J Huazhong Univ Sci Technol［Med Sci］
DOI 10.1007/s11596-012-0037-3
32(2):210-215,2012

Correlation between Osteoporosis and Degeneration of Intervertebral Discs in Aging Rats*

Yingang ZHANG (张银刚)[1], Junqing XIA (夏均青)[2], Yusheng QIU (邱裕生)[1], Yidong BAI (白益东)[3]
[1]*Department of Orthopedics, First Affiliated Hospital of Medical College of Xi'an Jiaotong University, Xi'an 710061, China*
[2]*Department of Orthopedics, Xi'an Traditional Chinese Medicine Hospital, Xi'an 710001, China*
[3]*Department of Cellular and Structural Biology, University of Texas Health Sciences Center, San Antonio, 78229, USA*

Summary: This study examined the correlation between osteoporosis and the degeneration of intervertebral discs. Sprague-Dawley rats were maintained up to 22 or 28 months. The femoral bone, tibial bone and lumbar vertebra were histologically studied and the expression of collagen type Ⅱ and Ⅹ in intervertebral discs was immunohistochemiscally determined. Several indices for the degeneration of intervertebral discs and osteoporosis and the correlation among them were then analyzed. Close correlations were found among the indices for the degeneration of intervertebral discs, including the relative area of the vascular bud, the ratio of the uncalcified and the calcified layers, the expression of collagen type Ⅱ and Ⅹ. The correlation with collagen type Ⅹ was negative. There existed positive correlations among the indices for osteoporosis, including the thickness ratio of cortical bone, the relative area of bone trabecula, the density of femoral and vertebral body bones, and the maximum stress and strain on bone. Analysis on the relationship of osteoporosis and the disease on disc showed that the indices of osteoporosis were negatively correlated with the indices of the degeneration of intervertebral discs but the expression of collagen type Ⅹ was positively correlated, with the density of vertebral body bones having the strongest dependence on collagen type Ⅹ. The maximum stress and strain bore no correlation with the degeneration of intervertebral discs. These results suggest that osteoporosis was negatively correlated with the degeneration of intervertebral discs.
Key words: osteoporosis; intervertebral disc; aging

Osteoporosis, a metabolic bone disease caused by the loss of bone matrix and bone minerals, is characterized by micro-architectural deterioration of bone tissues, reduced unit bone mass, and biomechanical changes[1]. It is caused by combined factors, including biological, mechanical and other unknown factors. The biological factors, such as calcium regulating hormones, growth regulating hormones, sex hormones, and other cellular and genetic factors, are associated with the incidence of osteoporosis[2–4]. The levels of calcium regulating hormones [25-(OH) vitamin D3, parathyroid hormone and calcitonin] and estrogen affect the regulation of bone metabolism; the two hormones may change with aging and organ functions[5, 6]. Human body development, growth, and aging are mainly regulated by the levels of various hormones that vary significantly at different stages of life. During the process of aging, estrogen can stimulate the differentiation of osteoblasts in an estrogen receptor (ER)-dependent manner to increase the bone matrix deposition and mineralization[7], and to reduce calcium regulating hormone and estrogen, leading to corresponding function changes in the target cells [8–10], and to reduce bone formation and increase bone resorption, resulting in a negative balance of bone metabolism, which eventually results in osteoporosis.

Not only is osteoporosis related to aging, but also disc degeneration is a natural physiological process related to aging and is caused by a series of cascade reactions in the process of aging. Normally, a person may have begun to experience intervertebral disc degeneration at his twenties[11]. With aging, the bone tissues are under abnormal stresses and therefore, the proteoglycan content in vertebral cartilage endplate decreases[12, 13], the nucleus pulposus active cells gradually reduce[14, 15], degenerated cells increase and present anti-differentiation characteristics[16], and the intervertebral disc elastic protein contents decrease significantly. The fibbers may exhibit as wear, fractures, irregular cavities and rupture with their flexibility weakening or even losing, which leads to the destruction of the overall structure of intervertebral discs and further degeneration of intervertebral discs[17, 18].

