

# Molecular basis of degenerative spinal disorders from a proteomic perspective (Review)

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**Abstract.** Intervertebral disc degeneration (IDD) and ligamentum flavum hypertrophy (LFH) are major causes of degenerative spinal disorders. Comparative and proteomic analysis was used to identify differentially expressed proteins (DEPs) in IDD and LFH discs compared with normal discs.

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**Abbreviations:** ADORA3, adenosine receptor A3; AF, annulus fibrosus; AGE-RAGE, advanced glycation endproducts-receptor for advanced glycation endproducts; APO, apolipoprotein; CHAD, chondroadherin; COL1, type I collagen; COL2, type II collagen; COL3, type III collagen; COL2A1,  $\alpha$ -1 type II collagen; COMP, cartilage oligomeric matrix protein; CSF, cerebrospinal fluid; DEP, differentially expressed protein; FAR1, fatty acyl-CoA reductase 1; FN1, fibronectin 1; G6PD, glucose-6-phosphate 1-dehydrogenase; GAG, glycosaminoglycan; GNAI2, guanine nucleotide-binding protein G(i) subunit  $\alpha$ -2; HSPA8, heat shock cognate 71-kDa protein; HTRA1, serine protease HTRA1; IDD, intervertebral disc degeneration; IVD, intervertebral disc; KCND3, potassium voltage-gated channel subfamily D member 3; KEGG, Kyoto Encyclopedia of Genes and Genomes; LBP, lower back pain; LDH, lumbar disk herniation; LF, ligamentum flavum; LFH, ligamentum flavum hypertrophy; LPA, lysophosphatidic acid; LPPR2, lipid phosphate phosphatase-related protein 2; LSS, lumbar spinal stenosis; MSC, mesenchymal stem cell; NC, notochordal cell; NP, nucleus pulposus; OA, osteoarthritis; PG, proteoglycan; SLRP, small leucine-rich proteoglycan; SOD, superoxide dismutase; SOX9, SRY-related protein 9; TGF- $\beta$ , transforming growth factor- $\beta$ ; TMEM51, transmembrane protein 51; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; UBAC1, ubiquitin-associated domain-containing protein 1

**Key words:** degenerative spinal disorders, intervertebral disc degeneration, ligamentum flavum hypertrophy, gene ontology term enrichment analysis, Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis

Subsequent gene ontology term enrichment analysis and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis of the DEPs in human IDD discs or LFH samples were performed to identify the biological processes and signaling pathways involved in IDD and LFH. The PI3K-AKT signaling pathway, advanced glycation endproducts-receptor for advanced glycation endproducts signaling pathway, p53 signaling pathway, and transforming growth factor- $\beta$  signaling pathway were activated in disc degeneration. This review summarizes the recently identified DEPs, including prolargin, fibronectin 1, cartilage intermediate layer protein, cartilage oligomeric matrix protein, and collagen types I, II and IV, and their pathophysiological roles in degenerative spinal disorders, and may provide a deeper understanding of the pathological processes of human generative spinal disorders. The present review aimed to summarize significantly changed proteins in degenerative spinal disorders and provide a deeper understanding to prevent these diseases.

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## 1. Introduction

Lower back pain (LBP) and other clinical symptoms occur as a result of degenerative spinal disorders, which includes disc degeneration, facet joint degeneration, and adjacent segment disease (1). Intervertebral disc degeneration (IDD)

and ligamentum flavum hypertrophy (LFH) are most common degenerative spinal disorders (2,3). Intervertebral discs (IVDs) are elastic joint tissues between vertebral bodies that bear the load of daily activities and are more susceptible to degeneration because of the upright posture of humans (4). IDD is a major cause of LBP and sciatica, with the severity of LBP depending on the pathological grading of IDD because the pathology depresses the normal function of IVDs (5,6). LFH and IDD are exacerbated by increased cellular apoptosis and senescence, and the upregulation of pro-inflammatory cytokines and proteins, which results from the turnover of the matrix in IVDs and the ligamentum flavum (LF) (7,8). Furthermore, aging (especially over the age of 50) (2) and several environmental factors (such as oxygen, mechanical stress and osmotic pressure) have been reported to trigger the onset and progression of IDD (9). Nevertheless, the pathophysiological mechanisms of IDD and LFH remain poorly understood.

A number of high-throughput proteomic analysis techniques, such as isobaric tags for relative and absolute quantification (iTRAQ), have been used to profile the proteomic map of the annulus fibrosus (AF) and nucleus pulposus (NP; Table I). NP is a gel-like tissue which is surrounded by AF, a layered cartilaginous structure. The present review summarizes the results of quantitative proteomic studies of the LF, AF, and NP, as well as body fluids, including cerebrospinal fluid (CSF) and serum from patients with degenerative spinal disorders. The aim of this review was to identify the crucial proteins mediating the onset and progression of IDD and LFH, which may facilitate the development of novel potential therapies for these disorders.

## 2. Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis

**Methods.** To conduct KEGG pathway analysis, 54 differentially expressed proteins (DEPs) from LF tissue, 15 DEPs from the AF (soluble, the supernatant of samples), 10 DEPs from AF (insoluble, the lyophilized pellet of samples), 21 DEPs from NP (soluble), and 7 DEPs from NP (insoluble) were selected based on previous proteomic studies (Table SI) (10,11); patient information is provided in Table SII. Briefly, for the LF, the DEPs were selected according to the protein expression ratio between lumbar spinal stenosis (LSS) and the control (individuals with disc herniation; protein expression ratio=LSS/control,  $\geq 2$  or  $\leq 0.5$ ) (11). For the AF and NP, genes which encoded DEPs that were significantly increased or decreased in the degenerative samples compared with control samples were selected ( $P < 0.05$ ) (10).

The KEGG pathway analysis database (<http://www.genome.jp/kegg>) was used to identify signaling pathways enriched by genes which encoded DEPs and the KEGG Orthology-Based Annotation System (KOBAS) version 3.0 (<http://kobas.cbi.pku.edu.cn>) was used to investigate gene/protein functional annotation and gene set enrichment (12-14). The module of KOBAS called 'Gene List Enrichment', based on the first gene set enrichment method, overrepresentation analysis, was used for KEGG pathway analysis. Statistical analysis was performed using a hypergeometric test and Fisher's exact test.  $P < 0.05$  was considered to indicate a statistically significant difference.

**Results.** KEGG pathway analysis indicated that multiple pathways were involved in LFH and the degeneration of AF and NP. Some were the same in LF, AF and NP, such as ECM-receptor interaction and focal adhesion. The total enrichment pathways are presented in Table SIII. The top 10 KEGG pathways enriched with DEPs, including protein digestion and absorption, ECM-receptor interaction and focal adhesion, are presented in Figs. 1B and 2. The p53 signaling pathway, advanced glycation endproduct-receptor for advanced glycation endproducts signaling pathway (AGE-RAGE), PI3K/AKT signaling pathway, and transforming growth factor (TGF)- $\beta$  signaling pathway were enriched by these DEPs (Table II). These four signaling pathways were not in the top 10 pathways in LF, but they were still enriched by DEPs (Table SIII). This review combines past proteomic analyses with present analysis to provide a deeper understanding of the molecular mechanisms of degenerative diseases of the spine.

## 3. Proteomic analysis of the human LF

**Structural proteomic analysis of the LF.** Hypertrophy and ossification of the LF are major causes of LSS (15,16). However, the proteins involved in hypertrophy of the LF remain unknown. To clarify the molecular events during LSS disease progression and to identify targets for treatment, LF proteomic analysis was employed using 2-dimensional image converted analysis of liquid chromatography (2DICAL)-based label-free proteomics. A set of small leucine-rich proteoglycans (SLRPs), including asporin, decorin, and fibromodulin were identified, in addition to the large proteoglycans (PGs), versican and aggrecan (11). These protein components suggest that the LF structure shares common features with other elastic tissues and that within normal physiology the LF is more elastic compared to other ligaments and tendons (17). This is demonstrated through the increased presence of fibulins (FBLN 1/2/3/5), elastin, and microfibril-associated protein 4 (10). Redox proteins present in the LF, including lysyl oxidase homolog 1 and superoxide dismutase (SOD) 3, are considered to be involved in the formation and regulation of these elastic fibers (18,19). The proteins identified in the human LF were classified into 24 cellular components by gene ontology (GO) term enrichment analysis (Fig. 1A) (11).

**Comparative proteomic analysis of the LF.** A number of proteins were identified through Selective Reaction Monitoring/Multi Reaction Monitoring in LFH samples (20) (Table I). Notably, vasculature is thought to be implicated in LFH owing to the significantly upregulated expression of plasma proteins, including fibrinogen, apolipoproteins (APOs), and transthyretin. Chondrometaplasia is also observed in degenerated LF (20); with multiple proteins involved in chondrometaplasia consistently upregulated in LFH, including chondroadherin (CHAD), prolargin, cartilage intermediate layer proteins, and aggrecan (21). These proteins have been reported to be associated with the ossification of the LF (21).

CHAD is a leucine-rich repeat (LRR) protein that is highly expressed in cartilaginous tissues (22). LRR proteins are involved in promoting interactions with other extracellular matrix (ECM) molecules and collagen fibrillogenesis (23), and are often regulated by TGF- $\beta$  (24). In addition, prolargin

