S100A8 and S100A9 homodimers but not the heterocomplex stimulate catabolic pathways in chondrocytes and may play a role in cartilage degeneration in osteoarthritis

Introduction: Progressive erosion of articular cartilage is a central feature of osteoarthritis (OA). Early loss of aggrecan associated with excessive proteolysis by ADAMTS enzymes reduces the load carrying capacity of cartilage. Subsequent degradation of the type II collagen network by matrix metalloproteinases (MMPs) results in irreparable disruption of cartilage. S100A8 and S100A9 calcium binding proteins are highly expressed in inflammatory arthritis, and can form a heterocomplex, known as calprotectin, that is a marker of inflammation. Chondrocytes express S100A8 and S100A9, with significant upregulation by interleukin-1 (IL-1) and oncostatin M (1). To-date, the effect of S100A8, S100A9 or their complex on chondrocyte metabolism and their potential role in OA, as opposed to inflammatory arthritis, has not been examined. We evaluated whether: (1) S100A8, S100A9 or their complex regulate chondrocyte expression of matrix proteins and proteinases implicated in OA; (2) S100A8 or S100A9 expression and protein are altered in in vitro models of cartilage degradation and in in vivo models of OA and inflammatory arthritis.

Methods: Articular chondrocytes were obtained from 4-year-old ovine knee cartilage and incubated for 24 hrs +/- 10 or 100nM recombinant human S100A8, S100A9 or the complex. The expression of aggrecan, type II collagen, ADAMTS-1, -4 & -5, MMP-1, -3, -13 & -14 and TIMPs-1, -2 & -3, was determined using quantitative real time RT-PCR. Femoral head cartilage was isolated from 24-day-old C57B6 WT mice and cultured +/- retinoic acid (RetA) or IL-1 for 2 & 4 days to stimulate cartilage catabolism and the expression of all S100 genes relative to the control cultures at each time point was determined by microarray. Expression of all S100 mRNA in OA cartilage was evaluated by microarray of micro-dissected mouse articular cartilage 1, 2 & 6 weeks after medial meniscal destabilization (MMD). Immunolocalization of S100A8 and S100A9 was performed on knee joints of: (A) mice 2, 4, 8 & 16 weeks after sham or MMD surgery; and (B) 7 and 28 days after induction of antigen-induced arthritis (AIA).

Results: Chondrocyte gene expression in response to S100A8 and S100A9: No genes were significantly regulated by the heterodimeric complex. S100A8 and S100A9 homodimers both down-regulated aggrecan expression whereas ADAMTS-1, -4 and -5 mRNA were all increased (Fig 1). Collagen type II mRNA was decreased by S100A8 and S100A9, while MMP-1, -3 and -13 were upregulated. MMP-14, TIMP-1 and TIMP-3 mRNA were unchanged and TIMP-2 mRNA was decreased by both S100A8 and S100A9 (Fig 1).

Regulation of S100A8 and S100A9 expression in mouse femoral head cartilage in vitro: IL-1 down regulated S100A4 (7 fold) at 2 and 4 days, while S100A8 and S100A9 were upregulated at day 4 (10 and 9 fold, respectively). At both times, S100A8 and S100A9 were down regulated (8 and 9 fold, respectively) by RetA. Consistent with the gene expression, S100A8 and S100A9 protein localization were increased in articular chondrocytes at day 4 by IL-1, but not RetA. Interestingly, despite the decrease in mRNA expression in RetA cultures, immunostaining for S100A8 and S100A9 showed little change.

In vivo expression of S100 genes in normal and OA articular cartilage chondrocytes: The expression of S100A5, S100A6, S100A8, S100A9, S100A11 and S100B was regulated in chondrocytes following MMD surgery. The most highly regulated members were S100A8 and S100A9, and unlike other S100 family members, they showed differential regulation in early (week 1 and 2) and later (week 6) stages of OA, compared with later (week 6) stage OA. S100A8 and S100A9 mRNAs were upregulated (7-14 fold) at weeks 1 and 2, and their expression decreased at 6 weeks such that it was below (7-18 fold) sham-operated levels.

Local immunolocalization of S100A8 and S100A9 proteins in OA and AIA: MMD induced a progressive deterioration of the articular cartilage in the medial femorotibial joint where full thickness erosion of non-calcified cartilage to cover over 50% of the joint surface was seen by week 16. In normal joints (non- or sham-operated), articular chondrocytes throughout the non-calcified cartilage showed positive staining for S100A8. A loss of cellular S100A8 immunoreactivity at all time points was found in the non-calcified cartilage of the central weight bearing region of MMD compared to the corresponding sham operated joint. S100A9 immunostaining was restricted to cells in the bone marrow with little or no staining observed in the cartilage in the normal or OA joints. In contrast with OA, articular chondrocyte S100A8 reactivity remained positive in AIA joints at both 7 and 28 days, with levels similar to that of the saline injected (non-inflamed) control joints, despite joint-wide proteoglycan loss with AIA. Furthermore, chondrocytes in AIA became immunopositive for S100A9 at 7 days and remained positive at 28 days despite resolution of the inflammation.

Discussion: These findings highlight differences in the pathogenesis of cartilage destruction in OA versus inflammatory joint diseases. The change in S100A8 and S100A9 was restricted to the cartilage in OA, whereas their localization in other tissues (bone and meniscus) was unaltered. This may indicate that local factors such as mechanical overloading rather than humoral agents, play a significant role in regulating the metabolism of these proteins in OA. Increased expression and release (and thus loss of cellular immunostaining) of homodimeric S100A8 or S100A9 into the extracellular environment may contribute to cartilage degradation in OA by upregulating MMPs and ADAMTS while decreasing aggrecan and collagen expression. As extracellular S100A8/S100A9 complex does not regulate chondrocytes genes, an imbalance in synthesis or release of the individual S100A8 or S100A9 proteins may be important. New efforts to prevent the progression of OA might include strategies that target S100A8 and S100A9.

Acknowledgements: This study was supported by grants from the Australian Research Council, the National Health & Medical Research Council of Australia and the Ulysses Club. We are grateful to Professor Carolyn Geczy for the anti-S100 antibodies.