Original Paper

N-Cadherin Maintains the Healthy Biology of Nucleus Pulposus Cells under High-Magnitude Compression

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Key Words

Disc degeneration • N-cadherin • Nucleus pulposus • Matrix • Compression • Apoptosis

Abstract

Background/Aims: Mechanical load can regulate disc nucleus pulposus (NP) biology in terms of cell viability, matrix homeostasis and cell phenotype. N-cadherin (N-CDH) is a molecular marker of NP cells. This study investigated the role of N-CDH in maintaining NP cell phenotype, NP matrix synthesis and NP cell viability under high-magnitude compression. **Methods:** Rat NP cells seeded on scaffolds were perfusion-cultured using a self-developed perfusion bioreactor for 5 days. NP cell biology in terms of cell apoptosis, matrix biosynthesis and cell phenotype was studied after the cells were subjected to different compressive magnitudes (low- and high-magnitudes: 2% and 20% compressive deformation, respectively). Non-loaded NP cells were used as controls. Lentivirus-mediated N-CDH overexpression was used to further investigate the role of N-CDH under high-magnitude compression. **Results:** The 20% deformation compression condition significantly decreased N-CDH expression compared with the 2% deformation compression and control conditions. Meanwhile, 20% deformation compression increased the number of apoptotic NP cells, up-regulated the expression of Bax and cleaved-caspase-3 and down-regulated the expression of Bcl-2, matrix macromolecules (aggrecan and collagen II) and NP cell markers (glypican-3, CAXII and keratin-19) compared with 2% deformation compression. Additionally, N-CDH overexpression attenuated the effects of 20% deformation compression on NP cell biology in relation to the designated parameters. **Conclusion:** N-CDH helps to restore the cell viability, matrix biosynthesis and cellular phenotype of NP cells under high-magnitude compression.

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Introduction

Due to the serious impacts of intervertebral disc degeneration (IDD) on the health care system and patients' life quality, an increasing number of researchers is exploring its underlying mechanisms and seeking to develop effective treatments (IDD) [1, 2]. Despite numerous breakthroughs in this field in the past decades [3, 4], the many unknowns about disc degeneration require further investigation.

Disc degeneration first occurs in the disc nucleus pulposus (NP) region and is characterized by a decrease in cell viability, attenuation of NP matrix anabolism and alteration of the normal NP cell phenotype [5, 6]. As a load-absorbing element of the spine motion unit, the intervertebral disc (IVD) is subjected to various types and magnitudes of mechanical stress *in vivo* [7, 8]. Previous studies found an increase in NP cell apoptosis and a decrease in NP matrix production as a result of overloaded mechanical compression [9, 10]. Similarly, several studies have demonstrated that mechanical stimulation plays an important role in regulating disc biology and have indicated that mechanical overloading is a risk factor for disc degeneration [11, 12]. Therefore, additional studies of the high-magnitude compression-induced pathological alterations of NP cells will contribute to a better understanding of mechanical load-related disc degeneration.

N-cadherin (N-CDH) is an important molecule in the development of the neural crest and the formation of neuronal connections [13, 14]. Recently, several studies have demonstrated that N-CDH is a specific molecule that is highly expressed in normal disc NP cells compared with degenerative NP cells, adjacent disc annulus fibrosus cells and cartilage endplate cells [15, 16]. In addition, N-CDH-mediated signaling helps to maintain the normal juvenile NP phenotype and NP matrix synthesis *in vitro* [17, 18]. Finally, N-CDH can regulate cell behaviors, cell phenotypes and other pathological phenomena in other tissues [13, 14]. In light of the loss of the normal NP cell phenotype, the decrease in NP matrix production and the attenuation of NP cell viability under conditions of mechanical compression overload [5, 9, 11], we hypothesize that N-CDH may play a positive role in maintaining normal NP cell biology under high-magnitude compression.

The main objective of this study was to investigate the role of N-CDH in maintaining the healthy biology of NP cells in terms of cellular phenotype, matrix biosynthesis and cell viability under high-magnitude compression. To achieve this, a self-developed perfusion bioreactor was used to apply different compressive magnitudes to the three-dimensional (3D) cultured NP cells. The main parameters evaluated were NP cell apoptosis, NP cell phenotype and NP matrix biosynthesis.

Materials and Methods

Ethical statement

All animal experiments performed in this study were in accordance with the relevant guidelines [SYXK (YU) 2012-0012] of the Ethics Committee at the First Hospital affiliated with Jilin University.

NP cell isolation and culture

Briefly, after the rats (male, 250 g and 6-8 weeks old) were sacrificed with carbon dioxide, the thoracic and lumbar discs were harvested according to previously described method [19, 20]. After the innermost NP tissue was isolated under a dissecting microscope, NP cell pellets were harvested using sequential digestion with 0.25% trypsin (Gibco, USA) for 5-10 minutes and 0.25% Type I collagenase (Sigma, USA) for 10-15 minutes. Then, the NP cell pellets were re-suspended in DMEM/F12 medium (HyClone, USA) containing 10% (v/v) fetal bovine serum (FBS, Gibco, USA) and 1% (v/v) penicillin-streptomycin (Gibco, USA) under standard conditions (37°C, 20% O₂ and 5% CO₂). To alleviate cell passage-induced effects on NP cell degeneration, passage 2 (P2) NP cells were used in the present study.

NP cell transfection

The recombinant lentiviral vector LV5-N-CDH (GenePharma, Shanghai, China) was used to enhance N-CDH expression in NP cells (NP-N-CDH). NP cells transfected with negative vectors (LV5-N-CDH-NC) were used as controls (NP-N-CDH-NC). According to the manufacturer's instructions, the transfected cells were further selected using puromycin. Transfection efficacy was verified by using real-time PCR and Western blotting assays.

Dynamic compression on NP cells

A previous study demonstrated that NP cells cultured on decalcified bone matrix (DBM) are metabolically active and express types I and II collagen and aggrecan [21]. Therefore, NP cells suspended in collagen solution (1 mg/mL, Shengyou, China) were seeded in prepared DBM (10 mm×10 mm×5 mm, 1×10⁷ cells per DBM) scaffolds as previously described [22]. The DBM scaffolds were provided free of charge by the Tissue Engineering Center of Jilin University. After 2 days of static preculture, the NP cell-seeded scaffolds were placed in the culture chambers of a substance exchanger-based perfusion bioreactor [22] and subjected to mechanical compression for 5 days. The DMEM/F12 medium was circulated at a rate of 5 mL/min. During this phase, unconfined axial compression was applied to NP cells in a distance-controlled manner. The designed groups were the control group (non-compression) and compression group (2% and 20% compressive deformation at a frequency of 1.0 Hz for 4 hours per day). The compression magnitudes were defined according to the disc height alteration in one day (20%-25%) and previous studies [23, 24].

Detection of cell apoptosis using flow cytometry

After compression, the NP cells seeded in the scaffold were harvested using sequential digestion with 0.05% trypsin and 0.1% collagenase I and washed with phosphate buffer solution (PBS). Then, NP cell apoptosis was evaluated using flow cytometry with Annexin V/PI staining according to the manufacturer's instructions (Beyotime, China). These Annexin V positive-stained and PI-negative-stained cells and double positive-stained cells were regarded as apoptotic cells.

Real-time PCR analysis

After compression, the DBM scaffolds containing NP cells were homogenized on ice. Then, the RNA was extracted using Trizol reagent (Invitrogen, USA) and synthesized into cDNA with a reverse transcription kit (Roche, Switzerland). The real-time PCR assay was then performed on the reaction system containing cDNA, primers (Table 1) and SYBR Green mix (Dongsheng Biotech, China). β -actin was used as the reference gene, and the relative expression of target genes was expressed as $2^{-\Delta\Delta Ct}$.

Western blotting assay

After compression, total protein was extracted from the DBM scaffolds with RIPA reagent (Beyotime, China), and protein concentration was measured using a protein BCA detection kit (Beyotime, China). The protein samples from each group were subjected to SDS-PAGE and transferred to the PVDF membrane as previously described [25]. After incubation with primary antibodies (β -actin: Proteintech, 60008-1-Ig; N-CDH, Abcam, ab18203; aggrecan: Santa Cruz, sc-16492; collagen II: Abcam, ab34712. Cleaved-caspase 3, Cell Signaling Technology, #9661; Bcl-2: Proteintech, 12789-1-AP; Bax: Proteintech, 50599-2-Ig; Glypican-3: Proteintech, 11145-1-AP; PTN: Proteintech, 10821-1-AP; CA XII: Proteintech, 15180-1-AP; Keratin-19: Proteintech, 10712-1-AP. All diluted 1:1000) and the corresponding HRP-conjugated secondary antibodies, the protein bands on the PVDF membrane were visualized using the SuperSignal West Pico Trial Kit (Thermo). Protein expression normalized to β -actin was analyzed using ImageJ software (National Institutes of Health, USA).

Table 1. Primers of the target genes

Gene	Accession number	Forward (5'-3')	Reverse (5'-3')
β -actin	NM_031144.3	CCGCGAGTACAACCTTCTTG	TGACCCATACCCACCATCAC
N-CDH	NM_031333.1	AAGAGGCCGAGACTTGTGAA	CACTGGGGATAAGGGAAGGT
Aggrecan	XM_002723376.1	ATGGCATTGAGGACAGCGAA	GCTCGGTCAAAGTCCAGTGT
Collagen II	NM_012929.1	GCCAGGATGCCCCGAAAATTAG	CCAGCCTTCTCGTCAAATCCT
Keratin 19	NM_199498.2	ATGAGCTGAACACCCAGGTC	ATGCTGAGCTGAGACTGCAA
PTN	NM_017066.2	GAAAATTTGCAGCTGCCTTC	TTGCCATTCTCCACAGTCAG
Glypican-3	NM_012774.1	AGTGCTTTGCCAGGCTACAT	TTCTTCATCCCATTCTCTGC
CA XII	NM_001014006.1	TACTGCGACCTGGAACAGTG	GGACAGAGACGGGAAGTGA

Statistical analysis

All the numerical data in this study are expressed as the means $\pm$ SD, and each experiment was performed in triplicate. After the homogeneity test for variance, comparisons between groups were performed with one-way analysis of variance (ANOVA) using SPSS 13.0 software, and the post hoc test was determined by the LSD test. A significant difference was indicated when the p-value was <0.05.

Results

High-magnitude compression significantly decreased N-CDH expression in NP cells

To investigate the role of N-CDH in the effects of mechanical compression on NP cells, we first analyzed N-CDH expression under different compressive magnitudes. As shown in Fig. 1, N-CDH expression in the 20% deformation compression group was significantly lower than in the 2% deformation compression group and the control group at both the gene and protein levels. However, N-CDH expression in the 2% deformation compression group was higher than in the control group.

Fig. 1. High-magnitude compression significantly decreased the N-CDH expression of nucleus pulposus (NP) cells. A: Real-time PCR analysis of N-CDH mRNA expression in NP cells under different compression magnitudes (2% and 20% deformation). B: Western blotting analysis of N-CDH protein expression in NP cells under different compression magnitudes. Data are expressed as the mean $\pm$ SD, n=3. *: Indicates a significant difference between two groups. #: Indicates a significant difference from the control group.

Fig. 2. N-CDH overexpression in NP cells. A: General observation showed that successfully transfected NP cells were tagged with green fluorescent protein. B: Verification of N-CDH overexpression in NP cells using real-time PCR and Western blotting. C: N-CDH expression under high-magnitude compression (20% deformation) after the enhancement of N-CDH expression in NP cells. Data are expressed as the mean $\pm$ SD, n=3. *: Indicates a significant difference between two groups. #: Indicates a significant difference from the control group.

Fig. 3. N-CDH overexpression attenuated the effects of high-magnitude compression on nucleus pulposus (NP) cell apoptosis. **A:** Apoptotic NP cells were increased in the high-magnitude (20% deformation) group compared with the control group and the low-magnitude (2% deformation) group, whereas N-CDH overexpression decreased apoptotic NP cells under high-magnitude (20% deformation) compression. **B:** High-magnitude compression increased pro-apoptotic molecule (Bax and Cleaved-caspase 3) expression and decreased anti-apoptotic molecule (Bcl-2) expression compared with the control condition and low-magnitude compression (2% deformation). However, N-CDH overexpression decreased pro-apoptotic molecule (Bax and Cleaved-caspase 3) expression and increased anti-apoptotic molecule (Bcl-2) expression under high-magnitude (20% deformation) compression. Data are expressed as the mean $\pm$ SD, $n=3$. *: Indicates a significant difference between two groups. #: Indicates a significant difference when compared with the control group or the 20% deformation group.

