



8-16-2013

Amyloid β -Peptide (1–42)-Induced Oxidative Stress in Alzheimer Disease: Importance in Disease Pathogenesis and Progression

D. Allan Butterfield

University of Kentucky, dabcons@uky.edu

Aaron M. Swomley

University of Kentucky, aaron.swomley@uky.edu

Rukhsana Sultana

University of Kentucky, rsult2@uky.edu

Follow this and additional works at: https://uknowledge.uky.edu/chemistry_facpub

 Part of the [Chemistry Commons](#)

[Right click to open a feedback form in a new tab to let us know how this document benefits you.](#)

Repository Citation

Butterfield, D. Allan; Swomley, Aaron M.; and Sultana, Rukhsana, "Amyloid β -Peptide (1–42)-Induced Oxidative Stress in Alzheimer Disease: Importance in Disease Pathogenesis and Progression" (2013). *Chemistry Faculty Publications*. 8.

https://uknowledge.uky.edu/chemistry_facpub/8

This Article is brought to you for free and open access by the Chemistry at UKnowledge. It has been accepted for inclusion in Chemistry Faculty Publications by an authorized administrator of UKnowledge. For more information, please contact UKnowledge@lsv.uky.edu.

Amyloid β -Peptide (1–42)-Induced Oxidative Stress in Alzheimer Disease: Importance in Disease Pathogenesis and Progression

Digital Object Identifier (DOI)

<http://dx.doi.org/10.1089/ars.2012.5027>

Notes/Citation Information

Published in *Antioxidants and Redox Signaling*, v. 19, no. 8, p. 823-835.

This is a copy of an article published in *Antioxidants and Redox Signaling* (c), 2013, copyright Mary Ann Liebert, Inc.; *Antioxidants and Redox Signaling* is available online at: <http://online.liebertpub.com>.

FORUM REVIEW ARTICLE

Amyloid β -Peptide (1–42)-Induced Oxidative Stress in Alzheimer Disease: Importance in Disease Pathogenesis and Progression

D. Allan Butterfield,^{1–3} Aaron M. Swomley,^{1–3,*} and Rukhsana Sultana^{1–3,*}

Abstract

Significance: Alzheimer disease (AD) is an age-related neurodegenerative disease. AD is characterized by progressive cognitive impairment. One of the main histopathological hallmarks of AD brain is the presence of senile plaques (SPs) and another is elevated oxidative stress. The main component of SPs is amyloid beta-peptide ($A\beta$) that is derived from the proteolytic cleavage of amyloid precursor protein. **Recent Advances:** Recent studies are consistent with the notion that methionine present at 35 position of $A\beta$ is critical to $A\beta$ -induced oxidative stress and neurotoxicity. Further, we also discuss the signatures of oxidatively modified brain proteins, identified using redox proteomics approaches, during the progression of AD. **Critical Issues:** The exact relationships of the specifically oxidatively modified proteins in AD pathogenesis require additional investigation. **Future Directions:** Further studies are needed to address whether the therapies directed toward brain oxidative stress and oxidatively modified key brain proteins might help delay or prevent the progression of AD. *Antioxid. Redox Signal.* 19, 823–835.

Introduction

ALZHEIMER DISEASE (AD) is an age-related neurodegenerative disorder that affects a large and ever-growing population of Americans 65 years of age or older, a number that current estimates place at ~5.1 million and that may grow to nearly 20 million by the year 2050 due to an aging Baby Boomer population (63). Pathologically, AD is characterized by a loss of synapses, an increase in the number of extracellular amyloid beta-peptide ($A\beta$)-rich senile plaques (SPs) formed from the amyloidogenic processing of amyloid precursor protein (APP) (discussed later), and an increase in intracellular neurofibrillary tangles (NFTs) composed of aggregated hyperphosphorylated Tau, a microtubule stabilizing protein (81). In the last decade or so, strong evidence has been put forth linking AD to an increase in oxidative stress due in part to both the increased production of reactive oxygen and nitrogen species (ROS and RNS, respectively) and a loss of function of many antioxidant defense enzymes (3,13,20,21,84,94,95,110,153,162).

Alzheimer Diagnosis and Staging

AD is clinically characterized by a decline in episodic memory that is often mistaken for normal cognitive deficiencies due to aging. Because the pathology remains hidden within the brain tissue, clinical diagnosis during the early stages remains inherently error prone and subjective, though advances have been made to aid a physician in making a correct diagnosis (100,167). To date, physicians have access to tools designed to help with diagnosis such as the Mini Mental State Evaluation (MMSE), which is used to track a patient's cognitive prowess (a 30-point scale is used with >25 being normal and <25 as probable AD), and also imaging alternatives such as magnetic resonance imaging (MRI) and positron emission tomography (PET) scans, which visualize both the potential hippocampal, sulci, and gyri degeneration and decreased glucose utilization (42,48,71,150).

Progression of typical AD can be stratified into four main stages: preclinical AD (PCAD), mild cognitive impairment (MCI), early AD (EAD), and late-stage AD (LAD). PCAD is

¹Department of Chemistry, ²Center of Membrane Sciences, and ³Sanders-Brown Center on Aging, University of Kentucky, Lexington, Kentucky.

*These authors contributed equally to this work.

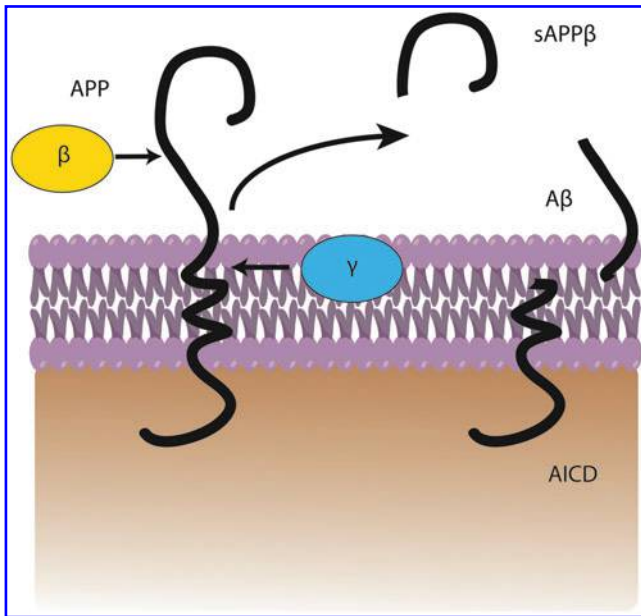


FIG. 1. A general depiction of amyloidogenic processing. Amyloid precursor protein (APP) is cleaved by β -secretase followed by γ -secretase within the bilayer to produce a fragment of amyloid-beta peptide ($A\beta$), $sAPP\beta$, and AICD. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

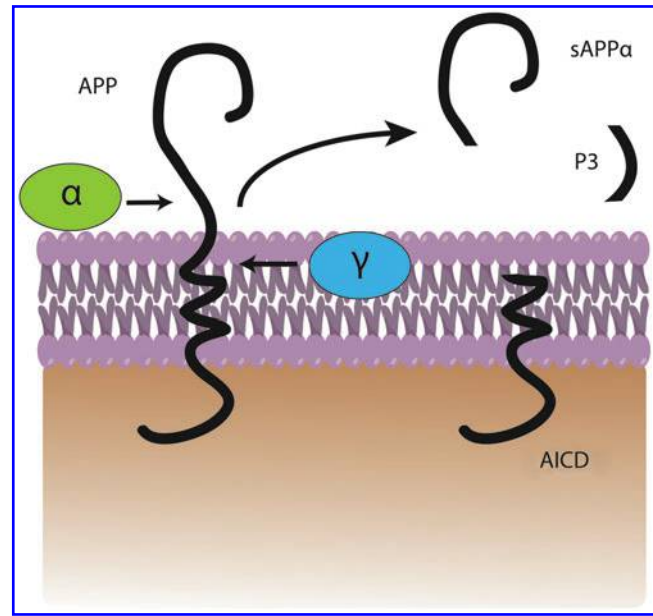


FIG. 2. A general depiction of non-amyloidogenic processing. APP is cleaved by α -secretase followed by γ -secretase within the bilayer to produce a fragment of P3, $sAPP\alpha$, and APP intracellular domain (AICD). To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars.

defined as the potential stage of AD in which the patient presents as a fully functional individual in cognitive exams such as MMSE, yet the growing pathology within the brain tissue is present, but likely unknown precluding early death from a non-neurodegenerative means (141,144,166). MCI has been described as being the transition stage between normal cognition and EAD, and is subdivided into both amnesic MCI (aMCI) and non-amnesic MCI, the former of the two presenting with memory deficits and maintains a 10%–15% conversion rate per year to AD (123,124,150). Pathologically, each stage differs in that both amyloid plaques and NFTs increase in distribution and density from MCI to LAD, though non-demented individuals have also been known to possess both types of pathology while maintaining normal cognition (159). Through use of imaging techniques such as MRI, all stages of clinical AD described demonstrate varying degrees of degeneration, with MCI presenting relatively small degeneration affecting the hippocampus, sulci, and gyri, whereas a larger degree affects the same brain regions in LAD accompanied by additional atrophy of the frontal lobe and ventricular widening in EAD and LAD (45,47,71). Additionally, research conducted using PET scans concluded that regional glucose utilization within the brain, including the temporal lobe, was significantly reduced in AD patients, a trend that was shown to remain for possible PCAD and MCI patients, indicating a severe energy deficiency, as glucose is known to be the predominant source of energy for the brain (34,35,42,66,125).

APP Processing

The main component of SPs is a 4-kDa protein called $A\beta$ (61,99). $A\beta$ is generated by the proteolytic cleavage of APP, a type I transmembrane protein suggested to play an important role in neurite outgrowth, neuronal protein trafficking, signal

transduction, calcium metabolism, and others. (176). There are three major alternate splicing variants with APP⁷⁷⁰, APP⁷⁵¹, and APP⁶⁹⁵.

