METABOLISM OF SMALL LEUCINE-RICH PROTEOGLYCANS IN AN OVINE MODEL OF OSTEOARTHRITIS

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INTRODUCTION: Osteoarthritis (OA) is characterized by progressive breakdown of articular cartilage. This degeneration of cartilage is associated with changes in the ultrastructure and composition of the extracellular matrix of the tissue. While one of the earliest cartilage changes in disease is proteolysis and loss of the large proteoglycan aggrecan, later disruption of the type II collagen fibrillar network may represent the point of irreversible damage. An expanding family of small leucine-rich proteoglycans (SLRPs) have been shown to be important in the formation and maintenance of the collagen fibrillar network, in addition to serving roles in growth factor modulation and direct regulation of cell growth [1]. Furthermore, it has been suggested that SLRPs bound to the surface of collagen fibrils in AC may act to stericly hinder access of the collagenolytic MMPs and therefore modulate collagen breakdown [2]. Synthesis and catabolism of members of the SLRP family may, therefore, play an important role in the disease process of OA. The aim of the present study was to assess the changes occurring in cartilage mRNA expression and protein levels and extent of catabolism of four SLRPs, decorin, biglycan, fibromodulin and lumican in an established sheep model of OA induced by lateral meniscectomy.

METHODS: Animal Model - Six 7-year-old female pure-bred Merino sheep underwent open lateral meniscectomy of both stifle joints (MEN), while 6 others were used as non-operated controls (NOC). Animals were maintained together on pasture for 6 months prior to sacrifice. Cartilage was harvested separately from the medial and lateral tibial plateaux of randomly selected left or right joints of each animal for RT-PCR and western blot analysis. The contralateral joint was used for histology.

Histology - Coronal osteochondral slabs were prepared through each tibial plateau and processed to paraffin. Sections (4µm) were cut and stained with Toluidine blue. Two independent assessors evaluated each section using a Modified Mankin Score (MMS) with a range of 0 to 29, the value increasing with severity of AC degeneration [3].

RESULTS: Differences between gro-

† The predominant fibromodulin band (50kDa), which was smaller than expected for full-length core protein and likely represents an N-terminally processed catabolite [2] was unchanged or slightly increased in MEN compared with NOC. In addition a small (~28kDa) fibromodulin fragment was detected in MEN.

There was little change in staining intensity of intact lumican core protein but an increase in size in MEN (from 60 - 64kDa), possibly due to increased glycosylation [4]. Furthermore, there was a marked increase in a 47kDa lumican fragment in MEN. In general the distribution of SLRP core proteins in human OA cartilage was similar to that in MEN.

DISCUSSION: Studies have demonstrated up-regulated mRNA expression and protein of all four SLRPs in late stage human OA [4], while in a model of OA in dogs biglycan and fibromodulin protein were increased while decorin was decreased [5]. Differential expression of the SLRPs is believed to contribute to their role in cartilage homeostasis and regulation may occur through competition for the same binding sites [6]. Our study demonstrated a strong up-regulation of one of each class investigated (biglycan and lumican), with either a decrease or no change in the partner molecule (decorin and fibromodulin, respectively). These expression changes were confined to the cartilage in the affected compartment and were therefore unlikely to be initiated by systemic factors or synovial inflammation, but local changes such as increased mechanical loading which may modulate decorin and biglycan synthesis. It is unclear whether the changes in SLRP expression in arthritis precede or are a consequence of cartilage damage. Despite the increased expression for biglycan and lumican, there was not a dramatic increase in intact core protein in the cartilage. It seems likely that the lack of accumulation of these molecules is associated with increased catabolism, and evidence of significant proteolysis of biglycan and lumican in particular, was evident. The cleavage sites and proteinases responsible for extracellular SLRP breakdown in arthritic cartilage are yet to be identified, and are the subject of further investigation. Understanding the relationship between SLRP metabolism and progressive cartilage breakdown in OA may provide novel targets for diagnostic markers as well as treatment.


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