

Gene expression profiles of disc tissues and peripheral blood mononuclear cells from patients with degenerative discs

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Abstract The objective of this study was to analyze gene expression profiles of intervertebral disc samples and peripheral blood mononuclear cells (PBMCs) from patients with degenerative discs using Agilent's Human 1A Oligo microarray. RNA samples from disc tissue and PBMCs were obtained from patients with degenerative discs and from subjects in a control group. RNA samples were reverse-transcribed into Cy5-labeled cRNA, combined with a Cy3-labeled reference and hybridized to oligonucleotide microarrays. Microarrays were scanned by GenePix 4000B and data were analyzed using GenePixPro 3.0 software. The microarray data were validated in the same RNA samples by qRT-PCR analysis of selected genes. For the disc tissue, the mRNA expressions of 522 genes changed obviously in the degeneration group, accounting for approximately 2.64% of all analyzed transcripts. These included transcription-related, ion channel and transport protein, receptor, protein synthesis and modifying, growth factor, etc. For PBMCs, the expressions of 62 genes changed obviously in the patients in the degeneration group. These changes included ion channel, transport protein, transcription-related, DNA synthesis and repair, metalloprotease, immune globulin-related, growth factor-related, extracellular matrix-related, adhesion molecule, etc. Analyzed on the association of the differential

expression of genes between disc tissue and PBMCs, some genes were not compatible. The course of intervertebral disc denegation is a complicated dynamic process, however, and may mainly be local pathogenesis. These findings furnish new data for the mechanistic investigation of degenerative discs.

Keywords Intervertebral disc · Degeneration · Microarray · Peripheral blood mononuclear cells · mRNA

Introduction

Intervertebral disc (IVD) degeneration and related diseases cause low back pain. The pathogenesis of intervertebral disc degeneration (IDD) has been studied for many years, but is still not clear. IVD has a complex structure, consisting of two discrete components: nucleus pulposus (NP) and annulus fibrosus (AF). At the molecular level, degeneration of the IVDs manifests as decreasing diffusion, cell viability, proteoglycan synthesis, and alteration in collagen distribution. Thus, loss of normal cells and abnormal matrix syntheses may account for disc degeneration [1]. Disc degeneration is a multifactorial process, which includes both environmental and genetic effects [2]. To our knowledge, to date, the exact cause for these changes in disc composition remains largely unknown, despite many years of extensive research. Therefore, it is critical to study the etiology of IDD, which may shed light on the prevention of and therapeutic intervention in this disease.

These changes of mRNA expression in the composition of the IVDs may provide further insights into understanding the mechanisms of IDD and the development of innovative therapeutic strategies for its treatment. Previous

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attempts to measure gene expression in IVD cells and tissue have used semi-quantitative or quantitative polymerase chain reaction (PCR) methods [3], but these techniques are highly subjective and their sensitivities are very low [4]. Microarrays can allow biological researchers to examine the expression of tens of thousands of genes at once. However, few researchers have used this technology to study human IVD. One likely reason is that the human IVD has relatively few cells (about 5% total mass). The technology of linear amplification of cRNA can help to make the study on human IVD using microarrays into a reality.

Peripheral blood mononuclear cells (PBMCs), which can transfuse into all kinds of tissues in human body, comprise 3–7% of the leucocytes in the blood and are precursor cells of osteoclasts [5, 6]. Some researchers have found that the mice which were devoid of mononuclear cells could have osteopetrosis [7] and did not lose bone even after ovariectomy [8]. In addition, PBMCs can secrete multiple cell cytokines acting on osteoclasts and osteoblasts, such as IL-1, IL-6 and TNF [9, 10]. With growth and the onset of menopause, the number of mononuclear cells and the amount of secreted growth factors mentioned above also increase [11, 12]. The percentage of estrogen receptor (ER)—positive monocytes decreases with increasing age [13]. Monocytes move to the bone surface through the circulatory system, then grow and differentiate, ultimately become osteoclasts [14–16]. To prevent and treat disc degeneration, it is important to investigate the roles of PBMCs during IDD.

In our study, we used the Agilent Human 1A Oligo Array, which includes many relevant anabolic and matrix genes—for example collagen types I, II and III; aggrecan, biglycan, decorin, osteonectin, and osteopontin—as well as catabolic gene products such as MMP-1, MMP-2, MMP-3, and MMP-13, and important intercellular regulators and their receptors (i.e. relevant cytokines and growth factors such as IL-1 α , IL-6). The aims of the present study were to analyze gene expression profiles of IVD samples and PBMCs in patients with degenerative discs using gene microarrays.

Materials and methods

Study groups

Experiment 1

Experiment 1 was about disc tissue. The experimental group was comprised of 10 individuals (6 female, 4 male) with IVD herniation and severe clinical symptoms. Magnetic resonance imaging (MRI) confirmed the degeneration

of discs with apparent decrease in the T2-weighted signal. Ten herniated lumbar IVD specimens were obtained from the group. All patients were undergoing primary surgical lumbar discectomy for persistent radicular symptoms, and had a minimum of 6 weeks of conservative nonoperative treatment before electively undergoing surgery. The age range was 21–43 years (mean 30.2). Of the 10 herniated discs, one was from the L3 to L4 disc space, five were from L4 to L5, and four were from the L5 to S1. The mean duration of radicular symptoms before surgery was 9 weeks, ranging from 6 to 16 weeks. The herniated disc material was obtained through a small window that was made in the posterior longitudinal ligament. Extruded disc material and any loose disc fragments within the IVD space were harvested. There was no transligamentous disc herniation in the study group. Patients with degenerative spinal stenosis, tumors, infections, and previous lumbar disc surgery were excluded from the study.

