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Introduction: Excessive mechanical and inflammatory stress triggers an imbalance of anabolic and catabolic processes in disc tissue which will lead to degeneration. The increase in catabolism is mediated by activation of the Nuclear Factor - kappaB (NF-κB) signaling pathway, which, among other actions, upregulates matrix metalloproteinase (MMP) activity and break down of extracellular matrix in intervertebral discs resulting in disc degeneration (IDD). Gene therapy using the anti-catabolic factor Tissue Inhibitor of Metalloproteinase -1 (hTIMP1) has shown promising efficacy in treating IDD1. However, a major limitation of the current gene therapy technique is the use of a constitutive promoter to drive transgene expression that results in uncontrolled and excessive expression of therapeutic gene product which could result in undesirable side effects, e.g., osteophyte formation when bone morphogenetic protein 2 (BMP2) transgene is used. To overcome this important limitation, we successfully developed a new gene therapy recombinant adeno-associated viral (rAAV) vector, rAAV-NFkB-hTIMP1, in which the therapeutic recombinant human TIMP1 gene is placed under the control of a promoter containing the NF-κB element. The rAAV-NFkB-hTIMP1 construct is designed to produce the therapeutic gene product TIMP1 only when the cells experience stress which activates the NF-κB pathway. Hence we expect using this strategy that hTIMP1 will be selectively expressed in cells under inflammatory conditions typically found in active degenerating discs and not under normal conditions, in which the therapeutic gene was not needed. In this study we tested out hypothesis that rabbit annulus fibrosis cells transfected with a serotype 2 of rAAV vector carrying a hTIMP1 gene will not express high level of hTIMP1 unless stimulated with the pro-inflammatory cytokine IL-1b.

Methods: Cultured Rabbit annulus fibrosis (rAF) cells were divided into six groups; “Control” (cell only); “rAAV-CMV-hTIMP1(cells transfected with AAV-CMV-hTIMP1 plasmid DNA)” and “rAAV-NFkB-hTIMP1(cells transfected with AAV-NFkB-hTIMP1 plasmid DNA)” treated with and without IL-1b (n=4 for each group). The transfection efficiency was estimated using a CMV-GFP construct, and NF-κB activation after IL-1b stimulation was verified by NF-κB nuclear translocation. Production of hTIMP1 was determined by ELISA and RT-PCR.

Results: The estimated transfection efficiency was over 50% and the NF-κB activation after IL-1b stimulation was almost 100%. RT-PCR analysis demonstrated that the level of hTIMP1 transcription from cells transfected with rAAV-NFkB-hTIMP1 construct was 20 fold greater in the IL-1b stimulated condition compared to nonstimulated control(Figure 1). Consistent with the PCR results, ELISA assay showed 5
fold greater hTIMP1 concentration in culture media of rAAV-NFkB-hTIMP1 transfected cells stimulated with IL-1b than unstimulated cells. As expected, cells transfected with rAAV-CMV-hTIMP1 produced high levels of hTIMP1 protein with or without IL-1b stimulation. This level of hTIMP1 is comparable to that seen in rAAV-NFkB-hTIMP1 transfected cells stimulated with IL-1b (Figure 2).

Discussion: Both RT-PCR and ELISA results showed cells transfected with AAV-NFkB-hTIMP1 plasmid DNA had significant increase in the hTimp-1 mRNA and protein expression after stimulation with IL-1b. These results demonstrate the novelty of this strategy in which the NF-kB element containing promoter ensures that the transgene hTIMP1 is expressed highly only under conditions of inflammation typically found in injured and degenerating discs which would act to block MMP-mediated matrix proteolytic...
destruction. The low basal hTIMP1 expression caused by endogenous TIMP1 gene or a few leaking of NFkB promoter, under normal unstimulated condition is also an important consideration because unnecessary production of a gene product would be energetically costly and undesirable for an environment of limited nutritional supply and metabolic capacity such as the disc.

**Significance:** This novel plasmid DNA of AAV-NFkB-hTIMP1 construct represents a feasible inducible system of transgene delivery and is unique in that the induction of the transgene is not dependent on exogenous treatments, but on endogenous factors that are present only in the cells requiring the gene therapy product. This is an elegant solution to minimize the concerns of the side effects caused by sustained constitutive transgene overexpression that arises when the transgene product may be unnecessary for disc cells that do not require therapy.

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