NEURAL REGENERATION RESEARCH
Volume 7, Issue 5, February 2012
www.nrronline.org

Cite this article as: Neural Regen Res. 2012;7(5):369-375.

Protein expression of sensory and motor nerves

Two-dimensional gel electrophoresis and mass spectrometry☆

Zhiwu Ren[1, 2], Yu Wang[1], Jiang Peng[1], Li Zhang[1], Wenjing Xu[1], Xiangdang Liang[3], Qing Zhao[4], Shibi Lu[1]

1*Key Laboratory of People's Liberation Army, Institute of Orthopedics, Chinese PLA General Hospital, Beijing 100853, China*
2*Medical College of Nankai University, Tianjin 300071, China*
3*Department of Orthopedics, Chinese PLA General Hospital, Beijing 100853, China*
4*Department of Orthopedics, First Affiliated Hospital of Chinese PLA General Hospital, Beijing 100037, China*

Abstract

The present study utilized samples from bilateral motor branches of the femoral nerve, as well as saphenous nerves, ventral roots, and dorsal roots of the spinal cord, to detect differential protein expression using two-dimensional gel electrophoresis and nano ultra-high performance liquid chromatography electrospray ionization mass spectrometry tandem mass spectrometry techniques. A mass spectrum was identified using the Mascot search. Results revealed differential expression of 11 proteins, including transgelin, Ig kappa chain precursor, plasma glutathione peroxidase precursor, an unnamed protein product (gi|55628), glyceraldehyde-3-phosphate dehydrogenase-like protein, lactoylglutathione lyase, adenylate kinase isozyme 1, two unnamed proteins products (gi|55628 and gi|1334163), and poly(rC)-binding protein 1 in motor and sensory nerves. Results suggested that these proteins played roles in specific nerve regeneration following peripheral nerve injury and served as specific markers for motor and sensory nerves.

Key Words: differential protein expression; mass spectrometry; motor nerve; peripheral nerve-specific regeneration; two-dimensional gel electrophoresis; sensory nerve

Zhiwu Ren☆, Studying for doctorate, Key Laboratory of People's Liberation Army, Institute of Orthopedics, Chinese PLA General Hospital, Beijing 100853, China; Medical College of Nankai University, Tianjin 300071, China

Corresponding author: Jiang Peng, Doctor, Associate researcher, Key Laboratory of People's Liberation Army, Institute of Orthopedics, Chinese PLA General Hospital, Beijing 100853, China
pengjiang301@126.com

Received: 2011-11-11
Accepted: 2012-01-05
(NY20111104001/H)

Ren ZW, Wang Y, Peng J, Zhang L, Xu WJ, Liang XD, Zhao Q, Lu SB. Protein expression of sensory and motor nerves: two-dimensional gel electrophoresis and mass spectrometry. Neural Regen Res. 2012;7(5):369-375.

www.crter.cn
www.nrronline.org

doi:10.3969/j.issn.1673-5374.2012.05.008

INTRODUCTION

Peripheral nerves are nervous system structures that link sensory and motor terminals to the central nervous system. The nerves comprise sensory and/or motor axons associated with particular glial cell types, such as Schwann cells, in connective tissue[1]. Functional recovery of peripheral nerve lesions is dependent on accurate axonal regeneration to original target end-organs, and misdirection of regenerating axons leads to poor results following nerve injury and repair[2]. Preferential regeneration of motor and sensory peripheral nerves is influenced by respective end-organs of muscle and skin[3-4]. Schwann cells also express distinct sensory and motor phenotypes associated with regeneration in a phenotype-specific manner[5]. To date, the exact molecular mechanisms by which sensory and motor nerves differentiate remain poorly understood. The identification of these molecules could facilitate specific functional reconstruction of sensory and motor nerves. The rat femoral nerve bifurcates below the inguinal ligament into a motor branch, which leads to the quadriceps muscle, and a terminal sensory branch, which is the saphenous nerve[6]. These two branches are the typical motor and sensory nerves utilized in peripheral nerve research. The motor branch has been utilized as a source of motor nerve grafts, and the sensory branch has been harvested for sensory grafts. In addition, dorsal and ventral spinal roots contain different types of axons. Dorsal roots of spinal nerves contain central arms of pseudounipolar neurons, and these bodies are located in dorsal root ganglia. Axonal peripheral arms are clustered within the peripheral nerves, and ventral roots consist predominantly of efferent axons (somatic and autonomic motor), and the bodies are present in the ventral horn of spinal cord gray matter. Therefore, dorsal and ventral roots are ideal sources of relatively pure sensory and motor axons.

Quantitative and qualitative assessment of the proteome is achieved by two-dimensional gel electrophoresis (2DGE), which generates a high-resolution, quantitative protein expression map; mass spectrometry is used to identify regulated proteins[7-8]. These procedures have been further modified for the identification of

peripheral nerve proteins[9].

The present study was designed to analyze differentially expressed proteins between the motor branch of the femoral nerve and saphenous nerve, as well as between ventral and dorsal roots, using the European Molecular Biology Laboratory silver staining and modified nano ultra-high performance liquid chromatography electrospray ionization mass spectrometry tandem mass spectrometry (NanoUPLC-nano-ESI-MS/MS).

RESULTS

2DGE maps and comparative proteomic analysis

(Figure 1)

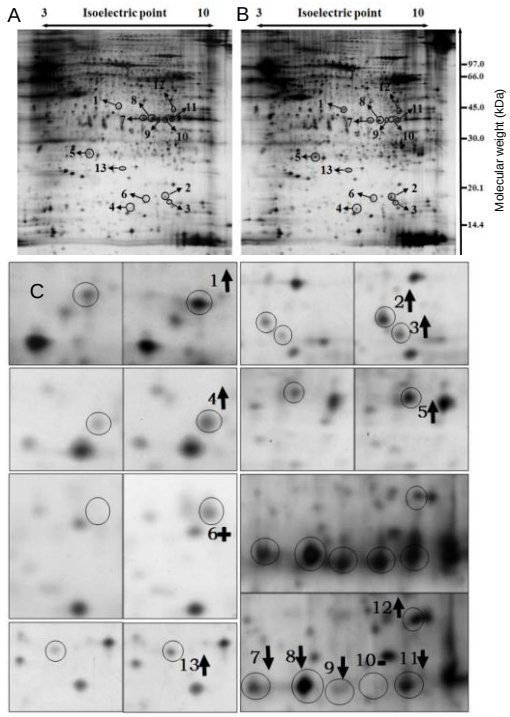

Figure 1 Silver nitrate staining results from two-dimensional gel electrophoresis (2DGE) of branches of femoral nerves and saphenous nerves.

Compared to motor branches of the femoral nerve, 13 protein spots exhibit significant differential expression in saphenous nerves, including seven up-regulated, four down-regulated, one peculiarly expressed and one with no expression.

(A) 2DGE map of motor branches of femoral nerve; (B) 2DGE map of saphenous nerve; (C) details of spot comparisons between motor branches of femoral nerve and saphenous nerve. ↑, ↓, + , – represents up-regulated, down-regulated, peculiar, and no expression in saphenous nerves, respectively.

A total of three 2DGE maps with good separation and clear protein spots were obtained from motor branches of the femoral nerve, saphenous nerves, ventral roots, and dorsal roots. ImageMaster 2D Platinum analysis revealed 1 010.67 ± 80.98, 1 122.67 ± 101.93, 972.33 ± 54.56, and 859.33 ± 96.67 protein spots in maps of motor branches of the femoral nerve, saphenous nerves, ventral roots, and dorsal roots, respectively, with a mean intragroup matching rate > 80%. A total of 13 protein spots exhibited significant differential expression between motor branches of the femoral nerve and saphenous nerves, and values were distributed from 5.0 to 9.0 (isoelectric point) and 20.0 to 60.0 kDa (molecular mass). Compared to motor branches of the femoral nerve, seven spots were upregulated, four spots were downregulated, one spot was specificially expressed, and one spot was not expressed in saphenous nerve tissues (Figure 1). In addition, 16 protein spots exhibited significant differential expression between ventral and dorsal roots, and values were distributed from 4.0 to 9.0 for isoelectric point and 10.0 kDa to 60.0 kDa for molecular mass. Compared to ventral roots, six spots were upregulated, seven spots were downregulated, and three spots were not expressed in the dorsal roots (Figure 2). The 29 protein spots with significant differential expression were selected for NanoUPLC-nano-ESI-MS/MS analysis.

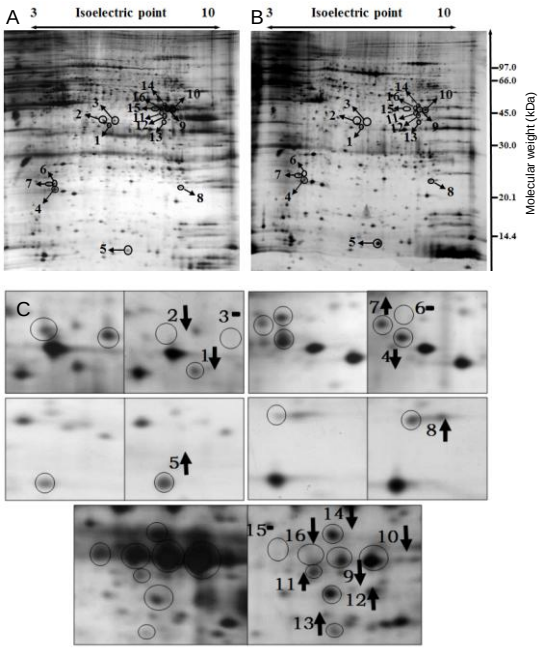

Figure 2 Silver nitrate staining results from two-dimensional gel electrophoresis (2DGE) of ventral roots and dorsal roots.

Compared to ventral roots, 16 protein spots exhibit significant differential expression in dorsal roots, including six up-regulated, seven down-regulated, and three with no expression

(A) 2DGE map of ventral rootl; (B) 2DGE map of dorsal root; (C) details of comparison of spots between ventral root and dorsal root. ↑, ↓, – represent up-regulated, down-regulated, and no expression in dorsal roots, respectively).

Protein identification by nanoUPLC-nano-ESI-MS/MS analysis

A total of 26/29 protein spots with significant differential

expression were successfully detected using nanoUPLC-nano-ESI-MS/MS analysis, and all contained peptides with matching scores > 34 ($P < 0.05$). The peptide segment coverage rate was 1–64%. After excluding the most frequently observed contaminant peptides derived from keratin[10] and repetition, 12 proteins were differently expressed in sensory and motor nerve tissues (Figure 3).

Protein comparisons of motor branches of the femoral nerve revealed down-regulated expressions in saphenous nerve tissues of transgelin, Ig kappa chain precursor, plasma glutathione peroxidase precursor, an anonymous protein product (gi|55628) were upregulated, and aldose reductase, a glyceraldehyde-3-phosphate dehydrogenase (GAPDH)-like protein (Table 1). Comparisons of ventral roots revealed up-regulated expressions of lactoylglutathione lyase, adenylate kinase isozyme 1, two anonymous proteins products (gi|55628 and gi|1334163), and down-regulated expression of poly(rC)-binding protein 1 (PCBP1). Expression of rCG31027 was absent in dorsal root tissues (Table 2).

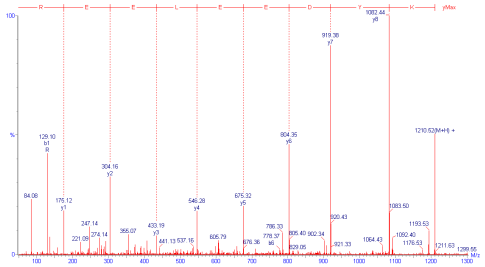

Figure 3 Peptide mass data of transgelin in two-dimensional gel electrophoresis map of saphenous nerves.

x-axis represents mass-to-charge ratio (m/z), whereas the y-axis represents relative intensity.

Peptide mass data were searched in the National Center for Biotechnology Information (NCBI) using the Mascot MS/MS Ions search engine and Rattus species category. The peptide sequence was KYDEELEER.

The protein spot was identified as transgelin, with protein sequence coverage of 64% (64% of peptide sequence is in accordance with the transgelin protein sequence).

Table 1 NanoUPLC-nano-ESI-MS/MS analysis results from protein spots with significant differential expression in 2DGE maps of saphenous nerves and motor branches of femoral nerves

Spot No.*	NCBI accession No.	Protein name	Molecular weight (Da)	pI	Protein score	Sequence coverage (%)	Expression variation in saphenous nerve*
1	gi|55628	Unnamed protein product	68 674	6.09	153	12	Up
2	gi|13928744	Transgelin	22 588	8.87	680	64	Up
3	gi|13928744	Transgelin	22 588	8.87	82	9	Up
5	gi|92401	Ig kappa chain precursor	26 381	5.22	38	5	Up
6	gi|13928744	Transgelin	22 588	8.87	345	49	Peculiar
7	gi|6978491	Aldose reductase	35 774	6.26	226	43	Down
8	gi|6978491	Aldose reductase	35 774	6.26	546	59	Down
9	gi|6978491	Aldose reductase	35 774	6.26	133	21	Down
10	gi|62653546	Predicted: glyceraldehyde-3-phosphate dehydrogenase-like	35 760	8.44	292	37	None
11	gi|62653546	Predicted: glyceraldehyde-3-phosphate dehydrogenase-like	35 760	8.44	596	45	Down
13	gi|6723180	Plasma glutathione peroxidase precursor	25 425	8.26	140	20	Up

Spots from No. 4 and 12 in 2DGE maps were identified and excluded, and the most frequently observed contaminant peptides were derived from keratin. * Expression variation in saphenous nerve was compared to motor branches of femoral nerves. Coverage: Ratio of protein sequence covered by matched peptides; pI: isoelectric point; 2DGE: two-dimensional gel electrophoresis; NanoUPLC-nano-ESI-MS/MS: nano ultra-high performance liquid chromatography electrospray ionization mass spectrometry tandem mass spectrometry.

Table 2 NanoUPLC-nano-ESI-MS/MS analysis results from protein spots with significant differential expression in 2DGE maps of ventral and dorsal roots

Spot No.	NCBI accession No.	Protein name	Molecular weight (Da)	pI	Protein score	Sequence coverage (%)	Expression variation in dorsal roots*
3	gi|149024688	rCG31027, isoform CRA_b	24 935	6.54	125	22	None
7	gi|46485429	Lactoylglutathione lyase	20 806	5.12	71	9	Up
8	gi|8918488	Adenylate kinase isozyme 1	21 588	7.71	162	31	Up
9	gi|149036630	Poly(rC)-binding protein 1	35 490	7.03	493	52	Down
10	gi|6978661	Creatine kinase M-type	42 992	6.58	65	10	None
11	gi|55628	Unnamed protein product	68 674	6.09	37	3	Up
12	gi|1334163	Unnamed protein product	39 134	6.78	431	35	Up
14	gi|149036630	Poly(rC)-binding protein 1	35 490	7.03	264	44	Down
16	gi|149036630	Poly(rC)-binding protein 1	35 490	7.03	55	10	Down

Spots from No. 1, 2, and 13 in 2DGE maps were not successfully identified. Spots from No. 4, 5, 6, and 15 in 2DGE maps were identified and excluded as contaminant peptides derived from keratin. *Expression variation in dorsal roots was compared to ventral roots. Coverage: Ratio of protein sequence covered by matched peptides; pI: isoelectric point; 2DGE: two-dimensional gel electrophoresis; NanoUPLC-nano-ESI-MS/MS: nano ultra-high performance liquid chromatography electrospray ionization mass spectrometry tandem mass spectrometry.

DISCUSSION

With the characterization of sensitive, accurate, and specific quantitative methods, nanoUPLC-nano-ESI-MS/MS has been developed and widely used in fields of biology[11], medicine[12], pharmacology[13], and food safety[14-15]. The present study employed proteomic techniques using nanoUPLC-nano-ESI-MS/MS to separately screen two groups of over-expressed proteins from proximal and distal segments of motor and sensory nerves. A total of 12 protein types were differentially expressed in sensory and motor nerve tissues, including several novel proteins specific to sensory or motor nerves.

Three protein spots of transgelin, with significant differential expression in 2DGE maps, was expressed at higher levels in the saphenous nerve compared with the motor branch of the femoral nerve. Transgelin, also termed Sm22-alpha, is a transformation-sensitive and rapidly gelled actin that was initially discovered in chicken gizzard smooth muscle[16-17], followed by smooth muscle cells in the aorta, uterus, lung, and intestine[18]. Transgelin expression is not restricted to smooth muscles, because the protein is also present in normal mesenchymal cells and secondary cultures of mouse and rat embryo fibroblasts, but is absent in many apparently normal fibroblast cell lines[19]. Transgelin down-regulation may, therefore, be an early and sensitive marker for the onset of transformation[19]. Transgelin, which often exhibits diminished expression in cancer, also regulates matrix metalloproteinases 9 expression, likely *via* interference with extracellular signal-regulated kinase 1 and 2 signaling[20].

In the motor branch of the femoral nerve, expression of aldose reductase and a GAPDH-like protein was significantly increased compared to other proteins in the saphenous nerve. Aldose reductase is a monomeric 5,10-methylenetetrahydrofolate reductase-dependent oxidoreductase with wide substrate specificities for carbonyl compounds; it is generally used as the first step in fructose synthesis from glucose[21]. GAPDH catalyzes an important energy-yielding step in carbohydrate metabolism[22]–the reversible oxidative phosphorylation of glyceraldehyde-3-phosphate in the presence of inorganic phosphate and nicotinamide adenine dinucleotide. Although GAPDH has been commonly used as a housekeeping gene due to its stably and constitutively high level expression, researchers have reported varying regulation under specific conditions[23]. Increased expression of aldose reductase and GAPDH in motor branches could indicate that energy metabolism in motor nerves prefers the glycolysis and gluconeogenesis pathway, which takes place without ATP.

A protein spot, which was expressed relatively greater in dorsal root 2DGE maps than in ventral roots, was identified as adenylate kinase isozyme 1. Adenylate kinase is a phosphotransferase enzyme that catalyzes reversible conversion of magnesium adenosine triphosphate + adenosine monophosphate to magnesium adenosine monophosphate + adenosine monophosphate, and also plays an important role in cellular energy homeostasis[24]. Adenylate kinase 1 is the cytosolic isozyme of adenylate kinase and is present in skeletal muscle, brain, and erythrocytes[25]. Up-regulation of this enzyme in sensory nerves could further reveal differences between energy metabolism pathways in motor and sensory nerves, although its exact role remains to be determined.

In ventral root 2DGE maps, three protein spots were expressed greater than in dorsal roots and were identified as PCBP1. PCBP1 is a member of the heterogeneous ribonucleoprotein family and participates in transcription and translation regulation[26]. In the prespliceosomal complex, PCBP1 and small nuclear ribonucleoprotein U1 bind together and are associated with silencing of pseudoexon splicing[27]. In addition to serving as an important RNA-binding protein[28], PCBP1 acts as a DNA-binding protein[29]. In a recent study, transcripts targeted by the lack of PCBP1 were identified, and some identified transcripts were grouped into neuronal categories. For example, microtubule-associated protein tau, roundabout homolog 2, slit homolog 2, and semaphoring 6A were grouped into axonogenesis, neurogenesis, axon guidance, neurite morphogenesis, and neuron morphogenesis during differentiation[30]. Consistent with the present ventral root results, PCBP1 could serve as a specific protein marker for motor nerves.

It should be noted that although differentially expressed proteins in motor and sensory nerves were observed, consistency was low between proximal and distal nerve segments. Only an unnamed protein product (gi|1334163) was detected with high expression in the saphenous nerve and ventral roots. These results were likely due to different cells and extracellular matrix of proximal and distal nerve segments. *In situ* immunohistochemistry, as well as *in vitro* pure cell proteome analysis, could help to identify these mechanisms of action.

In conclusion, through the use of 2DGE and nanoUPLC-nano-ESI-MS/MS analysis, comparisons between motor branches of the femoral nerve and saphenous nerve, as well as ventral and dorsal roots, several proteins were identified for the first time to be differentially expressed in sensory and motor nerves. These proteins could play roles in specific nerve regeneration. Based on these results, functional analyses of these differentially expressed proteins, as well as the differences between sensory and motor nerves following injury, should be performed in the further.

MATERIALS AND METHODS

Design

A proteomics experiment.

Time and setting

The experiments were performed at the Institute of Orthopedics, Chinese PLA General Hospital and National Center of Biomedical Analysis, China from June 2010 to March 2011.

Materials

Nine female, specific pathogen-free, Wistar rats, weighing 250–280 g, were provided by the Experimental Animal Center of Academy of Military Science, China. The rats were housed at 26°C with 45–55% relative humidity, and were maintained in a 12-hour light/12-hour dark cycle. The rats were allowed free access to food and water.

Methods

Sensory and motor nerve harvesting

Following general phenobarbital anesthesia (40 mg/kg, intraperitoneal), the bilateral inguen was incised to expose the femoral nerve bifurcation. Motor branch segments to the quadriceps muscles and saphenous nerves, respectively, which were distal to the bifurcation, were carefully separated and transected using microinstruments. A midline skin incision was made in the lumbar region to expose lumbar vertebrae at the L_{1-5} level, and the lamina were subsequently removed. Dura mater and arachnoid membranes were also incised, and segments of ventral and dorsal roots were gently and sequentially removed and carefully transected from the spinal cord using fine forceps.

Protein sample preparation

Total protein from two couples of sensory and motor nerve tissues were extracted according to a previously described method[31]. Briefly, nerve segments were homogenized in liquid nitrogen, transferred to an Eppendorf tube on ice, and further homogenized in buffer (40 mM Tris-base, 7 M urea, 2 M thiourea, 2% CHAPS, 1% dithiothreitol, and 1 mM ethylenediamine tetraacetic acid) at a ratio of 1: 10 (v/v). Subsequently, a 10-μL cocktail of protease inhibitors (0.7 mg/mL pepstatin A, 0.5 mg/mL leupeptin, 0.3 mg/mL EDTA-Na_2, and 100 mg/mL phenylmethyl sulfonylfluoride; Sigma, St. Louis, MO, USA) was added to the Tris buffer. Following sonication, 5 μL of 10 mg/mL DNA and RNA enzyme (Sino-American Biotechnology, Montgomery, Pennsylvania, USA) were added to the sample. The homogenate was maintained on ice for 20 minutes, followed by centrifugation at 40 000 × *g* for 20 minutes at 4°C. The supernatant was collected and stored at −80°C. Protein was quantified using the Bradford method[32].

2DGE and image analysis

A 2DGE protocol was performed as previously described[33] and was repeated three times. Briefly, 160 μg of nerve tissue proteins was loaded onto a ceramic immobilized pH gradient gel strip holder using the in-gel rehydration mode. The IPG gel was rehydrated for 12 hours under a low voltage of 30 V at 20°C. Isoelectric focusing was performed under the following parameters: 500 V for 1 hour, 1 000 V for 1 hour, and 8 000 V for 10 hours. Focused strips were placed in sodium dodecyl sulfate-balanced solution twice at room temperature for 15 minutes each, followed by 13% sodium dodecyl sulfate polyacrylamide gel vertical electrophoresis using the PROTEN II xi Cell system (Bio-Rad, Hercules, CA, USA) in a circulating bath at 16°C with 40 mA for 40 minutes and 60 mA for 5 hours, respectively. To ensure reliability, each type of nerve tissue was pooled and run on three separate gels. The gels were stained with silver using a mass spectrometry compatible method for clearer visualization[34-35] and were scanned using an image scanner. A total of three images from each nerve were analyzed using Imagemaster 2D platinum software (Amersham Pharmacia Biotech, Staffanstor, Sweden). Statistical comparisons (analysis sets using Student's *t*-test, $P < 0.05$) were performed between motor branches of femoral nerves and saphenous nerves, as well as ventral roots and dorsal roots. Protein spots with > 2-fold relative expression intensity differences and identical changes in repeated experiments were regarded as differential protein spots.

In-gel digestion

The stained protein spots were excised from the gel and digested using trypsin[36]. Briefly, after the gel pieces were rinsed three times with Milli-Q water, the samples were treated incubated in 50 mM NH_4HCO_3 and 50% acetonitrile. The spots were then dehydrated for 10 minutes in 100% acetonitrile, dried at room temperature, and then rehydrated in a digestion buffer of 25 mM NH_4HCO_3 containing 0.01 μg/μL modified trypsin. The gel slices were completely covered by digestion buffer and incubated overnight at 4°C. The tryptic peptides were extracted from the gel at room temperature, rinsed once in water, twice in 5% trifluoroacetic acid, and vortexed in 50% acetonitrile. The samples were finally concentrated by drying under vacuum conditions.

NanoUPLC-nano-ESI-MS/MS analysis

NanoUPLC-nano-ESI-MS/MS analysis was performed on a Waters ACQUITY UPLC system coupled with a Waters Micromass Q-tof microTM Synapt High Definition Mass Spectrometer equipped with electrospray ionization (Waters Corporation, Milford, MA, USA). For reversed-phase UPLC analysis, an ACQUITY UPLC™ BEH C_{18} column (75 μm × 250 mm, 1.7 μm) was used. Column temperature was maintained at 35°C; flow rate of the mobile phase was 200 nL/min; injection volume was fixed at 2.0 μL. Mobile phase A consisted of 0.1% formic acid in acetonitrile, and mobile phase B consisted of 0.1% formic acid in water. The column was eluted with a linear gradient of 1–40% mobile phase B initially for 80 minutes, followed by 40–80% mobile phase B for 80–90 minutes, and maintained at 80% mobile phase B for 10 minutes. The column was then returned to 1% mobile phase B for 100–105 minutes and maintained for 15 minutes, followed by at 80% mobile phase B for 105–120 minutes. For noaoUPLC-nano-ESI-MS/MS analysis,

the optimal conditions were as follows: capillary voltage of 2 500 V, sample cone voltage of 35 V, source temperature of 90°C. Data were acquired from 350 to 1 600 Da for MS mode, as well as from 50 to 2 000 Da for MS/MS mode.

Data processing and protein identification

Mass spectrometry data were imported into Markerlynx 4.1 software for peak detection and alignment. All data were normalized to summed total ion intensity per chromatogram, and resultant data matrices were introduced to data analysis software PLGS v2.3. Protein identification based on raw MS/MS data was searched in the NCBInr database using the Mascot MS/MS Ions search engine (http://www.matrixscience.com). The sequence database was the Rattus category of the 20110312 and 20110409 release of the NCBInr database. At most, one missed cleavage was allowed. Methionine residues present in the proteolytic peptides were empirically regarded as partially oxidized. Cysteine residues were identified as partially alkylated by iodoacetamide. MS and MS/MS tolerance was 0.5 Da. Individual ions scores > 34 indicated identity or extensive homology ($P < 0.05$).

Author contributions: Zhiwu Ren was responsible for experimental conditions, study design, study performance, data integration, and manuscript revision. Jiang Peng and Shibi Lu were responsible for selection of study methods and technical support, and were also responsible for funding. The remaining authors were responsible for experimental conduction.

Conflicts of interest: None declared.

Funding: The study was supported by the Key Projects in the National Science & Technology Pillar Program, No. 2009BAI87B02; the National Natural Science Foundation of China, No. 31100696; the National Basic Research Program of China (973 Program), No. 2012CB518106.

Ethical approval: All surgical and animal care procedures were conducted according to the local guidelines of the experimental animal committee.

Acknowledgments: We thank Hongli Wang and other staff at the National Center of Biomedical Analysis, China for technical support.

REFERENCES

[1] Fields RD, Stevens-Graham B. New insights into neuron-glia communication. Science. 2002;298(5593):556-562.
[2] de Ruiter GC, Malessy MJ, Alaid AO, et al. Misdirection of regenerating motor axons after nerve injury and repair in the rat sciatic nerve model. Exp Neurol. 2008;211(2):339-350.
[3] Brushart TM. Motor axons preferentially reinnervate motor pathways. J Neurosci. 1993;13(6):2730-2738.
[4] Robinson GA, Madison RD. Developmentally regulated changes in femoral nerve regeneration in the mouse and rat. Exp Neurol. 2006;197(2):341-346.
[5] Hoke A, Redett R, Hameed H, et al. Schwann cells express motor and sensory phenotypes that regulate axon regeneration. J Neurosci. 2006;26(38):9646-9655.
[6] Brushart TM. Preferential reinnervation of motor nerves by regenerating motor axons. J Neurosci. 1988;8(3):1026-1031.
[7] Clauser KR, Hall SC, Smith DM, et al. Rapid mass spectrometric peptide sequencing and mass matching for characterization of human melanoma proteins isolated by two-dimensional PAGE. Proc Natl Acad Sci U S A. 1995;92(11):5072-5076.
[8] Shevchenko A, Jensen ON, Podtelejnikov AV, et al. Linking genome and proteome by mass spectrometry: large-scale identification of yeast proteins from two dimensional gels. Proc Natl Acad Sci U S A. 1996;93(25):14440-14445.
[9] Qualtieri A, Le Pera M, Urso E, et al. Two-dimensional gel electrophoresis of peripheral nerve proteins: Optimized sample preparation. J Neurosci Methods. 2007;159(1):125-133.
[10] Ding Q, Xiao L, Xiong S, et al. Unmatched masses in peptide mass fingerprints caused by cross-contamination: an updated statistical result. Proteomics. 2003;3(7):1313-1317.
[11] Yin W, Zhou X, Qiao J, et al. Study the pharmacokinetics of AV-45 in rat plasma and metabolism in liver microsomes by ultra-performance liquid chromatography with mass spectrometry. Biomed Chromatogr. in press.
[12] Zhang H, Young SP, Auray-Blais C, et al. Analysis of glycosaminoglycans in cerebrospinal fluid from patients with mucopolysaccharidoses by isotope-dilution ultra-performance liquid chromatography-tandem mass spectrometry. Clin Chem. 2011;57(7):1005-1012.
[13] Huang C, Gao J, Miao L. Simultaneous determination of flucloxacillin and ampicillin in human plasma by ultra performance liquid chromatography-tandem mass spectrometry and subsequent application to a clinical study in healthy Chinese volunteers. J Pharm Biomed Anal. in press.
[14] Fusch G, Choi A, Rochow N, et al. Quantification of lactose content in human and cow's milk using UPLC-tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci. 2011;879(31):3759-3762.
[15] Li Y, Wang Z, De Saeger S, et al. Determination of deoxynivalenol in cereals by immunoaffinity clean-up and ultra-high performance liquid chromatography tandem mass spectrometry. Methods. in press.
[16] Lees-Miller JP, Heeley DH, Smillie LB, et al. Isolation and characterization of an abundant and novel 22-kDa protein (SM22) from chicken gizzard smooth muscle. J Biol Chem. 1987;262(7): 2988-2993.
[17] Nishida W, Kitami Y, Abe M, et al. Gene cloning and nucleotide sequence of SM22 alpha from the chicken gizzard smooth muscle. Biochem Int. 1991;23(4):663-668.
[18] Solway J, Seltzer J, Samaha FF, et al. Structure and expression of a smooth muscle cell-specific gene, SM22 alpha. J Biol Chem. 1995;270(22):13460-13469.
[19] Lawson D, Harrison M, Shapland C. Fibroblast transgelin and smooth muscle SM22alpha are the same protein, the expression of which is down-regulated in many cell lines. Cell Motil Cytoskeleton. 1997;38(3):250-257.
[20] Nair RR, Solway J, Boyd DD. Expression cloning identifies transgelin (SM22) as a novel repressor of 92-kDa type IV collagenase (MMP-9) expression. J Biol Chem. 2006;281(36): 26424-26436.
[21] Nishimura C, Matsuura Y, Kokai Y, et al. Cloning and expression of human aldose reductase. J Biol Chem. 1990;265(17): 9788-9792.
[22] Lebherz HG, Rutter WJ. Glyceraldehyde-3-phosphate dehydrogenase variants in phyletically diverse organisms. Science. 1967;157(793):1198-1200.
[23] Barber RD, Harmer DW, Coleman RA, et al. GAPDH as a housekeeping gene: analysis of GAPDH mRNA expression in a panel of 72 human tissues. Physiol Genomics. 2005;21(3): 389-395.
[24] Bowman JE, Frischer H, Ajmar F, et al. Population, family and biochemical investigation of human adenylate kinase polymorphism. Nature. 1967;214(5093):1156-1158.
[25] Singer JD, Brock DJ. Half-normal adenylate kinase activity in three generations. Ann Hum Genet. 1971;35(1):109-114.

