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Original Article

Differential proteomics analysis of mononuclear cells in cerebrospinal fluid of Parkinson's disease

Lifei Xing[1], Dongtao Wang[2], Lihong Wang[3], Wenjie Lan[3], Suyue Pan[4]

[1]Department of Neurology, Inner Mongolia North Heavy Industries Group Corp. Ltd Hospital, (Third Affiliated Hospital of Baotou Medical College), Baotou 014030, Inner Mongolia, China; [2]Department of Thoracic Surgery, The Central Hospital, Baotou 014040, Inner Mongolia, China; [3]Department of Image, Inner Mongolia North Heavy Industries Group Corp. Ltd Hospital, (Third Affiliated Hospital of Baotou Medical College), Baotou 014030, Inner Mongolia, China; [4]Department of Neurology, Nanfang Hospital, Southern Medical University, Guangzhou 510515, Guangdong, China

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Abstract: Parkinson's disease (PD) is one common neurodegenerative disease featured with degeneration of dopaminergic neurons in substantia nigra. Multiple factors participate in the pathogenesis and progression of PD. In this study, we investigated the proteomics profiles of mononuclear cells in cerebrospinal fluids from both PD patients and normal people, in order to explore the correlation between disease factors and PD. Cerebrospinal fluid samples were collected from both PD and normal people and were separated for mononuclear cells *in vitro*. Proteins were then extracted and separated by 2-dimensional gel electrophoresis. Proteins with differential expressions were identified by comparison to standard proteome expression profile map, followed by software and database analysis. In PD patients, there were 8 proteins with consistent expression profile and 16 proteins with differential expressions. Those differential proteins identified include cytoskeleton proteins (actin, myosin), signal transduction proteins (adenosine cyclase binding protein 1, calcium binding protein, talin) and anti-oxidation factor (thioredoxin peroxide reductase). PD patients had differential protein expressional profiles in the mononuclear cells of cerebrospinal fluids compared to normal people, suggesting the potential involvement of cytoskeleton and signal transduction proteins in apoptosis of neuronal apoptosis and PD pathogenesis.

Keywords: Parkinson's disease, cerebrospinal fluid, proteomics, mononuclear cells

Introduction

Parkinson's disease (PD) is featured with progressive degeneration and dysfunction of dopaminergic (DA) neurons in substantia nigra, thus enhancing thalamus-cortex inhibition via dopamine receptor D1 and D2, leading to motor dysfunctions [1, 2]. Currently available medication cannot reverse the progressive loss of DA neurons [3]. The exact reason causing PD is still unclear so far, as multiple factors including environment, genetics, age and cellular oxidative stress may all play certain roles [4-6]. Both animal and clinical studies have suggested significantly elevated oxidative stress, mitochondrial dysfunction and inflammatory cytokine levels in the cerebrospinal fluid of PD. Whether these changes are the cause or consequence of PD, however, is still inconclusive so far. Recent studies have suggested the involvement of cytoskeleton and anti-oxidative reactive enzymes in PD pathogenesis. Certain connection may exist between neural protective role and cellular oxidative stress in PD. Moreover, cell apoptosis also plays certain roles in PD, so dose permeable transport pore of mitochondria. Under oxidative stress, a series of changes may occur inside cells including calcium overload, increased permeability of mitochondria, releasing of small molecule, all of which may induce neuronal apoptosis. Serine-threonine kinase, thioredoxin peroxide reductase I and voltage-dependent anion channel protein consist the major component of anti-oxidation system, which may also clear free radicals for protecting brain tissues [7]. In this study, we utilized proteomics technology to study the differential expression of proteins in

Table 1. Clinical features of all research objects

	PD (N=17)	Control (N=15)
Age (years)	56.5	57.2
Male (%)	58%	49%
Disease period (years)	6.1	
L-DOPA taker	3	
Dopamine receptor agonist	1	
L-DOPA + Dopamine receptor agonist	8	

mononuclear cells in the cerebrospinal fluid from both healthy and PD patients, in order to study the possible mechanism of PD.

Materials and methods

Research objects

A total of 17 patients who have been diagnosed as PD in the department of neurology in Inner Mongolia North Heavy Industries Group Corp. Ltd Hospital from February 2012 to December 2014 were recruited in this study in accordance with diagnostic criteria. Meanwhile 15 healthy volunteers were enrolled in the control group. This study has been approved by the ethical committee and has obtained written consents from all individuals. Clinical parameters of two groups were listed in **Table 1**.