The control group was comprised of 10 cadavers of brain trauma (5 female, 5 male) with the age ranged from 28 to 55 years (mean 35.6). They had no anamnesis evidence for pre-existing spinal disorders, disc degeneration or previous spine-related operations. Disc tissue was removed within 5 h after death. The levels affected were L2–S1. Of the ten discs, one was from the L2 to L3 disc space, two were from L3 to L4, four were from L4 to L5, and three were from the L5 to S1. Disc tissue was obtained through a window made in the lateral aspect of the annulus fibrosus. A mixture of nucleus pulposus and annulus fibrosis was obtained.

After resection, the entire tissue (nucleus and annulus together) was rinsed with phosphate buffer solution (PBS, pH 7.2), snap-frozen in nitrogen and stored at -80°C .

Experiment 2

Experiment 2 was about PBMCs. The two groups' blood samples ($n = 20$) were collected after approval by the patients. 10 ml blood was withdrawn from the median cubital vein. All samples were incubated in the PAX gene Blood RNA tube for 24 h at room temperature prior to extraction. PBMCs were isolated using the Sigma AccuspinTM system (Sigma–Aldrich Ltd.) according to the manufacturer's instructions and stored at -80°C in 1 ml of Trizol reagent (Invitrogen, Carlsbad, CA, USA).

Tissue and blood were obtained according to methods approved by the local Ethics Committee.

RNA isolation and amplification

Total RNA was isolated from whole IVD tissue and whole blood tissue using Trizol reagent (Invitrogen, Carlsbad, CA, USA); the protocol was performed according to kit

instructions. The yield and quality of extracted total RNA samples were estimated using a NanoDrop ND-1000 UV–Vis Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and electrophoresis on 1.5% native agarose gels. Purified RNA samples were stored at -80°C until required for gene expression studies.

Total RNA from samples was amplified once using the RiboAmp linear amplification kit (Arcturus Engineering, Mountainview, CA, USA) to generate sufficient quantities of RNA for hybridization to the Agilent Oligo microarrays.

Oligo microarrays

Agilent's Human 1A Oligo microarray (V2) is comprised of 20,173 (60-mer) oligonucleotide probes, which span conserved exons across the transcripts of the targeted full-length genes. These probes represent 18,716 well-characterized, full-length, human genes from RefSeq and Incyte's Foundation.

We used a two-channel labeling system with two differently labeled RNA samples hybridized to the same microarray. Control samples were labeled with Cy5. Experimental samples were labeled with Cy3.

Hybridization and microarray scanning

The cRNA was suspended in a hybridization buffer and hybridized to microarrays for 17 h at 65°C according to the Agilent protocol. The glass slides were then washed. After immediately removing the wash buffer by centrifugation, the glass slides were scanned using Gene-Pix 4000B (Axon Instruments, Inc, CA, USA) containing a 532 nm laser for Cy3 measurement and a 635 nm laser for Cy5 measurement.

Normalization and analysis of microarray data

Sixteen-bit TIFF images produced by the Axon scanner were analyzed using the GenePixPro 3.0 (Axon Instruments, Inc, CA, USA) software package. After obtaining Cy3 and Cy5 grayscale images, each pseudo-color image was overlaid, and all spots in the ratio image were defined by accessing the gene list file that described the location of each gene on the microarray. The average of the signal intensity was subtracted from the median of background intensity and output with the UniGene and GenBank descriptors to a Microsoft Excel data spreadsheet. Relative expression levels were calculated by global normalization between two samples using all detected genes. The remaining genes were analyzed by the *t* test with *P* values lower than 0.05.

Quantitative real-time polymerase chain reaction

DNase I-treated total RNA (0.5 μg) was reverse transcribed with oligo(dT) primer using the ThermoScriptTM reverse transcriptase (RT)–PCR System (Invitrogen Carlsbad, CA, USA) for cDNA synthesis. RT reaction (2.0 μL) was amplified in triplicate by real-time PCR (ABIPRISM 7000) in a final volume of 25 μL using SYBR Green Master Mix reagent at a final concentration of $1\times$ (Applied Biosystems, CA, USA). Glyceraldehyde phosphate dehydrogenase (GAPDH), a constitutively expressed housekeeping gene, was also amplified under the same conditions and used to normalize reactions. To ensure the specificity of the reaction, the size of the PCR product for each gene was verified by 1.5% agarose gel electrophoresis. Results were obtained using sequence detection software (ABI PRISM 7000) and evaluated by using Microsoft Excel. The primers for human specific genes were designed according to the published sequences available in GenBank using Primer Express (Applied Biosystems CA, USA) as shown in Table 1. Genes were selected from two categories: (1) ECM components: aggrecan and collagen III; (2) catabolic genes: matrix metalloprotease-3 (MMP-3) and interleukin1 β (IL-1 β). For the analysis of each sample, gene expression levels were calibrated using GAPDH expression levels as an internal control. For gene expressed quantitation, the comparative Ct method was used. The differences between the mean Ct values of the gene of interest and the housekeeping gene were denoted ($\Delta - \text{Ct}$) and the difference between $\Delta - \text{Ct}$ and the Ct value of the calibrator sample was labeled $\Delta - \Delta - \text{Ct}$. The $\log(\Delta - \Delta - \text{Ct})$ gave the relative quantitation value of gene expression with intact NP gene expression designated as one. Results of quantitative RT–PCR were assessed by *t* test.

