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The role of the IGF/IGFBP system in the proliferation and differentiation of chondrocytes

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The major systemic hormones regulating longitudinal bone growth during childhood are growth hormone (GH), insulin-like growth factor (IGF)-I, thyroid hormones and glucocorticoids. Because IGF-I is an important chondrocyte growth factor, we sought to investigate, how it regulates the expression of the IGF binding proteins (IGFBPs), and to examine the intracellular mechanisms, by which exerts two of its pivotal effects, stimulation of proliferation and differentiation.

The bioactivity of IGF-I in the cellular microenvironment is modulated both by inhibitory and stimulatory IGFBPs whose production is under partial control of IGF-I. However, little is known on the IGF-mediated regulation of these IGFBPs in the growth plate. We therefore studied the effect of IGF-I on IGFBP synthesis in rat growth plate chondrocytes in primary culture and the involved distinct intracellular signaling pathways. Under baseline conditions, growth plate chondrocytes expressed mRNA species for IGFBP-2 to -6, determined by RT-PCR. Incubation with IGF-I enhanced IGFBP-3, IGFBP-4 and IGFBP-5 in conditioned cell culture medium in a dose- and time-dependent manner. Coincubation of IGF-I with specific inhibitors of the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK)1/2 pathway (PD098059 or U0126) completely abolished IGF-I-stimulated IGFBP-3 gene expression, quantified by RNase protection assay. In contrast, inhibition of the phosphatidylinositol-3 kinase (PI-3 kinase) signaling pathway by LY294002 abrogated both IGF-I-stimulated IGFBP-3 and -5 gene expression. For comparison, IGF-I-driven cell proliferation was mediated both through the MAPK/ERK1/2 and PI-3 kinase pathway. These data suggest that IGF-I modulates its activity in juvenile rat growth plate chondrocytes by the synthesis of both inhibitory (IGFBP-3, IGFBP-4) and stimulatory

(IGFBP-5) binding proteins in a feed-back manner. The finding that IGF-I uses different and only partially overlapping intracellular signaling pathways for the regulation of two IGFBPs with opposing biological functions might be important for the regulation of IGF bioactivity in the cellular microenvironment.

IGF-I promotes both proliferation and differentiation of growth plate chondrocytes *in vitro* and *in vivo*. In order to investigate the pathways involved in the IGF-regulation, we used the mesenchymal chondrogenic cell line RCJ3.1C5.18 (RCJ), which progresses spontaneously to differentiated growth plate chondrocytes. This differentiation process could be enhanced by exogenous IGF-I. Pharmacological inhibition of PI-3 kinase by LY294002, the MAPK/ERK1/2 by U0126 and the protein kinase A (PKA) pathway by H-89 completely surpressed IGF-I-stimulated cell proliferation as assessed by [^{3}H]thymidine incorporation, while blockade of the protein kinase C (PKC) pathway by Bisindolylmaleimide (BIS) had no significant effect. In contrast, IGF-I-induced early cell differentiation, as assessed by collagen type II gene expression, was not affected by MAPK/ERK1/2 pathway inhibition, but almost abolished by inhibition of the PI-3 kinase and PKA pathways. Moreover, middle to late differentiation of chondrocytes in response to IGF-I, as assessed by alcian blue and alkaline phosphatase activity assay, was only interrupted by PI-3 kinase pathway inhibition. The phosphorylation state of Akt downstream of PI-3 kinase was enhanced by inhibition of PKC and/or PKA, whereas PI-3 kinase or PKC inhibition had no effect on the MAPK cascade, as assessed by the ERK1/2 phosphorylation state. The respective protein content of distinct PKC and PKA subunits increased during differentiation. These data suggest that the two crucial cellular responses of chondrocytes to IGF-I, proliferation and differentiation, are mediated by parallel and partially overlapping signaling pathways. Whereas the PI-3 kinase, MAPK/ERK1/2 and PKA pathways subserve the mitogenic action of IGF-I, IGF-I-stimulated cell differentiation is mainly signaled through the PI-3 kinase pathway. Hence, IGF-I exerts its differential effect on chondrocyte proliferation vs. differentiation through the use of at least four partially interacting intracellular signaling pathways, whose activity is temporarily regulated.

Since we have shown previously that intact IGFBP-5 in the presence of IGF-I stimulates chondrocyte proliferation, we decided to examine the role of IGFBP-5 on chondrocyte differentiation, using the RCJ cell line. RCJ cells undergoing spontaneous differentiation markedly upregulated IGFBP-5 synthesis. Transient IGFBP-5 overexpression in RCJ cells in the absence of IGF-I did not promote the expression of the chondrocyte differentiation markers collagen type II and proteoglycan, indicating that IGFBP-5 on its own does not stimulate chondrocyte differentiation. However, IGFBP-5 overexpression enhanced the process of IGF-I-mediated differentiation of RCJ cells. A potential mechanism for this effect is the specific increase of Akt phosphorylation in IGFBP-5 overexpressing cells in the presence of IGF-I, indicating an increased activity of the PI-3 kinase pathway, while the MAPK/ERK1/2 cascade, was rather downregulated. Furthermore, IGF-I increased IGFBP-5 expression in differentiating chondrocytes by use of three signaling pathways (PI-3 kinase, PKA, and PKC pathway), which are also operative for IGF-I-mediated cell differentiation. The IGF-I-induced IGFBP-5 gene expression required *de novo* mRNA transcription and *de novo* protein synthesis. These data demonstrate that IGFBP-5 enhances growth plate chondrocyte differentiation through an IGF-I-dependent mechanism and imply a role for IGFBP-5 in upregulating IGF action during chondrocyte differentiation *in vivo*.