様式第３号

論　文　要　旨

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論文の要旨

To determine the effect of high molecular weight hyaluronic acid (HA) on matrix metalloproteinase 13 (MMP13) expression induced by tumor necrosis factor α (TNF-α) in chondrocytes. Human chondrocytic C28/I2 cells were incubated with TNF-α and HA. In some experiments, the cells were pre-incubated with a CD44 function-blocking monoclonal antibody (CD44 mAb) prior to addition of TNF-α and HA. The expression of MMP13 was determined by real-time reverse-transcription polymerase chain reaction (RT-PCR) and an enzyme linked immunosorbent assay, while the phosphorylation of signaling molecules was measured by western blot analysis. The transcriptional activity of activator protein 1 (AP-1) was analyzed by a reporter assay. To further clarify the molecular mechanisms of HA in MMP13 regulation, the expression level of dual-specificity protein phosphatase 10 (DUSP10)/mitogen-activated protein kinases phosphatase 5 (MKP5) in HA-treated chondrocytes was assessed by real-time RT-PCR, western blotting, and immunofluorescence microscopy. HA decreased MMP13 mRNA and protein expression induced by TNF-α. Blockage of HA-CD44 binding by CD44 mAb suppressed HA-mediated inhibition of MMP13. HA inhibited transient phosphorylation of p38 mitogen-activated protein kinase (MAPK) and c-jun NH_2-terminal kinase (JNK) induced by TNF-α. Reporter assay findings also revealed that pre-treatment with HA inhibited the transcriptional activity of AP-1 mediated by TNF-α. Moreover, HA induced the expression of DUSP10/MKP5, a negative regulator of p38 MAPK and JNK pathways. These results indicate that HA-CD44 interactions down-regulate TNF-α-induced MMP13 expression via regulation of DUSP10/MKP5, suggesting that HA plays an important role as a regulatory factor in cartilage degradation.