In the amyloidogenic pathway, APP is cleaved by β -secretase (also referred to as β -site APP-cleaving enzyme) (163) at position 671, resulting in the release of a large N-terminal derivative called β -secretase-cleaved soluble APP (β -sAPP) (Fig. 1). The β -sAPP differ from α -secretase-cleaved soluble APP (α -sAPP) (produced from non-amyloidogenic processing, Fig. 2) by lacking the $A\beta$ (1–16) regions at its C-terminus, but it has been reported to function as a death receptor 6 ligand and also mediate axonal pruning and neuronal cell death (114). The toxicity of C-terminal fragment (CTF) may possibly be mediated by the end products of γ - and/or caspase-cleavage including APP intracellular domain (AICD), C31, and Jcasp. Caspases can cleave APP at position Asp664 resulting in the formation of 31-amino-acid peptide of APP referred to as C31. C31 has been shown to induce cytotoxicity. Further, cleavage by γ -secretase generates JCasp (52,119); however, JCasp has been reported to play a minor role in cytotoxicity. In a transgenic mouse, mutation at caspase cleavage site in APP prevented AD-associated changes suggesting that caspase cleavage of APP might be crucial for $A\beta$ -mediated neurotoxicity. In the next step, the 99-amino-acid CTF of APP (C99) is cleaved by the γ -secretase complex releasing free peptides ranging from 38 to 43 amino acids referred to as $A\beta$, P83 fragment, and AICD (Fig. 2). Hence, the γ -cleavage is critical for the amount and type of $A\beta$ produced.

$A\beta$ 40 represents the most abundant form of $A\beta$ in the brain, while $A\beta$ 42 shows a significant increase with certain forms of AD (112). $A\beta$ 42 has two extra hydrophobic amino acids compared to $A\beta$ 40, which promotes greater fibrillar formation in $A\beta$ 42 and is known to be more toxic (Fig. 3). The evidence of $A\beta$ toxicity was provided by molecular pathology, human

FIG. 3. Amino acid sequence of beta-amyloid peptides. Red color indicates the two additional hydrophobic amino acids that are present in beta-amyloid (1–42), which is critical for higher aggregation rate of beta-amyloid (1–42), and its associated neurotoxicity (please see text for more details). To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

Beta-Amyloid (1-40)

H₂N-Asp¹-Ala²-Glu³-Phe⁴-Arg⁵-His⁶-Asp⁷-Ser⁸-Gly⁹-Tyr¹⁰-Glu¹¹-Val¹²-His¹³-His¹⁴-Gln¹⁵-Lys¹⁶-Leu¹⁷-Val¹⁸-Phe¹⁹-Phe²⁰-Ala²¹-Glu²²-Asp²³-Val²⁴-Gly²⁵-Ser²⁶-Asn²⁷-Lys²⁸-Gly²⁹-Ala³⁰-Ile³¹-Ile³²-Gly³³-Leu³⁴-Met³⁵-Val³⁶-Gly³⁷-Gly³⁸-Val³⁹-Val⁴⁰-COOH

Beta-Amyloid (1-42)

H₂N-Asp¹-Ala²-Glu³-Phe⁴-Arg⁵-His⁶-Asp⁷-Ser⁸-Gly⁹-Tyr¹⁰-Glu¹¹-Val¹²-His¹³-His¹⁴-Gln¹⁵-Lys¹⁶-Leu¹⁷-Val¹⁸-Phe¹⁹-Phe²⁰-Ala²¹-Glu²²-Asp²³-Val²⁴-Gly²⁵-Ser²⁶-Asn²⁷-Lys²⁸-Gly²⁹-Ala³⁰-Ile³¹-Ile³²-Gly³³-Leu³⁴-Met³⁵-Val³⁶-Gly³⁷-Gly³⁸-Val³⁹-Val⁴⁰-Ile⁴¹-Ala⁴²-COOH

genetics, and discoveries from cell biology (13,16,38,169). The increased hydrophobicity of A β 42 possibly allows this peptide to integrate within the lipid bilayer initiating the process of cell damage. Schmidt *et al.* using mass-per-length measurements and electron cryomicroscopy with 3-dimensional reconstruction on an A β (1–42) amyloid fibril morphology showed that the A β (1–42) fibril morphology has only one protofilament, in contrast to A β (1–40) fibril forms two protofilaments. Further, A β (1–42) showed pairs of β -sheets at the cores of the two protofilaments making up a fibril (135).

Once A β is produced, individual amyloid peptides (A β 42 in particular) aggregate to form small assemblies of dimers, trimers, oligomers, protofibrils, and large insoluble fibrils. Studies showed poor correlation between plaque load and cognitive function (113). Recently, the role of A β has been amended to suggest that soluble A β oligomers are the more toxic species. Further research has indicated that the soluble oligomers and not the plaques correlate well with cognitive decline (44,53,54,117,165,168). Moreover, A β levels and temporal NFT density have been shown to be elevated to a higher degree in LAD when compared with MCI and EAD, which are likewise elevated compared with control (9,11,58,108,159). The relationship between A β -containing SPs and NFT formation has been debated, but recently Jin *et al.* reported that with the addition of soluble A β dimers, tau became hyperphosphorylated before cytoarchitectural disruption was observed, followed by subsequent neuritic degeneration. Interestingly, this process was exacerbated with the overexpression of human tau and prevented with the knockdown of human tau (74). Soluble A β has also been shown to modulate the pro-survival PI3K/AKT-GSK3 β pathway, inhibiting various neurotrophin effects including that of α -sAPP (73). These lines of evidence provide insight into the progression of AD and a potential causal relationship between two known pathological hallmarks of this disease.

Genetic Evidences for A β Toxicity

The importance of APP and consequently A β in AD pathogenesis has emanated from genetic evidence of patients with familial AD (FAD) and Down syndrome (DS). After the cloning of the APP gene, a mutation causing FAD (autosomal dominant) was found at codon 717, close to the C-terminus of the A β domain of APP (55). Today, there are at least seven known APP mutations causing FAD (56,138). Interestingly, all APP mutations are located in or near the A β region of APP, close to the secretase sites. To date, 32 mutations in APP have been reported, and based on their locations they are grouped

into three main classes: the Swedish mutation, located adjacent to the β -cleavage site of APP; London mutations, Flemish mutation, located near the γ -site of APP; the Arctic, Dutch, and the Iowa mutations, located within the A β sequence itself. All the APP mutations are found to alter the proteolytic processing of APP, resulting in either increased production of total A β or a selective increase in the 42-amino-acid form of A β (56,138). In addition to mutations of APP, 177 mutations in presenilin 1 (PS1) (392 families) and 14 mutations in PS2 (23 families) has been identified in FAD, which further support the role of altered APP metabolism in AD pathogenesis. The evidence of involvement of A β (1–42) in AD pathogenesis is largely derived by observation of increased A β load and increased oxidative stress in FAD. Individuals with FAD mutations consistently show increases in the ratio of A β 42/40, suggesting that elevated levels of A β 42 is critical for AD pathogenesis (72,134).

DS patients have three copies of chromosome 21, and the APP gene is present on this chromosome; hence, if these patients live long enough they develop neuropathological features indistinguishable from AD. Further, DS patients had increased accumulation of intracellular A β preceding extracellular plaque formation, and the level of intraneuronal A β decreases as the extracellular A β plaques accumulate (59,109). Further, DS brain also has elevated oxidative stress (32,78,120,121).

A β and ROS/RNS in AD

In AD brain, increased levels of A β were found in the affected regions; however, A β 42 is also the predominating form of A β in SPs (106), while shorter A β proteins predominate in both vascular amyloid and in cerebral spinal fluid (CSF) (106,164). Further, AD CSF showed reduced levels of A β 42 compared with A β 40 suggesting that the deposition of the protein in SPs in brain leads to reduced levels of A β in the CSF. In AD plasma, the levels of A β is controversial, one study found an increase in plasma A β 42 (102). Most of the studies did not find any change in plasma A β 42 between AD patients and age-matched controls (70,106). Current data do not provide clear-cut evidence that A β protein in plasma/CSF reflects the amount of A β deposited in the brain or that plasma/CSF A β 42 has a potential as a biomarker for AD. Further studies are needed to develop biomarkers for AD diagnosis and therapeutic efficacy.

Several lines of evidence indicate that A β induces oxidative stress. Oxidative stress that occurs within the bilayer, hypothesized in the A β -induced oxidative stress hypothesis in

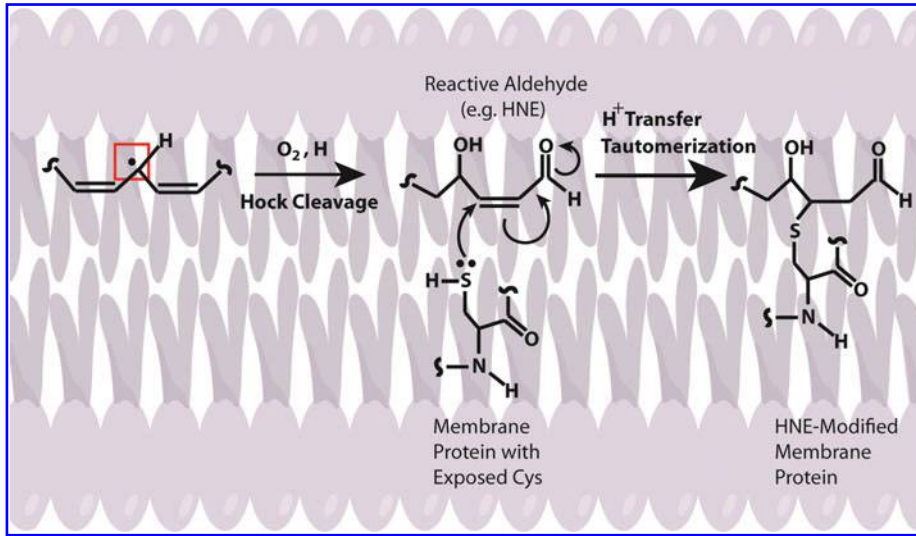


FIG. 4. Schematic illustration of HNE-modified protein. Upon formation of a radical centered allylic carbon on a fatty acid chain, the lipid may interact with molecular O_2 that freely diffuses through the bilayer because of its lack of dipole moment, to initiate the lipid peroxidation process that eventually, by way of a proposed Hock cleavage, generates an α/β unsaturated reactive aldehyde [e.g., 4-hydroxy-nonenal (HNE), malondialdehyde, and acrolein]. Membrane-bound proteins may then, by way of nucleophilic side chains such as Cys, Lys, and His, covalently bind the aldehyde that alters the structure and function of the target protein. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

which $A\beta_{1-42}$ inserts as oligomers into the bilayer and serves as a source of ROS, has been shown to initiate lipid peroxidation (Figs. 4 and 5) (16,17,93,94,101). For a comprehensive review on oxidative/nitrosative stress in the cell, the reader is referred to the following articles (28,29,151).