Sample collection and electrophoresis

20 mL cerebrospinal fluids were collected from all participants, and were extracted for mononuclear cells by gradient centrifugation. Total proteins were extracted and quantified by test kits. Isoelectric focusing system was used in combined with bidirectional gel electrophoresis and fixed pH gradient method to separate equal volume of protein samples in each well. After electrophoresis, gel was rinsed in neutralizing buffer for 15 min, followed by ethidium bromide-containing buffer for 15 min. Vertical SDS-PAGE was then used to separate proteins (5~7 hours), followed by coomassie brilliant blue staining.

Data analysis

Labscan software was used to acquire gel images, which were further analyzed by PD Quest software according to previously documented repetitive calculation method [8]. Targeted protein dots were separated for mass spectrometry analysis by DE STR 4307 MALDI-TOF-MS equipment (Voyager, US). Using internal standard, peptide fingerprint map was corrected and searched in online database. Analysis was performed in conjunction with bidirectional gel electrophoresis to reveal molecular weight, isoelectric spot and matching peptide length. The property of candidate protein fragments was then determined to reveal related proteins.

Results

2-D gel electrophoresis image analysis

Total protein in each sample was tested in triplicates and has obtained similar 2-D electrophoresis images. By scanning and analysis, 423 protein spots were found in PD group, while 436 spots were revealed in control group. By comparison, 5 proteins were up-regulated in PD patients while 9 proteins were down-regulated. Three protein candidates were not identified in PD group. **Figure 1** showed expressional profile map of both groups. Data were analyzed by Log2 scale and Wilcoxon test (**Figure 2A** and **2B**), suggesting candidate proteins involving cytoskeleton, signal transduction and anti-oxidation proteins.

Mass spectrometry analysis of differentially expressed proteins

Trypsin was used to digested protein spots with differential expressions. Meanwhile MALDI-TOF-MS approach was used to detect peptide fingerprint spectrum. MASCOT software was also used to search for protein database. We found a total of 26 protein spots including 18 proteins. They belonged to ubiquitin proteinase, cytoskeleton protein and anti-oxidation enzyme family. 21 protein spots were found to have differential expressions, revealed a total of 18 proteins. In PD group, up-regulated proteins included prion protein, while voltage-dependent anion channel, serine-threonine kinase, thioredoxin peroxide reductase I and Omega family protein were down-regulated as shown in **Table 2**.

Discussion

Proteomics can be used to study the pathogenesis and mechanism of various diseases [9]. In this study, we utilized 2-D gel electrophoresis to

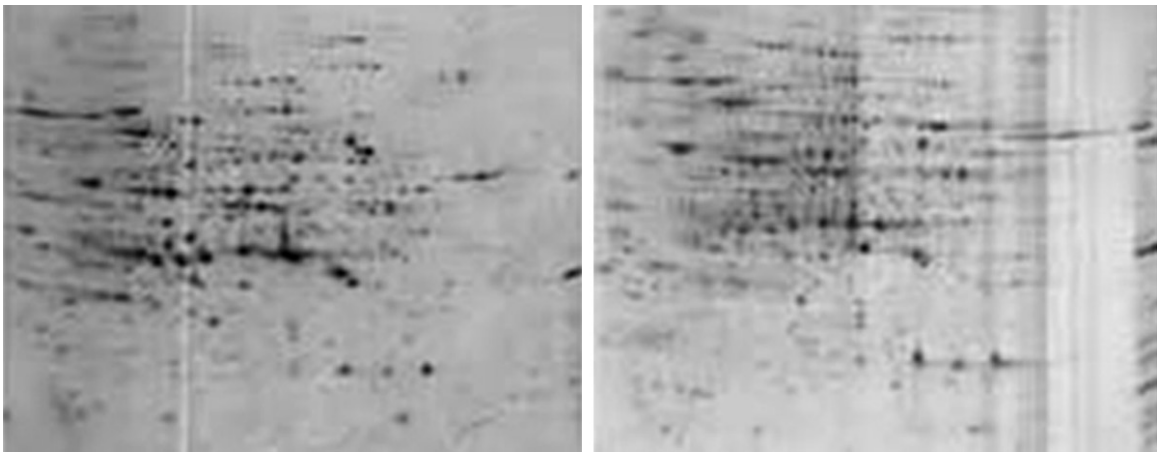

Figure 1. Electrophoresis images of normal (left) and PD (right) group.

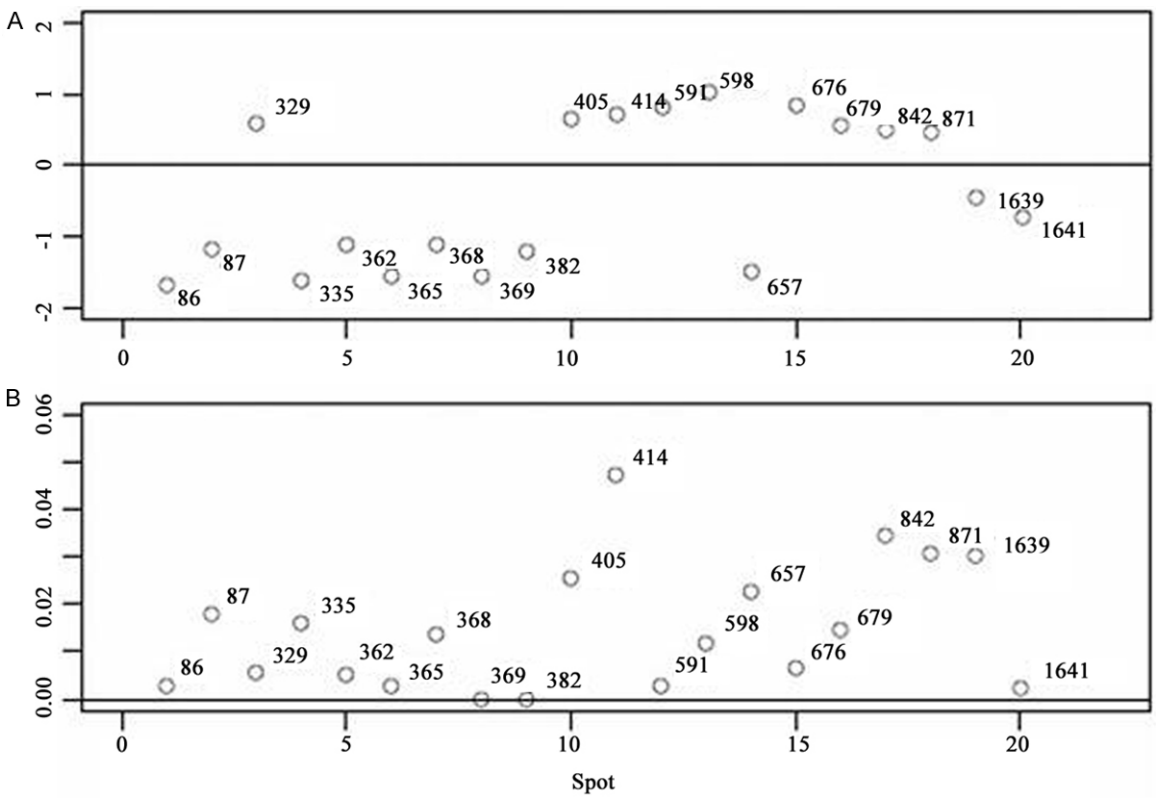

Figure 2. Fold change of protein expression level by Log2 scale (A) and Wilcoxon test (B).

establish proteomics in cerebrospinal fluid cells in PD patients, by comparison with protein database. Proteins with consistent and differential expression between PD and healthy individuals were identified, providing further information regarding PD pathogenesis.

Pathogenesis of PD is still unclear yet, perhaps involving interaction among genetic, environmental factor, aging, oxidative stress, mitochondrial dysfunction and inflammatory factors. Various clinical studies have found significantly elevated oxidative stress, mitochondrial dysfunction and inflammatory cytokine levels in both serum and cerebrospinal fluid of PD patients [6-9]. Recent study has found the participation of prion protein in cellular oxidative stress. Prion protein is one membrane glyco-

Table 2. Differential protein identification in mononuclear cells

Access No.	Protein name	MwPPI	Peptide match	PD
Q5T6W2	Tubulin alpha 1C-chain	5. 43P42009	12P33	*
Q53QU3	Actin related protein 3	6. 85P36824	8P25	*
Q5VVZ8	Cellular Prion protein	5. 69P29703	10P26	@
JX0232	Stress induced phosphoprotein	4. 80P19740	7P11	*
ACP1	T-complex protein 1 subunit alpha	8. 12P51795	7P20	@
AAA61237	GST01	6. 60P42215	18P43	@
Q96AF9ZYX	Serine Pthreonine kinase, Akt	7. 56P51694	8P27	@
CAI15802	Heat shock cognate 71 kDa protein	7. 67P28017	8P19	*
S68455	VDAC	8. 30P51802	9P25	@

Note: *, down-regulation; @, up-regulation.

protein with conserved sequence, and is widely distributed in human tissues including neurons and glial cells in central nervous system. It can participate in cellular processes including anti-apoptosis and anti-oxidative stress. Recent investigations of structure and functions of prion protein revealed its novel roles. Some studies pointed the participation of prion protein in neural protection and certain connection with cellular oxidative stress. During the pathogenesis of PD, apoptosis plays certain roles, in addition with mitochondrial permeable transport pores. Under oxidative stress stimulus, a series of changes may occur inside cells including calcium overload, increased permeability of mitochondria, releasing of small molecule, all of which may induce neuronal apoptosis [10]. In this process, voltage-dependent anion channel protein is one important component [11]. This study has identified down-regulation of voltage-dependent anion channel proteins in PD group, suggesting the potential correlation.

Serine threonine kinase (Akt) is one protein kinase in the form of heterodimers, and mainly exerts phosphorylation functions on serine or threonine residues of downstream signal molecules for transducing extracellular signal and affecting gene transcription. The major ligand of Akt is transforming growth factor-βs (TGF-βs) family, including TGF-β1 to β5. These members have similar structures and exert pluripotent functions including inhibiting cell proliferation, stimulating extracellular matrix synthesis, potentiating bone formation, and chemotactic attraction of cells and inducing embryonic development, dependent on specific cell type. By inducing a series of signal transduction, phosphoinositide-3 kinase (PI3K) related factors were imitated [12]. Many studies have revealed the enhanced cell survival by blocking Akt in order to shut down the induced cell death [13-15]. We found the down-regulation of Akt in PD, suggesting the correlation between signal transduction and pathogenesis. The down-regulation of Akt suggested its lower function in anti-apoptotic signaling pathway. As one important component of cytoskeleton, actin has been suggested to be related with PD pathogenesis by this study [16-19].

Thioredoxin peroxidase I is one member of sulfur specific anti-oxidative protein family, and constitutes one important anti-oxidation system with thioredoxin and thioredoxin reductase. It can also clear free radicals to protect brain tissues to certain extents. It is well known that free radicals are toxic to body as it can break down large biomolecules thus compromising cell viability. The body anti-oxidative system mainly composes of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-px). Thioredoxin peroxidase I can regulate intercellular H_2O_2 concentration, further modulating signal transduction of growth factors. Our study found down-regulation of this protein in cerebrospinal fluid of PD patients, suggesting the participation of this enzyme in PD pathogenesis under oxidative stress.

Members of Omega family are widely distributed in various body tissues, and are related with arsenic metabolism and neural degenerative disease. One of its family member, GST01, has certain activity of GSH-dependent thioltransferase and dehydrogenated ascorbic acid reductase, in addition to inhibition of cell apoptosis to certain extents [17, 18, 20]. Our results found elevated expression of GST01 in PD

patients, suggesting the response of anti-oxidation and anti-apoptosis activity.

In summary, this study established 2-D gel electrophoresis map of mononuclear cells in PD patients, and revealed differential protein expressions between PD and normal people, thus providing new clues for elucidation of the correlation between systematic change and central nervous degeneration in PD, although further studies are required to validate our proposed model.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Suyue Pan, Department of Neurology, Nanfang Hospital, Southern Medical University, 1838 Guangzhou Avenue, Guangzhou 510515, Guangdong, China. Tel: +86-21-62786261; Fax: +86-21-62786261; E-mail: pansuyue33@sina.com

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