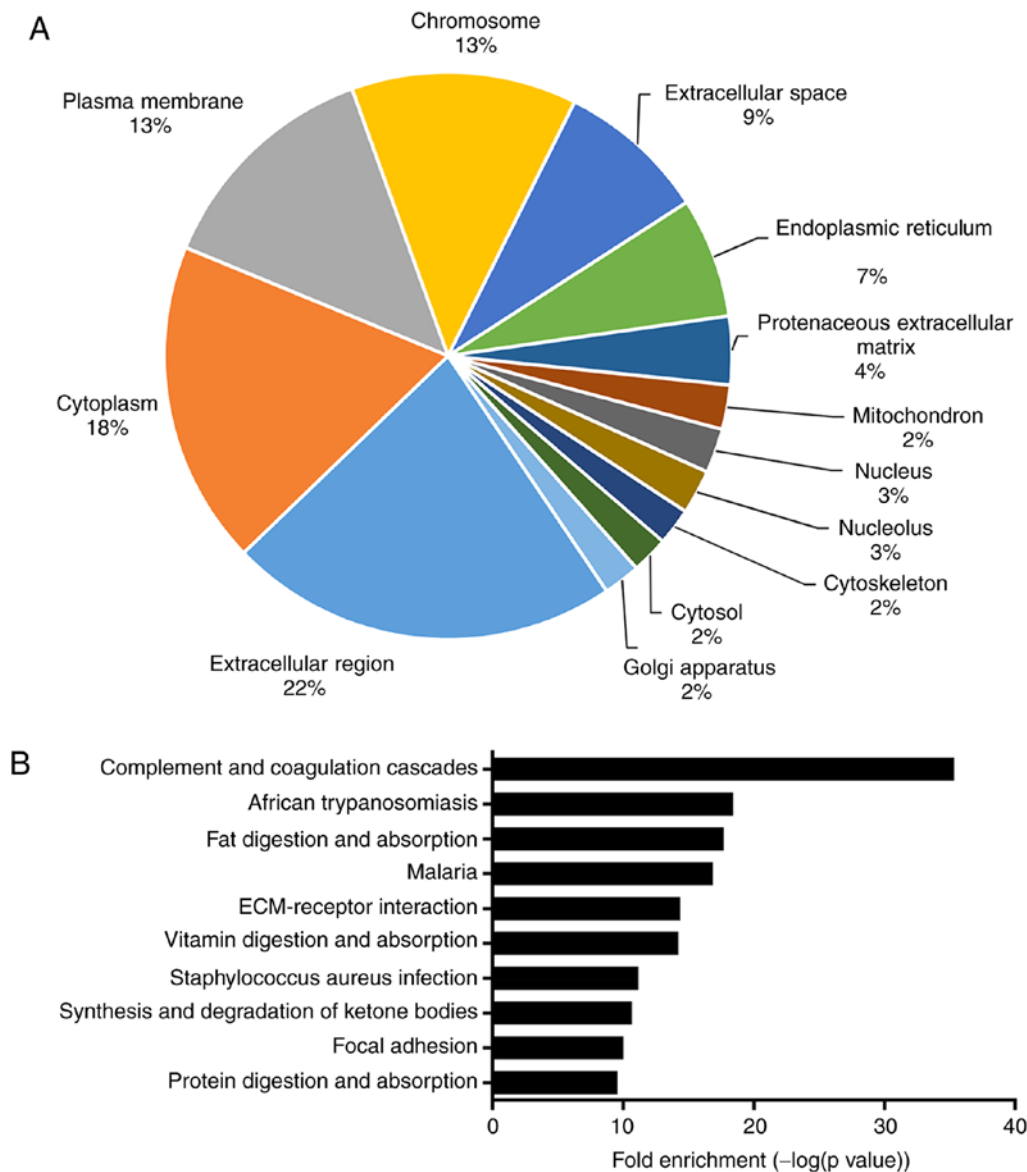


Figure 1. Proteomic characterization of the human ligamentum flavum in lumbar spinal stenosis. (A) Gene Ontology term enrichment analysis of unique proteins. (B) Top 10 Kyoto Encyclopedia of Genes and Genomes pathways enriched by differentially expressed proteins (54 proteins). ECM, extracellular matrix.

and SLRPs isolated from mice lacking decorin are reportedly involved in collagen fiber assembly and fibril abnormalities (25). Thus, the interactions between molecules in the ECM and collagen fibrillogenesis may directly influence the structure of the LF, and may be involved in the process of degeneration.

*In vitro* studies have demonstrated that cartilage intermediate layer protein (CILP) modulates TGF- $\beta$  signaling (26), and TGF- $\beta$  has been detected in the early stages of degenerative hypertrophy of the LF (27). Furthermore, the expression of lysophosphatidic acid (LPA), and its receptor, LPA receptor 1 (LPAR1), are significantly upregulated in samples isolated from LFH specimens (11). Previous studies from Japan and Finland have reported that LPA is closely related to the process of IDD (26,28), and that upon LPA interacting with LPAR1, the protein can promote LF cell proliferation and further induce LFH, through the LPAR1/AKT signaling pathway (29).

The expression levels of fibronectin 1 (FN1), tenascin, and serine protease HTRA1 (HTRA1) are positively correlated

with LFH, whereas asporin expression is negatively correlated in LFH (11). The level of peptides derived from FN1 is influenced by HTRA1, and the HTRA1 mutation causes diseases such as cerebral autosomal recessive arteriopathy and leukoencephalopathy (30). In addition, HTRA1 upregulation is observed in many degenerative disorders, including age-related macular degeneration, osteoarthritis (OA), and lumbar disc degeneration (31-33), with a previous study reporting that FNS is regulated by HTRA1 in joints affected by OA (34).

