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REVIEW PAPER

Proteomics in Human Parkinson's Disease: Present Scenario and Future Directions

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Abstract

Parkinson's disease (PD) is an age-related, threatening neurodegenerative disorder with no reliable treatment till date. Identification of specific and reliable biomarker is a major challenge for disease diagnosis and designing effective therapeutic strategy against it. PD pathology at molecular level involves abnormal expression and function of several proteins, including alpha-synuclein. These proteins affect the normal functioning of neurons through various post-translational modifications and interaction with other cellular components. The role of protein anomalies during PD pathogenesis can be better understood by the application of proteomics approach. A number of proteomic studies conducted on brain tissue, blood, and cerebrospinal fluid of PD patients have identified a wide array of protein alterations underlying disease pathogenesis. However, these studies are limited by the types of brain regions or biofluids utilized in the research. For a complete understanding of PD mechanism and discovery of reliable protein biomarkers, it is essential to analyze the proteome of different PD-associated brain regions and easily accessible biofluids such as saliva and urine. The present review summarizes the major advances in the field of PD research in humans utilizing proteomic techniques. Moreover, potential samples for proteomic analysis and limitations associated with the analyses of different types of samples have also been discussed.

Keywords Biomarker · Human · Parkinson's disease · Proteomics

Abbreviations

PD	Parkinson's disease
UCHL1	Ubiquitin carboxy-terminal hydrolase L1
LRRK2	Leucine-rich repeat kinase 2
PINK1	PTEN-induced kinase 1
LNs	Lewy neurites
LBs	Lewy bodies
LC	Locus coeruleus
SN	Substantia nigra
Prx	Peroxiredoxin
CSF	Cerebrospinal fluid
GGH	Gamma glutamyl hydrolase
SOD	Superoxide dismutase
AD	Alzheimer's disease
Apo	Apolipoprotein
TIMP1	Metalloproteinase inhibitor 1
APLP1	Amyloid-like protein 1
LRP1	Prolow-density lipoprotein receptor-related protein 1
CSF1R	Macrophage colony-stimulating factor 1 receptor
EPHA4	Ephrin type-A receptor 4
PRNP	Major prion protein
HSPG2	Heparan sulfate proteoglycan 2
MEGF8	Multiple EGF-like domains 8
NCAM1	Neural cell adhesion molecule 1
2-DE	Two-dimensional gel electrophoresis
2-D DIGE	Two-dimensional difference gel electrophoresis
MALDI-TOF/TOF-MS	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
MALDI-IMS	MALDI imaging mass spectrometry
LC-ESI-MS/MS	Liquid chromatography-electrospray ionization tandem mass spectrometry

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ESI-Q-TOF MS/MS	Electrospray-quadrupole-time-of-flight tandem mass spectrometry
ESI-MS	Electrospray ionization mass spectrometry

Introduction

Parkinson's disease (PD) is an age-related chronic and progressive neurodegenerative disorder with no reliable treatment till date. The disease is mainly characterized by the degeneration of dopaminergic neurons in substantia nigra pars compacta region of the midbrain, reduced dopamine level in the striatum, and the formation of intracytoplasmic alpha (α)-synuclein protein aggregates, known as Lewy bodies (LBs). Major motor symptoms of disease are resting tremor, rigidity, bradykinesia/akinesia, and postural instability. Various non-motor symptoms such as dementia, depression, sleep disturbance, anxiety, dysphagia, and constipation can appear in the PD patients during the early stages and can be aggravated with advancement of disease (Chaudhuri et al. 2006). Three primary causative factors of PD are age, environmental factors, and genetic predisposition (Dauer and Przedborski 2003). Incidence of disease occurrence increases with age with a frequency of 1% for those over the age of 60 years to 4% after 80 years. The environmental factors such as pesticide usage and heavy metal exposure have been found to be associated with increased risk of PD (Yadav et al. 2012). Familial PD caused by mutations in the genes encoding α-synuclein (SNCA), ubiquitin carboxy-terminal hydrolase L1 (UCHL1), and leucine-rich repeat kinase 2 (LRRK2) exhibits autosomal dominant inheritance while mutations in the genes encoding Parkin, PTEN-induced kinase 1 (PINK1) and DJ-1 exhibit autosomal-recessive pattern of inheritance (Gasser 2007).

PD pathology progresses through six distinct stages with the participation of different brain regions during each stage (Braak et al. 2003). Stage 1 and 2 involve mainly dorsal motor nucleus of the vagal nerve and locus coeruleus, stage 3 and 4 include brain stem and substantia nigra regions of the midbrain, and in final stages 5 and 6, pathology reaches to cortical area along with the aggravation of the damage occurred during the previous stages. Each stage is marked by the development of characteristic inclusion bodies in the form of thread-like Lewy neurites (LNs) within cellular processes and granular Lewy bodies (LBs) aggregates in the cell body of the affected neurons (Braak et al. 2003, 2004). Clinical diagnosis of PD is difficult at early stages due to unavailability of any reliable biomarker or diagnostic test for the disease. Physical examination of the motor symptoms is useful for PD diagnosis, but these symptoms are visible in patients who have already lost around 70% of dopaminergic neurons (Breen et al. 2011). Early diagnosis of PD by using specific biomarker is an utmost requirement in order to design therapeutic strategy for this debilitating disease.

PD is associated with a complex disease mechanism characterized by pathological expression and function of a number of proteins related to oxidative stress, inflammation, and ubiquitin proteasomal system (UPS) pathways (Licker et al. 2009). The proteotoxic stress due to the accumulation of misfolded or abnormal α-synuclein protein aggregates in PD is associated with impaired lysosomal and proteasomal clearance mechanisms causing further protein accumulation, disruption of cellular processes, and ultimately neuronal death (Olanow and McNaught 2011). This indicates the crucial role of protein homeostasis in PD pathology. Proteomics is the study of proteome of an organism which helps in quantitative comparison of changes in protein profiles under the influence of different factors such as stress, drug treatment, or as a result of aging and disease (Pienaar et al. 2008; Pienaar et al. 2010). Proteomics of brain tissue and biofluids has widely contributed in understanding the PD pathogenesis by providing evidence of dysfunctional mitochondrial electron transport, protein folding, UPS, and antioxidant defense system in the diseased sample (Dixit et al. 2013; Licker and Burkhard 2014; Kasap et al. 2017). Current advancement of the mass spectrometer in sensitivity, speed, and throughput potentially allows a much higher spatiotemporal resolution of the analysis down to (1) near single cell level to encompass cell type-specific proteome changes, and (2) large-scale screening of cerebrospinal fluid (CSF)/blood for biomarker discovery. A number of mass spectrometry (MS) techniques such as electrospray ionization (ESI) MS and laser-based MS have been optimized for single-cell analysis (Yin et al. 2019), while high spatial and mass resolution can be achieved with the help of MS imaging technique (Buchberger et al. 2018). However, the development of systematic and defined therapeutic plan for PD still seems unapproachable as the identified pathways and targets do not represent the complete picture of PD development. This could be due to proteomic studies conducted on PD patients till now have used limited types of samples, and little attention has been paid to other potential brain regions and biofluids. Moreover, a specific biomarker for assessment of PD progression could not be identified yet possibly because of inaccessibility of the sample, failure in correlating it with the disease stage, or poor reproducibility of the results. Therefore, detailed proteomic analyses of PD-affected brain regions and easily accessible biofluids are needed for better understanding of the disease mechanism(s) and identification of biomarkers.

This review aims to summarize the recent advances in proteomics of different brain regions, subcellular structures, and biofluids obtained from PD patients followed by significance of other potential samples for proteomic analysis. The

limitations associated with proteomic analysis of different types of samples have also been discussed at the end of this review.

Proteomics of Different Brain Regions Involved in PD Pathology

Locus Coeruleus (LC)

The first appearance of Lewy neurites in early stage and extensive neuronal loss along with norepinephrine depletion at later stages of PD is found in the LC region of the brain (Braak et al. 2003). A single proteomic study conducted on LC region of the PD-affected brain demonstrated protein alterations related to mitochondrial function, oxidative stress, inflammation, cytoskeleton, and protein folding pathways. The identification of two novel proteins regucalcin (regulates intracellular calcium homeostasis) and kinectin (involved in transport of cellular components) in this study provided new insights into PD pathogenesis (van Dijk et al. 2012). However, further research is needed to validate the role of LC proteins in PD pathogenesis.

Olfactory Bulb

Olfactory dysfunction and smell impairment are considered as early premotor symptoms of PD (Doty 2012). Olfactory bulb atrophy and α-synuclein inclusions in olfactory structures are also reported in PD patients with respect to controls (Braak et al. 2003). A recent proteomic analysis of olfactory bulb region obtained from PD brain demonstrated the disruption of olfactory MAPK, PDK1/PKC, and MKK3-6/p38 MAPK signaling pathways (Lachén-Montes et al. 2019) indicating the important role of olfactory bulb proteostasis in PD pathogenesis.

Substantia Nigra (SN)

Dopaminergic neuronal loss and formation of LBs in SN are associated with the middle stages of PD (Braak et al. 2003). Proteomic analysis of SN region of human brain demonstrated downregulation of neurofilament-L/M chains and upregulation of peroxiredoxin (Prx) 2, mitochondrial complex III, ATP synthase D, complexin I, profilin, L-type calcium channel δ-subunit, and fatty acid-binding protein in PD patients in comparison to control subjects. These results provide the evidence of disrupted mitochondrial and antioxidant function in PD (Basso et al. 2004). Two other proteomic studies utilizing SN region of PD patients also confirmed the involvement of mitochondrial dysfunction, cytoskeleton impairment, and oxidative stress in PD pathogenesis (Kitsou et al. 2008; Licker et al. 2012). A recent proteomic study conducted on SN and ventral tegmental area (VTA) of brain has shown that the level of prohibitin protein, involved in maintenance of mitochondrial integrity, was decreased in PD patients in comparison to controls (Dutta et al. 2018). Additionally, proteins (calmodulin, γ-enolase, and myelin basic protein) identified in the SN region have shown an overlap with the proteins previously reported in CSF (Kitsou et al. 2008). Licker and colleagues have found that the expression of a novel protein, cytosolic non-specific dipeptidase 2, was increased in SN region of PD patients. The protein could be associated with protein aggregation in PD (Licker et al. 2012). Many other proteins related to mitochondria, oxidative stress, and energy metabolism were also found to be differentially regulated in this study (Table 1). Dysregulation of proteins involved in various pathways such as retinoid metabolism, L-DOPA methylation, redox metabolism, iron metabolism, glial activation along with few novel proteins such as adenosylhomocysteinase (methylation), aldehyde dehydrogenase 1, and cellular retinol-binding protein 1 (aldehyde metabolism), was found in the SN region of the PD brain in comparison with normal controls. The study also showed that there was no change in the expression pattern of familial PD-related proteins DJ-1 and UCHL1 between controls and PD patients (Werner et al. 2008). A recent proteomic study of nigral tissues of PD patients identified 1795 proteins and out of them, 204 proteins were found to be differentially expressed in PD patients in comparison to controls (Licker et al. 2014). Two novel proteins, nebulette and gamma glutamyl hydrolase (GGH), were found to be upregulated and downregulated, respectively, in PD samples in comparison to control subjects. Upregulation of nebulette has been correlated with impaired cytoskeleton and downregulation of GGH, a key enzyme in folate metabolism, could be due to loss of iron-containing neurons in the SN region during PD (Licker et al. 2014). Overall, proteomics of the SN region of PD sufferer's brain has confirmed the central role of mitochondrial dysfunction, oxidative stress, and protein aggregation in PD pathology along with identification of many novel proteins (Table 1).

