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REVIEW ARTICLE

Protein Targets of Oxidative Damage in Human Neurodegenerative Diseases with Abnormal Protein Aggregates

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Abstract

Human neurodegenerative diseases with abnormal protein aggregates are associated with aberrant post-translational modifications, solubility, aggregation and fibril formation of selected proteins which cannot be degraded by cytosolic proteases, ubiquitin–protesome system and autophagy, and, therefore, accumulate in cells and extracellular compartments as residual debris. In addition to the accumulation of "primary" proteins, several other mechanisms are involved in the degenerative process and probably may explain crucial aspects such as the timing, selective cellular vulnerability and progression of the disease in particular individuals. One of these mechanisms is oxidative stress, which occurs in the vast majority of, if not all, degenerative diseases of the nervous system. The present review covers most of the protein targets that have been recognized as modified proteins mainly using bidimensional gel electrophoresis, Western blotting with oxidative and nitrosative markers, and identified by mass spectrometry in Alzheimer disease; certain tauopathies such as progressive supranuclear palsy, Pick disease, argyrophilic grain disease and frontotemporal lobar degeneration linked to mutations in tau protein, for example, FTLD-tau, Parkinson disease and related α-synucleinopathies; Huntington disease; and amyotrophic lateral sclerosis, together with related animal and cellular models. Vulnerable proteins can be mostly grouped in defined metabolic pathways covering glycolysis and energy metabolism, cytoskeletal, chaperoning, cellular stress responses, and members of the ubiquitin–proteasome system. Available information points to the fact that vital metabolic pathways are hampered by protein oxidative damage in several human degenerative diseases and that oxidative damage occurs at very early stages of the disease. Yet parallel functional studies are limited and further work is needed to document whether protein oxidation results in loss of activity and impaired performance. A better understanding of proteins susceptible to oxidation and nitration may serve to define damaged metabolic networks at early stages of disease and to advance therapeutic interventions to attenuate disease progression.

INTRODUCTION

In most aerobic cell types the mitochondrial respiratory chain is one of the main sources of generation of reactive oxygen species (ROS) under physiologic conditions (6, 45, 46, 68, 83, 112). In addition to mitochondria, peroxisomes, endoplasmatic reticulum, microsomes, nucleus and plasma membrane oxidases are potential sources of ROS. Univalent oxygen reduction by the mitochondrial respiratory chain, as well as metal-ion-catalyzed reactions, generates a wide diversity of highly reactive metabolites of oxygen and nitrogen. These products mainly include superoxide anion (O^{-}_{2}), hydrogen peroxide (H_2O_2), hydroxyl radical (HO·), which can be formed from either the O^{-}_{2} and H_2O_2 (Haber–Weiss reaction) or from metal ion (Fe^{2+}, Fe^{3+}) and H_2O_2 (Fenton reaction), peroxyl radical ($RO\cdot_2$), alkoxyl radical (RO·), hydroperoxyl radical ($HO\cdot_2$), hypochlorous acid (HOCl), hypobromous acid (HOBr) and singlet oxygen (O_2) (50).

ROS plays a vital signaling role in physiologic conditions (50, 66, 120). However, ROS surpassing antioxidant cellular stress responses can be considered a significant source of endogenous structural damage to other cellular macromolecules, including DNA, RNA, carbohydrates, lipids and proteins, finally producing cytotoxic effects.

Nitric oxide (NO·) is produced by the oxidation of one of the terminal guanidonitrogen atoms of L-arginine catalyzed by different isoforms of nitric oxide synthase. NO plays a crucial role in physiologic conditions, such as autoimmunity, muscular relaxation and neurotransmission.

Nevertheless, NO· is also a source of harmful reactive nitrogen species (RNS). Main RNS are nitrogen dioxide ($\cdot NO_2$), nitrous acid (HNO_2), nitrosyl cation (NO+), nitrosyl anion (NO^-), dinitrogen tetroxide (N_2O_4), dinitrogen trioxide (N_2O_3), peroxynitrite (ONOO-), peroxynitrous acid (ONOOH), alkyl peroxynitrites (ROONO), nitronium cation ($NO+_2$) and nitryl chloride (NO_2Cl) (66).

All amino acid residues are susceptible to oxidation, but ion-catalyzed oxidation of some residues may result in the production of protein carbonyl derivatives (37, 108, 111). Characteristic products are glutamic semialdehyde and aminoadipic semialdehyde, which are derived from arginine/proline and lysine, respectively (38, 102). Because the magnitude of protein carbonylation is higher than any other primary change resulting from oxidation, carbonylation of proteins is currently used as a marker of protein oxidation in variegated settings (1, 15, 35, 60, 110).

In addition to direct effects, protein oxidative modifications may also occur following the reaction of distinct reactive carbonyl species (RCS) as glyoxal, glycoaldehyde, methylglyoxal, malondialdehyde (MDA) and 4-hydroxynonenal (HNE), derived from the oxidation of carbohydrates and lipids. Carbonyl species react with lysine, arginine and cysteine residues leading to the formation of advanced glycation and lipoxidation end-products (AGE/ALEs) in proteins. Typical AGEs/ALEs adducts are MDA-lysine (MDAL), carboxymethyl-lysine (CML) and carboxyethyl-lysine (CEL), among many others (82, 85, 122, 124).

Regarding RNS, NO damage to thiols, amines and hydroxyls leads to nitrosative damage. Reactions with RNS lead to the formation of 3-nitrotyrosine (nitration) and to oxidation of distinct substrates. As an example, reactive peroxinitrite is able to nitrate tyrosine residues and to oxidize methionine residues of proteins (55, 99, 121).

Cells have developed different mechanisms to prevent oxidative molecular damage. Antioxidant enzymes are superoxide dismutases, including cytosolic Cu,Zn-superoxide dismutase (SOD1), matrix mitochondrial Mn-superoxide dismutase (SOD2) and extracellular superoxide dismutase 3 (SOD3); catalase; glutathione peroxidase; peroxiredoxin; and some molecular chaperones. Non-enzymatic systems composed of different proteins such as ferritin (binds iron in the cytoplasm of mammalian cells) and ceruloplasmin (binds copper in plasma) have the capacity to bind transition metals in oxidation reactions. Finally, α-tocopherol (vitamin E), ascorbic acid (vitamin C), glutathione (L-γ-glutamyl-L-cysteinyl-glycine), flavonoids and carotenoids may act as antioxidants (50).

The concept of oxidative stress has been applied to the imbalance between the generation of ROS/RNS/RCS, and the cellular antioxidant defense mechanisms (4, 49). This may result in oxidative damage to varied molecules including DNA, RNA, lipids and proteins. Oxidative damage increases in aging (15, 47, 60, 97, 109–111). The nervous system is particularly susceptible to oxidative stress because of the abundance of Polyunsaturated fatty acids (PUFA) content, especially arachidonic and docosahexaenoic acids, the high oxygen consumption rate, and the relatively low levels of antioxidant pathways (7, 14, 31). The presence of increased oxidative stress and oxidative damage in neurodegenerative diseases has been recognized for years, and it has been the subject of hundreds of papers and reviews (25, 39, 41, 44, 61–63, 67, 75, 80, 81, 90, 98, 113, 127–129). However, little is known about the specific protein targets of oxidative damage in human neurodegenerative diseases.

The term proteomics is used to define the analysis of the whole proteins expressed by a genome. Redox proteomics is used to name the analysis of proteins modified by oxidation and nitration (16, 34). The term *redox proteomics* is instrumental as it serves to identify proteins that are damaged as a result of oxidation as well as the methods used to recognize modified proteins (36).

The objectives of the present review are (i) to list proteins modified by oxidation/nitration identified so far in neurodegenerative diseases covering Alzheimer disease (AD), tauopathies, Parkinson disease (PD) and related α-synucleinopathies, Huntington disease (HD), and amyotrophic lateral sclerosis (ALS), and related animal and cellular models; (ii) to give information about the methods used to identify those proteins in the different studies; (iii) to identify vulnerable metabolic pathways in individual diseases and vulnerable proteins common to different neurodegenerative disorders; (iv) to investigate the effects of oxidative stress on protein targets at early stages of neurodegenerative diseases to learn whether oxidative damage to proteins is an early event in degenerative diseases of the nervous system; (v) to find out whether studies dealing with protein damage resulting from oxidation/nitration have been accompanied by studies focused on associated loss of function; (vi) to clarify whether information is available regarding the involvement of particular cell types; (vii) to discuss limitations of redox proteomics; and (viii) to comment on aspects that may help to improve the use and results of redox proteomics applied to the study of neurodegenerative diseases.

IDENTIFICATION OF OXIDIZED AND NITRATED PROTEINS

Several methods are currently used to identify oxidative stress and oxidative damage in tissues (36, 37). Yet the majority of studies dealing with the identification of proteins modified by oxidation in human neurodegenerative diseases are based on bidimensional gel electrophoresis of paired gels run in parallel—one of them is used to transfer proteins to membranes to carry out Western blotting with specific oxidative damage markers, and the other serves to pick up selected spots for protein identification by mass spectrometry. Common antibodies utilized to identify modified proteins are anti-AGE, anti-CEL, anti-CML, anti-3-NTyr, anti-MDAL and anti-HNE. The recognition of spots of modified proteins in Western blots is conducted in the parallel gels stained with Coomassie blue, SYPRO Ruby or silver. This is followed by in-gel digestion of the selected spots, analysis of fingerprints by MALDI mass spectrometry and identification of proteins using a database. To detect carbonyls, samples are derivatized to hydrazones with 2,4-dinitrophenylhydrazyne (DNPH) usually before the separation of proteins.

Several variables are introduced in different studies, including the characteristics of buffers of homogenates and the solutions used for protein loading. These aspects may have implications on the range and type of proteins finally transferred to membranes for Western blotting labeling (see section Pitfalls and limitations).

The effects of post-mortem delay in the study of oxidized/nitrated proteins in human brain has been analyzed by freezing part of the sample immediately 1 or 2 h after death, and storing pieces of the remaining sample at –4°C (thus mimicking corpse preservation) and then freezing them at 2, 6, 8, 12, 18, 24 and 48 h at –80°C until use. Monodimensional gel electrophoresis and Western blot-

ting to anti-MDAL, anti-HNE, anti-CEL, anti-CML and anti-3-NTyr antibodies has demonstrated good preservation up to 12–18 h. Yet reduction or enhancement of the intensity of previous bands and appearance of new bands occurs from this time onward (43).

The relevant methodological aspects in individual studies, including use of total homogenates or subfractions, buffers, regions examined, gel staining, methods employed for mass spectrometry and software characteristics, are shown in Tables S1–S3 (Supporting Information).

ALZHEIMER DISEASE

Excellent reviews of oxidatively damaged proteins in AD and related models, identified by researchers of the University of Kentucky, have recently appeared (20, 118). Yet other groups have significantly contributed to identify proteins modified by oxidation. For these reasons, the present review updates and complements the list by adding important observations made in other centers. To facilitate understanding, vulnerable proteins have been grouped in defined metabolic pathways covering glycolysis and energy metabolism, mitochondrial electron transport chain and oxidative phosphorylation, structural proteins, chaperones, stress proteins, ubiquitin–proteasome system components, and other proteins.

Glycolysis and energy metabolism

Aldolase A, which catalyzes D-fructose 1,6-bisphosphate to glyceraldehyde 3-phosphate and dihydroxyacetone phosphate, is modified by oxidation and nitration at middle and advanced stages of AD (57, 101); oxidative damage to aldolase C has also been detected in advanced stages of AD (58). Triose phosphate isomerase, which catalyzes the reversible interconversion of glyceraldehyde 3-phosphate and dihydroxyacetone phosphate, is also modified by oxidation and nitration at middle and advanced stages (24, 101, 115). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), involved in the two-step reaction that transforms glyceradehyde 3-phosphate to D-glycerate 1,3-bisphosphate, is modified by S-glutathionylation (77) and nitration (117) in AD.

Interestingly, GAPDH is carbonylated in the brain of Wistar rats following intracerebral injection of amyloid-beta$_{1–42}$ (A$\beta_{1–42}$) (12), and in neuronal cultures treated with A$\beta_{1–42}$ (116).

Phosphoglycerate kinase (PGK) catalyzes the reaction of D-glycerate 1,3-bisphosphate with adenosine diphosphate (ADP) to form adenosine triphosphate (ATP) and 3-phosphoglycerate. Oxidized PGK, as detected using anti-4-HNE, has been shown at middle stages of AD-related pathology to be clinically manifested as mild cognitive impairment (100).

Phosphoglycerate mutase (PGM) catalyzes an internal transfer of a phosphate group from 3-phosphoglycerate to 2-phosphoglycerate. Isoform B (PGM-1) is more oxidized and nitrated in AD cases than in age-matched controls (101, 115).

Similarly, PGM-1 was more carbonylated in 3-month-old male Wistar rats following intracerebral injection of A$\beta_{1–42}$ when compared with controls (12).

Enolases, which modulate the reaction of 2-phosphoglycerate to phosphoenolpyruvate in the next-to-last step of glycolysis, are targets of oxidative and nitrosative damage in AD. α-Enolase has increased carbonylation, lipoxidation, S-glutathionylation and nitration levels in advanced stages of AD and in cases of mild cognitive impairment (19, 23, 24, 77, 84, 100, 101, 114, 115). Likewise, increased oxidized α-enolase has been found in the brain of mutant Tg2576 mice which bear the Swedish APP mutation causative of familial AD (105).

Increased modification of enolase, as revealed with anti-DNPH, anti-MDAL and anti-3NTyr antibodies, has been described in sporadic AD and in cases with familial AD linked with mutations in presenilin-1 (18, 24, 84).

The last enzyme of glycolysis is pyruvate kinase (PK), which catalyzes the step from phosphoenolpyruvate to pyruvate, thus transferring phosphate to ADP to form ATP. Increased oxidation of isoform PK-M2 was reported in human brain samples of cases with mild cognitive impairment by using anti-DNPH and anti-HNE antibodies (19, 100). In the same line, increased PK oxidation occurred in neuronal cultures from rat fetuses exposed to A$\beta_{1–42}$ (116).

Two enzymes of the Krebs cycle appear to be targets of oxidation in AD and related models. Pyruvate dehydrogenase, which catalyzes the step from pyruvate to acetyl-CoA, is more carbonylated in the brain of rats treated with intracerebral injection of A$\beta_{1–42}$ (12). Malate dehydrogenase, which catalyzes the interconversion of malate and oxaloacetate using nicotinamide adenine as a coenzyme, has increased levels of carbonylation in primary rat neuronal cultures treated with A$\beta_{1–42}$ (116). Moreover, similar results have been obtained in transgenic *Caenorhabditis elegans* (13).

In addition to enzymes involved in glycolysis and Krebs cycle, several proteins linked to variegated metabolic reactions have been shown to be targets of oxidative damage in AD. These include carbonyl reductase 1, an oxoreductase enzyme related to arachidonic acid metabolism (100), carbonyl anhydrase (58), carbonic anhydrase II (CA II) (114, 117), and glutamate dehydrogenase, which converts glutamate to α-ketoglutarate (101). Increased carbonyl levels in GDH have also been discovered in gerbil synaptosomes exposed to A$\beta_{1–42}$ (11).

Related to nitrogen metabolism, glutamine synthetase (GS), which catalyzes glutamate and ammonia to form glutamine, is more oxidized in AD cases when compared with age-matched controls (19, 22). Increased oxidation of glutamate-ammonia ligase, a transferase enzyme, occurs after intracerebral injection of A$\beta_{1–42}$ in rat brain (12). Finally, lactate dehydrogenase B (LDH 2), which participates in the interconversion of pyruvate and lactate, is more oxidized in cases of AD presenting as mild cognitive impairment compared with controls (100).

Electron transport chain, oxidative phosphorylation and other mitochondrial components

Complex V or ATP synthase catalyzes the synthesis of ATP from ADP and inorganic phosphate with a flow of protons from the intermembrane space to the matrix side. Several studies have found lipoxidized and nitrated ATP synthase in middle and advanced stages of AD (84, 100, 117). ATP synthase oxidative damage is a very early event in AD, as ATP synthase has been found oxidized and its function reduced in the entorhinal cortex in asymptomatic cases with Braak II AD-related pathology (119).

In the same line, ATP synthase is more carbonylated in stable transgenic *C. elegans* strain CL 2337 when compared with wild worms (13).

Ubiquinol–cytochrome c reductase complex core protein I is a component of the complex III, which helps to link the complex between cytochromes c and c1. This protein is more lipoxidated in the frontal cerebral cortex of advanced AD when compared with control samples (84).

Evidence of increased oxidative damage in creatine kinase BB (CK BB) derives from the observation that specific protein carbonyl content is higher in AD cases when compared with controls (2, 3, 22), and in AD-related mice models (26).

Voltage-dependent anion-channel protein-1 (VDAC-1) is a porin that forms a channel through the mitochondrial outer membrane and the plasma membrane. It helps the transport of a variety of purine nucleotides (responsible for ATP/ADP exchange) and allows the diffusion of small hydrophilic molecules. VDAC-1 also has an important role as a regulator of mitochondrial function. Nitrated VDAC-1 is significantly increased in AD (117).

Structural proteins

Cytoskeletal proteins are targets of oxidative damage in AD. Increased β-actin carbonylation has been found in sporadic AD and in familial AD due to mutations in presenilin-1 (3, 18). Similar changes occur at earlier stages of AD corresponding with clinical symptoms of mild cognitive impairment (100). Transgenic *C. elegans* expressing human $A\beta_{1-42}$ also show β-actin oxidative damage (13). Increased β-actin oxidation also occurs in synaptosomes of Mongolian gerbils exposed to $A\beta_{1-42}$ (11, 12).

Oxidative damage to α-tubulin 1, as revealed with anti-MDAL antibodies, has been reported in AD (84). Tubulins are also targets of oxidative damage in the brains of rats following intracerebral injection of $A\beta_{1-42}$ (12).

By using a different approach, it has been shown that high molecular neurofilament proteins are substrates of adduction by HNE (123). Carbonyl-related modifications of neurofilament protein have been shown in neurofibrillary tangles in AD (106).

Glial fibrillary acidic protein (GFAP) is oxidized in the normal aged brain, but GFAP oxidative damage increases in AD (57, 84) and related animal models (11).

Chaperones, stress proteins and stress responses

There is cumulative evidence of increased oxidation of several chaperones including HSC-71 (23) and HSP-70 (100) in AD, and HSP-60 in experimental models (12, 26). αB-crystallin is also a target of S-glutathionylation in AD (77).

Pin-1 is a protein within the peptidyl-prolyl isomerase family with chaperone activity involved in several cellular functions, including the modulation of assembly and folding of several proteins. Increased oxidized Pin-1 level, using anti-DNPH antibodies, has been found in the hippocampus in AD (114).

Regarding oxidative stress responses, SOD1 is oxidatively damaged in AD (28).

Ubiquitin–proteasome system

Ubiquitin carboxy-terminal hydrolase L-1 (UCHL-1) belongs to a family of proteases with high specificity for ubiquitinated substrates. Increased levels of carbonylated and oxidized UCHL-1 have been reported in AD (22). Oxidative modifications of UCHL-1 in AD have also been reported by independent groups (27).

Additional targets of oxidative damage in AD

Other proteins are oxidatively damaged or are targets of nitration in AD cases. Most of the descriptions refer to unique reports that should be validated by further studies. Moreover, some of them appear as isolated molecules within a particular metabolic pathway. A list of altered proteins in AD is shown in Table 1. In addition to AD, details are also provided for several experimental models including intracerebral injection of $A\beta_{1-42}$ in rats and gerbils, transgenic mice, transgenic *C. elegans* and transfected cell lines.

TAUOPATHIES

Studies in tauopathies including progressive supranuclear palsy (PSP), Pick disease (PiD), argyrophilic grain disease (AGD) and familial frontotemporal lobar degeneration linked to *MAPT* mutations (FTLD-tau) are still very limited.

Energy metabolism enzymes phosphoglycerate kinase 1 (PGK-1) and aldolase A have been shown to be oxidatively modified in frontal cortex in terminal PSP stages (65). In addition, GFAP has been identified as a major target of oxidative damage in the striatum in conventional PSP and in cases with PSP-like pathology consistent with early pre-symptomatic stages of the disease (103).

Increased oxidation of GFAP is also encountered in the amygdala in AGD (103), and in the cerebral cortex of FTLD-tau (64) and PiD (54, 74). Oxidative damage to GFAP also occurs, although to a lesser extent, in the cerebral cortex in FTLD with ubiquitin-positive, tau-negative inclusions (FTLD-U) and in FTLD associated with motor neuron disease FTLD-MND (64).

Other proteins that are targets of oxidative damage in PiD are listed as follows: vesicle-fusing ATPase, cathepsin D precursor isoforms, carbonyl reductase NADPH1 isoforms, GAPDH and HSP-7054.

Additional data of studies dealing with protein oxidative damage in selected tauopathies is shown in Table 2.

PARKINSON DISEASE AND RELATED α-SYNUCLEINOPATHIES

Several proteins are damaged by oxidation in PD and dementia with Lewy bodies (DLB). One of them is α-synuclein, which is oxidized in the substantia nigra even at very early stages of PD (32). α-synuclein oxidative damage also occurs in the frontal cortex in PD and DLB, but also at preclinical (pre-motor) stages in which neuropathologic features (Lewy bodies) are restricted to selected nuclei of the brain stem at the time of the post-mortem study (incidental PD) (32). Other proteins, the mutations of which are causative of familial or sporadic PD, are also targets of oxidative damage in sporadic PD, such as DJ-1, which is modified by carbonylation (29). Parkin is S-nitrosated in the brain of PD (30, 126). These aspects have functional implications as oxidative stress-induced aggregation of parkin is followed by decreased parkin E3 ligase activity and impaired proteasome function (59). Down-regulation and increased oxidation of UCHL-1 has also been reported in PD (27).

Table 1. Alzheimer disease.

Reference	Disease/stage	Protein	Function	Detection antibody	Degree of oxidation in relation to age-matched controls	Enzymatic activity	Total protein levels	Comments
Aksenov *et al* (2)	AD	CK BB	Energy transduction	Anti-DNPH antibody	↑	↓	↓	—
Aksenov *et al* (3)	AD	β-tubulin	Cytoskeleton	Anti-DNPH antibody	=	NA	NA	—
		β-actin	Cytoskeleton		↑	NA	NA	—
		CK BB	Energy transduction		↑	NA	NA	—
Castegna *et al* (22)	AD	CK BB	Energy transduction	Anti-DNPH antibody	↑	NA	NA	—
		UCHL-1	UPS		↑	NA	NA	—
		GS	Amino acid biosynthesis		↑	NA	NA	—
Castegna *et al* (23)	AD	DRP-2	Pyrimidine metabolism	Anti-DNPH antibody	↑	NA	NA	—
		α-enolase	Glycolysis		↑	NA	NA	—
		HSC-71	Chaperone		↑	NA	NA	—
Castegna *et al* (24)	AD	α-Enolase	Glycolysis	Anti-3-NTyr antibody	↑	NA	NA	—
		Triosephosphate isomerase	Glycolysis		↑	NA	NA	—
		Neuropolypeptide h3	Phospholipid binding protein		↑	NA	NA	—
		β-actin	Cytoskeleton		=	NA	NA	—
		LDH	Glycolysis		=	NA	NA	—
		γ-enolase	Glycolysis		=	NA	NA	—
Choi *et al* (27)	AD	UCH-L1	UPS	Anti-DNPH antibody	↑	NA	↓ 50%	identification of oxidation sites
Choi *et al* (28)	AD	SOD1	Antioxidant response	Anti-DNPH antibody	↑	NA	↑	IHC
Pamplona *et al* (84)	AD Stages V-VI/C	Neurofilament triplet L	Cytoskeleton	Anti-MDAL-antibody	↑ (3-fold)	NA	NA	—
		Vimentin	Cytoskeleton		=	NA	NA	—
		β-tubulin 2	Cytoskeleton		=	NA	NA	—
		α-tubulin 1	Cytoskeleton		↑ (9-fold)	NA	NA	—
		α-tubulin 4	Cytoskeleton		=	NA	NA	—
		α-tubulin 6	Cytoskeleton		=	NA	NA	—
		β-actin	Cytoskeleton		=	NA	NA	—
		γ-actin	Cytoskeleton		=	NA	NA	—
		GFAP	Cytoskeleton		↑ (8-fold)	NA	NA	—
		γ-enolase	Glycolysis		↑ (2-fold)	NA	NA	—
		α-enolase	Glycolysis		=	NA	NA	—
		Ubiquinol-cytochrome *c* reductase complex core protein I	Electron transport chain		↑ (4-fold)	NA	NA	—
		ATP synthase (β chain)	Oxidative phosphorylation		↑ (4-fold)	NA	NA	—
		CK BB	Energy transduction		=	NA	NA	—
		GS	Amino acid biosynthesis		=	NA	NA	—
		Glutamate dehydrogenase 1	Amino acid biosynthesis		=	NA	NA	—
		Guanine nucleotide-binding protein G(I)/G(S)/G(T) β	Signal transduction		=	NA	NA	—
		60-kDa HSP	Chaperone		=	NA	NA	—
		Dihydropyrimidinase-related protein-2	Pyrimidine metabolism		=	NA	NA	—
Korolainen *et al* (57)	AD	GFAP	Cytoskeleton	Anti-DNPH antibody	↑	NA	↑	—
		α-actin	Cytoskeleton		↑	NA	NA	—
		β-actin	Cytoskeleton		↑	NA	NA	—
		Enolase	Glycolysis		↑	NA	NA	—
		CK BB	Energy transduction		↑	NA	NA	—
Korolainen *et al* (58)	AD Braak stages V-VI	CA II	CO_2 metabolism	Anti-DNPH antibody	↑	NA	=	Oxp/tp =
		MDH1a	Kreb's cycle		↓	NA	↑	Oxp/tp ↓
		MDH1b	Kreb's cycle		↓	NA	=	Oxp/tp ↓
		Aconitase	Kreb's cycle		↓	NA	=	Oxp/tp =
		Glutamate dehydrogenase	Amino acid biosynthesis		=	NA	=	Oxp/tp ↓
		14-3-3 protein zeta/delta	Cell signaling		↓	NA	=	Oxp/tp =
		Aldolase C	Glycolysis		↓	NA	=	Oxp/tp =
		Aldolase A	Glycolysis		↓	NA	=	Oxp/tp =
		ATP synthase	Oxidative phosphorylation		=	NA	↑	Oxp/tp =

Table 1. *Continued.*

Reference	Disease/stage	Protein	Function	Detection antibody	Degree of oxidation in relation to age-matched controls	Enzymatic activity	Total protein levels	Comments
Butterfield *et al* (19)	AD MCI Braak stages III, V, VI	Enolase 1	Glycolysis	Anti-DNPH antibody	↑	↓	NA	IP, IHC
		GS	Energy transduction		↑	↓	NA	IP, IHC
		Pyruvate kinase M2	Glycolysis		↑	↓	NA	—
		PIN 1	Chaperone		↑	↓	NA	—
Butterfield *et al* (18)	Familial AD with mutations in PS1	γ-enolase	Glycolysis	Anti-DNPH antibody	↑	NA	NA	—
		Actin	Cytoskeleton		↑	NA	NA	—
		DMDMAH-1	Nitric oxide metabolism		↑	NA	NA	—
		UCHL-1	UPS		↑	NA	NA	—
Sultana *et al* (115)	AD Hippocampus	Pin 1	Chaperone	Anti-DNPH antibody	↑	NA	↓	IP
		DRP-2	Pyrimidine metabolism		↑	NA	↓	—
		PGM 1	Glycolysis		↑	NA	↓	—
		CA II	CO_2 metabolism		↑	↓	↑	—
		Enolase 1	Glycolysis		↑	↓	↑	—
		TPI	Glycolysis		↑	=	↑	—
		UCHL-1	UPS		↑	↓	↑	—
Sultana *et al* (114)	AD	Pin 1	Chaperone	Anti-DNPH antibody	↑	↓	↓	—
Sultana *et al* (117)	AD	CA II	CO_2 metabolism	Anti-3-NTyr antibody	↑	NA	↑	IP: VDAC-1
		α-enolase	Glycolysis		↑	NA	↓	—
		GAPDH	Glycolysis		↑	NA	↓	—
		ATP synthase	Oxidative phosphorylation		↑	NA	↑	—
		VDAC	Ion transporter		↑	NA	=	—
Choi *et al* (29)	AD	DJ-1	Antioxidant response	Anti-DNPH antibody	↑	NA	↑	Identification of oxidation sites
Newman *et al* (77)	AD	α-enolase	Glycolysis	Anti-GSH antibody	↑	↓	NA	individual spots in 2D blots normalized with density of corresponding spots in 2D gels
		GAPDH	Glycolysis		↑	↓	NA	—
		Deoxyhemoglobin			↑	NA	NA	—
		αB-crystallin	Chaperone		↑	NA	NA	—
Santpere *et al* (104)	AD Braak stage V CAA	14-3-3 protein gamma	Chaperone, signal transduction	Anti-CEL antibody Anti-MDAL antibody	↑ PHF	NA	NA	—
		14-3-3 protein zeta	Signal transduction		↑ TH in AD, CAA ↑ PHF in AD	NA	NA	—
Reed *et al* (101)	AD Braak stage V	Peroxiredoxin 2	Antioxidant response	Anti-3-NTyr antibody	↑	NA	NA	—
		TPI	Glycolysis		↑	NA	NA	—
		GDH	Amino acid biosynthesis		↑	↓	NA	—
		Neuropolypeptide h3	Phospholipid binding protein		↑	NA	NA	—
		H+ transporting ATPase	Oxidative phosphorylation		↑	↓	NA	—
		α-enolase	Glycolysis		↑	NA	NA	—
		Aldolase 1ldolase mponsites easome system	Glycolysis		↑	NA	NA	—
		PGM1	Glycolysis		↑	NA	NA	—

Table 1. *Continued.*

Reference	Disease/stage	Protein	Function	Detection antibody	Degree of oxidation in relation to age-matched controls	Enzymatic activity	Total protein levels	Comments
Reed *et al* (100)	AD MCI Braak stages III, IV, V, VI	Hippocampus		Anti-HNE antibody				
		Neuropolypeptide h3	Phospholipid binding protein		↑	NA	NA	—
		Carbonyl reductase 1	Antioxidant response		↑	NA	NA	—
		LDH	Glycolysis		↑	↓	NA	—
		PGK	Glycolysis		↑	NA	NA	—
		HSP70	Chaperone		↑	NA	NA	—
		ATP synthase	Oxidative phosphorylation		↑	↓	NA	IP
		α-enolase	Glycolysis		↑	NA	NA	—
		IPL		Anti-HNE antibody				
		β-actin	Cytoskeleton		↑	NA	NA	—
		Pyruvate kinase	Glycolysis		↑	↓	NA	—
		ATP synthase	Oxidative phosphorylation		↑	↓	NA	—
		eIF-α	Protein synthesis		↑	NA	NA	—
		EF-Tu	Protein synthesis		↑	NA	NA	—
Terni *et al* (119)	AD Braak stages I/II	ATP synthase	Oxidative phosphorylation	Anti-HNE antibody	↑	↓ complex V	=	Treatment with 10 mM NaBH4 for 30 min
Choi *et al* (26)	ApoE-deficient mice	GFAP	Cytoskeleton	Anti-DNPH antibody	↑ Hippocampus = cortex	NA	NA	In-strip DNP derivatization
		CK BB	Energy transduction		↑ Hippocampus = cortex	NA	NA	—
		Glucose regulated protein, Erp61	Chaperone		↑ Hippocampus = cortex	NA	NA	—
		Chaperonin subunit 5	Chaperone		↑ Hippocampus = cortex	NA	NA	—
		Dihydropyrimidinase-related protein 2	Pyrimidine metabolism		↑ Hippocampus = cortex	NA	NA	—
		Mortalin, Grp70	Chaperone		↑ Hippocampus = cortex	NA	NA	—
Shin *et al* (105)	Tg2576 mice bearing the APP Swedish mutation	Anti-DNPH antibody		Anti-DNPH antibody Anti-3NTyr antibody				—
		α-enolase	Glycolysis		↑	NA	NA	—
		Laminin receptor 1 Anti-3NTyr antibody	Neurite growth		↑	NA	NA	—
		Atp5b	Oxidative phosphorylation		↑	NA	NA	—
		Calpain 12	Cytoskeleton remodeling processes, cell diferentiation, apoptosis, signal transduction		↑	NA	NA	—
		α-enolase	Glycolysis		↑	NA	NA	—
Boyd-Kimball *et al* (12)	Inracerebral injection of Aβ (1–42) to 3-month-old Wistar rats	NBM		Anti-DNPH antibody				Sonication in re-hydration buffer on ice IP of GAPDH
		14-3-3 ζ	Signal transduction		↑	NA	NA	—
		HSP-60	Chaperone		↑	NA	NA	—
		Cortex		Anti-DNPH antibody				
		Glutamate-ammonia ligase	Amino acid biosynthesis		↑	NA	NA	—
		Tubulin β chain 15/α-tubulin	Cytoskeleton		↑	NA	NA	—
		Hippocampus		Anti-DNPH antibody				
		β-synuclein	Regulator of α-synuclein aggregation		↑	NA	NA	—
		14-3-3 ζ	Signal transduction		↑	NA	NA	—
		GAPDH	Glycolysis		↑	NA	NA	—
		Pyruvate dehydrogenase	Glycolysis-Kreb's cycle		↑	NA	NA	—
		Phosphoglycerate mutase 1	Glycolysis		↑	NA	NA	—

Table 1. *Continued.*

Reference	Disease/stage	Protein	Function	Detection antibody	Degree of oxidation in relation to age-matched controls	Enzymatic activity	Total protein levels	Comments
Boyd-Kimball *et al* (11)	Intracerebral injection of Aβ (1–42) to *Mongolian gerbils*. Study of synaptosomal fractions	γ-actin	Cytoskeleton	Anti-DNPH antibody	↑	NA	NA	—
		β-actin	Cytoskeleton		↑	NA	NA	—
		GFAP	Cytoskeleton		↑	NA	NA	—
		H+-transporting two-sector ATPase	Oxidative phosphorylation		↑	NA	NA	—
		Syntaxin binding protein 1	Synaptic vesicle exocytosis		↑	NA	NA	—
		GDH	Amino acid biosynthesis		↑	NA	NA	—
		Dihydropyrimidinase-related protein-2	Pyrimidine metabolism		↑	NA	NA	—
		EF-Tu	Protein synthesis		↑	NA	NA	—
Boyd-Kimball *et al* (13)	*C.elegans*	Strain CL 4176 (Aβ 1–42)		Anti-DNPH antibody				Sonication in rehydration buffer on ice
		Medium-chain acyl-CoA dehydrogenase	Fatty acid metabolism		↑	NA	NA	—
		Short-chain acyl-CoA dehydrogenase	Fatty acid metabolism		↑	NA	NA	—
		Translation elongation factor EF	Protein synthesis		↑	NA	NA	—
		Malate dehydrogenase	Kreb's cycle		↑	NA	NA	—
		Arginine kinase	Energy transduction		↑	NA	NA	—
		RACK1 ortholog	Anchoring activated protein kinase C		↑	NA	NA	—
		Myosin regulatory light chain	Cytoskeleton		↑	NA	NA	—
		Actin	Cytoskeleton		↑	NA	NA	—
		Adenosine kinase	Purine biosynthesis		↑	NA	NA	—
		Nematode specific protein	Presynaptic development		↑	NA	NA	—
		Lipid binding protein	Metabolism		↑	NA	NA	—
		Transketolase	Calvin's cycle and pentose phosphate pathway		↑	NA	NA	—
		Proteasome alpha subunit	UPS		↑	NA	NA	—
		Proteasome beta subunit	UPS		↑	NA	NA	—
		Glutathionine S-transferase	Antioxidant response		↑	NA	NA	—
		Strain CL 2337		Anti-DNPH antibody				
		ATP synthase α chain	Oxidative phosphorylation		↑	NA	NA	—
		Nematode-specific protein	Presynaptic development		↑	NA	NA	—
		Glutamate dehydrogenase	Amino acid biosynthesis		↑	NA	NA	—
		Proteasome beta subunit	UPS		↑	NA	NA	—
		Strain XA 1440		Anti-DNPH antibody				
		20S proteasome subunit PAS-4	UPS		↑	NA	NA	—
Sultana *et al* (116)	Primary neuronal cultures treated with Aβ (1–42)	GAPDH	Glycolysis	Anti-DNPH antibody	↑	NA	NA	Sonication on ice pretreatment of the neuronal cells with D609 reduces protein oxidation
		14-3-3 zeta	Cell signaling		↑	NA	NA	—
		Pyruvate kinase	Glycolysis		↑	NA	NA	—
		MDH	Glycolysis		↑	NA	NA	—

Table 2. Tauopathies.

References	Disease/stage	Protein	Localization/function	Detection antibody	Degree of oxidation in relation with age-matched controls	Enzymatic activity	Total protein levels	Comments
Muntané *et al* (74)	PiD	GFAP	Cytoskeleton	Anti-AGE, anti-CEL, anti-CML, anti-HNE, anti-MDAL antibodies	↑	NA	NA	—
Ilieva *et al* (54)	PiD	Vesicle-fusing ATPase	Energy metabolism	Anti-DNPH antibody	↑	NA	NA	—
		GFAP	Cytoskeleton		↑	NA	NA	—
		Cathepsin D precursor isoform	Proteolysis		↑	NA	NA	—
		Carbonyl reductase NADPH1 isoforms	Antioxidant response		↑	NA	NA	—
		GAPDH	Glycolysis		↑	NA	NA	—
		HSP 70	Chaperone		↑	NA	NA	—
Martínez *et al* (65)	PSP	PGK-1	Glycolysis	Anti-HNE antibody	↑	NA	=	—
		Aldolasa A	Glycolysis		↑	NA	=	—
Santpere and Ferrer (103)	AGD	GFAP (anti-AGE)	Cytoskeleton	Anti-MDAL, anti-AGE, anti-CEL anti-CML antibodies	↑ Striatum in PSP ↑ Amygdala in AGD	NA	↑	—
Martínez *et al* (64)	PSP, early-PSP							
	FTLD-tau	GFAP	Cytoskeleton	Anti-HNE antibody	↑	NA	↑	IHC
	FTLD-U							Variations depending on the type of FTLD. More marked in FTLD-tau
	FTLD-MND							

In addition, increased oxidation of several glycolytic enzymes, the intensity of which increases with disease progression, has been found in PD and related diseases. Aldolase A, enolase 1 and GAPDH were oxidized, as revealed with anti-HNE antibodies, in the frontal cortex in the majority of cases of incidental PD and in all cases of PD and DLB when compared with control samples (48). Subunits of complex I have been observed to be oxidatively damaged, functionally impaired and misassembled in PD brains (56). Other proteins that are vulnerable to oxidative stress in PD are β-synuclein and SOD2 (33).

Transgenic mice overexpressing A30P mutant α-synuclein are also at risk of increased oxidative protein damage. Enolase, LDH and CA II show significantly higher carbonyl levels when compared with controls (95).

Details of studies dealing with protein targets of oxidative damage in PD and related α-synucleinopathies are found in Table 3.

HUNTINGTON DISEASE

Aldolase C, aconitase, GFAP, tubulin, peroxiredoxin 1/2/6, glutathione peroxidase and αB-crystallin were discovered as targets of oxidative modification by showing higher carbonyl levels using DNPH as a marker (107).

Increased carbonyl levels have also been shown in total homogenates of r6/2 strain transgenic HD mice, neuron-specific enolase, HSP90, aconitase, creatine kinase and VDAC have been identified as oxidized proteins (89).

AMYOTROPHIC LATERAL SCLEROSIS

Oxidative stress seems crucial in the pathogenesis of ALS (5, 53, 86). Yet practically nothing is known about protein targets of oxidative stress in ALS. GAPDH is conformationally and functionally altered in association with oxidative stress in mouse models of amyotrophic lateral sclerosis (92). Similarly, oxidative modification of SOD1, translationally controlled tumor protein, UCHL-1 and αβ-crystallin were evidenced in a mouse model of the disease (96). Transgenic mice expressing human SOD1 gene with a G93A mutation presented oxidized HSP70 and α-enolase in spinal cords, as revealed with anti-HNE antibody, and high levels of carbonyls in αβ-crystallin (88).

COMMON AND SPECIFIC PROTEIN TARGETS IN DIFFERENT PATHOLOGIES

Observations carried out by different groups have reached similar results in AD, the most studied disease, by using redox proteomics, thus giving support to the reliability of proteins vulnerable to oxidative and nitrosative stress. β-actin, β-tubulin, GFAP, α-enolase, γ-enolase, aldolase A, glutamate dehydrogenase, glutamine synthetase, ATP synthase, pyruvate kinase, UCHL-1, CK BB and Pin 1 have been identified as targets of oxidative/nitrosative damage, at least, in two different studies (see Table 1).

Also important is the evidence that several proteins linked to glycolysis and energy metabolism are targets of oxidative damage in distinct neurodegenerative diseases. Oxidative damage to aldolase A, α-enolase, LDH, UCHL-1, SOD1, DJ-1 and GAPDH have been reported in AD and PD (14, 19, 22–24, 26–29, 48, 77, 84, 100,

Table 3. Synucleopathies.

Reference	Disease/stage	Protein	Localization/function	Detection antibody	Degree of oxidation in relation to age-matched controls	Enzymatic activity	Total protein levels	Comments
Choi *et al* (26)	PD	UCHL-1	UPS	Anti-DNPH antibody	↑ (10-fold)	NA	NA	Identification of oxidation sites
Chung *et al* (30)	PD	Parkin	UPS	Anti-S-nitro-sylated parkin antibody	↑	NA	=	Increase of S-nitrosylated proteins measured by the Saville method parkin *S*-nitrosylated by the biotin switch method
Yao *et al* (126)	PD	Parkin	UPS	Anti-S-nitroso-parkin antibody	↑	NA	=	Detection of S-nitrosylated proteins by the biotin switch method
Choi *et al* (28)	PD	SOD1	Antioxidant response	Anti-DNPH antibody	↑	NA	↑	Identification of oxidation sites
Dalfó *et al* (33)	iPD	β-synuclein	Regulator of α-synuclein aggregation	Anti-MDAL antibody	↑	NA	NA	—
		SOD2	Antioxidant response		↑	NA	NA	—
Choi *et al* (29)	PD	DJ-1	Antioxidant response	Anti-DNPH antibody	↑	NA	↑	Identification of oxidation sites
Keeney *et al* (56)	PD	Mitochondrial complex I	Electron transport chain	Anti-DNPH antibody	↑	↓	=	Immunocapture to isolate the complete complex I in isolated mitochondria
Dalfó and Ferrer (32)	iPD	α-synucleinnd	Synapsis	Anti-MDAL antibody	↑	NA	NA	IP anti-MDAL antibody
Gómez and Ferrer (48)	iPD	Aldolase A	Glycolysis	Anti-HNE antibody	↑	NA	NA	—
	PD							
	DLB							
		Enolase 1	Glycolysis		↑	NA	NA	—
		GADPH	Glycolysis		↑	NA	NA	—
Poon *et al* (95)	A30P α-synuclein transgenic mice	LDH	Glycolysis	Anti-DNPH antibody	↑	↓	NA	—
		Enolase	Glycolysis		↑	↓	NA	—
		Carbonic anhydrase	CO_2 metabolism		↑	↓	NA	—

101, 115, 116). Increased oxidative damage to GFAP occurs in AD, tauopathies (PiD, PSP, FTLD-tau and AGD) and HD (54, 57, 65, 84, 103, 107).

Mitochondrial proteins are also vulnerable to oxidative/nitrosative stress in different conditions, although vulnerability of particular proteins appears to be disease-dependent. ATP synthase (complex V) is a target of oxidative damage in AD (84, 100, 117, 119), whereas subunits of complex I have been observed to be oxidatively damaged, functionally impaired and misassembled in PD (56).

These examples will serve to emphasize that certain proteins are damaged by oxidative stress in different pathologies whereas other proteins are selectively damaged in one degenerative disease but apparently not in another. Mitochondrial proteins are paradigms of this assumption.

Available evidence clearly indicates that only a small fraction of proteins exhibit discernible oxidative modifications, suggesting selective vulnerability. Obviously, more studies are needed to evaluate structural/functional factors shared by these proteins (if any) in order to explain this "specificity." ROS probably act in a random fashion; however, the sensitivities and proximities of potential targets differ. The factors that can affect selectivity of oxidative damage to proteins could include the presence of a metal-binding site, molecular conformation, rate of proteolysis, relative abundance of amino acid residues susceptible to metal-catalyzed oxidation, or even protein abundance (91), among others. In this line, it is clear that modifications present in predominant proteins are easier to detect than modifications in proteins that are less abundant. Studies geared to analyze proteins that are represented at low levels in the brain will improve our understanding of selective vs. non-selective vulnerability.

However, disease-related specificities in protein vulnerability have been demonstrated as well. This is best exemplified with selective vulnerability of certain subunits of the complexes of the respiratory chain in AD and PD. ATP synthase is consistently oxidatively damaged in early stages of AD-related pathology, mild cognitive impairment with AD pathology and advanced stages of AD. In contrast, complex I is consistently altered in PD.

OXIDATIVE CHANGES IN POST-MORTEM BRAIN ARE PRINCIPALLY PRIMARY

Primary or secondary oxidative damage in post-mortem brain is difficult to ascertain as several pre-mortem factors may have produce oxidative damage. However, several complementary data support a primary origin of the observed protein modifications in post-mortem human brain. It is important to stress that targets of oxidative damage are similar in human neurodegenerative diseases, in a case-control approach, and in several animal models covering intracerebral injection of $A\beta_{1-42}$ in rats and gerbils, and transgenic mice and worm models bearing human mutations of APP. Similar profiles have been reproduced in primary cortical cultures treated with $A\beta_{1-42}$.

Together these data point to the likelihood that at least many proteins identified as oxidized in the post-mortem human brain are not modified as a consequence of pre-mortem agonic state but, rather, those modifications are directly linked to the degenerative process.

PROTEIN OXIDATION IS AN EARLY EVENT IN NEURODEGENERATIVE DISEASES

Pioneering studies stressed oxidative damage as an early event in AD (78, 79). In agreement with those predictions, several proteins have been identified as targets of oxidation and nitration in cases clinically manifested as mild cognitive impairment and pathologically verified as middle (IV) or early advanced (V of Braak) stages of AD-related pathology (17, 19, 20, 100, 101). More impressive, oxidative damage of ATP synthase and its associated loss of function has been observed in the entorhinal cortex in asymptomatic cases with neuropathologic AD-related pathology restricted to the entorhinal and perirhinal cortices (stage II of Braak), thus representing the earlier oxidative damage to proteins reported in AD (119).

Protein oxidative damage has also been investigated in other conditions. Protein oxidative damage was increased in brain cortex from ALS patients with lumbar debut (53). Increased oxidative damage of α-synuclein has been found in the substantia nigra at pre-clinical or pre-motor stages of PD (stages II and III of Braak) also known as incidental PD (32). Importantly, increased oxidative damage of α-synuclein, β-synuclein, SOD2, aldolase A, enolase 1 and GADPH has been shown in the cerebral cortex in incidental PD (in addition to PD and DLB) (32, 33, 48). This indicates that the cerebral cortex in PD is involved at very early stages of the disease and that oxidative damage to enzymes linked to energy metabolism and glycolysis, oxidative stress responses, and synucleins is already present at these early stages of the disease and not associated with Lewy pathology (42).

Further studies are needed to unveil oxidative damage at pre-clinical stages in tauopathies and other diseases with abnormal protein aggregates. However, recent studies have shown increased GFAP oxidative damage at pre-clinical stages of cases with PSP-like pathology (103).

OXIDATIVE DAMAGE AND LOSS OF FUNCTION

Enzymatic activity decline has been noted in AD with disease progression. Yet loss of activity may be due to reduced number of cells or specific cellular types, as well to cell redistribution, reduced amount of the enzyme or to modifications in the protein that led to protein dysfunction. All these scenarios are probably at work in advanced stages of neurodegenerative diseases. Among these possibilities, it is well known that oxidative damage of proteins has consequences in cell function (21).

Unfortunately, the majority of studies dealing with oxidized proteins in neurodegenerative diseases are not accompanied by functional studies. Perhaps historical events may account for this situation as pioneering works focused on the mere presence of increased oxidative stress and oxidative damage in aging and degenerative conditions. This was followed by the identification of DNA, RNA, lipids and proteins as targets of oxidative damage. Subsequent studies have been centered on the identification of particular proteins.

The enzymatic activities of certain oxidatively damaged proteins have been analyzed in parallel in a few studies. Increased oxidative damage accompanied by decreased activity has been shown for CK BB, enolase 1, glutamine synthetase, Pin-1, CA II, UCHL-1, α-enolase, GAPDH, GDH, H+ transporting ATPase, LDH, ATP synthase and pyruvate kinase in AD (2, 19, 77, 100, 101, 114, 115, 119). However, decreased activity can be related to lower total levels of the protein; therefore, the value of reduction due to oxidation or to the total amount of the particular protein cannot be solved in those works.

Only a few studies have included the identification of the oxidatively damaged protein, the quantification of total protein levels and the reduction of enzymatic activity (2, 114, 115, 119).

No similar data are available in PD, but oxidative damage to LDH, enolase and CA II anhydrase is associated with the corresponding decreased enzymatic activities in transgenic mice overexpressing the human A30P α-synuclein mutation (95).

CLINICAL IMPLICATIONS

Reduced oxygen uptake and impaired glycolysis have been recognized by means of neuroimaging functional studies at relatively early stages of AD manifested as mild cognitive impairment, and in advanced stages of AD. Impaired energy metabolism in the cerebral cortex has also been reported in pre-clinical stages of individuals with familial AD (8, 10, 40, 51, 69–73, 93, 94). Unfortunately, no functional neuroimaging and neuropathologic studies have been performed in the same cases. As a result, the cause of impaired energy metabolism in these cases is not known.

However, preserved protein expression levels, together with decreased enzymatic function associated with oxidative damage of relevant energy metabolism enzymes and components of the respiratory chain, have been found in a few well-documented studies (2, 114, 115, 119). These examples are particularly illuminating as they demonstrate that reduced oxygen uptake and impaired energy metabolism may be a result of oxidative damage to selected proteins rather than a consequence of neuronal loss, at least at early stages of AD-related pathology.

In the same line, mitochondrial dysfunction and impaired energy metabolism in the cerebral cortex has been demonstrated by several convergent neurologic, neuroimaging and biochemical studies in PD (42). These observations reinforce a causal link between oxidative modifications of selected proteins and functional impairment of energy metabolism in PD.

Prognostic implications of these observations are obvious because oxidative damage is subject of therapeutic intervention as oxidatively damaged molecules can be substituted by new ones whereas neuronal loss is not.

CELLULAR LOCALIZATION OF OXIDATIVELY DAMAGED PROTEINS

Seminal immunohistochemical studies carried out several years ago showed that neurons and glial cells were putative sources of oxidative stress in the nervous system as they were labeled with antibodies that recognize oxidative adducts. Moreover, certain oxidative stress responses are particularly robust in astroglia.

In contrast with these observations, it is assumed that oxidatively damaged proteins, as detected by redox proteomics, are predominantly neuronal proteins. In fact there is little evidence that a particular damaged protein is neuronal or glial, unless the localization of the protein is known in advance. It is logical to interpret that

oxidatively damaged neurofilaments are localized in neurons, whereas oxidized glial fibrillary protein is localized in astrocytes. In some examples, HNE adducts co-localize with GFAP in astrocytes, as revealed by double-labeling immunofluorescence and confocal microscopy, at the time that HNE-modified GFAP is identified by bidimensional gel electrophoresis, Western blotting, in-gel digestion and mass spectrometry (64).

It is clear that further studies are needed to elucidate the localization of damaged molecules in neurons and glial cells to understand the implications of the abnormalities in definite cellular types. This is not only valid considering neurons vs. glial cells, but also among different neuronal types. The fact that similar molecules are oxidatively damaged in variegated degenerative diseases does not prove that the same neurons are affected in the different conditions.

PITFALLS AND LIMITATIONS

With no doubt, redox proteomics is a useful tool for detecting damaged proteins resulting from oxidative/nitrosative damage. Yet available studies have also detected limitations and pitfalls.

First of all, information about post-mortem delay is important as certain proteins might be affected by oxidation/nitration with post-mortem delay. On the other hand, degradation of proteins with post-mortem delay may minimize the abundance of oxidized proteins due to non-specific post-mortem degradation. Several available studies take into consideration this aspect whereas this information is lacking in many others. A caveat derived from this observation is that identification of oxidized/nitrated proteins in human neurodegenerative diseases is feasible provided that tissue samples are examined within a time not surpassing thresholds of protein degradation and vulnerability to oxidative damage which are variable from one protein to another.

A second point is the overrepresentation of most abundant proteins whereas oxidative damage of minority proteins is probably underdetected. This may lead to an oversimplification of damaged metabolic pathways and, therefore, to the putative neglect of damaged crucial components.

A third point is the lack of information regarding cellular types involved, including particular neuronal types in different neurodegenerative disorders. We barely understand the reasons for selective cell vulnerability in general terms, and this shortage may also be applied to selective vulnerability of individual molecules in particular cell types.

Another source of possible confusion is based on the convergence of abnormalities in particular metabolic pathways that may obscure the real impact of oxidative damage in determined cellular functions. As an example, abnormal mitochondrial function in AD may be the result of several components, including increased mitochondrial DNA deletions, abnormal fusion and fission of mitochondria, and decreased expression of certain complexes of the respiratory chain, such as complex IV (45, 52, 68, 87, 125). Mitochondrial alterations result in increased oxidative stress. Oxidative damage to ATP synthase (complex V), VDAC, ubiquinol–cytochrome c reductase complex core protein I, H+ transporting ATPase and Atp5b (11, 13, 58, 84, 100, 105, 117, 119) may, in turn, increase mitochondrial dysfunction.

Similar considerations can be applied to the cerebral cortex in PD (42, 76).

REFINING METHODS TO IMPROVE REDOX PROTEOMICS

The large majority of studies in human brain and animal models have been carried out by analyzing total homogenates of a particular region. This is with no doubt an adequate approach although damage of minority proteins can be easily underrepresented. The study of cellular fractions may improve the resolution of the study by increasing the total amount of a particular protein. In this line, sarkosyl-insoluble fractions have been used to recover proteins in paired-helical filament-enriched fractions (104), mitochondria-enriched fractions to recover proteins principally related with mitochondria (30, 48), or synaptosomal-enriched fractions to reveal abnormalities of synapsis-related proteins (11).

Another important point is the use of different protocols and buffers to increase the capture of different proteins. The combination of different buffers to grind or to focus samples is a strategy to improve the reproducibility at the acidic or alkaline extremes of the electrophoresis gel and, likewise, to solubilize different proteins receiving a better number of spots and resolution (9). This observation, originally applied to general bidimensional gel electrophoresis methodology, may be of considerable interest in redox proteomics to optimize spots detected as oxidized proteins. We should also take into account that membrane proteins are difficult to detect by current bidimensional gel electrophoresis, thus probably accounting for the low numbers of membrane proteins identified as targets of oxidation.

Finally, detection of specific residues of oxidative modifications may increase understanding of specific oxidation sites and their relevance to protein function. However, this approach is time-consuming and a combination of different methods is needed. By using MALDI-TOF/MS and HPLC-ESI/MS/MS techniques, oxidation sites have been identified only in UCH-L1, SOD1 and DJ-1 in AD and PD as yet (27, 28).

CONCLUDING COMMENTS

Neurodegenerative diseases with abnormal protein aggregates are associated with modifications of solubility, aggregation and fibril formation of selected proteins. Mutant proteins resulting from DNA mutations are causative in familial and certain sporadic settings. More commonly, post-translational modifications of proteins are involved in the majority of sporadic cases. Understanding the mechanisms involved in such modifications is crucial from a mechanistic perspective, but it is also essential for the delineation of therapeutic strategies. Oxidative stress plays a functional role in physiologic conditions as it switches on vital cellular responses. Yet imbalance between oxidative stress sources and antioxidant responses may cause a net flux of oxidative damage to DNA, RNA, carbohydrates, lipids and proteins, and, in most cases, concomitant loss of function. Oxidative stress increases with age largely because of progressive mitochondrial dysfunction and impairment or loss of cellular repair mechanisms. In addition, neurodegenerative diseases are associated with higher levels of oxidative damage and higher levels of direct and indirect protein modifications resulting from increased oxidation, nitrosation and nitration when compared with those

occurring in age-matched individuals with no diseases of the nervous system.

Vulnerable proteins can be grouped in defined metabolic pathways covering glycolysis and energy metabolism, mitochondrial proteins, cytoskeleton, chaperones, and members of the ubiquitin–proteasome system, among many others. Some proteins are affected in different degenerative diseases whereas others appear to be disease-specific. Importantly, many damaged proteins in human neurodegenerative diseases are also damaged in experimental models, transgenic mice and worms, and cell culture paradigms. These findings indicate that oxidative stress observed in post-mortem brains is a primary event linked to degeneration rather than a secondary effect resulting from pre-mortem agonic states.

Since oxidative damage may result in impaired function, protein oxidative damage may have important consequences on the nervous system thus resulting in abnormal glycolysis and energy metabolism, abnormal responses to protein folding and oxidative stress responses, cytoskeletal abnormalities, and impaired protein degradation, in addition to damage to relevant proteins as α-synuclein in PD and related α-synucleinopathies. Some of these abnormalities are reflected *in vivo* by using sophisticated metabolic and neuroimaging methods. Thus, abnormal energy metabolism has been observed in the cerebral cortex not only in AD but also in patients with mild cognitive impairment and in patients with PD in whom impaired metabolism cannot be ascribed to neuron loss. Therefore, it is reasonable to think that part of the metabolic disturbances observed at early stages of degenerative processes are related to oxidative damage of selected proteins rather than to neuron loss. However, much work has to be done as the majority of redox proteomics studies are not accompanied by functional analysis of oxidatively damaged proteins.

Available information points to the fact that vital metabolic pathways are hampered by protein oxidative damage in several human degenerative diseases at very early stages of the disease. A better understanding of proteins susceptible to oxidation and nitration may serve to define damaged metabolic networks at early stages of disease and to procure therapeutic interventions to attenuate disease progression.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1. Alzheimer disease.
Table S2. Tauopathies.
Table S3. Parkinson disease and related α-synucleinopathies.

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