#### 4. Proteomic analysis of the human AF

*Structural proteomic analysis of the AF.* The spine resists multi-directional loading from the radial, axial, and circumferential directions, and the upright posture of humans imposes greater mechanical loading and accelerates the process of IDD (35). IDD pathological features are accompanied by NP fibrosis, AF fissuring, and protein structure disorganization (36). Type II

collagen (COL2), chondroitin sulfate, and PGs are produced by AF cells (37), and in healthy IVDs, the AF contains 65–70% water; with the dry weight composed of 20% PGs, ~60% collagen, and 2% elastin (38). Type I collagen (COL1) extracted from AF cells is also significantly upregulated compared to COL1 extracted from NP cells, but the level of chondroitin-6-sulfated PGs demonstrates the opposite pattern (39). In IDD, changes in the level of PGs can be detected. In the early stages, AF cells proliferate with increasing biosynthetic processes, whereas in degenerated IVDs, the level of aggrecan is decreased, and the levels of decorin, biglycan, and fibromodulin (which are small PGs) are upregulated in AF cells (40). Tenomodulin levels were also reported to be increased in degenerated AF cells (41). Other genes that are correlated with AF cells in degenerated IVDs have been identified, including the gene encoding pleiotrophin, which increases in the AF with age (42); increased vascularization in the degenerated AF tissues may also be present (43). The proteome of a normal IVD from a 35-year old patient (male) has been established (10), and the cellular components from the AF (soluble and insoluble) determined by GO term enrichment analysis are presented in Fig. 3A and B. Compared with the AF (soluble), ECM proteins are present at a higher proportion in the AF (insoluble), and nuclear proteins only exist in the AF (soluble).

*Comparative proteomic analysis of the AF.* A previous study has identified a total of 759 proteins in non-degenerative AF tissue (44). DEPs of importance in the degenerated AF include the ubiquitin-associated domain-containing protein 1 (UBAC1), aspartyl transfer RNA synthetase, potassium voltage-gated channel subfamily D member 3 (KCND3), structural proteins, and signaling factors such as Indian hedgehog protein (44) (Table I). UBAC1 serves a prominent role in lysosomal and proteasomal degeneration (45,46), and KCND3, a voltage-activated A-type potassium ion channel, is involved in degenerative diseases such as spinocerebellar ataxia (47).

Semi-quantitative analysis of silver-stained 2D electrophoresis gels of AF cells isolated from normal and degenerated IVDs has demonstrated that the expression levels of glucose-6-phosphate 1-dehydrogenase (G6PD), heat shock cognate 71-kDa protein (HSPA8), and protocadherin-23 are decreased, whereas SOD, transmembrane protein 51 (TMEM51), guanine nucleotide-binding protein G(i) subunit  $\alpha$ -2 (GNAI2), 26S protease regulatory subunit 8, adenosine A3 receptor (ADORA3), fatty acyl-CoA reductase 1 (FAR1), and lipid phosphate phosphatase-related protein type 2 (LPPR2) expression levels are increased (48).

HSPA8 is a heat shock cognate protein that represses pre-mRNA splicing and forms an essential part of the spliceosome, where it is thought to be involved in spliceosome assembly (49). Thus, the low expression of HSPA8 suggests that the reduced repression of pre-mRNA splicing is a potential regulatory mechanism in the degenerated AF. G6PD is a member of the dehydrogenase family that provides pentose phosphates and serves as a reductant in fatty acid and nucleic acid synthesis (50). A significant decrease in G6PD expression in the AF suggests an important role for oxidative stress in the process of degeneration. These findings suggest that oxidative stress may be a major contributor to the process of degeneration (51). The main function of protocadherin-23 is to provide adhesion and it is involved in morphogenesis during development (52). However, the correlation

between protocadherin-23 and IDD remains to be elucidated. GNAI2 may serve as a signal transducer or modulator in multiple types of transmembrane signaling systems, as the  $\alpha$ -subunit GTP activating protein, GNAI2 is a regulator for the effector interaction (53). ADORA3 is an adenosine receptor and serve a role in duplication (54). Both GNAI2 and ADORA3 are associated with G-proteins (55), thus, G-proteins of the transmembrane signaling systems may be involved in the process of AF degeneration. FAR1 reduces saturated fatty acids into fatty alcohols (56). In addition, fatty alcohols accumulated in specific cell lines defective in plasmalogen biosynthesis (56). Thus, the upregulation of FAR1 suggests that plasmalogen biosynthesis may be involved in the AF degenerative process. The function of LPPR2 is similar to that of other lipid phosphate phosphatase superfamily members, which serve roles in signal transduction and extracellular concentrations of lipid phosphate esters (57); TMEM51 is a member of the multipass membrane proteins (58). However, the roles of LPPR2 and TMEM51 in AF cells are unknown, but the data suggest that these proteins may influence the process of AF cell degeneration.

## 5. Proteomic analysis of the human NP

*Structural proteomic analysis of the NP.* NP cells are commonly described as ‘chondrocyte-like’ or ‘stem cell-like’ owing to their morphology and the cell markers that they synthesize; the main constituents of the NP are PGs, collagen, elastin and water (38,59). The PGs absorb the water in the NP, and account for 35–65% of the dry weight (60), whereas COL2 fibrils form an incompact frame structure that holds the NP tissues together. A previous study demonstrated that NP water levels decrease with age, and a similar decrease may occur in PGs (38), which would decrease the size of the NP by ~50%. Although the morphology of the NP is closer to a solid form than a fluid structure due to the dehydration (61), the proteome of the NP (soluble and insoluble) is similar to the AF (Fig. 3C and D). Nevertheless, PGs are more abundant in the NP (soluble and insoluble) compared with the AF (10).

