Supplementation of chondroitin sulfate proteoglycan stimulates anabolic turnover in bovine and human nucleus pulposus cells through gelsolin/actin pathway under physiologically relevant culture conditions *in vitro*

Kunihiko Miyazaki, Yusuke Aoki, James D Kang, Shuichi Mizuno
Department of Orthopaedic Surgery, Brigham and Women's Hospital, Boston, MA

Disclosures: Kunihiko Miyazaki (N), Yusuke Aoki (N), James D Kang (N), Shuichi Mizuno (N)

INTRODUCTION

We reported that chondroitin sulfate proteoglycan (CSPG) defined as matrix associated osmotic pressure (OP) and high-osmolality medium balanced with NaCl stimulated anabolic turnover in bovine nucleus pulposus (bNP) cells under repetitive cyclic hydrostatic pressure (HP) followed by constant HP *in vitro* mimicking day-night spinal movement. But it was unclear what's gateway of changes in these physicochemical conditions: OP and HP alter signal pathway. We hypothesize that changes in the physiologically relevant conditions alter location or distribution of gelsolin/actin in NP cells and stimulate synthetic turnover. We tested this hypothesis using normal bovine nucleus pulposus (bNP) cells and human NP (hNP) cells on metabolic turnovers in these cells evaluated via measuring genetic expression of matrix-related molecules and immunohistologically using antibodies corresponding to these molecules.

METHODS

CSPG was extracted from bNP with 4M guanidine and lyophilized after extensive dialysis (MWCO 30 kD) and validated with SDS-PAGE.
Bovine NP (bNP) tissues were harvested from tails (6 repetitions). 1.0×10^5 bNP cells/25 µl isolated enzymatically were seeded with 0, 4, or 16 mg/ml CSPG into pouches made of PVDF semipermeable membrane (MWCO 500 kD).
Human NP (hNP) tissues were collected from patients who underwent cervical discectomy or cervical fusion surgery (ages 20–70 years, both sexes, Pfirrmann grade 2, 3; n = 6 and grade 4; n = 6). 1.0×10^5 hNP cells isolated enzymatically were seeded with 0, 4 and 16 mg/ml CSPG into pouches.
The pouches were incubated under two culture conditions: 1) Control (no HP): atmospheric pressure; and 2) HP: cyclic HP (0.2-0.7 MPa, 0.5 Hz) for 2 days followed by constant HP (0.3 MPa) for 1 day, repeated 6 times over 12 days mimicking physiological relevant culture conditions (Fig. 1). The pouches were collected at 3 and 12 days and subjected to gene expression assay *(Acan, Col-2, Csgalnact1, Mmp13, Timp2, and Gsn)* in bNP cells and *ACAN, COL-2, CSGALNACT1, MMP13, TIMP2, and GSN)* in hNP cells using RT-PCR, regular microscopy and laser confocal microscopy. Immunohistochemical staining was performed at 3 and 12 days to confirm the accumulation of keratan sulfate (KS) and MMP13 in the bNP and hNP cells/cluster. Gene expression was analyzed with two-way ANOVA, and statistical difference was defined as $p < 0.05$.

RESULTS

bNP cells with CSPG, anabolic molecules: *Acan*, *Col-2*, and *Csgalnact1* were upregulated approximately threefold by 12 days compared to bNP cells without CSPG ($P<0.01$, $P< 0.05$, $P<0.05$, Fig. 2). The levels of upregulation with CSPG at 4 or 16 mg/ml showed similar trends. With CSPG and under HP, the anabolic molecules and *Gsn* were upregulated approximately threefold compared to the control (without CSPG or HP). Degenerative molecule: *Mmp13* declined significantly ($P<0.05$, Fig. 2) compared to control (without CSPG and HP). Immunohistological staining showed more KS accumulation with time and there were similar trends between concentrations of CSPG and the presence/absence of HP (Fig.3).
hNP cells isolated from moderately degenerated discs (Pfirrmann grade 2 or 3) and severely degenerated discs (Pfirrmann grade 4) were evaluated. hNP cells from grade 2-3 with CSPG with/without HP upregulated anabolic molecules (*ACAN, COL-2,* and *CSGALNACT1*), but no clear difference was observed between cells with and without HP. On the other hand, hNP cells from grade 4 showed trends similar to those seen in hNP from grade 2-3 (Fig.2).
Genetic expression of other molecules showing no significant difference are not presented due to space limitations.

DISCUSSION

We revealed that
Immunohistological images indicated distinctively different locations and shapes of gelsolin in NP cells between HP and no HP. These trends were consistent with gene expression profiles, which are consistent to upregulation of aggrecan (*Agg/AGG*) and chondroitin sulfate N-acetylgalactosaminyl-transferase 1 (*CSGALNACT1*). Thus, gelsolin (GSN) is a key molecule with actin filaments to indicate NP regeneration under physiological movement. Bovine bNPs supplemented with CSPG under HP showed increased anabolic turnover in a dose-dependent manner compared without CSPG. Thus supplementation of CSPG to the IVD has the potential to stimulate regeneration in bNP under daily spine movement. Since anabolic gene expression increased and higher concentrations of CSPG may be more effective. In addition, human hNP cells from grade 2-3 supplemented with CSPG showed increased anabolic turnover compared to no CSPG and similar trend under HP; whereas hNP from grade 4 showed diminished anabolic turnover. Thus, severally damaged hNP cells altered their original characteristics. Higher upregulation of MMP-13 under HP was seen in hNP cells. These contrasting gene expression profiles between normal bNP cells and degenerated hNP cells suggest that future therapeutic strategies should include not only promotion of anabolic turnover but also prevention of degeneration.

SIGNIFICANCE:

Supplementation of CSPG has the potential to promote accumulation of newly synthesized matrix in moderately degenerated nucleus pulposus if degeneration by MMP can be diminished under circadian spinal movement. Other options to promote NP regeneration include supplementation of cells producing CSPG.

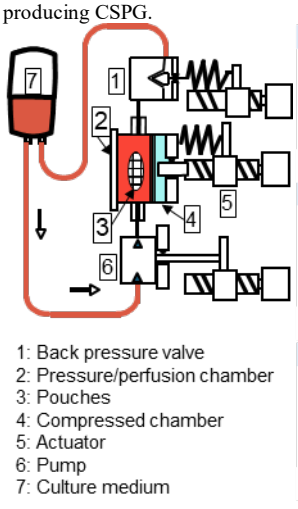

Fig. 1 HP/perfusion culture system

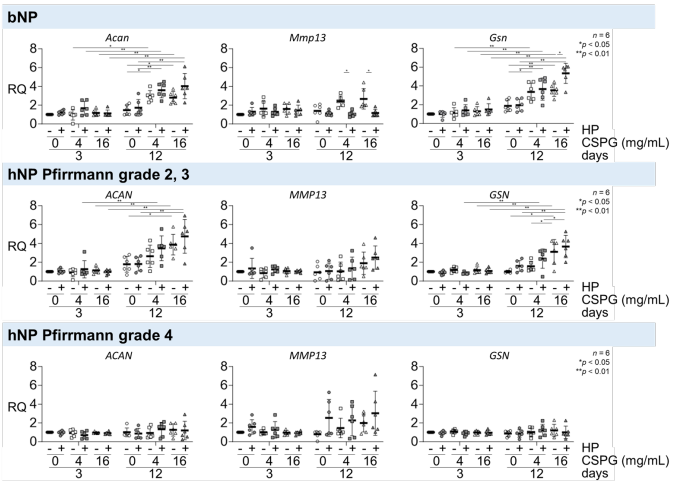

Fig. 2 Gene expressions of selected molecules in NP cells. Relative quantity (RQ)

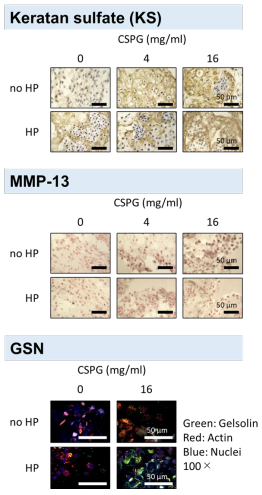

Fig. 3 Immunohistology in bNPs at 12 days