QUANTIFICATION OF TYPE II COLLAGEN COLLAGENASE-CLEAVAGE EPITOPE AND TYPE X COLLAGEN IN ARTICULAR CARTILAGE

INTRODUCTION

Cleavage of the type II collagen (TIC) matrix by collagenases is increased in osteoarthritis. Collagenases cleave at the Gly\(^{975}\)-Leu\(^{976}\) bond on the \(\alpha\)-chain, \(\frac{3}{4}\) of the way from the N-terminus resulting in the production of \(\frac{3}{4}\) and \(\frac{3}{4}\) length collagen fragments. We have generated an antibody, Hst1, which recognizes a neoepitope on the \(\frac{3}{4}\) collagen fragment. We aim to develop an immunohistochemical assay, using frozen cartilage sections and recombinant active MMP13 to localize and quantify both endogenous and induced Hst1 levels in articular cartilage. This assay could be employed to study spatial localization of cleaved collagen and the collagenases involved in this process.

METHODS

All samples were taken with prior ethical approval. Human articular cartilage from osteoarthritic (OA) donors (patients undergone total knee replacement surgery) and post-mortem donors (normal, no history of OA) were snap-frozen in liquid nitrogen within 48 hours of excision. Cryostat sections of full-depth cartilage were cut at 7 \(\mu\)m thickness onto Superfrost plus microscope slides (VWR International).

**Hst1 detection:** Sections were fixed in 4 % formaldehyde. Following treatment with 0.0125 units of chondroitinase per 50 \(\mu\)l chondroitinase ABC (Sigma) sections were incubated overnight with 1.1 ng/ml Hst1 antibody. Hst1 is an affinity-purified, rabbit antibody directed against a 9-amino acid peptide sequence detected in cleaved type II collagen. The secondary antibody was sheep anti-rabbit IgG (Serotec). Detection was with 3,3-diaminobenzidine (DAB; Dako).

**Active MMP13 treatment:** MMP13 was activated for 2.5 hours at 37\(^\circ\)C with 1 mM APMA (Sigma). Sections were then treated with 0.75 ug/ml active MMP13 or 1 mM APMA alone for 21 hours at 37 \(\circ\)C. Solution was applied directly to the section on the slide. Treated sections were then stained for Hst1, as described above.

**Type X collagen (TXC) detection:** Sections were fixed in cold acetone. Following antigen retrieval with 0.02 mg/ml protease XXVI (Sigma) and 2 mg/ml hyaluronidase (Sigma) sections were incubated overnight with 5 \(\mu\)g/ml mouse anti-TXC antibody (Sigma). Detection was with DAB (Dako).

**Quantification:** Quantification of Hst1 staining was using Clarient Chromavision ACIS (Automated Cellular Imaging) software.

RESULTS

Hst1 antibody showed no immunoreactivity against native type II collagen. Immunoreactivity was observed against the \(\frac{3}{4}\) –fragment of collagenase-cleaved type II collagen, which increased with increasing time of cleavage (data not shown).

Endogenous levels of type II collagen cleavage, as reported by Hst1 staining, were greater in OA than in PM cartilage and staining intensity was increased in areas of damaged cartilage (Fig.1A-C).

APMA is an activator of latent MMP’S. APMA treatment of cartilage sections resulted in Hst1 staining in all zones of OA cartilage. In intact PM cartilage this was only seen in the upper zones while damaged PM cartilage showed greater Hst1 immunoreactivity than undamaged cartilage (Fig. 2).

DISCUSSION

We have characterised the use of a type II collagen neoepitope antibody, Hst1, for use in articular cartilage by immunohistochemistry. We have used this antibody to demonstrate that levels of latent MMP’S capable of generating the Hst1 neoepitope are increased with damage to the cartilage. Moreover, the distinctive pattern of type II collagen cleavage (as reported by Hst1 staining) obtained following APMA treatment is reproduced on active MMP13 treatment. This indicates that collagenases are likely responsible for the APMA effects.

In addition, we have demonstrated differences in collagen types in the deep zone of PM and OA cartilage (a switch from type II to type X collagen). Type X collagen is normally expressed by differentiating, hypertrophic chondrocytes of the endochondral ossification pathway. This study suggests that chondrocytes in articular cartilage acquire a phenotype similar to that of hypertrophic chondrocytes, at histologically early stages of disease. This implicates chondrocyte hypertrophy and cartilage mineralisation in the pathogenesis of OA.

REFERENCES


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