Non-viral Transfer of Sox-trio Gene to Adipose Tissue-Derived Mesenchymal Stem Cells Using a Microporator

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Introduction
The adipose tissue has attracted attention on account of its easy accessibility and abundance. It has been reported that adipose tissue-derived mesenchymal stem cells (ATMSCs) obtained from lipoaspirates have the multilineage potential to differentiate into adipogenic, chondrogenic, myogenic and osteogenic cells. Since the pioneering studies demonstrated chondrogenic differentiation from ATMSCs subsequent investigations including one from the authors’ group did not achieve comparable results using the combination of growth factors currently known to induce chondrogenesis from BMMSCs.

Many lines of evidence, both in vitro and in vivo, have shown that SOX (sex determining region Y-type high mobility group box) proteins are necessary for chondrogenesis. This study tested the hypothesis that sox-trios (sox-5,6,9) enhance chondrogenesis and suppress hypertrophic changes during chondrogenesis from ATMSCs (adipose tissue derived mesenchymal stem cells).

Materials and 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 pEGFPC1 mammalian expression vector (Clontech, Palo Alto, CA). The microporator™ (Invitrogen) and the buffer system were utilized for gene delivery. Microporation is a non-viral method used to transfer genes into living cells by means of high voltage electric pulses. Approximately 3 x 10^5 ATMSCs and 0.5 µg plasmid DNA were used for a test of microporation. After microporation, in-vitro pellet cultures were carried out using 2.5 x 10^5 ATMSCs in chondrogenic medium. Flow cytometry revealed that 85~98% of cells infected with non-viral vector, non-viral-sox5,6,9, and non-viral-sox-trio respectively. After three weeks, cells were analyzed for DNA contents, GAG amount, real time PCR and safranine-o staining. ATMSCs cultured under the condition as non-viral gene transfer group was used negative control. And 5 ng/ml of TGF-β2 and 100ng/ml of BMP-7 group was used as positive control.

Results
DNA contents did not increase in sox-trio. But GAG contents were greatest when a combination of sox-trio was used. Real time PCR analysis showed COL1A1 mRNA decreased to almost a quarter in growth factor treated ATMSCs. The mRNA levels of COL2A1 increased several fold in ATMSCs after sox-trio treatment, whereas the gene expression of Col10A1 further decreased when treated with sox-trio. The mRNA level of SOX-9 did increase dose-dependently in ATMSCs. When cultured in pellets with chondrogenic media for 3 weeks, ATMSCs treated with the sox-trio exhibited an accumulation of proteoglycan-rich matrix, whereas those treated each sox alone did not.

Discussion and Conclusions
The findings from real-time RT-PCR showed that the genes collagen type II, a marker of chondrogenesis, showed increased expression with sox-trio overexpression. In contrast, the expression of collagen type I, a marker of bone or undifferentiated mesenchymal tissue, and collagen type X, a marker of hypertrophy change, showed decreased with sox-trio overexpression. In conclusion, although ATMSCs have different chondrogenic potential to BMMSCs, sox-trio might induce adequate chondrogenesis. These results lend a further support to the application of ATMSCs for tissue cartilage engineering.