Results

Differential gene expression of IVD tissues

Compared to individuals in the control group, the expressions of 522 genes changed obviously in the degeneration group, accounting for approximately 2.64% of all analyzed transcripts. Of these, 498 pieces increased significantly while 24 pieces decreased significantly. Grouping of these genes according to their biological functions identified significant changes in all functional categories, including transcription-related, ion channel and transport protein, receptor, protein synthesis and modifying, growth factor, molecules involved in signal transduction, extracellular matrix-related, DNA synthesis and repair, oncogene-related, growth-related, cytoskeleton and motion, cycle phase-related, G-protein-related, apoptosis-related, interleukin,

Table 1 Forward and reverse primers used for quantitative real-time polymerase chain reaction

Gene	Sequence	GeneBank accession no.
Aggrecan	GCTACGGAGACAAGGATGAGTTC CGTAAAAGACCTCACCTCCAT	L38480
Collagen III	GCTGAAGGGCAGGGAACAA AAGAGCGAGACGAAGTAGGG	D1281
IL-1 β	GCCGATGGTCCCAATTACAT ACAAGACCTGCCGGAAGCT	M26295
MMP3	ACACCGGATCTGCCAAGAGA CTGGAGAACGTGAGTGGAGTCA	M25664
GAPDH	GGAGAAAGCTGCTAA ACGACCTGGTCCTCGGTGTA	L23961

IL interleukin, MMP matrix metalloprotease, GAPDH glyceraldehyde phosphate dehydrogenase

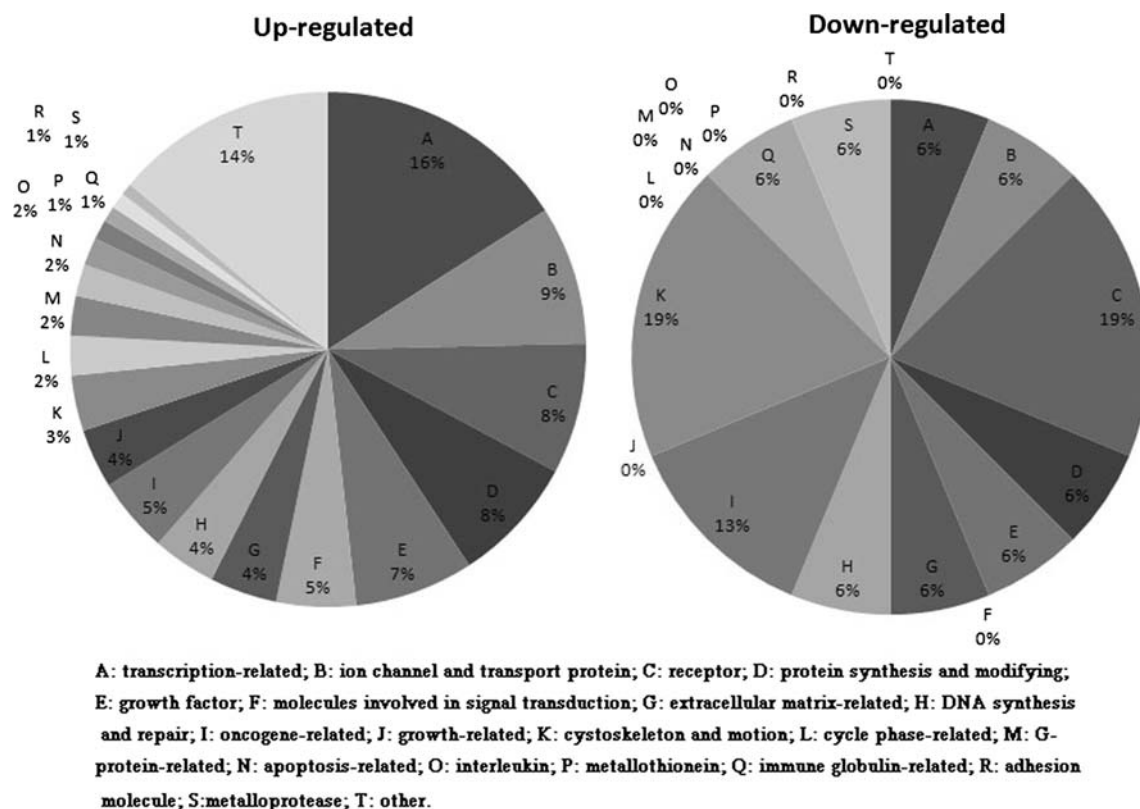


Fig. 1 Functional classification of the differentially regulated genes in the degenerated disc. Numbers represent the percentage (%) of genes differentially regulated in degenerated disc with respect to the

metallothionein, immune globulin-related, adhesion molecule, metalloprotease and other (Fig. 1).