Oxidative Stress at Different Stages of AD

Oxidative stress and its effects have been found as early as MCI in the progression toward AD. Studies conducted in our laboratory and others have found that oxidative stress markers for protein oxidation/nitration, such as protein carbonyls and 3-nitro-tyrosine, are elevated in brains from subjects with aMCI (6–8,25,83). More recently, it has been shown that the phosphorylation profile of proteins such as heme-oxygenase-1 and biliverdin reductase A have been altered in MCI and AD indicating the possibility of aberrant signaling in at least this one critical antioxidant pathway. Increased levels of 8-OHdG, 8-OHG, 5-hydroxycytosine, 2,6-diamino-4-hydroxy-5-formamidopyrimidine, and 4,6-diamino-5-formamidopyrimidine, all markers of nucleic acid oxidation, were found in both mitochondrial DNA and nuclear DNA indicating nucleic acid oxidation in MCI (105,171). 8-OHdG (also found elevated in CSF of AD patients), 8-OHA, and 5-OHU were found in AD brain regions demonstrating that though DNA is more protected from oxidation than RNA, oxidation still occurs (2,50,104,150).

Significant RNA oxidation has been shown to exist in AD, as has been found in the earlier stages of the disease. A high percentage (30%–70%) of mRNA in the frontal cortex was shown to be oxidized in AD brain (139). In EAD brain, 8-OHG was found to be elevated in the cytoplasm of AD hippocampus, frontal, and occipital neocortex, which correlated with the β -amyloid load (89,115,116,140). Ribosomal RNA oxidation was observed in the superior middle gyri and inferior parietal lobule (IPL) of AD brain (43). 8-OHG levels decreased with increased $A\beta$ and NFT levels, a finding that suggests that at the early stages of AD, oxidative damage to RNA may be an early event in AD progression (115).

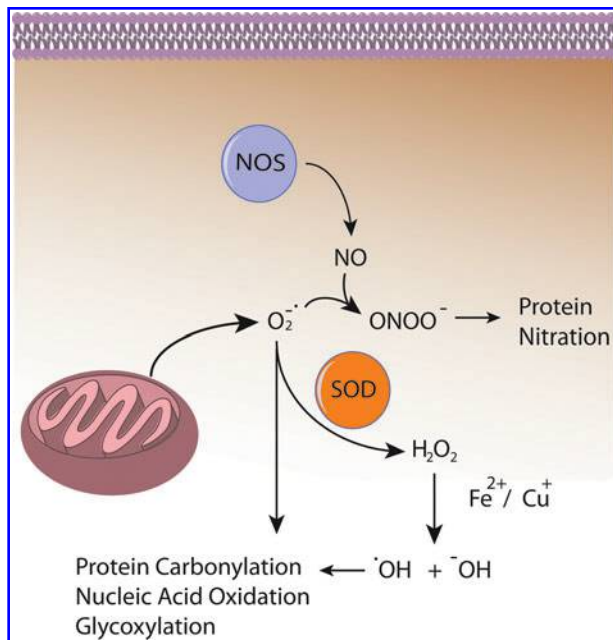


FIG. 5. Some consequences of elevated ROS and RNS. Reactive oxygen species (ROS) leaked from mitochondria (e.g., $O_2^{\cdot -}$) interact with nitric oxide (NO) produced by nitric oxide synthase (NOS) to produce reactive nitrogen species such as $ONOO^-$, which covalently modify proteins. $O_2^{\cdot -}$ can also directly oxidize proteins, lipids, and carbohydrates. $O_2^{\cdot -}$ may also be dismutated to H_2O_2 by superoxide dismutase (SOD) enzymes in an attempt to mitigate $O_2^{\cdot -}$ induced damage. However, hydrogen peroxide (H_2O_2) in the presence of Fe^{2+} or Cu^+ undergoes Fenton chemistry to produce the reactive ROS $\cdot OH$ and $\cdot OH$, which also cause protein, nucleic acid, and carbohydrate oxidation. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

Increased protein-bound 4-hydroxy-nonenal (HNE) and free HNE, TBARS, and MDA were found, and a higher isoprostane (F₂isoP) level in plasma, urine, and CSF in MCI when compared with healthy controls (83,96,173). There have been high levels of free and protein-bound HNE found in AD brain (16,23,86,88,97,103,122). In addition to lipid peroxidation, protein carbonyls were found to be increased in regions of the brain heavily associated with AD, including the hippocampus and parietal cortex, while leaving the cerebellum relatively untouched (64). Moreover, another index of protein oxidation, protein nitration, was also found to be increased in the CSF and AD brain in regions such as the IPL, neocortical regions, and the hippocampus (7,31,65,143,155). Increased protein nitration and protein-bound HNE were found in brains of subjects with EAD (130,131). Inversely correlated to the increase in oxidation observed was the activity of antioxidant systems (both enzymatic and nonenzymatic) found by Sultana *et al.* and Guidi *et al.* while no changes in total protein levels were observed, which may be a result of, and contribute to, the observed increase in free radicals during the progression of AD (57,153).

Redox Proteomics Studies of MCI, EAD, and AD

Redox proteomics is a method of identification of oxidatively modified proteins pioneered by our laboratory that employs redox-specific antibodies, two-dimensional polyacrylamide gel electrophoresis, and tandem-mass spectrometry (MS/MS) with the identification of specific proteins based on their tryptic peptide amino acid sequence after interrogation of protein databases such as SwissProt (41,67,68). Our laboratory has identified proteins in MCI, EAD, and AD brain that are vital to cellular function as being oxidatively modified and dysfunctional; however, for the sake of this review only a select few will be discussed. For a discussion of oxidatively modified proteins discovered by our laboratory using redox proteomics, the reader is referred to articles cited here (4,24,27,41,150).

Sultana *et al.* found that the important protein regulator Pin1 is oxidized and activity decreased (148). Recently, there has been much interest in the area of regulation *via* the phosphorylation specific peptidyl-prolyl *cis-trans* isomerase (PPIase), Pin1, and its role in neuronal cell cycle checkpoints and cellular phosphorylation status in diseases such as AD and cancer (5,14,46,82). Pin1 recognizes the specific motif of phosphorylated serine or threonine on the amino-terminal side of an adjacent proline (pSer/Thr-Pro) and catalyzes the isomerization of the peptide bond (90,128). This regulation has been shown to be important in the phosphorylation status of both APP and Tau, and some kinases and phosphatases that act on those target proteins, giving Pin1 both a direct and indirect regulation of two key pathological hallmarks of AD (14,85,87,92).

Another link between the stages of AD is the presence of oxidatively modified proteins important to cellular energy production (150). Three enzymes, α -enolase, adenosine-triphosphate-synthase, and lactate dehydrogenase were implicated as being oxidatively modified in brains of subjects with aMCI and AD, while α -enolase in particular was found to be modified in EAD as well (25,30,31,122,129,149,152,155,156). Additionally, enolase was identified by redox proteomics as oxidatively modified in brains of subjects with FAD (19).

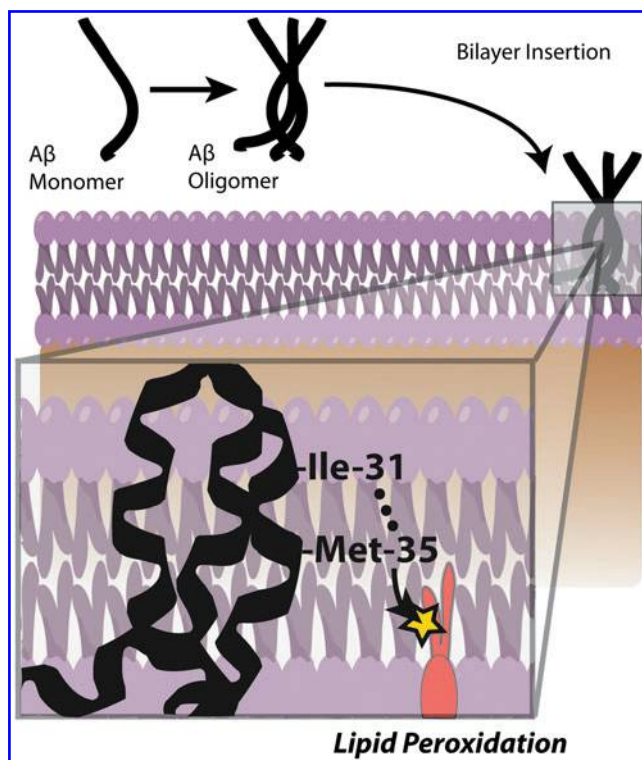


FIG. 6. A pictorial representation of A β oligomerization and insertion into the bilayer. When inserted into the bilayer, A β forms an α -helix that allows the peptide backbone carbonyl of Ile-31 to come within Van der Waals distance of the sulfur atom on Met-35, as explained by the *i*+4 rule of α -helices. This interaction allows for the formation of a sulfuranyl radical that leads to a catalytic lipid peroxidation process. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

The activity of α -enolase as a glycolytic protein is well understood. Consequently, the oxidative modification and subsequent loss of activity may significantly hinder energy production (150). α -Enolase however, possesses nonglycolytic activities in signaling pathways important to cell survival and in A β clearance (22). Evidence also suggests that α -enolase may be a neurotrophic factor, play a role in hypoxic stress regulation, and have transcription factor capabilities (1,62,147,158).

The examples of Pin1 and α -enolase were selected to demonstrate the power of redox proteomics in identifying specific links in cell signaling pathways that are damaged and dysfunctional as opposed to global tissue oxidation, and free radical induced oxidative stress of enzymatic proteins with multifunctional roles that may have far-reaching effects. In using redox proteomics, researchers may identify proteins that are more susceptible to oxidative modification and from this information garner insight regarding the progression and possibly even potential treatment for diseases such as AD.

Role of Methionine in A β -Induced Oxidative Stress

Studies from our laboratory and others showed Met-35 of A β peptides is critical for A β -associated toxicity and oxidative stress (15,160,174,175). Met can undergo two-electron oxidation to form methionine sulfoxide (MetSOx) (127,137). Oxidation of Met to the sulfoxide might play an important role in the regulation of protein function or cellular defense

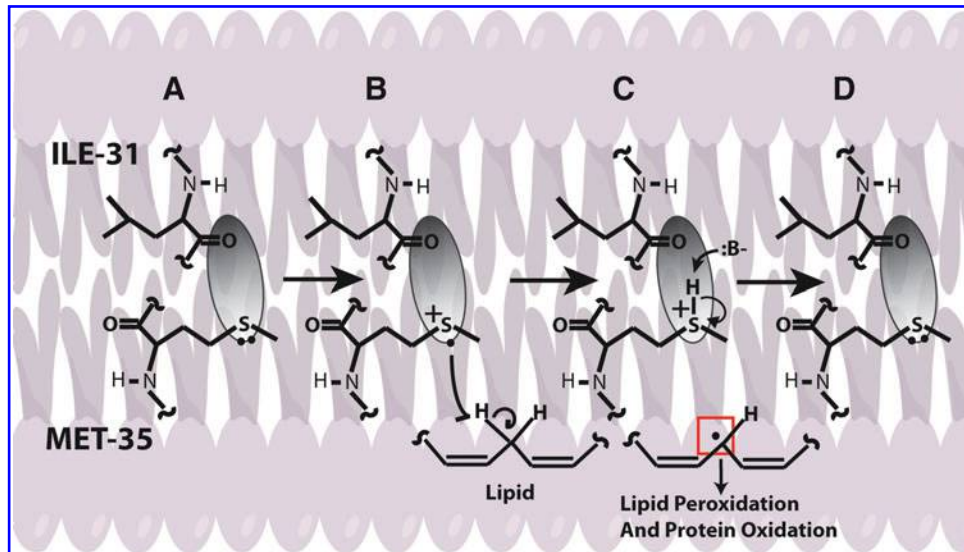


FIG. 7. A proposed mechanism for the $A\beta$ -induced free radical stress hypothesis. As shown, the electron density surrounding the sulfur atom of Met-35 is pulled away by the more electronegative oxygen of the carbonyl located on the peptide backbone at the position of Ile-31. As discussed, the carbonyl is within Van der Waals distance to the sulfur, which primes the lone pair on the sulfur for one-electron oxidation, forming the sulfuranium radical. Because this occurs within the bilayer, unsaturated lipids are present, allowing for an allylic hydrogen atom abstraction by the sulfuranium radical to eventually form a reduced Met-35 that recycles back upon deprotonation to the starting conditions for another cycle. The carbon centered radical may then go on to undergo peroxidation to create reactive aldehydes or may directly interact with another protein or lipid in a radical propagation step. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

mechanism (145). Further, the presence of methionine sulfide reductase (MSR), which catalyzes the conversion of MetSOx to Met (91,98,146), suggests that MSR might play an antioxidant role. Interestingly, in AD brain the activity of MSR is less, and a significant fraction of SP-resident $A\beta$ peptide has Met in the form of MetSOx (112), suggesting that Met oxidation might play an important role AD progression and pathogenesis (49). However, *in vitro* studies showed that $A\beta$ with

MetSOx is less toxic at a shorter incubation time (160), this could possibly be related to altered production of toxic $A\beta$ oligomers (75,107).

In addition, $A\beta$ -resident Met in the lipid bilayer can undergo one-electron oxidation forming sulfuranium free radical [MetS⁺]. Since, $A\beta$ is generated from cleavage of APP, a transmembrane protein as discussed above, we proposed that $A\beta$ once produced can insert as small oligomers into the lipid bilayer adopting an α -helical conformation (9,175). According to the α -helix conformation rule of $i+4$ rule, that is, every fourth amino acid interacts; hence, the Met-35 S-atom would interact with carbonyl oxygen of Ile-31 (79,80,137) (Fig. 6). Since oxygen of Ile31 is more electronegative than sulfur it will pull the electron density toward it, making the S-atom in Met-35 more vulnerable to one-electron oxidation to form sulfuranium free radical [MetS⁺] on Met (79,136,160) (Fig. 7). The substitution of Ile-31 by proline, an α -helix breaker, abrogates the oxidative stress and neurotoxicity associated with $A\beta(1-42)$ (80), suggesting that the secondary structure of $A\beta(1-42)$ contributes to reactivity of the neurotoxic peptide. However, until now the source of oxidant that triggers this event largely remains unknown. It is proposed that either molecular oxygen or Cu²⁺ might be key in the oxidation of Met to the sulfuranium radical. In the absence of oxygen, $A\beta$ s cannot lead to free radical production (161). Prior studies showed that $A\beta(1-42)$ has Cu/Zn SOD-like properties (39), and that amyloid plaques had high levels of copper (33). *In vitro* studies showed that $A\beta(1-42)$ can promote the reduction of peptide-bound Cu²⁺ to Cu⁺ and form hydrogen peroxide (H₂O₂). Further Cu⁺, can react with the H₂O₂ to form highly reactive, hydroxyl free radicals (69,76). Further, chelation of copper by clioquinol (CQ, 5-chloro-7-iodoquinolin-8-ol), hydroxyquinoline antibiotic that has nanomolar affinity

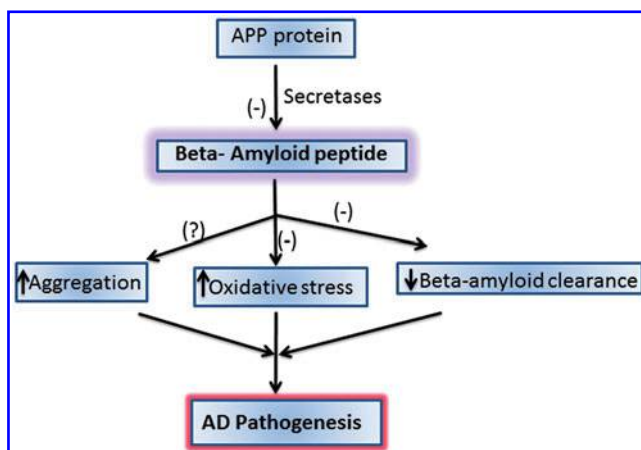


FIG. 8. Potential therapeutic targets for AD. There are various potential targets to prevent Alzheimer disease (AD) progression and pathogenesis that include inhibiting the beta-amyloid formation or increasing its clearance from the brain or inhibiting the oxidative stress induced by beta-amyloid peptide. (-) indicates potential targets to combat AD. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/ars

for Cu²⁺ (118), reduced the formation of H₂O₂ by A β (12,142,172). *In vivo* studies showed that oral administration of the clioquinol in Tg2576 mice reduced amyloid levels. Further, Phase 2 clinical trial showed that CQ slowed the rate of cognitive decline and reduced the plasma A β 42 levels in moderately severe AD patients (132). The importance of copper in A β -induced toxicity is suggested by a study where Met35 was substituted by Val that showed to increase the toxicity (36), suggesting that this substitution might lead to a change in the conformation of A β from α -helix to a mixture of α -helical and β -sheet conformations, thereby increasing the binding of Cu²⁺ and subsequently its associated toxicity. Further, substitution of His 6,13,14 in A β (1–42) by Tyr, which binds Cu²⁺ with less affinity than His, showed that it did not affect the oxidative stress and neurotoxicity further emphasizing the importance of Met-35 in the A β -induced toxicity and oxidative stress (10,160). Further research is needed to understand the role of copper in A β .

Once MetS⁺ radical is generated it can abstract allylic H atoms from the acyl chains of unsaturated fatty acids in the lipid bilayer to initiate the process of lipid peroxidation (60), and consequently affect the lipid bilayer. The products of oxidation further diffuse through the membrane affecting other cellular compartments, greatly amplifying the effect of the original A β -centered free radical, eventually leading to cell loss and AD. Consistent with this model, we substituted Gly at residue 37 of A β (1–42) by aspartic acid. The effect of this negatively charged amino acid was to remove the Met-35 residue from the bilayer, and no oxidative stress was observed in neuronal cultures (93). Vitamin E, a chain-breaking antioxidant blocks the chain reaction in the mechanism of lipid peroxidation, preventing oxidative stress to neurons (80). However, clinical trials conducted using vitamin E for the most part did not show beneficial effects in AD, which could be due to experimental design (77).

The earliest study using transgenic *Caenorhabditis elegans* expressing human A β (1–42) showed increased oxidation that correlated with the phenotypic expression (*e.g.*, paralysis) of the worm (170,175), which was confirmed by others (44). However, when the Met-35 was substituted by Cys no oxidative stress was found, but the deposition of modified A β (1–42) was not altered (175). Consistent with the role of Met, an *in vitro* study demonstrated that when the sulfur atom of methionine in A β (1–42) was substituted by a methylene moiety [A β (1–42)M35NLE] that has the same side chain length and hydrophobicity as Met (175), A β loses its associated free radical formation, oxidative stress, and toxicity (37,40,111). In contrast, some studies suggested that the 33–35 region of A β (25–35) is critical for the aggregation and neurotoxic properties of A β peptide, but substitution of Met by norleucine did not reduce the toxicity associated with this peptide (126). However, the chemistry of C-terminal Met is entirely different than Met within the peptide chain.

A recent study from our laboratory used for the first time an *in vivo* mammalian model to show that A β -resident Met-35 is critical to oxidative stress and neurotoxicity (18). In this study the PDAPP mouse, with Swedish and Indiana familial mutations of APP, has a third mutation introduced: substitution of leucine in APP at M631, corresponding to Met-35 of A β (1–42) (18). These mice were referred to as PDAPPM631L mice. In contrast, to the brain from PDAPP mice, which demonstrate oxidative stress, brain from PDAPPM631L mice

showed no *in vivo* oxidative stress. In addition, punctate deposits of A β (1–42) were found in the latter brain compared to frank amyloid deposits in the brain of PDAPP mice, suggesting that A β (1–42)-resident Met not only affects *in vivo* oxidative stress but also affects plaque formation. Interestingly, Met substitution in A β (1–42) did not rescue spatial learning and memory deficits at 6 months of age as assessed by the Morris water maze test. Given that APP is processed to produce toxic sAPP β and other toxic fragments of APP, this result may not be surprising. Other, more sensitive cognitive tests are needed to better understand the effect of the loss of Met on learning and memory. Proteomics analysis on brain from PDAPPM631L mice showed reduced oxidation of key proteins that are critical in regulating cellular pathways such as energy metabolism, cellular defense, protein degradation, and pH regulation compared to PDAPP mice (133,157). The decreased oxidation in general and reduced oxidation of key proteins like Pin1 (Pin1, discussed earlier) might play an important role in preventing AD pathogenesis (157).

Conclusion

The overproduction and accumulation of A β are key to the progression and pathogenesis of AD. Hence, the use of treatments to reduce A β formation or the downstream oxidative stress associated with A β could be helpful in preventing, treating, or delaying the progression of AD (51). There are various approaches that could be potential candidates to reduce A β levels: inhibiting A β production (by inhibiting secretase enzymes) or increasing the clearance of A β or using compounds that bind A β to impair aggregation (Fig. 8). Dissolving the extant SP may not be a good idea to combat this devastating disease, since oligomeric A β , the likely main toxic species of this peptide would be elevated by simple equilibrium considerations. Studies are in progress in our laboratory and others to further delineate the mechanism of A β -associated toxicity and develop a regimen to treat, slow, or hopefully one day prevent AD.

