INTRODUCTION
Chondrocyte viability is essential for the maintenance of articular cartilage as they constitute the only cell type in articular cartilage. Apoptosis is a form of programmed cell death that is processed by specific intracellular signaling cascades and thus has been an attractive therapeutic target for diseases in which apoptosis is an integral part of its pathogenesis. Apoptosis of chondrocytes has been suggested as an important part of the pathogenesis in osteoarthritis. Transglutaminase 2 (TGase 2) serves as an enzyme catalyzing Ca2+-dependent protein cross-linking. TGase 2 has been shown to be induced and activated during apoptosis. We have previously shown that TGase 2 expression is increased in human chondrocytes undergoing apoptosis. Furthermore, inhibition of TGase 2 by monodansylcadaverine (MDC); a competitive substrate of TGase 2; and TGase 2 siRNA have increased chondrocyte apoptosis. These findings suggested a possible protective role of TGase 2 in chondrocyte apoptosis. Retinoic acid (RA) and its various synthetic analogs affect mammalian cell growth, differentiation, and apoptosis. RA consistently induces TGase 2 expression and activation, and it was recently shown that increased TGase 2 expression protected NIH3T3 cells from apoptosis. The purpose of this study was to demonstrate whether TGase 2 is induced in human chondrocytes with RA and explore the role of TGase 2 in human chondrocyte apoptosis.

METHODS
1. Primary culture of human chondrocytes and apoptosis assay
Human chondrocyte culture, apoptosis induction and analysis of TGase 2 expression was performed as previously described. Briefly, human chondrocytes were obtained from the articular cartilage of patients undergoing total knee arthroplasty and cultured in monolayer. Chondrocyte apoptosis was induced by treating with H2O2 (1mM) for 24 hours. Apoptosis was assessed by two methods, biochemically by Annexin-V FACS analysis, and morphologically by nuclear staining for 4’-6-Diamidine-2’-phenylindole (DAPI).

2. RA treatment
Human chondrocytes were grown in medium containing 10% bovine serum and 1% streptomycin at 37°C in a humidified incubator with 5% CO2. The cells were starved in medium containing 1% serum for 48 hours prior to the treatment with 5 µM RA.

3. TGase 2 assay
The expression and enzyme activity of TGase 2 was examined with Western blot and immunocytochemistry. Total cellular proteins were isolated and Western blotted as described previously. Enzyme activity was evaluated by determining the incorporated biotinylated pentamidine using horseradish peroxidase-conjugated streptavidin.

RESULTS
1. RA up-regulated TGase 2 expression and enzyme activity in human chondrocytes
Human chondrocytes treated with RA resulted in up-regulation of TGase 2 protein as shown by the Western blot and immunocytochemistry (Fig 1A and 1B). RA-induced TGase 2 expression increased time dependent (Fig 1A). The enzyme activity of TGase 2 was also increased in RA treated cells compared with normal human chondrocyte cells (Fig 1B).

DISCUSSION
We have previously reported that endogenous TGase 2 expression was increased in human chondrocytes undergoing apoptosis. Inhibition of TGase 2 by MDC and TGase 2 siRNA was also shown to increase apoptosis and suggest a possible protective role of TGase 2 in chondrocyte apoptosis. The protective role of TGase 2 was further validated in this study as the RA-induced overexpression of TGase 2 decreased apoptosis of human chondrocytes. These results implicate a protective role of TGase 2 against apoptosis in human chondrocytes. Our findings provide new insights into the possibility of TGase 2 as a potential modulator of osteoarthritis.

REFERENCES

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Figure 1A: Western blot shows up-regulation of TGase 2 protein in time-dependent manner. The chondrocytes were incubated with 5 µM of RA for 12 hours, 1day, 2days, and 3days.

Figure 1B: Immunocytochemistry shows increased TGase 2 protein (green) expression and enzyme activity (red) in RA and RA+H2O2 treated cells. The chondrocytes were incubated with 5 µM of RA for 48 hours in the presence (1mM) or absence of H2O2 for 24 hours.

Figure 2: The chondrocytes were incubated with 5 µM of RA for 48 hours, in the presence (1mM) or absence of H2O2 for 24 hours. Apoptotic cells were quantified by Annexin V FACS analysis. Representative FACS plot (Fig 2A) and quantification of apoptosis (Fig 2B) of triplicate experiments are shown. A: Representative Annexin V FACS analysis. B: Quantification of apoptotic cells. *Difference from negative control (no treatment), P< 0.05. **Difference from H2O2- treated group, P< 0.05.