Among the genes differentially expressed in degenerated discs, we selected for further study those related to loss of cells and to abnormalities of matrix in degenerated discs. The list includes 70 genes related to the control of cell and matrix in discs, of which 63 were deemed to be up-regulated (Table 2) and only 7 down-regulated (Table 3). A number of these genes have previously been established

total number of up-regulated (left pie chart) and down-regulated (right pie chart) analyzed transcripts

through conventional methods to be altered in degenerated discs. Among these, several growth factor/cytokines and their activity regulator genes were found to be significantly up-regulated. For example, insulin-like growth factor 1 (IGF-1) was up-regulated to 2.11, while insulin like growth factor binding protein 2 was up-regulated to 2.74. In extracellular matrix (ECM) component genes, Alpha 1 subunit of type I collagen and Alpha 2 subunit of type I collagen, and Alpha 1 subunit of type X collagen were also

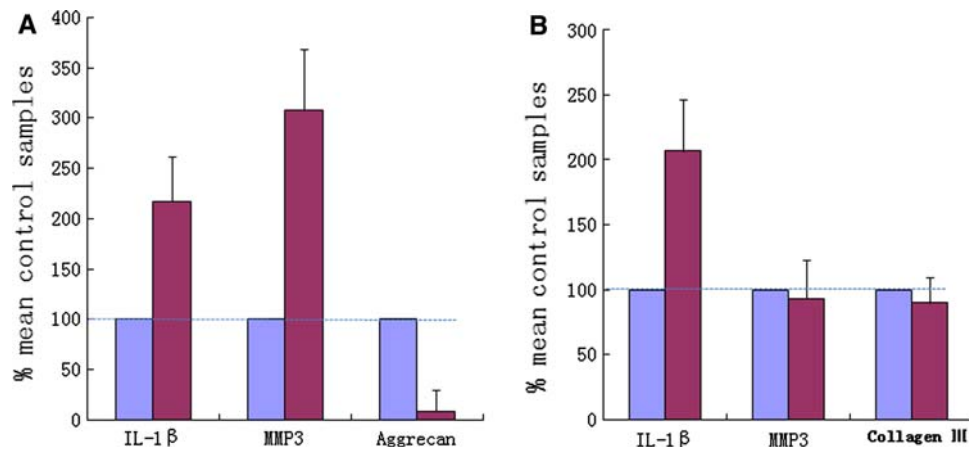


Fig. 2 qRT-PCR analysis of disc and PBMCs samples from degenerated (red bar) and control (blue bar) groups. The longitudinal axis is the relative expression level of disc or PBMC samples, compared with the controls. For disc samples (a), two up-regulated genes (IL-1 β , MMP3) and one down-regulated gene (aggrecan) were arbitrarily

selected for the validation of microarray data. For PBMC samples (b), one up-regulated gene (IL-1 β) and two unchanged genes (MMP3, collagen III) were chosen. There were statistically significant differences at *P* level 0.01 for all genes, as compared with control samples. Data are expressed as means \pm SEM

up-regulated. Matrix metalloproteinase, a key ECM degradation enzyme, was also up-regulated. Tissue inhibitor of metalloproteinase 4 and 3, a key molecule involved in increased ECM production, was significantly up-regulated. In this study, we also detected a number of genes that have been implicated in apoptotic cell death, for example the apoptosis gene TNFRSP11 up-regulated to 3.65 in the discs. Among the down-regulated genes, the mRNA encoding secretory granule proteoglycan 1 (serglycin, proteoglycan core protein) was down-regulated to 0.12 (see Table 3).

Differential gene expression of PBMCs

The expressions of 62 genes changed obviously in the degeneration group, compared with the control group. Of these, 39 pieces increased while 24 pieces decreased significantly. Grouping of these genes according to their biological functions identified significant changes in all functional categories, including ion channel, transport protein, transcription-related, DNA synthesis and repair, metalloprotease, immune globulin-related, growth factor-related, extracellular matrix-related, adhesion molecule, etc. Genes found to be significantly expressed between PMBCs in patients and controls are shown in Tables 4 and 5.

Our results identified several groups of up-regulated genes in the patients. One group is genes which are related with transcription, consisting of FLJ14345, C21orf81 and SUPV3L1. A second group consists of IGLL1, IGHA1, IGKV1D-39 and MYOM2; IGLL1 is critical for B-cell development. Another group consists of CCR4, BMPRI1A. CCR4 is a receptor for chemokine and BMPRI1A is a receptor for BMP. Human NK cells express CCR4 and

respond to thymus and activation-regulated chemokine, macrophage-derived chemokine.

Compared to the gene expression differences of IVD tissue, the expression pattern of PMBCs were different, that is, the majority of IVD tissue did not show in PMBCs; only a small number of genes in both tissues were differentially expressed. Analysis of some of them showed they were not compatible (Table 6). For example, TNFSF11 was up-regulated to 3.65 in disc tissue while down-regulated to 0.34 in PMBCs. FGF22 was up-regulated to 4.43 in disc tissue while down-regulated to 0.16 in PMBCs.

