The Gene Expression Profile of ADAMTS-5 in the Synovium of Experimental Osteoarthritis and Gene Silencing Using Small Interfering RNA

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Introduction: The molecular biological mechanisms underlying joint disorders are gradually being clarified. Recent studies have showed proteinases including aggrecanase and metalloproteinase closely related pathogenesis of osteoarthritis (OA). Since it was reported that the progression of experimental OA in a disintegrin and metalloproteinase with thrombospondin-like motifs-5 (ADAMTS-5; aggrecanase-2) knock out mice was significantly milder than that of wild-type mice [1], ADAMTS-5 has attracted attention as a key mediator of cartilage degeneration in OA. Synovial tissue inflammation is one of the pathological features of OA, and it was reported that ADAMTS-5 was expressed in the synovium from osteoarthritic joint [2]. However, the role of ADAMTS-5 in synovitis is not sufficiently clarified. The aim of this study is to investigate the time course expression of ADAMTS-5 in the synovium of the experimental OA and examine the regulation of gene expression in synovial cells using small interfering RNA (siRNA).

Materials and Methods: OA was induced in the left knee joint of Wister rats by performing unilateral transection of the anterior cruciate ligament (ACL T). In sham-operated animals, only arthrostomy was performed. One, 2, 3, 4, 7 and 10 weeks after surgery, synovial tissue of knee joints were collected and total RNA was extracted. The gene expression of ADAMTS-5 was evaluated by real-time RT-PCR. Cartilage was harvested from the evaluated knees and examined histologically.

Synovial fibroblasts were isolated from synovial tissues of knee joints of rats. Three strands of siRNA specific for ADAMTS-5 (siADAMTS-5) or unrelated siRNA were transduced into synovial fibroblasts by using siPORT Amine (Ambion). Two days after transfection, total RNA was extracted, and the expression of ADAMTS-5 in the synovial fibroblasts was evaluated by real-time RT-PCR.

Results: The histological examination showed mild degeneration of cartilage until 4 weeks after ACL T, while severe OA was observed after 7 weeks. Until 4 weeks after ACL T, ADAMTS-5 showed increased expression in synovium. The expression was gradually reduced according to the progression of OA. One, 3 and 4 weeks after surgery, ADAMTS-5 expressions of ACL T knees were significantly higher than that of sham-operated knees. In contrast, the expressions were not significantly different in 7 and 10 weeks after surgery.

Discussion: Although it was considered that ADAMTS-5 is a key aggrecanase in OA pathogenesis, the expression of ADAMTS-5 in the cartilage and synovium of the end-stage OA which undergone joint replacement surgery was lower than that of normal cartilage [3]. It was reported that aggrecanase was mainly involved in degradation of cartilage in the early-stage of OA, metalloproteinase played main role in the end-stage of OA [4]. In this study, ADAMTS-5 expression in the synovium of ACL T group was significantly higher than that of sham-operated group within a month after operation. However, the expression reduced in the end-stage of disease. These findings suggest that ADAMTS-5 in synovium related degradation of cartilage in the early-stage of OA.

RNA interference (RNAi) is a potent and convenient gene silencing technique, and siRNA has been expected as a next-generation therapeutic agent. In the present study, siADAMTS-5 suppressed the expression of ADAMTS-5 in synovial fibroblasts effectively. This finding suggests that siRNA is one of the potent tools for the suppression of disease responsible genes in synovial cells. We have already established in vivo siRNA transduction method into joint synovium [5, 6]. Therefore, to suppress the expression of ADAMTS-5 in the synovium using siRNA could be applied as a new therapeutic strategy of OA.


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