Interaction Between HIF-1α and Notch Signaling Pathways in Nucleus Pulposus Cells of Patients with Modic Changes

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Modic changes, HIF-1α, Notch signaling pathway, nucleus pulposus, endplates degeneration
Abstract
Background The HIF-1α/Notch signaling pathway has been shown to regulate proliferation, apoptosis, and metabolism in the intervertebral disc (IVD). The NP is an important structure adjacent to the disc. However, the roles of HIF1α and Notch signaling pathways in NP cells of patients with different Modic changes (MCs) are unclear. The purpose of this research was to assess the expression and association of HIF-1α and components of the Notch pathway in nucleus pulposus (NP) tissue of patients with various MCs.
Methods Eighty-five surgical NP tissue samples were obtained from patients undergoing microdiscectomy procedures for the treatment of low back and root pain caused by prolapse of the IVD. The NP tissues were divided into four groups based on the adjacent endplate degeneration: MC I, II, III, and negative MC groups. The expression of HIF-1α and Notch-related components were measured and compared.
Results The expression of HIF-1α, Notch1, and Notch2 were gradually increased in the MC I and MC II groups compared with that of the negative MC group. Meanwhile, HIF-1α and Notch-related components were rarely detected in MC III group.
Conclusions The expression of HIF-1α/Notch is increased in the NP cells of patients with MC I and MC II. Application of the association between HIF-1α/Notch signaling pathway could be promising target for clinical diagnosis and treatment of disc degeneration in MC patients.

Background
Modic changes (MCs) are changes of vertebral body marrow and endplate lesions that appear are visual signals on magnetic resonance images (MRI). In 1988, this phenomenon was first described and validated by Modic et al [1, 2]. In their study, these changes were classified into 3 general types. The different Modic types may represent different stages of the same pathological process: Modic type 1 changes (MC I) are associated with fissuring of the cartilaginous endplate corresponding to endplate edema. Modic type 2 changes (MC II) reflect fatty replacement of the adjacent marrow. Modic type 3 lesions (MC III) are observed in vertebral bodies with sclerotic changes.

Previous studies of MCs have focused mainly on their pathogenesis and the clinical significance of
changes between different types of MCs, as well as their relationship with low back pain (LBP) [3, 4]. The main cause of MCs has been attributed to minor trauma of the endplate as a result of repetitive loading [5] or to recurrent disc injury which causes an inflammatory reaction within the nucleus pulposus (NP). This initiates endplate changes and intervertebral disc (IVD) degeneration [6]. Therefore, investigating whether or not there is an association between IVD degeneration and MCs was desired. In order to clarify the correlation between IVD degeneration and MCs, we have focused on their change of hypoxic biomarkers.

Hypoxia-inducible factor (HIF) is a master transcription factor induced upon hypoxia and directs coordinated cellular responses to hypoxic environments. The HIF family of proteins comprises several distinct HIF proteins: HIF-1, HIF-2, and HIF-3. Each of these consist of an α-subunit and a constitutively expressed β-subunit known as aryl hydrocarbon receptor nuclear translocator [7, 8]. Risbud et al. [9] examined the expression of HIF-1α in rat, human, and sheep NP cells under both hypoxia and normoxia (2% and 21% oxygen); they found that NP cells consistently expressed functionally active HIF-1α protein under hypoxia. Thus, we apply HIF-1α as an index of the ischemia and anoxia of NP cells of the IVD. Therefore, it is reasonable to predict that HIF1-α may be a potential target for the prevention and treatment of IVD degeneration.

Notch is a hypoxia-sensitive receptor protein that can widely regulate proliferation of progenitor cells. This protein has four species of Notch receptors, including Notch1, Notch2, Notch3, and Notch4. Several studies have investigated the relationship between HIF-1 and Notch in physiological and pathological conditions [10–12]. In short, hypoxia activates the Notch signaling pathway to maintain IVD cell proliferation, accelerating catabolism. Therefore, HIF-1 and Notch may play an important role in the pathological process of disc degeneration. We predict that HIF-1/Notch might be an important signaling pathway in the maintenance of disc cell proliferation, and thus offers a therapeutic target for the restoration of cell numbers during degenerative disc disease.

In this study, we investigated the expression of HIF-1α and Notch in the bulgy discs adjacent to end plates with MCs and discuss the relationship between MCs and disc degeneration through imaging, biochemical, immunohistochemical methods to determine whether the expression of HIF-1α and
Notch is helpful for the diagnosis and treatment of degenerative disc diseases.

Materials And Methods

Human Tissue Collection

The Ethics Committee of the Hospital and Medical College approved this study and waived the requirement for informed consent. Eighty-five surgical IVD tissue samples were obtained from patients undergoing micro discectomy procedures for the treatment of LBP and root pain caused by prolapse of the IVD from January 2013 to January 2016. Each sample was obtained from the protrusive region of the IVD. The average LBP intensity was reported by the patient on a 0–10 numerical rating scale (NRS): 0 = no pain, 10 = worst possible pain. (Table 1)

| Modic Type   | Sex | Population | Age     | Sample Level | Population | NRS score |
|--------------|-----|------------|---------|--------------|------------|-----------|
| Control Group | M   | F          | 5 15    | 48.81 ± 9.93 | L1/2       | 2         | 3.3 ± 2.1 |
| Modic Type 1 | M   | F          | 9 6     | 42.6 ± 12.88 | L1/2       | 2         | 4.2 ± 1.6 |
| Modic Type 2 | M   | F          | 10 30   | 55.69 ± 11.03 | L1/2       | 2         | 3.6 ± 1.7 |
| Modic Type 3 | M   | F          | 4 6     | 64.6 ± 7.3   | L2/3       | 1         | 4.3 ± 1.7 |

Values are presented as the mean ± SD.

As shown in Fig. 1, patients were included with MC 1, MC2, and MC3 according to the inclusion criteria for MCs on MRI. The exclusion criteria were as follows: mixed MCs, ankylosing spondylitis, scoliosis, vertebral fractures, lumbar spine infection, spinal tumors, metastatic lesions, and other spine-related diseases; diabetes, hypertension, and other relevant medical history; history of spinal surgery, smoking, alcoholism, or drug use; psychological disorders, mental disorders, and other systemic disorder.

Isolation Of Np Cells And Treatments

NP cells from three patients of each group (L4/5, mean age of 55.16 years, 4 males and 8 females) were isolated using a previously described method.[13] After isolation, cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) with 10% fetal bovine serum (FBS) supplemented with
antibiotics. For hypoxic culture, NP cells were cultured in a tri-gas incubator (Huaxi Electronics Technetronic Co, ltd) under the condition of a mixture of 1% O₂, 5% CO₂, and 94% N₂ for 8 h.

Protein Isolation And Western Blotting
NP tissues from three patients of each group were placed on ice immediately and washed with pre-cooled PBS buffer solution, followed by homogenization in RIPA (Aspen, China) supplemented with phenylmethanesulfonyl fluoride, as well as protease and phosphatase inhibitors (Aspen, China). Tissue lysates were sonicated on ice, and protein concentrations were determined using the BCA protein assay kit (Sigma). The extracted proteins were resolved on 10% SDS-PAGE gels and blotted onto PVDF membranes (EMD Millipore Corporation, US), which was blocked with 5% non-fat dry milk in TBST at room temperature for 1 h. Membranes were then incubated overnight 4 °C with primary antibodies. The membrane was then washed with TBST three times, and the protein bands were developed using horseradish peroxidase (HRP)-conjugated secondary antibody (Proteintech) at room temperature for 1 h. Resulting banding was detected using enhanced chemiluminescent detection reagents.

Rna Extraction And Real-time Polymerase Chain Reaction (rt-pcr)
MC tissue was frozen in liquid nitrogen and ground into a fine powder under liquid nitrogen using a mortar and pestle. Total RNA was extracted using the RNeasy Mini kit (Qiagen, Valencia, CA) according to the manufacturer’s protocols. The quantity of the total RNA was measured by a spectrophotometer. Complementary DNA was synthesized using the First Strand cDNA Synthesis Kit (TAKARA, Japan) according to the manufacturer’s instructions. The forward and reverse primers for the target genes are listed in Table 2. RT-PCR was performed on a 96-well plate ABI Prism 7500 (Applied Biosystems, Foster City, CA) using KAPA SYBR FAST qPCR Kit Master Mix. Relative expression was calculated using the 2 – ΔCt method normalized to GAPDH.
Table 2
Primer sequences for Real-Time Polymerase Chain Reaction (RT-PCR)

| Gene   | Primer sequence                  |
|--------|----------------------------------|
| Notch 1| Forward: GCGACAAACGCTACCTCTG    |
|        | Reverse: AAGCCATGTAGCCGGCTCC     |
| Notch 2| Forward: TCAGCGCGGATACCTATGAG    |
|        | Reverse: CTGGCAGTGTCCCTGGAATGT   |
| Notch 3| Forward: AACGCTGCTTTTCTA         |
|        | Reverse: GAGCAGCTGTCACATCTCG     |
| Notch 4| Forward: GGAGAGGGGCTGTGGAAT      |
|        | Reverse: GCAGGGTCAGGAACTGG       |
| HES 1  | Forward: GAGTGCACTGAACGAGGTGAC   |
|        | Reverse: GGTCAATGGCAATGATCTGG    |
| HIF1α  | Forward: ACCACCTATGACCTGCTGG     |
|        | Reverse: TATCCAGGCTGTGCAGTCG     |
| GAPDH  | Forward: GCACCGTCAAGGCTGAGAAC    |
|        | Reverse: TGGTGAAGACGCCAGTGGA     |

Immunohistochemical Analysis

Immunohistochemistry was used to confirm and localize production of HIF-1α and Notch1 in IVDs. Tissue sections were dewaxed and rehydrated, endogenous peroxidases were quenched, and, following heat antigen retrieval, blocked in goat serum. Sections were incubated overnight at 4 °C with rabbit polyclonal antibodies against human HIF-1α and Notch1 (1:200). Pre-immune rabbit IgG (Abcam) was used as a negative control. After washing, sections were incubated with biotinylated goat anti-rabbit antiserum (1:400) and binding was detected by the formation of streptavidin-biotin complex (Vector Laboratories, Peterborough, UK) with 3,3-diaminobenzidine tetrahydrochloride solution (Sigma-Aldrich). Sections were counterstained with Mayer’s Hematoxylin (Leica Microsystems, Milton Keynes, UK), dehydrated, cleared, and mounted in Pertex (Leica Microsystems). Sections were visualized and images captured using an Olympus BX60 microscope and QCapture Pro software (version 8.0, Media-Cybernetics, Marlow, UK). Random fields of view were assessed for immunopositive and immunonegative cells until a total of 200 NP cells were counted in each section; the number of immunopositive cells were expressed as a percentage of the total count.

Statistical Analysis

All measurements were performed in triplicate. Data were analyzed using the Graphpad Prism statistical software (version 7.00). Unpaired t-test was used for comparison between different groups. Differences between groups were assessed by analysis of variance. P values less than 0.05 were considered statistically significant.

Results

Histological Analysis
We assayed HIF-1α expression related to Notch signaling in human NP tissues isolated from degenerated IVDs. The results showed that the NP tissues from MC I and MC II groups expressed higher level of HIF-1α protein compared with that of the control group. The expression of HIF-1α protein did not show any significant change in the MC III group (Fig. 2a). Additional statistical analysis showed that the percentage of cells immunopositive for HIF-1α in the MC I and MC II groups was significantly higher than that in the control group (MC I: 33.96% vs. 9.8%, MC II: 41.52% vs. 9.8%, P < 0.01; Fig. 2b). In accordance with the immunoactivity for HIF-1α, the Notch1 intracellular domain (NICD) expression was correlated to elevated HIF-1α level in MC I and MC II groups (MC I: 29.75% vs. 18.32%, MC II: 41.96% vs. 18.32%, p < 0.01; Fig. 2c).

Gene Expression
An increase in Notch1 and Notch2 mRNA levels were seen in MC I and MC II groups, which positively correlated with HIF-1α upregulation. However, the activities of those genes in patients with MC III showed no statistically significant difference compared with that of the control group (Fig. 3a-c). Additionally, there was no significant difference in the expression of Notch3 in patients with MC I and MC III compared with that of the control group (Fig. 3d). The transcriptional level of Notch3 receptor in the MC II group increased moderately compared to that of the control group. The transcriptional activities of Notch4 in MC groups displayed no statistically significant difference compared with that of the control group (Fig. 3e). Moreover, a significantly increased expression of HES1 (Fig. 3f), the target gene of Notch signaling, was detected in MC I and MC II groups when compared with that in the control group.

Correlation Analysis
The expression of HIF-1α and Notch1 (Fig. 3g; p = 0.0001), HIF-1α and Notch2 (Fig. 3h; p = 0.0077), and HIF-1α and Notch3 (Fig. 3i; p = 0.0011), and HIF-1α and Notch4 (Fig. 3j; p = 0.0077) in patients with MC II were significantly correlated with one another (Table 3). No significant correlations were observed between NRS back pain and HIF-1α or Notch receptors in any MC groups (Table 4).
Table 3
Correlation of mRNA expression between HIF-1α and Notch receptors in different groups. The p values and R² of Pearson’s correlation are provided.

|       | Ctrl | MC I | MC II | MC III |
|-------|------|------|-------|--------|
|       | p    | R²   | p     | R²     | p      | R²     |
| Notch1| 0.09 | 0.15 | 0.16  | 0.14   | <0.01  | 0.33   | 0.01  | 0.72   |
| Notch2| 0.18 | 0.10 | 0.68  | 0.01   | 0.01   | 0.17   | 0.46  | 0.07   |
| Notch3| 0.08 | 0.16 | 0.05  | 0.27   | <0.01  | 0.25   | 1.00  | <0.01  |
| Notch4| 0.13 | 0.12 | 0.67  | 0.01   | 0.01   | 0.17   | 0.72  | 0.02   |

Table 4
Correlation of mRNA expression between NRS scores and HIF-1α/Notch receptors in different groups

|       | HIF-1α | Notch1 | Notch2 | Notch3 | Notch4 |
|-------|--------|--------|--------|--------|--------|
| MC I  | 0.66   | 0.20   | 0.99   | 0.84   | 0.89   |
| MC II | 0.67   | 0.54   | 0.83   | 0.92   | 0.12   |
| MC III| 0.63   | 0.79   | 0.74   | 0.84   | 0.77   |

p values are provided.

Protein Expression Of Isolated Np Cells

Figure 4 shows that NP cells display a HIF-1α-dependent increase in protein levels of Notch1 and Notch2. The expression levels of NICD and HES1 were prominently increased in MC I and MC II groups compared with those in the control group, which was consistent with the trend observed for HIF-1α. These results indicated that the expression of HIF-1α correlated with the Notch signaling pathway to a great extent in MC tissues.

Discussion

In this research, the expression of HIF-1α was elevated in the NP cells of patients with MC I and MC II compared with that of the cells of patients with pure disc herniation. First of all, HIF-1α is an indicator of anaerobic condition. In response to hypoxic conditions, cells up-regulate the synthesis of HIF proteins [14]. HIF-1α plays an important role in the regulation of the biological behaviors of NP cells [7, 15]. Therefore, the increased expression of HIF-1α indicates conditions of degeneration or ischemia and hypoxia. Furthermore, other studies found that the HIF-1α/Notch signaling pathway plays an important role in the anoxic pathologic process such as tumor and neural degeneration disease. Based on the above results, we hypothesize that there is a correlation between Notch and hypoxia (through HIF-1α) in the patients with IVD herniation. Our study shows that the expression of NICD in the NP cells of the patients with IVD protrusion was higher, and it was positively correlated with HIF-1α. Therefore, we can speculate that in the NP cells of the disc herniation patients HIF-1α
may work through Notch signaling and change the downstream products.

Based on the interesting observation in our immunohistochemical results above, we assessed the mRNA expression of major components of the Notch signaling pathway in lumbar disc cells of different MC groups. There was an increased expression of Notch1, NICD, and HES1 in MC I and MC II groups, which was consistent with the histological results obtained. The evident correlation between HIF-1α and the Notch signaling pathway strengthens the point that HIF-1α regulates disc regeneration through activation of the Notch-HES1 pathway. Specifically, HIF-1α may promote recruitment of the NICD to the CSL-binding motifs in the HES1 promoter and maintain the homeostasis of the ECM in NP.

Furthermore, to illustrate the association between MCs and lumbar disc degeneration, a Spearman’s rank correlation analysis between NRS score and gene expression of HIF-1α/Notch receptors was conducted. Contrary to the imaging methods to evaluate the degree of IVD degeneration [18], neither of the biochemical markers above are positively correlated with the clinical symptoms of LBP in different MC groups. This inconsistent result is likely because the Notch-HES1 pathway is not involved in the initiation of LBP. A large study showed that there were significant correlations between LBP and inflammatory factors, such as IL-6, IL-8, PGE2, TNFα, etc [19, 20]. Most of them have been successfully used to activate Notch signaling in NP [21, 22]. In addition, HIF-1α expression was significantly increased in the IL-1β-stimulated NP cells under hypoxic condition [23]. We therefore speculate that the overexpressed inflammatory factors participate in consistent activation of the Notch and HIF-1α pathways and subsequent initiation of IVD degeneration in MCs patients, especially MC I and MC II on MRI [24].

Most studies have demonstrated crosstalk between HIF-1α and Notch signaling pathways in IVD [17]. Our study is the first to elucidate the different co-expression pattern of HIF-1α and the Notch signaling pathway in patients with different MCs. Specifically, hypoxia-induced Notch receptors and downstream molecules were highly expressed only in patients with MC I and MC II, not MC III, as detected by RT-PCR, western blotting, and immunohistochemistry. Notch1 and Notch2 mRNA transcriptional levels were markedly elevated in the NP, while Notch3 and Notch4 were not altered as
a result of the change of $PO_2$ in pathophysiological progress in IVD with MCs.

We can conclude that HIF-1α and Notch signaling pathways play an important role in the degeneration process of IVDs. Therefore, the treatment should proceed from the etiology, especially for a patient not suitable for surgery. These patients may get clinical benefit through intervention of HIF-1α and Notch pathways, and this may offer a new therapeutic target for the treatment of degenerative disc disease with MCs. For future clinical application, we should further investigate the interaction between HIF-1α and Notch signaling and the influence of downstream products.

This study has several limitations. First of all, the sample size was so small within MC I and MC III groups that they were inclusive when conducting Spearman’s rank correlation analysis. This may lead to a statistical uncorrelation between HIF-1α and Notch1/Notch2 in MC I and MC III groups. Second, given that the annulus fibrosus (AF) and endplate (EP) parts of the IVD samples were far too small to proceed with the follow-up analysis, they were carefully excluded from NP tissue. We did not evaluate the change of the above related genes and proteins in AF and EP tissues. Thus, evaluation of the study results in AF and EP tissues is needed in the future. Meanwhile, the samples used for western blotting must have strong proliferative ability in vitro. The enrolled samples from L4/5 of each group may lead to a large margin of selection bias.

