Efficient and stable gene transfer of growth factors into chondrogenic cells and primary articular chondrocytes using a VSV.G pseudotyped retroviral vector.

Abstract:

Since efficient transfer of foreign genes into primary articular chondrocytes (CC) is difficult, a VSV.G pseudotyped retroviral vector (Bullet) was developed for marker and growth factor gene transfer. Transduction efficiency was analysed by FACS. BMP2 production was determined by specific hBMP2-ELISA. BMP2 effect on cells regarding proteoglycan production was measured by alcian blue staining and dye quantification. Alkaline phosphatase activity was determined by enzymatic reaction with p-nitrophenyl phosphate at OD 405nm and proliferation rate was analysed by MTT-assay. ATDC5 cells (98.3+/-0.6%SD) were transduced to express the reporter gene eGFP. After 52 weeks 94.7+/-0.6%SD of cells were positive. Retroviral transduction efficiency for nlslacZ exceeded 92.3+/-6.1%SD in rabbit CC and expression remained high after 15 weeks (75.7+/-14.2%SD). ATDC5 cells and CC expressed the growth factor gene hBMP2 after retroviral transduction at different time-points. BMP2 led to an increase in proteoglycan and alkaline phosphatase production. Initially, the proliferation rate detected by MTT-assay increased in both the cell types; afterwards the proliferation rate was similar to controls. The described
retroviral vector system achieved high initial transduction rates in ATDC5 cells and CC. Gene transfer was very stable over the time period analysed, rendering it a useful tool for future in vitro and in vivo studies on cartilage remodelling.