*Comparative proteomic analysis of the NP.* Similar to the LF, cartilage oligomeric matrix protein (COMP), prolargin, FN1, and clusterin expression were upregulated in both soluble and insoluble fractions of the NP from IDD specimens (10) (Table I). Furthermore, the expression of SLRPs, such as biglycan and decorin, and extracellular SOD, were increased in the soluble fraction (10). However, Erwin *et al* reported that SLRPs were intact in both chondrodystrophic canines that developed early disc degeneration and non-chondrodystrophic animals (62). As previously discussed, COL2 and CHAD are closely associated with collagen fibrillogenesis and are observed to be down-regulated with age (11), thus indicating that substantial matrix remodeling is involved in NP degeneration. The majority of abnormal changes in proteins are similar to those observed in the LF, except for COL1. COL1, the major component of the insoluble fraction of the degenerative NP, was present in increased amounts (10). It has been reported that COL1 is capable of achieving cross-linking, mediated by enzymatic or non-enzymatic processes (10), which is consistent with the increasing trends in the insoluble fraction of the degenerative NP. These data suggested that COL1 may be a major contributor to reducing protein solubility in degenerative discs.

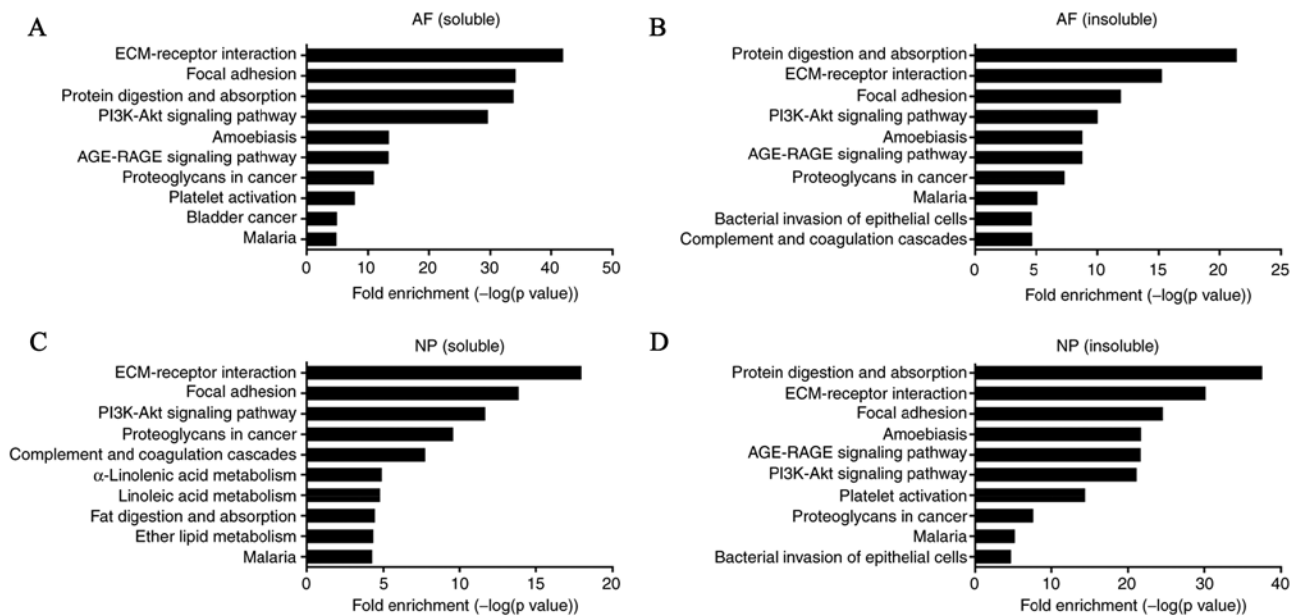


Figure 2. Top 10 KEGG pathways enriched by DEPs identified in (A) AF soluble (15 proteins), (B) AF insoluble (10 proteins), (C) NP soluble (21 proteins) and (D) NP insoluble (7 proteins) fractions. ECM, extracellular matrix; AGE-RAGE, advanced glycation endproducts-receptor for advanced glycation endproducts; AF, annulus fibrosus; NP, nucleus pulposus.