Acknowledgment

This research was supported by NIH grants to D.A.B. [AG-05119].

References

1. Aaronson RM, Graven KK, Tucci M, McDonald RJ, and Farber HW. Non-neuronal enolase is an endothelial hypoxic stress protein. *J Biol Chem* 270: 27752–27757, 1995.
2. Abe T, Tohgi H, Isobe C, Murata T, and Sato C. Remarkable increase in the concentration of 8-hydroxyguanosine in cerebrospinal fluid from patients with Alzheimer's disease. *J Neurosci Res* 70: 447–450, 2002.
3. Aksenov MY, Tucker HM, Nair P, Aksenova MV, Butterfield DA, Estus S, and Markesbery WR. The expression of key oxidative stress-handling genes in different brain regions in Alzheimer's disease. *J Mol Neurosci* 11: 151–164, 1998.
4. Aluise CD, Robinson RAS, Cai JA, Pierce WM, Markesbery WR, and Butterfield DA. Redox proteomics analysis of brains from subjects with amnesic mild cognitive impairment compared to brains from subjects with preclinical Alzheimer's disease: insights into memory loss in MCI. *J Alzheimers Dis* 23: 257–269, 2011.

5. Balastik M, Lim J, Pastorino L, and Lu KP. Pin1 in Alzheimer's disease: multiple substrates, one regulatory mechanism? *Biochim Biophys Acta* 1772: 422–429, 2007.
6. Barone E, Di Domenico F, Cenini G, Sultana R, Cini C, Preziosi P, Perluigi M, Mancuso C, and Butterfield DA. Biliverdin reductase—a protein levels and activity in the brains of subjects with Alzheimer disease and mild cognitive impairment. *Biochim Biophys Acta* 1812: 480–487, 2011.
7. Barone E, Di Domenico F, Cenini G, Sultana R, Coccia R, Preziosi P, Perluigi M, Mancuso C, and Butterfield DA. Oxidative and nitrosative modifications of biliverdin reductase-A in the brain of subjects with Alzheimer's disease and amnesic mild cognitive impairment. *J Alzheimers Dis* 25: 623–633, 2011.
8. Barone E, Di Domenico F, Sultana R, Coccia R, Mancuso C, Perluigi M, and Butterfield DA. Heme oxygenase-1 post-translational modifications in the brain of subjects with Alzheimer disease and mild cognitive impairment. *Free Radic Biol Med* 52: 2292–2301, 2012.
9. Boyd-Kimball D, Castegna A, Sultana R, Poon HF, Petroze R, Lynn BC, Klein JB, and Butterfield DA. Proteomic identification of proteins oxidized by Aβ(1–42) in synaptosomes: implications for Alzheimer's disease. *Brain Res* 1044: 206–215, 2005.
10. Boyd-Kimball D, Mohammad Abdul H, Reed T, Sultana R, and Butterfield DA. Role of phenylalanine 20 in Alzheimer's amyloid beta-peptide (1–42)-induced oxidative stress and neurotoxicity. *Chem Res Toxicol* 17: 1743–1749, 2004.
11. Boyd-Kimball D, Poon HF, Lynn BC, Cai J, Pierce WM, Jr., Klein JB, Ferguson J, Link CD, and Butterfield DA. Proteomic identification of proteins specifically oxidized in *Caenorhabditis elegans* expressing human Aβ(1–42): implications for Alzheimer's disease. *Neurobiol Aging* 27: 1239–1249, 2006.
12. Bush AI. Drug development based on the metals hypothesis of Alzheimer's disease. *J Alzheimers Dis* 15: 223–240, 2008.
13. Butterfield DA. beta-Amyloid-associated free radical oxidative stress and neurotoxicity: implications for Alzheimer's disease. *Chem Res Toxicol* 10: 495–506, 1997.
14. Butterfield DA, Abdul HM, Opii W, Newman SF, Joshi G, Ansari MA, and Sultana R. Pin1 in Alzheimer's disease. *J Neurochem* 98: 1697–1706, 2006.
15. Butterfield DA, and Boyd-Kimball D. The critical role of methionine 35 in Alzheimer's amyloid beta-peptide (1–42)-induced oxidative stress and neurotoxicity. *Biochim Biophys Acta* 1703: 149–156, 2005.
16. Butterfield DA, Castegna A, Lauderback CM, and Drake J. Evidence that amyloid beta-peptide-induced lipid peroxidation and its sequelae in Alzheimer's disease brain contribute to neuronal death. *Neurobiol Aging* 23: 655–664, 2002.
17. Butterfield DA, Drake J, Pocernich C, and Castegna A. Evidence of oxidative damage in Alzheimer's disease brain: central role for amyloid beta-peptide. *Trends Mol Med* 7: 548–554, 2001.
18. Butterfield DA, Galvan V, Lange MB, Tang H, Sowell RA, Spilman P, Fombonne J, Gorostiza O, Zhang J, Sultana R, and Bredesen DE. *In vivo* oxidative stress in brain of Alzheimer disease transgenic mice: requirement for methionine 35 in amyloid beta-peptide of APP. *Free Radic Biol Med* 48: 136–144, 2010.
19. Butterfield DA, Gnjec A, Poon HF, Castegna A, Pierce WM, Klein JB, and Martins RN. Redox proteomics identification of oxidatively modified brain proteins in inherited Alzheimer's disease: an initial assessment. *J Alzheimers Dis* 10: 391–397, 2006.
20. Butterfield DA, Howard B, Yatin S, Koppal T, Drake J, Hensley K, Aksenov M, Aksenova M, Subramaniam R, Varadarajan S, Harris-White ME, Pedigo NW, Jr., and Carney JM. Elevated oxidative stress in models of normal brain aging and Alzheimer's disease. *Life Sci* 65: 1883–1892, 1999.
21. Butterfield DA, and Kanski J. Methionine residue 35 is critical for the oxidative stress and neurotoxic properties of Alzheimer's amyloid beta-peptide 1–42. *Peptides* 23: 1299–1309, 2002.
22. Butterfield DA, and Lange ML. Multifunctional roles of enolase in Alzheimer's disease brain: beyond altered glucose metabolism. *J Neurochem* 111: 915–933, 2009.
23. Butterfield DA, and Lauderback CM. Lipid peroxidation and protein oxidation in Alzheimer's disease brain: potential causes and consequences involving amyloid beta-peptide-associated free radical oxidative stress. *Free Radic Biol Med* 32: 1050–1060, 2002.
24. Butterfield DA, Perluigi M, Reed T, Muharib T, Hughes CP, Robinson RA, and Sultana R. Redox proteomics in selected neurodegenerative disorders: from its infancy to future applications. *Antioxid Redox Signal* 17: 1610–1655, 2012.
25. Butterfield DA, Poon HF, Clair DS, Keller JN, Pierce WM, Klein JB, and Markesbery WR. Redox proteomics identification of oxidatively modified hippocampal proteins in mild cognitive impairment: insights into the development of Alzheimer's disease. *Neurobiol Dis* 22: 223–232, 2006.
26. This reference has been deleted.
27. Butterfield DA, and Sultana R. Redox proteomics identification of oxidatively modified brain proteins in Alzheimer's disease and mild cognitive impairment: insights into the progression of this dementing disorder. *J Alzheimers Dis* 12: 61–72, 2007.
28. Calabrese V, Cornelius C, Mancuso C, Lentile R, Stella AM, and Butterfield DA. Redox homeostasis and cellular stress response in aging and neurodegeneration. *Methods Mol Biol* 610: 285–308, 2010.
29. Calabrese V, Cornelius C, Rizzarelli E, Owen JB, Dinkova-Kostova AT, and Butterfield DA. Nitric oxide in cell survival: a janus molecule. *Antioxid Redox Signal* 11: 2717–2739, 2009.
30. Castegna A, Aksenov M, Thongboonkerd V, Klein JB, Pierce WM, Booze R, Markesbery WR, and Butterfield DA. Proteomic identification of oxidatively modified proteins in Alzheimer's disease brain. Part II: dihydropyrimidinase-related protein 2, alpha-enolase and heat shock cognate 71. *J Neurochem* 82: 1524–1532, 2002.
31. Castegna A, Thongboonkerd V, Klein JB, Lynn B, Markesbery WR, and Butterfield DA. Proteomic identification of nitrated proteins in Alzheimer's disease brain. *J Neurochem* 85: 1394–1401, 2003.
32. Cenini G, Dowling AL, Beckett TL, Barone E, Mancuso C, Murphy MP, Levine H, 3rd, Lott IT, Schmitt FA, Butterfield DA, and Head E. Association between frontal cortex oxidative damage and beta-amyloid as a function of age in Down syndrome. *Biochim Biophys Acta* 1822: 130–138, 2012.
33. Cerpa WF, Barria MI, Chacon MA, Suazo M, Gonzalez M, Opazo C, Bush AI, and Inestrosa NC. The N-terminal copper-binding domain of the amyloid precursor protein protects against Cu²⁺ neurotoxicity *in vivo*. *FASEB J* 18: 1701–1703, 2004.
34. Chetelat G, Desgranges B, de la Sayette V, Viader F, Eustache F, and Baron JC. Mild cognitive impairment: can FDG-PET predict who is to rapidly convert to Alzheimer's disease? *Neurology* 60: 1374–1377, 2003.