N-CDH overexpression increased N-CDH expression in NP cells under high-magnitude compression

To investigate the role of N-CDH in maintaining the healthy biology of NP cells under high-magnitude compression, we enhanced the N-CDH expression in NP cells (Fig. 2A-B). Subsequently, we found that N-CDH overexpression significantly increased N-CDH expression in NP cells under 20% deformation compression (Fig. 2C).

Fig. 4. N-CDH overexpression attenuated the inhibitory effects of high-magnitude compression on matrix biosynthesis in nucleus pulposus (NP) cells. A: High-magnitude (20% deformation) compression decreased the protein expression of matrix macromolecules (aggrecan and collagen II) in NP cells compared with the control condition and low-magnitude compression (2% deformation). B: N-CDH overexpression increased the protein expression of matrix macromolecules (aggrecan and collagen II) in NP cells under high-magnitude (20% deformation) compression. Data are expressed as the mean $\pm$ SD, n=3. *: Indicates a significant difference between two groups. #: Indicates a significant difference when compared with the control group or the 20% deformation group.

N-CDH overexpression abolished the effects of high-magnitude compression on NP cell apoptosis

Compared with 2% deformation compression group and the control condition, 20% deformation compression significantly increased the number of apoptotic NP cells, up-regulated the expression of pro-apoptotic molecules (Bax and Cleaved-caspase 3) and down-regulated the expression of anti-apoptotic molecules (Bcl-2). Although no significant differences were found in the number of apoptotic NP cells and the expression of Bax, the 2% deformation compression group showed significantly increased Bcl-2 expression and decreased cleaved-caspase-3 expression compared with the control group. However, when

Fig. 5. N-CDH overexpression helped to maintain the nucleus pulposus (NP) cell phenotype under high-magnitude compression. **A:** High-magnitude (20% deformation) compression decreased the gene and protein expression of NP cell-specific makers (glypican-3, CA XII and keratin 19) compared with the control condition and low-magnitude (2% deformation) compression. **B:** N-CDH overexpression increased the gene and protein expression of NP cell-specific makers (glypican-3, CA XII and keratin 19) under high-magnitude (20% deformation) compression. Data are expressed as the mean $\pm$ SD, n=3. *: Indicates a significant difference between two groups. #: Indicates a significant difference when compared with the control group or the 20% deformation group.

N-CDH expression was enhanced in the NP cells, the effects of 20% deformation compression on the NP cell apoptosis rate and the expression of pro-/anti-apoptotic molecules were attenuated (Fig. 3). Together, these results indicate that N-CDH can inhibit NP cell apoptosis under high-magnitude compression.

N-CDH overexpression attenuated the inhibitory effects of high-magnitude compression on matrix biosynthesis in NP cells

The expression of matrix macromolecules (aggrecan and collagen II) was investigated as a reflection of the biosynthesis of NP cells. The results showed that 20% deformation compression significantly down-regulated the expression of aggrecan and collagen compared with 2% deformation compression and the control condition at both the gene and protein levels. Conversely, N-CDH overexpression in NP cells significantly increased the expression of aggrecan and collagen II under 20% deformation compression (Fig. 4), suggesting that N-CDH can promote matrix biosynthesis in NP cells under high-magnitude compression.

N-CDH overexpression helped to maintain the NP cell phenotype under high-magnitude compression

Loss of the normal NP cell phenotype is usually reported during disc degeneration. Previous studies demonstrated that keratin 19, PTN, glypican-3 and CAXII are typical markers of NP cells [26, 27]. Here, the effects of compression on the expression of NP-specific markers (keratin 19, PTN, glypican-3 and CAXII) were evaluated. The results showed that expression of keratin 19, glypican and CAXII in the 20% deformation compression group was higher than that in the 2% deformation compression group and the control group. Moreover, N-CDH overexpression up-regulated the expression of keratin 19, glypican and CAXII in NP cells under 20% deformation compression. In addition, mechanical compression and N-CDH overexpression had no significant effects on PTN expression (Fig. 5). Collectively, these findings indicate that N-CDH expression can inhibit mechanical overloading-induced down-regulation of NP cell-specific markers.

