Gene Transfer of the Catabolic Inhibitor TIMP-1 Increases Proteoglycan Synthesis in Nucleus Pulposus Cells from Degenerated Human Discs

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Introduction:
Degenerative disc disease is characterized in part by a progressive decline of the proteoglycan content within the nucleus pulposus, and subsequent alterations in the water content and biomechanical properties of the disc. The reduced proteoglycan concentration reflects an imbalance in the homeostatic functions of the nucleus pulposus cells, resulting from decreased synthesis, increased catabolism, or a combination of these processes.

Research with gene therapy as a potential means to replenish the diminished proteoglycan content has resulted in successful up-regulation of proteoglycan synthesis by adenoviral mediated delivery of the cDNA of anabolic growth factors, such as TGF-β1. Although several anabolic growth factors are capable of increasing matrix synthesis, the use of catabolic inhibitors to slow the degenerative process within the intervertebral disc has yet to be explored.

Biochemical analysis of degenerated discs has demonstrated elevated levels of the catabolic enzymes, matrix metalloproteinases, compared to non-degenerated discs - suggesting an intimate role of such catabolic agents in the degenerative process. In this study, we assessed the effect of inhibiting these catabolic enzymes on measured proteoglycan synthesis by delivering the gene encoding tissue inhibitor of metalloproteinase-1 (TIMP-1) via an adenoviral vector at different viral concentrations to nucleus pulposus cells from degenerated human discs in-vitro.

Methods:
Human intervertebral discs from eight (8) patients were harvested during elective surgical repair of degenerated cervical (4) or lumbar (4) discs by one surgeon (JDK). Nucleus pulposus cells were isolated under sterile conditions via enzymatic digestion and cultured in monolayer. Experimental groups were pooled and normalized for cell number prior to transduction with the adenoviral-TIMP-1 construct at the concentrations 50, 100, and 150 multiplicity of infection (MOI). A separate group of pooled lumbar cells was additionally transduced with an adenoviral-BMP-2 construct at the same concentrations.

The vectors used in this experiment were first generation E1/E3 deleted type 5 replication defective adenoviruses with the cDNA encoding TIMP-1 inserted into the E1 region under the regulation of the human cytomegalovirus promoter. Experimental groups were allowed to recover from transduction for 48 hours before cells were incorporated into a three dimensional “pellet” culture system, at 150,000 cells/pellet. After an additional 48 hours of incubation, active proteoglycan synthesis was assessed with 35S radioactive sulfate incorporation using chromatography and scintillation count. Results were normalized to DNA content and general linear modeling was used to determine the p value for pair-wise comparison of individuals with control.

Transduction rate was assessed with an identical procedure using an adenoviral vector encoding the Lac-Z marker gene, and percentage of cells synthesizing β-galactosidase was determined.

Results:
Transduction efficiency - Approximately 70% of cells exposed to the Ad-LacZ virus expressed transgene synthesis at the highest MOI evaluated ~ 32% at 50 MOI, 52% at 100 MOI and 68% at 150 MOI. Cultured cells demonstrated an increase in successful transduction with an increasing multiplicity of infection.

Proteoglycan synthesis – Gene delivery of TIMP-1 resulted in increase proteoglycan synthesis for all concentrations assessed. An MOI of 50 demonstrated 139% of the proteoglycan synthesized by controls, an MOI of 100, 279%, and an MOI of 150, 419%. (Figure 2)

Discussion:
Degenerative disc disease remains an increasingly common cause of patient morbidity and health care resource utilization. Unfortunately, conservative treatment is often unsuccessful at alleviating patients’ symptoms or their reoccurrence, while surgical modalities are invasive, costly, and associated with significant potential complications (non-union, failure, infection, etc.). Interestingly, current treatment options are aimed at minimizing the clinical symptoms of disc disease, as opposed to addressing its underlying cause. As a result, gene therapy has been explored as a potential therapeutic modality to restore the basic biological and biomechanical properties of the intervertebral disc by replenishing the diminished proteoglycan content in the extracellular matrix of the nucleus pulposus.

Several anabolic factors have previously demonstrated promise in up-regulating proteoglycan synthesis in both in-vitro and in-vivo models. In this study, we explored inhibition of the elevated catabolic processes occurring in the degenerated disc. Our study demonstrates not only the successful transfer of the anti-catabolic gene, TIMP-1, to cultured cells from degenerated discs, but also, TIMP-1’s capacity to increase the proteoglycan content by inhibiting proteoglycan degradation in the extracellular matrix. Additionally, we have demonstrated a clear dose response pattern of proteoglycan synthesis up-regulation with increasing viral concentrations of the adenoviral-BMP-2 construct.

We propose that the inhibition of catabolism provides an exciting new avenue for research in the potential treatment of degenerative disc disease by gene therapy, and one that is complementary to ongoing approaches with anabolic factors. Future studies will explore combination gene therapy, using the cDNA of both anabolic factors and catabolic inhibitors to optimize biologic modification, while minimizing the required viral load.