Abbreviations

IVD
Intervertebral disc
MCs
Modic changes
HIF-1α
Hypoxia inducible factor-1α
NP
Nucleus pulposus
MRI
Magnetic resonance images
LBP
Low back pain
NICD
Notch1 intracellular domain
AF
Annulus fibrosus
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Contributions
Design of the experiment: ZX, and JD; collection of human samples: ZX, JD, and JGZ; cell experiments: ZX, and SY; data assessment: ZX, and JD; statistical analysis: ZX; writing of the manuscript: ZX, and JD; editing and final approval of the manuscript: XG, and JZ. All authors read and approved the final manuscript.
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**Ethics declarations**

**Ethics approval and consent to participate:** The Ethics Committee of the Hospital and Medical College approved this study and waived the requirement for informed consent. The manuscript submitted does not contain information about medical device(s)/drug(s). The requirement for an informed consent form was waived by the Ethics committee.

**Consent for publication:** Not applicable.

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**References**

1. Modic MT, Steinberg PM, Ross JS, Masaryk TJ, Carter JR: Degenerative disk disease: assessment of changes in vertebral body marrow with MR imaging. Radiology 1988, 166(1 Pt 1):193-199.

2. Modic MT, Masaryk TJ, Ross JS, Carter JR: Imaging of degenerative disk disease. Radiology 1988, 168(1):177-186.

3. Vital JM, Gille O, Pointillart V, Pedram M, Bacon P, Razanabola F, Schaelderle C, Azzouz S: Course of Modic 1 six months after lumbar posterior osteosynthesis. Spine 2003, 28(7):715-720.

4. Schmid G, Witteler A, Willburger R, Kuhnen C, Jergas M, Koester O: Lumbar disk herniation: Correlation of histologic findings with marrow signal intensity changes in vertebral endplates at MR imaging. Radiology 2004, 231(2):352-358.

5. Hansson T, Roos B: Microcalluses of the trabeculae in lumbar vertebrae and their relation to the bone mineral content. Spine 1981, 6(4):375-380.

6. Crock HV: Internal disc disruption. A challenge to disc prolapse fifty years on. Spine 1986, 11(6):650-653.

7. Boskey AL: Signaling in Response to Hypoxia and Normoxia in the Intervertebral Disc. Arthritis Rheum 2008, 58(12):3637-3639.
8. Semenza GL: Signal transduction to hypoxia-inducible factor 1. Biochem Pharmacol 2002, 64(5-6):993-998.

9. Risbud MV, Guttapalli A, Stokes DG, Hawkins D, Danielson KG, Schaer TP, Albert TJ, Shapiro IM: Nucleus pulposus cells express HIF-1 alpha under normoxic culture conditions: A metabolic adaptation to the intervertebral disc microenvironment. J Cell Biochem 2006, 98(1):152-159.

10. Hiyama A, Skubutyte R, Markova D, Anderson DG, Yadla S, Sakai D, Mochida J, Albert TJ, Shapiro IM, Risbud MV: Hypoxia Activates the Notch Signaling Pathway in Cells of the Intervertebral Disc Implications in Degenerative Disc Disease. Arthritis Rheum 2011, 63(5):1355-1364.

11. Zou J, Li P, Lu F, Liu N, Dai JJ, Ye JJ, Qu X, Sun XL, Ma DX, Park J et al: Notch1 is required for hypoxia-induced proliferation, invasion and chemoresistance of T-cell acute lymphoblastic leukemia cells. J Hematol Oncol 2013, 6:13.

12. Wang XM, Mao XO, Xie L, Greenberg DA, Jin KL: Involvement of Notch1 signaling in neurogenesis in the subventricular zone of normal and ischemic rat brain in vivo. J Cereb Blood Flow Metab 2009, 29(10):1644-1654.

13. Choi H, Merceron C, Mangiavini L, Seifert EL, Schipani E, Shapiro IM, Risbud MV: Hypoxia promotes noncanonical autophagy in nucleus pulposus cells independent of MTOR and HIF1A signaling. Autophagy 2016, 12(9):1631-1646.

14. Wenger RH, Gassmann M: Oxygen(es) and the hypoxia-inducible factor-1. Biol Chem 1997, 378(7):609-616.

15. Risbud MV, Schipani E, Shapiro IM: Hypoxic Regulation of Nucleus Pulposus Cell Survival From Niche to Notch. Am J Pathol 2010, 176(4):1577-1583.