Discussion

Mechanical load is an important and common external risk factor for disc degeneration [28-33]. It is well known that aggravated NP cell apoptosis, attenuated NP matrix anabolism and loss of the normal NP cell phenotype are typical characteristics of the NP region during disc degeneration [5, 34]. Due to the key role of NP tissue in disc mechanical function, a clear and accurate understanding of NP mechanobiology is important. Our results demonstrated that high-magnitude compression harms healthy NP cell biology by promoting NP cell apoptosis, decreasing NP matrix biosynthesis and suppressing the expression of NP cell-specific markers, whereas N-CDH over-expression in NP cells partly reversed the effects of high-magnitude compression on NP cell biology. This study reported for the first time that N-CDH is beneficial for maintaining healthy NP cell biology in terms of cell viability, matrix biosynthesis and normal NP cell phenotype under high-magnitude compression, indirectly providing a new strategy for retarding mechanical overloading-induced disc degeneration.

Several studies have identified N-CDH expression in NP cells at either the transcriptional or protein level, regardless of animal species [15-18]. It is also worth mentioning that N-CDH expression decreases with disc aging and disc degeneration and that the decrease in N-CDH expression coincides with those degenerative disc changes [15, 16]. Hence, several research teams have proposed and verified that N-CDH-mediated signaling is necessary for maintaining normal NP biology [17, 18]. In this study, high-magnitude (20% deformation) compression significantly down-regulated N-CDH expression compared with low-magnitude (2% deformation) compression. In light of the reported destructive effects of mechanical overloading on disc NP biology [9, 11, 29, 31], we deduce that N-CDH down-regulation may participate in this process.

Due to its avascular character, the disc NP region has a relatively low cell density (approximately 6×10^3 cell/mm³) [35]. Notably, the number of NP cells continues to decrease during disc degeneration because certain pathological factors, such as mechanical overloading, limited nutrient supply and increased inflammation processes, induce NP cell apoptosis [5, 36-38]. When the number of NP cells decreases to an adequately low level, NP matrix homeostasis is obviously destroyed, and the disc degeneration process is initiated and further exacerbated [24]. In this study, high-magnitude (20% deformation) compression significantly promoted NP cell apoptosis compared with low-magnitude (2% deformation) compression. This is consistent with previous reports [9, 39, 40]. However, N-CDH overexpression significantly inhibited NP cell apoptosis under high-magnitude compression, indicating that N-CDH is helpful for maintaining NP cell viability under mechanical compression.

NP matrix homeostasis is another key event during disc degeneration. Because proteoglycans (PGs) and collagen II are the main macromolecules within the NP tissue [41], the expression of aggrecan and collagen II were evaluated in this study. Our results showed

that high-magnitude (20% deformation) compression significantly decreased NP matrix synthesis compared with low-magnitude (2% deformation) compression. This confirms the findings of previous studies that reported that mechanical overloading promoted matrix catabolism [11]. Previous studies have also indicated that N-CDH-mediated signaling promoted NP cell matrix production, whereas the blockage of N-CDH-mediated signaling resulted in the loss of NP cell matrix production [17, 18]. In line with this, we found that N-CDH overexpression partly promoted NP matrix biosynthesis under high-magnitude (20% deformation) compression, indicating that N-CDH plays an important role in resisting high-magnitude compression-induced NP matrix catabolism.

Several previous studies have investigated specific markers for NP cells [42-45]. In this study, high-magnitude (20% deformation) compression down-regulated the expression of these NP markers (keratin 19, glypican and CA XII) compared with low-magnitude (2% deformation) compression. Down-regulation of NP-specific markers may lead to the loss of the normal NP phenotype and thus the alteration of extracellular matrix composition. Numerous studies have demonstrated that mechanical overloading can induce degenerative changes in NP cells [9, 29, 39], indirectly indicating that the loss of the normal NP phenotype may be one mechanism behind the mechanical overloading-induced decrease in matrix production. N-CDH is unique to normal NP cells, regardless of animal species [15]. A previous study revealed that N-CDH-mediated signaling preserved the expression of NP-specific markers [17]. Similarly, we found that N-CDH overexpression partly maintained the expression of these NP markers under high-magnitude compression, indicating that N-CDH may be able to inhibit the mechanical overloading-induced loss of the normal NP phenotype.

