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Expressions of IL-1α and MMP-9 in degenerated lumbar disc tissues and their clinical significance

P.-B. LI[1], W.-J. TANG[2], K. WANG[1], K. ZOU[1], B. CHE[1]

[1]Department of Orthopedics, General Hospital of the Yangtze River Shipping and Wuhan Brain Hospital, Wuhan, Hubei Province, China
[2]Radiology Department of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei Province, China

Pengbin Li and WenjuanTang contributed to this work equally

Abstract. – OBJECTIVE: To observe the expression levels of interleukin-1α (IL-1α) and matrix metalloproteinase-9 (MMP-9) in degenerated lumbar disc tissues and to investigate their clinical significance.

PATIENTS AND METHODS: Fifty patients with lumbar disc degeneration received the operative treatment were divided into three groups according to the magnetic resonance imaging (MRI) results: protrusion group, extrusion group, and free group. The degenerated intervertebral disc tissues were taken, and the normal intervertebral disc tissues of 20 patients received the operative treatment due to lumbar bursting fracture, and were selected as the control group. The bone mineral densities of all patients were measured. The mRNA and protein expression levels of MMP-9 and IL-1α were detected via Real-time polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA), respectively.

RESULTS: The bone mass of patients with lumbar disc degeneration was significantly decreased compared with that of the control group, and the bone mass was decreased more significantly in a free group than that in protrusion group. The mRNA and protein levels in IL-1α and MMP-9 of patients with lumbar disc degeneration were significantly increased compared with those in control group; the mRNA and protein levels in IL-1α and MMP-9 of extrusion group and free group were significantly higher than those in protrusion group, and the mRNA and protein levels in IL-1α and MMP-9 of free group were significantly higher than those in extrusion group.

CONCLUSIONS: The levels of IL-1α and MMP-9 in degenerated lumbar disc tissues are higher than normal levels, and the increasing levels are positively correlated with the disease condition.

Key Words:
Lumbar disc degeneration, Interleukin-1α, Matrix metalloproteinase-9.

Introduction

Intervertebral disc degeneration (IDD) refers to the premature hypofunction of intervertebral disc due to a variety of factors and the decline in functional cells in intervertebral disc, whose clinical manifestations are spinal instability, protrusion of intervertebral disc, spinal stenosis, etc[1]. An epidemiological survey showed that 80% of the population suffers from pains in waist and legs in different degrees, showing a younger trend[2]. In recent years, the incidence rate of lumbar disc degeneration has also been increasing year by year, also showing a younger trend. Its clinical symptoms are pains in waist and legs, seriously affecting people's quality of life[3]. The mechanism of lumbar disc degeneration is not fully clear, which involves age, obesity, heredity, smoking and other factors at present[4-6]. Changes in inflammatory mediators in intervertebral disc and activity of matrix metalloproteinases (MMPs) can aggravate the progression of intervertebral disc degeneration[7,8]. In this work, the expression levels of matrix metalloproteinase-9 (MMP-9) and interleukin-1α (IL-1α) in degenerated lumbar disc tissues were observed to investigate the relationship between inflammatory cytokines and MMPs and lumbar disc degeneration and search new targets for the prevention and treatment of lumbar disc degeneration, thereby delaying the progression of lumbar disc degeneration.

Patients and Methods

Patients

A total of 70 participants were enrolled in the present study. 20 patients who received the

Corresponding Author: Biao Che, MD; e-mail: 568414248@qq.com

operative treatment in our Department due to lumbar bursting fracture were selected as the control (n=20), including 12 males and 8 females with an average age of 44.26±11.58 years old. Another 50 patients who received operative treatment due to lumbar disc herniation in our Department from January 2016 to January 2017 were selected, including 28 males and 22 females with an average age of 46.13±10.07 years old. All patients were diagnosed with lumbar disc herniation via magnetic resonance imaging (MRI) before operation. All patients suffered from significant nerve compression symptom, and most were accompanied by low back pain. Patients with a history of acute and chronic infection or rheum immune systemic diseases were excluded. According to the results of MRI, patients were divided into protrusion group (n=20), extrusion group (n=17) and free group (n=13). The degenerated intervertebral disc tissues were taken during operation and cryopreserved in liquid nitrogen. The normal intervertebral disc tissues were taken during operation and cryopreserved in liquid nitrogen. Inclusion criteria of patients in control group: patients with no histories of lumbar disc herniation and spinal surgery, and no history of spinal diseases and rheumaimmune systemic diseases; no lumbar disc herniation was found in lumbar Computed Tomography (CT) or MRI, and no protrusion of intervertebral disc was found in fracture site during operation. There were no significant differences in gender and age of patients among the four groups (p>0.05). The current study was approved by the Ethics Committee of General Hospital of the Yangtze River Shipping and Wuhan Brain Hospital. All patients signed written informed consents.

Experimental Apparatus and Reagents

Dual-energy X-ray bone densitometer (DEXA, New York, NY, USA); ultra-micro ultraviolet spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA); continuous-wavelength multi-functional microplate reader (Tecan, Salzburg, Austria); Real-time fluorescence quantitative polymerase chain reaction (PCR) instrument (Eppendorf, Hamburg, Germany); TRIzol (Invitrogen, Carlsbad, CA, USA); reverse transcription kit (Toyobo, Osaka, Japan); IL-1α, MMP-9 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers (Sangon, Shanghai, China); SYBR Green PCR Master mix (TaKaRa, Otsu, Shiga, Japan); IL-1α and MMP-9 ELISA kits (R&D, Minneapolis, MN, USA).

Measurement of Bone Mineral Density

Bone mineral densities of all patients were measured before operation.

1) Parameter setting: resolution of 1.0×1.0 mm, scanning speed of 60 mm/s and scanning width of 5.0 cm.; 2) to evaluate the accuracy of measurement, 1 patient was randomly selected and the bone mineral density was measured for 5 consecutive times at the same position; 3) the coefficient of variation (CV) was used to express the accuracy. Specimen collection: lumbar disc tissues were separated and removed from all patients enrolled and repeatedly washed with normal saline immediately to remove the contaminated blood. Then tissues were immediately cryopreserved in liquid nitrogen.

RNA Extraction and Reverse Transcription of Lumbar Disc Tissues

Lumbar disc tissues were taken and added with appropriate amount of TRIzol (Invitrogen, Carlsbad, CA, USA) to extract the total RNA according to the instructions of TRIzol (Invitrogen, Carlsbad, CA, USA). The RNA concentration was determined via ultraviolet spectrophotometer, and the concentration and purity of RNA met the experimental requirements. RNA reverse transcription was performed: 2 μg RNA was taken, degenerated via heat at 65°C for 5 min and immediately placed on ice for cooling; 4 μL 5×RT Master Mix was added and the water treated by diethylpyrocarbonate (DEPC) was also added until the total volume reached 20 μL. Reaction parameters: 37°C for 15 min, 52°C for 5 min and 98°C for 5 min.

Detection of mRNA Expression Levels of IL-1α and MMP-9 in Lumbar Intervertebral disc via Real-time PCR

Primer sequences of IL-1α, MMP-9 and GAPDH are shown in Table I. Reaction system: 2 μL cDNA, 0.5 μL forward primer and 0.5 μL reverse primer, 12.5 μL 2×SYBR Green PCR Master Mix, pure water, and the total volume of 25 μL. Then, amplification was performed using the Real-time fluorescence quantitative PCR instrument. Reaction parameters: 95°C for 30s, 95°C for 5 s and 60°C for 30 s, a total of 40 cycles. The amplification curve and Ct value of each reaction were read. With GAPDH as the control, the expression difference of each gene was compared using the relative quantitative $2^{-\Delta\Delta Ct}$ method.

Table I. Primer sequence.

Gene	Amplification length	Primer sequence
IL-1α	147bp	Forward: 5'- AGATGCCTGAGATACCCAAAACC -3' Reverse: 5'- CCAAGCACACCCAGTAGTCT -3'
MMP-9	139bp	Forward: 5'- GGGACGCAGACATCGTCATC-3' Reverse: 5'- TCGTCATCGTCGAAATGGGC-3'
GAPDH	116bp	Forward: 5'- TGTGGGCATCAATGGATTTGG-3' Reverse: 5'- ACACCATGTATTCCGGGTCAAT-3'

Table II. Comparison of bone mineral density in each group.

Group	Case	Bone mineral density
Control group	20	0.1579±0.010
Protrusion group	20	0.1463±0.009**
Extrusion group	17	0.1418±0.008**
Free group	13	0.1325±0.011**##

Note: Compared with control group, **p<0.01; compared with protrusion group, ##p<0.01.

Detection of Protein Contents in IL-1α and MMP-9 in Lumbar Disc Tissues Via ELISA

The appropriate number of lumbar disc tissues was taken and added with normal saline for homogenate, followed by centrifugation at 3000 rpm at 4°C for 15 min. The supernatant was isolated for ELISA. After the loading and treatment strictly according to the instructions of kit, the optical density (OD) value was measured using the continuous-wavelength multi-functional microplate reader and the standard curve was drawn. The protein contents in IL-1α and MMP-9 in each specimen were calculated.

Statistical Analysis

The experimental results were presented as $\bar{x}$ ± s, and Statistical Product and Service Solutions (SPSS) 20.0 statistical software (IBM, Armonk, NY, USA) was used for statistical analysis. The independent sample *t*-test was used for comparison between the two groups, and the one-way analysis of variance followed by LSD (Least Significant Difference) test was used for comparison among groups. p<0.05 suggested that the difference was statistically significant.

Results

Comparison of Bone Mineral Density in Each Group

The bone mineral densities of patients in protrusion group, extrusion group and free group were significantly decreased compared with that in control group, and the difference was statistically significant (p<0.01); the bone mineral density was decreased more significantly in free group than that in protrusion group, and the difference was statistically significant (p<0.01) (Table II).

Comparison of Bone Morphology in Each Group

Compared with those in control group, the static bone morphological indexes (BV/TV and Tb.N) of patients in protrusion group, extrusion group and free group were significantly decreased (p<0.01), but the osteoclast index (Oc.No/BV) and trabecular separation (Tb.Sp) were significantly increased (p<0.01). Compared with those in protrusion group, the static bone morphological indexes (BV/TV and Tb.N) of patients in free group were significantly decreased (p<0.01), but the osteoclast index (Oc.No/BV) and trabecular separation (Tb.Sp) were significantly increased (p<0.05 or p<0.01) (Table III).

Table III. Comparison of bone morphology in each group.

Group	Case	BV/TV (%)	Tb.N (/mm)	Tb.Sp (μm)	Oc.No/BV (/mm²)
Control group	20	22.35±3.26	3.20±0.41	223.57±46.08	9.21±1.15
Protrusion group	20	15.52±3.75**	2.66±0.38**	307.16±53.91**	11.94±1.28**
Extrusion group	17	13.48±4.02**	2.41±0.46**	337.74±58.46**	13.20±1.43**
Free group	13	9.79±3.20**##	1.95±0.40**##	370.08±61.22**#	14.65±1.36**##

Note: Compared with control group, **p<0.01; compared with protrusion group, ##p<0.01.

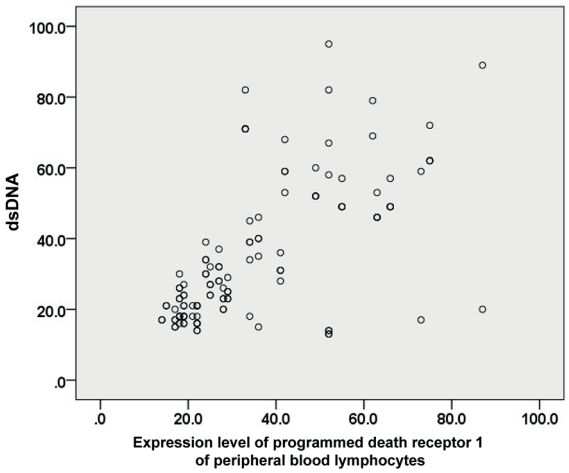

Figure 1. Comparison of mRNA expression level in IL-1α in each group. The results show that the mRNA expression levels in IL-1α in protrusion group, extrusion group and free group are significantly increased compared with that in control group ($p<0.01$); the mRNA expression levels in IL-1α in extrusion group and free group are significantly increased compared with that in protrusion group ($p<0.05$ or $p<0.01$). The mRNA expression level in IL-1α in free group is significantly increased compared with that in extrusion group ($p<0.01$). $^{**}p<0.01$, compared with control group; $^{\#\#}p<0.01$, compared with protrusion group; $^{\Delta\Delta}p<0.01$, compared with extrusion group.

