Immunohistological Detection of COMP in Biomechanically Loaded Articular Cartilage Explants – Comparison with Murine and Human OA Cartilage

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ABSTRACT INTRODUCTION:
Disparity between the in vivo contact stress and the local cartilage biomechanical properties probably acts as a mechanical trigger for cartilage degeneration in osteoarthritises (OA). In response to mechanical stimuli applied, chondrocytes are known to regulate their own metabolic activities. We previously reported that mechanical loading can induce degenerative metabolic changes in articular cartilage explants similar to those seen in OA [1,2]. Monitoring subtle changes in the expression and localization of several extracellular matrix components by immunohistology might help to identify the onset of pathological events. Among those, e.g. the cartilage oligomeric matrix protein (COMP) seems promising, as various studies support its role as a molecular indicator of events in OA.

The aim of the presented histological study was 1) to characterize (immuno)histologically mechanically stimulated bovine cartilage explants for their ability to mimick an OA-like appearance and tissue reactivity and, 2) to compare these mechanically challenged tissue with cartilage from human knee OA as well as from a murine in vivo-model of OA.

METHODS:

Biomechanical stimulation of cartilage explants: Full-thickness bovine articular cartilage discs were intermittently loaded for a period of 1 to 9 days using a mechanical loading device designed and constructed to load living cartilage explants over extended time periods under sterile conditions in a normal bench top incubator at 37°C, 5% CO₂ and 95% humidity (Fig. 1) [3]. The cyclic loads (0.5 MPa) were applied with a sinusoidal waveform of 0.1-Hz frequency for 10 s followed by a period of unloading lasting 100 s. Unloaded cartilage discs of the same condyle served as controls. Each experiment was repeated five times (N=6).

Fig. 1 Drawing of a loading chamber used to load cultured cartilage explants under sterile conditions.

Murine and human OA cartilage: The STR-1N mouse is a strain that consistently develops severe spontaneous OA in a short time, which is most pronounced in the knee joint. For comparison with our mechanically manipulated cartilage explants, murine knee cartilage with an age of 7 weeks were selected, which displayed moderate OA alterations. Human articular cartilage were obtained from knee-joint replacement surgeries. Approval of the institutional review boards and informed consent of patients were obtained.

Immunohistology: The OA status of all cartilage samples was staged by staining with Safranin-O / Fast Green using a modified Mankin-score. In addition, formalin-fixed and paraffin-embedded sections of all samples were subjected to immunohistology using antibody against bovine COMP and the alkaline-phosphatase method with Fast Red as substrate.

RESULTS:

Mechanical loading of cartilage explants induced time-dependently the expression of COMP. Samples loaded for up to 3 days did not show remarkable differences to their unloaded controls. Loading for 6 days led to distinct changes in the expression of COMP with an OA-like appearance. Intermittent loading of explants for 9 days further enhanced the expression of COMP.

The expression and localization of COMP appears to be similar to those seen in human OA cartilage as well as in osteoarthritic mice. In all cases, the COMP immunolocalization is complementary to the distribution pattern of the Safranin-O staining, thus becoming more pronounced with an increasing OA morphology.

DISCUSSION:

COMP expression and localization within articular cartilage appears to be influenced by biomechanical factors. Most interestingly, the expression pattern of COMP by loaded bovine explants are in accordance with human as well as murine OA-cartilage. In addition, our histological results presented here are in accordance with our previously reported OA-like proteoglycan and fibronectin metabolism of mechanically challenged bovine explants [1,2].

Further ongoing studies will proof whether these biomechanically challenged bovine cartilage explants can be used for pharmacological studies as a novel in vitro-model of OA-like cartilage degeneration.

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REFERENCES: