Journal of Neuroimmunology 250 (2012) 71–76

Contents lists available at SciVerse ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Decreased serum levels of nucleolin protein fragment, as analyzed by bead-based proteomic technology, in multiple sclerosis patients compared to controls

Jianghong Liu [a,1], Linlin Yin [b,*,1], Huiqing Dong [a], Erhe Xu [a], Lan Zhang [b], Yuchen Qiao [a], Yuan Liu [a], Lin Li [b,**], Jianping Jia [a,**]

[a] Department of Neurology, Xuan Wu Hospital of Capital Medical University, 45 Changchun Street, Beijing, 100053, PR China
[b] Department of Pharmacology, Xuan Wu Hospital of Capital Medical University, Beijing Geriatric Medical Research Center, Key Laboratory for Neurodegenerative Disease of Ministry of Education, 45 Changchun Street, Beijing, 100053, PR China

ARTICLE INFO

Article history:
Received 20 January 2012
Received in revised form 1 May 2012
Accepted 2 May 2012

Keywords:
Relapsing–remitting multiple sclerosis
Proteomic technology
Nucleolin protein

ABSTRACT

In order to investigate the biomarkers associated with relapsing–remitting multiple sclerosis (RRMS), we analyzed 72 patients with RRMS and 65 healthy controls using proteome technology. Peptides in sera were purified using magnetic beads, and analyzed by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry and ClinProTool software. Thirteen peptides were significantly different between patients with RRMS and healthy controls. Furthermore, a pattern of peaks was selected for genetic algorithm (GA), supervised neural network (SNN) and quick classifier (QC) model building. Among these three models, GA method was best with 93.49% of recognition capability and 82.66% of cross-validation and discriminated the proteomic spectra in patients with RRMS from healthy controls, with a sensitivity of 80% and a specificity of 91.3%. Meanwhile, the first peptide with m/z 2023.3 was identified as fragment of nucleolin protein. There is a possible relationship between the fragment peptide of nucleolin and the trigger of relapse in MS. Sera nucleolin may serve as a possible biomarker of RRMS.

1. Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the human central nervous system (CNS) with heterogeneous pathophysiological and clinical manifestations and a very complicated etiology (Kornek and Lassmann, 2003; Hafler, 2004), which is possibly triggered by a complex interplay of multiple genetic (International Multiple Sclerosis Genetics Consortium et al., 2007), infectious (Steiner et al., 2001) and environmental factors (Koning et al., 2007). However, at present, the true triggering mechanisms of the disease have not been clearly defined (Hohlfeld and Wekerle, 2004).

In most MS patients, this disorder is characterized by a relapsing–remitting course (Polman et al., 2010). Therefore, it is clinically useful to find biomarkers that correlate with relapsing–remitting MS (RRMS), because this would enable the prediction of relapses and the early initiation of therapeutic interventions. Many studies have investigated serum or cerebrospinal fluid (CSF) biomarkers in relation to RRMS (Mahad et al., 2003; Kuenz et al., 2005; Aranami et al., 2006; Frisullo et al., 2006; Kanesaka et al., 2006). However, the development of biomarkers in MS has not reached a mature stage and there are no identified candidates used in clinical. Therefore, more reliable methods need to be developed.

Matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry is being widely applied to analyze serum samples for the diagnosis of human diseases and for the identification of potential biomarkers (Qiu et al., 2009; Liang et al., 2010). Peptidome analysis based on mass spectrometric screening methods have been developed and offered a high-throughput approach to discover new potential biomarkers in various body fluids, which could be useful in the diagnosis and treatment of disease (Diamandis, 2004; Hortin, 2006). In the present study, we used affinity bead purification and nano-liquid chromatography/electrospray ionization-mass spectrum to detect serum markers in patients with RRMS and identified the first peptide (2023.3 m/z). Furthermore, we validated the decreased serum levels of nucleolin peptides by immunoblotting method.

2. Materials and methods

2.1. Patients and blood sample preparation

Fasting serum samples were obtained from a total of 72 patients who had been diagnosed with RRMS from the Department of Neurology, Xuan Wu Hospital of Capital Medical University included from February 2010 till November 2010. Serum samples were collected at the time of initial relapsing phase. We examined all the MS patients' clinical data including past medical history, medication, stage at diagnosis, immunological and brain and spinal cord magnetic resonance image (MRI) data obtained at the day we collected the patients' serum samples. Age, gender, disease

* Corresponding author. Tel.: +86 10 8319 8881; fax: +86 10 8315 4745.
** Corresponding authors.
E-mail address: yinll913@126.com (L. Yin).
[1] Both authors made equal contributions to this work.

0165-5728/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneuroim.2012.05.002

course and duration, expanded disability status scale (EDSS) and a number of relapses were all recorded. All patients (21 men and 51 women) fulfilled the revised McDonald criteria of MS (Polman et al., 2010). The healthy control group consisted of 65 individuals, all from our hospital healthy check center. Table 1 provides information pertaining to the patients utilized for this study. The samples were all collected, processed, and stored in a similar fashion according to standard protocol. After sample collection, the vacuum serum (collected in a red-top tube containing no preservatives or anticoagulant) was allowed to clot or to sediment at room temperature (about 25 °C) for at least 2 h and then centrifuged at 3000 *g* for 15 min. Samples were divided in aliquots of 50 μl immediately and frozen at −80 °C refrigerator until use.

All participants gave informed consent to enter into the study, which was conducted according to the provisions of the Helsinki Declaration and approved by the ethics committee of Xuan Wu Hospital.

2.2. Serum pretreatment with magnetic beads

All the serum samples were fractionated using weak cation exchange magnetic beads (MB-WCX) according to the manufacturers' instructions through a standard protocol (ClinProt™, Bruker Daltonics). Samples were purified and isolated through three steps: binding, washing, and elution. Firstly, 10 μl beads, 10 μl MB-WCX binding solution (BS) and 5 μl serum samples were added in a tube and mixed carefully and incubated for 5 min. The tube was placed on the magnetic bead separation device (Bruker Daltonics) and the beads were collected at the tube wall for 1 min. Then, the supernatant was removed and 100 μl magnetic bead washing solution (WS) was added and these were mixed thoroughly. After washing for three times, the supernatant was removed, another 5 μl magnetic bead eluting solution (ES) was added, and the beads at the tube wall in the separation device for 2 min were collected. Finally, the clear supernatant into a fresh tube was transferred, 5 μl magnetic bead stabilizing solution (SS) to the eluate was added and these were mixed intensively, the sample tube was stored in a refrigerator (−20 °C).

2.3. Anchor chip spotting and protein profiling

The eluted sample was diluted 1:10 in matrix solution α-cyano-4-hydroxycinnamic acid (0.3 g/l in ethanol: acetone 2:1), which was daily prepared. For example, 1 μl of eluate was added to 10 μl matrix solution. Then 1 μl of the mixture was spot onto a MALDI-TOF mass spectrometry target (AnchorChip™, Bruker Daltonics) and dried at room temperature before analysis. MALDI-TOF mass spectrometry measurements were performed using an Autoflex TOF instrument (Bruker Daltonics). Eleven peptides were used as external standard preparation and the average molecular weight deviation was no more than 100 μg/g. Before data acquisition of every eight samples, the standard preparation would be calibrated. Additionally, 13 reference sera were added as external standard too. The coefficient of variability less than 30% indicated that the system ran well. Profile spectra were acquired from an average of 400 laser shots per sample. The peak m/z values or intensities in the mass range of 1000–10,000 Da were determined.

Table 1
Clinical characteristics of patients with relapsing–remitting multiple sclerosis (RRMS) and healthy controls used in model building and validation.

	Training set	Testing set
RRMS		
Mean age (range)	40.5 (17–63)	38.7 (21–60)
Sex (men/women)	16/31	5/20
Mean disease duration (years)	3.5 (range 0–17)	2.1 (range 0–13)
EDSS (number of subjects)		
0–3.5	31	17
4.0–5.5	10	7
6.0–8.0	6	1
Mean EDSS (range)	3.2 (0–7.0)	2.9 (0–6.0)
Treatments within last 3 months		
Corticosteroids	19	12
Globulin-r	5	1
Interferon-β	1	0
Mitoxantrone	4	1
Traditional Chinese herbs	2	3
Other treatments (vitamin B, aspirin, antibiotics)	4	8
No treatment	16	4
Healthy controls		
Mean age (range)	37.3 (16–67)	37.9 (20–61)
Sex (men/women)	18/24	11/12

2.4. Peptide sequence

Experiment for 2023.3 m/z peptide identification was performed using a nano-liquid chromatography–electrospray ionization–tandem mass spectrometry (nano-LC/ESI–mass spectrometry/mass spectrometry) system consisting of an Aquity UPLC system (Waters) and a LTQ Obitrap XL mass spectrometer (Thermo Fisher) equipped with a nano-ESI source. The peptide solutions were loaded to a C18 trap column (nanoACQUITY) (180 μm × 20 mm × 5 μm (symmetry)). The flow rate was 15 μl/min. Then the desalted peptides were analyzed by C18 analytical column (nanoACQUITY) (75 μm × 150 mm × 3.5 μm (symmetry)) at a flow rate of 400 nl/min. The mobile phases A (5% acetonitrile, 0.1% formic acid) and B (95% acetonitrile, 0.1% formic acid) were used for analytical columns. The gradient elution profile was as follows: 5%B–50%B–80%B–80%B–5%B–5%B in 100 min. The MS instrument was operated in a data-dependent model. The range of full scan was 400–2000 m/z with a mass resolution of 100,000 (m/z 400). The eight most intense monoisotope ions were the precursors for collision induced dissociation. Mass spectrometry/mass spectrometry spectra were limited to two consecutive scans per precursor ion followed by 60 s of dynamic exclusion.

2.5. Bioinformatics and identification of protein markers

The obtained chromatograms were analyzed with Bioworks Browser 3.3.1 SP1 and the resulting mass lists were used for database search using Sequest™ (IPI Human (3.45)). Parameters for generating peak list were as follows: parent ion and fragment mass relative accuracy were set at 50 μg/g and 1 Da, respectively.

2.6. Statistical analysis

ClinProTools (ClinProt software version 2.0, Bruker Daltonics) was used to subtract baseline, normalize spectra (using total ion current) and determine peak m/z values and intensities in the mass range of 1000 to 10,000 Da. The signal-to-noise (S/N) ratio should be higher than five. To align the spectra, a mass shift of no more than 0.1% was determined. The peak area was used as quantitative standardization. Genetic algorithm (GA), supervised neural network (SNN) and quick classifier (QC) contained in this software suite were used to establish the pattern for identifying MS from healthy controls. After each profile was generated, a 20% leave out cross-validation process was performed within the software. Comparison of relative peak intensity levels between classes was also calculated within the software suite. Student's *t* test was used for analysis of normally distributed continuous data, while Wilcoxon test for non-normally distributed continuous data. Chi-square test was used for categorical data analysis. $P<0.05$ was considered statistically significant.

2.7. Western blot analysis for validation

Serum samples were denaturalized and protein concentrations were measured using bicinchoninic acid (BCA) kit assay. Equal amounts were electrophoresed on 4–20% SDS-polyacrylamide gradient gels

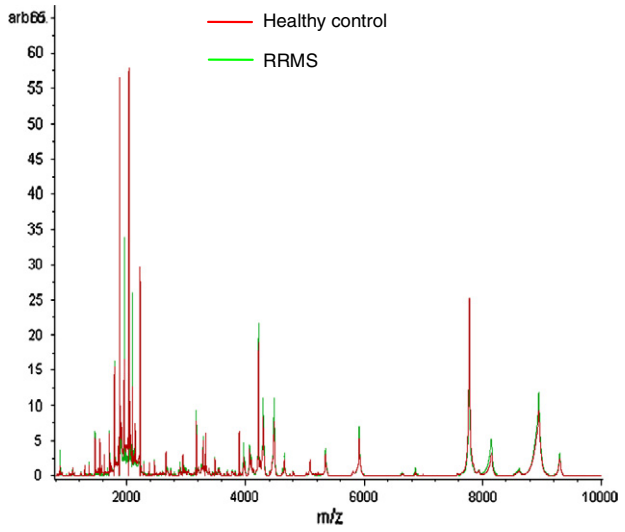

Fig. 1. Average spectrum profiles of training set. Average spectrum profiles of patients with relapsing–remitting multiple sclerosis (RRMS) (green) and healthy controls (red). x-axis: molecular mass (m/z); y-axis: relative intensity.

(Amersham) and transferred onto Hybond™ nitrocellulose membranes (Amersham). Primary antibody for anti-nucleolin protein (Abcam, 1:1000) was used for immunoblotting, followed by secondary antibody (1:2000 HRP conjugated anti-rabbit; Santa Cruz). Blots were developed with 1:1 solution of Super Signal West Pico Chemiluminescent Substrate and Luminol/Enhancer (Thermo). Blots of loading control were probed for β-actin (1:2000, Santa Cruz). The optical density of bands was quantified using BioRad Quantity One analysis software and the ratio to β-actin was analyzed and expressed as values for the graphs.

3. Results

3.1. MALDI-TOF mass spectrometry analysis of peptides in serum from RRMS patients and healthy control

In order to screen serum biomarkers of interest for RRMS, 72 patients with RRMS and 65 healthy control serum samples were analyzed by MALDI-TOF mass spectrometry. 47 patients with RRMS and 42 healthy controls were assigned randomly to a training set and the remaining was used as testing set. The average spectrum profiles of training set were shown in Fig. 1. We used ClinProTools software to discover 85 peaks in all training set samples. Thirteen of these peaks were significantly different between patients with RRMS and healthy controls (Table 2).

3.2. Model building and blind test

As demonstrated in Table 3, a pattern of peaks was selected for genetic algorithm (GA), supervised neural network (SNN) and quick classifier (QC) model building. In GA model, the result of the cross-validation was 82.66%. However, the cross-validations of SNN and QC were 75.52% and 79.74%, respectively. Among these three, model built by GA method was best with 93.49% of recognition capability and 82.66% of cross-validation. Then GA model were selected to discriminate the proteomic spectra in patients with RRMS from the healthy control, with a sensitivity of 80.0% and specificity of 91.3%. The top 2 significant peaks with m/z 2023.3 Da (x axis) and 2039.2 Da (y axis) were plotted. Values were represented peptide abundance ratio and showed significant difference between patients and healthy controls (Fig. 2).

3.3. Identification of nucleolin protein fragments by proteome analysis

With a nano-LC/ESI–mass spectrometry/mass spectrometry detection, the first peptide sequence of 2023.3 m/z was identified. The Sequest search reported that the peptide was a partial sequence of nucleolin protein with the sequence SISLYYTGEKGQNQDYR. Parameters were as follows: false discovery rate (FDR) 0.000499<0.01; delta Cn 0.413; XCorr 2.506. Nucleolin protein played an important role in autoimmune diseases (Storck et al., 2007) and a representative sequencing result of m/z 2023.3 was shown in Fig. 3. Table 4 showed the identification of specific b and y ions for nucleolin peptide sequence.

3.4. Validation decreased serum levels of nucleolin protein fragment by immunoblot

Next, we sought to determine the level of nucleolin protein in blood samples by western blot analysis. Among blood samples from all cases, nucleolin immunoreactive band was consistently observed, however, only weak or no bands were seen in MS cases (Fig. 4A). Quantification of bands from western blot analysis showed decreased level of nucleolin protein in blood samples from some MS cases, relative to age-matched control samples significantly (Fig. 4B).

Table 2
Different peaks between patients with RRMS and healthy controls in training set.

Mass (m/z)	PAD[a]	PTTA[b]	PWKW[c]	Ave 1[d]	Ave 2[e]	SD 1[f]	SD 2[g]
2023.3	0.0000803	0.00000479	0.00000566	470.64	185.03	270.17	129.36
2039.18	<0.000001	0.00000582	0.0000285	99.23	40.72	55.96	33.15
2083.16	<0.000001	0.00164	0.000243	92.35	192.42	95.37	133.57
1935.73	0.00000286	0.000074	0.000278	76.79	33.41	49.56	21.67
1946.07	<0.000001	0.000508	0.00176	122.7	232.96	65.03	150.93
8602.94	<0.000001	0.135	0.00846	69.58	114.87	89.42	98.54
1532.61	<0.000001	0.138	0.00846	35.61	21.41	32.55	27.15
1279.44	<0.000001	0.146	0.0214	14.67	10.48	9.12	9.07
1012.26	0.00000673	0.00863	0.022	4.65	2.92	2.77	1.49
861.66	<0.000001	0.0486	0.0228	11.61	25.09	11.1	30.03
8134.95	<0.000001	0.0309	0.0384	237.7	430.16	161.92	383.24
4285.26	<0.000001	0.35	0.0384	146.22	211.11	182.29	215.6
6853	<0.000001	0.0486	0.0384	17.48	32.9	18.88	31.3

[a] P-value was calculated with Anderson–Darling test; values higher than 0.05 indicated normal distribution.
[b] P-value was calculated with *t*-test; values lower than 0.05 indicated statistical relevance.
[c] P-value was calculated with Wilcoxon/Kruskal Wallis test; values lower than 0.05 suggest statistical relevance.
[d] Average area of peak of healthy controls.
[e] Average area of peak of patients with relapsing–remitting multiple sclerosis (RRMS).
[f] SD of the peak area of healthy controls.
[g] SD of the peak area of patients with relapsing–remitting multiple sclerosis (RRMS).

Table 3
Profiles of the algorithm models.

Model generation	Peak selection (m/z)	Recognition capability	Sensitivity	Specificity	Cross-validation
Genetic algorithm (GA)	5172.65, 2023.3, 4127.36, 4069.86, 5354.29	93.49%	89.36%	97.62%	82.66%
Supervised neural network (SNN)	2128.88, 5172.65, 5112.34, 4251.38	86.90%	100%	73.81%	75.52%
Quick classifier (QC)	2023.3, 2039.18	81.59%	89.36%	73.81%	79.74%

4. Discussion

In the present study, we used bead-based proteomic technology to find potential biomarkers for RRMS. Our data showed that nucleolin fragments detected in blood of patients were less frequently than that in healthy individuals (Fig. 3, Tables 2 and 4). Western blotting analysis using the human serum peptides showed decreased serum levels of nucleolin protein fragment from RRMS patients compared with healthy controls (Fig. 4). Our findings demonstrated that nucleolin protein fragments might be a candidate blood biomarker for RRMS. The spectra of protein fragments were used to generate disease model that successfully classified blinded samples from patients with RRMS and healthy controls with high sensitivity and specificity. Our results demonstrated that GA model could discriminate the proteomic spectra in patients with RRMS from the healthy control, with a sensitivity of 80.0% and a specificity of 91.3% (Table 3).

MALDI-TOF mass spectrometry is being widely applied to analyze serum samples for the diagnosis or prognosis of human diseases and peptide sequence identification of the potential biomarkers. Recent advances in mass spectrometry have enabled the identification of hundreds of low molecular weight peptides which have previously been difficult to detect in human serums (Villanueva et al., 2006). In this study, we used weak cation exchange magnetic beads (MB-WCX) to fractionate serum samples. Although some advantages of this method are high-throughput capability, needing of small sample sizes for analysis, exquisitely sensitive and high-resolution for peptide detection, and monitoring disease progression accuration, some biases generating from using this method preferentially identifies a subset of peptides and ignores other peptides with different physicochemical properties. We sought to minimize the influences by standardizing the collection and fractionation protocol, optimizing parameters of MALDI-TOF mass spectrometry and reagents.

Serum samples from 72 patients with RRMS were collected at the time of initial relapsing phase. We endeavored to find a putative blood biomarker for RRMS and compared serum samples between patients with RRMS and healthy controls. Thus, nucleolin fragments, a candidate biomarker we found in this study, might be a putative biomarker for RRMS. Serum samples from MS patients who weren't undergoing an attack of MS at the time of blood collection that served as another control would be better. Further investigations on serum biomarkers of RRMS should be done.

Our data showed that nucleolin fragments detected in blood of MS patients were less frequently than in healthy individuals. We have validated these results by western blot analysis. Human nucleolin is a 707-amino acid protein consisting of an acidic histone-like N terminus, a central domain containing four RNA binding domains, and a C terminus that is rich in arginine and glycine. This multidomain structure reflects the diverse roles of nucleolin in cell growth, proliferation and death (Ginisty et al., 1999; Srivastava and Pollard, 1999; Shang et al., 2011). Although nucleolin has been generally considered a predominantly nucleolar protein, it appears to be very mobile and can also be present in the nucleoplasm and cytoplasm and on the cell surface (Daniely and Borowiec, 2000; Wang et al., 2001). In fact there are several reports describing redistribution of nucleolin within the cell in response to a number of stimuli including T cell activation (Gil et al., 2001).

Some autoimmune diseases are thought to develop, at least in part, because of defection in apoptotic responses. The presence of nucleolin in apoptotic bodies has a number of clinical implications. In healthy individuals, apoptotic bodies are engulfed by macrophages or neighboring cells and cleared from the circulation. However, under conditions of excessive apoptosis, apoptotic bodies may be released into the circulation and could potentially be detected in plasma or serum (Eguchi, 2001). Acute MS relapse is characterized by altered expression of genes responsible for apoptosis and cell survival, and that the joint occurrence of both

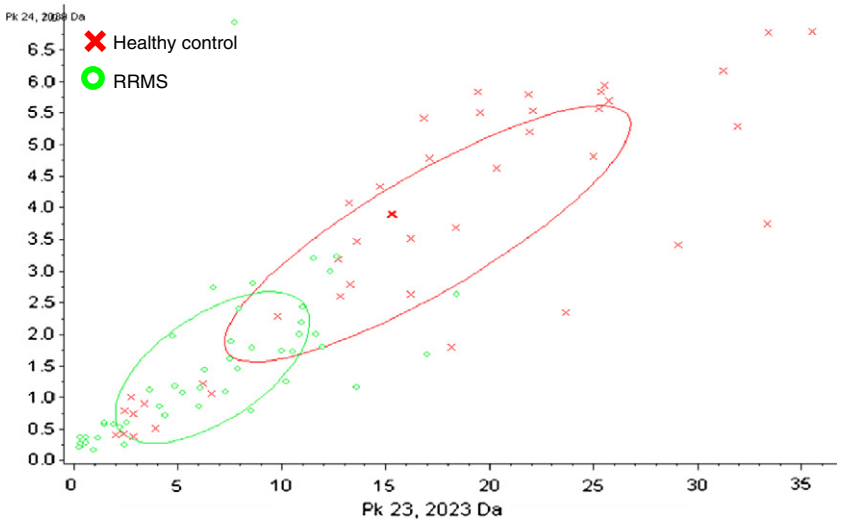

Fig. 2. Discrimination features of peptides with m/z 2023.3 (x-axis) and 2083.2 (y-axis) between the patients with relapsing–remitting multiple sclerosis (RRMS) (green circle) and healthy control group (red cross). Values were represented peptide abundance ratio.

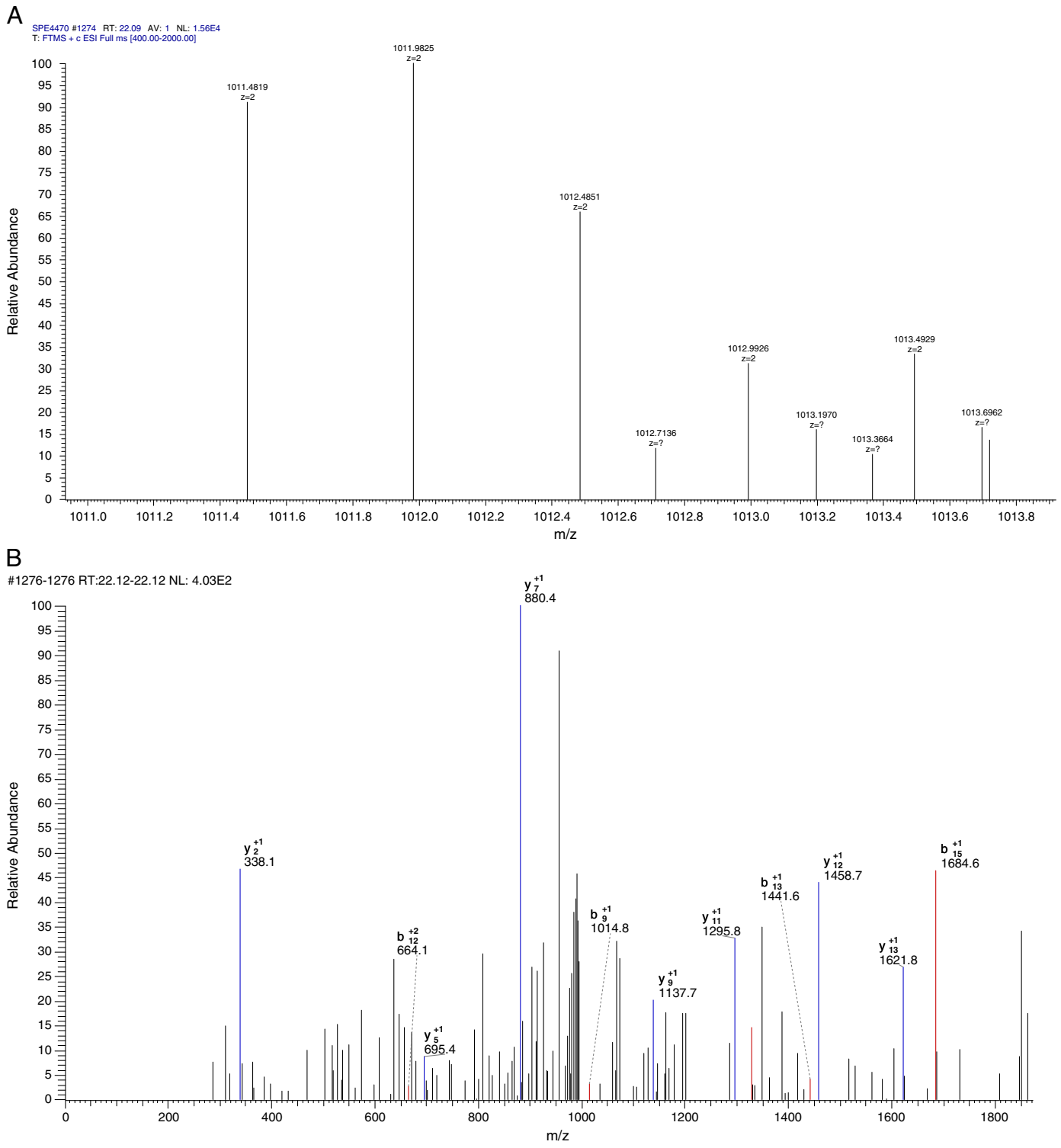

Fig. 3. A representative nano-LC/ESI–mass spectrometry/mass spectrometry sequencing result. A peptide that specifically occurred in patients with relapsing–remitting multiple sclerosis (RRMS), which is a fragment of nucleolin protein with sequence SISLYYTGEKGQNQDYR and molecular weight 2021.9563 (average). (A) Representative mass spectrum. (B) Mass spectrum of the ions.

mechanisms leads to apoptotic suppression (Achiron et al., 2007). Apoptosis suppression could prevent apoptotic bodies releasing into serum accompanied by decreased nucleolin level. Mi et al. (2003) reported that apoptosis in leukemia cells is accompanied by alterations in the levels and locations of nucleolin and identified nucleolin as an important component of the apoptotic pathway in leukemia cells. MS was characterized by the development of autoreactive T cells against self-antigens and nucleolin autoantibodies may appear in sera of MS patients at acute phase, which may neutralize nucleolin proteins and represent decreased sera level as our observation.

As shown in Table 1, some patients were treated with corticosteroids and other drugs such as interferon-β, mitoxantrone and traditional Chinese herbs and so on within 3 months. Because of different washout period of patients, it is impossible to exclude a treatment effect underlying

Table 4
Identification of specific b and y ions for nucleolin peptide sequence.

Amino acid	b+	b2+	y+	y2+
S	88.04	44.52	–	–
I	201.12	101.07	1934.92	967.97
S	288.16	144.58	1821.84	911.42
L	401.24	201.12	1734.81	867.91
Y	564.3	282.66	1621.72	811.37
Y	727.37	364.19	1458.66	729.83
T	828.41	414.71	1295.6	648.3
G	885.44	443.22	1194.55	597.78
E	1014.48	507.74	1137.53	569.27
K	1142.57	571.79	1008.49	504.75
G	1199.59	600.3	880.39	440.7
Q	1327.65	664.33	823.37	412.19
N	1441.7	721.35	695.31	348.16
Q	1569.75	785.38	581.27	291.14
D	1684.78	842.89	453.21	227.11
Y	1847.84	924.43	338.18	169.59
R	–	–	175.12	88.06

some of the possible differences between patients and healthy controls. MS is a chronic and disabling inflammatory disease of the CNS affecting more than 2.5 million people worldwide (Noseworthy et al., 2000; Frohman et al., 2006). A major inflammatory neurodegeneration of the central nervous system in MS is thought to result from the activation of immune cells of the autoimmune system, such as lymphocytes, macrophages and microglia (Kim et al., 2005). Although we have not yet elucidated the complex process of leukemia apoptosis and the precise role of nucleolin protein, nucleolin protein may play a role in the processing of acute MS relapse. Our data demonstrated that there is a possible relationship between the nucleolin fragment and the initiation of relapse in MS. Mass spectrometry may be useful in detecting small peptides and providing new insights concerning the pathophysiology of a variety of neurological disorders, including MS.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (No. 81001656, 30973513), Beijing Administration of Traditional Chinese Medicine Foundation (QN2010-4) and Beijing Natural Science Foundation (No. 7112061).

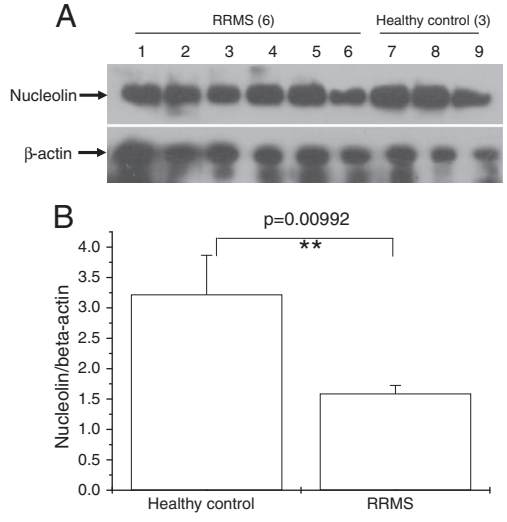

Fig. 4. Decreased nucleolin fragment expression in serum peptides analyzed by western blot. (A) Representative graphs of nucleolin fragment and internal control β-actin. (B) Densitometry comparison of nucleolin fragment relative to β-actin as determined by western blot analysis in figure A. Student's *t*-test was used to derive the P value indicated on the graph. All cases were detected and some representative graphs were shown.

References

Achiron, A., Feldman, A., Mandel, M., Gurevich, M., 2007. Impaired expression of peripheral blood apoptotic-related gene transcripts in acute multiple sclerosis relapse. Ann. N. Y. Acad. Sci. 1107, 155–167.
Aranami, T., Miyake, S., Yamamura, T., 2006. Differential expression of CD11c by peripheral blood NK cells reflects temporal activity of multiple sclerosis. J. Immunol. 177, 5659–5667.
Daniely, Y., Borowiec, J.A., 2000. Formation of a complex between nucleolin and replication protein A after cell stress prevents initiation of DNA replication. J. Cell Biol. 149, 799–810.
Diamandis, E.P., 2004. Mass spectrometry as a diagnostic and a cancer biomarker discovery tool: opportunities and potential limitations. Mol. Cell Proteomics 3, 367–378.
Eguchi, K., 2001. Apoptosis in autoimmune diseases. Intern. Med. 40, 275–284.
Frisullo, G., Angelucci, F., Caggiula, M., Nociti, V., Iorio, R., Patanella, A.K., Sancricca, C., Mirabella, M., Tonali, P.A., Batocchi, A.P., 2006. pSTAT1, pSTAT3, and T-bet expression in peripheral blood mononuclear cells from relapsing–remitting multiple sclerosis patients correlates with disease activity. J. Neurosci. Res. 84, 1027–1036.
Frohman, E.M., Racke, M.K., Raine, C.S., 2006. Multiple sclerosis—the plaque and its pathogenesis. N. Engl. J. Med. 354, 942–955.
Gil, D., Gutiérrez, D., Alarcón, B., 2001. Intracellular redistribution of nucleolin upon interaction with the CD3epsilon chain of the T cell receptor complex. J. Biol. Chem. 276, 11174–11179.
Ginisty, H., Sicard, H., Roger, B., Bouvet, P., 1999. Structure and functions of nucleolin. J. Cell Sci. 112, 761–772.
Hafler, D.A., 2004. Multiple sclerosis. J. Clin. Invest. 113, 788–794.
Hohlfeld, R., Wekerle, H., 2004. Autoimmune concepts of multiple sclerosis as a basis for selective immunotherapy: from pipe dreams to (therapeutic) pipelines. Proc. Natl. Acad. Sci. U. S. A. 101 (Suppl. 2), 14599–14606.
Hortin, G.L., 2006. The MALDI-TOF mass spectrometric view of the plasma proteome and peptidome. Clin. Chem. 52, 1223–1237.
International Multiple Sclerosis Genetics Consortium, Hafler, D.A., Compston, A., Sawcer, S., Lander, E.S., Daly, M.J., De Jager, P.L., de Bakker, P.I., Gabriel, S.B., Mirel, D.B., Ivinson, A.J., Pericak-Vance, M.A., Gregory, S.G., Rioux, J.D., McCauley, J.L., Haines, J.L., Barcellos, L.F., Cree, B., Oksenberg, J.R., Hauser, S.L., 2007. Risk alleles for multiple sclerosis identified by a genomewide study. N. Engl. J. Med. 357, 851–862.
Kanesaka, T., Mori, M., Hattori, T., Oki, T., Kuwabara, S., 2006. Serum matrix metalloproteinase-3 levels correlate with disease activity in relapsing–remitting multiple sclerosis. J. Neurol. Neurosurg. Psychiatry 77, 185–188.
Kim, R.H., Smith, P.D., Aleyasin, H., Hayley, S., Mount, M.P., Pownall, S., Wakeham, A., You-Ten, A.J., Kalia, S.K., Horne, P., Westaway, D., Lozano, A.M., Anisman, H., Park, D.S., Mak, T.W., 2005. Hypersensitivity of DJ-1-deficient mice to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyrindine (MPTP) and oxidative stress. Proc. Natl. Acad. Sci. U. S. A. 102, 5215–5220.
Koning, N., Bö, L., Hoek, R.M., Huitinga, I., 2007. Downregulation of macrophage inhibitory molecules in multiple sclerosis lesions. Ann. Neurol. 62, 504–514.
Kornek, B., Lassmann, H., 2003. Neuropathology of multiple sclerosis—new concepts. Brain Res. Bull. 61, 321–326.
Kuenz, B., Lutterotti, A., Khalil, M., Ehling, R., Gneiss, C., Deisenhammer, F., Reindl, M., Berger, T., 2005. Plasma levels of soluble adhesion molecules sPECAM-1, sP-selectin and sE-selectin are associated with relapsing–remitting disease course of multiple sclerosis. J. Neuroimmunol. 167, 143–149.
Liang, T., Wang, N., Li, W., Li, A., Wang, J., Cui, J., Liu, N., Li, Y., Li, L., Yang, G., Du, Z., Li, D., He, K., Wang, G., 2010. Identification of complement C3f-desArg and its derivative for acute leukemia diagnosis and minimal residual disease assessment. Proteomics 10, 90–98.
Mahad, D.J., Lawry, J., Howell, S.J., Woodroofe, M.N., 2003. Longitudinal study of chemokine receptor expression on peripheral lymphocytes in multiple sclerosis: CXCR3 upregulation is associated with relapse. Mult. Scler. 9, 189–198.
Mi, Y., Thomas, S.D., Xu, X., Casson, L.K., Miller, D.M., Bates, P.J., 2003. Apoptosis in leukemia cells is accompanied by alterations in the levels and localization of nucleolin. J. Biol. Chem. 278, 8572–8579.
Noseworthy, J.H., Lucchinetti, C., Rodriguez, M., Weinshenker, B.G., 2000. Multiple sclerosis. N. Engl. J. Med. 343, 938–952.
Polman, C.H., Reingold, S.C., Banwell, B., Clanet, M., Cohen, J.A., Filippi, M., Fujihara, K., Havrdova, E., Hutchinson, M., Kappos, L., Lublin, F.D., Montalban, X., O'Connor, P., Sandberg-Wollheim, M., Thompson, A.J., Waubant, E., Weinshenker, B.G., Wolinsky, J.S., 2010. Diagnostic criteria for multiple sclerosis: 2010 revisions to the "McDonald Criteria". Ann. Neurol. 69, 292–302.
Qiu, F., Liu, H.Y., Zhang, X.J., Tian, Y.P., 2009. Optimization of magnetic beads for MALDI-TOF MS analysis. Front. Biosci. 14, 3712–3723.
Shang, Y., Kakinuma, S., Nishimura, M., Kobayashi, Y., Nagata, K., Shimada, Y., 2011. Interleukin-9 receptor gene is transcriptionally regulated by nucleolin in T-cell lymphoma cells. Mol. Carcinog., http://dx.doi.org/10.1002/mc.20834
Srivastava, M., Pollard, H.B., 1999. Molecular dissection of nucleolin's role in growth and cell proliferation: new insights. FASEB J. 13, 1911–1922.
Steiner, I., Nisipianu, P., Wirguin, I., 2001. Infection and the etiology and pathogenesis of multiple sclerosis. Curr. Neurol. Neurosci. Rep. 1, 271–276.
Storck, S., Shukla, M., Dimitrov, S., Bouvet, P., 2007. Functions of the histone chaperone nucleolin in diseases. Subcell. Biochem. 41, 125–144.
Villanueva, J., Shaffer, D.R., Philip, J., Chaparro, C.A., Erdjument-Bromage, H., Olshen, A.B., Fleisher, M., Lilja, H., Brogi, E., Boyd, J., Sanchez-Carbayo, M., Holland, E.C., Cordon-Cardo, C., Scher, H.I., Tempst, P., 2006. Differential exoprotease activities confer tumor-specific serum peptidome patterns. J. Clin. Invest. 116, 271–284.
Wang, Y., Guan, J., Wang, H., Wang, Y., Leeper, D., Iliakis, G., 2001. Regulation of DNA replication after heat shock by replication protein a–nucleolin interactions. J. Biol. Chem. 276, 20579–20588.