TRANSFER OF OSTEOGENIC PROTEIN-1 GENE BY GENE GUN SYSTEM PROMOTES MATRIX SYNTHESIS IN BOVINE INTERVERTEBRAL DISC AND ARTICULAR CARTILAGE CELLS

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**INTRODUCTION**

Therapeutic approaches to restore intervertebral disc (IVD) damage in degenerative diseases are limited. Gene therapy is one of the most attractive tools for inducing the expression of growth factors. A non-viral gene delivery system is an alternative that avoids the risks of insertional mutagenesis of retroviruses, immunogenicity of adenoviruses and acquiring replication competence. However direct introduction of genes into the IVD has been considered difficult, in part due to the presence in the tissue of an extracellular matrix composed of a dense collagen framework and proteoglycan (PG). We have recently reported the potential of a non-viral gene-mediated gene transfer method for efficient transfection [1] of the IVD using a reporter gene.

The purposes of this study are (1) to compare the efficiency of transfection of different IVD [annulus fibrosus (AF) and nucleus pulposus (NP)] cells and articular cartilage (AC) cells using the gene-gun transfection system and (2) to induce by transfection of the human osteogenic protein-1 (OP-1) gene specific metabolic changes in IVD cells (NP and AF cells) and AC cells.

**MATERIALS AND METHODS**

**Cell and Tissue Preparation:** AF and NP tissues were isolated from IVD of tails, and AC from the metacarpophalangeal joints of 14-18 month bovine steer. Cells were isolated by sequential digestion with 0.2% pronase and 0.025% collagenase plus 0.24% DNAase. The cells were seeded at a density of 50,000 cells/well in a 12-well plate and cultured for 2 days prior to transfection. Tissues of IVD and AC were also prepared for gene gun transfection. After the transfection, cells and tissues were cultured in DMEM/F12 medium containing 10% FBS plus 10% horse serum.

**Reporter Gene and OP-1 Expression Vector:** pCMV-β-galactosidase (Clontech) served as a reporter gene, and transgene expression was assessed using the In Situ β-galactosidase staining kit (Stratagene). Human OP-1 expression vector, pW24, was a generous gift from Dr. John C. Lee.

**Gene Transfer:** At the time of gene transfer, a pulse of high pressure helium gas (125 psi) was released from a helium tank through the Gold-Coat tubing, accelerating the DNA-coated gold particles on the inside of the tubing cartridge to penetrate the target cells. The gene gun was positioned at a minimal distance from the petri dish and tissue, and a single bombardment was carried out.

**Assessment of Transfection Efficiency:** After 3 days, the transfection efficiency of a Lac reporter gene construct (pCMV-β-galactosidase) in the primary monolayer cultures of normal bovine NP, AF, and AC cells was assessed using an In Situ β-galactosidase staining kit.

**Measurement of Metabolic Activity of Transfected Cells:** The DNA content and the total PG content were measured in the cell layer to assess metabolic activity [2]. PG synthesis was also measured using [35S]-sulfate labeling, followed by rapid filtration [2] and was compared between the OP-1-transfected (pW24) and the control (vacant vector) groups. Statistical analyses were performed by one-way ANOVA with Fisher’s PLSD test as a post hoc test.

**RESULTS**

The gene transfer of β-galactosidase was performed to probe the efficiency of transfection in the three different cell sources. Analysis of X-gal staining demonstrated an efficiency of 10.1% in normal NP cells, 6.2% in AF cells and 5.2% in the AC cells (Figure 1). The DNA content and rate of PG synthesis in the three cell types did not differ significantly when the pCMV-β-gal transfected and non-treated groups were compared. This suggested that the gene gun procedure does not have a significant adverse effect on cell metabolism.

To study whether gene transfection can alter the metabolism of IVD and AC cells, the human OP-1 gene was transfected using a pW24 vector. On day 3 after transfection, there were no significant differences in the DNA content and PG content of the cell layer in any group. On the other hand, in the OP-1 transfected group, the rate of PG synthesis was significantly higher in all cell types [AC (120%), AF (124%), and NP (144%) cells (Figure 2)]. NP cells were more responsive than AC and AF cells to the transfection of the OP-1 gene.

**DISCUSSION**

The results of this study reveal, for the first time, that transfection of the OP-1 gene by the gene gun system to both IVD and AC cells in vitro can alter the metabolism of these cells. Both the efficiency study and the metabolic study provide evidence that the NP cells might be the best target for the transfection.

Although it remains to be proven that OP-1 production was enhanced after gene transfer, this study suggested that gene therapy with the OP-1 expression vector can be a useful method for inducing the regeneration of IVD and AC tissues. Additional studies are now ongoing to determine if the transfection of the OP-1 gene into the IVD and AC tissues can be achieved and if it can influence the metabolism of tissues.

**REFERENCES:**

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