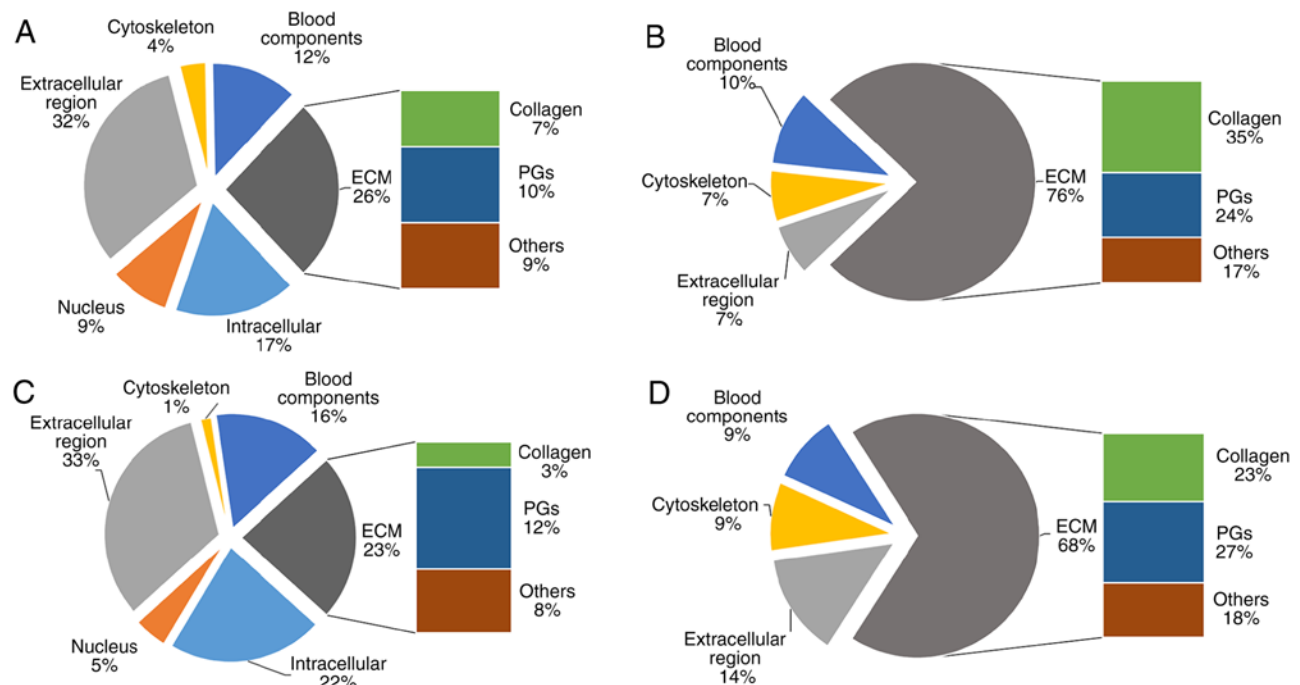


Figure 3. Structural proteomic analysis of a normal intervertebral disc from a 35-year old sample determined by GO analysis. (A-D) GO term enrichment analysis of the (A) 71 unique cellular proteins detected in the AF (soluble), (B) 24 unique proteins detected in the AF (insoluble), (C) 64 unique proteins detected in the NP (soluble), and (D) 18 unique proteins detected in the NP (insoluble). GO, gene ontology; AF, annulus fibrosus; NP, nucleus pulposus, PGs, proteoglycans; ECM, extracellular matrix.

The function of local or migratory cells in IVD is not completely understood. Nevertheless, self-repair induced by local or migratory cells has been observed in dogs with IDD induced by enzymatic digestion (63). A number of studies have transplanted bone marrow-derived mesenchymal stromal cells (BM-MSCs) and stem-like or progenitor-like cells in IDD models (64,65). The transplantation of these cells activates a set

of native, uncharacterized cells, which express both  $\alpha$ -1 COL2 (COL2A1) and SRY-related protein 9 (SOX9) (66), which postponed the onset of IDD in humans and sheep (64,65). However, this evidence is not sufficiently robust to support cellular transplantation as a clinical therapy. In fact, another study reported that there was no clinically significant difference between MSC and sham treatment for IDD, regardless

Table I. Significantly differentially expressed proteins in degenerative disc disease.

Author, year	Sample source	Increased protein expression	Decreased protein expression	(Refs.)
Kamita <i>et al</i> , 2015	Ligamentum flavum	Chondroadherin, cartilage intermediate layer protein, lysophosphatidic acid receptor 1, SLRPs, prolargin, FN1, HTRA serine peptidase 1, tenascin	Asporin	(11)
Johnson <i>et al</i> , 2006	Annulus fibrosus	Ubiquitin-associated domain-containing protein 1, potassium voltage-gated channel subfamily D member 3	NA	(39)
Battié <i>et al</i> , 2008		FN1, clusterin, aggrecan, decorin, prolargin,	COL2, type XI collagen, COL1, COL6	(9)
Yee <i>et al</i> , 2016		Guanine nucleotide-binding protein G(i) subunit $\alpha$ -2, transmembrane protein 51, adenosine receptor A3, FAR1, lipid phosphate phosphatase-related protein type 2	Heat shock cognate 71-kDA protein, glucose-6-phosphate dehydrogenase, protocadherin-23	(10)
Battié <i>et al</i> , 2008	Nucleus pulposus	Prolargin, FN1, COMP, clusterin, SLRPs	COL1	(9)
Honsho <i>et al</i> , 2010	Notochordal cell conditioned medium	Laminin B1, glycosaminoglycan, SOX9, COL2, transforming growth factor $\beta$ 3	Type III collagen	(56)
Elliott and Setton, 2001	Murine intervertebral discs	FN1, prolargin, COMP, COL6, type XII collagen, type XV collagen, SOX9, COL2	NA	(61)
Markolf and Morris, 1974	Cerebrospinal fluid	APO A-IV, vitamin D-binding protein, neurofilament triplet L protein, tetranectin, hemoglobin, immunoglobulin G	ProSAAS, prostaglandin D2 synthase, creatine kinase B, superoxide dismutase 1, peroxiredoxin 2	(63)
Yang <i>et al</i> , 2009	Serum	APO L1	apolipoprotein M, tetranectin	(66)

