INTRODUCTION: Low back pain is one of the most common musculoskeletal disorders. Frequently, this condition is associated with intervertebral disc (IVD) degeneration. One potential approach for treating disc degeneration utilizes growth factors for enhancing disc repair. Our group has recently explored the role of myostatin (GDF-8) in musculoskeletal tissue regeneration. Myostatin is a distinctive member of the TGF-β superfamily which negatively regulates skeletal muscle development, growth and regeneration (1). GDF-8 binds the type IIB activin receptor (ActRIIB) and type I co-receptor (Alk4/5) to regulate the expression of downstream target genes such as myogenic differentiation protein (MyoD) and myogenic factor-5 (Myf-5) via a TGF-β signaling pathway (2,3). Interestingly, our earlier studies indicated a role for myostatin in bone formation (4), fracture healing (5), and ACL repair (6). GDF-8 treatment of cells isolated from mouse tendon (7) and human anterior cruciate ligament (6) increases fibroblast proliferation and extracellular matrix protein expression including collagen and proteoglycans. These interesting findings suggest that myostatin might have significant effects on other musculoskeletal tissues besides muscle. Based on these findings, we investigated the hypothesis that myostatin is a pro-fibrogenic, anti-myogenic factor that might promote fibroblast proliferation and ECM synthesis in annulus fibrosus (AF) and nucleus pulposus (NP) cell of intervertebral discs.

MATERIAL AND METHOD
Cell isolation & culture: IVDs used in this investigation were obtained from lumbar discs of skeletal mature rabbits. Cells from AF and NP tissues were isolated by 1 hour digestion in 0.05% Pronase (Boehringer Mannheim), followed by overnight digestion in 0.2% collagenase (Worthington Biologicals) for AF cells and 3hrs for NP cells using F-12 medium (Hyclone). The cells were plated onto tissue culture plates. Stimulation of AF and NP cells with GDF-8: AF and NP cells were cultured on 96 well plates for cell proliferation assay and 24 well plates for ECM gene analysis. The cells were stimulated by GDF-8 (50ng/ml, 100ng/ml) for 24hr, 48hr and 72hrs for cell proliferation assay and 24hrs (100ng/ml GDF-8) for ECM mRNA analysis in serum free media. Cell proliferation assay: Cells were washed twice with PBS and medium (Hyclone). The cells were then incubated for 3 h at 37°C in a humidified, 5% CO2 incubator. Optical density (OD) was read at 490 nm. RNA isolation: Total RNA was isolated from AF and NP tissues for constitutive GDF-8 expression and stimulated culture (GDF-8) using a TRIZOL reagent. Total RNA concentration and its purity were determined by a spectrophotometer. Reverse transcriptase was used to prepare cDNA and the samples were assayed using gene specific primers against rabbit GDF-8, ECM genes (Collagen type II and aggrecan) and housekeeping genes (18S and GAPDH).

RESULTS: We analyzed the expression of GDF-8 mRNA in AF and NP tissue of rabbit disc tissue by real time–PCR, using the gene specific primers. We found that the amplified DNA band corresponding to 83 bp as expected size for the amplification product of GDF-8 which confirms the specificity. The annular fibrous cells showed 2.89 fold greater expression of myostatin than nucleus pulposus cells (Fig.1).

GDF-8 treatment of primary cells isolated from rabbit disc showed a dose-dependent increase in fibroblast proliferation after 24 h (Fig.2). The lower dose of myostatin significantly increased proliferation after both 48 and 72h (Fig 2a). The higher myostatin dose also significantly increased fibroblast proliferation relative to controls at 48 and 72h, but this increase was similar to that observed with the lower myostatin dose in annular cells. The nucleus pulposus cells showed the greatest cell proliferation @ 72h at the higher dose (100ng/ml) (Fig.2b).

Table (1) shows the percentage increase in cell number compared to control.

To analyze the effect of myostatin on ECM synthesis, we did real time PCR following the treatment of myostatin (24hr treatment) on important anabolic genes collagen type II and aggrecan. Our results indicate that myostatin treatment increased expression of collagen type II 2.3 and 2.9 fold in AF and NP, respectively, and 2.05 and 1.62 fold of aggrecan in AF and NP, respectively (Fig.3a&b).

DISCUSSION: Our study is the first, to our knowledge, that reports a role of GDF-8 in the IVD cells. Myostatin treatment increased the proliferation of AF and NP cells as well as the expression of genes involved in ECM synthesis. Other growth and differentiation factor members have previously shown promising effects on disc regeneration. It is reported that in vivo injection of GDF-5 protein and virus transfection in a disc degeneration model in rabbits promoted regeneration of the disc, with increased cell proliferation and production of matrix(8). GDF-6 also increases the proteoglycan synthesis rate and chondrocytic phenotype of disc cells (9). It is clear from our experiments that myostatin, like other GDFs, can significantly affect disc cells, and our data further suggest that exogenous delivery of recombinant myostatin may have therapeutic potential for intervertebral disc regeneration.

REFERENCES
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