Published in final edited form as:

Expert Rev Proteomics. 2016 ; 13(3): 259–274. doi:10.1586/14789450.2016.1149470.

Clinical implications from proteomic studies in neurodegenerative diseases: lessons from mitochondrial proteins

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Abstract

Mitochondria play a key role in eukaryotic cells, being mediators of energy, biosynthetic and regulatory requirements of these cells. Emerging proteomics techniques have allowed scientists to obtain the differentially expressed proteome or the proteomic redox status in mitochondria. This has unmasked the diversity of proteins with respect to subcellular location, expression and interactions. Mitochondria have become a research ‘hot spot’ in subcellular proteomics, leading to identification of candidate clinical targets in neurodegenerative diseases in which mitochondria are known to play pathological roles. The extensive efforts to rapidly obtain differentially expressed proteomes and unravel the redox proteomic status in mitochondria have yielded clinical insights into the neuropathological mechanisms of disease, identification of disease early stage and evaluation of disease progression. Although current technical limitations hamper full exploitation of the mitochondrial proteome in neurosciences, future advances are predicted to provide identification of specific therapeutic targets for neurodegenerative disorders.

Keywords

Proteomics; redox proteomics; mitochondria; Alzheimer disease; Parkinson disease; neurodegeneration; clinical biomarkers

Introduction

Mitochondria are key organelles of eukaryotic cells, as their roles in metabolism and cellular processes guide cell life. Aside from apoptotic cellular death, mitochondria modulate ionic homeostasis, oxidize carbohydrates and fatty acids, and participate in numerous other pathways. Consistent with their apparent prokaryotic ancestry, the mitochondrial proteome is

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Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

relatively less complex than the cellular proteome, yet well defined, as mitochondria preserve their double membrane structure, their own circular genome, transcription and translation machinery. The number of mitochondrial proteins extrapolated from human genomic studies is around 2000, among which only 600 have been identified [1]. For all these reasons, the mitochondrial proteome can be legitimately exploited for proteomics studies.

The involvement of mitochondria in the mechanisms of pathogenesis typical of neurodegenerative disorders is well established [2,3]. However, classical studies on mitochondria from different neurodegeneration models or affected subjects have been applied on single proteins, by using enzymatic or electrophoretic methods. In contrast, proteomics allow separation and detection of a variety of proteins at once, providing a global glance of the whole proteome status. Furthermore, recent advances in proteomics have allowed more in-depth studies of proteins, such as protein expression levels, posttranslational modifications, localization and interaction. For these reasons, joint efforts by genomic, mass spectrometry and bioinformatics studies have increased the number of identified human mitochondrial proteins, providing new tools not only to investigate mitochondrial and cellular function but also to understand the pathogenesis of diseases in which mitochondrial dysfunctions are known to play a role. Indeed, proteomics approaches on mitochondria allow identification of candidate biomarkers for (early) diagnosis and staging of disease. This review summarizes the features of the mitochondrial proteome and the progress in the comparative mitochondrial proteomic studies with respect to Alzheimer disease (AD), Parkinson disease (PD) and Down syndrome (DS), highlighting the clinical significance of the findings in terms of protein expression and oxidation. Present challenges and future perspectives of the role of the mitochondrial proteome in neurodegeneration are also discussed.

The Mitochondrial Proteome

Mitochondria, aside from their function in bioenergetics, can regulate cell death, modulate ionic homeostasis, oxidize carbohydrates and fatty acids, and participate in numerous other catabolic and anabolic pathways. It is far from unlikely then that mitochondrial dysfunction can have grave consequences that range from defects in energy metabolism to etiologically complex diseases with a mitochondrial association [1].

Properties of the mitochondrial proteome

Size and dynamic range—Pagliarini and coworkers [4] defined a high-confidence mitochondrial compendium of 1098 genes, called MitoCarta; these scientists combined their data of discovery and subtractive MS/MS spectra obtained from matched crude and highly purified mitochondria collected from ten tissues with computation, microscopy, and previous literature to produce their compendium [5]. This is the most complete mitochondrial catalog at present and contains ~1100 gene loci encoding for mitochondrial proteins. As protein abundance is lately becoming a key parameter describing a proteome, it is crucial to define the abundance of the mitochondrial proteome, which can span a broad dynamic range. Five or six orders of magnitude of abundance have been previously reported [6]. The two most

abundant inner (IM) and outer mitochondrial membrane (OM) proteins, based on 2D gels, are adenine nucleotide translocator (ANT, in the IM) and voltage-dependent anion channel (VDAC, in the OM) [7]. Also, based on estimates of protein abundance across 14 mouse tissues, the five most abundant mitochondrial proteins are ATP5A1, ATP5B, ACO2, ANT1, and ANT2 [4].

Mitochondrial localization of proteins—Recent studies suggest that around 15% of mitochondrial proteins are dual-localized [8]. Although mass spectrometry-based proteomics is often employed to characterize the protein composition of organelle-enriched fractions [9], the high sensitivity of mass spectrometers and the difficulties related to the purification of organelles to homogeneity, often pose a difficulty in distinguishing organellar proteins from those that of contaminating components. However, protein correlation profiling (PCP) [10], in which mass spectrometric intensity profiles from organelle marker proteins were used to define consensus profiles obtained from density centrifugation gradients, in direct analogy to Western blotting profiling of gradient fractions, may ease or potentially eliminate this difficulty. Confidently detection of multiple locations for 39% of all proteins and 16% of mitochondrial proteins has been achieved [6].

Also data derived from yeast are available in which 60% of the *S. cerevisiae* proteome has been epitope-tagged. Using directed topoisomerase I-mediated cloning strategies and genome-wide transposon mutagenesis, Kumar et al. have determined the subcellular localization of more than 2000 yeast proteins by immunolocalization of tagged gene products [11]. These researchers found evidence of dual localization for 11% of all proteins and 15% of proteins with mitochondrial staining.

The high number of dually localized mitochondrial proteins poses interesting questions with respect to the meaning. This is probably necessary to accomplish similar functions in different compartments of the cell with distinct regulatory properties. These proteins are usually products from a single gene locus that are present in multiple compartments. This dual localization can be attained through several mechanisms, such as alternative splicing or alternative start sites in order to produce two distinct proteins, one of which has a mitochondrial import presequence. Moreover, like the proapoptotic protein BID, proteins may also post-translationally change cellular locations upon a stimulus: following a death stimulus the COOH-terminal domain of BID, cleaved by Caspase-8 indeed does translocate from the cytosol to the mitochondrion where it triggers cytochrome c release [12].

Mitochondrial metabolic pathways—Among the ~1100 proteins in the mitochondrial proteome found in the Pagliarini et al. [4] compendium approximately 300 proteins have no known function, resulting in no association with any biological process from Gene Ontology: this might indicate that novel mitochondrially resident components for pathways are waiting for discovery. An additional 300 proteins have only domain annotations based on sequence similarity through phylogenetic profiling [4,13]. These proteins could be new components of well-studied pathways such as those involved in energy metabolism, like OXPHOS and the TCA cycle, or part of additional pathways, such as heme biosynthesis, fatty acid/amino acid oxidation, pyrimidine biosynthesis, calcium homeostasis, or apoptosis. Or, alternatively and surprisingly, these additional proteins may

represent components of novel pathways, meaning pathways not previously appreciated even though they reside in mitochondria.

