its function at the neuromuscular junction. It is responsible for synapse formation via binding to the MuSK and LRP4 receptor complex, leading to Acetylcholine receptor aggregation. Mice deficient of Agrin die perinatally as a result of respiratory failure. Agrin is also expressed in other cell types including chondrocytes of the developing growth plate, and agrin-deficient embryos in which agrin expression had been rescued at the neuromuscular junction develop skeletal abnormalities 1. Therefore it is possible that Agrin may play a role in cartilage homeostasis.
Aims: The aim of this project is to determine if agrin plays a functional role in the homeostasis of the articular cartilage and in osteoarthritis.
Methods: Human adult articular cartilage explants were obtained from preserved or damaged cartilage from individuals undergoing joint replacement for osteoarthritis. Experimental osteoarthritis was induced in 10 week old mice by destabilization of the medial meniscus2. Sham-operated controlateral knees were used as controls. Knees were collected 8weeks after surgery, decalcified and embedded in paraffin. Bovine primary articular chondrocytes (BPAC) were isolated by