35. Chetelat G, Eustache F, Viader F, De La Sayette V, Pelerin A, Mezenge F, Hannequin D, Dupuy B, Baron JC, and Desgranges B. FDG-PET measurement is more accurate than neuropsychological assessments to predict global cognitive deterioration in patients with mild cognitive impairment. *Neurocase* 11: 14–25, 2005.
36. Ciccotosto GD, Tew D, Curtain CC, Smith D, Carrington D, Masters CL, Bush AI, Cherny RA, Cappai R, and Barnham KJ. Enhanced toxicity and cellular binding of a modified amyloid beta peptide with a methionine to valine substitution. *J Biol Chem* 279: 42528–42534, 2004.
37. Clementi ME, Pezzotti M, Orsini F, Sampaolese B, Mezzogori D, Grassi C, Giardina B, and Misiti F. Alzheimer's amyloid beta-peptide (1–42) induces cell death in human neuroblastoma via bax/bcl-2 ratio increase: an intriguing role for methionine 35. *Biochem Biophys Res Commun* 342: 206–213, 2006.
38. Copani A, Koh JY, and Cotman CW. Beta-amyloid increases neuronal susceptibility to injury by glucose deprivation. *Neuroreport* 2: 763–765, 1991.
39. Curtain CC, Ali F, Volitakis I, Cherny RA, Norton RS, Beyreuther K, Barrow CJ, Masters CL, Bush AI, and Barnham KJ. Alzheimer's disease amyloid-beta binds copper and zinc to generate an allosterically ordered membrane-penetrating structure containing superoxide dismutase-like subunits. *J Biol Chem* 276: 20466–20473, 2001.
40. Dai XL, Sun YX, and Jiang ZF. Attenuated cytotoxicity but enhanced betafibril of a mutant amyloid beta-peptide with a methionine to cysteine substitution. *FEBS Lett* 581: 1269–1274, 2007.
41. Dalle-Donne I, Scaloni A, and Butterfield DA. *Redox Proteomics: From Protein Modifications to Cellular Dysfunction and Diseases*. Hoboken, NJ: John Wiley and Sons, 2006.
42. de Leon MJ, George AE, Ferris SH, Rosenbloom S, Christman DR, Gentes CI, Reisberg B, Kricheff II, and Wolf AP. Regional correlation of PET and CT in senile dementia of the Alzheimer type. *AJNR Am J Neuroradiol* 4: 553–556, 1983.
43. Ding QX, Markesbery WR, Cekarini V, and Keller JN. Decreased RNA, and increased RNA oxidation, in ribosomes from early Alzheimer's disease. *Neurochem Res* 31: 705–710, 2006.
44. Drake J, Link CD, and Butterfield DA. Oxidative stress precedes fibrillar deposition of Alzheimer's disease amyloid beta-peptide (1–42) in a transgenic *Caenorhabditis elegans* model. *Neurobiol Aging* 24: 415–420, 2003.
45. Drayer BP, Heyman A, Wilkinson W, Barrett L, and Weinberg T. Early-onset Alzheimer's disease: an analysis of CT findings. *Ann Neurol* 17: 407–410, 1985.
46. Driver JA, and Lu KP. Pin1: a new genetic link between Alzheimer's disease, cancer and aging. *Curr Aging Sci* 3: 158–165, 2010.
47. Farrow TF, Thiyagesh SN, Wilkinson ID, Parks RW, Ingram L, and Woodruff PW. Fronto-temporal-lobe atrophy in early-stage Alzheimer's disease identified using an improved detection methodology. *Psychiatry Res* 155: 11–19, 2007.
48. Folstein MF, Folstein SE, and McHugh PR. "Mini-mental state." A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 12: 189–198, 1975.
49. Gabbita SP, Aksenov MY, Lovell MA, and Markesbery WR. Decrease in peptide methionine sulfoxide reductase in Alzheimer's disease brain. *J Neurochem* 73: 1660–1666, 1999.
50. Gabbita SP, Lovell MA, and Markesbery WR. Increased nuclear DNA oxidation in the brain in Alzheimer's disease. *J Neurochem* 71: 2034–2040, 1998.
51. Ganjei JK. Targeting amyloid precursor protein secretases: Alzheimer's disease and beyond. *Drug News Perspect* 23: 573–584, 2010.
52. Gervais FG, Xu D, Robertson GS, Vaillancourt JP, Zhu Y, Huang J, LeBlanc A, Smith D, Rigby M, Shearman MS, Clarke EE, Zheng H, Van Der Ploeg LH, Ruffolo SC, Thornberry NA, Xanthoudakis S, Zamboni RJ, Roy S, and Nicholson DW. Involvement of caspases in proteolytic cleavage of Alzheimer's amyloid-beta precursor protein and amyloidogenic A beta peptide formation. *Cell* 97: 395–406, 1999.
53. Geula C, Mesulam MM, Saroff DM, and Wu CK. Relationship between plaques, tangles, and loss of cortical cholinergic fibers in Alzheimer disease. *J Neuropathol Exp Neurol* 57: 63–75, 1998.
54. Glabe CC. Amyloid accumulation and pathogenesis of Alzheimer's disease: significance of monomeric, oligomeric and fibrillar A β . *Subcell Biochem* 38: 167–177, 2005.
55. Goate A. Segregation of a missense mutation in the amyloid beta-protein precursor gene with familial Alzheimer's disease. *J Alzheimers Dis* 9: 341–347, 2006.
56. Goate A, and Hardy J. Twenty years of Alzheimer's disease-causing mutations. *J Neurochem* 120 Suppl 1: 3–8, 2012.
57. Guidi I, Galimberti D, Lonati S, Novembrino C, Bamonti F, Tiriticco M, Fenoglio C, Venturelli E, Baron P, Bresolin N, and Scarpini E. Oxidative imbalance in patients with mild cognitive impairment and Alzheimer's disease. *Neurobiol Aging* 27: 262–269, 2006.
58. Guillozet AL, Weintraub S, Mash DC, and Mesulam MM. Neurofibrillary tangles, amyloid, and memory in aging and mild cognitive impairment. *Arch Neurol* 60: 729–736, 2003.
59. Gyure KA, Durham R, Stewart WF, Smialek JE, and Troncoso JC. Intraneuronal A β precedes development of amyloid plaques in Down syndrome. *Arch Pathol Lab Med* 125: 489–492, 2001.
60. Halliwell B, and Gutteridge JM. Biologically relevant metal ion-dependent hydroxyl radical generation. An update. *FEBS Lett* 307: 108–112, 1992.
61. Hardy J, and Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* 297: 353–356, 2002.
62. Hattori T, Takei N, Mizuno Y, Kato K, and Kohsaka S. Neurotrophic and neuroprotective effects of neuron-specific enolase on cultured neurons from embryonic rat-brain. *Neurosci Res* 21: 191–198, 1995.
63. Hebert LE, Scherr PA, Bienias JL, Bennett DA, and Evans DA. Alzheimer disease in the US population—prevalence estimates using the 2000 census. *Arch Neurol* 60: 1119–1122, 2003.
64. Hensley K, Hall N, Subramaniam R, Cole P, Harris M, Aksenov M, Aksenova M, Gabbita SP, Wu JF, Carney JM, Lovell M, Markesbery WR, and Butterfield DA. Brain regional correspondence between Alzheimers-disease histopathology and biomarkers of protein oxidation. *J Neurochem* 65: 2146–2156, 1995.
65. Hensley K, Maidt ML, Yu ZQ, Sang H, Markesbery WR, and Floyd RA. Electrochemical analysis of protein nitrotyrosine and dityrosine in the Alzheimer brain indicates region-specific accumulation. *J Neurosci* 18: 8126–8132, 1998.
66. Herholz K, Salmon E, Perani D, Baron JC, Holthoff V, Frolich L, Schonknecht P, Ito K, Mielke R, Kalbe E, Zundorf G, Delbeuck X, Pelati O, Anchisi D, Fazio F, Kerrouche N, Desgranges B, Eustache F, Beuthien-Baumann B, Menzel C, Schroder J, Kato T, Arahata Y, Henze M, and Heiss WD. Discrimination between Alzheimer dementia and controls