There have been elegant studies that highlighted uncharacterized proteins, now shown to reside in the mitochondrion, tightly connected to modules of known function. For example eukaryotes appear to have a highly conserved mitochondrial pathway for fatty acid synthesis (FAS), which is completely independent of the cytosolic FAS machinery. The activities of this mitochondrial complex are catalyzed by soluble enzymes, and the pathway is reminiscent of its prokaryotic counterparts [14]. One already well-defined end product of this pathway is octanoic acid, which is the direct precursor for lipoic acid synthesis, and possibly for fatty acids that are incorporated into the mitochondrial lipids. Also, Nilsson et al. discovered such unknown components of heme biosynthesis by combining MitoCarta informations with large-scale coexpression analysis [15]. They identified SLC25A39, SLC22A4, and TMEM14C, which are mitochondrial transporters, as well as C1orf69 and ISCA1, which are iron-sulfur cluster proteins, to be required for the eight enzymatic reactions that originate in mitochondria and continue in the cytosol leading to heme biosynthesis.

Not least large-scale proteomic surveys also have revealed a large number of mitochondrial proteins that appear to be involved in reversible phosphorylation and acetylation [16,17], suggesting the existence of a large signaling network within the organelle and a high level of regulation of mitochondrial proteins. Further studies will be needed though for a better understanding of tissue-specific changes in mitochondrial protein post translational modifications for a range of physiological and pathophysiological conditions.

Mitochondrial protein expression in tissues—Starting from the whole population of the organelle in the cell, eukaryotic cells contain mitochondria variable in number and shape, which in turn contain multiple copies of a small compact genome (mtDNA) whose expression and function is strictly coordinated with the nuclear one. In response to overall metabolic and bioenergetics demands, mtDNA copies and total mitochondrial number vary raising a multiple-fold difference between different cell or tissues types, and they also may vary as a consequence or cause of specific pathological conditions [18]. Regulation of mtDNA copy number seems to be linked with the epigenetic methylation of the nuclear-encoded mitochondria DNA polymerase gamma catalytic subunit (POLG) in a tissue specific manner [19]. Knockdown of mitochondria DNA polymerase POLG in mouse embryonic stem cells (ESCs) results in reduced expression of the pluripotency transcription factor OCT4 and slightly increased levels of some of the mesodermal markers [20].

During cell differentiation, different tissues need higher amounts of energy necessary to sustain specialized functions whereas a lower proliferation capacity and necessity of anabolic precursors allow for a more efficient conversion of metabolic substrates into ATP [21]. The reprogramming of patient fibroblasts with mitochondrial defects provides a great model for this: for example, Cooper et al. [22] generated induced pluripotent stem cells (iPSCs) from neurodegenerative diseases patients carrying mutations in mitochondrial protein like PINK1 and LRRK2. Researchers found that neural cells differentiated from patient-specific iPSCs could be phenotypically rescued by certain chemicals [23].

It seems that almost half of the ~1100 mitochondrial proteins are central components found in virtually all tissues, whereas the remaining half are distributed in a tissue-specific manner [4]. This appears to be reasonable since mitochondria are geared to suit the metabolic and signaling needs of each cell type.

Consistent with the considerations above, mitochondria from mouse brain, heart, kidney, and liver have been explored in their molecular composition by Mootha et al. [24], combining the results with existing gene annotations. These workers indicate tissue-specific differences in organelle composition, based on the large concordance of protein expression data with large-scale surveys of RNA abundance, and both measure RNA expression profiles across tissues revealed networks of mitochondrial genes that share functional and regulatory mechanisms.

Some core pathways show rather surprising patterns of tissue diversity. The only direct evidence of tissue specificity is for cytochrome c oxidase. Indeed, unlike complexes I, II, III, and V that seem to be expressed in high abundance in all tissues, complex IV is an interesting outlier. Different subunits of the enzyme have different migrations in different tissues [25]. Furthermore all of the nuclear encoded subunits of complex IV appeared to show tissue specific as well as adult-fetal differences in immunological reactivity [26]. Also the mitochondrial ribosome is one of the most different of all large macromolecular complexes among tissues. The mitochondrial ribosome is required for translating the 13 mtDNA-encoded proteins in all tissues, but many of its subunits appear to be expressed in a tissue-specific manner [27].

Another critical aspect involves cell defense. Since a functional mitochondrial network must be maintained to avoid cell damage [28], the cell removes misfolded, denatured or oxidized proteins through the activity of mitochondrial proteases that remove proteins resident in the mitochondrial milieu [29,30], and on the activity of the cytosolic ubiquitin–proteasome system (UPS), which in turn recognizes and disrupt mistargeted or misfolded mitochondrial proteins before they reach the organelle. Importantly, the mitochondria control UPS-dependent is regulated at different levels and one of them depends on tissue specificity of E3 ligases transcripts [31,32].

Finally, the shift between fusion and fission is one of major regulatory mechanism that takes part in the dynamism of the mitochondrial network; indeed complex and divergent stimuli influence the mitochondrial appearance [33,34].

Ganglioside-induced differentiation associated protein 1 (GDAP1) has been recently found to be a component of the outer mitochondrial membrane and together with other proteins regulates the balance between fused and fragmented mitochondria, the last most likely derived to mitochondrial fission. Its COOH-terminal part is essential for organelle targeting and the localization of this protein into mitochondria is required for activity. It is not surprising that an increase of GDAP1 mRNA expression was found in neural-differentiated P19 cells and during development of the mouse brain [35]: the precise regulation of mitochondrial dynamics indeed seems to be crucial for the integrity of the peripheral nervous system (PNS). Therefore the proper localization and the modulating activity on

mitochondria of GDAP1 appear to be very crucial for the functional integrity of myelinated peripheral nerves [36].

All these data reveal that a deeper analysis of tissue-specific expression of mitochondrial proteins conceivably could provide a framework for understanding the organelle's contribution to human disease.

Recent findings have shown that neurotropic viruses (such herpes virus) attack and disrupt the nervous system by taking control of cell's mitochondria. This occurs through the intervention of the mitochondrial protein Miro, which under the effect of intracellular Ca^{2+} increase, promotes blockade of the organelles in the synapse, a mechanism very specific in neuronal cells, in such a way that mitochondria can provide energy as the cell passes the signal along to the next neuron. Viral infection floods the cell with Ca^{2+} , which can be detected by Miro, and this stops mitochondria by the axon and causes them to shed motor proteins. It is suggested that the virus then interacts with kinesin-1 to freely move within the infected cell and spread into the nervous system [37].

Strategies to identify mitochondrial proteins

Mitochondrial Targeting signal sequence—It was originally believed that all mitochondrial precursor proteins are imported through the so-called presequence pathway, in which the classical mitochondrial targeting signals are the cleavable presequences. However, noncleavable targets and other sorting signals, which are located in the mature regions of mitochondrial proteins, have also been described, suggesting the existence of different routes for proteins in the sorting process within mitochondria. The presequence pathway requires an outer and inner membrane translocase and needs precursor proteins to have amino-terminal presequences that form positively charged amphipathic α helices. These, in turn, would act as classical targeting signals directing proteins into mitochondria.

Later studies demonstrated that the precursors of metabolite carrier proteins of the inner mitochondrial membrane can rely on different signals for a different sorting route, suggesting that there are two pathways for mitochondrial import: the presequence pathway and the carrier pathway. Fewer than 5 years ago, the entire view of mitochondrial protein biogenesis changed very rapidly because numerous new import components and two new import pathways were identified. New mechanistic principles, such as redox-regulated import, formation of supercomplexes with components of the respiratory chain [38], and two-membrane coupling of translocases, were discovered. The mechanisms by which precursor protein translocases are built up has been deciphered. Basically, these translocases are composed of modular units that dynamically cooperate with each other [39].

The discussion above demonstrates the versatility of the protein import apparatus of this organelle. Despite this complexity, three principles can be distinguished in the targeting signals for mitochondrial proteins: (1) single linear targeting signals at the amino terminus or internal positions; (2) multiple noncontiguous signals that direct translocation in a loop formation; and (3) redox-regulated signals that undergo transient covalent interaction with the corresponding import receptor [40].

Utilizing multiple proteomic approaches (iTRAQ and two-dimensional-differential in-gel electrophoresis) proteomic alterations have been reported, associated with nuclear encoded mitochondrial protein import dysfunction [41].

Prediction of mitochondrial localization can rely on different algorithms [4], which represent a potential but still weak instrument to in silico identify protein localization in mitochondria [4].

Mass spectrometry-based proteomics—Mitochondria are ideal targets for whole proteome analysis studies because they have a slight difficult level of complexity as a consequence of their apparent prokaryotic origin [1]. Early studies initially separated mitochondrial proteins by 2D gel electrophoresis and used peptide mass fingerprinting or MS/MS to identify 46 proteins in mitochondria from human placenta [42]. Recently Pagliarini et al. analyzed mitochondrial extracts from 14 different mouse tissues and detected a total of 3881 proteins [4].

Histograms of the distribution of protein pI values over the 4–11 range also have been performed on the human heart proteome, based on similar approaches that had been used previously for proteomes of several prokaryotes with fully sequenced genomes [43]. It was found that integral membrane proteins make clusters around pI 8.5–9.0, whereas cytosolic proteins clustered around pI values of 5–6. Interestingly, there was also a third cluster with pI values around 7.0 in eukaryotes. An overrepresentation of basic proteins may represent both the cationic characteristics necessary for efficient import into their mitochondrial locations and the high proportion of integral membrane proteins identified [1]. The latter have a higher proportion of basic residues to promote stability through favorable electrostatic interactions [43].

Since well annotated proteins likely represent the more abundant proteins, in agreement with analysis by traditional biochemical approaches, the estimates most probably represent a lower bound on the mitochondrial proteome. In the future mitochondrial proteomics should make efforts to expand the inventory of mitochondrial proteins through benefits from higher dimensional chromatography and improved sample preparation, more sensitive and quantitative mass spectrometry technologies [9], and hopefully by use of genetic strategies [44]. Combining data with genome-wide expression microarrays, may lead to reconstruction of pathways in a more comprehensive way within the mitochondrion and to determine the extent to which mitochondrial diversity occurs in other cell types and to lower abundant gene products [24].

It should be highlighted that not all proteins detected in mitochondrial extracts actually represent proteins truly localized into mitochondria. The high sensitivity of new generation instruments indeed show that many of detected proteins are contaminants from co-purification, which represent up to 75% of proteins detected by the most sensitive MS/MS experiments. However, one experimental approach that should be performed to distinguish contaminants is protein correlation profiling (PCP), which first separates subcellular organelles through a sucrose gradient and then compares profiles of peptides across gradient fractions to profiles of marker proteins for each organelle [6]. Also, comparing MS/MS

abundance measurements of each protein across many cellular compartments could be an alternative approach to assign the mitochondrial localization of the detected proteins in a more certain way [45].

Fluorescence Microscopy—Another experimental approach in the study of the mitochondrial proteome is based on microscopy in order to establish mitochondrial localization. Tagging endogenous proteins with epitopes such as GFP, or transfecting cells with exogenous tagged genes can facilitate this approach, by immunofluorescence of native proteins using antibodies [27].

A new public resource for the storage and investigation of mitochondrial proteomics data, called MitoMiner, that exploits a model to describe the proteomics data and associated biological information, has been developed. Proteomics data of 33 publications from both mass spectrometry and green fluorescent protein tagging experiments were imported in the database and integrated with protein annotation from UniProt and genome projects [46]. Currently, there are a total of 321 human proteins and 166 mouse mitochondrial proteins evidenced through microscopy according to the MitoMiner database.

Clinical Insights From Mitochondrial Proteomics

Proteomic technologies are emerging as powerful tools to produce high throughput data, enabling comprehensive analysis of structure and function at a “single protein” detail [47]. This approach represents a valid platform for providing molecular insights into several aspects of neurodegenerative diseases.

A strong body of evidence points to mitochondrial dysfunction as a key player in aging-related neurodegenerative diseases including Alzheimer’s disease (AD), Parkinson’s disease (PD), Down syndrome (DS) among others. Proteomic studies on mitochondrial proteins are then crucial to the most complete understanding of the pathogenetic mechanisms underlying neurodegenerative disorders. Since a thousand different polypeptides are estimated to reside in mitochondria [48], mitochondrial prefractionation is required to identify of mitochondrial-specific low-abundant proteins as well as hydrophobic proteins [49]. However, proteomic data from crude sample have identified some targets of mitochondrial localization.

In this section we will describe the pathogenetic mechanisms linking mitochondria to neurodegenerative diseases (such as AD, PD, and DS) and describe the most significant proteomic works with respect to identification of clinically significant mitochondrial targets. Since studies are reported on proteins displaying changing levels of expression and oxidation in these disorders, an introduction on protein oxidation opens the following section.

Redox biology and protein oxidation

Oxidative stress is a condition that arises when free radical production exceeds biochemical means of scavenging these free radicals, and this is traditionally considered a cause of damage [50]. However, studies over the last 20 years in different organisms point to a different view with respect to the role of ROS, which are no longer considered as damaging

but also as signalling molecules, involved in regulation of different important pathways, such as proper cellular differentiation, tissue regeneration, and prevention of aging (i.e. redox biology) [51]. In pathological conditions production of ROS might be excessive or temporally/spatially unrelated to its physiological signalling role, leading to attack on biomolecules, such as proteins [51–53].

Oxidative modifications on proteins have been sometimes associated to protein loss of function [50,53]. Protein oxidation has also been linked to the execution of signalling mechanisms [54], which need to be coherent with that signalling pathway being activated. Oxidative stress is then a very significant event not only with respect to protein inactivation by ROS damage, but also to protein “gain of function”, which underlines the perturbation of redox biological signals due to prolonged or unbalanced ROS production, leading to unsynchronized, hyper- (or hypo-) activated and then pathological events for cell function [51]. How these harmful mechanisms concur to neurodegenerative disorders is far from being understood. Redox proteomics represent a valuable tool to provide insights with this respect. Analysis of protein oxidation relies on detection of protein carbonyls, which arise as a consequence of complex reactive processes involving free radical reactions on amino acid side chains [50].

Several classes of proteins can be differently oxidized. An intriguing question arises on which feature renders a protein more susceptible to protein oxidation and if a relationship between protein expression and protein oxidation exists. Evidence suggesting a relationship between protein oxidation and protein expression come from our seminal redox proteomics studies in AD brain. CK and GS are the more extensively studied proteomic targets in AD. Both proteins undergo oxidative modifications and display reduced activity. Interestingly, the expression of both proteins is reduced in AD, as revealed by 2D-PAGE maps [53,55]. This might evidence the fact that oxidative targets of protein oxidation might precipitate as insoluble matter, suggesting an inverse relationship between protein oxidation and the amount of soluble protein [56]. This can be extended to some but not all oxidized proteins.

Considering the susceptibility of a protein to a given insult, it is notable that recurrent identification of oxidation targets such as GADPH, α -enolase, UCH-L1, V-DAC and subunits of the ATP synthase, indicating that protein abundance might discriminate which target is more susceptible of oxidative modification. Protein primary sequence might also influence oxidation. Free protein thiol groups also correlate to the extent of protein oxidation [57]. Oxidation of methionine, which is a known feature of protein oxidation, occurs in a very specific way, as it depends on the neighboring amino acids. At physiological pH, methionine is more easily oxidized in an acidic amino acid environment. Additionally, the extent of methionine oxidation depends on the used oxidant [52,58]. Whether this holds true for oxidative modifications on other amino acids is still to be clarified.

Alzheimer disease

Mitochondrial dysfunctions in AD—AD is the most common cause of memory and cognitive impairment among elderly people. The three major characteristic neuropathological hallmarks of AD include the presence of senile plaques (SP, composed of a core of amyloid β -peptide ($A\beta$) surrounded by dystrophic neurites with several associated

proteins), neurofibrillary tangles (NFT, composed of hyperphosphorylated tau protein) [59], and the loss of synapses in brain parenchyma [60]. A β , a 39–43 amino acid peptide, is formed by proteolytic cleavage of amyloid precursor protein (APP) by β -secretase and γ -secretase [50].

Accumulating evidence indicates that mitochondrial abnormalities and oxidative damage take place early in AD brain [61,62]. Indeed the mitochondrial hypothesis has been proposed that causally links mitochondrial dysfunction and A β deposition, synaptic degeneration, and formation of neurofibrillary tangles [63] in AD brain. Also, alternative approaches focused on manipulations of mitochondria and energy metabolism, both of which are altered in the brains of AD patients, have been proposed [64].

Mitochondrial metabolism is lowered in AD brain. In fibroblasts and post mortem brain from AD subjects the activities of the TCA enzymes (pyruvate dehydrogenase, isocitrate dehydrogenase and α -ketoglutarate dehydrogenase) are reduced [47,65]. Additionally the respiratory chain complexes I, III and IV are reduced in platelets and lymphocytes from AD patients and postmortem brain tissue [66–69]. Mitochondrial dynamics and biogenesis also are impaired in AD brain [70–72]. AD fibroblasts display alterations in mitochondria morphology and distribution [28], which has been linked to a decrease in dynamin- like protein 1 (DLP1), a regulator of mitochondrial fission and distribution [73].

Oxidative stress and mitochondria are strongly linked. In the Tg2576 mouse A β plaque deposition follows H₂O₂ production and loss of cytochrome oxidase activity, suggesting that oxidative stress is an early event in AD pathophysiology [74]. A significant decrease in the levels of HNE-lipoic acid in the AD brain was found compared to that of age-matched controls. Lipoamide dehydrogenase (LADH) activity was measured in AD, and found to be decreased compared to control brains. Additionally, LADH activity was measured after *in-vitro* HNE-treatment to mice brains. Both LADH expression level and activity were found to be significantly reduced in AD brain compared to age-matched control [75].

Mitochondrial proteomic studies in AD—Several proteomic studies on mitochondrial proteins have been performed, either identifying mitochondrial targets or directly on isolated mitochondrial fractions. A comprehensive ICAT-based proteomic study from mitochondria isolated from MCI and AD brain identified several proteins displaying an increase in MCI compared to control and a further increase from MCI to AD. These proteins belong to different functional groups: TCA related enzymes (citrate synthase, aconitase and malate dehydrogenase); ATP-related proteins (ATP synthase and ATP/ADP translocase 1); aminoacid metabolic enzymes (glutaminase, aspartic aminotransferase); and OXPHOS components (cytochrome C oxidase and reductase, NADH-ubiquinone oxidoreductase) [76]. These findings suggest a strong metabolic rearrangement in different classes of metabolites in AD brain that dynamically accompanies the transition from MCI to AD.

Transgenic mice models of AD have been also extensively studied. A proteomic work on cerebral cortices of 6-month-old male 3 $\times$ Tg-AD (which harbor mutations in three human transgenes) analyzed before deposition of significant amyloid plaques and neurofibrillary tangles found derangement in protein expression in TCA enzymes and oxidative

phosphorylation subunits, suggesting an early role of mitochondrial dysfunction in AD development [77]. A β incubation with primary rat cortical neuron cultures led to alteration of several proteins, among which Na⁺/K⁺-transporting ATPase, cofilin, dihydropyrimidinase, pyruvate kinase and VDAC1 compared to untreated cells were identified [78]. Synaptosomes from Tg2576 mice over-expressing mutant human amyloid precursor protein (K670N, M671L) and from their non-transgenic littermates, exhibited changes in the subunit composition of the respiratory chain complexes I and III compared to non transgenic animals, consistent with the notion of the toxic role of A β on mitochondrial functions [79].

Extensive redox proteomic studies have been performed with this respect. Identification of targets of protein oxidation, correlation with protein expression and enzyme activity has clearly provided clues into the clinical implications of oxidative stress, which is known to take place in AD brain [50]. Although data on activity changes related to oxidation are not present for each protein, it is likely that protein oxidation could mediate processes of activity loss, increased turnover, or gain of function [80,81]. From here a clear link between oxidation related protein abnormalities and disease pathogenetic mechanisms can be established. Our group has pioneered that technology starting from AD and MCI specimens [53,82–85] as well as to several animal models of AD and other neurodegenerative disorders [86–92].

GAPDH, aconitase, VDAC, ATP synthase-alpha chain, lactate dehydrogenase (LDH), beta-actin, and alpha-tubulin, which are either mitochondrial proteins or are known to interact with mitochondria, have been identified as oxidatively-modified in AD brain [50, 93–95]. VDAC is a part of the outer mitochondrial membrane and a component of the mitochondrial permeability transition pore (MPTP). VDAC participates in synaptic communication, and is involved in the early stages of apoptosis [96]. VDAC-1 were reported to be significantly decreased in AD brain [94,95] and oxidation and dysfunction of this protein correlated well with the impairment of learning and memory as reported in AD [97]. Oxidation of VDAC-1 may trigger apoptosis [98,99]. All these findings point to a role of oxidized VDAC in modulating synaptic transmission and plasticity and induce cell death in AD brain through apoptosis.

Oxidation of GAPDH, a glycolytic enzyme that catalyzes the reversible phosphorylation of glyceraldehyde-3-phosphate to 1,3-bisphosphoglycerate, might impair energy generation [100–101]. GAPDH oxidation correlated with the reported decreased activity of GAPDH [100]. The decreased level of GAPDH correlates well with the decreased cerebral glucose utilization reported in AD brain [100]. Moreover, decreased activity of GAPDH would lead to elevation of trioses, such as methylglyoxal, a reactive aldehyde capable of modifying proteins [101,102].

Beta-actin and alpha-tubulin play a structural role in mitochondria [103,104]. Their oxidation is likely to lead to mitochondrial structural abnormalities that may eventually lead to malfunction of mitochondria, contributing to the AD pathogenesis. For example mitochondrial fission and fusion require cytoskeletal remodeling, so oxidation of mitochondrial proteins would be predicted to negatively modulate the dynamic nature of mitochondria.

ATP synthase α -chain is a part of complex V of the electron transport chain of the inner mitochondrial membrane, involved in oxidative phosphorylation and fundamental for ATP synthesis and release. Increased oxidation associated with a decreased level of ATP synthase in AD brain [68,95,105,106] together with the activity-changing association of ATP synthase to both APP and A β [107] point to a role of ATP synthase oxidative modification in the observed decrease in brain glucose metabolism in AD and MCI.

LDH is also found in the mitochondria and is important in the utilization of lactate to generate ATP, and this takes place in both neurons and in astrocytes [108]. Since the energy demand of brain is high, a decline in the process of utilization of lactate to generate energy due to LDH oxidation which is detrimental to brain function.

Studies on MCI brain identified a number of overlapping mitochondrial oxidized proteins overlapping with AD including, ATP synthase alpha, lactate dehydrogenase and beta-actin [82,84,93–95]. Additionally, malate dehydrogenase (MDH), which catalyzes the reversible oxidation of malate to oxaloacetate by NAD⁺ in the TCA cycle, was found to be specifically oxidized in MCI brain [94]. The significance of increased activity of MDH in MCI brain is unclear, but conceivably this observation may reflect a compensatory response of the brain to oxidative damage to other key energy-related proteins in this prodromal stage of AD. A recent quantitative proteomic study in AD hippocampus identified oxoglutarate/malate carrier (SLC25A11) as upregulated in AD hippocampus [109].

Deeper insights into the clinical potential of mitochondrial proteomics have been provided by our work on mitochondria isolated from lymphocytes of patients affected by mild cognitive impairment (MCI), in which a correlation between increased oxidative stress with altered levels of a number of vitamin E components is present [110]. In order to elucidate whether targets of mitochondrial protein oxidation in the peripheral system may potentially reflect the brain damage and could potentially serve as a biomarker for progression, diagnosis, or treatment of AD we identified several markers of mitochondrial protein dysregulation. These proteins from lymphocyte mitochondria that were identified as altered between controls and MCI are grouped into 4 categories: *cellular energetics* that include - glyceraldehyde 3-phosphate dehydrogenase (GADPH), lactate dehydrogenase B chain (LDH), and ATP synthase subunit beta; *structural proteins*: annexin, beta-centractin, and myosin light polypeptide 6; *cell signaling*-Rho GDP-dissociation inhibitor 2 (RhoGDI); and *cellular defense*: thioredoxin-dependent peroxide reductase/peroxiredoxin III (PDXIII) [111]. These results point to a potential role of mitochondria from peripheral lymphocytes, in addition to plasma and CSF, in contributing to a potential biomarker assessment for AD [111] and MCI [110]. Indeed elevated indices of oxidative damage to mitochondria isolated from lymphocytes inversely correlated with performance on measures of cognitive function in both AD and MCI, supporting the hypothesis that mitochondria isolated from peripheral lymphocytes potentially could be part of a biomarker for AD and its earlier forms.

Platelet studies have also provided clues with respect to the role of mitochondrial proteins as biomarkers associated with AD. Reduced OXPHOS complex I, III, and IV activities have been reported in platelets and lymphocytes from patients with AD and in postmortem brain tissue [66–68]. The neurotransmitter-degrading enzyme monoamine oxidase Mao-B has

been investigated with this respect due to the observation that Mao-B activities in brain and platelets and mRNA in brain [112–113] are elevated and correlate positively with ageing [112].

Parkinson's Disease

Mitochondrial dysfunctions in PD—Parkinson's disease (PD) is an age-related, neurodegenerative motor disorder characterized by progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta and by the presence of α -synuclein-containing protein aggregates. The notion that PD pathogenesis is linked to mitochondrial function is clearly established for 25 years [114]. The discovery of a syndrome reminiscent of PD by the drug 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) a mitochondrial complex I inhibitor [115], led to the development of a MPTP animal model of PD, strongly linking PD pathogenesis to mitochondrial dysfunction [116].

There have been several reports of mtDNA mutations in rare maternally-inherited pedigrees of parkinsonism, including the 12SrRNA gene in one family with parkinsonism, deafness and neuropathy [117]. More recently, mutations in DNA polymerase γ (POLG), a nuclear encoded mitochondrial gene and multiple mitochondrial deletions, were reported in parkinsonism associated with progressive external ophthalmoplegia [118].

The subcellular localization of DJ1 [119], a protein which mutations were identified in two European families with early-onset ARPD [120] was recently clarified. The distribution of the endogenous protein is primarily cytoplasmic but a smaller pool localizes to mitochondria [119]. DJ1 was detected in both neurons and glia. DJ1 is a member of the ThiJ/PfpI/DJ1 superfamily. Among its functions the most relevant in terms of the pathogenesis of PD is its potential role in oxidative stress response, either as a redox sensor or antioxidant protein [121,122]. To support this hypothesis, in mammalian cells exposed to an oxidative stressor, such as paraquat or H₂O₂, DJ1 has been shown to undergo an acidic shift in pI-value by modifying its cysteine residues, quenching ROS and protecting cells against stress-induced death.

The levels of the mitochondrial proteins prohibitin, ATP synthase and superoxide dismutase 2 (SOD2) are altered in the substantia nigra and frontal cortex tissue of PD patients compared to controls [123]. Mutations of different genes involved in the genetic forms of PD are strongly linked to altered mitochondrial function leading to oxidative stress [2,124,125]. Among these, the PTEN-induced kinase 1 (PINK1) is a mitochondria-targeted serine/threonine kinase, which is linked to autosomal recessive familial Parkinson's disease [125]. Mutations in the mitochondrial Ser/Thr kinase PTEN-induced kinase 1 (PINK1) are associated with an autosomal recessive familial form of early-onset PD. Recent studies have suggested that PINK1 plays important neuroprotective roles against mitochondrial dysfunction by phosphorylating and recruiting Parkin, a cytosolic E3 ubiquitin ligase, to facilitate elimination of damaged mitochondria via autophagy-lysosomal pathways. Loss of PINK1 in cells and animals leads to various mitochondrial impairments and oxidative stress, culminating in dopaminergic neuronal death in humans [126]. PINK1 is supposed to be related with ψ m through the involvement of the electron transport chain or, more likely, the mitochondrial pro-apoptotic pathways. In response to stress both the voltage-dependent

anion channel (VDAC) and the adenine nucleotide translocator (ANT) are phosphorylated and this might represent a potential area for PINK1 involvement [126]. PINK1 has been associated OMI/HTRA2 with respect to mitochondrial response to cellular stress and modulation of apoptosis. OMI/HTRA2 (high temperature requirement protein A2) protein is an example of a procaspase that is released from the intermembrane space by the opening of the mPTP. It is implicated in neurodegeneration, and recently has been associated with PD19. It does localize to the mitochondrial intermembrane space, and it could be released into the cytosol during apoptosis to relieve the inhibition of caspases by binding to inhibitor of apoptosis proteins (IAPs) [127]. OMI/HTRA2 can most probably act as a downstream target for PINK1, which initiates apoptosis when levels of oxidative stress become too high.

Furthermore, 5–6% of cases with a positive family history of PD have been estimated to associate on LRRK2 mutations [128]. This protein encodes a kinase capable of autophosphorylation associated with the outer mitochondrial membrane (OMM) [129,130] and of binding parkin [131]. Indeed, overexpression of mutated forms was sufficient to induce neuronal degeneration in mouse primary cortical neurons [126,131].

Mitochondrial proteomic studies in PD—Proteomic studies on PD are extensive [132]. Here we focused on those more directly related to or performed on the mitochondrial proteome.

Proteomic studies performed in human brain tissue from PD patients [133–136] and compared to those from healthy controls showed up-regulation of peroxiredoxin II, mitochondrial Co-III, ATP synthase D chain, complexin I, profilin, L-type calcium channel α subunit and fatty-acid binding protein. Other differentially regulated proteins were aldehyde dehydrogenase 1, annexin V, β -tubulin cofactor A, co-actosin-like protein and V-type ATPase subunit 1 [136]. These results are consistent with the view that mitochondrial function is impaired in PD [137].

One of the few proteomics studies on mitochondrial proteins from PD brain [138] used multidimensional protein identification technology (MudPIT) [139,140]. A total of 119 proteins were identified, among which mortalin, and several subunits of Complex I are notable. Mortalin belongs to the heat shock protein 70 family and plays a role in the control of cell proliferation but can also act as a chaperone [141] and associate with the protein translocation system [142,143]. Protein quality control within the cell implies intervention of many molecular chaperones and proteases [144]. When this quality control system is disrupted due to protein oxidation, misfolding, inactivity and aggregation can occur. Changes in mortalin might reflect this event.

In contrast to studies on human brain, proteomics investigations of animal models of PD are extensive. A proteomics study on 6-OHDA rat model of PD [145] identified α -enolase as upregulates, suggesting that glycolytic changes occur as a compensatory effect of energy depletion. However, enolase is more than a glycolytic enzyme: major pro-survival pathways are regulated by enolase, that become dysregulated in oxidative stress conditions. Evaluation of the differences in expressed brain proteome and phosphoproteome between 6-month-old PINK1-deficient mice and wild-type mice suggests that defects in signaling networks,

energy metabolism, cellular proteostasis, and neuronal structure and plasticity are involved in the pathogenesis of familial PD [125].

By applying a redox proteomic approach, three significantly oxidized proteins in brains of symptomatic A30P alpha-synuclein were identified compared to control mice: carbonic anhydrase 2 (Car2), alpha-enolase (Eno1), and lactate dehydrogenase 2 (Ldh2). Analysis of the activities of these proteins demonstrates decreased functions of these oxidatively modified proteins in brains from the A30P compared to control mice. These findings suggest that proteins associated with impaired energy metabolism and mitochondria are particularly prone to oxidative stress associated with A30P-mutant alpha-synuclein [90]. Carbonic anhydrase is quite interesting. Anaplerotic reactions often require CO₂ addition to pyruvate via pyruvate carboxylase, forming oxaloacetate. CA2 clearly affects the CO₂/HCO₃⁻ equilibrium and would be expected to be involved in CO₂ formation needed for anaplerotic reactions. Oxidation of CA2 conceivably could significantly affect efficiency of anaplerotic reactions in PD.

Since dopamine's oxidation to its reactive metabolites, ROS and DA quinone (DAQ), may also contribute to the oxidative stress and mitochondrial dysfunction seen in PD by altering mitochondrial respiration and inducing permeability transition in brain mitochondria [146,147], exposure of isolated mitochondria to dopamine quinone was subjected to proteomic studies, which found dysregulated proteins such as mitochondrial creatine kinase, mitofilin, mortalin, the 75 kDa subunit of NADH dehydrogenase, and superoxide dismutase 2. This suggests that following dopamine oxidation, expression changes might indicate derangements in energy production, redox homeostasis, mitochondrial morphology and stability and cell proliferation [148].

In a study conducted by Alberio et al. it is investigated the effect of dopamine on the expression pattern of mitochondrial proteins in the undifferentiated human catecholaminergic neuroblastoma cell line SH-SY5Y, a used cellular model that reproduces impaired dopamine homeostasis, which is a possible pivotal aspect in the pathogenesis of PD [149,150]. In this work mitochondria enriched fractions were analyzed with the two main approaches: peptide- and protein-based (two-dimensional electrophoresis and shotgun proteomics). This led to identification of specific pathways respectively perturbed by dopamine and the mitochondrial toxin MPP⁺, which mimics Parkinsonism in animal models. Twentyseven proteins were exclusively altered by MPP⁺, which included the MPP⁺ target complex I itself, the component NADH dehydrogenase [ubiquinone] iron-sulfur protein 3. An upregulation of prohibitin has been shown through the shotgun analysis after both treatments, whereas prohibitin 2 was increased only by MPP⁺ treatment, probably as an effect of ATP depletion following complex I inhibition [151]. However, a 25 kDa prohibitin fragment identified by 2-DE displayed discordant changes after the two treatments. These data might be due to a different post-translational processing at the mitochondrial level that most certainly reflect an alteration of the mitochondrial function and dynamics. It is known indeed that prohibitins regulate key participants in the quality control proteins [152].

The combination of orthogonal proteomic approaches also led to some discrepancies that need more investigation in the quality control of mitochondria [153]. The voltage-dependent

anion channel isoform 2 (VDAC2), for instance appeared to be upregulated in the shotgun analysis by both treatments, but 2-DE analysis highlighted a significant reduction of the spot identified as VDAC2 after DA exposure. Upregulation of proteins linked to mitochondrial disease including VDAC1 and ADP/ATP translocases, and components of the mitochondrial protein synthesis machinery, such as the mitochondrial ribosomal protein S22, the elongation factor Tu, together with the heat shock proteins was also noted.

Down Syndrome

Mitochondrial dysfunction in DS—Down syndrome (DS), the result of trisomy of chromosome 21, is one of the most common genetic causes of intellectual disability characterized by multiple pathological phenotypes, among which neurodegeneration is a key feature. The neuropathology of DS is complex and likely results from impaired mitochondrial function, increased oxidative stress, and altered proteostasis, but also display a strong metabolic component as demonstrated by the evident metabolic derangements typical of the disease [154] due to overexpression of metabolic enzymes whose gene is located on chromosome 21. After the age of 40 years, many (most) DS individuals develop a type of dementia that closely resembles that of Alzheimer's disease with deposition of senile plaques and neurofibrillary tangles, which is associated to critical events such as increased oxidative damage, accumulation of damaged/misfolded protein aggregates, and dysfunction of intracellular degradative systems [155]. Thus, after a critical age, DS neurodegeneration might be a human model of early Alzheimer's disease and could contribute to understanding the overlapping mechanisms that lead from normal aging to development of dementia.

In Down syndrome mitochondrial dysfunctions and ROS production are extensive [156]. The mitochondrial complexes I, III and V are defective in the cerebellar and brain regions of subjects affected by Down syndrome (DS) [156]. Moreover, reduced mitochondrial redox activity and membrane potential have been observed in DS astrocytes and neuronal cultures [157,158]. In the Ts1Cje mouse model for DS ATP production is defective [159]. This finding is confirmed in fetal DS fibroblasts, in which adenine nucleotide translocators, ATP synthase, and adenylate kinase display lower activities together with a deficit of complex I. Increased gene expression coding for specific cystathionine beta-synthase translates directly into biochemical aberrations, which result in a biochemical and metabolic imbalance of the methyl status [160]. This event is destined to impact mitochondrial function since methylation is a necessary event in mitochondria and relies on the availability and uptake of the methyl donor S-adenosylmethionine by the SAM mitochondrial carrier (SAMC) [161]. We demonstrated that a strong methyl imbalance is present in lymphoblastoid cells from DS patients, which associates to overexpression of the SAMC carrier and hypomethylation of mitochondrial DNA [162]. This represents a first link between mitochondrial dysfunctions and metabolic unbalance in DS [163–164].

Mitochondrial Proteomic studies in DS—Our recent brain redox proteomic study of young DS subjects ($\pm$ 24 years old) compared to age matched controls [165] carbonylation (normalized to expression levels) of showed increased oxidation of six proteins, among which V0-type proton ATPase subunit B, brain isoform (V0-ATPase) and succinyl-CoA:3-ketoacid-coenzyme A transferase 1 mitochondrial (SCOT-1), pointing to the mitochondrial

molecular metabolic pathways perturbed by oxidative stress, which may play a key role in the neurodegenerative phenomena occurring in DS. In the effort to identify HNE-bound proteins as a function of AD-like dementia in DS and control brains, several mitochondrial HNE-modified targets were identified in DS/AD versus DS (GDH1, cytochrome b-c1, and aconitase) and in DS versus control subjects (cytochrome b-c1 and GDH1)[166].

Clinical Implications of Mitochondrial Proteomic Studies

Emerging proteomics approaches offer a broad spectrum of information that provides insights into the mechanisms of diseases in which mitochondrial dysfunctions are present. Advances in the application of proteomics techniques permit solving many of the issues associated with the manipulation of proteins. Indeed protein mixtures require purification and separation in order for a single protein to be evaluated. Since no amplification techniques exist for proteins evaluation of very low expressed proteins is technically hampered. Use of proteomic data for clinical purposes requires standardization of the proteomic experiments in terms of sample collection, storage and processing as well as bio-informatics and statistical analysis between different laboratories [167]. Additionally, a further level of difficulty derives from focusing on mitochondrial proteins, in which critical issues are present and need to be addressed. In the next section we will focus on the limitations regarding mitochondrial proteins.

Mitochondrial proteins: feasibility and clinical implications

Relatively few proteomics studies have been performed on isolated mitochondria. Purification of mitochondria or preparation of mitochondrial-enriched fractions prior to proteomic analysis are crucial to produce a higher yield of mitochondrial targets. This requirement needs a robust method to isolate purified mitochondria. The sucrose density gradient ultracentrifugation is the classical method to obtain highly enriched fractions of mitochondria from tissues and cells, although this is a very time-consuming method [1]. Alternatively, several kits are commercially available for mitochondrial isolation, making the procedure more efficient in terms of time and yield without jeopardizing the mitochondrial purity [168].

Protein separation techniques need also to be tailored to meet mitochondrial proteomic features. Two-dimensional polyacrylamide electrophoresis (2D-PAGE), which is the classical technique to separate complex protein samples, might fail to provide good coverage of low abundant proteins. Additionally, proteins with very low or very high pI might be underrepresented. Indeed mitochondrial proteins often display a very basic pI, making isoelectric focusing resolution quite difficult due to endo-osmotic effects in the pH gradient [169]. For instance, cytochrome c and most of the solute mitochondrial carriers have pIs higher than 10 [170]. Recently emerging technologies not based on 2D-PAGE [171] demonstrate that this issue can be partly overcome.

Once the separation technique is chosen, one of the difficulties encountered by scientists is the limited protein coverage of a given methodological approach, which often requires utilization of parallel approaches. These strategies often result in improvement in the overall

coverage [172,173] although it requires considerable efforts in terms of time and technology implementation.

Mitochondrial protein identification requires suitable mitochondrial protein databases. Researchers can rely on different mitochondrially-specific protein databases (Mitoproteome, MITOMAP, mtDB, MigDB, hmtDB, MitoP2) [174–176], which provide large coverage of mitochondrial proteins, but are still not comprehensive. Consequently, it is important to search on more than one database, also to avoid problems in protein identification due to multiple matching [177]. For these reasons development of comparison tools for results from different search engines are needed.

From a clinical point of view, mitochondrial proteomic studies hold the promise to provide novel insights into the pathogenesis of neurodegenerative diseases in which mitochondrial dysfunctions are demonstrated to be related to pathogenesis. The issues above described still hamper the identification of new mitochondrial targets and mediators of the disease and represent a limitation that is difficult to overcome. Filling the gaps in the human mitochondrial proteome will pave the way for identification of new targets, increasing our understanding of how mitochondrial proteins function together in pathways and complexes, which is fundamental for translating proteomic information into insights into pathogenesis. Additionally, many mitochondrial proteins found in proteomic studies are associated with various mitochondrial-associated signaling pathways, including apoptosis, cell cycle, and DNA repair [44,178–179]. Bioinformatic studies to predict interaction together with validation experiments are strongly awaited to provide a more comprehensive view on the role of mitochondrial function on disease pathogenesis [44].

Our and others' work on mitochondrial proteins differentially expressed [110–111,180] in peripheral cells from AD patients suggest that mitochondrial proteins might be targets for biomarker identification, potential indicators of therapy sensitivity, selectivity disease progression, and prognosis evaluation [181]. However these data have yet to achieve the diagnostic power, sensitivity, and reproducibility necessary for widespread use in a clinical setting. Reproducibility issues may arise in part due to different analytical methodologies and sample treatment [182]. Therefore, standardization using a valid universal protocol for sample handling and analytical technique is essential before assessing which proteins are, in fact, reproducible biomarkers of AD [183–184].

Conclusions

As outlined in this review article, mitochondria play key roles in neurodegenerative disorders such as AD, PD and DS. Proteomics approaches, including redox proteomics, have provided significant insights into specific mitochondrial proteins and their role in aberrant mitochondria structure and function in AD, PD and DS.

That similar mitochondrial proteins were identified in peripheral tissues as in brain in AD suggests that, potentially, proteomics identification of altered mitochondrial proteins from peripheral tissues could become part of a panel of putative biomarkers for earlier detection

of neurodegenerative diseases and monitoring of therapeutic efficacy. Much more research is required to reach this goal.

Expert commentary

As authors of the first use of redox proteomics in brain of a major neurodegenerative disorder [50], we are gratified to see how proteomics and redox proteomics have advanced since this first study. Proteomics has provided new insights into several neurodegenerative disorders [27,82–85 134]. Altered expression and/or posttranslational modification of mitochondrial proteins, identified by proteomics, are consistent with the notion that such proteins are key players in the pathogenesis of AD, PD, and other neurodegenerative disorders. It is our view that proteomics methods will continue to be important tools in the armamentarium of approaches required to gain additional insights into the molecular basis of mitochondrial involvement in neurodegenerative disorders and therapeutic strategies to attack these devastating diseases.

Five-year view

Defective mitochondria, associated with amyloid β -peptide in the case of AD and with α -synuclein in the case of PD, are intimately involved with neurodegenerative processes in these disorders. We predict that better analytical methods for selective purification of mitochondria from brain and peripheral cells, combined with advances in instrumentation and software, will enable proteomics studies of AD and PD, as well as other neurodegenerative disorders to progress significantly. We predict that such studies, combined with advances in other complementary methods, for example stable isotope-resolved metabolomics, and with new animal models having high fidelity to human disease neuropathology, behavior, and biochemistry, will lead to definitive understanding of altered mitochondrial pathways involved in these disorders. Such insights will significantly enhance the chances to identify specific therapeutic targets that, if successfully modified, will result in slowing the progression of these important neurodegenerative disorders that currently have such large negative impacts on the quality of life for millions of persons worldwide.

Acknowledgments

This work was supported in part by an NIH grant (NS094891) to DA Butterfield.

Abbreviations

SAMC	s-adenosylmethionine carrier
SAM	s-adenosylmethionine
met	methionine
hcys	homocysteine
Cys	cysteine
5CH₂-THF	methylene tetrahydrofolate

THF	tetrahydrofolate
SOD2	superoxodismutase 2
GOT	glutamate oxaloacetate transaminase
GLS	glutaminase
GDH	glutamate dehydrogenase
asp	aspartate
OAA	oxaloacetate
glu	glutamate
gln	glutamine
LADH	Dihydrolipoamide dehydrogenase
LDH	lactic dehydrogenase
PC	pyruvate carboxylase
SCOT1	Succinyl-CoA-3-ketoacid-coenzyme A transferase
MDH	malate dehydrogenase
IDH	isocitrate dehydrogenase
α-kg	α -ketoglutarate
VDAC	voltage-dependent anion-selective channel protein 1
ANT1	adenosine nucleotide translocase 1
cyt C	cytochrome C

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Key issues

- Neurodegenerative disorders such as AD and PD are the major cause of dementia worldwide. The etiologies of these disorders remain unclear.
- Mitochondrial activities are known to play a role in the pathogenesis of these disorders, as both morphological and functional impairments of mitochondria are associated with these disorders.
- Mitochondrial proteins display specific features, and recent advances in proteomics have provided new tools to detect and identify mitochondrial proteins and confirm their mitochondrial localization.
- Due to the identified role of mitochondria in mediating neurodegeneration, extensive proteomic studies have been performed in brain of subjects with neurodegenerative disorders and animal models thereof, leading to the identification of many targets of protein dysregulation and oxidation.
- Integration of mitochondrial proteomics data with clinical information has provided important clues into the pathological events mediating neurodegeneration at molecular levels. These advances make the mitochondrial proteome an ideal target of studies with clinical implications in AD, PD, DS, and other neurodegenerative disorders.
- Current technical limitations hamper exploiting the mitochondrial proteome in neurosciences. Strong efforts are needed to overcome limitations with respect to mitochondrial purification, mitochondrial proteome coverage and mitochondrial proteome database availability.
- Seminal findings on mitochondrial proteins of peripheral cells have demonstrated that mitochondrial proteins could be conceivably be part of a panel of potential biomarkers of disease. However, diagnostic power, sensitivity, and reproducibility necessary for widespread use in a clinical setting is still lagging.

Figure 1. Strategies to study the mitochondrial proteome

Targeting the mitochondrial proteome can be achieved by isolating purified mitochondria or from crude biological samples. Progresses have been made with respect to protein subcellular localization, especially with advances in mass spectrometry, protein sequence bioinformatics and fluorescence microscopy, and protein identification, the latter achieved with 2D-PAGE- or MudPit-based technologies. These advances have enriched our knowledge of mitochondria both in terms of structure and function and the identity of mitochondria-resident proteins. Additional information can be gathered from redox proteomics studies, a branch of comparative proteomics designed to identify protein targets of oxidation. Generation of several new mitochondrial protein databases, coupled to the strong effort in producing comparative mitochondrial proteomic studies in health and disease states, have provided clinical insights into neurodegenerative diseases.

Figure 2. Mitochondrial dysfunctions from proteomics studies in AD, PD and DS

Consistent with the notion that mitochondrial dysfunction is associated with AD, PD and DS, several mitochondrial proteins are altered in terms of expression or oxidation in specimens from affected subjects or animal models. Thereof this comprehensive model depicted in this figure each protein target is indicated with relevance to AD (altered targets ■; oxidatively-modified targets ▲), PD (altered targets ★; oxidatively-modified targets ★▲) and/or DS (altered targets ■▲; oxidatively-modified targets ■▲★).