APO, apolipoprotein; COL1, type I collagen; COL2, type II collagen; COL6, type VI collagen; COMP, cartilage oligomeric matrix protein; FN1, fibronectin 1; NA, not applicable; SLRP, small leucine-rich proteoglycan; SOX9, SRY-related protein 9; ProSAAS, proprotein convertase subtilisin/kexin type 1 inhibitor.

of allogeneic or autologous transplantation methods (67). Therefore, the development of progenitor cell therapies and the identification of specific biomarkers will require a deeper understanding of progenitor cells.

Notochordal cells (NCs), which are potential progenitor cells, can induce the differentiation of MSCs to NP cells by synthesizing PGs and resisting the expression and hypertrophy of collagen fiber; this is noted through the increased production of glycosaminoglycans (GAG), laminin B1, and type III collagen (COL3) observed in human MSCs cultured in porcine notochordal conditioned medium (68). Furthermore, following MSC transplantation in animals, NCs in the native tissue promoted the upregulation expression of SOX9, COL2,

and transforming growth factor  $\beta$ 3 (TGF- $\beta$ 3), which are also detected in healthy NP (69-71). These data suggest that laminin B1, GAG, COL3, SOX9, and TGF- $\beta$ 3 may serve vital roles in the transformation of MSCs into NP cells (72).

## 6. Comparative proteomic analysis of IDD model mice

Previous studies have established SM/J and LG/J mouse models of IDD. The former display cellular and matrix changes in IVDs similar to those in degenerative human IVDs, whereas the latter maintain abundant vacuolated NC-like cells in the NP (72). FN1, Prolargin, and COMP upregulated in SM/J mice, which is consistent with observations in degenerative human

Table II. Differentially expressed proteins enriched in the PI3K/AKT, AGE-RAGE, p53 and TGF- $\beta$  signaling pathways.

Tissue	Enriched signaling pathway	Increased protein expression	Decrease protein expression
LF	PI3K/AKT	Type VI collagen, FN1, COMP, chondroadherin	COL1A1, COL1A2
	AGE-RAGE	FN1	COL1A1, COL1A2
	p53	Insulin-like growth factor-binding protein	NA
AF (soluble)	PI3K-AKT	THBS1, FN1	COL1A2, COL2A1, COL1A1, $\alpha$ -2 type VI collagen, $\alpha$ -3 type VI collagen
	AGE-RAGE	FN1	COL1A2, COL1A1
	p53	THBS1	NA
	TGF- $\beta$	THBS1	NA
AF (insoluble)	PI3K/AKT	COMP, FN1	COL2A1
	AGE-RAGE	A-1 type III collagen, FN1	NA
	TGF- $\beta$	DCN	NA
NP (soluble)	PI3K/AKT	COMP, FN1	Chondroadherin, COL2A1
	AGE-RAGE	FN1	NA
	TGF- $\beta$	DCN	NA
NP (insoluble)	PI3K/AKT	COL1A2, FN1, COMP, COL1A1	COL2A1
	AGE-RAGE	COL1A2, FN1, COL1A1	COL2A1

AGE-RAGE, advanced glycation end products-receptor for advanced glycation endproducts; AF, annulus fibrosus; THBS1, thrombospondin 1; COL1A1,  $\alpha$ -1 type I collagen; COL1A2,  $\alpha$ -2 type I collagen; COL2A1,  $\alpha$ -1 type II collagen; COMP, cartilage oligomeric matrix protein; FN1, fibronectin 1; LF, ligamentum flavum; NA, not applicable; NP, nucleus pulposus; TGF- $\beta$ , transforming growth factor  $\beta$ ; DCN, decorin.

IVDs (73). In addition, the upregulation of collagen, such as  $\alpha$ -1 type VI and type V collagen expression, is observed in SM/J mice (73). These changes indicate ECM enrichment in SM/J mice, with processes such as chondrogenic differentiation and fibrillogenesis likely to be taking place. Notably, chondrocyte markers such as SOX9 and COL2A1 are detected at the edge of the NP region, close to the AF, which has been observed in other mouse strains (74). Thus, chondrocyte markers may serve an important role in the process of IVD degeneration in mice.