This study has several limitations. First, the environment of NP cells is rich in laminin and collagen II but not collagen I. Although the NP cells used in this study were metabolically active and expressed NP matrix macromolecules (aggrecan and collagen II), differences in the surface chemistry and texture between DBM scaffolds and other biomimetic scaffolds may lead to differences in cellular behavior in response to the scaffold material. Second, this NP cell scaffold culture model may not be physiologically relevant. Culturing a segment of the thoracic or lumbar region and applying mechanical compression may solve this problem. However, the present mechanical application system could not meet these needs. In the future, this drawback should be overcome to acquire more physiologically relevant results.

Based on the findings of this study, we can conclude that N-CDH is able to restore healthy NP cell biology in terms of cell viability, matrix biosynthesis and normal cell phenotype under high-magnitude compression. This study provides a new intervention target for retarding mechanical overloading-induced disc degeneration.

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Disclosure Statement

The authors do not have any conflicts of interest related to this work.

References

- 1 Ma JF, Zang LN, Xi YM, Yang WJ, Zou D: MiR-125a Rs12976445 Polymorphism is Associated with the Apoptosis Status of Nucleus Pulposus Cells and the Risk of Intervertebral Disc Degeneration. *Cell Physiol Biochem* 2016;38:295-305.

- 2 Yan N, Yu S, Zhang H, Hou T: Lumbar Disc Degeneration is Facilitated by MiR-100-Mediated FGFR3 Suppression. *Cell Physiol Biochem* 2015;36:2229-2236.
- 3 Hu B, Shi C, Tian Y, Zhang Y, Xu C, Chen H, Cao P, Yuan W: TGF-beta Induces Up-Regulation of Chondroitin Sulfate Synthase 1 (CHSY1) in Nucleus Pulposus Cells Through MAPK Signaling. *Cell Physiol Biochem* 2015;37:793-804.
- 4 Liu Z, Zhou K, Fu W, Zhang H: Insulin-Like Growth Factor 1 Activates PI3k/Akt Signaling to Antagonize Lumbar Disc Degeneration. *Cell Physiol Biochem* 2015;37:225-232.
- 5 Zhao CQ, Wang LM, Jiang LS, Dai LY: The cell biology of intervertebral disc aging and degeneration. *Ageing Res Rev* 2007;6:247-261.
- 6 Wang SZ, Rui YF, Lu J, Wang C: Cell and molecular biology of intervertebral disc degeneration: current understanding and implications for potential therapeutic strategies. *Cell Prolif* 2014;47:381-390.
- 7 Neidlinger-Wilke C, Galbusera F, Pratsinis H, Mavrogomatou E, Mietsch A, Kletsas D, Wilke HJ: Mechanical loading of the intervertebral disc: from the macroscopic to the cellular level. *Eur Spine J* 2014;23:S333-343.
- 8 Hwang D, Gabai AS, Yu M, Yew AG, Hsieh AH: Role of load history in intervertebral disc mechanics and intradiscal pressure generation. *Biomech Model Mechanobiol* 2012;11:95-106.
- 9 Li P, Gan Y, Wang H, Zhang C, Wang L, Xu Y, Song L, Li S, Li S, Ou Y, Zhou Q: Dynamic Compression Effects on Immature Nucleus Pulposus: a Study Using a Novel Intelligent and Mechanically Active Bioreactor. *Int J Med Sci* 2016;13:225-234.
