THE ROLE OF MECHANICAL STRAIN IN THE FORMATION OF DIARTHRODIAL JOINTS: A POSSIBLE MECHANISM CONTROLLING HYALURONAN PRODUCTION

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Introduction: The interaction between hyaluronan (HA) and its cell surface binding proteins (HABPs) is believed to play an important role in the development of diarthrodial joints (1,2). Indeed, disruption of HA:HABP interactions prevents functional joint cavity formation (3). It is also well established that immobilisation of embryos prevents formation of functional joint cavities and the joint fusions evident in such animals are associated with decreases in HA synthetic ability and HABP expression (4). Whilst these results provide phenomenological evidence for the removal of movement-induced stimuli in mediating HA:HABP interactions, they do not directly address the role of mechanical stimuli in mediating HA synthesis and HA:HABP interactions in developing joints. In addition, it is known that the actin cytoskeleton and the actin capping protein moesin may play a role in controlling HA:HABP interactions during joint development and that the phosphorylation of specific amino acid residues in CD44 and possibly moesin may facilitate changes in cell:matrix interactions during development and matrix metabolism (3). Therefore, the aim of this study was to assess the effect of mechanical stimuli applied to cells isolated from developing chick articular surfaces on HA synthesis and HABP expression and to to examine changes in phosphorylation of CD44 and moesin in strained cells.

Methods: Chick articular fibrocartilage cells isolated from Stage 42 tibio-tarsi by collagenase digestion were grown to confluence on plastic strips in DMEM containing 5% chick serum. Confluent cells were serum deprived for 16 hours then subjected to uniaxial 4 point bending at 3800 micro strain for 10 minutes at 1Hz (n = 15 strips). Controls comprised cells subjected to pertubation of culture medium alone (flow) and unperturbed (static) cells. At various time points up to 24 hours, medium samples were assayed for HA concentration using an ELISA plate-based assay. At 24 hours, cells were immunolabelled with antibodies for CD44, reacted for UDPGD activity (an indicator of HA synthetic ability) and labelled with biotinylated HA to assess HA binding ability. Alkaline phosphatase end-products for CD44 and bHA were measured semi quantitatively and UDPGD reaction products were measured quantitatively using microdensitometry. To assess changes in amino acid phosphorylation, samples were extracted in TBS (pH 7.5) containing 0.5% Triton X-100. Detergent insoluble pellets were run on 10% SDS-PAGE, electrotransferred to nitrocellulose and probed with antibodies to CD44, moesin, phospho-serine, phospho-threonine and phospho-tyrosine.

Results: Ten minutes post-strain there was no significant increase in HA release in strained samples compared with static and flow controls ($p>0.05$, students t-test). At 6 hours post-treatment, there was a significant increase in media HA concentration in strained samples compared with staic ($p<0.001$) and flow ($p<0.001$) samples. Similarly, at 24 hours there was a significant increase in media HA concentration in strain samples compared with the static and flow controls ($p<0.001$ in both cases). There was no significant difference in media HA concentration between static and flow samples at any time point. In addition, at 24 hours UDPGD activity was significantly increased in strained samples compared with static ($p<0.0001$) and flow ($p<0.001$) controls. CD44 labelling was also significantly increased at 24 hours in strained samples compared with flow ($p<0.001$) and static ($p< 0.001$) controls. Consistent with increased CD44 immunolabelling at 24 hours, binding of bHA to hyaluronidase pre-treated cells (total HA binding sites) was significantly increased in strained samples compared with flow ($p<0.001$) and static ($p<0.001$) controls. Using detergent insoluble extracts, 2 distinct bands were visible in control samples probed with anti-CD44 antibody with an apparent molecular mass of 80-85 kDa. Upon the application of strain, the higher molecular mass band was not apparent. When probed with antibody to the actin capping protein moesin a band of 70 kDa was apparent in static, detergent insoluble samples which was not apparent in strained extracts. Labelling of extracts with antibodies to phosphorylated amino acid residues revealed the doublet CD44 band of static samples to be phospho-serine, phospho-threonine and phospho-tyrosine positive whereas the 70kDa band identified as moesin was only labelled with antibody to phospho-serine. After strain, the single CD44 positive band did not label with antibodies to phosphorylated amino acid residues.

Discussion: These experiments indicate that embryonic fibrocartilage cells respond directly to mechanical strain by increasing CD44 expression, bHA binding ability and HA release into the media and that these changes are associated with changes in the phosphorylation of CD44. Thus, the application of strain in vitro induces a number of HA-related characteristics associated with the development of the joint in ovo including increased UDPGD activity, CD44 expression and bHA binding (2, 3). These results, in addition to those indicating immobilisation-induced changes in HA synthesis and CD44 expression (4) and HA oligosaccharide-induced failure to cavitate (3), endorse the concept that HA:HABP interactions play a pivotal role in the mechanism of diarthrodial joint morphogenesis and that this mechanism is mediated by mechanical stimuli. In addition, the increases apparent in HA release, UDPGD activity and CD44 expression are associated with changes in the phosphorylation of CD44 and moesin, suggesting that complex signalling events control both CD44 expression and HA synthesis and that the synthesis of and binding to HA are closely coordinated.

References: (1) Edwards et al. (1994) J.Anat. 185, 355 (2) Pitsillides et al. (1995) J.Histochem.Cytochem . 43,263 (3) Dowthwaite et al. (1997) Trans. ORS. 22, 59 (4) Pitsillides (1998) in Biology of the Synovial Joint.

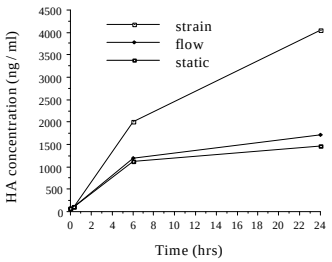

Figure 1: HA release into media over 24 hours following the application of strain.

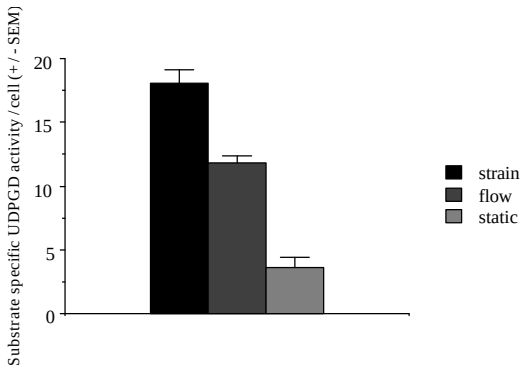

Figure 2: UDPGD activity 24 hours after the application of strain.

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