Comparisons of mRNA Expression Levels in IL-1α and MMP-9 in Each Group

The mRNA expression levels in IL-1α and MMP-9 in protrusion group, extrusion group and free group were significantly increased compared with those in control group ($p<0.01$); the mRNA expression levels in IL-1α and MMP-9 in extrusion group and free group were significantly increased compared with those in protrusion group ($p<0.05$ or $p<0.01$). The mRNA expression levels in IL-1α and MMP-9 in free group were significantly increased compared with those in extrusion group ($p<0.01$) (Figures 1-2).

Comparisons of Protein Expression Levels in IL-1α and MMP-9 in Each Group

The protein expression levels in IL-1α and MMP-9 in protrusion group, extrusion group and free group were significantly increased compared with those in control group ($p<0.05$ or $p<0.01$); the protein expression levels in IL-1α and MMP-9 in extrusion group and free group were significantly increased compared with those in protrusion group ($p<0.05$ or $p<0.01$). The protein expression levels in IL-1α and MMP-9 in free group were significantly increased compared with those in extrusion group ($p<0.05$ or $p<0.01$) (Figures 3-4).

Discussion

The intervertebral disc is the motion segment of spine, responsible for the bending and twisting of the spine. Human lumbar intervertebral disc mainly consists of the outer annulus fibrosis, cartilage endplate on upper and lower intervertebral surfaces and nucleus pulposus tissue. After adulthood, the blood is lost directly in intervertebral disc, and the source of nutrition is mainly from the permeation of fiber ring and vertebral endplate cartilage. When the bone mineral density is reduced, the bone strength is decreased, causing tiny fractures in the bone endplate and vertebral body, thus destroying the metabolic cycle and nutritional supply of intervertebral disc, which can accelerate the disc degeneration[9-11]. This investigation found that the decreased lumbar vertebral bone mineral density is a risk factor of IDD, and the vertebral bone density is significantly reduced in patients with lumbar disc herniation[12]. We also found that the bone mineral density is significantly reduced in patients with lumbar disc degeneration, which will be decreased more significantly with the exacerbation of disease. The levels of a variety of cytokines change in IDD development

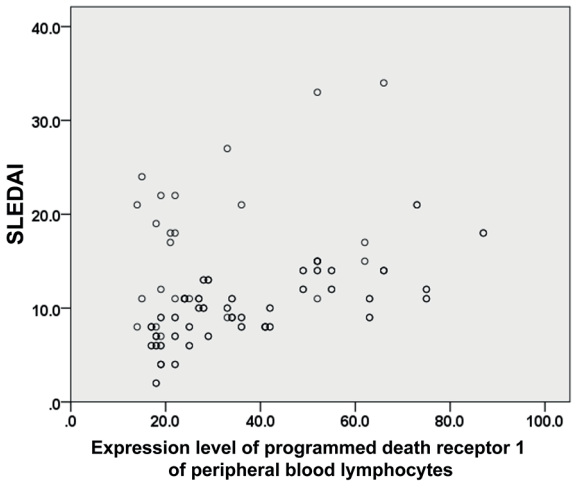

Figure 2. Comparison of mRNA expression level in MMP-9 in each group. The results show that the mRNA expression levels in MMP-9 in protrusion group, extrusion group and free group are significantly increased compared with that in control group ($p<0.01$); the mRNA expression levels in MMP-9 in extrusion group and free group are significantly increased compared with that in protrusion group ($p<0.01$). The mRNA expression level in MMP-9 in free group is significantly increased compared with that in extrusion group ($p<0.01$). $^{**}p<0.01$, compared with control group; $^{\#\#}p<0.01$, compared with protrusion group; $^{\Delta\Delta}p<0.01$, compared with extrusion group.

