Introduction
Interleukin-1β (IL-1β) has been suggested to be involved in the pathogenesis of osteoarthritis (OA) by increasing catabolic enzymes or enhancing apoptosis.

Recently it has been reported that SIRT1, a histone deacetylase that regulates gene expression and protein function by deacetylating histone and nonhistone proteins, inhibits apoptosis and promotes synthesis of extracellular matrix in human chondrocytes, suggesting that SIRT1 plays a protective role in human chondrocytes. Nevertheless, precise roles of SIRT1 in human chondrocytes are not yet fully understood. In this study, we examined the effect of overexpression of SIRT1 on IL-1β-treated human chondrocytes to explore protective roles of SIRT1 in human chondrocytes.

Methods
Normal Human Articular Chondrocytes-knee (NHAC-kn) was purchased (Cambrex, USA) and used as normal human chondrocytes. NHAC-kn was transfected with either control plasmid or SIRT1 expression plasmid by a lipofection technique. After the transfection, NHAC-kn was treated with 10ng/ml IL-1β for 24 hours. The effects of IL-1β treatment and the overexpression of SIRT1 were examined by real-time PCR and western blotting. Interaction of SIRT1 and p65 (a member of NF-κB) were examined by immunoprecipitation.

Results
1. Overexpression of SIRT1 inhibited IL-1β-induced MMPs and ADAMTS-5.
We first examined the expression of SIRT1 1 by western blotting and confirmed efficiently overexpressed SIRT1 proteins by the SIRT1 expression plasmid (Figure 1). We next examined the effect of overexpression of SIRT1 under the stimulation of IL-1β. The stimulation of IL-1β significantly upregulated MMP-1, -2, -9, -13 and ADAMTS-5. The overexpression of SIRT1 significantly inhibited the upregulation of MMP-1, -2, -9, -13 and ADAMTS-5 caused by the stimulation of IL-1β (Figure 2). Consistently, the overexpression of SIRT1 decreased the increased protein levels of MMP-13 and ADAMTS-5 caused by the stimulation of IL-1β (Figure 3).

2. Overexpression of SIRT1 reduced acetylation of p65 induced by IL-1β.
Since it has been reported that the expression of MMPs are regulated by the NF-κB pathway, we examined the effect of the overexpression of SIRT1 on the NF-κB pathway. The stimulation of IL-1β increased total p65 and acetylated p65. The overexpression of SIRT1 markedly reduced IL-1β-induced acetylation of p65 while it did not much affect total p65 protein level (Figure 4).

3. SIRT1 physically interacted with p65 in human chondrocytes
To further examine the interaction of SIRT1, we performed immunoprecipitation. Endogenous SIRT1 in human chondrocytes was immunoprecipitated with an antibody for human SIRT1. Western blotting analysis using anti-p65 antibody showed the presence of p65 in the immunoprecipitates (Figure 5).

Discussion and Conclusions
We found that SIRT1 overexpression counteracted against the upregulations of expressions of catabolic factors induced by IL-1β. In addition, we found that SIRT1 bound to p65 and the overexpression of SIRT1 reduced acetylated p65 induced by IL-1β. Since it has been reported that the expression of MMPs are regulated by the NF-κB pathway, our observations suggested that SIRT1 counteracted against IL-1β by at least partially modulating the NF-κB pathway. Taken together, our observations suggested that SIRT1 can play a protective role for chondrocytes and SIRT1 overexpression might be a new therapeutic approach for OA.

References