MONOCLONAL ANTIBODIES RECOGNISING KERATAN SULPHATE "STUBS" AND SULPHATION MOTIFS USED IN ELISA TO QUANTIFY CHANGES IN PROTEOGLYCAN METABOLISM IN MUSCULOSKELETAL DISEASES

**Harris, A M; **Kotwal, R; *Wright, A E; *Hodgson, P; *Hughes, C E; **Roberts, S; **Richardson, J; *Dent, C; *Caterson, B
*School of Biosciences, Cardiff University, Wales, UK
HarrisAM1@cf.ac.uk

Introduction: Monoclonal antibodies (mAbs) recognizing linear sulphation motifs in keratan sulphate (KS) were first developed in the early 1980’s (1) and soon after quantitative ELISAs were developed to quantify KS metabolism as biomarkers in serum, synovial fluids and tissue obtained from patients (and animals) with a variety of musculoskeletal diseases (2). The prototype mAb for these studies was 5-D-4. This mAb, and now others exist, which recognize linear monosulphated or disulphated KS disaccharide motifs of 6 - 8 sugar units in length that occur within KS proteoglycan GAG chains covalently attached to KS GAG chains. Competitive ELISAs were developed using both mAbs 5-D-4 and BKS-1. The slopes on inhibition curves obtained with these two mAbs and their relative 50% inhibition points (using the chondroitinase ABC and keratanase digested bovine corneal proteoglycan mix as competing antigen-standard for comparison) were similar for both 5-D-4 and BKS-1 (see figure 1). Also, repeated analyses over several days/wells showed low co-efficients of variation. When used in analyses of human OA cartilage samples, obtained from patients with different stages of degenerative joint disease and different ages, the assays showed the most interesting results when the ratio of linear KS sulphated epitope (5-D-4) to keratananase-generated neoepitope (BKS-1) was used to discriminate between KS present on immature tissue (i.e. this neoepitope was not seen in early OA compared to 3.0-4.0 in a patient with late-stage disease, this difference most likely reflecting differences in the type (immature versus mature) of KS sulphation motifs and substitutions on an aggrecan molecule or metabolite.

Results & Discussion: The specificity and utilization of mAb 5-D-4 (an IgG) has been described in numerous publications. mAb BKS-1 specifically recognizes only a keratanase-generated neoepitope (GlcNAc-6S) present at the non-reducing terminal of KS GAG chains that had been digested with keratanase; i.e. this neoepitope was not present on native KS GAG chains (from either type I or type II KS), nor on KS GAG chains that had been digested with keratanase II or endo-beta-galactosidase, two other enzymes that are capable of digesting GAG chains. Competitive ELISAs were developed using both mAbs 5-D-4 and BKS-1. The slopes on inhibition curves obtained with these two mAbs and their relative 50% inhibition points (using the chondroitinase ABC and keratanase digested bovine corneal proteoglycan mix as competing antigen-standard for comparison) were similar for both 5-D-4 and BKS-1 (see figure 1). Also, repeated analyses over several days/wells showed low co-efficients of variation. When used in analyses of human OA cartilage samples, obtained from patients with different stages of degenerative joint disease and different ages, the assays showed the most interesting results when the ratio of linear KS sulphated epitope (5-D-4) to keratananase-generated neoepitope (BKS-1) was used to discriminate between KS present on immature tissue (i.e. this neoepitope was not seen in early OA compared to 3.0-4.0 in a patient with late-stage disease, this difference most likely reflecting differences in the type (immature versus mature) of KS sulphation motifs and substitutions on an aggrecan molecule or metabolite.

Materials & Methods: Patient tissue samples, synovial fluid and/or serum were derived from human surgical waste material using procedures approved by the Regional Ethics Committee and the NHS at University of Wales Hospital, Cardiff, U.K. Monoclonal antibody 5-D-4 was produced and characterized (1) and then ELISA assays developed (2) according to now well-established procedures. However, for the ELISAs described below, 96-well plates were coated with 0.5 µg/ml chondroitinase ABC and keratanase digested bovine corneal proteoglycan antigen mix in Na2CO3 buffer overnight at 4°C. A chondroitinase ABC and keratanase digested bovine corneal proteoglycan mix was used to facilitate adequate presentation of the linear KS sulphation motif epitope (the 5-D-4 epitope) and keratananase-generated neoepitope (the BKS-1 neoepitope – see below). This "antigen mix" was also used as the standard for comparison in the ELISAs for BKS-1 & 5-D-4. A mAb (BKS-1) that specifically recognizes a keratananase-generated neoepitope of N-acetyl-glucosamin-6-sulphate (GlcNAc-6S) at the non-reducing terminus of "KS-stubs" present on the keratanase digested KS GAG chains covalently attached to KS-proteoglycan metabolites was produced using keratanase treated "bovine KS-peptides" purified from bovine nasal cartilage aggrecan as the immunizing antigen. Screening procedures using keratanase-treated bovine corneal KS-proteoglycans were used to specifically identify mAbs that recognized this keratananase-generated neoepitope (i.e. non-reducing terminal GlcNAc-6S) on "KS-stubs". This mAb (BKS-1) was used to develop an ELISA for quantifying keratanase-generated "KS-stubs" using methods described for 5-D-4 above.

Conclusion: This presentation describes the production and use of a new mAb that recognises keratanase-generated "KS-stubs" on KS-metabolites resulting from KS-proteoglycan turnover in musculoskeletal tissues. This mAb (BKS-1) has been used in conjunction with a well-established anti-KS mAb (5-D-4) to develop quantitative ELISAs that can be used to discriminate between KS present on immature tissues (e.g. those from aggrecan synthesised by developing or repair/regenerating tissue) and those present in mature cartilage. These assays will prove useful in better discrimination of KS-metabolite biomarkers in serum, synovial fluids and tissue samples of patients suffering a variety of musculoskeletal diseases; e.g. degenerative joint and disc diseases.

References:

**Robert Jones & Agnes Hunt Orthopaedic Hospital, Oswestry, UK

Figure 1: Competitive ELISA for keratan sulphate proteoglycans from bovine cornea with mAbs BKS-1 (●) and 5-D-4 (▲).

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