Quantitative real-time polymerase chain reaction

The microarray data were validated using the same RNA samples by qRT-PCR analysis on selected genes found up-regulated (IL-1 β , MMP3), down-regulated (Aggrecan) in the microarray analysis for disc tissue, and up-regulated (IL-1 β), unchanged (Collagen III, MMP3) for PMBCs (Fig. 2).

Discussion

To the best of our knowledge, the current study is the first to investigate gene expression change profiles in both disc tissue and blood from degenerative disc disease patients using DNA microarrays. The results of this study have shed light on the expression of various genes that may be involved in IVD. Given the inherent drawbacks of this technique, such as limitations in sensitivity and quantification, in our study we used a real-time quantitative reverse transcription PCR technique to validate the microarray

Table 2 Up-regulated genes associated with loss of cells and abnormalities of matrix in degenerated discs

Genes	Accession no.	Symbol	Fold-range
Paraspeckle protein 1	NM_018282.1	PSP1	3.43
Microtubule aggregate protein 44	BC022870.1	IFI44	2.20
Toll-like receptor 4	U93091.1	TLR4	2.38
Matrix metalloproteinase 3	X05232.1	MMP3	3.08
Matrix metalloproteinase 7	BC003635.1	MMP7	2.78
Matrix metalloproteinase 2	BC002576.1	MMP2	2.00
Matrix metalloproteinase 19	U37791.1	MMP19	3.70
Matrix metalloproteinase 11	X57766.1	MMP11	2.08
Nuclear factor I-X	L31881.1	NFIX	2.25
POU domain class 4 transcription factor 2	NM_004575.1	POU4F2	2.25
Tissue inhibitor of metalloproteinase 4	BC010553.1	TIMP4	4.34
Tissue inhibitor of metalloproteinase 3	S78453.1	TIMP3	3.28
A disintegrin and metalloproteinase domain 10	AF009615.1	ADAM10	3.91
A disintegrin and metalloproteinase domain 15	BC014566.1	ADAM15	2.75
Matrix metalloproteinase 13	X81334.1	MMP13	2.58
Growth differentiation factor 1	M62302.1	GDF1	2.36
Alpha 1 subunit of type XI collagen	NM_080629.1	COL11A1	3.22
Collagen type IX alpha 2 chain	M95610.1	COL9A2	3.28
Neuronal pentraxin I	U61849.1	NPTX1	3.31
Dentin matrix acidic phosphoprotein 1	U89012.1	DMP1	2.38
Creatine kinase (mitochondrial 2, sarcomeric)	BC029140.1	CKMT2	2.22
Mitogen-activated protein kinase kinase kinase 12	U07358.1	MAP3K12	2.15
Alpha 1 subunit of type X collagen	X60382.1	COL10A1	2.55
Alpha 2 subunit of type I collagen	J03464.1	COL1A2	2.04
Alpha 1 subunit of type I collagen	BC036531.1	COL1A1	3.48
Guanine nucleotide binding protein alpha transducing 2	BC000233.1	GNAT2	2.96
Exonuclease 1	AF084974.1	EXO1	2.02
Polymerase delta-interacting protein 1	BC036228.1	PDIP1	2.02
ADP-ribosyltransferase 3	NM_001179.2	ART3	2.52
G protein alpha transducin subunit 1	X63749.1	GNAT1	2.25
WW domain binding protein 2	BC010616.1	WBP2	2.70
Proline-rich protein HaeIII subfamily 1	K03203.1	PRH1	2.68
Epsin 3	NM_017957.1	EPN3	2.14
Interleukin 13 receptor alpha 2	Y08768.1	IL13RA2	2.26
Interleukin 1 receptor-like 1	NM_016232.4	IL1RL1	2.41
Interleukin-1 receptor antagonist homolog 1	AF186094.1	IL1F5	2.45
Interleukin 6 (interferon-beta 2)	BC015511.1	IL6	2.48
Interleukin 1 alpha	BC013142.1	IL1A	3.14
Interleukin 1 beta	BC008678.1	IL1B	2.17
Interleukin 8	Y00787.1	IL8	2.24
SRY (sex determining region Y)-box 9 (SOX9)	BC007951.1	SOX9	2.54
SRY (sex determining region Y)-box 6 (SOX6)	NM_033326.1	SOX6	2.03
Cadherin-8	AB035305.1	CDH8	2.25
Vesicle-associated membrane protein 4	BC005974.1	VAMP4	2.23
Bone morphogenetic protein 2	M22489.1	BMP2	2.03
Bone morphogenetic protein 4	M22490.1	BMP4	2.10
Bone morphogenetic protein 7	X51801.1	BMP7	2.36
Clusterin (apolipoprotein J)	BC010514.1	CLU	2.03

