LOCALIZATION OF MMP- AND AGGREGANASE-GENERATED NEO-EPITOPES IN OA ARTICULAR CARTILAGE

Purpose: MMP- and aggreganase-mediated degradation of the cartilage matrix, and aggrecan in particular, have been extensively studied in cartilage explants as these two families of proteases are the most important proteolytic enzymes involved in cartilage turnover. Catabolic stimulated bovine explants cultures have been reported to release aggrecan-fragments carrying aggrecanase-mediated neo-epitopes (e.g. the amino acid sequence 342FFGVG) in the early phases, while the release into the supernatant of fragments expressing the MMP-mediated neo-epitopes (such as 342GGVG...) is delayed until the later stages. We therefore wanted to investigate if this separation in time was reflected in the differential localization of the aggrecan fragments in the cartilage tissue. The overall aim of the study was to determine the localization of aggrecanase and MMP-mediated neo-epitopes in human sections of articular cartilage.

Methods: Human OA articular cartilage was obtained from knee replacement surgery. Full depth biopsies were isolated from areas proximal to lesions, followed by fixing in paraformaldehyde, decalcification and paraffin embedding. Sequential cartilage sections were immunohistochemically stained for presence of MMP and aggrecanase-mediated neo-epitopes using monoclonal antibody AF28, BC-3 and 1H11 recognizing the 342FFGVG... the 374 ARGSV... and the NITEGE 373, respectively.

Results: IHC staining of the cartilage sections aggrecanase-mediated aggrecan fragments in the proximaty of the chondrocytes in the upper zones. However, aggrecan fragments generated by aggrecanases – BC-3 and 1H11 were found at the interface between the upper zone and the superficial layer. Since the superficial layer is still present at this part of the biopsies (low degree of erosion) it could depict the presence of aggrecanases-mediated fragments at earlier stages of disease. In contrast, MMP-mediated fragments were mainly observed in the uppermid zone where the superficial layer was lost (high degree of erosion), specifically in the surrounding of clusters.

Conclusions: Our immunohistochemistry results support earlier reports, that the release of aggrecan fragments into the supernatant of the catabolic stimulated explants cultures showed a bi-phasis pattern with aggrecanase-mediated release at early time points and MMP-mediated release of aggrecan fragments at the later stages. We speculate that MMP and aggrecanases activity is related to disease states more than to specific sites. Furthermore that MMP-mediated degradation is related to areas with high cellular activity (e.g. clusters). The molecular mechanism and sequence of events is still unclear, but current study gives some direction to which path to follow.