REGULATION OF TENASCIN-C IN HUMAN ARTICULAR CHONDROCYTES OF OSTEOARTHRITIS

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Introduction: Osteoarthritis (OA), the most common cause of ability in the elderly, is characterized by injury in articular cartilage with loss of cartilage matrix. TN-C is an extracellular glycoprotein having important functions in tissue remodeling processes, such as wound repair and embryogenesis including cartilage formation. In the normal adult cartilage, tenascin-C (TN-C) is barely expressed, whereas an increased expression of TN-C in OA cartilage is observed. It is generally accepted that proinflammatory cytokines, such as interleukin-1 (IL-1), tumor necrosis factor (TNF-α) and interleukin-6 (IL-6) produced in OA joints cause the cartilage damage. It is reported that TN-C expression is upregulated by IL-1 in chondrocytes of OA cartilage. TNF-α induces the production of IL-6 in chondrocytes of OA. We hypothesized that TN-C production of chondrocytes may be regulated by TNF-α and IL-6 as well as IL-1. In this study we examined whether TNF-α and IL-6 seen in OA upregulates TN-C expression in vitro.

Materials and Methods: Chondrocyte isolation and culture: Chondrocytes were isolated from human articular cartilage during knee replacements under sterile conditions. The cells were cultured in DMEM/F12 containing 10% FBS.

Immunofluorescence: For immunofluorescent staining, chondrocytes were cultured in 0.1% BSA medium and then 100 ng/ml of TNF-α or IL-6 was added to the medium. The cells were treated with 1 μM monensin before fixation to block secretion and thus cause accumulation of proteins in the cytoplasm. The chondrocytes were incubated with the anti-TN-C antibody (4F10TT) at 350 kDa and 210 kDa, respectively. Western blot analysis: Chondrocytes were placed in a fresh 0.1% BSA medium without FBS, and 100 ng/ml of TNF-α or IL-6 was added. After 24 h of incubation, the conditioned medium was collected. The proteins were subjected to SDS-PAGE after analysis for normalization of protein content, and then transferred to membrane by a semidry trans-blotting system. The membrane was incubated with the appropriate HRP labeled secondary antibody. The signal of the conditioned medium was visualized using ECL detection reagents by the chemiluminescence method.

Quantitative real-time PCR: The total cellular RNA was isolated from chondrocytes cultured with TNF-α (1, 10, and 100 ng/ml) or IL-6 (1, 10 and 100 ng/ml). cDNA synthesis was performed with single-strand cDNA synthesis kit for RT-PCR. Quantitative analysis of TN-C mRNA was performed by real-time RT-PCR. TN-C mRNA levels were normalized by the GAPDH level of each sample. The levels are expressed as an x-fold induction compared with non-treated cells.

Statistical analysis: Association with the variables was determined by Mann-Whitney U-test. Differences were considered significant for p<0.05.

Results: Immunofluorescence: When chondrocytes were cultured in the medium containing 0.1% BSA, immunofluorescence staining of TN-C showed low immunoreactivity (Fig.1A). In the chondrocytes treated with TNF-α at 100ng/ml concentrations, fluorescence was remarkably strong (Fig.1B). However, Immunofluorescence staining of TN-C results showed no effect of IL-6 (Fig.1C).

Western blot analysis: Both large and small TN-C variants could be detected by the anti-TN-C antibody (4F10TT) at 350 kDa and 210 kDa, respectively. Addition of TNF-α to chondrocytes for 24h significantly increased expression of TN-C protein compared with untreated chondrocytes, whereas IL-6 treated cells was similar to TN-C expression of untreated condition (Fig.2).

Quantitative real-time PCR: Quantitative analysis by real time PCR demonstrated that TN-C mRNA level was significantly upregulated, being 1.9 ± 0.7 and 2.9 ± 1.5 times after treatment 1 and 10 ng/ml of TNF-α, respectively, compared with non-treated cells (n=7, p < 0.001) Treatment of 100ng/ml TNF-α (3.0 ± 1.3) was similar to the results treated with 10ng/ml TNF-α (Fig.3A). However, we did not find an effect of IL-6 on mRNA of TN-C expression in any concentrations (n=6) (Fig.3B).

Discussion: In this study using human articular chondrocytes, we have demonstrated that TNF-α stimulates the expression of TN-C in both protein and mRNA levels in vitro, whereas IL-6 does not affect.

Previous studies showed that inflammatory cytokines such as IL-1β, TNF-α and IL-6 were increased in OA cartilage and stimulated different catabolic pathways by activating the transcription factor NFκB and several other signal cascades. In recent studies, it showed that IL-1β increased the expression of TN-C in OA chondrocytes. TNF-α also stimulated the expression of TN-C. These inflammatory cytokines stimulate expression of matrix metalloproteinases and aggrecanase, thereby contributing to the dysregulation of chondrocyte function that leads to the progressive degradation of the cartilage matrix and loss of joint function.

TNF-α might regulate TN-C production in articular chondrocytes in OA. Whereas upregulated TN-C could imply an important role in the pathogenesis of OA, bidirectional effects of NFκB signaling, cell death or survival, have been well demonstrated. Further investigations on TN-C functions in OA should be warranted.