Given that both osteoporosis and the degeneration

Yingang ZHANG, E-mail: zyingang@mail.xjtu.edu.cn
*The study was supported by grants from the National Natural Science Foundation of China (No. 81171761, 30973063) and the Fundamental Research Funds for the Central University (No. 08143045).

of intervertebral discs are aging-related pathology, previous investigators have suggested that the two processes might be correlated and have similar pathogenesis[19]. However, some epidemiological investigations have revealed that the incidence of osteoporosis and degeneration of intervertebral discs were negatively correlated[20, 21]. Over-stressing caused by walking upright and excessive activities increased not only bone mineral density but also intervertebral disc over-strain, resulting in degeneration[22]. In addition, the biomechanical studies have shown that, under conditions of the same load, discs with subchondral bone compact are likely to experience more and more mechanical pressure, and, on the contrary, the osteoporotic bones could act as a soft pad to protect against and lessen pressure on cartilage to slow down intervertebral disc degeneration[23]. The results support negative correlation between osteoporosis and the degeneration of intervertebral discs. The correlation, positive or negative, warrants more evidence. In our previous studies, we found that the relative area of endplate vascular buds, the ratio of the uncalcified and calcified layers, and the expression of collagen type Ⅱ and type Ⅹ could be used to evaluate the aging-related degeneration of intervertebral discs[24]. In this study, the correlations between these indices and osteoporosis were evaluated to determine the relationship between osteoporosis and the degeneration of intervertebral discs in the process of aging.

1 MATERIALS AND METHODS

1.1 Establishment of the Aging Rat Model

Healthy female new born Sprague Dawley rats (n=100), from the Experimental Animal Center of Xi'an Jiaotong University, Xi'an, China, were maintained on a standard free-feeding lab diet and allowed free access to distilled drinking water for 22 or 28 months, when the rats weighed from 220 to 310 g. Fifty infertile rats sacrificed at 22 months were included in the "early-stage aging rat group", while the other 50 rats sacrificed at 28 months were assigned to the "advanced-stage aging rat group".

The animals were anesthetized with 20% urethane solution and sacrificed by exsanguinations of celiac artery blood. The tissues from the femur, tibia, and lumbar spine in bilateral hind-limbs were taken out and fixed in 4% paraformaldehyde, or were stored at –20ºC until analysis. L1 and L2 vertebral bodies and left femurs were used for mineral density testing, which was followed by biomechanical examination. The right lower limb and L3 and L4 vertebral bodies were used for histological examination.

1.2 Pathology Examination with HE Staining

L3–L4 vertebral segments, including the 3rd and 4th lumbar vertebrae and the L3–L4 disc, the middle-section of the femur, and the mid-upper tibia (including knees), were fixed in 4% paraformaldehyde, and decalcified with 8% neutral EDTA for 40 days. The tissues were then embedded in paraffin, and glass slides were processed with 9% polylysines. Then each tissue piece was sliced into 6 to 8 μm and HE-stained.

1.3 Immunohistochemistry and Quantitation of Type Ⅱ and Ⅹ Collagens

Immunohistochemical staining with monoclonal antibodies was performed to determine the amount of type Ⅱ and type Ⅹ collagens in intervertebral disc cartilage. The disc slices were de-waxed with xylene, grade-hydrated with ethanol, and then incubated with 2 mg/mL hyaluronidase (Sigma, USA) for 30 min at room temperature. Type Ⅱ and type Ⅹ collagen was processed at room temperature with 1 mg/mL of Pronase (Sigma, USA) and 0.02 mg/mL of protease for 30 min, respectively. The samples were incubated with the first antibodies (mouse monoclonal antibody, provided by the Experimental Center for Molecular Biology of the University of Erlangen-Numberg, Germany) at 4°C overnight, and then incubated with the second antibody (biotin-labeled alkaline phosphatase anti-mouse IgG) for 30 min. The colorimetric reaction was developed with 3-hydroxy-2-napthylacid 2, 4 dimethylanilid (Sigma, USA) at room temperature. The nucleolus was re-stained with hematoxylin and cemented with a water-soluble tablet for observation. Positive expression presented as red particles around the matrix and the cells. The semi-automatic color image analysis system (Beihang Biomedicine, Beijing, China) was applied in gray scale scanning mode. The nucleolus in the scanned slices was not re-stained.

1.4 Measurement of the Relative Area of Endplate Vascular Buds in Cartilage

With HE staining, the number of vascular buds in end-plate cartilage (the number of buds in front and caudal/2) was counted under an optical microscope at 40× magnification. The squared method was used to show the relative vascular area covering the endplate region (fig. 1). Ten specimens were taken from each group, with each specimen being consecutively sliced into 2–8 μm transections. The images were collected under the microscope. After size calibration, graphical data were collected, and an automatic analysis was performed by using a medical image analysis software package (Biomedicine Company, Beihang University, Beijing, China).