Table 2 continued

Genes	Accession no.	Symbol	Fold-range
Nuclear receptor subfamily 2 group F member 6	BC002669.1	NR2F6	2.31
Insulin like growth factor binding protein 2	BC012769.1	IGFBP2	2.74
Insulin-like growth factor 1	M27544.1	IGF1	2.11
Insulin-like growth factor II receptor	Y00285.1	IGF2R	2.52
Insulin-like growth factor I receptor	X04434.1	IGF1R	2.19
Transforming growth factor beta 2	M19154.1	TGFB2	2.21
Transforming growth factor beta receptor I	L11695.1	TGFBR1	2.37
Homo sapiens transforming growth factor, alpha (TGFA)	NM_003236.1	TGFA	2.51
Connective tissue growth factor	X78947.1	CTGF	4.34
Osteoblast specific factor 2	D13666.1	OSF-2	5.11
BCL2 associated X protein	NM_004324.2	BAX	5.55
Fas-activated serine threonine kinase	BC011770.1	FASTK	2.73
Tumor necrosis factor (ligand) superfamily member 11	AF053712.1	TNFSF11	3.65
Tumor necrosis factor induced protein 6	BC030205.1	TNFAIP6	5.25
Fibroblast growth factor 22	AB021925.1	FGF22	4.43

This list includes genes associated with loss of cells and abnormalities of matrix in discs according to the SOURCE database. Genes are identified according to the encoded protein, gene symbol and GenBank accession number. Numbers represent the fold increase in the genes in which the expression was significantly ($P < 0.05$) up-regulated in degenerated discs as compared with control discs

Table 3 Down-regulated genes associated with loss of cells and abnormalities of matrix in degenerated discs

Genes	Accession no.	Symbol	Fold-range
Annexin A3 (lipocortin III)	BC000871.1	ANXA3	0.38
Aggrecan 1	NM_013227.1	AGC1	0.09
Secretory granule proteoglycan 1 (serglycin, proteoglycan core protein)	BC015516.1	PRG1	0.12
Cathelicidin antimicrobial peptide	U19970.1	CAMP	0.06
Platelet factor 4	M25897.1	PF4	0.24
Ferritin heavy polypeptide 1	NM_002032.1	FTH1	0.44
Membrane protein palmitoylated 1	M64925.1	MPP1	0.27

This list includes genes associated with loss of cells and abnormalities of matrix in discs according to the SOURCE database. Genes are identified according to the encoded protein, gene symbol and GenBank accession number. Numbers represent the fold increase in the genes in which the expression was significantly ($P < 0.05$) up-regulated in degenerated discs as compared with control discs

results of selected genes. The study demonstrated a wide range of differentially expressed genes in degenerated human IVDs.

The current study on IVD tissues showed the expressions of 522 genes changed obviously in the degeneration group, accounting for approximately 2.64% of all analyzed transcripts. These genes can be divided into several categories according to their biological functions, including transcription-related, ion channel and transport protein, receptor, protein synthesis and modifying, growth factor, molecules involved in signal transduction, extracellular matrix-related, DNA synthesis and repair, oncogene-related, growth-related, cytoskeleton and motion, cycle phase-related, G-protein-related, apoptosis-related, interleukin, metallothionein, immune globulin-related, adhesion

molecule, metalloprotease and so on. At the molecular level, degenerative changes in the IVD include decreases in diffusion, cell viability, proteoglycan synthesis, and alteration in collagen distribution, but loss of cells and abnormal matrix are key events in disc degeneration. Based on this, we selected those related to loss of cells and abnormalities of matrix in degenerated discs as subjects for further study. Those included 70 genes related to the control of cell and matrix in disc, of which 63 were deemed to be up-regulated and only 7 down-regulated. These could be grouped into four categories: (1) ECM components: collagen type Ia and IIa, aggrecan; (2) catabolic genes: matrix metalloprotease-3 (MMP-3), inducible nitric oxide synthase (iNOS), and interleukin1 β (IL-1 β); (3) anabolic growth genes: bone morphogenetic protein-2, -7 (BMP-2,

