Int J Clin Exp Med 2017;10(12):16172-16180
www.ijcem.com /ISSN:1940-5901/IJCEM0054199

Original Article

Differential protein expression between normal and degenerated human nucleus pulposus cells by proteomic and bioinformatics analyses

Dongping Ye[1], Weiguo Liang[1], Libing Dai[1], Yicun Yao[1], Honghui Chen[1], Yi Zhou[2], Jiake Xu[3]

[1]*Guangzhou Institute of Traumatic Surgery, Guangzhou Red Cross Hospital, The Fourth Affiliated Hospital of The Medical College, Jinan University, Guangzhou, China;* [2]*Research Centre for Regenerative Medicine, Guangxi Medical University, Guangxi 530021, China;* [3]*School of Pathology and Laboratory Medicine, The University of Western Australia Perth, WA 6009, Australia*

Received March 30, 2017; Accepted September 4, 2017; Epub December 15, 2017; Published December 30, 2017

Abstract: Low back pain is a chronic disease associated with the degeneration of lumbar intervertebral disc (IVD). The molecular and cellular basis of degenerate IVD disorder remains largely unclear. Using 2-D electrophoresis, mass spectrometric analyses, database searching and western blot assay, we examined differential protein expression between normal surrogates and degenerated human nucleus pulposus (NP) cells. Ten protein spots with most altered differential expression levels between normal surrogates and degenerated NP cells were identified. Among these, five proteins showed decreased expression in degenerated NP cells, including SID 1 transmembrane family member 2, isoform 1 of proteasome activator complex subunit 3, transaldolase 1, isoform 1 of 26S protease regulatory subunit 8 and guanylate cyclase soluble subunit beta-1. Conversely, five proteins showed increased expression in degenerated NP cells including peroxiredoxin 2, superoxide dismutase, isoform 2 of neutrophil gelatinase-associated lipocalin, peroxiredoxin-4 and isoform 4 of Calumenin. Further, bioinformatics analysis hints that these differentially expressed proteins bear characteristics of membrane-like molecules with antioxidant function. Collectively, differentially expressed proteins might serve as biomarkers of the degenerate IVD and help us to understand the pathogenesis of IVD.

Keywords: Nucleus pulposus cells, degeneration, mass spectrometric analysis, proteome.

Introduction

Lumbar intervertebral disc (IVD) degeneration is a major cause of chronic low back pain, accompanied by altered mechanical loading responses [1]. At the cellular and molecular levels, IVD age-related degeneration is characterized by dysfunctional nucleus pulposus (NP) and annulus fibrosus (AF) with significant changes in matrix structure, composition and cell population, leading to the loss of proteoglycans and limited self-repair capacities [1-3]. To date, treatment options for degenerate IVD are very limited due to poor understanding of the complex pathogenesis of IVD. Thus, identifying molecules that are involved in the process of degenerated IVD may facilitate the design of novel therapeutic strategies for the treatment of this common medical condition.

The nucleus pulposus (NP) tissue consists of multilayer cellular structures, transferring from central jelly-like gel to peripheral fibrosus-like tissue. The proteoglycan, collagen II and water content of NP tissue are the major components of NP, but are decreased in degenerative IVD disorder with aging [4]. Loss of water, degradation of aggrecan molecules and collagen II are commonly observed in NP tissues in pathological IVD conditions [5]. Poor functional consequences of IVD could result from the pathological alteration in structure and composition of the extracellular matrix of the NP tissue [6]. However, molecules that are involved in the degeneration processes of the NP cells in IVD remain poorly understood.

In this study, we conducted comparative proteomic analysis using high-resolution 2-D elec-

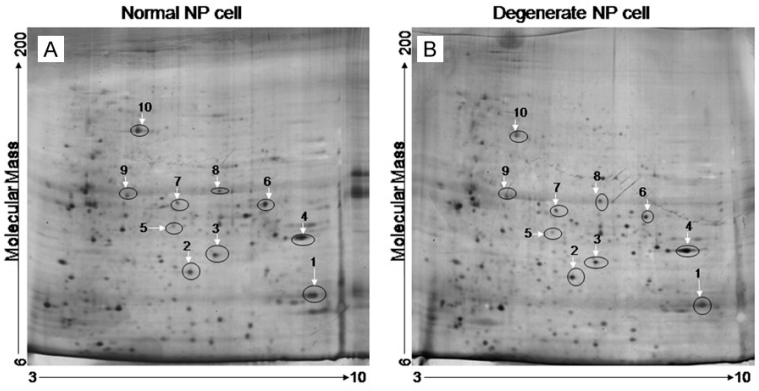

Figure 1. Protein profile differences between normal and degenerated nucleus pulposus (NP) cells. The pictures are representative silver-stained 2-D electrophoresis maps of proteins from normal and degenerated NP cells. Ten differentially expressed spots identified by MALDI-TOF MS and tandem mass spectrometry scanning are marked with numbers.

trophoresis gels, mass spectrometric analyses, and protein database searching. We identified several proteins that exhibit significantly altered expression levels between normal surrogate and degenerated NP cells. Collectively, these proteins represent membrane-like proteins and antioxidant functions of NP cells. These results might help improving our understanding of the pathogenesis of NP cell degeneration at the molecular level. The key molecules identified from this work might also serve as therapeutic targets for the development of treatment strategies for degenerated IVD disorders.

