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Role of Oxidative Stress in the Progression of Alzheimer's Disease

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Abstract. Alzheimer's disease (AD) is a progressive neurodegenerative disorder that is characterized pathologically by the presence of senile plaques, neurofibrillary tangles, and synapse loss. Increasing evidence supports a role of amyloid β-peptide (Aβ)-induced oxidative stress in the progression and pathogenesis of AD. In this review, we summarize evidence for a role of oxidative stress in the progression of AD by comparing the appearance of the same oxidized brain proteins from subjects with mild cognitive impairment (MCI), early AD (EAD), and late-stage AD, and relating these findings to the reported AD pathology. The identification of oxidized brain proteins in common in MCI, EAD, and AD brain suggest that certain key pathways are triggered and may be involved in the progression of AD. Exploring these pathways in detail may provide clues for better understanding the pathogenesis and progression of AD and also for the development of effective therapies to treat or delay this dementing disorder.

Keywords: Alzheimer's disease, amyloid, early Alzheimer's disease, mild cognitive impairment, oxidative stress, proteomics

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease characterized by dementia, cognitive impairment, and memory loss. Pathologically, AD is characterized by the presence of senile plaques and neurofibrillary tangles, which contain aggregated amyloid β-peptide (Aβ) and highly phosphorylated tau proteins, respectively, in addition to synapse loss [1]. Currently the only way to unequivocally diagnose AD is via histopathology analysis performed at autopsy. Probable AD patients are identified by the Mini Mental State Evaluation (MMSE) test, which assesses cognitive decline and is often coupled to imaging modalities [2]. Persons with AD typically have MMSE scores lower than 25 out of a maximum 30 points, which gets worse with time, reflecting increasing dementia.

Based on histopathology, imaging, and MMSE scores, mild cognitive impairment (MCI) and early AD (EAD) are considered early stages in the progression of AD. MCI is the intermediary stage between cognitively-intact brain and EAD, and EAD is the intermediary stage between MCI and late-stage AD. The symptoms of EAD mirror the disease advancement between the two phases. Based on memory issues, MCI is further divided into two broad subtypes: amnestic (memory-affecting) MCI or non-amnestic MCI (memory is not affected) [3,4]. The rate of amnestic MCI conversion to AD is roughly 10–15% per year; however, in some cases MCI individuals can revert to normal [5].

As noted above, magnetic resonance imaging (MRI) is being used a diagnostic tool for AD in addition to MMSE scores. By MRI, MCI brain shows mild degeneration of the hippocampus, sulci, and gyri [6], whereas late-stage AD patients demonstrate greater degen-

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eration in hippocampus, sulci, and gyri. EAD brain shows frontal lobe atrophy [7] and ventricular widening, changes that are well documented in late-stage AD [8]. The brain morphological alterations observed by MRI reflect changes observed in the assessment of cognitive function of MCI, EAD, and AD patients. Further, brains from subjects with EAD showed increased number of neurofibrillary tangles compared to MCI patients in the frontal and temporal lobes [9] and also demonstrate synapse loss [10,11].

Cognitive impairment in AD does not show a consistent correlation with distribution and density of both diffuse and neuritic Aβ plaques. However, the levels of soluble Aβ are directly correlated with the decline in cognitive impairment, suggesting that small oligomers of Aβ are the actual toxic species of this peptide rather than fibrillar Aβ [12–18]. Further, Aβ_{1-40} and Aβ_{1-42} are found to be elevated in AD brain, and a number of *in vitro* and *in vivo* studies showed that Aβ_{1-42}, a primary component of senile plaques, is more toxic than Aβ_{1-40} [19–23]. The amounts of Aβ and senile plaques were reported to be lower in MCI and EAD conditions compared to AD [24], but cases of MCI with Braak stage V/VI are known. Nevertheless, the exact mechanism by which Aβ might produce synaptic loss and neuronal death is still controversial; however, previous studies from our laboratory showed that the presence of vitamin E or other antioxidant compounds block Aβ-induced neurotoxicity in neural cell culture and in *in vivo* and *ex vivo* AD models, consistent with a role of oxidative stress in AD progression and pathogenesis [25,26].

A large number of hypotheses have been put forward to explain the pathogenesis of AD including: excitotoxicity, inflammation, oxidative stress, etc. Our laboratory has provided strong support of the Aβ-induced oxidative stress hypothesis of AD pathogenesis. Oxidative stress has been implicated in the pathogenesis of a number of diseases including ischemia, cancer, neurodegenerative disorders, etc. [27–29]. Oxidative stress occurs due to an imbalance in the oxidant and antioxidant systems due to certain environmental factors, stressors, or disease. Oxidants, such as reactive oxygen species (ROS) and reactive nitrogen species (RNS), are produced at low levels in all aerobic organisms as a part of normal physiological process. ROS include superoxide radical anion ($O_2\cdot^-$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\cdot$ OH), among others. $O_2\cdot^-$ can be dismutated to H_2O_2 and oxygen by superoxide dismutase (SOD), an antioxidant enzyme. H_2O_2 can cross biological membranes and act as an intracellular messenger. Superoxide, when protonated, forms neutral HO_2, which can penetrate lipid bilayers. Further, $O_2\cdot^-$ can act as an intermediate in the generation of more reactive ROS like hypocholorous acid, OH, and by reaction with nitric oxide (NO) forms the RNS, peroxynitrite. H_2O_2 is converted to water and O_2 by catalase or glutathione peroxidases (GPx). However, in AD brain antioxidant systems are reported to be less functional, which might lead to further increased ROS and RNS that may react with biomolecules including proteins, lipids, carbohydrates, DNA and RNA [30], thereby causing oxidative damage of these biomolecules.

Oxidative modification of biomolecules generally has been shown to lead to loss of its function [31–34]. One way to measure oxidative stress in a biological samples is to determine the level oxidative stress markers, such as protein carbonyls, 3-nitrotyrosine (3-NT), thiobarbituric acid reactive substance (TBARS), free fatty acid release, iso- and neuroprostane formation, acrolein, 4-hydroxy-2-nonenal (HNE), carbohydrate-mediated advanced glycation end products, and 8-OH-2'-deoxyguanosine and 8-oxo-7,8-dihydroguanosine (8-OHG) and other oxidized bases, and altered DNA repair mechanisms [29,33,35–56].

OXIDATIVE STRESS IN AD

As noted above, oxidative stress in AD brain is well documented. The levels of antioxidants enzymes were found to be altered in AD brain and further there is a clear evidence of increased levels of oxidative stress markers in AD brain compared to age-matched by controls. Studies have shown increased protein carbonyls in the hippocampus and parietal cortex, the regions of the brain that are severely affected in AD, but not in the cerebellum where there is less significant AD pathology [47]. Further, increased levels of dityrosine and 3-NT levels were found in hippocampus, inferior parietal lobule (IPL), and neocortical regions of the AD brain and also in the cerebrospinal fluid (CSF) [38,40,48, 55]. Immunohistochemistry studies showed the presence of increased levels of 3-NT specifically in neurons from AD brain [40,56]. High levels of free HNE were reported in amygdala, hippocampus, parahippocampal gyrus, and ventricular CSF [50,52,53]. In addition to increased levels of free HNE, elevated levels of protein-bound HNE were also reported in AD brain [29,46, 49,54,57]. *In vitro* and *in vivo* studies using Aβ_{1-42} clearly showed a significant increase in protein carbonyls, HNE, 3-NT levels in synaptosomes and neu-

ronal cells [32,49], consistent with the notion that Aβ might be involved in AD associated oxidative stress. Oxidative modification of the proteins may lead to alterations in the structure and function of proteins [49, 58] as demonstrated by studies from our laboratory and others that consequently leads to the progression and/or pathogenesis of AD.

RNA is more susceptible to oxidative damage as it is a single stranded nucleic acid and, unlike DNA, its bases are not protected by hydrogen bonding or histones. In AD hippocampus, frontal and occipital neocortex high levels of 8-OHG were reported from the cytoplasmic RNA, and this correlated with the Aβ load and suggested that RNA damage is an early event in AD [59–62]. Shan and Lin [63] showed that 30–70% oxidation of the mRNAs in the frontal cortex of the AD brain [63]. Further, an increase level of 8-OHG was also reported in frontal cortex of familial AD subjects [64]. In addition to the oxidation of mRNA, an increase in rRNA oxidation has also been shown in the AD superior middle gyri and IPL [65]. The markers of DNA damage like 8-oxo-7,8-dihydro-2-deoxyguanosine (8-OHdG), 8-hydroxyadenine (8-OHA), and 5-hydroxyuracil (5-OHU) were found to be elevated in temporal, parietal, and frontal lobes in AD [66,67]. An increase in 8-OHdG has been identified not only in brain tissue but also in CSF from AD patients [68]. The oxidation of mtDNA is approximately 10-fold higher than nDNA bases. High levels of mitochondrial DNA oxidation support the reported mitochondrial abnormalities in the AD brain [69], that may contribute to the increase superoxide (O_2^-) leakage ultimately leading to elevated oxidative stress. The oxidation of RNA and DNA in the AD brain could impair protein synthesis, DNA repair, transcription etc., that may eventually lead to cell death and AD pathogenesis [65].

OXIDATIVE STRESS IN MCI

Decreased protein levels and activity of enzymatic and non-enzymatic antioxidants were reported in MCI brain with no alterations in the total protein levels [70,71]. This decrease in the antioxidant enzyme activity may lead to increased production of free radicals during the progression from MCI to EAD or MCI to AD. Studies from our laboratory and others showed elevated protein carbonyls, protein-bound HNE, free HNE, TBARS, and MDA and 3-NT in brain of MCI subjects compared to age-matched controls [72–76]. MCI patients also showed higher levels of isoprostanes (F_2isoP) in plasma, urine, and cerebrospinal fluid compared to those of healthy subjects [77]. Further, increased oxidative damage was reported in nuclear and mitochondrial DNA in MCI, as indexed by increased levels of 8-OHdG, 2,6-diamino-4-hydroxy-5-formamidopyrimidine (fapyguanine), 8-OHG, 4,6-diamino-5-formamidopyrimidine (fapyadenine), and 5-hydroxycytosine (5-OHC) [78,79]. Studies conducted up to now are consistent with the notion that oxidative stress initiated in MCI brain is an early event in AD and may contribute significantly to the progression of AD.

OXIDATIVE STRESS IN EAD

The information involving oxidative stress in brain of EAD is limited. Since on average there is an 8-year period from diagnosis of AD to death at late-stage AD, this dearth of information in EAD may result from the requirement that subjects with EAD die from other causes, an event that then provides brain for scientific investigations. A significant increase of 8-OHG was reported in cytosol of EAD brain that decreased as Aβ peptide and neurofibrillary tangle burden increased suggesting the oxidative damage to RNA is an early event in the progression of AD [60]. Brains from EAD subjects also showed increased levels of protein nitration, indicative of increase levels of RNS [80], and elevated protein-bound HNE [81], indicative of lipid peroxidation. These results suggest a role of oxidative stress in the progression of AD [80,81].

Taken together, increased levels of oxidative stress were observed in all the three stages of AD, i.e., MCI, EAD, and late-stage AD, supporting the concept that oxidative stress may be one of the mechanism(s) operating in common at different stages of AD. Further, our laboratory applied redox proteomics approaches to identify common targets of protein oxidation in each condition. Such studies may provide insight into AD pathogenesis and to develop disease markers. These studies may also potentially lead to development of therapeutic targets to treat or delay the onset of AD.

A COMPARISON OF REDOX PROTEOMICS IDENTIFIED OXIDATIVELY MODIFIED BRAIN PROTEINS IN AD, MCI, AND EAD

Redox proteomics

Proteomics techniques can be used to identify specifically oxidized proteins, altered protein levels, and oth-

Table 1
Mass spectrometry search engines for peptide mass fingerprinting

Search engine URL
Mascot http://www.matrixscience.com
MOWSE http://www.hgmp.mrc.ac.uk/Bioinformatics/Webapp/mowse
Profound http://prowl.rockefeller.edu/profound_bin/WebProFound.exe
MS-fit http://prospector.ucsf.edu/ucsfhtml4.0/msfit.htm
Peptident http://ca.expasy.org/tools/peptident.html

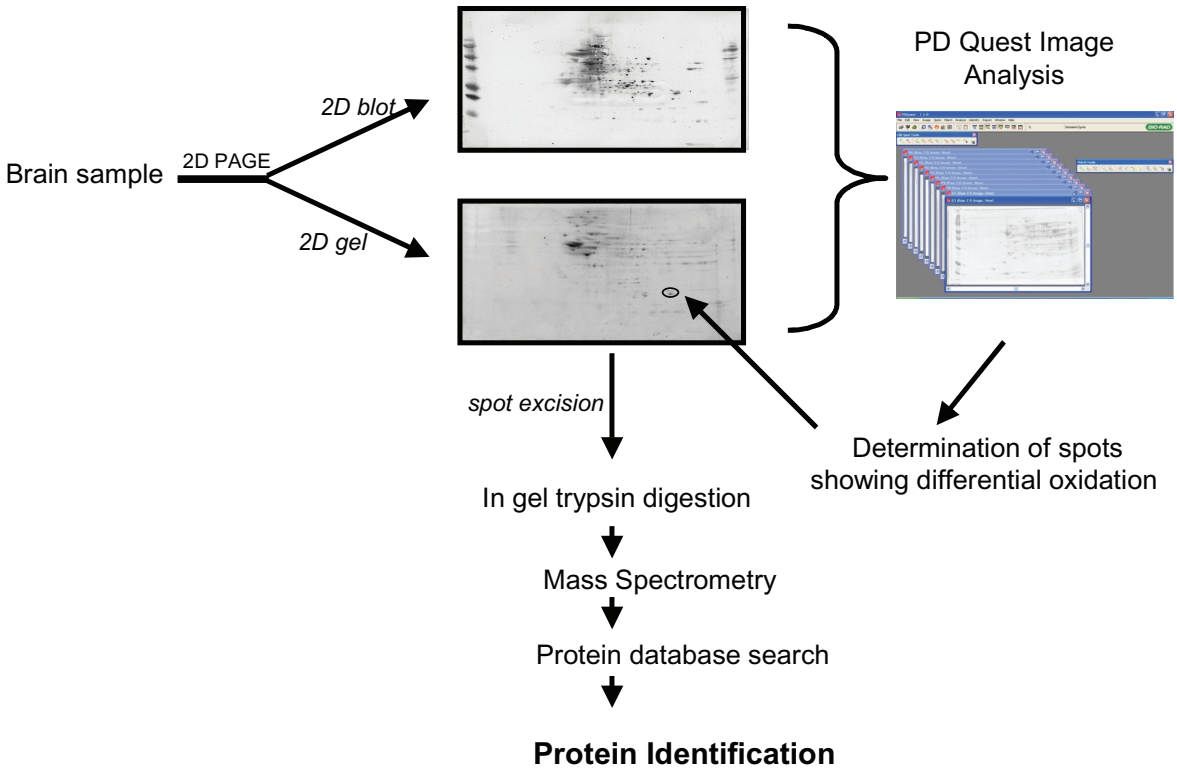

Fig. 1. Protocol followed in our laboratory for the detection of oxidatively modified brain proteins.

er posttranslational protein modifications. A redox proteomics technique couples two-dimensional (2D) gel electrophoresis techniques and 2D-Western blotting with mass spectrometry (MS) that allows simultaneous visualization of large number of protein spots, followed by their identification. Some of the limitation of this technique include solubilization of membrane proteins [82], highly basic proteins, and inability to detect low-abundance proteins. Our laboratory used redox proteomics approaches [44] to identify brain proteins with post-translational protein modifications such as protein carbonyls, protein-bound HNE, protein- resident 3-NT, and glutathionylation, etc. in oxidative stress-related diseases and their models (Fig. 1). A detailed description of redox proteomics is provided elsewhere [42,44,73]. Briefly, biological samples are split into equal aliquots and separated by isoelectric focusing (IEF) followed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The gels are stained with Sypro Ruby and scanned at the appropriate wavelengths for total protein detection; the proteins from a second gel are transferred to a nitrocellulose membrane for 2D Western blot analysis for the detection of the post-translational modification of interest. Following sophisticated computer-mediated image analysis, protein spots showing differential intensity are excised, digested in-gel with trypsin and subjected to mass spectrometry. The peptide mass fingerprints obtained from tryptic digests are characteristic of a specific protein, which allows correct identification of a particular protein using a suitable database that compares the experimental masses with theoretical mass-

Table 2
Oxidatively modified proteins identified in MCI, EAD, and AD brain

Protein function	MCI	EAD	AD
Energy dysfunction/mitochondrial alterations	$\alpha-$enolase, glucose regulated protein precursor, aldolase, MDH, pyruvate kinase, ATP synthase, LDH, phosphoglycerate kinase	$\alpha-$enolase, TPI, PGM1, Fructose 1,6-bisphosphate aldolase, H+ transporting ATPase	$\alpha-$enolase, TPI, PGM1, CK, γ-enolase, LDH GAPDH, aconitase, aldolase, VDAC, ATP synthase (α-chain)
Proteosomal dysfunction and synaptic dysfunction			UCHL-1, GAPDH, HSC 71
Neuritic abnormalities	DRP-2, β-actin, Fascin 1, syntaxin binding protein 1		DRP-2, β-actin, α-tubulin
Excitotoxicity	Glutamine synthetase	Glutamate dehydrogenase	Glutamine synthetase, EAAT2
Lipid abnormalities and cholinergic dysfunction	Neuropolypeptide h3	Neuropolypeptide h3	Neuropolypeptide h3
pH buffering and CO_2 transport	CA II		CA II
Cell cycle; tau phosphorylation; Aβ production	Pin-1		Pin-1
Synaptic abnormalities and LTP			$\gamma-$SNAP
Antioxidant defense/detoxification system	Peroxiredoxin 6, MRP3 protein, GSTM3, HSP70, carbonyl reductase	Peroxiredoxin 2	MnSOD, peroxiredoxin 6, GST, MRP-1,
Cell signaling dysfunction	14-3-3 gamma		
Protein synthesis alterations	Initiation factor alpha, elongation factor Tu		

MDH: malate dehydrogenase; LDH: lactate dehydrogenase; DRP-2: dihydroxypyrimidine related protein-2; CA II: carbonic anhydrase II; Pin-1: peptidyl- prolys *cis/trans* isomerase; MRP3: multidrug resistant protein 3; GSTM3: glutathione S-transferase Mu 3; HSP70: heat shock protein 70; TPI: triose phosphate isomerase; PGM1: phosphoglycerate mutase 1; CK: creatine kinase; GAPDH: glyceraldehydes-3-phosphate dehydrogenase; VDAC: voltage dependent anion channel; UCHL-1: ubiqutin C-terminal hydrolase L-1; HSC 71: heat shock cognate 71; EAAT2: excitatory amino acid transporter 2; SNAP: gamma-soluble N-ethylmaleimide-sensitive factor (NSF) attachment protein; MnSOD: manganese superoxide dismutase; GST: glutathione S-transferase; MRP-1-multidrug resistant protein 1.

Table 3
Oxidatively modified proteins identified in MCI and AD brain

Protein function	MCI	AD
Energy dysfunction/ mitochondrial alterations	$\alpha-$enolase, aldolase, ATP synthase, LDH	$\alpha-$enolase, aldolase, ATP synthase, LDH
Neuritic abnormalities	DRP-2, β-actin	DRP-2, β-actin
Excitotoxicity	Glutamine synthetase	Glutamine synthetase,
Lipid abnormalities and cholinergic dysfunction	Neuropolypeptide h3	Neuropolypeptide h3
pH buffering and CO_2 transport	CAII	CAII
Cell cycle; tau phosphorylation; Aβ production	Pin-1	Pin-1
Antioxidant defense/detoxification system	PR VI, GSTM3, MRP-3 protein,	PR VI, GST, MRP-1,

LDH: lactate dehydrogenase; DRP-2: dihydropyrimidinase-related protein-2; CA II: carbonic anhydrase II; Pin-1: peptidyl prolys *cis/trans* isomerase; MRP-3: multidrug resistant protein 3; PR VI: peroxiredoxin 6; GSTM3: glutathione-S-transferase Mu 3; GST: glutathione-S-transferase; MRP-1: multidrug resistant protein 1.

es of trypsin-generated protein sequences. The protein sequence database SwissProt is the most commonly used database for protein identification that is based on computer algorithms [83]. In addition to SwissProt there are various other protein search databases that are available online (listed in Table 1).

Our laboratory focuses on identifying oxidatively modified proteins in MCI, EAD, and AD brain that may provide a basis to explain pathology, biochemistry, and the progression of AD, may select potential targets for therapy, and may develop biomarkers for detection of disease prior to development of symptoms.

The brain proteins that were identified as oxidatively modified in AD, MCI, and EAD are involved in different cellular functions such as energy metabolism, cellular defense, protein degradation, etc. (Table 2). It is interesting to note that some identified proteins, such as enolase, are found in all the three stages of the AD. Further, a number of other brain proteins in similar functional classes were identified as oxidatively modified in MCI, EAD, and AD, whose oxidation impedes their cellular function and consequently may be involved in the progression of AD (Tables 2, 3 and 4). In this review, we discuss the brain proteins that were oxidized in common in different stages of AD.

Table 4
Oxidatively modified proteins identified in EAD and AD brain

Protein function	EAD	AD
Energy dysfunction/ mitochondrial alterations	$\alpha-$enolase, TPI, PGM1, Fructose 1,6-bisphosphate aldolase, H+ transporting ATPase	$\alpha-$enolase, TPI, PGM1, aldolase, ATP synthase (α-chain)
Lipid abnormalities and cholinergic dysfunction	Neuropolypeptide h3	Neuropolypeptide h3
Antioxidant defense/detoxification system	Peroxiredoxin 2	MnSOD, PR VI peroxiredoxin 6, GST, MRP-1,

PR VI: peroxiredoxin 6; TPI: triose phosphate isomerase; PGM1: phosphoglycerate mutase 1; MnSOD: manganese superoxide dismutase; GST: glutathione S-transferase; MRP-1: multidrug resistant protein 1.

COMPARISON OF OXIDATIVELY MODIFIED PROTEINS IN MCI AND AD BRAIN

A large number of proteins were found to be oxidatively modified in the MCI brain compared to age-matched controls, which suggests the involvement of oxidative stress at an early stage of AD in dysfunction of glucose utilization, neuritic length, excitotoxicity, lipids, cholinergic neurons, pH buffering and CO_2 transport, regulation of the cell cycle, amyloid-β protein precursor (AβPP) processing, and tau hyperphosphorylation, antioxidants, cell signaling and protein synthesis [35,73,84]. Comparing the functional groups between AD and MCI, we observed an overlap of a large number of functional groups with the exception of two main functional categories of proteins, i.e., cell signaling and protein synthesis (Table 2) [35,38,42,55, 57,73,84–86].

The appearance of common functional categories of specifically oxidatively modified proteins between AD and MCI (Table 3) suggests that these brain proteins are oxidized at an initial stage of AD (e.g., MCI) and may play key roles in the progression of MCI to AD. Among all the functional categories, peptidyl prolys *cis/trans* isomerase (Pin-1), ATP synthase, enolase, lactate dehdyrogenase (LDH), dihydropyrimidinase related protein-2 (DRP-2), β-actin, glutamine synthetase (GS), neuropolypeptide h3, carbonic anhydrase II (CA II), and peroxiredoxin 6 (PR VI) are found be oxidatively modified in both AD and MCI brain (Table III).

Pin-1 regulates the function of some of the proteins that are involved in cell cycle regulations such as cell cycle dependent protein kinase 5 (CDK5). In addition to its role in the cell cycle, Pin-1 also regulates other biological functions such as protein assembly, folding, intracellular transport, intracellular signaling, transcription, and apoptosis [87–89]. Two additional important functions of Pin-1 that were revealed recently were its ability to regulate the function of both AβPP and tau [87,90–92]. Pin-1 has been shown to bind to AβPP and regulate the production of Aβ [89,91], and via its action on both kinases and phosphatases, Pin-1 also regulates the phosphorylation of tau protein [89, 93]. Further, Pin-1 has been shown to co-localize with phosphorylated tau in AD brain and showed an inverse relationship to levels of tau [94,95]. Decreased levels and oxidative dysfunction of Pin-1 might not only alter cell cycle machinery [96], but can also promote tangle formation and Aβ production that may eventually lead to synapse loss [36,42,73,89,94,97], i.e., the major pathological hallmarks of AD. These considerations are consistent with the notion that Pin-1 is pivotal in the pathogenesis of AD, which is further strengthened by the fact that Pin-1 can protect neurons against age-related neurodegeneration [92].

Brain mainly depends on glucose for ATP production. ATP is crucial for normal cellular functions including maintenance and function at nerve terminals for normal neural communication. Three proteins, i.e, ATP synthase, enolase, and lactate dehydrogenase (LDH), each involved in energy metabolism, were found to oxidized in common between MCI and AD brain [35,38, 41,42,55,57,73,84–86]. Enolase belongs to the glycolytic pathway of glucose metabolism, and the final product of glycolytic pathway under aerobic conditions is pyruvate. Under anaerobic conditions pyruvate is converted to lactate by the action of a reversible enzyme LDH. Pyruvate is then further converted to carbon dioxide and water, and in this process more ATP molecules are produced. Hence, oxidative modification of the proteins involved in the glycolytic pathway will impact glucose metabolism and ultimately to total cellular energetics. The resulting decreased levels of ATP may directly or indirectly lead to changes in cell potentials, consequently leading to altered action potentials, opening of voltage-gated Ca^{+2} channels, failure to maintain ion gradients, and also to exposure of phosphatidylserine to the outer membrane leaflet, a signal for apoptosis [98,99]. Altered cellular energetics may also lead to alterations in ion-motive ATPases, glucose and glutamate transporters, protein synthesis, cholinergic neurotransmission, cholesterol homeosta-

sis, and signal transduction, etc., eventually leading to neuronal loss and consequently to cognitive decline in AD patients. Essentially each of these functions is compromised in AD brain, consistent with the concept that oxidative dysfunction of energy-related enzymes contributes to these functional alterations in AD. In addition to its role in glycolysis, enolase also is known to perform various other functions, such as roles associated with plasminogen activator protein, heat shock protein (HSP), autoimmunity, hypoxia inducible factor, etc. [100]. Enolase has been reported to be involved in many other diseases such as ischemia, autoimmune and neurodegenerative disorders [100,101]. Further, oxidative modification of ATP synthase as reported in AD and MCI [55,57] may lead to perturbed function of mitochondria, adding to further decreases in total cellular energetics. ATP synthase α-chain is a component of complex V that plays a key role in energy production; hence, oxidative mediated functional impairment of this protein may also contribute to the loss of cellular energetics in AD and MCI [55]. The identification of oxidatively modified energy-related proteins in AD and MCI brain correlated with the reported decrease glucose utilization and altered activity of enzymes involved in glucose metabolism [73,84,102–104]. In addition to identification of enolase, and ATP synthase, as oxidatively modified brain proteins in subjects with AD and MCI, these proteins also were found to be oxidatively modified in Aβ-related animals models of AD [19,38,41,42,55,57,73,84,86,105,106], which suggests a role of Aβ in the oxidative modification of brain proteins and consequent pathogenesis in AD.

DRP-2 and β-actin are cytoskeletal proteins that were identified by proteomics to be oxidatively-modified proteins in both MCI and AD brain [57,84, 86]. Neurocytoskeletal proteins are crucial for maintaining proper neuronal structure, connections, and axonal transport. The oxidative modification and altered function of these proteins could contribute to the reported loss of interneuronal connections, shortened dendritic length, impaired axonal transport, and loss of neuronal structural integrity in AD brain [107–109]. These cytoskeletal proteins were also found to be oxidatively modified in *in vitro* and *in vivo* Aβ models of AD that again suggest a role of Aβ in the oxidation of these proteins and to the consequent development or progression of AD [19,106,110]. Moreover, the role of Aβ in the oxidative modification of DRP2 is supported by a study conducted in Down patients, who have a trisomy of chromosome 21 (the *AβPP* gene also is localized on chromosome 21), and showed decreased levels of UCH L-1 [111]. This observation, further suggests a role of the *AβPP* gene and Aβ in impaired neuritic abnormalities in AD brain [109,111].

Decreased total brain mass due to loss of brain cells in AD has been reported [1]. One of the mechanism(s) that is suggested to contribute to the loss of neuronal cell is excitotoxicity. In both AD and MCI brain, the glutamate-related enzyme, glutamine synthetase (GS), is found to oxidatively modified [73,85], and this oxidative modification renders this protein less active. GS is important in the conversion of extracellular glutamate to glutamine. Hence, oxidative modification and altered function of this protein may lead to continuous excitation of post-synaptic neurons, Ca^{+2} accumulation, free radical formation, all contributing to impairment of neurotransmission and excitotoxic neuronal cell death [47,112–114].

AD is associated with cholinergic neuronal loss [115–119]. The loss of cholinergic neurons is consistent with the reported decreased levels of acetylcholine, an important neurotransmitter, in AD brain. One of the enzymes involved in the production of acetylcholine is neuropolypeptide h3, which is involved with production of acetylcholine via choline acetyltransferase. Neuropolypeptide h3 is also known as RAF kinase inhibitor (RKIP), hippocampal cholinergic neurostimulating protein (HCNP), and phosphatidylethanolamine binding protein (PEBP). PEBP may also play an important role in maintaining phospholipid asymmetry. That neuropolypeptide h3 is found to be oxidatively modified protein in both AD and MCI brain [38,73] suggests altered activity of this enzyme to carry out its function and could contribute to the consequent decreased acetylcholine levels in AD brain [115–117,120,121]. Further, the level of PEBP is reported to be decreased in AD brain. Since PEBP regulates lipid asymmetry, oxidative modification of this protein in AD and MCI [38, 73] may lead to loss of lipid asymmetry that is consistent with the reported loss of lipid asymmetry in MCI and AD brain [122]. In addition, *in vitro* studies conducted with Aβ_{1-42}- or HNE-treated synaptosomes as a model of AD showed an antioxidant-inhibited loss of lipid asymmetry [98,99,123], suggesting a role for Aβ and its associated oxidative stress in the loss of lipid asymmetry and consequently in neuronal loss and AD pathogenesis.

For the proper function of proteins, maintenance of the correct cellular pH is crucial. CA II plays an important role in the maintenance of cellular pH as well as in electrolytic and water balance [124]. CA II is an oxidatively modified brain protein in MCI and AD

brain [41,73]. Consistent with other oxidatively modified proteins, CA II shows decreased activity in AD brain compared to age-matched controls [124]. The oxidation of CA II may lead to altered cellular pH that in turn may lead to altered enzyme activities, and/or impairment of the mitochondrial proton gradient for ATP synthesis and may also lead to increased aggregation of proteins as seen in AD [41,124,125].

Increased oxidative stress in AD brain could be related to decreased levels or oxidative modifications of antioxidant enzymes. PR VI is a common target of oxidation between MCI and AD brain [57,84], and others reported decrease activity of this protein [126]. Based on these findings we hypothesize that loss of functional activity of antioxidant proteins may be involved with the increased levels of oxidative stress that may contribute to the pathology and neuronal death observed in AD.

As noted, a large of number of brain proteins that are oxidatively modified in AD and MCI correlate directly or indirectly with AD pathology. Oxidation of most proteins leads to functional impairment, and this has been clearly documented in MCI and AD brain [42,47,49,55,57,73,80,84] with the exception of triose phosphate isomerase (TPI) and malate dehydrogenase (MDH). TPI did not show a decrease in activity compared to that in control brain, while MDH had increased activity, despite their oxidation. We assert that oxidative stress plays an important role in AD pathogenesis, and the consequent impaired cellular and biochemical processes resulting from oxidative modification of key proteins contribute to this dementing disorder [74]. Our research results are consistent with the oxidative stress hypothesis of AD and suggest a role of Aβ in the progression of AD. However, we did identify some proteins that were oxidized only at the MCI stage and which were not identified as oxidized either in EAD or late-stage AD. This finding requires additional studies to understand.

OXIDATIVELY MODIFIED BRAIN PROTEINS IN EAD AND COMPARISON TO AD

Proteins that were found to be oxidatively modified in brain of subjects with EAD suggest their involvement in energy dysfunction, excitotoxicity, lipid abnormalities and cholinergic dysfunction, and antioxidant defense [80]. Most of the proteins that were found to be oxidatively modified in brain of subjects with EAD were also reported in late-stage AD with the exception of fructose 1,6-bisphosphate, glutamate dehydrogenase, and peroxiredoxin 2 (Tables 2 and 4).

PET studies showed that there is decreased glucose uptake in EAD brain, and the proteomics-facilitated identification of proteins involved in glucose metabolism as oxidatively modified (Table 2) is consistent with the PET findings [80,127]. Alpha-enolase, TPI, phosphoglycerate mutase 1 (PGM1), and ATP synthase alpha are found as common targets of oxidation in late-stage AD and EAD brain [38,41,55,57, 80,86]. Alpha enolase, TPI and PGM1 are glycolytic intermediate, triose phosphate metabolizing enzymes. Alteration in the function of the above-mentioned enzymes may lead to increased production of methyl glyoxal (MG), a small ketoaldehyde compound, derived from the glycolytic intermediate, triosephosphate, that is electrophilic in nature. MG can react with lysine, arginine, histidine, and cysteine residues and glycate them to form advance glycation end products [128,129] and may ultimately lead to altered structure and function of proteins.

The proteins enolase, neuropolypeptide h3, ATP synthase, and aldolase appear to be oxidized in brain from subjects at each stage of AD: late-stage AD, MCI, and EAD [38,41,55,57,73,80,84,86]. Studies are in progress in our laboratory to decipher the roles of these proteins in AD pathogenesis.

CONCLUSIONS

The proteomics-identified oxidized brain protein in common in MCI, EAD, and AD suggest that certain key pathways are triggered and are maintained during the progression of AD. Further investigation of these pathways in detail may provide clues for better understanding of AD pathogenesis. Redox proteomics studies of brain from animal models of the different stages of AD would be interesting and may further aid in delineating mechanism(s) of AD pathogenesis and the development of effective therapies to treat or delay this dementing disorder. Such studies are in progress in our laboratory.

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