

Novel Chondroitin Sulphation Motifs As Putative Biomarkers of Articular Cartilage Chondroprogenitor Cells

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INTRODUCTION: The cellular mechanisms responsible for the zonal stratification of articular cartilage are still not fully understood. However, accumulating evidence indicates that the tissue is maintained by appositional growth occurring from the surface zone¹ which contains a population of chondroprogenitor cells². In this study, we demonstrate that monoclonal antibodies (mAbs) recognising novel chondroitin sulphation (CS) motifs in CS glycosaminoglycans (GAGs)^{3,4} can be used to identify and separate this chondroprogenitor cell sub-population.

METHODS: For immunohistochemistry, cryosections of articular cartilage (from the hock joints of 7 day bovines) were labeled by standard immuno-fluorescence procedures using mAbs 3B3(-), 7D4 and 4C3 which recognise novel sulphation motifs in CS GAGs^{3,4} before counterstaining with propidium iodide. Sections were photographed on a Leica epi-fluorescent microscope equipped with digital image acquisition. For FACS analysis, thin surface slices of articular cartilage were digested overnight at 37°C in 1.2U/ml dispase II (Roche # 295825) and 100U/ml type II collagenase (Worthington #X4N7639) to enrich for superficial zone chondrons (*i.e.* cell and pericellular matrix). Isolated chondrons were immuno-fluorescently labeled with mAbs 3B3(-), 4C3 and 7D4 as described above. Labeled suspensions of chondrons were analysed in a FACS Calibur instrument. Ten thousand events were registered per sample and analysis of whole chondrons was performed using appropriate scatter gates to avoid cellular debris and aggregates.

RESULTS: Monoclonal antibodies 3B3(-), 7D4 and 4C3 strongly immuno-located novel CS motifs at the cell membrane and pericellular matrix of chondrocytes in the superficial zone of articular cartilage (figure 1). FACS analysis of isolated chondrons demonstrated that the former two mAbs, but not 7D4, could be used to identify and isolate this surface zone population (figure 2).

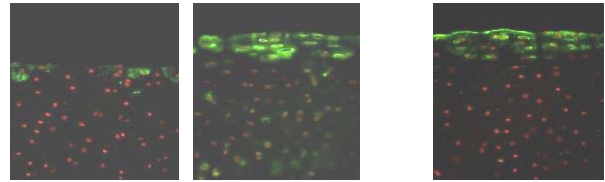


Fig. 1: Distribution of novel CS sulphation motifs in articular surface zone with mAbs 3B3(-), 4C3 & 7D4. (left to right)

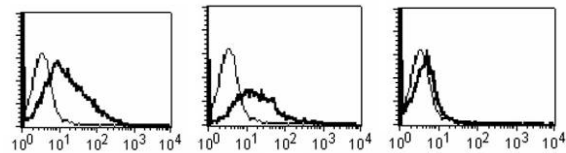


Fig. 2: FACS plots showing 3B3(-), 4C3 & 7D4 positive cells (left to right; bold line) compared to unlabelled controls.

DISCUSSION & CONCLUSIONS: In this study we show that the superficial zone cells of articular cartilage, recognized as a chondroprogenitor cell population², can be identified and separated by the presence of membrane-bound/pericellular novel CS glycosaminoglycan sulphation motif epitopes using monoclonal antibodies 3B3(-) and 4C3. Consistent with previous findings⁵, the CS epitopes recognised by mAb 7D4 appear to be lost during the enzymatic procedure used to isolate intact chondrons. However it is anticipated that they may be retained following a mechanical isolation procedure (currently being evaluated). The identification, isolation and characterisation of this chondroprogenitor cell sub-population will lead to a greater understanding of the role novel CS sulphation plays in articular cartilage development and will be of benefit to new cell-based techniques for cartilage repair.

REFERENCES:

- ¹Hayes *et al.* (2001) *Anat. & Embryol.* **203**:469-479; ²Dowthwaite *et al.* (2004) *J. Cell Sci.* **117**:889-97; ³Sorrel *et al.* (1990) *J. Histochem. Cytochem.* **38**:393-402; ⁴Caterson *et al.* (1990) *J. Cell Sci.* **97**:411-412; ⁵Lee *et al.* (1997) *Arthritis & Cartilage.* **5**: 261-274