Materials and methods

Culture of normal and degenerated NP cells

Normal surrogate NP cells were isolated from three patients who were performed orthopaedic surgery for scoliosis (one patient was 15 years old and two patients were 13); degenerated NP cells were received from three patients who were performed orthopaedic surgery for lumbar disc herniation (one patient was 70 years old and two patients were 65). The degeneration grade of all samples were histologically assessed with the method of Sive *et al.* [7]. Three degenerated discs were defined as histological grades 8, 9, and 10 while three normal discs were defined as histological grade 1 according to the method of Sive *et al.* [7]. The NP tissues were soaked in saline containing penicillin-streptomycin antibiotics for 10 min right after removed from patients. Using the ophthalmic scissors, NP tissues were cut into 1 × 1 × 1 mm size, then digest by 10-mL 2% collagenase II, and stirred for 60 min. NP tissues were centrifuged at 1000 rpm for 10 min. The supernatant was removed and added 1-mL Dulbecco's modified Eagle's medium containing 10% fetal calf serum to disperse the cells. Then NP cells were cultured with 6-mL Dulbecco's modified Eagle's medium containing 10% fetal bovine serum at 37°C, saturated humidity, and 5% CO_2 in a T25 tissue culture flask for 3 days. This work was consent by the patients and approved by the ethics committee of Guangzhou Red Cross Hospital (Gzrc.0127).

RPMI medium 1640 (GIBCO, USA), North American fetal bovine serum (GIBCO, USA), RIPA cracking solution (Bebb, Shanghai, China), BRADFORD Protein Quantification Kit (Bio-Rad, USA), Penicillin Streptomycin Solution double antibody (GIBCO, USA), Purine (ACROS, USA), 0.25% trypsin (SIGMA, USA), Acetone (-20 C pre cold) (Guangzhou chemical reagent factory, China), dithiothreitol (SIGMA, USA), IPG Buffer (Bio-Rad,USA),Bromine phenol blue (BPB)(Shanghai Shi Feng Biotechnology Co., Ltd., China), 30% acrylamide monomer storage (SIGMA, USA), Ammonium sulfate (AP) (SIGMA, USA), N, N, N', N'-four methyl ethylenediamine (TEMED) (SIGMA, USA), Water saturated butanol (Guangzhou chemical reagent factory, China), Tris-HCl (pH 8.8) (SIGMA, USA), Iodoacetamide IAA (SIGMA, America), Ethanol (Tianjin chemical reagent factory, China), Glacial acetic acid (Guangzhou chemical reagent factory, China), $NaS_2O_3 \bullet 5H_2O$ (Guangzhou chemical reagent factory, China), Silver nitrate (Guangzhou chemical reagent factory, China) were used for the experiment.

Preparation of isoelectric focusing

150 μg of protein was taken from two groups, added into 250 μL of buffer and fully mixed. After the refrigeration, dry rubber strip was rewarmed. The adhesive tape was removed from the samples with isoelectric focusing

Table 1. Identification results of proteins differentially expressed in normal and degenerated nucleus pulpusus cells

Spot no[a]	Protein name	UniProtKB accession no.	Theoretical MW(KDa)/PI	Measured MW(KDa)/PI	Peptide count	Protein score (CI)	-Fold change[b]
1	SID 1 transmembrane family member 2 (SIDT2)	E9PMC3	12.51/9.63	12.50/9.63	11	90 (100%)	-4.2
2	Peroxiredoxin 2, isoform CRA-a (PRDX2)	A6NIW5	15.14/5.83	17.46/6.12	18	228 (100%)	2.3
3	Superoxide dismutase (SOD)	B3KUK2	19.73/7.81	19.83/7.41	6	81 (100%)	2.9
4	Isoform 2 of Neutrophil gelatinase-associated lipocalin (NGAL2)	P80188-2	22.46/9.34	22.56/9.33	7	239 (100%)	2.1
5	Isoform 1 of proteasome activator complex subunit 3 (PSME3)	P61289-1	29.51/5.69	29.60/5.69	6	266 (100%)	-2.9
6	Transaldolase (TALDO)	F2Z393	35.33/9.07	35.53/8.81	10	78 (100%)	-3.8
7	Peroxiredoxin-4 (PRDX4)	Q13162	30.54/5.86	36.11/5.96	4	68 (100%)	3.2
8	Isoform 4 of Calumenin (CALU4)	O43852-4	38.17/4.41	39.17/4.61	6	206 (100%)	2.0
9	Isoform 1 of 26S protease regulatory subunit 8 (PRS8)	P62195-1	45.63/7.11	42.93/7.52	6	72 (99.4%)	-5.3
10	Guanylate cyclase soluble subunit beta-1(ß1-sGC)	B7Z426	68.16/5.22	68.98/5.23	6	225 (100%)	-6.6