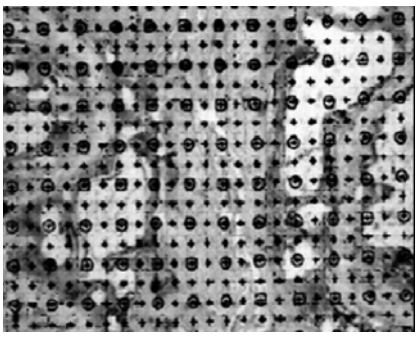

Fig. 1 The relative area of blood vessels (HE×40)
The ratio of the point number of vascular buds to total points counted under an optical microscope

1.5 Measurement of the Thickness of Calcified and Uncalcified Layers on Cartilage Endplate

The slices were HE-stained, and the tidemark was considered to be the demarcation line. Under a 40× magnification, the thickness of the calcified and uncalcified

layers at the anterior 1/3, middle point and posterior 1/3 of the sagittal plane of the middle intervertebral disc endplate cartilage was determined. Three average values were used to calculate the ratio of non-calcified/calcified layers (fig. 2).

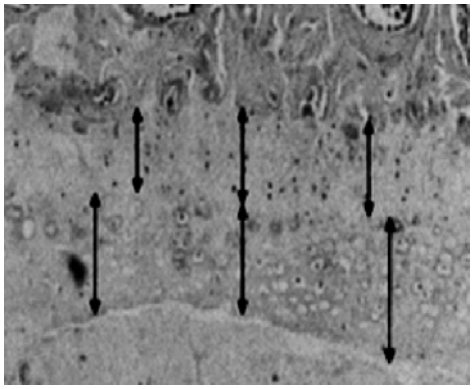

Fig. 2 The measurement of cartilage endplate (HE×40)
The tidemark is considered to be the separatrix. The thickness of the calcified (arrows on the upper line) and uncalcified layers (arrows on the lower line) at the anterior 1/3, middle point and posterior 1/3 of the sagittal plane of the middle intervertebral disc endplate cartilage was determined. Three average values were used to calculate the ratio of non-calcified / calcified layer.

1.6 Determination of the Index of Cortical Bone Thickness

The slices of middle femoral transections were HE-stained, and the index of cortical thickness was measured and counted under an optical microscope. The calculating method was shown in fig. 3.

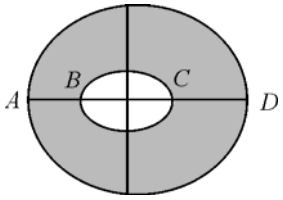

Fig. 3 The index of bone cortical thickness
The calculating method was as follows: the thickness of cortical bone index= (AB+CD)/AD
The AB, CD, AD are described as in the diagram. The mean values were obtained based on the values of 4–8 sites

1.7 Measurement of the Relative Area of Trabecular Bones

After the paraffin slices from the tibias were HE-stained, they were measured by using a semi-automatic color image analysis system (Beihang Medicine, Beijing, China). In the cancellous bone area, 1 mm below the articular cartilage, each slice was measured in three visual fields (at 40× magnification, with observed area equal to 274 753 μm^2). The mean value was used to determine the relative trabecular area.

1.8 Measurement of Bone Mineral Density

The mineral density of the four limbs and lumbar vertebral bones was measured by using a QDR-2000 HOLOJAC two-photon bone densitometer (PE Co., USA). The radioactive source was americium^{241}. The error was less than 3%. When measuring the bone *in vitro*, it was placed into a plexiglass box, with each box containing 7 specimens. The regional high resolution QDR 4500 software for small animal analysis was used.

1.9 Biomechanical Determination

The right femur was evaluated using a three-point mechanical test by a computer-controlled electronic universal testing machine (PE Co., USA) to observe the maximum stress and strain experienced by the bone. When the external force was applied to the bone, it would resist the force by internal impedance generated from deformation, which was called bone stress. The maximum load was used to show the stress (g/cm^2). The strain refers to the bone deformation under an external force, which can be presented as the maximum distance displaced (cm) during the time from the beginning of the stress to fracture. When measuring, the distance between the two constrained stents was adjusted to 20 mm. The rat femurs (with relatively well-proportioned density) were placed on the stent. The short axis of the femur was placed parallel with the vertical direction. Then, grade pressure was applied until the bone fractured. The load leading to fracture was recorded. The specimens were maintained in normal saline prior to analysis. The measuring accuracy of load was 0.1 Newton (N) and the accuracy of deformation was within 0.001 mm.

1.10 Statistical Method

The relative Pearson's analysis for variables was performed with the SPSS 10.0 for windows. The results were presented as $\bar{x}\pm s$. The statistical difference was set as 95% confidence interval (CI), and the test standard was $\alpha=0.05$, $P<0.05$.

2 RESULTS

2.1 Morphological Changes

The aged rats in both groups exhibited signs of osteoporosis such as trabecular bone sparsity, disorders in the lining, increased gaps between trabecular bones, and increased ratios of trabecular bone area. The tissue samples also showed signs of intervertebral disc degeneration, including reduced vascular buds on the cartilage endplate and irregular order of annulus fibrosus, which was more serious in advanced-stage aging rat group than in the early-stage aging rat group. The results are shown in tables 1.

2.2 Correlation Analyses among Various Indices

The analysis of early-stage aging rat group and advanced-stage aging rat group demonstrated that there was a moderate to strong positive correlation among the indices of osteoporosis, including the bone cortical thickness, relative area of bone trabecula, femoral bone mineral density, vertebral body bone mineral density, maximum load (N), and maximum deformity (mm). There also existed a moderate or strong positive correlation among the indices of degeneration of intervertebral discs, including the relative area of endplate vascular buds, the ratio of the uncalcified layer/calcified layer, and the expression of type Ⅱ collagen. However, a negative correlation was found between indices of degeneration of intervertebral discs and expression of type Ⅹ collagen (table 2).

Table 1 The values of relevant indices of osteoporosis and degeneration of intervertebral discs in the two groups of aging rats ($\bar{x}\pm s$)

Indices	*n*	Early-stage	Advanced-stage
Osteoporosis indices			
Cortical thickness	50	0.07±0.01	0.065±0.01
Relative area of trabecular bone	50	9.95±4.12	9.45±4.20
Femoral bone mineral density	50	0.0643±0.0070	0.0621±0.0060
Vertebral body bone mineral density	50	0.0793±0.0131	0.0752±0.010
Maximum load (N)	50	134.41±23.09	133.65±25.25
Maximum deformity (mm)	50	1.0800±0.1204	1.0700±0.1321
Indices of degeneration of intervertebral discs			
Relative area of endplate vascular buds	50	24.07±8.80	23.66±8.70
Ratio of uncalcified layer/ calcified layer	50	0.82±0.23	0.84±0.26
Expression of type II collagen (nucleus pulposus)	50	338.41±31.70	336.32±32.80
Expression of type Ⅹ collagen (uncalcified layer)	50	88.41±3.72	89.56±3.68

Table 2 Correlation analysis of the indices of osteoporosis and the degeneration of intervertebral discs in both aging rats

	Groups	RAT	DF	DV	ML	MD	VA	RDC	QⅡ	QⅩ
IBC	Early-stage	0.654*	0.601*	0.733*	0.521*	0.571*	–0.413*	–0.545*	–0.642*	0.621*
	Advanced-stage	0.655*	0.602*	0.732*	0.519*	0.572*	–0.412*	–0.547*	–0.646*	0.625*
RAT	Early-stage		0.821*	0.809*	0.567*	0.445*	–0.521*	–0.534*	–0.687*	0.567*
	Advanced-stage		0.821*	0.804*	0.566*	0.443*	–0.523*	–0.532*	–0.685*	0.568*
DF	Early-stage			0.867*	0.601*	0.623*	–0.612*	–0.589*	–0.701*	0.498*
	Advanced-stage			0.866*	0.603*	0.622*	–0.611*	–0.584*	–0.702*	0.498*
DV	Early-stage				0.525*	0.498*	–0.834*	–0.823*	–0.767*	0.891*
	Advanced-stage				0.524*	0.494*	–0.839*	–0.821*	–0.762*	0.798*
ML	Early-stage					0.754*	–0.012	–0.043	–0.009	0.098
	Advanced-stage					0.751*	–0.015	–0.048	–0.008	0.096
MD	Early-stage						–0.008	–0.012	–0.008	0.017
	Advanced-stage						–0.008	–0.012	–0.009	0.017
VA	Early-stage							0.872*	0.796*	–0.564*
	Advanced-stage							0.874*	0.794*	–0.566*
RDC	Early-stage								0.657*	–0.761*
	Advanced-stage								0.658*	–0.768*
QⅡ	Early-stage									–0.654*
	Advanced-stage									–0.655*