Table 4 Up-regulated genes in the PBMCs from degenerated discs

Gene	Accession no.	Symbol	Fold-range
Protein with high similarity to zinc finger protein 234 (human ZNF234)	NM_024733.1	FLJ14345	2.78
Guanine deaminase (guanine aminohydrolase)	AB033084.1	GDA	2.00
Protein with weak similarity to the myotubularin family of dual specificity protein phosphatases	NM_019061.2	3PAP	3.70
Dual oxidase 1	AF213465.1	DUOX1	2.08
Werner helicase interacting protein	AF218313.1	WHIP	2.25
Retired, was disintegrin and metalloproteinase domain 28	NM_021777.1	ADAM28	2.25
Protein with strong similarity to dispatched homolog 1 (mouse Disp1)	NM_032890.2	DISPA	4.34
Transmembrane gamma-carboxyglutamic acid protein 4	BC010052.1	TMG4	3.28
Immunoglobulin lambda-like polypeptide 1	BC012293.1	IGLL1	3.91
Protein with high similarity to immunoglobulin heavy chain constant region alpha 1 (human IGHA1)	BC032249.1	MGC27165	2.75
Interleukin 1 beta	BC008678.1	IL1B	2.07
Retired, was protein with high similarity to immunoglobulin gamma 2b (mouse Igh-1)	CAA40950.1(GenPept)		2.36
Myomesin (M-protein) 2 (165kD)	X69089.1	MYOM2	3.22
Protein with high similarity to mouse Igk-V8	BC030813.1		3.28
Retired, was protein with high similarity to immunoglobulin lambda (mouse 1810027O01Rik)	CAA40950.1(GenPept)		3.31
Protein of unknown function, has strong similarity to uncharacterized mouse AF064782	NM_032042.2	DKFZP564D172	2.38
Homo sapiens hypothetical protein MGC26568 (MGC26568)	NM_152402.1	TRAM1L1	2.22
Homo sapiens leucine-rich repeat LGI family, member 4 (LGI4)	NM_139284.1	LGI4	2.15
Chemokine (C-C motif) receptor 4	X85740.1	CCR4	2.55
Bone morphogenetic protein receptor (type IA)	BC028383.1	BMPR1A	2.04
Protein with high similarity to odorant receptor S25 (mouse Ors25)	AB065837.1		3.48
Member of the keratin B2 high sulfur family	AJ406936.1	KRTAP4-4	2.78
Protein with high similarity to cardiac myosin heavy chain (mouse Myhca)	AB023217.2		2.00
Cyclin B1, regulatory subunit of the CCNB1	BC006510.1	CCNB1	3.70
Rad50-interacting protein 1	NM_021930.2	FLJ11785	2.08
Zinc finger protein 195	NM_007152.1	ZNF195	2.25
Zinc finger protein 25	NM_016220.1	ZFD25	2.25
RAB-like protein 2B	NM_007081.1	RABL2B	4.34
Hepatitis B virus x associated protein	AF227948.2	HBXAP	3.28
Hemoglobin gamma G	NM_000184.2	HBG2	3.91
SPARC-like 1 (hevin)	BC033721.1	SPARCL1	2.75
Suppressor of var1 S	BC036112.1	SUPV3L1	3.22
Protein containing five C2H2 type zinc finger domains	AL832801.1		3.28
Neuroblastoma-related (N-myc) oncogene	BC002712.1	MYCN	2.03
Kappa casein	BC010935.1	CSN3	5.14
Homo sapiens chromosome 14 open reading frame 79	NM_174891.1	C14orf79	2.51
Annexin A3 (lipocortin III)	BC000871.1	ANXA3	2.38
Cathelicidin antimicrobial peptide	U19970.1	CAMP	2.18
Protein of unknown function	NM_152288.1	MGC13024	2.16

This list includes genes in discs according to the SOURCE database. Genes are identified according to the encoded protein, gene symbol and GenBank accession number. Numbers represent the fold increase in the genes in which the expression was significantly ($P < 0.05$) up-regulated in degenerated discs as compared with control discs

Table 5 Down-regulated genes in the PBMCs from degenerated discs

Genes	Accession no.	Symbol	Fold-range
Bcl-2-associated transcription factor	AF249273.1	BTF	0.29
Frequently rearranged in advanced T-cell lymphomas 2	NM_012083.1	FRAT2	0.14
Melanoma antigen family A 6	BC041599.1	MAGEA6	0.37
Bcl2 associated athanogene 1	NM_004323.2	BAG1	0.37
Myeloblastosis oncogene-like 1	X13294.1	MYBL1	0.27
CD79A antigen (immunoglobulin-associated alpha)	M80462.1	CD79A	0.26
Chaperonin containing T-complex 1 subunit 4 (delta)	AF026291.1	CCT4	0.25
Ribosomal protein S17	BC022370.1	RPS17	0.30
Tumor necrosis factor (ligand) superfamily member 11	AF053712.1	TNFSF11	0.34
Fibroblast growth factor 22	AB021925.1	FGF22	0.16
Exonuclease 1	AF084974.1	EXO1	0.33
Matrix metalloproteinase 19	U37791.1	MMP19	0.10
A disintegrin and metalloproteinase domain 10	AF009615.1	ADAM10	0.30
Osteoblast specific factor 2	D13666.1	OSF-2	0.14
Tumor necrosis factor receptor superfamily member 9	U03397.1	TNFRSF9	0.32
Matrix metalloproteinase 10 (stromelysin 2)	BC002591.1	MMP10	0.39
A disintegrin and metalloproteinase domain 17	U69611.1	ADAM17	0.32
Fibroblast growth factor 4	NM_002007.1	FGF4	0.38
Homo sapiens insulin-like growth factor 2	NM_000612.2	IGF2	0.30
Histocompatibility class II DM alpha	BC011447.1	HLA-DMA	0.35
Homo sapiens interferon regulatory factor 5 (IRF5)	NM_032643.2	IRF5	0.16
TERF1 (TRF1)-interacting nuclear factor 2	AF195512.1	TINF2	0.30
Mitogen-activated protein kinase kinase kinase kinase 4	NM_145686.1	MAP4K4	0.36
Human leukocyte antigen-C	NM_002117.3	HLA-C	0.15

This list includes genes in discs according to the SOURCE database. Genes are identified according to the encoded protein, gene symbol and GenBank accession number. Numbers represent the fold increase in the genes in which the expression was significantly ($P < 0.05$) down-regulated in degenerated discs as compared with control discs

Table 6 Non-compatible genes of the differential expression of genes between disc tissue and PBMCs