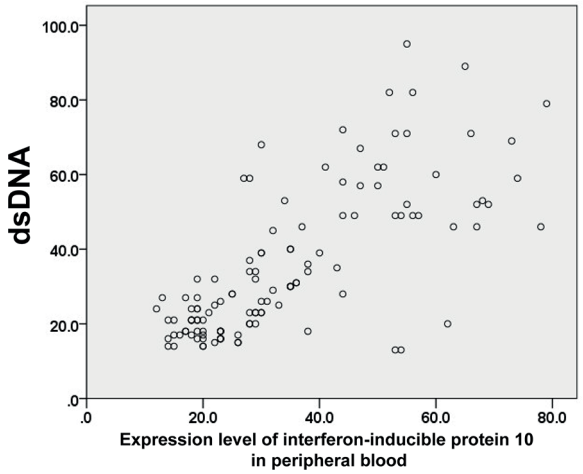

Figure 3. Comparison of protein level in IL-1α in each group. The results show that the protein levels in IL-1α in protrusion group, extrusion group and free group are significantly increased compared with that in control group (p<0.05 or p<0.01); the protein levels in IL-1α in extrusion group and free group are significantly increased compared with that in protrusion group (p<0.05 or p<0.01). The protein level in IL-1α in free group is significantly increased compared with that in extrusion group (p<0.01). $^{*}p$<0.05, $^{**}p$<0.01, compared with control group; $^{\#}p$<0.05, $^{\#\#}p$<0.01, compared with protrusion group; $^{\Delta\Delta}p$<0.01, compared with extrusion group.

process, among which IL family has a close relationship with IDD[13]. IL-1α is one of the important inflammatory mediators of the human body, which is mainly secreted by fibroblasts, monocyte-macrophages, chondrocytes and endothelial cells, etc. In the degenerated intervertebral disc, IL-1α can degrade the enzyme gene expression through promoting the matrix, especially in the degenerated nucleus pulposus-derived cells. At the same time, it can reduce the expression of cellular matrix molecular gene in intervertebral disc, finally leading to the imbalance of synthesis and degradation of intervertebral disc matrix and causing IDD[14]. Rannou et al[15] found that IL-1α exists in the intervertebral disc of patients with lumbar disc-derived back pain, and IL-1α can stimulate the lumbar disc cells to secrete prostaglandin E2 and other inflammatory substances. Moreover, IL-1α can enhance the sensitivity of bradykinin and directly stimulate the nerve root, thus participating in IDD-induced nerve root pain[16]. It was found in this study that the mRNA and protein levels of IL-1α in patients with lumbar disc degeneration were significantly increased; it is a process from lumbar disc herniation, lumbar disc extrusion to free lumbar disc with the progression of disease, indicating that the inflammatory cytokine IL-1α is closely related to the occurrence and development of lumbar disc degeneration. MMP is a class of proteolytic enzyme that contains Ca^{2+} and Zn^{2+} in the structure, which is an important enzyme that regulates the dynamic balance of extracellular matrix. It is derived from neutrophils, macrophages, chondrocytes and osteoclasts, etc., and can degrade almost all extracellular matrixes other than polysaccharides, playing an important role in the degradation and destruction of extracellular matrix[17]. MMP-9, also known as gelatinase B, is the 92KD gelatinase, and its molecular weight after activation is 82KD. MMP-9 is a key enzyme of extracellular matrix metabolism, which can not only directly degrade the proteoglycan and collagen in extracellular matrix, but also cause the "waterfall activation" of MMPs[18]. Sedowofia et al[19] first discovered gelatinase, neutral collagenase and elastase in human nucleus pulposus and fibrous ring of lumbar intervertebral disc, and found that there are significant differences in the activity of various enzymes in different regions of intervertebral disc. In the occurrence and development of IDD, the activity of MMPs is significantly increased, and extracellular matrix of intervertebral disc tissues is destroyed and decomposed, breaking the balance of extracellular

