ERK1/2 Inhibitor Promotes Chondrogenesis from Bone Marrow and Adipose Tissue-derived-mesenchymal Stem Cells.

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INTRODUCTION
This study tested the hypothesis that MAP kinase inhibitors enhance chondrogenesis and suppress hypertrophic changes during chondrogenesis from MSCs. The effect of PD98059 (an ERK1/2 inhibitor) and SB203580 (a p38 inhibitor), were tested on bone marrow-derived mesenchymal stem cells (BMMSCs) and adipose-tissue-derived mesenchymal stem cells (ATMSCs).

METHODS
To induce chondrogenesis, in-vitro pellet cultures were carried out using 2.5 x 10^3 MSCs in chondrogenic medium containing 5 ng/ml of TGF-β1; for BMMSCs, and 5 ng/ml of TGF-β; and 100 ng/ml of BMP-7 for ATMSCs. From the 14th day of culture, subsets of the pellets were additionally treated with PD98059 [0, 1μM, 10μM] or SB203580 [0, 1μM, 10μM]. After two more weeks of in-vitro culture, pellets were harvested for analysis.

RESULTS
Treatment of PD98059 increased DNA contents (Fig 1) and GAG amounts (Fig 1) in both BMMSCs and ATMSCs, whereas SB203580 had little effect. Real-time PCR analysis showed COL1A1 mRNA decreased to almost a quarter in PD98059 treated BMMSCs, but did not change in ATMSCs. The mRNA levels of SOX-9 and COL2A1 increased several fold in BMMSCs and ATMSCS after PD98059 treatment, whereas SB203580 had only a slight effect. The gene expression of Runx-2, and, to a lesser degree, COL10A1, decreased after PD98059 treatment in both BMMSCs and ATMSCs, whereas, SB203580 elevated their expressions in both cell types. Type I collagen expression was significantly declined by PD98059 in both BMMSCs and ATMSCs, but was not significantly changed by SB203580 in both cells (Fig 3). Safranin-O and type II collagen expression were increased in both BMMSCs and ATMSCs by PD98059. Whereas SB203580 had moderate increase in Safranin-O and type II collagen expression in BMMSC and no observable effect in ATMSCs. On the other hand type X collagen and Runx-2 protein expressions were reduced by PD98059 in both BMMSCs and ATMSCs, but were slightly increased by SB203580 in both cells (Fig 4).

DISCUSSION
Our study demonstrates the usefulness of the ERK 1/2 inhibitor, PD98059 for the promotion of chondrogenesis and the suppression of hypertrophic changes. This finding could be helpful for cartilage tissue engineering from MSCs.

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