- 10 Li P, Gan Y, Xu Y, Song L, Wang H, Zhang C, Wang L, Zhao C, Luo L, Zhou Q: Matrix homeostasis within the immature annulus fibrosus depends on the frequency of dynamic compression: a study based on the self-developed mechanically active bioreactor. *Biomech Model Mechanobiol* 2017;16:385-394.
- 11 Chan SC, Ferguson SJ, Gantenbein-Ritter B: The effects of dynamic loading on the intervertebral disc. *Eur Spine J* 2011;20:1796-1812.
- 12 Yan Z, Pan Y, Wang S, Cheng M, Kong H, Sun C, Hu K, Chen T, Dong Q, Chen J: Static Compression Induces ECM Remodeling and Integrin alpha2beta1 Expression and Signaling in a Rat Tail Caudal Intervertebral Disc Degeneration Model. *Spine (Phila Pa 1976)* 2017;42:E448-E458.
- 13 Bruses JL: N-cadherin signaling in synapse formation and neuronal physiology. *Mol Neurobiol* 2006;33:237-252.
- 14 Halbleib JM, Nelson WJ: Cadherins in development: cell adhesion, sorting, and tissue morphogenesis. *Genes Dev* 2006;20:3199-3214.
- 15 Lv F, Leung VY, Huang S, Huang Y, Sun Y, Cheung KM: In search of nucleus pulposus-specific molecular markers. *Rheumatology (Oxford)* 2014;53:600-610.
- 16 Minogue BM, Richardson SM, Zeef LA, Freemont AJ, Hoyland JA: Transcriptional profiling of bovine intervertebral disc cells: implications for identification of normal and degenerate human intervertebral disc cell phenotypes. *Arthritis Res Ther* 2010;12:R22.
- 17 Hwang PY, Jing L, Chen J, Lim FL, Tang R, Choi H, Cheung KM, Risbud MV, Gersbach CA, Guilak F, Leung VY, Setton LA: N-cadherin is Key to Expression of the Nucleus Pulposus Cell Phenotype under Selective Substrate Culture Conditions. *Sci Rep* 2016;6:28038.
- 18 Hwang PY, Jing L, Michael KW, Richardson WJ, Chen J, Setton LA: N-Cadherin-Mediated Signaling Regulates Cell Phenotype for Nucleus Pulposus Cells of the Intervertebral Disc. *Cell Mol Bioeng* 2015;8:51-62.
- 19 Li P, Gan Y, Wang H, Xu Y, Li S, Song L, Zhang C, Ou Y, Wang L, Zhou Q: Role of the ERK1/2 pathway in osmolarity effects on nucleus pulposus cell apoptosis in a disc perfusion culture. *J Orthop Res* 2017;35:86-92.
- 20 Li P, Gan Y, Xu Y, Li S, Song L, Li S, Li H, Zhou Q: Osmolarity affects matrix synthesis in the nucleus pulposus associated with the involvement of MAPK pathways: A study of ex vivo disc organ culture system. *J Orthop Res* 2016;34:1092-1100.
- 21 Brown RQ, Mount A, Burg KJ: Evaluation of polymer scaffolds to be used in a composite injectable system for intervertebral disc tissue engineering. *J Biomed Mater Res A* 2005;74:32-39.
- 22 Li ST, Liu Y, Zhou Q, Lue RF, Song L, Dong SW, Guo P, Kopjar B: A novel axial-stress bioreactor system combined with a substance exchanger for tissue engineering of 3D constructs. *Tissue Eng Part C Methods* 2014;20:205-214.
- 23 Lee DA, Knight MM, Bolton JF, Idowu BD, Kayser MV, Bader DL: Chondrocyte deformation within compressed agarose constructs at the cellular and sub-cellular levels. *J Biomech* 2000;33:81-95.