a, Spot numbers are shown in **Figure 1**. b, The spot intensities were quantified using PDQuest software (Bio-Rad). The average fold change of spot intensity for each protein was calculated from three independent experiments (degenerate nucleus pulposus cells compare to normal nucleus pulposus cells). -, decrease. *P* value between two groups were <0.05.

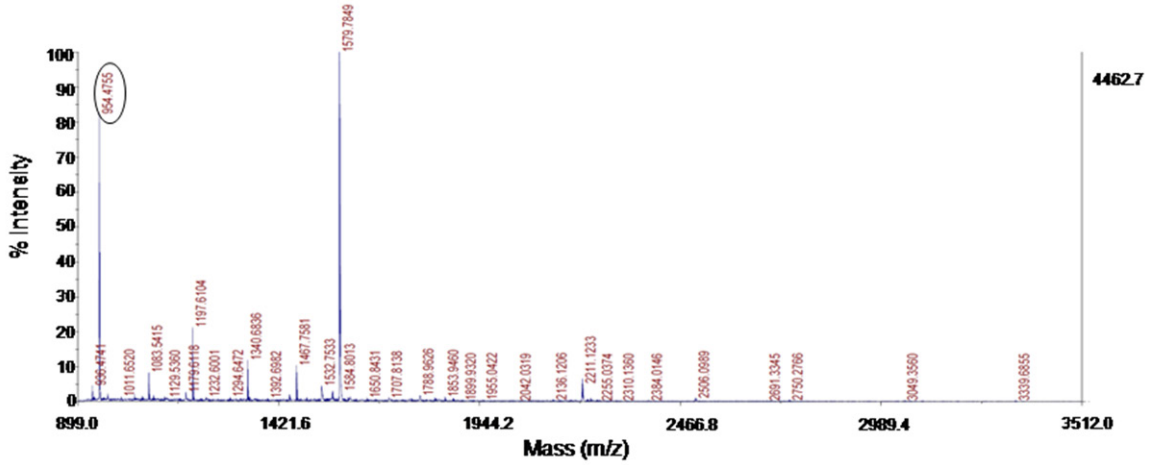

Figure 2. Identification of isoform 4 of Calumenin (spot 8) by MALDI-TOF MS analysis. MALDI-TOF MS spectrum of isoform 4 of Calumenin labelled with detected masses and peptide assignments. The precursor ion 954.476 m/z, highlighted by an open circle, was submitted for tandem mass spectrometry scanning.

groove, evenly covered with the bottom. The supported film was placed at the beginning of the acidic end (a word end), gently tear covering film. In the face down, the acidic end was placed at the top in the isoelectric focusing. A good adhesive was put after the addition of 1.2 mL cover oil on the support film moisture. The isoelectric focusing instrument was employed and the temperature was set to 20°C.

Electrophoresis

Electrophoresis tank was joined for the electrophoresis liquid, low melting point of fat sugar,

