Chondroitin sulphate motifs as biomarkers for the stem/progenitor cell niche in musculoskeletal tissues.

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INTRODUCTION: In the mid-1980's our laboratory produced and characterised several monoclonal antibodies [mAbs 3-B-3(-), 4-C-3, 6-C-3 & 7-D-4) that recognised unique 'native' sulphation motifs in chondroitin sulphate (CS) glycosaminoglycan (GAG) chains on connective tissue proteoglycans (PGs). These antibodies were shown to specifically locate CS-PGs in the peri-cellular regions surrounding putative sites where haemopoietic stem cells were undergoing lymphopoiesis in the Bursa of Fabricius of embroyonic chicks (Sorrell et al 1988, *J Immunol 140: 4263*; Caterson et al 1990, *J Cell Sci 97: 411*). In later studies, we also observed immunostaining for some of these mAbs [3-B-3(-) & 7-D-4] in chondrocyte clusters present in tissue sections from late-stage osteoarthritic cartilage from canine and human patients (Visco et al 1993, *Arthritis Rheum 36: 1718*; Slater et al 1995, *Arthritis Rheum 38: 655*). In a very recent study (Hayes et al 2008 *J. Histochem Cytochem 56: 125*) we have used these anti-CS GAG sulphation motif mAbs to identify proteoglycans with these specific GAG motifs in pericellular domains surrounding stem and/or chondroprogenitor cells located in the surface zone of hyaline articular cartilage.

METHODS: Our cartilage studies were performed using hyaline articular cartilage harvested from 1–2 week old bovine calves. Full thickness sections of this cartilage tissue were used for fluorescent and confocal immunohistochemical (IHC) analyses. In some instances these full depth cartilage pieces were carefully dissected into surface, middle and deep zone morphological regions and used for either 3D confocal microscopy analyses, extraction of proteoglycans followed by analyses using Western blot or Dot blot procedures or the isolation of chondrocytes from the different zones (using pronase & collagenase) for use with these mAbs in FACS analyses to sort and isolate chondro-progenitor cells for potential pluripotent cell enrichment in tissue engineering or tissue regeneration technologies. Sections from other musculoskeletal tisses (i.e. bovine tendon & developing rat intervertebral disc) and other animal organs (i.e. mouse gut & the developing chick eye) were also obtained and IHC analyses performed using these native CS GAG motif mAbs.

RESULTS & DISCUSSION: In our recent studies we have shown that our mAbs [3-B-3(-), 4-C-3 & 7-D-4] that recognise novel CS sulfation motif epitopes in GAG chains on proteoglycans can be used to identify metabolically distinct sub-populations of cells specifically within the superficial zone of hyaline articular cartilage, and that flow cytometric analysis can recognize these cell sub-populations. Fluorochrome co-localisation analysis suggest that these CS sulphation motifs are associated with a range of cell and extracellular matrix (ECM) PGs within the stem cell niche, that include perlecan and aggrecan, but not versican. We have also used several of these mAbs to identify stem/progenitor cells in different anatomical and functional regions of the tendon; i.e. where the tendon wraps around bone in compressed regions where the cells exhibit a more chondrogenic phenotype and also in the outer zones of the bovine tendon surrounding pericytes where vascularisation is present. In studies of the developing rat intervertebral some of these mAbs specifically recognise stem/progenitor cells at the interzone between the outer and inner anulus an also the boundary of the nucleus with the inner annulus, these results indicating their potential use for stem/progenitor cell identification and isolation in other musculoskeletal tissues. Interestingly, one of these mAbs (6-C-3) also immunostained the pericellular environment ("stem cell niche") in the crypts of the mouse gut and several mAbs the limbus of the chicken eye where stem cells reside.

CONCLUSIONS: The unique distributions of these CS motifs on PGs found within the pericellular micro-environment of superficial zone chondrocytes in cartilage is conistant with that reported from other studies (Dowthwaite et al 2004, *J Cell Sci 117: 889*) and they appear to identify early stages of stem/progenitor cell differentiation which is consistent with these molecules playing a functional role in regulating aspects of chondrogenesis. Collectively, this data suggests that these mAbs that recognise CS sulphation motifs can be used as biomarkers to identify stem cell niches in numerous tissues of the body and that they can be used for stem & progenitor cell isolation.

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