INTRODUCTION:
The concept of intradiscal gene therapy for the treatment of intervertebral disc degeneration has been in existence since the late 1990s. The viral transfer of growth factor genes (TGF-β, BMP-2) to the cells of the intervertebral has revealed promising initial results with increased proteoglycan production in vitro and in vivo [1,2]. However, we have observed that expression of transgenic growth factors outside the intervertebral disc in the event of a misdirected injection has potentially detrimental consequences (e.g., toxicity) [3]. To date, a safety system that allows the transgenes to be turned “off” or “on” has not been produced for intradiscal gene therapy.

The aim of this study is to describe the application of an “off” inducible system for intervertebral disc gene therapy, testing an adenoviral vector for gene delivery under control of a tetracycline (Tet)-regulated gene expression system. Our goal was to engineer the ability to regulate the amount of the target-gene produced by our adenoviral vector in nucleus pulposus cells (NPC). Therefore, we have performed experiments to establish the levels of the transgene expression under induced or uninduced conditions.

METHODS:
The Adenoviral construct used in this study (Ad/FasL-GFP<sup>TET</sup>) is constitutive of a transactivator (TAT) constitutively expressed from a strong cytomegalovirus promoter and by the tetracycline responsive promoter element (TRE) that regulates the expression of the Green Fluorescent Protein (GFP) and Fas Ligand (FasL) genes under the control of TAT. The TTA binding to the TRE depends on the tetracycline.

Human NPCs were transduced with Ad/FasL-GFP<sup>TET</sup>, at 0, 50, 100, and 200 multiplicity of infection (MOI). After one day cells were cultured in the presence of tetracycline (1, 10, 100mg/l) for three days and then cultured again without tetracycline. The transgene expression was evaluated either at 0, 3, and 6 day after starting tetracycline by imaging cultured again without tetracycline. The transgene expression was normalized to the percent of GFP positive at each time points.

RESULTS:
High levels of GFP expression in NPCs could be observed by fluorescent microscopy at 24 h after infection with Ad/FasL-GFP<sup>TET</sup> at an MOI of 50, 100, and 200. The expression of GFP was proportional to the MOI used (Figure 1A). The presence of tetracycline in the culture media inhibited the transgene expression of the transduced NPC at all MOIs. In fact, GFP was not observed three days after the tetracycline administration at all concentrations used (1, 10, 100mg/l). Six days after removal of tetracycline, the expression of GFP recovered at all the MOIs used and at all the tetracycline concentrations (Figure 1). To account for the observed decrease in GFP expression with time in controls, we normalized the flow cytometry data to the percent of GFP positive control cell in percent at each time points. It is possible to clearly observe the decrease of the transgene expression after the administration of tetracycline and also the recovery of the GFP signal after the stopping the administration of tetracycline (Figure 2). However, a low level of transgene expression was observed after 3 days of exposure to tetracycline.

DISCUSSION:
The transgene expressed by the transduced NPC was efficiently regulated by inclusion of tetracycline in culture media. The presence of tetracycline turns off the protein expression and the subsequent absence turns it on again, demonstrating the ability to control gene expression in NPCs. The clinical relevance of using a gene regulation system is that in the event of a misdirected injection, an inducible system for gene therapy would allow the option of turning off transgene expression, avoiding possible toxicity to surrounding healthy tissues, via administration of an oral drug. Therefore, the therapeutic effect derived from the continued production of a growth factor could be controlled by an antidote administrable in the worst-case scenario.

Therefore, we propose a tet-off inducible system as an efficient tool for modulating the transgene expression to avoid the toxicity that could derive from a misdirected injection.

REFERENCES:

ACKNOWLEDGMENTS:
An NIH Grant and The Pittsburgh Foundation supported this study.

IMPROVED SAFETY OF INTRIVERTEBRAL DISC GENE THERAPY USING A TRANSGENE REGULATION SYSTEM
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