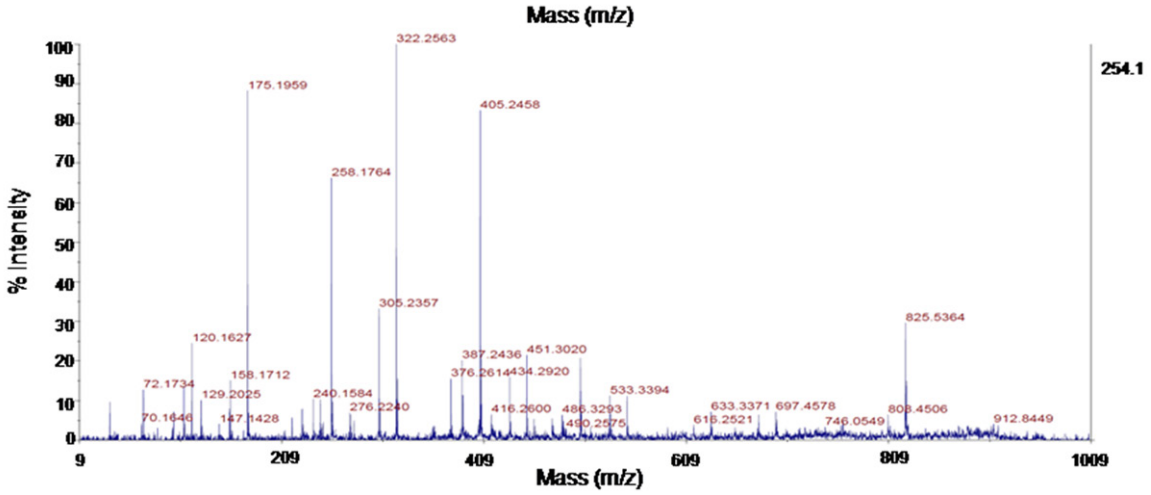

Figure 3. Tandem mass spectrometry spectra of the precursor ion 954.476 m/z for isoform 4 (CALU) of Calumenin show a characteristic profile of CALU.

and the upper layer of the upper layer electrophoresis. The glass plate was removed from the glue device, and placed into the electrophoresis tank. Electrophoresis tank was covered at the top with electrophoretic liquid before the middle clapboard, and connected to the electrode before the beginning of electrophoresis.

Fixation

A plastic ruler was used and the glass plate Alice was gently removed from the glue, pulling the rubber surface and agarose, a small angle was cut in the rubber surface acidic end with a plastic ruler. The glass plate surface was washed and the rubber surface with deionized water was transferred to the dyeing adhesive plate. The plate was placed on the table vibration level which was fixed, at room temperature for 2 h.

Sensitization

After the rubber block fixing was completed, the staining tray was removed, and then poured into the liquid, per disc with 250 ml sensitizing solution. After staining, the disc was arranged on a horizontal shaker vibration, at a room temperature sensitization for 1 h.

Rinse

After the completion of sensitization, the disc staining was removed, each dish was added for 250 ml of ultrapure water for 10 min. Vibration plate was arranged at the level of shaking, and then it was rinsed four times.

Silver stain

Silver staining solution was prepared to avoid the backup, after rinsing, the disc staining was removed, and 250 ml of silver staining solution was added, staining disc was arranged on a horizontal shaker vibration for 2 h.

According to the formula of color liquid, Formaldehyde Solution was added before use. The terminal solution was added to a color liquid and vibrated for 30 min, rinsed with water for 30min, and repeated twice.

Scanning

The rubber block was removed. Image scanner was placed in the direction of gel. The gule piece of plastic wrap was scaled and stored in the refrigerator at 4°C.

Image analysis

PDQuest™ 2-D (V 8.0; Bio-Rad) and quantity one software (Bio-Rad) for image analysis, image of the scan after editing, point detection, matching, strength analysis, corresponding gel was matched for comparison and analysis, we set gel strength 2 times threshold as significant differences.

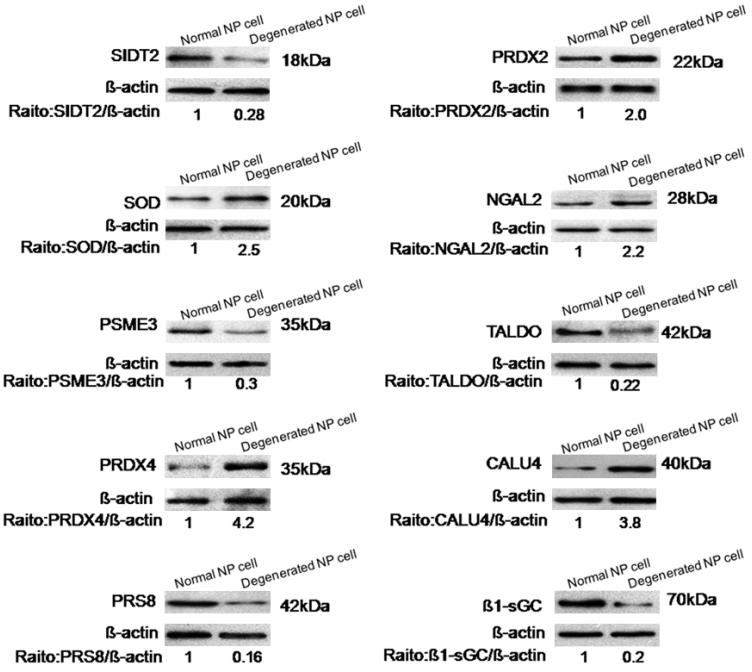

Figure 4. Western blot was used to confirm the MS/MS results. The results showed that the differential expression of the proteins in degenerated NP cells compared to normal NP cells was consistent with the MS/MS results.

