DOSE DEPENDENT TRANSDUCTION OF HUMAN INTERVERTEBRAL DISC CELLS BY ADENO-ASSOCIATED VIRUS

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INTRODUCTION

Recent advances have led to the potential use of gene therapy for the treatment of acquired diseases, including disorders of the musculoskeletal system. The rate-limiting step for successful gene therapy, however, is the ability to efficiently transfer the appropriate therapeutic gene(s) to the target tissues, therefore mandating comparison of different gene delivery techniques. The intervertebral disc has recently been studied as a potential target organ for gene therapy approaches for the treatment of degenerative disc disease. Adenovirus-mediated approaches have demonstrated efficient transfer of exogenous genes to intervertebral disc cells in-vitro (1, 2), and to human intervertebral disc cells in-vitro (3). However, the potential for an immune response to adenovirus remains a concern (4). Adeno-associated virus (AAV) has recently received favorable interest due to its low immunogenicity, but it has not previously been established that human disc cells are indeed susceptible to gene transfer using this vector. Accordingly, the objective of this study was to test the efficacy of the adeno-associated virus mediated gene transfer technique for transferring exogenous genes to human intervertebral disc cells in-vitro.

MATERIALS AND METHODS

All of the experimental protocols were approved by the human subjects Institutional Review Board at the University of Pittsburgh. Lumbar and cervical intervertebral disc tissue was obtained from five patients during surgical procedures performed for idiopathic scoliosis, disc herniation, and spinal stenosis. Isolation of disc cells was performed as previously described (5).

The cultures were organized into two groups: (1) Adeno-GFP (green fluorescent protein) gene group, and (2) AAV-GFP gene group. Three different concentrations of each virus-gene construct were used in order to determine optimal multiplicity of infection (MOI). In each well, 400 μl of HBSS (Hank’s Balanced Salt Solution) with a specific MOI of one of the virus-gene constructs was added to each of the culture wells. All cultures were incubated at 37°C for three hours with gentle agitation. Culture medium (600 μl) with 10% fetal bovine serum was then added to each well, and the cells were further incubated at 37°C for 23 hours. Culture media was then changed every three days. Gene expression was assessed at one week by determining the presence of GFP using fluorescence microscopy.

RESULTS

Both Adeno-GFP and the AAV-GFP constructs transduced human disc cells in a dose-dependent fashion, as shown in Figure 1. Adeno-GFP transgene expression was higher than AAV transgene expression for all MOI tested (Figure 2). Cell viability was high for all MOI tested.

DISCUSSION

Successful gene therapy depends upon both the rate of gene transfer to the cells and the duration of transgene expression. The current study has demonstrated that AAV is capable of transducing intervertebral disc cells in-vitro in a dose dependent manner. The high transduction rate—even to cells from degenerated discs and from different levels (cervical and lumbar)—indicates that this viral construct may be well-suited for potential treatment of a wide range of disc disorders. Having established the efficacy of in-vitro marker gene transfer to human intervertebral disc cells in the current study, the next step is to evaluate the efficiency of AAV-mediated gene transfer to human disc cells in an in-vivo setting.

In summary, we have established the efficacy of the adeno-associated virus mediated gene transfer technique for the transfer of exogenous genes to human intervertebral disc cells in-vitro. High transducibility with low immunogenicity makes adeno-associated virus a promising vector for future clinical applications of gene therapy for the prevention and treatment of degenerative disc disease.

[Image: FIGURE 1 and FIGURE 2]

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REFERENCES


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