- by automated analysis of multicenter FDG PET. *Neuroimage* 17: 302–316, 2002.
67. Hoogland C, Mostaguir K, Sanchez JC, Hochstrasser DF, and Appel RD. SWISS-2DPAGE, ten years later. *Proteomics* 4: 2352–2356, 2004.
 68. Hoogland C, Sanchez JC, Tonella L, Binz PA, Bairoch A, Hochstrasser DF, and Appel RD. The 1999 SWISS-2DPAGE database update. *Nucleic Acids Res* 28: 286–288, 2000.
 69. Huang X, Atwood CS, Hartshorn MA, Multhaup G, Goldstein LE, Scarpa RC, Cuajungco MP, Gray DN, Lim J, Moir RD, Tanzi RE, and Bush AI. The A beta peptide of Alzheimer's disease directly produces hydrogen peroxide through metal ion reduction. *Biochemistry* 38: 7609–7616, 1999.
 70. Ida N, Hartmann T, Pantel J, Schroder J, Zerfass R, Forstl H, Sandbrink R, Masters CL, and Beyreuther K. Analysis of heterogeneous A4 peptides in human cerebrospinal fluid and blood by a newly developed sensitive Western blot assay. *J Biol Chem* 271: 22908–22914, 1996.
 71. Jack CR, Jr., Petersen RC, Xu YC, O'Brien PC, Smith GE, Ivnik RJ, Boeve BF, Waring SC, Tangalos EG, and Kokmen E. Prediction of AD with MRI-based hippocampal volume in mild cognitive impairment. *Neurology* 52: 1397–1403, 1999.
 72. Jarrett JT, Berger EP, and Lansbury PT, Jr. The carboxy terminus of the beta amyloid protein is critical for the seeding of amyloid formation: implications for the pathogenesis of Alzheimer's disease. *Biochemistry* 32: 4693–4697, 1993.
 73. Jimenez S, Torres M, Vizuete M, Sanchez-Varo R, Sanchez-Mejias E, Trujillo-Estrada L, Carmona-Cuenca I, Caballero C, Ruano D, Gutierrez A, and Vitorica J. Age-dependent accumulation of soluble amyloid beta (A β) oligomers reverses the neuroprotective effect of soluble amyloid precursor protein-alpha (sAPP(alpha)) by modulating phosphatidylinositol 3-kinase (PI3K)/Akt-GSK-3beta pathway in Alzheimer mouse model. *J Biol Chem* 286: 18414–18425, 2011.
 74. Jin M, Shepardson N, Yang T, Chen G, Walsh D, and Selkoe DJ. Soluble amyloid beta-protein dimers isolated from Alzheimer cortex directly induce Tau hyperphosphorylation and neuritic degeneration. *Proc Natl Acad Sci U S A* 108: 5819–5824, 2011.
 75. Johansson AS, Bergquist J, Volbracht C, Paivio A, Leist M, Lannfelt L, and Westlind-Danielsson A. Attenuated amyloid-beta aggregation and neurotoxicity owing to methionine oxidation. *Neuroreport* 18: 559–563, 2007.
 76. Jomova K, Vondrakova D, Lawson M, and Valko M. Metals, oxidative stress and neurodegenerative disorders. *Mol Cell Biochem* 345: 91–104, 2010.
 77. Joshi YB, and Pratico D. Vitamin E in aging, dementia, and Alzheimer's disease. *Biofactors* 38: 90–97, 2012.
 78. Jovanovic SV, Clements D, and MacLeod K. Biomarkers of oxidative stress are significantly elevated in Down syndrome. *Free Radic Biol Med* 25: 1044–1048, 1998.
 79. Kanski J, Aksenova M, and Butterfield DA. The hydrophobic environment of Met35 of Alzheimer's A β (1–42) is important for the neurotoxic and oxidative properties of the peptide. *Neurotox Res* 4: 219–223, 2002.
 80. Kanski J, Aksenova M, Schoneich C, and Butterfield DA. Substitution of isoleucine-31 by helical-breaking proline abolishes oxidative stress and neurotoxic properties of Alzheimer's amyloid beta-peptide. *Free Radic Biol Med* 32: 1205–1211, 2002.
 81. Katzman R, and Saitoh T. Advances in Alzheimer's disease. *FASEB J* 5: 278–286, 1991.
 82. Keeney JT, Swomley AM, Harris JL, Fiorini A, Mitov MI, Perluigi M, Sultana R, and Butterfield DA. Cell cycle proteins in brain in mild cognitive impairment: insights into progression to Alzheimer disease. *Neurotox Res* 22: 220–230, 2012.
 83. Keller JN, Schmitt FA, Scheff SW, Ding Q, Chen Q, Butterfield DA, and Markesbery WR. Evidence of increased oxidative damage in subjects with mild cognitive impairment. *Neurology* 64: 1152–1156, 2005.
 84. Koppal T, Drake J, Yatin S, Jordan B, Varadarajan S, Bettenhausen L, and Butterfield DA. Peroxynitrite-induced alterations in synaptosomal membrane proteins: insight into oxidative stress in Alzheimer's disease. *J Neurochem* 72: 310–317, 1999.
 85. Landrieu I, Smet-Nocca C, Amniai L, Louis JV, Wieruszkeski JM, Goris J, Janssens V, and Lippens G. Molecular implication of PP2A and Pin1 in the Alzheimer's disease specific hyperphosphorylation of Tau. *PLoS One* 6: e21521, 2011.
 86. Lauderback CM, Hackett JM, Huang FF, Keller JN, Szweda LI, Markesbery WR, and Butterfield DA. The glial glutamate transporter, GLT-1, is oxidatively modified by 4-hydroxy-2-nonenal in the Alzheimer's disease brain: the role of A beta 1–42. *J Neurochem* 78: 413–416, 2001.
 87. Lim J, Balastik M, Lee TH, Nakamura K, Liou YC, Sun A, Finn G, Pastorino L, Lee VM, and Lu KP. Pin1 has opposite effects on wild-type and P301L tau stability and tauopathy. *J Clin Invest* 118: 1877–1889, 2008.
 88. Lovell MA, Ehmann WD, Mattson MP, and Markesbery WR. Elevated 4-hydroxynonenal in ventricular fluid in Alzheimer's disease. *Neurobiol Aging* 18: 457–461, 1997.
 89. Lovell MA, and Markesbery WR. Oxidatively modified RNA in mild cognitive impairment. *Neurobiol Dis* 29: 169–175, 2008.
 90. Lu PJ, Wulf G, Zhou XZ, Davies P, and Lu KP. The prolyl isomerase Pin1 restores the function of Alzheimer-associated phosphorylated tau protein. *Nature* 399: 784–788, 1999.
 91. Luo S, and Levine RL. Methionine in proteins defends against oxidative stress. *FASEB J* 23: 464–472, 2009.
 92. Ma SL, Pastorino L, Zhou XZ, and Lu KP. Prolyl isomerase Pin1 promotes amyloid precursor protein (APP) turnover by inhibiting glycogen synthase kinase-3beta (GSK3beta) activity: novel mechanism for Pin1 to protect against Alzheimer disease. *J Biol Chem* 287: 6969–6973, 2012.
 93. Mark RJ, Lovell MA, Markesbery WR, Uchida K, and Mattson MP. A role for 4-hydroxynonenal, an aldehydic product of lipid peroxidation, in disruption of ion homeostasis and neuronal death induced by amyloid beta-peptide. *J Neurochem* 68: 255–264, 1997.
 94. Markesbery WR. Oxidative stress hypothesis in Alzheimer's disease. *Free Radic Biol Med* 23: 134–147, 1997.
 95. Markesbery WR, and Carney JM. Oxidative alterations in Alzheimer's disease. *Brain Pathol* 9: 133–146, 1999.
 96. Markesbery WR, Kryscio RJ, Lovell MA, and Morrow JD. Lipid peroxidation is an early event in the brain in amnesic mild cognitive impairment. *Ann Neurol* 58: 730–735, 2005.
 97. Markesbery WR, and Lovell MA. Four-hydroxynonenal, a product of lipid peroxidation, is increased in the brain in Alzheimer's disease. *Neurobiol Aging* 19: 33–36, 1998.
 98. Mary J, Vouquier S, Picot CR, Perichon M, Petropoulos I, and Friguet B. Enzymatic reactions involved in the repair of oxidized proteins. *Exp Gerontol* 39: 1117–1123, 2004.
 99. Masters CL, Simms G, Weinman NA, Multhaup G, McDonald BL, and Beyreuther K. Amyloid plaque core protein in Alzheimer disease and Down syndrome. *Proc Natl Acad Sci U S A* 82: 4245–4249, 1985.
 100. Mattila J, Koikkalainen J, Virkki A, Simonsen A, van Gils M, Waldemar G, Soininen H, and Lotjonen J. A disease state fingerprint for evaluation of Alzheimer's disease. *J Alzheimers Dis* 27: 163–176, 2011.

101. Mattson MP. Cellular actions of beta-amyloid precursor protein and its soluble and fibrillogenic derivatives. *Physiol Rev* 77: 1081–1132, 1997.
102. Mayeux R, Tang MX, Jacobs DM, Manly J, Bell K, Merchant C, Small SA, Stern Y, Wisniewski HM, and Mehta PD. Plasma amyloid beta-peptide 1–42 and incipient Alzheimer's disease. *Ann Neurol* 46: 412–416, 1999.
103. McGrath LT, McGleenon BM, Brennan S, McColl D, McIlroy S, and Passmore AP. Increased oxidative stress in Alzheimer's disease as assessed with 4-hydroxynonenal but not malondialdehyde. *QJM-Monthly J Associaf Phys* 94: 485–490, 2001.
104. Mecocci P, MacGarvey U, and Beal MF. Oxidative damage to mitochondrial DNA is increased in Alzheimer's disease. *Ann Neurol* 36: 747–751, 1994.
105. Migliore L, Fontana I, Trippi F, Cognato R, Coppede F, Tognoni G, Nucciarone B, and Siciliano G. Oxidative DNA damage in peripheral leukocytes of mild cognitive impairment and AD patients. *Neurobiol Aging* 26: 567–573, 2005.
106. Miller DL, Papayannopoulos IA, Styles J, Bobin SA, Lin YY, Biemann K, and Iqbal K. Peptide compositions of the cerebrovascular and senile plaque core amyloid deposits of Alzheimer's disease. *Arch Biochem Biophys* 301: 41–52, 1993.
107. Misiti F, Clementi ME, and Giardina B. Oxidation of methionine 35 reduces toxicity of the amyloid beta-peptide(1–42) in neuroblastoma cells (IMR-32) via enzyme methionine sulfoxide reductase A expression and function. *Neurochem Int* 56: 597–602, 2010.
108. Mohammad Abdul H, Sultana R, Keller JN, St Clair DK, Markesbery WR, and Butterfield DA. Mutations in amyloid precursor protein and presenilin-1 genes increase the basal oxidative stress in murine neuronal cells and lead to increased sensitivity to oxidative stress mediated by amyloid beta-peptide (1–42), HO and kainic acid: implications for Alzheimer's disease. *J Neurochem* 96: 1322–1335, 2006.
109. Mori C, Spooner ET, Wisniewsk KE, Wisniewski TM, Yamaguchi H, Saido TC, Tolan DR, Selkoe DJ, and Lemere CA. Intraneuronal Abeta42 accumulation in Down syndrome brain. *Amyloid* 9: 88–102, 2002.
110. Munch G, Schinzel R, Loske C, Wong A, Durany N, Li JJ, Vlassara H, Smith MA, Perry G, and Riederer P. Alzheimer's disease—synergistic effects of glucose deficit, oxidative stress and advanced glycation endproducts. *J Neural Transm* 105: 439–461, 1998.
111. Murray MM, Bernstein SL, Nyugen V, Condrion MM, Teplow DB, and Bowers MT. Amyloid beta protein: Abeta40 inhibits Abeta42 oligomerization. *J Am Chem Soc* 131: 6316–6317, 2009.
112. Naslund J, Schierhorn A, Hellman U, Lannfelt L, Roses AD, Tjernberg LO, Silberring J, Gandy SE, Winblad B, Greenberg P, et al. Relative abundance of Alzheimer A beta amyloid peptide variants in Alzheimer disease and normal aging. *Proc Natl Acad Sci U S A* 91: 8378–8382, 1994.
113. Nelson PT, Alafuzoff I, Bigio EH, Bouras C, Braak H, Cairns NJ, Castellani RJ, Crain BJ, Davies P, Del Tredici K, Duyckaerts C, Frosch MP, Haroutunian V, Hof PR, Hulette CM, Hyman BT, Iwatsubo T, Jellinger KA, Jicha GA, Kovari E, Kukull WA, Leverenz JB, Love S, Mackenzie IR, Mann DM, Masliah E, McKee AC, Montine TJ, Morris JC, Schneider JA, Sonnen JA, Thal DR, Trojanowski JQ, Troncoso JC, Wisniewski T, Woltjer RL, and Beach TG. Correlation of Alzheimer disease neuropathologic changes with cognitive status: a review of the literature. *J Neuropathol Exp Neurol* 71: 362–381, 2012.
114. Nikolaev A, McLaughlin T, O'Leary DD, and Tessier-Lavigne M. APP binds DR6 to trigger axon pruning and neuron death via distinct caspases. *Nature* 457: 981–989, 2009.
115. Nunomura A, Perry G, Aliev G, Hirai K, Takeda A, Balraj EK, Jones PK, Ghanbari H, Wataya T, Shimohama S, Chiba S, Atwood CS, Petersen RB, and Smith MA. Oxidative damage is the earliest event in Alzheimer disease. *J Neuropathol Exp Neurol* 60: 759–767, 2001.
116. Nunomura A, Perry G, Pappolla MA, Wade R, Hirai K, Chiba S, and Smith MA. RNA oxidation is a prominent feature of vulnerable neurons in Alzheimer's disease. *J Neurosci* 19: 1959–1964, 1999.
117. Oda T, Wals P, Osterburg HH, Johnson SA, Pasinetti GM, Morgan TE, Rozovsky I, Stine WB, Snyder SW, Holzman TE, et al. Clusterin (apoJ) alters the aggregation of amyloid beta-peptide (A beta 1–42) and forms slowly sedimenting A beta complexes that cause oxidative stress. *Exp Neurol* 136: 22–31, 1995.
118. Opazo C, Luza S, Villemagne VL, Volitakis I, Rowe C, Barnham KJ, Strozzyk D, Masters CL, Cherny RA, and Bush AI. Radioiodinated clioquinol as a biomarker for beta-amyloid: Zn complexes in Alzheimer's disease. *Aging Cell* 5: 69–79, 2006.
119. Park SA, Shaked GM, Bredesen DE, and Koo EH. Mechanism of cytotoxicity mediated by the C31 fragment of the amyloid precursor protein. *Biochem Biophys Res Commun* 388: 450–455, 2009.
120. Perluigi M, and Butterfield DA. The identification of protein biomarkers for oxidative stress in Down syndrome. *Expert Rev Proteomics* 8: 427–429, 2011.
121. Perluigi M, and Butterfield DA. Oxidative stress and Down syndrome: a route toward Alzheimer-like dementia. *Curr Gerontol Geriatr Res* 2012: 724904, 2012.
122. Perluigi M, Sultana R, Cenini G, Di Domenico F, Memo M, Pierce WM, Coccia R, and Butterfield DA. Redox proteomics identification of 4-hydroxynonenal-modified brain proteins in Alzheimer's disease: role of lipid peroxidation in Alzheimer's disease pathogenesis. *Proteomics Clin Appl* 3: 682–693, 2009.
123. Petersen RC. Mild cognitive impairment: transition between aging and Alzheimer's disease. *Neurologia* 15: 93–101, 2000.
124. Petersen RC. Mild cognitive impairment as a diagnostic entity. *J Intern Med* 256: 183–194, 2004.
125. Petrie EC, Cross DJ, Galasko D, Schellenberg GD, Raskind MA, Peskind ER, and Minoshima S. Preclinical evidence of Alzheimer changes: convergent cerebrospinal fluid biomarker and fluorodeoxyglucose positron emission tomography findings. *Arch Neurol* 66: 632–637, 2009.
126. Pike CJ, Walencewicz-Wasserman AJ, Kosmoski J, Cribbs DH, Glabe CG, and Cotman CW. Structure-activity analyses of beta-amyloid peptides: contributions of the beta 25–35 region to aggregation and neurotoxicity. *J Neurochem* 64: 253–265, 1995.
127. Pogocki D, and Schoneich C. Redox properties of Met(35) in neurotoxic beta-amyloid peptide. A molecular modeling study. *Chem Res Toxicol* 15: 408–418, 2002.
128. Ranganathan R, Lu KP, Hunter T, and Noel JP. Structural and functional analysis of the mitotic rotamase Pin1 suggests substrate recognition is phosphorylation dependent. *Cell* 89: 875–886, 1997.
129. Reed T, Perluigi M, Sultana R, Pierce WM, Klein JB, Turner DM, Coccia R, Markesbery WR, and Butterfield DA. Redox proteomic identification of 4-hydroxy-2-nonenal-modified brain proteins in amnesic mild cognitive impairment: insight into