Matrix-assisted laser desorption/ionization time of flight mass spectrometry and MS/MS spectrometry analysis

For acquisition of mass spectra, 0.5-lL peptide solution was mixed with 0.5-lL matrix (4-mg/mL a-cyano-4-hydroxylcinnamic acid in 1% trifluoroacetic acid and 35% acetonitrile) before spotting onto the MALDI plate. Mass spectrometric analyses were performed on a MALDI-TOF/TOF MS/MS ABI 4700 proteomics analyser (Applied Biosystems, Shanghai, China). Briefly, MS/MS spectra were obtained by collecting 3000 laser shots with a default calibration. The MS/MS peaks were detected on a minimum signal-to-noise (S/N) ratio >3 and a cluster area S/N threshold >15 with smoothing. The MS peaks (MH+) were detected on a cluster area S/N threshold >25 and a minimum S/N ratio >20 without smoothing and raw spectrum filtering. The precursor ions with a minimum S/N ratio of 50 were selected for MS/MS scanning.

Western blotting

To confirm the expression of proteins, Western blotting was used. After separated in 10% sodium dodecyl sulphate polyacrylamide gel electrophoresis gel by electrophoresis (90 V, constant voltage) for 120 min, the proteins were transferred to a 0.45-mM PVDF membrane. Following transferring, the membranes were incubated with anti-SIDT2 (1:500 dilution), anti-PRDX2 (1:500 dilution), anti-SOD (1:500 dilution), anti-NGAL2 (1:500 dilution), anti-PSME3 (1:500 dilution), anti-TALDO1 (1:500 dilution), anti-PRDX4 (1:500 dilution), anti-CALU4 (1:500 dilution), anti-PRS8 (1:500 dilution), anti-β1-sGC (1:500 dilution), and anti-mouse-HRP (1:3000 dilution) antibodies, which were all purchased from Abcam (Cambridge, MA, USA). ECL reagents were used to detect the western blot signals.

Results

Ten most differentially expressed protein spots were obtained

We conducted comparative proteomic analysis to examine the differential expression of proteins between normal surrogate and degenerated human NP Cells. **Figure 1** shows a comparative layout of stained 2-DE gels with expressed protein spots of NP cells from the experimental group and control group; respectively (**Figure 1**). A total of 425 ± 33 protein spots were detected in normal surrogates and degenerated NP cells. Ten most differentially expressed protein spots in NP cells were identified in the gels between normal surrogates and degenerated NP cells (**Figure 1**), and selected for further MALDI-TOF MS analysis.

To examine the identity of these differentially expressed proteins, protein spots were isolated from the Coomassie Blue-stained gels and subjected to in-gel tryptic digestion. The digested peptides were quantitatively analyzed by MALDI-TOF MS. Protein database with the SEQUEST search program was used to match the MS/MS-identified candidates. We found that five proteins were decreased in the degenerated NP cells as compared to normal surro-

