The role of IGFBPs in the regulation of chondrocyte metabolism *in vitro*

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Summary

Insulin-like growth factors (IGFs) and inflammatory cytokines (eg. IL-1 and TNFα) affect cartilage metabolism in opposite ways. While the growth factors stimulate the anabolic processes such as proteoglycan synthesis, the inflammatory cytokines increase the degradation rate of these cartilage macromolecules and suppress their synthesis through the mechanism which is not fully understood. The actions of IGFs in biological systems are modulated by locally produced IGF binding proteins (IGFBPs). Recent reports of elevated levels of IGFBPs in osteoarthritic (OA) and rheumatoid arthritic (RA) joints and findings that OA chondrocytes increase the production of IGFBPs have indicated that those factors could play a critical role in regulating the metabolism of cartilage-specific macromolecules in pathophysiological conditions. Therefore, I decided to investigate effects of the IGFs and inflammatory cytokines on IGFBPs produced by chondrocytes and the subsequent interplay of these factors on proteoglycan production *in vitro*. To do this, a primary culture of ovine articular chondrocytes was used as an *in vitro* experimental model system.

The initial results of this study have shown that, in basal conditions, ovine articular chondrocytes produce IGFBP-2 and a 24-kDa IGFBP whose mobility on the SDS gel corresponds to that of non-glycosylated IGFBP-4. Furthermore, the IGFs and inflammatory cytokines stimulated the appearance of IGFBP-5 in chondrocyte primary culture, although through different mechanisms of action. Thus, while IL-1 induced the expression of IGFBP-5 mRNA, IGF-I upregulated IGFBP-5 predominantly at the post-translational level by protecting the binding protein from proteolytic degradation. The inhibitor profile of the chondrocyte-derived IGFBP-5 proteolytic activity points to a serine protease whose activity is inhibited in the presence of IGF-I. The two mechanisms of IGFBP-5 regulation acted synergistically

when chondrocytes were co-incubated with IGF-I and IL-1, which resulted in a considerably increased concentration of the binding protein.

IGF-I was ten times less potent in stimulating proteoglycan synthesis in ovine articular chondrocytes than its analogues with reduced affinities for IGFBPs, des(1-3)IGF-I and LR^3IGF-I, clearly demonstrating the negative effect of the locally produced IGFBPs on the IGF-I activity. A recombinant IGFBP-5 additionally suppressed the IGF-I-induced proteoglycan synthesis indicating that this binding protein contributes to the overall negative effect of the IGFBPs on the IGF-I bioactivity. These results imply that the upregulation of IGFBP-5 by IGF-I is a negative feedback mechanism by which the growth factor restricts its own bioactivity in cartilage. In addition, IL-1, which in the presence of IGF-I caused a substantial increase in IGFBP-5 protein level, significantly diminished IGF-I-stimulated proteoglycan synthesis whereas the activities of des(1-3)IGF-I and LR^3IGF-I remained unaffected. This again demonstrates the negative effect of the endogenous IGFBPs and strongly suggests that the suppressive effect of IL-1 on IGF-I-induced proteoglycan production in ovine articular chondrocytes is mediated through increased levels of IGFBP-5. A high level of IGFBP-5 most likely contributes to the IGF-I sequestration thus preventing the growth factor from interacting with the receptors.

In conclusion, the IGFBP-5-mediated decrease in proteoglycan synthesis could be a relevant *in vivo* mechanism by which IL-1 exerts its catabolic effect and disturbs the balance between the synthesis and degradation of cartilage matrix macromolecules in pathological conditions.