[26] Leffers H, Dejgaard K, Celis JE. Characterisation of two major cellular poly(rC)-binding human proteins, each containing three K-homologous (KH) domains. Eur J Biochem. 1995;230(2): 447-453.
[27] Akker SA, Misra S, Aslam S, et al. Pre-spliceosomal binding of U1 small nuclear ribonucleoprotein (RNP) and heterogenous nuclear RNP E1 is associated with suppression of a growth hormone receptor pseudoexon. Mol Endocrinol. 2007;21(10):2529-2540.
[28] Ostareck-Lederer A, Ostareck DH. Control of mRNA translation and stability in haematopoietic cells: the function of hnRNPs K and E1/E2. Biol Cell. 2004;96(6):407-411.
[29] Berry AM, Flock KE, Loh HH, et al. Molecular basis of cellular localization of poly C binding protein 1 in neuronal cells. Biochem Biophys Res Commun. 2006;349(4):1378-1386.
[30] Huo LR, Ju W, Yan M, et al. Identification of differentially expressed transcripts and translatants targeted by knock-down of endogenous PCBP1. Biochim Biophys Acta. 2010;1804(10): 1954-1964.
[31] Molloy MP, Herbert BR, Walsh BJ et al. Extraction of membrane proteins by differential solubilization for separation using two-dimensional gel electrophoresis. Electrophoresis. 1998;19(5): 837-844.
[32] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 1976;72:248-254.
[33] Ding Q, Wu Z, Guo Y, et al. Proteome analysis of up-regulated proteins in the rat spinal cord induced by transection injury. Proteomics. 2006;6(2):505-518.
[34] Shevchenko A, Wilm M, Vorm O, et al. Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels. Anal Chem. 1996;68(5):850-858.
[35] Jin LT, Hwang SY, Yoo GS, et al. A mass spectrometry compatible silver staining method for protein incorporating a new silver sensitizer in sodium dodecyl sulfate-polyacrylamide electrophoresis gels. Proteomics. 2006;6(8):2334-2337.
[36] Hellman U, Wernstedt C, Gonez J, et al. Improvement of an "In-Gel" digestion procedure for the micropreparation of internal protein fragments for amino acid sequencing. Anal Biochem. 1995; 224(1):451-455.

(Edited by Gu XS, Yang YM/Su LL/Wang L)