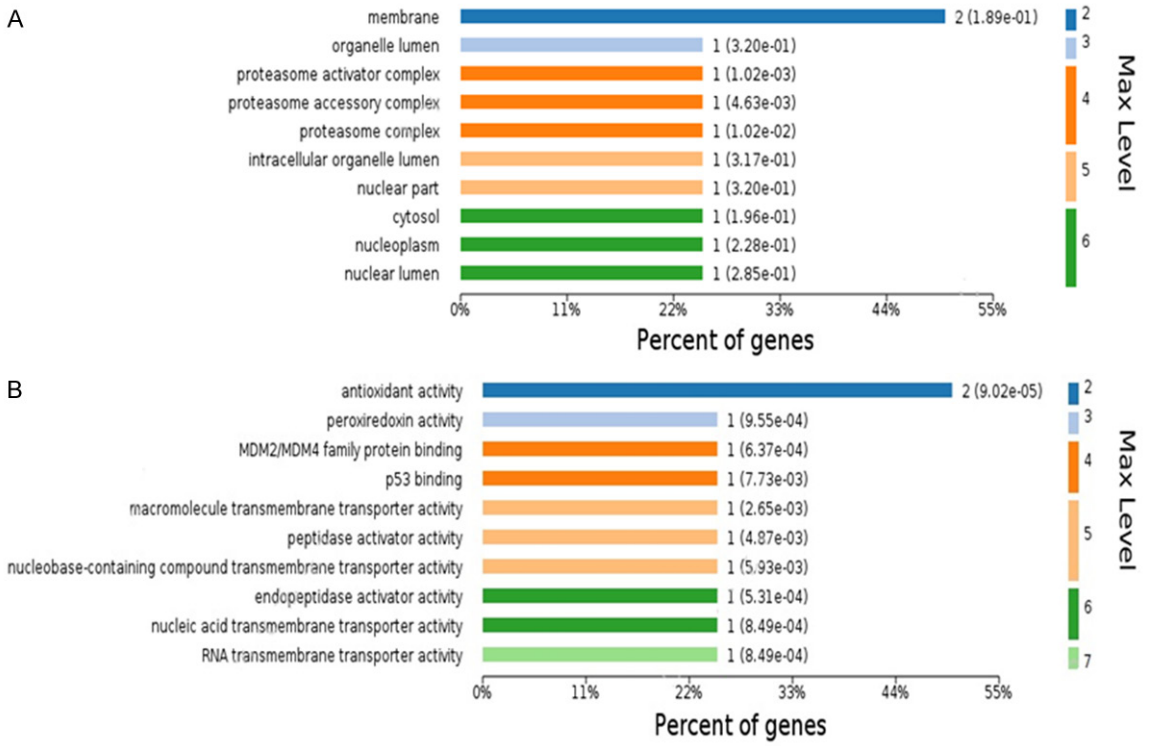

Figure 5. Bioinformatics analysis is performed to examine the cellular component and predicted molecule function of 10 identified proteins linked with the uniprot ID. (A) Levels of enriched cell component and (B) levels of enriched molecular function were recorded.

gate NP cells. These include SID 1 transmembrane family member 2, isoform 1 of proteasome activator complex subunit 3, transaldolase 1, isoform 1 of 26S protease regulatory subunit 8 and guanylate cyclase soluble subunit beta-1. Additionally, five proteins were increased in the degenerated NP cells as compared to normal surrogate NP cells. These include peroxiredoxin 2, superoxide dismutase, isoform 2 of neutrophil gelatinase-associated lipocalin, peroxiredoxin-4 and isoform 4 of Calumenin. The ten identified spots with their summary scores, relative volume, etc. are presented in **Table 1**.

MS/MS analysis of the ten proteins

All protein spots were then subjected to further analysis by using MALDI-TOF MS. As an example, **Figure 2** shows the characteristic profile of CALU (spot 8) by MALDI-TOF MS analysis, showing MALDI-TOF MS spectrum of CALU labelled with masses detected and peptide identity assignments. The precursor ion 954.476 m/z, highlighted by an open circle, was submitted for MS/MS scanning. **Figure 3** shows MS/MS spectra of the precursor ion 954.476 m/z for CALU. The differentially expressed proteins by fold changes from the MS/MS analysis were summarized in **Table 1**.

Western blot analysis of the ten proteins

To further confirm the differential expression of proteins, western blot analysis was used with specific antibodies to each of these identified proteins (**Figure 4**). The results showed that the differential expression of proteins in degenerated NP cells compared to normal surrogate NP cells were consistent with the MS/MS results (**Table 1**, **Figure 4**).

Bioinformatics analysis of the ten proteins

Bioinformatics analysis was performed to examine the cellular component and predicted molecule function of 10 identified proteins linked to the uniprot webpage with the uniprot ID. We could see that the membrane-like protein was accounted for 50%, and with the antioxidant functions. Organelle lumen protein, proteasome activator complex protein, proteasome accessory complex protein, intracellular organelle lumen protein, nuclear part protein, cytosol protein, nucleoplasm protein, nucleo-

plasm protein and nuclear lumen protein were accounted for 25%. The function of these proteins was included peroxiredoxin activity, MDM2/MDM4 protein binding, p53 binding, macromolecule transmembrane transporter activity, peptidase activator activity, nucleobase-containing compound transmembrane transporter activity, endopeptidase activator activity, nucleic acid transmembrane transporter activity, and RNA transmembrane transporter activity. The results revealed that membrane-like proteins and antioxidant functions of NP cells are predominantly affected in degenerated NP cells among these differentially expressed proteins (**Figure 5**).

Discussion

In this study, we identified that five proteins were decreased in degenerated NP cells as compared to normal surrogates NP cells, including SID 1 transmembrane family member 2, isoform 1 of proteasome activator complex subunit 3, transaldolase 1, isoform 1 of 26S protease regulatory subunit 8 and guanylate cyclase soluble subunit beta-1. In contrast, five proteins were increased in degenerated NP cells as compared to normal surrogates NP cells, including peroxiredoxin 2, superoxide dismutase, isoform 2 of neutrophil gelatinase-associated lipocalin, peroxiredoxin-4 and isoform 4 of Calumenin. Our study suggests the potential value of these differentially expressed proteins as potential biomarkers for the diagnosis of degenerative IVD. It also indicates the possible involvement of these proteins in the pathophysiological processes of IVD degeneration.