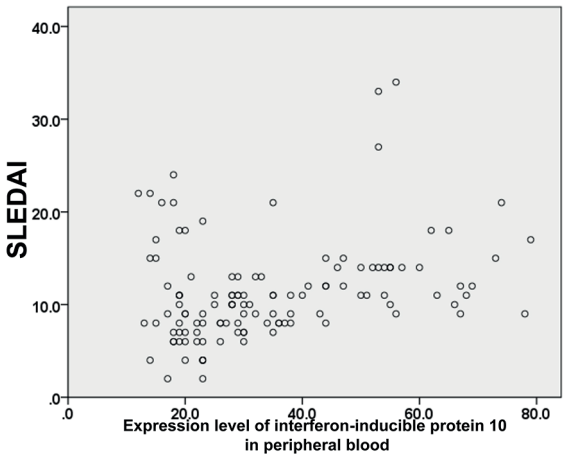

Figure 4. Comparison of protein level in MMP-9 in each group. The results show that the protein levels in MMP-9 in protrusion group, extrusion group and free group are significantly increased compared with that in control group (p<0.01); the protein levels in MMP-9 in extrusion group and free group are significantly increased compared with that in protrusion group (p<0.01). The protein level in IL-1α in free group is significantly increased compared with that in extrusion group (p<0.05). $^{**}p$<0.01, compared with control group; $^{\#\#}p$<0.01, compared with protrusion group; $^{\Delta}p$<0.05, $^{\Delta\Delta}p$<0.01, compared with extrusion group.

matrix, causing the loss of elasticity and water of nucleus pulposus, decreasing the biomechanics of intervertebral disc and promoting the development of IDD[20,21]. A number of studies have shown that the severity of intervertebral disc degeneration is positively correlated with the MMP-9 expression[22,23]. It was found in this paper that the mRNA and protein levels in MMP-9 in patients with lumbar disc degeneration were significantly increased, and it is a process from lumbar disc herniation, lumbar disc extrusion to free lumbar disc with the progression of disease, indicating that MMP-9 is closely related to the occurrence and development of lumbar disc degeneration.

Conclusions

We showed that with the occurrence and progression of lumbar disc degeneration, the bone mineral density in patients was decreased gradually, and the expression levels of inflammatory cytokines, IL-1α and MMP-9, were gradually increased, suggesting that the degree of lumbar disc degeneration has a certain correlation with the level of bone mineral density. With the development and progression of lumbar disc degeneration, many inflammatory cytokines accumulate in the lumbar disc tissues and the inflammatory response occurs. Meanwhile, the overexpression of MMP-9 leads to the damage of extracellular matrixes, resulting in decline in biomechanics of intervertebral disc and a series of clinical symptoms. IL-1α and MMP-9 levels can be used as indexes to judge the severity of lumbar disc degeneration, prognosis and drug efficacy.

Conflict of interest

The authors declare no conflicts of interest.