Cerebral Cortex

The cerebral cortex is found to be severely affected in later stages of PD. Several disease-associated features such as mitochondrial abnormalities and decreased glucose metabolism are reported in the cerebral cortex of PD patients (Ferrer 2009). Three enzymes aldolase A, enolase 1 and glyceraldehyde-3-phosphate dehydrogenase were found to be altered by oxidation in frontal cortex of PD patients, which provide evidence for impaired glycolysis and energy metabolism in PD (Gómez and Ferrer 2009). A number of antioxidant proteins in the brain, such as Prx, superoxide dismutase (SOD), catalase, and glutathione peroxidase, provide protection

Table 1 List of some important proteomic studies conducted on brain tissue and biofluids of PD patients

S. no.	Cell/tissue/region	Pathway/organelle affected	Major proteins altered	Level/modification in PD patients (in comparison to controls)	Techniques used	Reference
Brain regions						
1	LC	Calcium homeostasis and transport	Regucalcin	Upregulation	1-DE and nano-LC–MS/MS	van Dijk et al. (2012)
			Kinectin	Downregulation		
2	Olfactory bulb	Cell signaling	Phosphoinositide-dependent protein kinase 1, p38 α and β subunits, monomeric α-synuclein	Upregulation	MALDI-IMS	Lachén-Montes et al. (2019)
			ERK1/2, MKK3/6-p38 MAPK	Downregulation		
3	SN	Mitochondria, cytoskeleton	Prx2, mitochondrial complex III, ATP synthase D chain, complexin I, profilin, fatty acid-binding protein	Upregulation	2-DE and MALDI-TOF–MS	Basso et al. (2004)
			L and M neurofilament chains	Downregulation		
4	SN	Aldehyde metabolism, L-DOPA methylation, Glial activation, redox metabolism, iron metabolism	Aldehyde dehydrogenase A1 and cellular retinol-binding protein 1, S-adenosyl homocysteine hydrolase 1, glutathione-S-transferase (GST) M3, GST P1, GST O1, and SH3-binding glutamic acid-rich-like protein, glial fibrillary acidic protein, glial maturation factor β, galectin 1, and sorcin A, H-ferritin, β-tubulin cofactor A, Annexin V	Upregulation	2-DE and MALDI-MS	Werner et al. (2008)
			V-type ATPase A1	Downregulation		

Table 1 (continued)

S. no.	Cell/tissue/region	Pathway/organelle affected	Major proteins altered	Level/modification in PD patients (in comparison to controls)	Techniques used	Reference
5	SN	Mitochondria, oxidative stress, and energy metabolism	Cytosolic non-specific dipeptidase 2, guanine nucleotide binding protein G(I)/G(S)/G(T) subunit β, vacuolar protein sorting-associated protein 29, Ferritin L-chain	Upregulation	2-DE and MALDI-TOF/TOF–MS	Licker et al. (2012)
			cytochrome b–c1 subunit 2, and ATP synthase subunit D	Downregulation		
6	SN	Cytoskeleton, folate metabolism	γ-glutamyl hydrolase	Upregulation	Tandem mass tag labeling, LC–MS/MS	Licker et al. (2014)
			Nebulette	Downregulation		
7	SN and VTA	Mitochondrial integrity	Prohibitin	Downregulation	2-DE and MALDI-TOF/TOF	Dutta et al. (2018)
8	Cortex	Antioxidation	SOD 1	Carbonylation and oxidation	MALDI-TOF–MS and HPLC- ESI–MS/MS	Choi et al. (2005)
9	Cortex	Glycolysis	Aldolase A, enolase, glyceraldehyde dehydrogenase	Oxidation	2-DE and capLC nano-ESI-MS/MS	Gómez and Ferrer (2009)
10	Cortex	Antioxidation	Prx2 and Prx6	Upregulation	2-DE and MALDI-MS	Krapfenbauer et al. (2003)
			Prx3	Downregulation		
11	Cortex	Ubiquitination pathway	UCHL1	Carbonylation and Oxidation	2-DE, MALDI-TOF/MS, and HPLC-ESI/MS/MS	Choi et al. (2004)
12	Cortex	Oxidative stress	DJ-1	Upregulation, Carbonylation and Oxidation	2-DE, MALDI-TOF/MS, MALDI-TOF/TOF/MS/MS, and HPLC-ESI/MS/MS	Choi et al. (2006)
Subcellular structure						
13	Mitochondrial fraction (SN region)	Mitochondrial stress	Ubiquinol-cytochrome C reductase iron–sulfur subunit	Upregulation	Multidimensional Protein Identification Technology, Isotope Coded Affinity Tag and Stable Isotope Labeling by Amino acids in Cell culture	Jin et al. (2006)
			Mortalin/mthsp70/GRP75, NADH-ubiquinone oxidoreductase subunit B14.7, ubiquinone oxidoreductase	Downregulation		
Biofluids						
14	CSF	Oxidative stress, protein folding	DJ-1, α-synuclein	Downregulation	Gel filtration, MS, and Luminex assay	Hong et al. (2010)

Table 1 (continued)

S. no.	Cell/tissue/region	Pathway/organelle affected	Major proteins altered	Level/modification in PD patients (in comparison to controls)	Techniques used	Reference
15	CSF	Immune system, cell survival, antioxidation, lipoprotein metabolism	ApoE and autotaxin	Upregulation	2-DE and LC–MS/MS	Guo et al. (2009)
			Complement C3, C4α, isoforms of haptoglobin	Downregulation		
			SOD1	Oxidation		
16	CSF	Immune system	Complement proteins C3b, C4b, factor B	Downregulation	2-DE and MALDI-TOF/TOF	Finehout et al. (2005)
17	CSF	Hemoglobin/globin metabolism	Serum albumin precursor, serum albumin chain-A, proline-rich repeat 14, and serum transferrin N-terminal lobe	Upregulation	2-DE, MALDI-TOF, and LC–MS	Sinha et al. (2009)
			hemoglobin β fragment and mutant globin	Downregulation		
18	CSF	Iron transportation, lipoprotein metabolism	Ceruloplasmin, ApoH	Downregulation	Isobaric Tagging for Relative and Absolute protein Quantification and MS/MS	Abdi et al. (2006)
19	CSF	Inflammation, neurodegeneration	EPHA4 and LRP1	Upregulation	LC–MS/MS	Shi et al. (2015)
			SPP1, CSF1R and TIMP1	Downregulation		
20	CSF	Cell-surface adhesion protein Serum Glycoproteins	Neurexin-1	Upregulation	LC- MALDI-TOF/TOF	Pan et al. (2008)
			α-1-Acid glycoprotein	Downregulation		
22	CSF	Cell growth, lipoprotein metabolism, Immune system	Interleukin 8, β2-microglobulin, and vitamin D-binding protein	Upregulation	Multiplex assay	Zhang et al. (2008a, b)
			brain-derived neurotrophic factor, ApoA2 and ApoE	Downregulation		
23	CSF	Cell adhesion	Neural cell adhesion molecule-120, α-dystroglycan	Upregulation	2-DE and LC–MS/MS	Yin et al. (2009)
24	Blood Plasma	Hemoglobin clearance/metabolism	Haptoglobin-related protein precursor, truncated β-globin	Upregulation	2-DE and MALDI-TOF/TOF	Sinha et al. (2007)
25	Blood Plasma	Inflammation, phagocytosis, cell signaling	PRNP, HSPG2, MEGF8, and NCAM1	Upregulation	Selected reaction monitoring, N-glycocapture, and LC–MS	Pan et al. (2014)
26	Blood Plasma	Lipid metabolism, immunity, protein folding	Clusterin, complement C1r subcomponent, ApoA1 (exosomal proteins) fibrinogen γ-chain	Downregulation	2D-DIGE and MALDI-TOF/TOF/MS	Kitamura et al. (2018)

Table 1 (continued)

S. no.	Cell/tissue/region	Pathway/organelle affected	Major proteins altered	Level/modification in PD patients (in comparison to controls)	Techniques used	Reference
27	Blood Serum	Aggregation of proteins	Serum amyloid P component	Upregulation	2-DE and LC–MS/MS	Chen et al. (2011a, b)
28	Blood Serum	Lipid metabolism, intracellular transport, cell proliferation, and immunoregulation	Clusterin, transthyretin, immunoglobulin kappa-chain VK-1, Ig γ-3 chain C region, β-2-glycoprotein 1	Upregulation	2-DE and ESI-Q-TOF–MS/MS	Zhao et al. (2010)
			Serum amyloid A protein, haptoglobin, complex-forming glycoprotein HC, KIAA0325 protein, and myosin heavy chain IIx/d	Downregulation		
29	Blood Serum	Blood clotting	Fibrinogen γ-chain, full size inter-α-trypsin inhibitor heavy chain H4	Upregulation	2-DE and LC–MS/MS	Lu et al. (2014)
			fragmented ApoA4	Downregulation		
30	Blood Serum	Inflammation, immune response, lipoprotein metabolism	Transthyretin, ApoA1, complement factor H	Upregulation	2-DE and LC–MS/MS	Alberio et al. (2013)
			complement C3, haptoglobin, ApoE	Downregulation		
31	Blood Lymphocytes	Cytoskeleton, mitochondria	Cofilin-1, actin, mitochondrial ATP synthase β-subunit	Upregulation	2-DE and LC–MS/MS	Mila et al. (2009)
			Tropomyosin, γ -fibrinogen	Downregulation		
32	Tears	Immune response, lipid metabolism, and oxidative stress	Prx6, annexin-A5, and glutathione-S-transferase-A1, ApoD, ApoA4, ApoA1, α-2-macroglobulin	Upregulation	Bottom up LC–MS/MS	Boerger et al. (2019)
			α-1-antiproteinase, Profilin 1, lactotransferrin, clusterin, galectin 3, β-2 microglobulin	Downregulation		

1-DE one-dimensional gel electrophoresis, *2-DE* two-dimensional gel electrophoresis, *2-D DIGE* two-dimensional difference gel electrophoresis, *MALDI-TOF/TOF–MS* matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, *MALDI-IMS* matrix-assisted laser desorption/ionization-imaging mass spectrometry, HPLC–ESI–MS/MS high-performance liquid chromatography/electrospray ionization tandem mass spectrometry, *LC–MS/MS* liquid chromatography-tandem mass spectrometry, *ESI* electrospray ionization, *ESI-Q-TOF MS/MS* electrospray ionization-quadrupole-time-of-flight tandem mass spectrometry

against oxidative stress. Proteomic analysis of the cerebral cortex of the PD brain shows that the expression of Prx2 and Prx6 was increased while Prx3 was decreased in the frontal cortex of PD patient in comparison to control subjects. However, there was no significant change in the levels of Prx1, SOD2, and glutathione-S-transferase ω1 between both the groups (Krapfenbauer et al. 2003). Another proteomic study conducted in the frontal cortex region of brain of PD patients revealed that there is carbonylation and oxidation of Cys-146 to cysteic acid in the SOD1 protein (Choi et al. 2005). These studies demonstrate that the compromised antioxidant defense system is critical for PD pathogenesis.

Oxidative modification, carbonylation, or phosphorylation of familial PD-related proteins in cerebral cortex of PD patients could be responsible for their altered function in PD brain. The UCHL1 protein was found to be severely modified by carbonyl formation, methionine oxidation, and cysteine oxidation (Choi et al. 2004) while DJ-1 protein was found irreversibly oxidized by carbonylation and methionine oxidation in PD brains (Choi et al. 2006). A large-scale proteomic study conducted on prefrontal cortex sample of PD patients supported the involvement of mitochondrial pathways in PD pathology. Furthermore, with the application of Genome Wide Association Study, it was found that SNCA loci showed significantly increased protein level, but no alteration in RNA level, which again supports that the altered level of α-synuclein protein is involved in PD pathogenesis (Dumitriu et al. 2016). Another recent global quantitative proteomic analysis of two brain regions (frontal cortex and anterior cingulate gyrus) obtained from four different groups; healthy controls, Alzheimer's disease (AD), PD, and co-morbid AD/PD cases led to the identification of 127,321 total unique peptides which mapped to 11,840 unique protein groups. This dataset is useful for understanding the molecular signatures and pathways involved in the pathogenesis of both AD and PD (Ping et al. 2018).

Proteomics of Subcellular Organelle of PD Brain

Among various subcellular organelles, mitochondria are the most studied organelle for understanding PD pathogenesis and are known to play a central role in PD pathology. A number of proteins related to disease such as PINK1, DJ-1, and parkin are either localized inside or interact with mitochondria, and death of dopaminergic neurons is caused due to induction of oxidative stress and apoptotic pathways involving mitochondria (Nicotra and Parvez 2002; Dixit et al. 2013). However, only a single proteomic study has been conducted on the mitochondrial fraction of the SN region of the PD brain, which demonstrated that 119 out of 842 identified proteins were significantly different in their relative abundance in comparison to age-matched controls. Out of these proteins, a novel mitochondrial stress protein mortalin (mthsp70/GRP75) was found to be decreased in PD patients in comparison to controls. The study also showed that mortalin mediates rotenone-induced toxicity in cellular model of PD through enhanced oxidative stress, and mitochondrial and proteasomal dysfunctions (Jin et al. 2006).

Proteomics of Biofluids of PD Patients

Biofluids such as plasma, serum, and CSF are always preferred human samples than brain tissue for proteomic analysis due to their easy and disease stage-specific availability. As the genomic or transcriptomic study is not possible for these fluids, the proteomics approach is the suitable choice for the analysis of global changes taking place in these samples and for the discovery of diagnostic biomarkers (Veenstra et al. 2005).

CSF

CSF represents a useful source for the identification of PD biomarkers (Guo et al. 2009; Waybright 2013). Since CSF proteins are not present in a large amount as compared to their corresponding serum proteins, a sensitive analytical technique is required to analyze these proteins (Romeo et al. 2005). CSF can be obtained at any stage of PD which makes it possible to assess and monitor the protein alterations with the progression of disease. Various proteomic studies have been successfully conducted on CSF of PD patients in search of biomarker for the disease (van Dijk et al. 2010) (Table 1). The proteins such as neurexin-1, R-1-acid glycoprotein, and β-2-glycoprotein 1 were validated as biomarkers in the CSF of PD patients by applying targeted quantitative proteomics approach (Pan et al. 2008). Use of sensitive Luminex assay revealed that DJ-1 and α-synuclein protein levels were decreased in CSF of PD patients in comparison to normal controls and AD patients. However, no association was reported between DJ-1 and α-synuclein levels and PD severity (Hong et al. 2010). Guo et al. reported the decreased abundance of Apolipoprotein (Apo) E and autotaxin, and increased levels of complement C3, C4α, and three isoforms of haptoglobin along with oxidative modification of SOD1 in CSF samples of PD patients in comparison to controls (Guo et al. 2009). Similarly, another proteomic study showed alterations in complement protein isoforms C3b, C4b, and factor B in CSF of PD patients in comparison to controls suggesting compromised immunity in PD patients (Finehout et al. 2005). Sinha et al. have shown that hemoglobin β-fragment and mutant globin were decreased while serum albumin precursor, serum albumin chain-A, proline-rich

repeat 14, and serum transferrin N-terminal lobe were increased in CSF samples of PD patients in comparison to controls (Sinha et al. 2009).

Ceruloplasmin is a ferroxidase enzyme that protects tissues from oxidative damage by regulating iron metabolism. Increase in protein carbonylation and oxidation of ceruloplasmin was observed in the CSF of PD patients in comparison to AD, dementia with LBs (DLB), and control samples which might be responsible for aggravated oxidative stress in PD patients (Olivieri et al. 2011). A multiplex quantitative proteomic approach applied to CSF samples of PD, AD, and DLB patients identified 72 proteins. Out of these, ceruloplasmin, chromogranin B, and ApoH proteins were found to be important for differentiating PD patients from controls as well as from other neurological patients (Abdi et al. 2006). Validation of these results in CSF samples of control subjects, AD, and PD patients has revealed an increased expression of interleukin 8 and vitamin D-binding protein, and decreased expression of brain-derived neurotrophic factor, ApoA2, and ApoE proteins in neurodegenerative diseases as compared to control samples (Zhang et al. 2008a, b). Similarly, another study has reported the upregulation of neural cell adhesion molecule-120 and α-dystroglycan in CSF of AD and PD patients in comparison to normal controls (Yin et al. 2009). The level of these proteins can be useful to distinguish PD patients from normal neurological controls. However, other protein β2-microglobulin was found upregulated specifically in PD patients and could help in differentiating PD from other neurodegenerative diseases (Zhang et al. 2008a, b; Yin et al. 2009). The proteome profiling of CSF of PD, AD patients (diseased controls), and healthy controls revealed that a combination of two peptides belonging to proteins TIMP1 (metalloproteinase inhibitor 1) and APLP1 (amyloid-like protein 1) is significantly correlated with disease severity in PD and a panel of five peptides belonging to proteins SPP1, LRP1, CSF1R, EPHA4 (Osteopontin, Prolow-density lipoprotein receptor-related protein1, Macrophage colony-stimulating factor1 receptor, Ephrin type-A receptor4), and TIMP1 are important in differentiating PD from healthy and diseased (AD) controls (Shi et al. 2015). Another recent proteomic study conducted on CSF samples obtained from control subjects and patients suffering from PD and atypical parkinsonian syndrome showed that the levels of acute phase/inflammatory proteins and neuronal/synaptic proteins were, respectively, increased or decreased in atypical parkinsonism, while their levels in PD subjects were intermediate between controls and atypical parkinsonism (Magdalinou et al. 2017). This reveals that neurodegeneration in PD is comparatively slower than other forms of Parkinsonism.

Blood

Expression of blood proteins changes rapidly in response to any external factor or pathological condition, and blood contains proteins derived from other tissues as well. This makes the blood a rich source of information for analyzing disease progression (Ray et al. 2011). The blood represents the useful source for the discovery of protein biomarker for PD due to the presence of various pathological features such as increase in the level of hydroxyl radicals with duration of disease (Ihara et al. 1999) and mitochondrial complex I deficiency in blood platelets of PD patients (Parker et al. 1989). Comparative proteomics of serum samples of PD patients and controls revealed alterations in 21 proteins belonging to different categories such as cell degeneration, oxidative stress, and inflammation. Out of these, 11 proteins were abnormally expressed only in the patients having mild symptoms and 14 in moderate-to-severe PD stage which shows that the proteome component of blood responds to disease severity (Goldknopf et al. 2009). Similarly, in another study, comparison of serum proteome of Chinese PD patients with control subjects revealed 15 differentially displayed proteins related with antioxidation, lipid metabolism, intracellular transport, cell proliferation, and immunoregulation (Zhao et al. 2010) (Table 1). Lu and colleagues have reported that fibrinogen γ-chain and inter-α-trypsin inhibitor heavy chain H4 proteins show an increased abundance in serum samples of PD patients in comparison to controls while fragmented ApoA4 was present mainly in control samples. The level of these proteins may serve as diagnostic criteria for PD (Lu et al. 2014).

An automated literature analysis of studies conducted on the plasma samples of PD and control subjects revealed 9 proteins, including haptoglobin, transthyretin, ApoA1, serum amyloid P component, ApoE, complement factor H, fibrinogen γ, thrombin, and complement C3 as potential diagnostic proteins. Except serum amyloid P component, fibrinogen γ and thrombin, other 6 proteins were confirmed experimentally as markers of PD (Alberio et al. 2013). On the contrary, another proteomic study showed a significant increase in the level of serum amyloid P component in plasma of PD patients in comparison to controls which indicates the possibility of the protein to serve as a biomarker for PD (Chen et al. 2011a, b). An increase in haptoglobin-related protein precursor and truncated β-globin proteins in plasma of untreated PD patients and their restoration after L-DOPA treatment has been demonstrated by proteomic approach. Reduction in the abundance of truncated β-globin was observed in smoker PD patients pointing towards the protective role of smoking in PD (Sinha et al. 2007). A study conducted on plasma samples of the larger cohort (around 300 subjects) of PD patients along with healthy and diseased controls depicted

that a combination of several peptides derived from proteins PRNP, HSPG2, MEGF8, and NCAM1 (major prion protein, heparan sulfate proteoglycan 2, multiple EGF-like domains 8, neural cell adhesion molecule 1) is useful to distinguish PD from normal subjects, and a combination of two peptides derived from proteins, MEGF8 and ICAM1 (intercellular adhesion molecule 1), show significant correlation with PD severity (Pan et al. 2014). Proteome profiling of exosomes isolated from plasma samples of PD patients and healthy subjects demonstrated that expression of ApoA1 protein in exosomes correlates with the disease progression while the level of plasma protein fibrinogen γ-chain was decreased in PD patients in comparison to controls and could be important for initial PD screening (Kitamura et al. 2018).

Proteome profiling of blood cells has provided the significant insights into the protein alterations in PD patients receiving different therapies. Comparison of proteome profile of peripheral lymphocytes of PD patients under L-DOPA or subthalamic nucleus deep-brain stimulation therapy with normal controls revealed that cytoskeletal proteins such as cofilin-1, tropomyosin, and an actin isoform were altered in patients regardless of the therapy. However, three proteins, namely, tropomyosin variant, protein disulfide isomerase A3, and actin fragment were found to be altered differently between the two therapy conditions (Mila et al. 2009). Peripheral blood lymphocytes respond well towards dopaminergic treatment as shown by the proteomic study conducted on T-lymphocytes derived from PD patients under L-DOPA and/or dopamine agonist treatment. Expression of 2 proteins, ATP synthase subunit β and proteasome subunit β type-2, was linearly correlated with L-DOPA dose while 7 proteins, namely, prolidase, actin-related protein 2, F-actin-capping protein subunit β, tropomyosin α-3 chain, proteasome activator complex subunit 1, Prx6, and glyceraldehyde-3-phosphate dehydrogenase isoform were altered in patients receiving dopamine agonists. These results suggest that therapies involving dopaminergic stimulation may affect immunity of patients by altering T cell proteome (Alberio et al. 2012).

Tears

Dysfunction of lacrimal glands and reduction in tear secretion are common non-motor symptoms of PD (Bagheri et al. 1994). A recent proteomic analysis explored the potential of easily accessible tear fluid for PD diagnosis and demonstrated the dysregulation of several proteins related with immune response, lipid metabolism, and oxidative stress in PD patients in comparison to normal controls (Boerger et al. 2019). The study provided the candidate proteins to be further validated as biomarker for PD.

Potential Samples from PD Patients for Proteomic Analysis: Future Directions

As described above, proteomic studies conducted on specific brain regions and biofluids of PD patients have provided significant clues about its mechanism and possible therapeutic options. However, careful analysis of past and present literature related to PD research points towards the crucial role of other prospective brain regions as well as biofluids in PD pathology which in future could serve as a useful source for the biomarker discovery by applying proteomic approach (Fig. 1).

Brain Regions

Like SN, pathology of the striatum is also related to middle stages of PD progression and there is a 80% reduction in dopamine content in the striatum with the onset of motor symptoms in PD. Neurodegeneration in PD takes place through 'dying back' mechanism which means that neuronal terminals in striatum region are more prone to death than their cell bodies in the SN region (Dauer and Przedborski 2003). The study of protein profile of the striatum region of PD brain may solve many unanswered questions related to initiation of neurodegeneration process. Another important brain region is the cerebellum, which controls posture and motor functions (Wu and Hallett 2013). Akinesia has been correlated with abnormally increased blood flow in the cerebellum (Payoux et al. 2004). The levels of dopamine receptor, tyrosine hydroxylase, and α-synuclein were found to be decreased in the cerebellum of PD patients (Hurley et al. 2003). Future studies focusing on protein profiling of the cerebellum of PD sufferers may help to identify the proteins involved in the regulation of motor functions. Similarly, thalamus and hypothalamus regions of the PD patient's brain show a significant amount of neurodegeneration at advanced stages of PD and can be used for proteomic analysis (Prakash et al. 2016).

Biofluids

Abnormal salivation is one of the common non-motor symptoms of PD (Edwards et al. 1991). Additionally, Lewy pathology in the glands responsible for saliva secretion and altered expression of α-synuclein and DJ-1 is reported in the saliva of PD patients (Del Tredici et al. 2010; Devic et al. 2011). DJ-1 level in saliva has also been correlated with disease severity (Masters et al. 2015). Proteomics of saliva of PD patients represent a convenient and

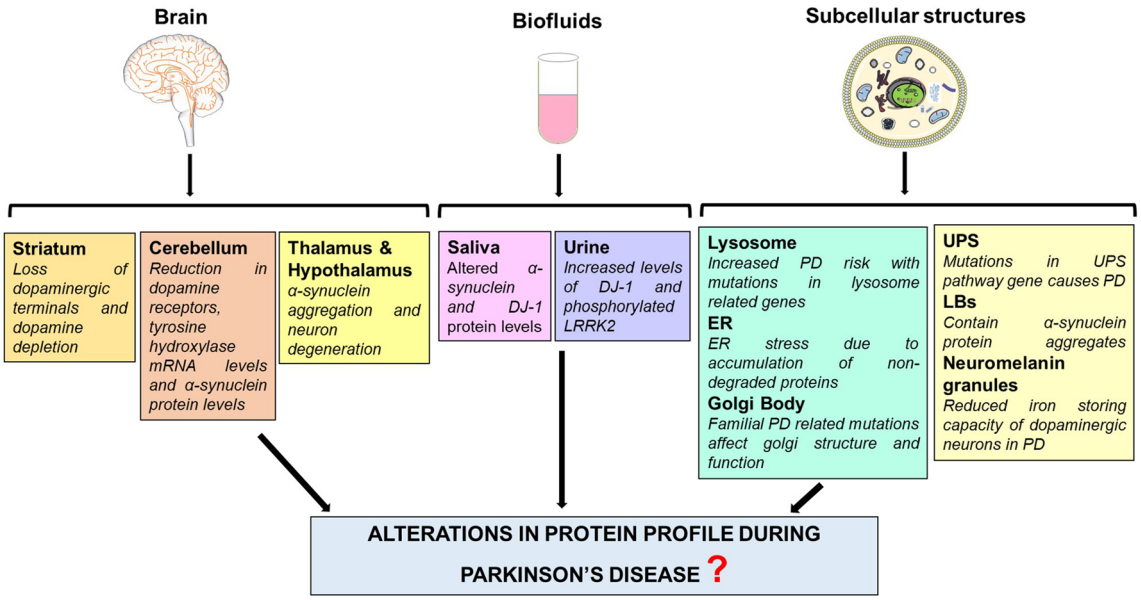

Fig. 1 Prospective samples for proteomic analysis of PD patients. Brain regions, biofluids, and subcellular structures derived from brain tissue have been selected on the basis of representation of disease-associated features/pathways in these samples

useful tool for biomarker discovery as the sample can be obtained in large quantity and at various time intervals. The use of saliva is also advantageous in comparison to blood and CSF due to its non-invasive sampling (Haas et al. 2012).

An increase in the level of 8-hydroxydeoxyguanosine, an indicator of oxidative stress, and DJ-1 protein was found in the urine of PD patients in comparison to control (Sato et al. 2005; Ho et al. 2014). Also, an elevated ratio of phosphorylated Ser-1292 LRRK2 to total LRRK2 in urine exosomes was found to be associated with PD in LRRK2 mutation carriers (Fraser et al. 2016). These studies indicate the strong possibility of discovering specific biomarker for PD in future with the help of protein profiling of urine.

Subcellular Structures

The central role of mitochondria in the pathophysiology of PD has been well documented (Jin et al. 2005; Pienaar et al. 2010); however, it does not provide a complete picture of PD mechanism. The involvement of endoplasmic reticulum (ER), Golgi body, lysosome, UPS, and important role of the proteins localized within these structures in PD pathology should not be ignored (Slodzinski et al. 2009; Ruan et al. 2010; Rabouille and Haase 2015; Domingues et al. 2008). Besides, detailed analysis of the mitochondrial proteome of SN and other brain regions is required to understand the role of mitochondrial dysfunction in PD. As neuronal cell death in PD involves intercommunication and participation of these subcellular structures (Domingues et al. 2008; Arduíno et al. 2009), identification of changes in their protein profile may fill the knowledge gaps associated with the mechanism of neurodegeneration in PD.

Subcellular structures such as LBs and neuromelanin granules are potential samples for future investigations of PD mechanism with the help of proteomics. LBs are the pathological hallmark of PD and contain primarily α-synuclein protein aggregates. Proteomic analysis of LBs obtained from brain tissue of patients with LB pathology revealed that the several kinases and ubiquitin ligases together with a novel deubiquitinating enzyme (otubain 1) were co-enriched with α-synuclein. (Xia et al. 2008). Another proteomic study conducted on porcine brain synaptosomes identified the conformation-specific interacting proteins of human α-synuclein monomers and oligomers (Betzer et al. 2015). These studies depict the role of interacting proteins in the formation of α-synuclein inclusions and its toxicity in neurodegenerative diseases. Leverenz and colleagues collected around 2500 cortical LBs from the temporal cortex of patients with cortical LB disease by utilizing laser capture microdissection technique. Proteomic analysis of these LBs identified 296 proteins including 14-3-3, α-synuclein, heat shock cognate 71 kDa protein, amyloid-β A4, and UCHL1 indicating the important role of these proteins in LB formation and neurodegeneration in PD (Leverenz et al. 2007).

Reduced capacity of neuromelanin to accumulate iron in the diseased state makes dopaminergic neurons more vulnerable to oxidative stress (Double et al. 2002). The iron-storing protein, L-ferritin, has been identified by proteomic approach in the neuromelanin granules of the SN region of

normal brain, which directly indicates the role of neuromelanin in iron homeostasis (Tribl et al. 2009). Neuromelanin granules can be isolated with the help of a sequential fractionation strategy or by laser microdissection and can be further used for proteomic analysis (Tribl et al. 2005; Plum et al. 2016). The latter technique uses a relatively reduced sample amount, i.e., 10-µm-thin sections of brain tissue, which is particularly important in human studies where the SN tissue sample is limited. Study of sub-proteome allows more specific and detailed analysis of various subcellular structures and helps to overcome the dominance of the most abundant proteins over those proteins that are distinctively expressed in disease-associated cells (Tribl et al. 2006).

Limitations

Proteomic analyses of human brain tissue, blood, and CSF provide evidence-based direct information about the pathological changes occurring in PD and has raised the hope for the development of specific biomarker and treatment plans for the incurable PD. However, there are vital challenges which need to be surpassed for obtaining more accurate results using proteomic technique. Major limitations of proteomics of human brain tissue include difficult accessibility and ethical concerns related to its use for the experimental purpose (Ward et al. 2009). Moreover, a human brain can be used only after the death of the patient, which will reflect pathological changes present at the last stage of PD. Sometimes, the long post-mortem delay can affect the expression level of proteins or lead to their degradation (Licker et al. 2009; Licker and Burkhard 2014).

Brain tissue sample prepared by traditional methods does not contain only neuronal cells of interest, but is a mixture of heterogeneous cell population, including glial cells, astrocytes, and other types of brain cells. The data obtained from whole brain tissue proteomics represent the low signal-to-noise ratio, which can be overcome by fractionation of brain tissue (Craft et al. 2013). However, analysis of sub-proteome further involves many challenges. Isolation of specific cell organelle suffers from the impurities of other cellular structures which may yield non-specific results. Fractionation of desired part of the tissue and further its subfractionation are crucial steps and require more sophisticated and reliable techniques. Moreover, this decreases the amount of protein available for the experiment. Since there is no amplification method available for protein sample, a highly sensitive technique is required for sub-proteome analysis of the brain tissue (Craft et al. 2013).

There are several limitations linked with proteomics of CSF or blood samples. Despite the easy availability of CSF samples from PD patients in comparison to brain tissue, it is very difficult to receive ethical approval for the lumbar puncture to collect CSF sample and it becomes even more challenging in case of normal individuals (Ward et al. 2009). Characterization of CSF proteome requires a careful approach in sample collection, storage, preparation, analysis, and data mining for reproducible results (Waybright 2013). Salt and blood contaminants should be completely removed from the CSF sample before analysis. Moreover, due to the low protein content of CSF, prefractionation of sample is necessary in some cases (Shi et al. 2009). Sample collection, storage time, and duration have a significant effect on blood proteome as well. The major limitation of blood proteomics is the presence of some proteins in a fairly large amount which masks the expression of other low abundant proteins (Anderson and Anderson 2002). Technical difficulties associated with proteomic analysis of saliva and urine samples include low protein concentration and high intra-individual and inter-individual variability (Thongboonkerd 2007).

Conclusions

In spite of the great revolution in the technology, it is still a dream to develop an efficient treatment strategy for the PD, a strategy which not only slows down its progression but also eradicates this fatal disease from the patient's body. Advanced as well as conventional techniques in the field of proteomics can offer a new vision to identify and develop the novel biomarkers and therapeutic plans for PD. Detailed analysis of global and subcellular proteome of PD-associated brain regions may decipher the cellular basis of disease mechanism along with role of specific brain regions in PD pathogenesis. Since the origin of PD is in the brain, the poorly accessible organ, the discovery of a PD biomarker from easily available sample is necessary for the diagnosis of the disease. Proteomic analysis of biofluids such as blood, saliva, tears and urine may lead to the discovery of such biomarkers which will help in early detection of PD.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they do not have any conflict of interest.

Ethical Approval All reported studies involving human participants/animals have been previously published and procedures performed in studies were in accordance with applicable ethical standards of the

institution and/or national research committee, international, national, and/or institutional guidelines, and 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the reported studies.

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