Among five proteins that display a decreased expression profile in degenerated NP cells, β1-sGC is the mammalian receptor of the endogenous messenger nitric oxide (NO) and catalyzes the formation of the second messenger cGMP from GTP after activation by NO binding expressed in different cell types. It plays an important role in the regulation of lung airway relaxation, vascular blood pressure, and immune response [8]. Change of the expression level of β1-sGC may suggest an involvement of G proteins of the transmembrane signaling systems in degenerated NP cells. SIDT2 functions as a channel capable of transporting dsRNA into cells in Drosophila cells [9]. The change of SID-1 may suggest an involvement of signal transport process in degenerated NP cells. PSME3 is the subunit of the 11S REG-gamma proteasome regulator associated with the proteasome and may be involved in cell cycle regulation [10, 11]. The decreased expression of PSME3 in the degenerated NP cells suggests that during the NP cells' degeneration, the proteasome-mediated apoptosis rate might be increased. Transaldolase (TALDO1) has been found in archaea, bacteria, and eukarya. It is an enzyme of the pentose phosphate pathway (PPP) which transfers a dihydroxyacetone group from donor compounds (fructose 6-phosphate or sedoheptulose 7-phosphate) to aldehyde acceptor compounds [12]. The decreased expression of TALDO1 in the degenerated NP cells might imply an important role of the balance of metabolites in the pentose-phosphate pathway in the NP cells degeneration.

Among five proteins that exhibited an increased differential expression profile in degenerated NP cells, PRDX2 belongs to a ubiquitous family of multifunctional antioxidant thioredoxin-dependent peroxidases which can reduce H_2O_2 and hydroperoxides into water and alcohol [13, 14]. The altered expression of the oxidation and antioxidant proteins in the degenerated NP cells suggests that responses to reactive oxygen species might contribute to the NP cell degenerative processes. PRDX4 regulates the activation of NF-kB in the cytosol through a modulation of I-kappa-B-alpha phosphorylation which is probably involved in redox regulation of the cell [15, 16]. Until now, there has been no report about the altered expression of these molecules in the degenerated NP cells. The increased expression of PRDX4 may reveal its possible involvement in the pathogenesis in degenerated NP cells. NGAL2 plays a role in innate immunity, possibly by sequestrating iron, leading to limitation of bacterial growth [17]. The altered expression of this protein may suggest an involvement of iron-trafficking machinery in degenerated NP [18]. Change of the expression level of CALU may alter the regulatory machinery in degenerated NP cells.

Conclusions

In summary, we identified differentially expressed proteins in degenerated NP cells. These molecules might play an important role in the pathogenesis of IVD degeneration, and potentially serve as biomarkers for the diagnosis of

degenerative IVD. These proteins appear to represent predominantly membrane-like proteins and antioxidant functions of NP cells based on bioinformatics analysis. Future studies integrating functional and mechanistic analyses are needed to improve our understanding of the role of these molecules involved in the pathogenesis of degenerated IVD.

Acknowledgements

This project was supported by the Fund of Medical and Health Project of Guangzhou City (20151A011013, 20161A011013, 20171A011249, 20171A011012), the Fund of Guangzhou Municipal Science and Technology Bureau (201607010051), the Fund of Guangdong science and Technology Department (2013B021800070) and the Guangdong Provincial Natural Science Fund of China (2015A030313736).

Disclosure of conflict of interest

None.

Authors' contribution

All authors prepared for the experiment, reviewed the literature, edited the manuscript, and approved the final version.

Address correspondence to: Dongping Ye and Weiguo Liang, Guangzhou Red Cross Hospital, The Fourth Affiliated Hospital of The Medical College, Jinan University, Guangzhou, China. E-mail: yedongping927@126.com (DPY); liangweiguo927@126.com (WGL); Jiake Xu, School of Pathology and Laboratory Medicine, The University of Western Australia Perth, WA 6009, Australia. E-mail: jiake.xu@uwa.edu.au

References

[1] Freemont AJ. The cellular pathobiology of the degenerate intervertebral disc and discogenic back pain. Rheumatology (Oxford) 2009; 48: 5-10.

[2] Tang X, Jing L and Chen J. Changes in the molecular phenotype of nucleus pulposus cells with intervertebral disc aging. PLoS One 2012; 7: e52020.

[3] Ciapetti G, Granchi D, Devescovi V, Leonardi E, Greggi T, Di Silvestre M and Baldini N. Ex vivo observation of human intervertebral disc tissue and cells isolated from degenerated intervertebral discs. Eur Spine J 2012; 21 Suppl 1: S10-19.

