INTRODUCTION

Irreversible destruction of cartilage is a feature of arthritis such as rheumatoid arthritis (RA) and osteoarthritis (OA). Inflammation pathways are involved in the catabolic processes of articular cartilage especially in RA and potentially in OA. Stimulation of proinflammatory cytokines in arthritis activates numerous transcriptional factors and induces expression of matrix metalloproteinases (MMPs), aggrecanases, and other catabolic factors. Among transcriptional factors activated by cytokines, we focused on the functions of the CCAAT/enhancer binding protein (C/EBP) family in arthritis. C/EBP is a family of basic leucine zipper transcriptional factors. In particular, C/EBPβ is induced in response to inflammatory stimulation in various cells and tissues. In fact, C/EBPβ is expressed in tissues of RA and OA. Therefore C/EBPβ may play important roles in various phenomena in arthritis. Thus we hypothesized that C/EBPβ mediates the expression of MMPs and aggrecanases in arthritis.

MATERIALS AND METHODS

**Immunohistochemistry**
Tissue samples of synovium and articular cartilage were obtained from patients with RA and OA at the time of total knee arthroplasty. Immunohistochemistry were performed by using the streptavidin-biotin-peroxidase method. **Cell culture** C-28/I2 cells, immortalized human chondrocytes, SW582 human synovial sarcoma cells and Human fibroblast-like synoviocytes (HFLS) that were derived from normal synovial tissue were cultured. **Transient transfection and gene over-expression in cell lines** cells were transfected with adenovirus expression vector for C/EBPβ-LAP or LacZ control for 24 hours. **Gene knockdown in cell lines** Cells were transfected C/EBPβ Silencer Pre-designed siRNA or negative control siRNA using Lipofectamine. Cell lines were cultured for 24 hours after transfection, and then treated with IL-1β at concentration of 2 ng/ml 5hours. For immunoprecipitation, anti-C/EBPβ antibodies and normal rabbit IgG were used. **Over-expression in organ culture** Cartilage tissue was obtained from the intercondyle notch of 3 young adult patients who underwent anterior cruciate ligament (ACL) reconstruction. The cartilage tissue was cultured with an adenovirus expression vector for C/EBPβ-LAP or LacZ control for 24 hours.

RESULTS

Immunohistochemistry showed localization of C/EBPβ and MMP-3 expression in synovium and cartilage. Western blot analysis revealed the expression of C/EBPβ, two major isoforms; LAP and LIP in both cell lines after treatment with IL-1β. In cells transfected with adenovirus expression vector for C/EBPβ, MMP-3, 13 and ADAMTS-5 expression were significantly increased (Fig1). C/EBPβ stimulated MMP-3 expression and induced matrix degradation in cartilage explants (Fig2). C/EBPβ knockdown with siRNA dramatically reduced the MMP3 and ADAMTS-5 expression induced by IL-1β (Fig3). Reporter assay was showed that C/EBPβ stimulates the MMP-3 and ADAMTS-5 promoters activity in a dose-dependent manner and C/EBPβ responsive element was located between -108bp and -100bp of the MMP-3 promoter. A ChIP assay showed that C/EBPβ directly bound to MMP-3 and ADAMTS-5 promoters.