CATABOLISM OF THE SMALL LEUCINE RICH REPEAT PROTEOGLYCANS (SLRPs) IN AN OVINE MODEL OF EXPERIMENTAL INTERVERTEBRAL DISC DEGENERATION

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INTRODUCTION
Earlier studies using the ovine annular lesion model of intervertebral disc degeneration demonstrated a focal down-regulation in the synthesis of aggrecan and its depletion from and catabolism in the experimental lesion sites accompanied by an up-regulation in decorin and biglycan production [1-3]. These changes are consistent with findings in degenerate human intervertebral discs [4, 5]. The present study extended these earlier observations and examined the catabolism of a number of the SLRPs to determine if this class of proteoglycans was affected by the tissue remodeling evident in this ovine model.

METHODS
The present study used controlled 4 mm deep and 10 mm wide anterolateral annular lesions in the outer third of the AF (lesion sheep group, n=32) at the L1L2 and L3L4 spinal levels [1, 6]. An additional sheep group (n=32) received the same surgical approach only (sham operated control sheep group). Discs of the lesion and sham sheep groups were collected at 3, 6, 12 and 26 mth post operation (PO) dissected into lateral halves of the AF and the NP, and the tissues extracted with 4M GuHCl containing protease inhibitors, the extract was subsequently dialysed and freeze dried. Western blotting was used to identify SLRP core protein fragments in the tissue extracts using specific carboxyl terminal antibodies to the C-terminal sequences of human, bovine and chick decorin (PR-84), biglycan (PR-85), lumican (PR-353), and fibromodulin (PR-184) core protein. Lesion and sham operated discs were also fixed ‘en-bloc’ in 10% neutral buffered formalin, de-calcified in 10% formic acid, processed into paraffin blocks and vertical sagittal micrometre (4 μm) sections prepared. These were stained for anionic proteoglycan using toluidine blue-fast green and collagen using Masson’s Trichrome and Picrosirus red. The sections were viewed under polarized light or by brightfield microscopy.

Figure 1. A. Diagrammatic illustration of the anterolateral annular defect used in this study. B. Focal loss of anionic proteoglycan associated with the lesion site (arrow) but not in the sham group.

RESULTS
Toluidine blue-fast green histology clearly delineated the annular defects (Fig 1) and the focal loss of anionic proteoglycan from the defect zones. Western blotting subsequently showed that two biglycan (17, 25 kDa) and two fibromodulin (28, 36 kDa) core protein fragments were specifically associated with the AF lesion zone tissue extracts but significantly, they were not present in the NP extracts or any disc tissues of the sham control sheep. Decorin fragmentation was also evident in the lesion zone samples 3-6mth PO and may be associated with remodeling of the lesion site. No lumican fragments were detected.

DISCUSSION
Negligible information exists on the physiological consequences of SLRP catabolism and the presence of SLRP core protein fragments in the ECM, however, given the diverse functions of the SLRPs, their catabolism could result in altered tissue responses to growth factors and cytokines, abnormal collagen fibril formation, cell adhesion and growth. The ovine annular lesion model of experimental disc degeneration represents a useful model for the further evaluation of the effect of ECM remodeling on SLRP catabolism. Identification of the cleavage sites on the SLRPs, raising of neoepitope antibodies and characterization of the proteinases responsible in their generation may uncover important diagnostic or prognostic bio-markers and therapeutic target molecules which will prove to be useful in the study of musculoskeletal disorders.

REFERENCES

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