Molecular Therapy for Intervertebral Disc Degeneration with Growth and Differentiation Factor-5

BACKGROUND CONTEXT: Musculoskeletal disorders of the spine and low back pain are the leading source of disability in people under 45 years of age and result in national economic losses of over 90 billion dollars per year. Current treatment options, including both conservative measures such as bed rest, anti-inflammatories, analgesia, and physical therapy as well as surgical measures, target the clinical symptoms of IVD disease rather than addressing the early pathologic processes occurring in the course of degeneration. However, recent advancements in molecular biology including gene cloning and gene transfer technology have made it possible to contemplate treating the IVD at the molecular level to prevent or delay the progression of disc degeneration.

PURPOSE: The aim of the present study was to investigate the effect of recombinant GDF-5 protein and GDF-5 cDNA on the metabolism of intervertebral disc cells in vitro and in vivo.

METHODS: Mouse disc cells in vitro were treated with recombinant GDF-5 protein. Mouse GDF-5 cDNA was cloned into an expression vector and was used to transfect mouse disc cells in vitro. Therapy with Ad-GDF-5, GDF-5 protein and cDNA was assessed by measuring cell proliferation, proteoglycan production, and extracellular matrix gene expression in vitro and evaluating histological changes, immunostaining and extracellular matrix production in a needle puncture-induced disc degeneration model.

RESULTS: Biochemical assays revealed an elevated GAG/DNA ratio in mouse IVD cells that were cultured in the presence of various concentrations of mGDF-5 protein. Real-time RT-PCR demonstrated that treating the cells with GDF-5 protein increased the expression of the collagen type II and aggrecan genes in a dose dependent manner while MMP-3 gene expression decreased. Immunohistochemistry showed an increase in the aggregation of mouse IVD cells that were treated with mGDF-5 in culture compared to the control group. The mouse GDF-5 gene was successfully cloned into an expression plasmid vector and GDF-5 protein production was confirmed by Western blot analysis. Type II collagen and aggrecan gene expression by the cells increased significantly in the cells that were transfected by Nucleofection with the GDF-5 plasmid compared with cells that were transfected with a control plasmid.

The production of exogenous genes could express and keep on certain strength at least 5 weeks after the injections. Quantitative measurements of %DHI and MRI image showed the Ad-GDF5 injection improves the water content and the restoration of disc height. The histology study demonstrated the chondroid cells proliferated from the annulus fibrosus (AF). In Ad-GF5 groups, the extensive and intense of cells proliferation are obviously stronger than Ad-Luc groups. The immunostaining showed more collagen II expressed in Ad-GDF5 groups. The biochemical analysis indicated the cell number of the IVD in Ad-Luc groups reduced since 4 weeks post operation and didn’t recover throughout the study.
CONCLUSIONS: This is the first report to clone the mouse GDF-5 gene and use the Nucleofection method to transfer DNA into IVD cells. The data suggests that both recombinant protein and the cDNA forms of GDF-5 can increase the expression of genes for extracellular matrix proteins in mouse IVD cells. Compare with the GDF-5 protein injection, Ad-GDF5 could provide a stable source of GDF-5 and prolong the time of keeping an effective concentration of GDF-5 in the specific location. Our research confirmed the therapeutic effect of Ad-GDF5 for intervertebral disc degeneration in vivo. Future attempts at gene therapy to treat degenerative disc disease with a novel ex vivo gene transfer technique are needed to develop a therapy that would alleviate the condition of patients with clinically relevant axial spine pain.