Nonviral Transfer of Sox-trio Gene to Adults Stem Cells using a Microporator

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Introduction
Many lines of evidence have shown that Sox (sex determining region Y-type high mobility group box) proteins are necessary for chondrogenesis. Sox9 is expressed in all chondro- progenitors and chondrocytes except hypertrophic chondrocytes. Sox9 binds to and activate chondrocyte-specific enhancer elements in Col2a1, Col9a1, Col11a2, and Aggrecan in vitro. Two other members of the Sox family, Sox5 and Sox6, are also required for complete chondrogenesis. In vitro studies have shown that Sox5 and Sox6 cooperate with Sox9 to activate the Col2a1 enhancer in chondrogenic cells. This study tested the hypothesis that nonviral gene transfer of Sox-trios (Sox-5,6,9) enhance chondrogenesis and suppress hypertrophic changes during chondrogenesis from bone marrow mesenchymal stem cells (MSCs).

Methods
To create non-viral expressing Sox5, Sox6, and Sox9, full-length human Sox5, Sox6, and Sox9 complementary DNA (cDNA) was amplified by polymerase chain reaction (PCR) and cloned into pEGFP-C1 mammalian expression vector (Clontech, Palo Alto, CA). The microporatorTM (Invitrogen) and the buffer system were utilized for gene delivery. Microporation transfers genes into living cells by means of high voltage electric pulses. Approximately 3 x 10^5 MSCs and 0.5 μg plasmid DNA were used for a single transfer. After microporation, in-vitro pellet cultures were carried out using 2.5 x 10^3 MSCs in chondrogenic medium (Fig 1). Flow cytometry revealed that 82.7-90% of cells infected with non-viral vector, non-viral Sox5,6,9, and non-viral-Sox-trio respectively (Table 1). After three weeks, cells were analyzed for DNA contents, GAG amount, real time PCR and safranine-O staining. MSCs to which empty vector was transferred was used as the negative control, and MSCs which were treated with 5 ng/ml of TGF-β group throughout the three weeks were used as the positive control.

Results
DNA contents did not change by gene transfer of any gene (Fig 1). GAG contents increased six folds when Sox-trio was transferred (Fig 2). Real-time PCR analysis showed that the mRNA levels of COL2A1 increased several fold in MSCs to which Sox-trio genes were transferred. COL1A1 or COL10A1 mRNA increased, but to a much less degree than when TGF-β was treated (Fig 3). Safranin-O staining demonstrated that MSCs to which the Sox-trio genes were transferred exhibited greater accumulation of proteoglycan-rich matrix than TGF-β treated ones (Fig 4).

Discussion
The nonviral method for gene transfer shown here demonstrated an high efficiency not preceded by other studies. Gene transfer of Sox-trio was effective in promoting chondrogenesis while increasing type, and X collagen to a much less degree than growth factors did. This nonviral gene transfer system for Sox-trio may provides a potent new means to achieve chondrogenesis from MSCs.

Reference

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Disclosure
Include commercial conflict of interest disclosure information here, or a statement that authors have nothing to disclose.