Note: The data in the table indicates the Pearson's correlation coefficient, *r*; *$P<0.05$

IBC: index of bone cortical thickness; RAT: relative area of bone trabecula; DF: femoral bone density; DV: vertebral body density; ML: max stress; MD: max strain; VA: relative area of vascular bud on the endplate; RDC＝the ratio of the uncalcified and the calcified layers; QⅡ: the quantitation of collagen type Ⅱ (nucleus pulposus); QⅩ: the quantitation of collagen type Ⅹ (uncalcified layer)

2.3 The Correlation between Osteoporosis and the Degeneration of Intervertebral Discs

The relative analysis demonstrated that there was a moderate or strong negative correlation among the indices of osteoporosis and the degeneration of intervertebral discs, including bone cortical thickness, the relative area of bone trabecula, femoral bone mineral density, vertebral body bone mineral density, the relative area of endplate vascular buds, the ratio of the uncalcified and the calcified layers, and the expression of type Ⅱ collagen, while there was strong positive correlation with the expression of type Ⅹ collagen. The strongest correlation was found between the vertebral body bone mineral density and the expression of type Ⅹ collagen on the cartilage endplate (fig. 4). The relative indices of degeneration of the intervertebral discs were not only correlated with the bone mineral density of the neighboring vertebral body, but also correlated with the bone mineral density of distant femoral bones. However, there was no correlation found between the biomechanical indices, including maximum deformity and maximum load and the indices of degeneration of intervertebral discs (table 2).

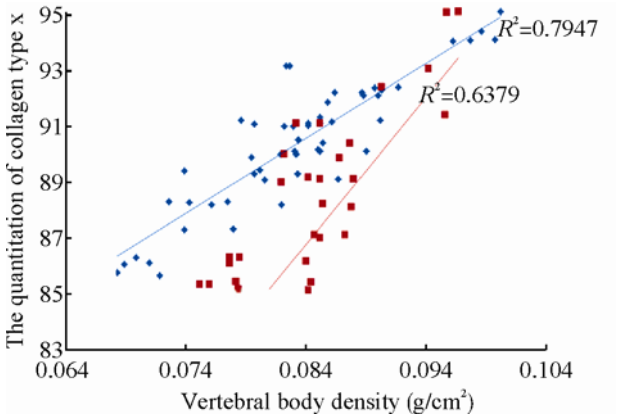

Fig. 4 Correlation analysis of the bone density of the vertebral body to the phenotypic expression of type X collagen on the cartilage endplate in both aging rats

Blue color represents the data of early-stage aging rats, and red color represents data of advanced-stage ones.

3 DISCUSSION

In this investigation, rats were used to eliminate effects of stress (from walking or standing as with humans) on the development of osteoporosis and the degeneration of intervertebral discs. The 22- or 28-month-old female rats were used to generate a model of aging-related osteoporosis and degeneration of intervertebral discs. The normal life-span of rats is 2 to 3 years, with animals reaching sexual maturity 3 months after birth. Therefore, a 22-month-old female rat may be considered an equivalent of a woman in her 50's or 60's in terms of age. For humans, this is the age when postmenopausal osteoporosis often occurs. In addition, the degeneration of intervertebral discs and related diseases tend to develop at this age. Because age is a factor that affects the results of correlative analysis of osteoporosis and degeneration of intervertebral discs, all of the rats used for the study in each group were of the same age.

The aging female rat model constructed in this experiment showed signs of osteoporosis in bone morphology. In order to evaluate the characteristics of osteoporosis, the histomorphometric indices of bone, bone mineral density, and bone biomechanics were determined and analyzed. The results showed that there was a moderate to strong correlation among the indices Among the indices for the degeneration of intervertebral discs, there were close correlations, and of them, only the correlation with collagen type Ⅹ was negative. These indices were histologically confirmed, and were consistent with the mechanism of aging of intervertebral discs[24].

