EXPRESSION OF 3 ISOFORMS OF HYALURONAN SYNTHASE(HAS) IN HUMAN RHEUMATOID SYNOVIAL CELLS

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Introduction
Hyaluronan (HA), a high molecular weight linear glycosaminoglycan (GAG) consisting of alternating units of D-glucuronic acid and N-acetylglucosamine, is a component of the extracellular matrix (ECM). Concentrations of HA are highest in musculoskeletal tissues, skin, embryological tissues, and synovial fluid, and HA plays an important part in wound healing, embryonic development, tumour growth, and synovial fluid viscosity. In joint disease, the viscosity of synovial fluid, which is mainly determined by the concentration and molecular weight distribution of HA, is changed. HA is synthesized via hyaluronan synthase (HAS) which is located at the plasma membrane. Recently, it has been reported that 3 isoforms of HAS, named HAS1, HAS2 and HAS3, have been cloned and that each of those plays a different role in HA production in vivo. Therefore, the purpose of this study was to compare the regulation of three hyaluronan synthase transcripts and HA production by transforming growth factor β (TGFβ1), tumour necrosis factor α (TNFα) and interleukin 1β (IL-1β) in human rheumatoid synovial cells.

Materials and Methods
Synovial fibroblasts were obtained from patients with rheumatoid arthritis at the time of surgery for total knee replacement. Tissue was minced finely, digested with collagenase for 6h, centrifuged and resuspended in DMEM supplemented with 10% FBS and penicillin/ streptomycin. Cells were passed upon confluence and were used 5th passage. Cultures of synovial cells were prepared in a free-serum medium for 12 hours and stimulated with TGFβ1(1ng/ml), TNFα(100u/ml), IL-1β(1ng/ml) for 3h, 6h, 12h and 24h. Total RNAs were prepared from cells by direct lysis of RT-lysis buffer and purification according to the procedure described for isolation of total RNA (RNeasy Total RNA kit, Qiagen). RNA was eluted from the columns in a volume of 30μl and was stored at –70°C. 1μg of total RNA from each dish was reverse transcribed to cDNA using MuLV reverse transcriptase primed with randomhexamers.

The cDNA for human Has1,Has2 and Has3 were generated using RT-PCR. The following primers were designed for RT-PCR: sense primer, 5’-ACTC-GGACACAAGGGTGGAC-3’, and anti-sense primer, 5’-ACGAGGGCGTCTCTGAGTAG-3’, for Hasl; sense primer, 5’-GGGACATGAAGATCATCTCT-3’, and anti-sense primer, 5’-ACGTGTTGCGAGCTTTCTTT-3’, for Has2; and sense primer, 5’-ACTGGTACCATCAGAAGTTC-3’, and anti-sense primer, 5’-ACGTGTTGCGAGCTTTCTTT-3’, for Has3. The cDNA for human Has1,Has2 and Has3 were generated using RT-PCR. The following primers were designed for RT-PCR: sense primer, 5’-ACTCGGACACAAGGGTGGAC-3’, and anti-sense primer, 5’-ACGAGGGCGTCTCTGAGTAG-3’, for Hasl; sense primer, 5’-GGGACATGAAGATCATCTCT-3’, and anti-sense primer, 5’-ACGTGTTGCGAGCTTTCTTT-3’, for Has2; and sense primer, 5’-ACTGGTACCATCAGAAGTTC-3’, and anti-sense primer, 5’-ACGTGTTGCGAGCTTTCTTT-3’, for Has3; and sense primer, 5’-ACTGGTACCATCAGAAGTTC-3’, and anti-sense primer, 5’-ACGTGTTGCGAGCTTTCTTT-3’, for GAPDH. The expressions of HAS1, HAS2 and HAS3 were assessed by mRNA analysis using a novel realtime quantitative polymerase chain reaction method with LightCycler (Roche) following the manufacturer’s protocol. Expression levels between dishes were calibrated with the amount of co-amplified GAPDH mRNA.

Concentrations of HA in culture supernatants of synovial cells with or without stimulation by TGFβ1, TNFα or IL-1β were measured by a sandwich binding protein assay kit (Hyaluronate Plate Chugai,Chugai Pharmaceutica).

Results
RT-PCR showed amplified bands of the expected sizes by using the primer sets for HAS1, HAS2 and HAS3 (data not shown). Using the LightCycler induction of HAS1, HAS2 and HAS3 mRNA content could be confirmed by quantitative PCR. When the cultures were stimulated with TGFβ1, maximum expression of HAS1 mRNA was observed after 6 hours, and HAS3 mRNA was up-regulated by IL-1β and TNFα, while HAS2 mRNA was not significantly changed by these cytokines.

Discussion
The results of the present study show that all three HAS isoforms are expressed in human rheumatoid synovial cells at the mRNA level. Several lines of evidence demonstrated that expression of HAS and synthesis of HA are regulated by a variety of mechanisms. Cytokines are actively involved in the pathogenesis of chronic inflammatory and degenerative joint diseases. High levels of proinflammatory cytokines such as IL-1β and TNFα have been found in the synovial fluid of patients with inflammatory joint disease. In joints of rheumatoid arthritis, the viscosity is generally decreased and molecular weight is low. Furthermore, recent observation demonstrated that HA with low molecular weight showed various biological activities including macrophage activation. In addition, analysis of the size distribution of HA generated in vitro demonstrated that HAS3 synthesized HA with a molecular mass of 2×105 to 3×105 Da, shorter than those synthesized by HAS1 and HAS2 which have molecular masses of 2×106 Da. In this study, HAS3 mRNA was up-regulated by IL-1β and TNFα, while HAS1 mRNA was over expressed by TGFβ1. Differences in expression pattern of Has1, Has2 and Has3 mRNA in human rheumatoid fibroblastic synovial cells suggest that 3 subtypes of HAS genes are regulated independently and synthesized hyaluronan may have a different function in human rheumatoid synovial joint. Further studies to investigate the expression of HAS3 genes and its relationship to inflammatory responses would help clarify the functions of HA in rheumatoid arthritis.

Figure 1.
Quantitative determination of Has1,Has2 and Has3 mRNA contents and HA production.

References
Haubeck HD et al., Arthritis Rheum, 38: 669-677, 1995

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