- 24 Urban JP: The role of the physicochemical environment in determining disc cell behaviour. *Biochem Soc Trans* 2002;30:858-864.
- 25 Li P, Xu Y, Gan Y, Wang L, Ouyang B, Zhang C, Luo L, Zhao C, Zhou Q: Estrogen Enhances Matrix Synthesis in Nucleus Pulposus Cell through the Estrogen Receptor beta-p38 MAPK Pathway. *Cell Physiol Biochem* 2016;39:2216-2226.
- 26 Bertolo A, Mehr M, Aebli N, Baur M, Ferguson SJ, Stoyanov JV: Influence of different commercial scaffolds on the *in vitro* differentiation of human mesenchymal stem cells to nucleus pulposus-like cells. *Eur Spine J* 2012;21:S826-838.
- 27 Choi H, Johnson ZI, Risbud MV: Understanding nucleus pulposus cell phenotype: a prerequisite for stem cell based therapies to treat intervertebral disc degeneration. *Curr Stem Cell Res Ther* 2015;10:307-316.
- 28 Gao X, Zhu Q, Gu W: Prediction of glycosaminoglycan synthesis in intervertebral disc under mechanical loading. *J Biomech* 2016;49:2655-2661.
- 29 Hsieh AH, Twomey JD: Cellular mechanobiology of the intervertebral disc: new directions and approaches. *J Biomech* 2010;43:137-145.
- 30 Zhu Q, Gao X, Gu W: Temporal changes of mechanical signals and extracellular composition in human intervertebral disc during degenerative progression. *J Biomech* 2014;47:3734-3743.
- 31 Hsieh AH, Lotz JC: Prolonged spinal loading induces matrix metalloproteinase-2 activation in intervertebral discs. *Spine (Phila Pa 1976)* 2003;28:1781-1788.
- 32 Lotz JC, Hsieh AH, Walsh AL, Palmer EI, Chin JR: Mechanobiology of the intervertebral disc. *Biochem Soc Trans* 2002;30:853-858.
- 33 Lotz JC, Staples A, Walsh A, Hsieh AH: Mechanobiology in intervertebral disc degeneration and regeneration. *Conf Proc IEEE Eng Med Biol Soc* 2004;7:5459.
- 34 Hsieh AH, Yoon ST: Update on the pathophysiology of degenerative disc disease and new developments in treatment strategies. *Open Access J Sports Med* 2010;1:191-199.
- 35 Maroudas A, Stockwell RA, Nachemson A, Urban J: Factors involved in the nutrition of the human lumbar intervertebral disc: cellularity and diffusion of glucose *in vitro*. *J Anat* 1975;120:113-130.
- 36 Gruber HE, Hanley EN, Jr.: Analysis of aging and degeneration of the human intervertebral disc. Comparison of surgical specimens with normal controls. *Spine (Phila Pa 1976)* 1998;23:751-757.
- 37 Ding F, Shao ZW, Xiong LM: Cell death in intervertebral disc degeneration. *Apoptosis* 2013;18:777-785.
- 38 Wang C, Yu X, Yan Y, Yang W, Zhang S, Xiang Y, Zhang J, Wang W: Tumor necrosis factor-alpha: a key contributor to intervertebral disc degeneration. *Acta Biochim Biophys Sin (Shanghai)* 2017;49:1-13.
- 39 Lotz JC, Chin JR: Intervertebral disc cell death is dependent on the magnitude and duration of spinal loading. *Spine (Phila Pa 1976)* 2000;25:1477-1483.
- 40 Walsh AJ, Lotz JC: Biological response of the intervertebral disc to dynamic loading. *J Biomech* 2004;37:329-337.
- 41 Clouet J, Vinatier C, Merceron C, Pot-Vaucel M, Hamel O, Weiss P, Grimandi G, Guicheux J: The intervertebral disc: from pathophysiology to tissue engineering. *Joint Bone Spine* 2009;76:614-618.
- 42 Fujita N, Miyamoto T, Imai J, Hosogane N, Suzuki T, Yagi M, Morita K, Ninomiya K, Miyamoto K, Takaishi H, Matsumoto M, Morioka H, Yabe H, Chiba K, Watanabe S, Toyama Y, Suda T: CD24 is expressed specifically in the nucleus pulposus of intervertebral discs. *Biochem Biophys Res Commun* 2005;338:1890-1896.
- 43 Minogue BM, Richardson SM, Zeef LA, Freemont AJ, Hoyland JA: Characterization of the human nucleus pulposus cell phenotype and evaluation of novel marker gene expression to define adult stem cell differentiation. *Arthritis Rheum* 2010;62:3695-3705.
- 44 Shapiro IM, Risbud MV: Transcriptional profiling of the nucleus pulposus: say yes to notochord. *Arthritis Res Ther* 2010;12:117.
- 45 Thorpe AA, Binch AL, Creemers LB, Sammon C, Le Maitre CL: Nucleus pulposus phenotypic markers to determine stem cell differentiation: fact or fiction? *Oncotarget* 2016;7:2189-2200.