[4] Roberts S, Evans H, Trivedi J and Menage J. Histology and pathology of the human intervertebral disc. J Bone Joint Surg Am 2006; 88 Suppl 2: 10-14.

[5] Tsai TT, Danielson KG, Guttapalli A, Oguz E, Albert TJ, Shapiro IM and Risbud MV. TonEBP/OREBP is a regulator of nucleus pulposus cell function and survival in the intervertebral disc. J Biol Chem 2006; 281: 25416-25424.

[6] Liu Y, Fu S, Rahaman MN, Mao JJ and Bal BS. Native nucleus pulposus tissue matrix promotes notochordal differentiation of human induced pluripotent stem cells with potential for treating intervertebral disc degeneration. J Biomed Mater Res A 2015; 103: 1053-1059.

[7] Sive JI, Baird P, Jeziorsk M, Watkins A, Hoyland JA and Freemont AJ. Expression of chondrocyte markers by cells of normal and degenerate intervertebral discs. Mol Pathol 2002; 55: 91-97.

[8] Yoo BK, Lamarre I, Martin JL and Negrerie M. Quaternary structure controls ligand dynamics in soluble guanylate cyclase. J Biol Chem 2012; 287: 6851-6859.

[9] Ren R, Xu X, Lin T, Weng S, Liang H, Huang M, Dong C, Luo Y and He J. Cloning, characterization, and biological function analysis of the SidT2 gene from siniperca chuatsi. Dev Comp Immunol 2011; 35: 692-701.

[10] Roessler M, Rollinger W, Mantovani-Endl L, Hagmann ML, Palme S, Berndt P, Engel AM, Pfeffer M, Karl J, Bodenmuller H, Ruschoff J, Henkel T, Rohr G, Rossol S, Rosch W, Langen H, Zolg W and Tacke M. Identification of PSME3 as a novel serum tumor marker for colorectal cancer by combining two-dimensional polyacrylamide gel electrophoresis with a strictly mass spectrometry-based approach for data analysis. Mol Cell Proteomics 2006; 5: 2092-2101.

[11] Yan Q, Sharma-Kuinkel BK, Deshmukh H, Tsalik EL, Cyr DD, Lucas J, Woods CW, Scott WK, Sempowski GD, Thaden JT, Rude TH, Ahn SH and Fowler VG Jr. Dusp3 and Psme3 are associated with murine susceptibility to Staphylococcus aureus infection and human sepsis. PLoS Pathog 2014; 10: e1004149.

[12] Al-Shamsi AM, Ben-Salem S, Hertecant J and Al-Jasmi F. Transaldolase deficiency caused by the homozygous p.R192C mutation of the TALDO1 gene in four Emirati patients with considerable phenotypic variability. Eur J Pediatr 2015; 174: 661-668.

[13] Sanchez-Font MF, Sebastia J, Sanfeliu C, Cristofol R, Marfany G and Gonzalez-Duarte R. Peroxiredoxin 2 (PRDX2), an antioxidant enzyme, is under-expressed in Down syndrome fetal brains. Cell Mol Life Sci 2003; 60: 1513-1523.

[14] Yoo SM, Choi SH, Jung MD, Lim SC and Baek SH. Short-term use of telmisartan attenuates

oxidation and improves Prdx2 expression more than antioxidant beta-blockers in the cardiovascular systems of spontaneously hypertensive rats. Hypertens Res 2015; 38: 106-115.

[15] Zhang Y, Emmanuel N, Kamboj G, Chen J, Shurafa M, Van Dyke DL, Wiktor A and Rowley JD. PRDX4, a member of the peroxiredoxin family, is fused to AML1 (RUNX1) in an acute myeloid leukemia patient with a t(X;21)(p22;q22). Genes Chromosomes Cancer 2004; 40: 365-370.

[16] Yan Y, Wladyka C, Fujii J and Sockanathan S. Prdx4 is a compartment-specific H2O2 sensor that regulates neurogenesis by controlling surface expression of GDE2. Nat Commun 2015; 6: 7006.

[17] Zhang Y, Du L, Xu R, Cui R, Hao J, Sun C and Li Y. Transcription factors SOD7/NGAL2 and DPA4/NGAL3 act redundantly to regulate seed size by directly repressing KLU expression in Arabidopsis thaliana. Plant Cell 2015; 27: 620-632.

[18] Vorum H, Liu X, Madsen P, Rasmussen HH and Honore B. Molecular cloning of a cDNA encoding human calumenin, expression in Escherichia coli and analysis of its Ca2+-binding activity. Biochim Biophys Acta 1998; 1386: 121-131.