## 7. Clinical proteomic analysis of body fluids from patients with degenerative spinal disorders

The majority of lumbar disk herniation (LDH) cases are caused by IDD (75); however, the pathophysiological mechanism of disc herniation is not fully understood. Owing to the compression of the NP on the nerve root, many of the DEPs in the CSF of patients with LDH are associated with neurons and pain; for example, Lin *et al* reported that a total of nine proteins were detected at high levels in the CSF of LDH patients, including cystatin C and APO A-IV, whereas five proteins were found to be decreased, including creatine kinase B-type and SOD1 (Table I) (76). In addition, APOL1, which exists in endothelial cells and is closely related to atherosclerotic iliac arteries, is regulated by tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and upregulated in the serum of people suffering from LDH (77,78). This higher expression of APOL1 may be responsible for the degeneration, because TNF- $\alpha$  is involved in the inflammation induced by LDH (79). Similarly, APOM which was downregulated in

serum of LDH patients is an immunity-associated gene adjacent to the TNF- $\alpha$  and lymphotoxin genes (80). However, the explanation behind the downregulation of APOM in the serum of LDH patients requires further study.

## 8. Differentially regulated proteins and their signaling pathways in degenerative spinal disorders

$\alpha$ -2 COL1 forms one of the chains for COL1 and the structural disturbance of this protein is involved in bone development (81). There is not enough evidence to prove the link between bone development and IDD except for the downregulation of COLA2. COL2 is a major component of cartilage, and mutations in this protein have been reported to contribute to type II collagenopathies (82). The expression levels of FN1, one of the major components of the ECM that binds a large number of molecules related to signal transduction and cell adhesion, is reportedly increased in degenerating discs, which is related to the change in the organizational structure (83,84). Furthermore, COMP, which is found in the ECM as an integral part of ligaments and tendons, is believed to serve a role in cellular proliferation and apoptosis, in addition to regulating cell movement and attachment (85). A previous study demonstrated that the abnormal expression of COMP and SOX9 is associated with cartilage degeneration in OA (86), and another study reported that alongside CILP and HTRA1, COMP serves as a biomarker that may be involved in the process of IDD (10).

A previous study indicated that downregulated microRNAs (including hsa-miR-125b-1-3p and hsa-miR-1184)

and upregulated genes (including AP2 associated kinase 1 and hemoglobin subunit  $\beta$ ) between normal and degenerative discs are involved in the PI3K/AKT signaling pathway (87). The activation of the PI3K/AKT pathway serves an important role in protecting cells from harmful physiological processes, such as cellular apoptosis, oxidative damage, and a hypoxic microenvironment, and has been found to protect against IDD (88). Furthermore, it was recently demonstrated that bone morphogenetic protein 2 can inhibit cellular apoptosis and suppress the synthesis of matrix proteins via the PI3K/AKT signaling pathway, which further alleviates IDD (89).

Activation of the AGE-RAGE signaling pathway in diabetic polyneuropathy, a complication of diabetes, is commonly observed, and increased AGE-RAGE signaling exacerbates degenerative disorders of peripheral neurons (90). In fact, a recent study reported no significant difference in the GAG content or histological features between discs from non-obese diabetic mice and euglycemic littermates, but noted increases in cellular apoptosis and matrix aggrecan fragmentation (91). To determine the link between diabetes and IDD, the role of the AGE-RAGE signaling pathway in IDD requires further research.

The p53 signaling pathway is an important indicator of cellular apoptosis, and decreased expression of wild-type p53-induced phosphatase 1 is closely related to p53 activation and neuronal apoptosis (92). During periods of replicative senescence, the p53/p21/retinoblastoma pathway is activated to alleviate telomere erosion and DNA damage response (93,94). Furthermore, the effects of small ubiquitin-related modifier 2 on the proliferation and senescence of NP cells has been investigated through the mediation of the p53 signaling pathway in rat models of IDD (95) which indicated that SUMOS was a potential target for IDD treatment.

TGF- $\beta$  signaling is an extensive pathway involved in developmental programs of cells, including proliferation, differentiation, homeostasis, and regeneration (96). In the IVD, TGF- $\beta$  is a major regulatory cytokine that maintains cellular differentiation and homeostasis (97). Previous research has reported that the upregulation of TGF- $\beta$  causes a decrease in NF- $\kappa$ B (98). The inhibition of NF- $\kappa$ B may play an important role in IDD (99). Furthermore, TGF- $\beta$ -dependent AF cell proliferation and progressive vertebral fusions due to the loss of filamin B was involved in IDD (100). Moreover, the increased expression of COL2 and aggrecan regulated by TGF- $\beta$ 1 alleviates the degeneration of IVDs (101).

## 9. Conclusions

Main components of the matrix such as prolargin, FN1, CILP, COMP, COL1 and COL2 are significantly changed in the degenerative LF, AF, and NP. COMP is involved in cartilage degeneration in OA (102), but it has not been fully studied in IDD. Moreover, The role of AGE-RAGE signaling pathway in IDD requires further research. Despite the limitations of GO and KEGG pathway analysis, proteomic analysis still provides novel targets that aid in understanding IDD pathophysiology.

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## Availability of data and materials

All data generated or analyzed during the present study are included in the article.

## Authors' contributions

CL conceived and designed the experiment, analyzed the data and wrote the manuscript. MY and LL collected the data and analyzed the data. YaZ, QZ, CH and HW performed data analysis and provided interpretation. YQZ and HL provided technical support and analyzed and interpreted the data. CQL and BH critically revised the article and interpreted the data. CF and YZ were involved in designing the experiment, analyzing the data, revising the manuscript for important intellectual content and final approval of the version to be published.

## Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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