16. Gustafsson MV, Zheng X, Pereira T, Gradin K, Jin S, Lundkvist J, Ruas JL, Poellinger L, Lendahl U, Bondesson M: Hypoxia requires notch signaling to maintain the
undifferentiated cell state. Developmental cell 2005, 9(5):617-628.

17. Liu Z, Li C, Meng X, Bai Y, Qi J, Wang J, Zhou Q, Zhang W, Zhang X: Hypoxia-inducible factor-lalpha mediates aggrecan and collagen Pi expression via NOTCH1 signaling in nucleus pulposus cells during intervertebral disc degeneration. Biochemical and biophysical research communications 2017, 488(3):554-561.

18. Xiao L, Ni CL, Shi JD, Wang ZR, Wang SC, Zhang JW, Lu AQ: Analysis of Correlation Between Vertebral Endplate Change and Lumbar Disc Degeneration. Med Sci Monitor 2017, 23:4932-4938.

19. Burke JG, Watson RWG, McCormack D, Dowling FE, Walsh MG, Fitzpatrick JM: Intervertebral discs which cause low back pain secrete high levels of proinflammatory mediators. J Bone Joint Surg-Br Vol 2002, 84B(2):196-201.

20. Risbud MV, Shapiro IM: Role of cytokines in intervertebral disc degeneration: pain and disc content. Nat Rev Rheumatol 2014, 10(1):44-56.

21. Wang H, Tian Y, Wang JR, Phillips KLE, Binch ALA, Dunn S, Cross A, Chiverton N, Zheng ZM, Shapiro IM et al: Inflammatory Cytokines Induce NOTCH Signaling in Nucleus Pulposus Cells IMPLICATIONS IN INTERVERTEBRAL DISC DEGENERATION. J Biol Chem 2013, 288(23):16761-16774.

22. Zheng YX, Liu CC, Ni L, Liu ZY, Mirando AJ, Lin J, Saijilafu, Chen D, Hilton MJ, Li B et al: Cell type-specific effects of Notch signaling activation on intervertebral discs: Implications for intervertebral disc degeneration. J Cell Physiol 2018, 233(7):5431-5440.

23. Kwon WK, Moon HJ, Kwon TH, Park YK, Kim JH: The Role of Hypoxia in Angiogenesis and Extracellular Matrix Regulation of Intervertebral Disc Cells During Inflammatory Reactions. Neurosurgery 2017, 81(5):867-875.

24. Ohtori S, Inoue G, Ito T, Koshi T, Ozawa T, Doya H, Saito T, Moriya H, Takahashi K:
Tumor necrosis factor-immunoreactive cells and PGP 9.5-immunoreactive nerve fibers in vertebral endplates of patients with discogenic low back pain and Modic Type 1 or Type 2 changes on MRI. Spine 2006, 31(9):1026-1031.

Figures

Figure 1

Representative T1- and corresponding T2-weighted images of different Modic change (MCs).

The red arrows indicate positions of MCs.
Figure 2

Immunohistochemistry of HIF1α and the Notch1 intracellular domain (NICD) in nucleus pulposus (NP) cells of different MC tissues and control group. Scale bar: 20 μm. Values are presented as the means ± S.E.M. *p < 0.05, **p < 0.01.
RT-PCR analyses of HIF-1α (A), Notch receptors (B-E), and HES1 (F) from different MC tissues. Spearman’s rank correlation analysis between HIF1α and Notch1 (G), HIF1α and Notch2 (H), HIF1α and Notch3 (I), and HIF1α and Notch4 (J). Values are presented as means ± S.E.M. *p < 0.05, **p < 0.01.
Figure 4

Representative expression of HIF1α and Notch receptors/target genes in NP cells isolated from different patients under hypoxia (1% O2). Anti-GAPDH was used as a loading control. Values are expressed as the mean ± SEM of three individual experiments. *p < 0.05, **p < 0.01; (n = 3)