References

1) Sive JI, Baird P, Jeziorsk M, Watkins A, Hoyland JA, Freemont AJ. Expression of chondrocyte markers by cells of normal and degenerate intervertebral discs. Mol Pathol 2002; 55: 91-97.
2) DePalma MJ, Ketchum JM, Saullo T. What is the source of chronic low back pain and does age play a role? Pain Med 2011; 12: 224-233.
3) Cohen SP, Chen Y, Neufeld NJ. Sacroiliac joint pain: a comprehensive review of epidemiology, diagnosis and treatment. Expert Rev Neurother 2013; 13: 99-116.
4) Cheung KM, Samartzis D, Karppinen J, Mok FP, Ho DW, Fong DY, Luk KD. Intervertebral disc degeneration: new insights based on "skipped" level disc pathology. Arthritis Rheum 2010; 62: 2392-2400.
5) Cui YZ, Yang XH, Liu PF, Wang B, Chen WJ. Preliminary study on diagnosis of lumbar disc degeneration with magnetic resonance T1p, T2 mapping and DWI quantitative detection technologies. Eur Rev Med Pharmacol Sci 2016; 20: 3344-3350.
6) Sun ZM, Miao L, Zhang YG, Ming L. Association between the -1562 C/T polymorphism of matrix metalloproteinase-9 gene and lumbar disc disease in the young adult population in North China. Connect Tissue Res 2009; 50: 181-185.
7) Rannou F, Richette P, Benallaoua M, Francois M, Genries V, Korwin-Zmijowska C, Revel M, Corvol M, Poiraudeau S. Cyclic tensile stretch modulates proteoglycan production by intervertebral disc annulus fibrosus cells through production of nitrite oxide. J Cell Biochem 2003; 90: 148-157.
8) Christgau S, Garnero P, Fledelius C, Moniz C, Ensig M, Gineyts E, Rosenquist C, Qvist P. Collagen type II C-telopeptide fragments as an index of cartilage degradation. Bone 2001; 29: 209-215.
9) Leung VY, Hung SC, Li LC, Wu EX, Luk KD, Chan D, Cheung KM. Age-related degeneration of lumbar intervertebral discs in rabbits revealed by deuterium oxide-assisted MRI. Osteoarthritis Cartilage 2008; 16: 1312-1318.
10) Hangai M, Kaneoka K, Kuno S, Hinotsu S, Sakane M, Mamizuka N, Sakai S, Ochiai N. Factors associated with lumbar intervertebral disc degeneration in the elderly. Spine J 2008; 8: 732-740.
11) Benneker LM, Heini PF, Anderson SE, Alini M, Ito K. Correlation of radiographic and MRI parameters to morphological and biochemical assessment of intervertebral disc degeneration. Eur Spine J 2005; 14: 27-35.
12) Margulies JY, Payzer A, Nyska M, Neuwirth MG, Floman Y, Robin GC. The relationship between degenerative changes and osteoporosis in the lumbar spine. Clin Orthop Relat Res 1996: 145-152.
13) Jimbo K, Park JS, Yokosuka K, Sato K, Nagata K. Positive feedback loop of interleukin-1beta upregulating production of inflammatory mediators in human intervertebral disc cells in vitro. J Neurosurg Spine 2005; 2: 589-595.
14) Le Maitre CL, Freemont AJ, Hoyland JA. The role of interleukin-1 in the pathogenesis of human intervertebral disc degeneration. Arthritis Res Ther 2005; 7: R732-R745.
15) Rannou F, Corvol MT, Hudry C, Anract P, Dumontier MF, Tsagris L, Revel M, Poiraudeau S. Sensitivity of anulusfibrosus cells to interleukin 1 beta. Comparison with articular chondrocytes. Spine (Phila Pa 1976) 2000; 25: 17-23.
16) Olmarker K, Nutu M, Storkson R. Changes in spontaneous behavior in rats exposed to experimental disc herniation are blocked by selective TNF-alpha inhibition. Spine (Phila Pa 1976) 2003; 28: 1635-1641.

17) Mott JD, Werb Z. Regulation of matrix biology by matrix metalloproteinases. Curr Opin Cell Biol 2004; 16: 558-564.

18) Raffetto JD, Khalil RA. Matrix metalloproteinases and their inhibitors in vascular remodeling and vascular disease. Biochem Pharmacol 2008; 75: 346-359.

19) Sedowofia KA, Tomlinson IW, Weiss JB, Hilton RC, Jayson MI. Collagenolytic enzyme systems in human intervertebral disc: their control, mechanism, and their possible role in the initiation of biomechanical failure. Spine (Phila Pa 1976) 1982; 7: 213-222.

20) Sakai D. Future perspectives of cell-based therapy for intervertebral disc disease. Eur Spine J 2008; 17 Suppl 4: 452-458.

21) Nishida K, Suzuki T, Kakutani K, Yurube T, Maeno K, Kurosaka M, Doita M. Gene therapy approach for disc degeneration and associated spinal disorders. Eur Spine J 2008; 17 Suppl 4: 459-466.

22) Anderson DG, Izzo MW, Hall DJ, Vaccaro AR, Hilibrand A, Arnold W, Tuan RS, Albert TJ. Comparative gene expression profiling of normal and degenerative discs: analysis of a rabbit annular laceration model. Spine (Phila Pa 1976) 2002; 27: 1291-1296.

23) Weiler C, Nerlich AG, Zipperer J, Bachmeier BE, Boos N. 2002 SSE award competition in basic science: expression of major matrix metalloproteinases is associated with intervertebral disc degradation and resorption. Eur Spine J 2002; 11: 308-320.