INTRODUCTION
The major components of the extracellular matrix of articular cartilage are often adversely affected during joint pathologies. Through degradation and/or down-regulation of synthesis, articular cartilage loses key components such as aggrecan and type II collagen, thereby increasing the risk of developing osteoarthritis and other cartilage pathologies in affected individuals. While the effects and modes of action of various cytokines and growth factors on post-transcriptional regulation of aggrecan expression has been extensively studied, there is relatively little information available on transcriptional mechanisms of aggrecan gene expression. The mitogen-activated protein kinase or extracellular signal-regulated protein kinase kinase (MEK) inhibitor PD98059 has previously been reported to stimulate aggrecan promoter activities [1], suggesting a role for the MEK/extracellular signal-regulated protein kinase (ERK) cascade in regulating expression of the gene. The objective of this study was to further characterize the role of the MEK/ERK signaling pathway in regulation of aggrecan gene expression. Both PD98059 and the more potent MEK inhibitor U0126 were used.

METHODS
Transfection of chondrocytic cell line. The rat chondrosarcoma cell line RCS (a gift of Dr. James Kimura, Henry Ford Hospital, Detroit, MI) was stably transfected simultaneously with pcDNA3-based expression constructs containing a 2.8-kb human aggrecan 5' flanking region (promoter and exon 1)-driven luciferase or a cytomegavirus (CMV) promoter-driven β-galactosidase reporter gene. The β-galactosidase construct was used to control for variations in cell number per experiment. Transfection of the cells was performed with Lipofectamine 2000 (Life Technologies, Inc, Grand Island, NY).

Treatment of transfected cells with MEK inhibitors. Transfected RCS cells were plated at a density of 3 x 10^5 cells/well in 6-well tissue culture plates and grown for 24 hours in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). The cells were then treated for 1 to 48 hours with graded levels (0 to 100 μM) of PD98059 or U0126 (n=8) as indicated in Fig. 1. Control cells were treated with DMSO (≤ 0.1%) equivalent to the amount present in the drug treated cell cultures. Reporter gene assays. After treatment, the cells were rinsed 2X with ice-cold Dulbecco’s PBS and lysed in 0.3 ml of 1X Promega Reporter Lysis Buffer (Promega Corporation, Madison, WI) per well. The activity of luciferase in each cell extract was then assayed using the Luciferase Assay System (Promega). β-Galactosidase activity was assayed using the GalacToLight Assay Kit (Tropix, Bedford, MA). The assays were performed on a Labsystems Fluoroskan Ascent FL dual fluorescence/luminescence plate reader.

Statistical analysis. The luciferase activity of each sample was normalized to its corresponding β-galactosidase activity. The data were then statistically analyzed, using the SAS statistical software system, by ANOVA and Fisher’s least significant difference procedure. Because of unequal variances, the data were log transformed before statistical analysis. The bar and error bar presented in Fig. 1 represent the mean ± SD of each sample.

RESULTS
Treatment of transfected RCS cells with graded levels of the MEK inhibitor PD98059 (n=8) (Fig. 1) dose-dependently increased aggrecan promoter activity by 2.7- to 4.1-fold relative to untreated controls (p<0.001, Fisher’s least significant difference test), consistent with previous observations [1]. In contrast to the stimulatory effects of PD98059 on aggrecan gene expression, U0126, at concentrations as high as 20 μM had no significant effects on aggrecan promoter activities (p>0.19, ANOVA). However, at 50 and 100 μM, there was a decreasing trend of aggrecan promoter activity in the presence of both PD98059 and U0126. Assessment of the early time effects of the MEK inhibitors on aggrecan promoter activities revealed that PD98059 has no significant effects until 2 hours or later. U0126, on the other hand, had no effects at all time points studied.

DISCUSSION
PD98059 is a widely used inhibitor of MEK in studies investigating the role of the MEK/ERK signaling pathways in regulation of cell activities. It inhibits activation of MEK1 and MEK2 by Raf, with greater inhibitory effects on MEK1 than on MEK2 [2]. U0126 is a newer, more potent inhibitor of the enzyme activities of both MEK1 and MEK2 [3]. The previously reported increase of aggrecan gene expression by PD98059 [1] suggested involvement of the MEK/signaling pathway in controlling aggrecan gene expression in chondrocytes. In an attempt to identify regulatory elements within the 5'-flanking region (promoter and exon 1) of the human aggrecan gene that may be responsive to the MEK/ERK signaling pathway, we discovered that the MEK inhibitors PD98059 and U0126 have differential effects on aggrecan promoter activities (Fig. 1). The stimulatory effects of PD98059 and the lack of effect of U0126 are suggestive of two possible mechanisms with regard to the role of the MEK/ERK pathway in regulation of aggrecan gene expression. Firstly, the data suggest that the PD98059 effect is mediated through mechanisms other than signaling through the MEK/ERK signaling pathway. This would mean that PD98059 affects aggrecan gene expression through some other processes that do not require MEK and/or ERK. The second possible mechanism suggested by the data is that the activities of MEK1 and MEK2 have differential effects on aggrecan gene expression and that the activity of at least one of these kinases is inhibitory to expression of the gene. Since PD98059 preferentially inhibits MEK1 activation, this alternative interpretation would mean that the activity of MEK1 is inhibitory to aggrecan gene expression. However, since it is MEK1 and MEK2 are likely to be inhibited by PD98059 at concentrations ≥ 10 μM and a differential effect still exists, the hypothesis that PD98059 affects aggrecan gene expression through a MEK/ERK-independent mechanism is the more likely explanation for the data presented here. DNA footprinting experiments are being performed to determine regions of the aggrecan gene that are affected by PD98059.

REFERENCES

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