Analysis on the relationship of osteoporosis and the disease on discs showed that the indices of osteoporosis were negatively correlated with the indices of the degeneration of intervertebral disc except that the expression of collagen type Ⅹ was positively correlated, with the density of vertebral body bones having the strongest dependence on collagen type Ⅹ. These findings demonstrated that bone mineral density was positively correlated with the degeneration of intervertebral discs, while osteoporosis was negatively correlated with degeneration of intervertebral discs. Theoretically, the bone mineral density should be positively correlated with maximum deformity and maximum load. However, there was no correlation between the biomechanical indices, including maximum deformity and maximum load, and the indices of degeneration of intervertebral discs. Considering that bone mineral density of vertebral body was subject to the regional environment, we also analyzed the correlation between the femoral bone mineral density and degeneration of intervertebral discs. These results also supported the negative correlation between osteoporosis and degeneration of intervertebral discs during the aging process. In this investigation, rats were used to obviously remove the potential effects of stresses induced by walking upright and strenuous activity that may affect studies of human. However, our study demonstrated that osteoporosis was still negatively correlated with degeneration of intervertebral discs. This finding indicated that the "stress theory" might not give a satisfactory explanation for the negative correlation between osteoporosis and degeneration of intervertebral discs.

Osteoporosis is considered to be changes of systemic skeletons during the aging process, while intervertebral disc degeneration can be attributable more to the changes of local tissues with aging[25]. However, the studies on degenerated intervertebral disc tissues by RT-PCR showed that more than 300 cell factors and their receptor genes and at least 10 kinds of cytokines are involved in the degeneration of intervertebral discs through the signaling pathways in different cells[26]. In addition, it has been recognized that the cytokines play an essential regulatory role during the process of osteoporosis. The cytokines generated from bone micro-environment include interleukin (IL)-1, interleukin-6, tumor necrosis factors, *etc*. IL-1 and TNF-α are known cytokines with strongest stimulation to bone resorption[27, 28]. Therefore, it deserves more investigations to clarify whether cytokines are responsible for the negative correlation between osteoporosis and degeneration of intervertebral discs.

Studies by other investigators have demonstrated that expression of IGF and TGF was high in osteoarthritic bone with high bone mineral density[29]. Moreover, genetic studies revealed that osteoporosis and osteoarthritis have a different genetic basis[30]. It is possible that different genetic causes underlie the pathogenesis of osteoporosis and the degeneration of intervertebral discs[31–33]. While our results indicate that these two processes are not related pathologically. This study was preliminary, and further investigation is needed to understand the genetic basis of the degeneration of intervertebral discs, and its relation with osteoporosis.

REFERENCES

1 Simon LS. Osteoporosis. Rheum Dis Clin North Am, 2007,33(1):149-176
2 Clunie G. Update on postmenopausal osteoporosis management. Clin Med, 2007,7(1):48-52
3 Shoback D. Update in osteoporosis and metabolic bone disorders. J Clin Endocrinol Metab, 2007,92(3):747-753
4 Albagha OM, Ralston SH. Genetics and osteoporosis. Rheum Dis Clin North Am, 2006,32(4):659-680
5 Tennekoon KH, Karunanayake EH. Serum FSH, LH, and testosterone concentrations in presumably fertile men: effect of age. Int J Fertil, 1993,38(2):108-112
6 Levine JP. Long-term estrogen and hormone replacement therapy for the prevention and treatment of osteoporosis. Curr Womens Health Rep, 2003,3(3):181-186
7 Chen XW, Garner SC, Anderson JJ. Isoflavones regulate interleukin-6 and osteoprotegerin synthesis during osteoblast cell differentiation via an estrogen-receptor-dependent pathway. Biochem Biophys Res Commun, 2002, 295(2):417-422
8 Bodine PV, Henderson RA, Green J, *et al*. Estrogen receptor-alpha is developmentally regulated during osteoblast differentiation and contributes to selective responsiveness of gene expression. Endocrinology, 1998, 139(4):2048-2057
9 Keeting PE, Scott RE, Colvard DS, *et al*. Lack of a direct effect of estrogen on proliferation and differentiation of normal human osteoblast-like cells. J Bone Miner Res, 1991,6(3):297-304
10 Scheven BA, Damen CA, Hamilton NJ, *et al*. Stimulatory effects of estrogen and progesterone on proliferation and differentiation of normal human osteoblast-like cells in

