**Introduction:** It is well established that IGF-I exerts positive anabolic effects on chondrocytes in vivo and in vitro. However, little is known on IGF-I mediated regulation of collagen II, matrix metalloproteinase-13 (MMP13) gene expression and the involved intracellular signaling pathway in endplate chondrocytes. In the present study, we studied the effect of IGF-I on collagen II and MMP13 mRNA expression and the role of PI3K and MAPK signaling pathways in the regulation of collagen II and MMP13 mRNA expression in rat endplate chondrocytes.

**Materials and Methods:** Primary chondrocytes were isolated from the cervical spine endplate cartilage as described by Wang Yongjun et al (1).

RNA was harvested using the RNAeasy kit per manufacturer’s protocol. PCR was carried out using the RotorGene real-time DNA amplification system. Gene expression was normalized to beta-actin. The result was illustrated as F value.

$F\text{ value} = \frac{\text{the concentration of target gene in sample}}{\text{the concentration of beta-actin in sample}} \div \frac{\text{the concentration of target gene in control}}{\text{the concentration of beta-actin in control}}$

Western Blot Analysis. The endplate chondrocytes of the second passage were lysed. Thirty micrograms of protein exact were separated by SDS-PAGE and then transferred to a PVDF membrane. The blots were probed overnight at 4 degree with either rabbit anti-rat phosphespecific Akt antibody or Phosphespecific ErK1/2 antibody. After washing, the membranes were incubated for 1 hour at 20 degree with goat anti rabbit-IgG. Immune complexes were detected using Odyssey infrared imaging system. Membranes were reprobed for beta-actin to confirm equal protein loading.

**Results:** IGF-1 increased the collagen II alpha mRNA expression in a time- and dose-dependent manner. With the treatment of IGF-1 (100 ng/ml), the expression of collagen II alpha mRNA in rat endplate chondrocytes reached the highest value at 24 h and then decreased gradually along with time. IGF-1 (100 ng/ml) increased collagen II alpha mRNA levels in rat endplate chondrocytes by 3.89 fold after treatment 24 h. Additionally, IGF-1 decreased MMP13 mRNA expression in rat endplate chondrocytes. IGF-1 (100 ng/ml) decreased MMP13 mRNA level by 0.55 fold after treatment 24 h. Furthermore, IGF-1 activated (phosphorylation) members of both the PI3K pathway and the ERK/MAPK pathway, Akt and ERK1/2. Coincubation of IGF-1 with PI3K inhibitor wortmannin significantly blocked the stimulatory effect of IGF-1 on collagen II alpha mRNA expression, but did not significantly inhibit IGF-induced repression of MMP13 mRNA expression. In contrast, the ERK/MAPK inhibitors PD98059 was able to block partially IGF-stimulated collagen II alpha mRNA expression, but significantly blocked IGF-induced MMP13 mRNA repression.

**Discussion:** The result suggested that IGF-1 stimulated Collagen2 alpha mRNA expression and inhibited MMP13 mRNA expression in endplate chondrocytes. In additional, IGF-1 stimulation of PI3K signaling pathway is responsible for the ability of IGF-1 to increase collagen II alpha mRNA expression, and IGF-1 stimulation of ERK/MAPK signaling pathway is responsible for the ability of IGF-1 to inhibit MMP13 mRNA expression.

**References:**

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