PAR-2 and we demonstrate that matriptase-dependent enhancement of collagenolysis from OA cartilage is blocked by PAR-2 inhibition.
**Conclusions:** This study assessed for the first time the role of the transmembrane serine protease matriptase in cartilage destruction in OA. Elevated matriptase expression in OA, its ability to activate selective proMMPs as well as induce collagenase expression make this serine proteinase a key initiator and inducer of cartilage destruction in OA. We propose that the indirect effects of matriptase are PAR-2-mediated and a more detailed understanding of these mechanisms may highlight important new therapeutic targets for OA treatment.

## 003

### STIMULATION OF MMPS AND CARTILAGE DEGRADATION BY S100A8 AND S100A9

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**Purpose:** Chondrocytes express the calcium binding proteins S100A8 and S100A9, and both are increased in cartilage in acute and chronic inflammatory arthritis; however their role in osteoarthritis (OA) is less clear. We recently demonstrated that chondrocyte *S100a8* and *S100a9* mRNA were co-ordinately increased during the onset of experimental OA, but both were significantly down-regulated as OA progressed, although asynchronously with *S100a9* > *S100a8*. S100A8 and S100A9, but not the heterodimer (S100A8/S100A9) decrease aggrecan and collagen-2 and induce MMP and ADAMTS expression in isolated primary articular chondrocytes. The aim of this study is to determine if similar effect(s) are seen using a more physiologically-relevant explant culture system and whether MMPs implicated in late stage cartilage breakdown in OA are activated.
**Methods:** Ovine articular cartilage explants were cultured for 4 days in serum-free media ± $10^{-7}$M or $10^{-8}$M human S100A8, S100A9 or S100A8/S100A9 (n=3/treatment). Gene expression of *S100a8*, *S100a9*, key cartilage proteins, metalloproteinases and their inhibitors was measured using real time RT-PCR. Collagen and aggrecan release were measured as hydroxyproline and glycosaminoglycan (GAG) respectively. Activity of MMP-2 and MMP-9 were evaluated by gelatin zymography, and MMP-13 using a fluorometric assay.
**Results:** Aggrecan and collagen-2 expression were not regulated by S100A8 or S100A9. *Adamts-1, Adamts-5*, *Mmp-1*, and *Mmp-3* mRNA levels were dose-dependently up-regulated by S100A8 ($p<0.01$) and S100A9 ($p<0.05$) while no change was seen in *Mmp-2*, *Mmp-9*, *Mmp-14*, *Timp-1, Timp-2* or *Timp-3* mRNA levels. The heterodimer did not regulate expression of any genes other than *Mmp-13*. *Mmp-13* was increased by S100A8 (≈100 fold; $p<0.05$) and S100A9 (≈20 fold; p=0.8), but down-regulated by S100A8/S100A9 (≈25 fold; $p<0.05$). *S100a9* expression was dose-dependently up-regulated by S100A8 ($p<0.01$) and S100A9 ($p<0.04$). *S100a8* expression was low but increased in response to $10^{-7}$M S100A8. There was no collagen release in any cultures. Only in response to $10^{-7}$M S100A8 we observed: increased GAG release (3-fold); activation of pro-MMP-2; secretion of pro-MMP-9 and pro-MMP-13 (no active MMP-9 or MMP-13 were detected in any cultures).
**Conclusions:** These results have confirmed that both S100A8 and S100A9 homodimers are implicated in chondrocyte-mediated cartilage degradation by increasing expression of MMPs and ADAMTS. Over the short time course of these experiments S100A8 was more pro-catabolic than S100A9, leading to greater up-regulation of metalloproteinase mRNA, release of pro-MMP-9 and pro-MMP-13, release and activation of MMP-2, and cartilage aggrecan loss. Longer-term cultures will determine whether these differences are maintained and if MMP-driven collagenolysis ultimately occurs. Chondrocyte *S100a9* and *S100a8* expression has been shown to be increased by IL-1, and the current data also suggests some degree of auto-regulation. Changes in cartilage explant gene expression by S100A8 and S100A9 largely matched those in isolated chondrocytes except that in the latter aggrecan and collagen-2 were significantly down-regulated by both homodimers. Of particular interest was the opposite effect on *Mmp-13* expression of both homodimers versus the heterodimer, implicating the uncoordinated regulation of the two S100 proteins in cartilage degradation.

## 004

### CALPAIN IS INVOLVED IN C-TERMINAL TRUNCATION OF HUMAN AGGRECAN

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**Purpose:** Aggrecan, a structural extracellular matrix protein, is degraded by proteolysis in joint injuries and in arthritis as an early key event. We here investigate the possible role of calpain digestion of human aggrecan, looking at the preferred order of cleavage and analyzing the *in vivo* content of calpain generated aggrecan fragments in knee cartilage and synovial fluid.
**Methods:** Human aggrecan fragments were purified by dissociative and associative CsCl density gradient centrifugations from: OA-pool (adults, n=10) and normal (youth, n=1) knee cartilage, and from OA-pool (n=47) and acute inflammatory arthritis (n=2) synovial fluid (SF). Cartilage A1D1 fractions were *in vitro* digested by m-calpain (EC nr 3.4.22.17; Calbiochem) for up to 2h. Aggrecan samples were deglycosylated, separated by SDS-PAGE, transferred to PVDF membranes and probed with antibodies against calpain generated neo-epitopes (PGVA, GDLS, and EDLS) or against aggrecan G1- and G3-domains. N- and C-terminal ends not verified by immunodetection were estimated using a calculation model.
**Results:** To study calpain digestion of aggrecan we produced neoepitope antibodies against the following human aggrecan calpain sites: -PGVA$^{709}$, in the keratan sulfate (KS) domain; -GDLS$^{954, 973, 1353, 1431}$ and -EDLS$^{1411,1472}$, in the chondroitin sulfate 1 (CS1) region. Neoepitope specificity of the GDLS and PGVA antibodies were confirmed by complete blocking of the Western blot immunoreactions by the immunogen peptides and by no blocking using spanning peptides. The EDLS antibody not only recognized the neoepitope but also the internal sequence. Western blot experiments of *in vitro* digested aggrecan (A1D1 from cartilage OA-pool) showed that calpain cuts human aggrecan in several sites in six separate regions (a–f, Fig. 1). Time dependent *in vitro* kinetic studies showed that calpain has a preferred order of cutting aggrecan, as follows: site-d, -b, -a, -e and site-f. Due to weak signals it was not possible to get kinetic data on cleavage site-c. Except that site-a and site-e was exchanged, the same preferred calpain cleavage order was observed in aggrecan prepared from normal cartilage as for the OA-pool. Calpain generated aggrecan fragments G1-PGVA (137 kDa), G1-EDLS (310 kDa) and AVPV-GDLS (225 kDa) were also detected *in vivo* in OA cartilage, and in similar amounts in normal cartilage. Also, a ARGS-EDLS (280 kDa) fragment, with an aggrecanase generated N-terminal, was detected in SF from patients with acute inflammatory arthritis but not in the SF OA-pool.

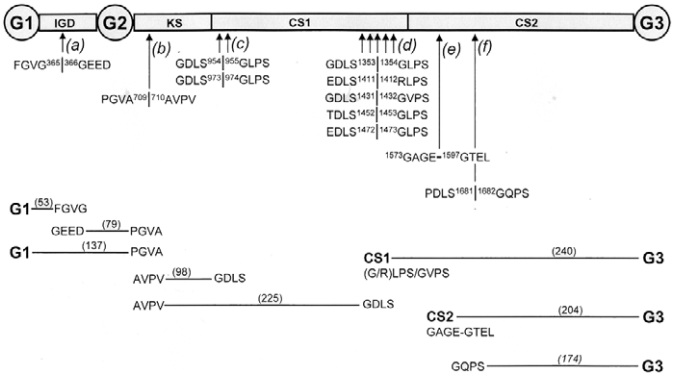


Figure 1. Six (a–f) calpain cleavage sites/regions were found in human aggrecan. OA cartilage (A1D1) was *in vitro* digested by calpain and fragments were visualized by Western blot. Illustration of human aggrecan, calpain cleavage sites and calpain *in vitro* generated fragments (Mw in kDA).

**Conclusions:** Our results indicate that calpain cuts human aggrecan in at least six specific sites/regions in a preferred order. The results further suggest that calpain has a function in digesting aggrecan in the CS region as a step in physiological C-terminal maturation. We can not rule out a role for calpain digestion of aggrecan also in joint diseases or injuries.