

P003 UGDH is a positive modulator of GAG production and chondrogenesis

**Clarkin C.E, Allen S, Wheeler B.T,
Wheeler-Jones C.P.D and Pitsillides A.A**

The Royal Veterinary College

Restoring the compressive resilience of cartilage once its glycosaminoglycan (GAG) content becomes compromised cannot be achieved until mechanisms regulating chondrocyte GAG synthesis have been fully elucidated. UDP-glucose dehydrogenase (UGDH) is a key enzyme catalysing the oxidation of UDP-glucose to UDP-glucuronic acid, an essential monosaccharide precursor required for hyaluronan (HA) and sGAG synthesis. Herein, we have used retroviral (RCAS) transfection techniques to examine the effects of UGDH overexpression on the behaviour of chick embryo articular surface (AS) cells, as well as chick marrow and limb bud micromass cultures. We found that media conditioned by UGDH-transfected AS cells contained significantly greater HA and sGAG levels (vs. RCAS-GFP controls). UGDH-overexpressing AS cells formed significantly larger HA-dependent pericellular coats, suggesting a rate-limiting role for UGDH in both HA and sGAG synthesis. In addition, an inhibitor of HAS activity (4-MU) blocked control and UGDH-dependent increases in HA release. Furthermore, we found that transfection with UGDH resulted in significant increases in Alcian blue-positive nodule size, but not number, in micromassed marrow culture, and increased chondrogenesis in chick limb bud micromass. These studies suggest that UGDH modulates chondrogenesis. To test UGDH involvement in the anabolic response to archetypal growth factors, consequent studies used AS cells and disclosed increases in UGDH mRNA and protein expression in response to TGF- β treatment that were associated with significant increases in sGAG production, HA release and pericellular coat formation. Our results provide evidence that sGAG and HA synthesis are limited by UGDH activity and suggest a potential direct positive role for UGDH in regulating chondrogenesis.