vitro. Biochem Biophys Res Commun, 1992,186(1):54-60
11 Adams MA, Roughley PJ. What is intervertebral disc degeneration, and what causes it? Spine (Phila Pa 1976), 2006,31(18):2151-2161
12 Holm S, Baranto A, Kaigle Holm A, *et al*. Reactive changes in the adolescent porcine spine with disc degeneration due to endplate injury. Vet Comp Orthop Traumatol, 2007,20 (1):12-17
13 Zhang YG, Guo TM, Guo X, *et al*. Clinical diagnosis for discogenic low back pain. Int J Biol Sci, 2009,5(7): 647-658
14 Zhang Y, Sun Z, Liu J, *et al*. Advances in susceptibility genetics of intervertebral degenerative disc disease. Int J Biol Sci, 2008,4(5):283-290
15 Zhang YG, Guo X, Xu P, *et al*. Bone mesenchymal stem cells transplanted into rabbit intervertebral discs can increase proteoglycans. Clin Orthop Relat Res, 2005, 430:219-226
16 Zhao CQ, Jiang LS, Dai LY. Programmed cell death in intervertebral disc degeneration. Apoptosis, 2006, 11(12):2079-2088
17 Videman T, Saran S, Battié MC, *et al*. The long-term effects of physical loading and exercise lifestyles on back related symptoms, disability and spinal pathology among men. Spine (Phila Pa 1976), 1995,20(6):699-709.
18 Roberts S, Evans H, Trivedi J, *et al*. Histology and pathology of the human intervertebral disc. J Bone Joint Surg Am, 2006, 88(Suppl 2):10-14.
19 Aoki J, Yamamoto I, Kitamura N, *et al.* End plate of the discovertebral joint: degenerative change in the elderly adult. Radiology, 1987,164(2):411-414
20 Miyakoshi N, Itoi E, Murai H, *et al*. Inverse relation between osteoporosis and spondylosis in postmenopausal women as evaluated by bone mineral density and semiquantitative scoring of spinal degeneration. Spine (Phila Pa 1976), 2003,28(5):492-495
21 Margulies JY, Payzer A, Nyska M, *et al*. The relationship between degenerative changes and osteoporosis in the lumbar spine. Clin Orthop Relat Res, 1996,324:145-152
22 Lotz JC, Colliou OK, Chin JR, *et al*. Compression-induced degeneration of the intervertebral disc: an *in vivo* mouse model and finite-element study. Spine (Phila Pa 1976), 1998,23(23):2493-506
23 Harada A, Okuizumi H, Miyagi N, *et al*. Correlation between bone mineral density and intervertebral disc degeneration. Spine (Phila Pa 1976), 1998,23(8):857-861; discussion 862
24 Zhang YG, Sun ZM, Liu JT, *et al*. Features of intervertebral disc degeneration in rat's aging process. J Zhejiang Univ Sci B, 2009,10(7):522-527
25 Zhang YG, Guo X, Sun ZM, *et al*. Gene expression profiles of disc tissues and peripheral blood mononuclear cells from patients with degenerative disc. J Bone Miner Metab, 2010,28(2):209-19
26 Freemont TJ, LeMaitre C, Watkins A, *et al*. Degeneration of intervertebral discs: current understanding of cellular and molecular events, and implications for novel therapies. Expert Rev Mol Med, 2001:1-10
27 Ishimi Y, Miyaura C, Jin CH, *et al*. Il-6 is produced by osteoblasts and induces bone resorption. J Immunol, 1990, 145(10):3297-3303
28 Thomson BM, Mundy GR, Chambers TJ. Tumor necrosis factors alpha and beta induce osteoblastic cells to stimulate osteoclastic bone resorption. J Immunol, 1987,138(3): 775-779
29 Irlenbusch U, Dominick G. Investigations in generalized osteoarthritis. Part 2: special histological features in generalized osteoarthritis (histological investigations in Heberden's nodes using a histological score). Osteoarthritis Cartilage, 2006,14(5):428-434
30 Ogata N, Shiraki M, Hosoi T, *et al*. A polymorphic variant at the Werner helicase (WRN) gene is associated with bone density, but not spondylosis, in postmenopausal women. J Bone Miner Metab, 2001,19(5):296-301
31 Zhang YG, Sun ZM, Zhang Z *et al*. Risk factors for lumbar intervertebral disc herniation in Chinese population. A case-control study. Spine (Phila Pa 1976), 2009,34(25): E918-E922
32 Sun ZM, Miao L, Zhang YG, *et al*. 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(3):181-185
33 Guo TM, Liu M, Zhang YG, *et al*. Association between Caspase-9 promoter region polymorphisms and discogenic low back pain. Connect Tissue Res, 2011,52(2):133-138

(Received Nov. 11, 2011)