INTRODUCTION:
COX-2 inhibitors have been prescribed for long-term treatment of osteoarthritis (OA) for their fewer side effects on gastrointestinal tract. The anti-inflammatory effects of COX-2 inhibitors are beneficial for preventing OA progression. However, the direct influences of COX-2 inhibitors on the functions of articular chondrocytes during the progress of OA were rarely investigated. On the other hand, it was indicated that one of mechanisms of OA is articular chondrocytes undergoing termini differentiation, mineralization and eventually apoptosis. These articular chondrocytes resume the phenotypic changes similar to those in epiphyseal growth plates, which express collagen type X, annexins and alkaline phosphatase, but eliminate the expression of collagen type II. Aza-C (5-azacytidine), suppressing the cytidine methylation on gene transcription, was reported to induce the terminal differentiation changes of cultured epiphyseal chondrocytes as they occur during endochondral ossification. We have developed a terminal differentiation model in a 3-dimensional human articular chondrocyte culture by treating Aza-C at the 5th day of induction. This effect was also reversed by celecoxib and DFU (10−6-10−5M) (Fig 1-3). The expression of the marker genes, SOX9, PTHrP, aggrecan, COL2a1 and COL10a1 were significantly suppressed after 1-5 days of induction (Fig 1), COL2a1 and aggrecan (Fig 3), and the content of sulfated glucosaminoglycan were measured by DMMB assay.

RESULTS:
The results showed that the mRNA expressions of SOX9 (Fig 1), PTHrP (Fig 2), COL2a1 and aggrecan (Fig 3), and the content of sulfated glucosaminoglycan (GAG) were significantly suppressed after 1-5 days of Aza-C induction. Our data further showed that the Aza-C caused suppressions of gene expression and GAG synthesis were reversed by both celecoxib and DFU (10−5−10−4M) (Fig 1-3). The expression of the marker gene of hypertrophic chondrocytes, COL10a1, was significantly increased by Aza-C at the 5th day of induction. This effect was also reversed by celecoxib and DFU at 10−5M, but not 10−4M (Fig 4).

DISCUSSION:
COX-2 was known to be increased in immune cells while inflammation. COX-2 selective NSAIDs are used to block this COX-2 promoted prostaglandin synthesis. Other than the beneficial effects of COX-2 inhibitors on anti-inflammation, the direct influence on chondrocytes were not well understood. Our previous results indicated that both COX-2 selective and non-selective NSAIDs inhibited proliferation of epiphyseal-articular chondrocytes of fetal rats. Chondrocytic death was also induced by non-selective NSAIDs, but not COX-2 inhibitors. In addition, we further found that COX-2 inhibitors suppressed the expressions of functional genes, but increased the expression of type X collagen in normal human articular chondrocytes. Surprisingly, in this study we found that celecoxib and DFU attenuated Aza-C induced terminal differentiation in normal human articular chondrocytes. Our results showed that both COX-2 inhibitors rescued the Aza-C induced chondrogenic gene suppressions (SOX9, Collagen type II, PTHrP and aggrecan) and type X collagen expression. From this result, we suggest that COX-2 inhibitors may attenuate the progress of terminal differentiation in articular chondrocytes in individual with early OA changes.

REFERENCES: