FRZB IS A CRITICAL MODULATOR OF CANONICAL WNT SIGNALING 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 variants on OA susceptibility. We have optimized the handling and processing of cartilage tissue for mass spectrometry (LC/MS) analysis and its potential application for OA disease research.

Methods: Our data showed that, in addition to the higher susceptibility to OA in acute induced models, Frzb/-/- mice are more prone to OA in a full transgenic model of the disease characterized by slowly progressive joint damage. Overexpression of Frzb stimulates chondrogenesis by its inhibitory role on Wnt/beta-catenin signalling.

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AGRIN EXPRESSION IS DOWNREGULATED IN OA AND IS REQUIRED FOR CHONDROCYTE DIFFERENTIATION

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Purpose: 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 receptor, which agrin expression had been rescued at the neuromuscular junction. It is responsible for synapse formation via binding to the MuSK receptor, which agrin expression had been rescued at the neuromuscular junction.

Methods: Human adult cartilage was 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 meniscus. Sham-operated controlateral knees were used as controls. Knees were collected 5 weeks after surgery, decalcified and embedded in paraffin. Bovine primary articular cartilages were isolated by

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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 deamination occurs spontaneously at a regular rate. However, deamination can only be observed when it occurs within the lifespan of a protein because newly synthesized proteins eventually replace deamidated proteins. Under these circumstances, deamination could be utilized to determine the half-lives of various 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 targets 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). We subsequently performed qualitative screening by 1D reverse phase LC coupled with tandem mass spectrometry in a DDA (Data Dependent Acquisition) mode of acquisition. 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.