Accession no.	Symbol	PBMCs (Fold-range)	Disc tissue (Fold-range)
AF053712.1	TNFSF11	0.34	3.65
AB021925.1	FGF22	0.16	4.43
AF084974.1	EXO1	0.33	2.02
U37791.1	MMP19	0.11	3.71
AF009615.1	ADAM10	0.31	3.91
U89012.1	DMP1	0.29	2.38
D13666.1	OSF-2	0.14	5.11
BC000871.1	ANXA3	2.38	0.38

This list includes genes in discs according to the SOURCE database. Genes are identified according to the encoded protein, gene symbol and GenBank accession number. Numbers represent the fold increase in the genes in which the expression was significantly ($P < 0.05$) down-regulated in degenerated discs as compared with control discs

BMP-7), transforming growth factor (TGF- β 1), and insulin-like growth factors-1(IGF-1); and (4) anti-catabolic gene: tissue inhibitor of metalloprotease-1 (TIMP-1).

We were surprised that not only catabolic but also some anabolic or anti-catabolic genes have higher expressions in the IVDs than in normal discs, for example MMP-3 and TIMP-1. TIMPs are highly specific for MMPs and form noncovalent complexes, blocking the access of their substrates to the MMP catalytic site [17]. TIMP-1, an endogenous inhibitor of matrix metalloproteinases which degrades the extracellular matrix of the IVD [18], inactivates MMP-3 by binding MMP-3 specifically in the molar ratio of 1:1. An imbalance in the ratio of MMP-3 to its inhibitor, TIMP-1, is observed in degenerating articular cartilage [19] and degenerating disc tissue [20]. In the past, investigators have suggested that an imbalance between the levels of activity of MMPs and TIMP was responsible for an increased degradation of cartilage in osteoarthritis [21]. An imbalance between degradation enzymes and their inhibitors could be one of the key mechanisms for human disc degeneration. In normal discs they keep a balance, but if extrinsic factors such as physical loading persist, the balance can be broken so that catabolic genes are predominant compared to the anabolic or anti-catabolic genes.

In order to keep the homeostasis of the IVDs microenvironment, the synthesis of the inhibitor of catabolic enzymes would increase and then would be accompanied by the up-regulation of other gene expressions.

The current study on PMBCs showed the expressions of 62 genes changed obviously in the patients of the degeneration group. These genes were divided into groups, according to their biological functions, of ion channel, transport protein, transcription-related, DNA synthesis and repair, metalloprotease, immune globulin-related, growth factor-related, extracellular matrix-related, adhesion molecule, etc. Our results identified several groups of up-regulated genes in the patients with degeneration of IVD. One group has genes which are related to transcription, consisting of FLJ14345, C21orf81 and SUPV3L1. Another group consists of IGLL1, IGHA1, IGKV1D-39 and MYOM2. IGLL1 is critical for B-cell development, IGHA1 may serve both to defend against local infection and to prevent access of foreign antigens to the general immunologic system and IGKV1D-39 is related to antigen binding. Another group comprises CCR4, BMPRI1A. CCR4 is a receptor for chemokine and BMPRI1A is a receptor for BMP. Human NK cells express CCR4 and respond to thymus and activation-regulated chemokine, macrophage-derived chemokine. In juvenile arthritis, genes involved in regulation of immune cell functions, receptor signaling, as well as protein metabolism and degradation were up-regulated [22]. And in rheumatoid arthritis, genes of IL-8 and IL-10 were up-regulated, while COX2 gene was down-regulated [23]. In arthritis patients, the following genes were more highly expressed: macrophage differentiation marker MND1, MRP8 and MRP14; signaling molecules JAK3 and MAP kinase p38; receptors TNFR2/p75, CCR1, CXCR4 and integrin beta1; and the cytokines/chemokines interleukin (IL) 1beta and IL-8 [32]. Heat shock 90 kDa protein 1, alpha; inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein; interleukin 13 receptor alpha 1; laminin, gamma 1; C-X-C motif ligand 4 and tumor necrosis factor alpha-induced protein 6 were significantly down-regulated in mild osteoarthritis [24]. Thus, there is relatively little overlap between the gene expression changes observed in these studies and in our study. The differences suggest the mechanism of degeneration of IVD may be different from the other cartilage-related diseases.

After comparing gene expression profiles in the blood with those in IVDs, we found that some genes were incompatible. For example, TNFRSF11B was up-regulated by 3.2847 in the disc, but down-regulated by 0.3236 in the blood. The changes of gene expressions from degeneration of human IVD mainly appear in disc tissue but not in other tissues, such as blood. It seems that degeneration of IVD could be only a local pathogenesis. It was convincing that

extrinsic factors such as physical loading may be the main suspected risk factor contribution to the initiation of disc degeneration, and that heredity may play a subordinate role in disc degeneration. Excessive compressive stress induced cellular apoptosis in the disc and the loss of the extracellular matrix including proteoglycans (PGs) and collagen [25, 26].

In summary, the occurrence of IDD is a complicated dynamic process. We verified that differences in gene expressions existed in the human IDD in our experiment. At the same time, we found that the expression of some genes in the tissue and blood were incompatible. Our results contribute to a better understanding of the complex molecular mechanisms underlying the degeneration of the human IVDs and to opening new avenues for the identification of a number of genes and pathways potentially relevant in the pathogenesis of this disease.

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