- the role of lipid peroxidation in the progression and pathogenesis of Alzheimer's disease. *Neurobiol Dis* 30: 107–120, 2008.
130. Reed TT, Pierce WM, Jr., Turner DM, Markesbery WR, and Butterfield DA. Proteomic identification of nitrated brain proteins in early Alzheimer's disease inferior parietal lobule. *J Cell Mol Med* 13: 2019–2029, 2009.
 131. Reed TT, Pierce WM, Markesbery WR, and Butterfield DA. Proteomic identification of HNE-bound proteins in early Alzheimer disease: insights into the role of lipid peroxidation in the progression of AD. *Brain Res* 1274: 66–76, 2009.
 132. Ritchie CW, Bush AI, Mackinnon A, Macfarlane S, Mastwyk M, MacGregor L, Kierns L, Cherny R, Li QX, Tammer A, Carrington D, Mavros C, Volitakis I, Xilinas M, Ames D, Davis S, Beyreuther K, Tanzi RE, and Masters CL. Metal-protein attenuation with iodochlorhydroxyquin (clioquinol) targeting Abeta amyloid deposition and toxicity in Alzheimer disease: a pilot phase 2 clinical trial. *Arch Neurol* 60: 1685–1691, 2003.
 133. Robinson RA, Lange MB, Sultana R, Galvan V, Fombonne J, Gorostiza O, Zhang J, Warriar G, Cai J, Pierce WM, Bredesen DE, and Butterfield DA. Differential expression and redox proteomics analyses of an Alzheimer disease transgenic mouse model: effects of the amyloid-beta peptide of amyloid precursor protein. *Neuroscience* 177: 207–222, 2011.
 134. Scheuner D, Eckman C, Jensen M, Song X, Citron M, Suzuki N, Bird TD, Hardy J, Hutton M, Kukull W, Larson E, Levy-Lahad E, Viitanen M, Peskind E, Poorkaj P, Schellenberg G, Tanzi R, Wasco W, Lannfelt L, Selkoe D, and Younkin S. Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased *in vivo* by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. *Nat Med* 2: 864–870, 1996.
 135. Schmidt M, Sachse C, Richter W, Xu C, Fandrich M, and Grigorieff N. Comparison of Alzheimer Abeta(1–40) and Abeta(1–42) amyloid fibrils reveals similar protofilament structures. *Proc Natl Acad Sci U S A* 106: 19813–19818, 2009.
 136. Schoneich C. Methionine oxidation by reactive oxygen species: reaction mechanisms and relevance to Alzheimer's disease. *Biochim Biophys Acta* 1703: 111–119, 2005.
 137. Schoneich C, Pogocki D, Hug GL, and Bobrowski K. Free radical reactions of methionine in peptides: mechanisms relevant to beta-amyloid oxidation and Alzheimer's disease. *J Am Chem Soc* 125: 13700–13713, 2003.
 138. Selkoe DJ. Alzheimer's disease: genes, proteins, and therapy. *Physiol Rev* 81: 741–766, 2001.
 139. Shan X, and Lin CLG. Quantification of oxidized RNAs in Alzheimer's disease. *Neurobiol Aging* 27: 657–662, 2006.
 140. Shan X, Tashiro H, and Lin CLG. The identification and characterization of oxidized RNAs in Alzheimer's disease. *J Neurosci* 23: 4913–4921, 2003.
 141. Small BJ, Fratiglioni L, and Backman L. Canaries in a coal mine: cognitive markers of preclinical Alzheimer disease. *Arch Gen Psychiatry* 58: 859–860, 2001.
 142. Smith DG, Cappai R, and Barnham KJ. The redox chemistry of the Alzheimer's disease amyloid beta peptide. *Biochim Biophys Acta* 1768: 1976–1990, 2007.
 143. Smith MA, Harris PLR, Sayre LM, Beckman JS, and Perry G. Widespread peroxynitrite-mediated damage in Alzheimer's disease. *J Neurosci* 17: 2653–2657, 1997.
 144. Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, Iwatsubo T, Jack CR, Jr., Kaye J, Montine TJ, Park DC, Reiman EM, Rowe CC, Siemers E, Stern Y, Yaffe K, Carrillo MC, Thies B, Morrison-Bogorad M, Wagster MV, and Phelps CH. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement* 7: 280–292, 2011.
 145. Stadtman ER. Cyclic oxidation and reduction of methionine residues of proteins in antioxidant defense and cellular regulation. *Arch Biochem Biophys* 423: 2–5, 2004.
 146. Stadtman ER, Moskovitz J, and Levine RL. Oxidation of methionine residues of proteins: biological consequences. *Antioxid Redox Signal* 5: 577–582, 2003.
 147. Subramanian A, and Miller DM. Structural analysis of alpha-enolase. Mapping the functional domains involved in down-regulation of the c-myc protooncogene. *J Biol Chem* 275: 5958–5965, 2000.
 148. Sultana R, Boyd-Kimball D, Poon HF, Cai J, Pierce WM, Klein JB, Markesbery WR, Zhou XZ, Lu KP, and Butterfield DA. Oxidative modification and down-regulation of Pin1 in Alzheimer's disease hippocampus: a redox proteomics analysis. *Neurobiol Aging* 27: 918–925, 2006.
 149. Sultana R, Boyd-Kimball D, Poon HF, Cai J, Pierce WM, Klein JB, Merchant M, Markesbery WR, and Butterfield DA. Redox proteomics identification of oxidized proteins in Alzheimer's disease hippocampus and cerebellum: an approach to understand pathological and biochemical alterations in AD. *Neurobiol Aging* 27: 1564–1576, 2006.
 150. Sultana R, and Butterfield DA. Role of oxidative stress in the progression of Alzheimer's disease. *J Alzheimers Dis* 19: 341–353, 2010.
 151. Sultana R, Mecocci P, Mangialasche F, Cecchetti R, Baglioni M, and Butterfield DA. Increased protein and lipid oxidative damage in mitochondria isolated from lymphocytes from patients with Alzheimer's disease: insights into the role of oxidative stress in Alzheimer's disease and initial investigations into a potential biomarker for this dementing disorder. *J Alzheimers Dis* 24: 77–84, 2011.
 152. Sultana R, Perluigi M, and Butterfield DA. Redox proteomics identification of oxidatively modified proteins in Alzheimer's disease brain and *in vivo* and *in vitro* models of AD centered around Abeta(1–42). *J Chromatogr B Analyt Technol Biomed Life Sci* 833: 3–11, 2006.
 153. Sultana R, Piroddi M, Galli F, and Butterfield D. Protein levels and activity of some antioxidant enzymes in hippocampus of subjects with amnesic mild cognitive impairment. *Neurochem Res* 33: 2540–2546, 2008.
 154. This reference has been deleted.
 155. Sultana R, Poon HF, Cai J, Pierce WM, Merchant M, Klein JB, Markesbery WR, and Butterfield DA. Identification of nitrated proteins in Alzheimer's disease brain using a redox proteomics approach. *Neurobiol Dis* 22: 76–87, 2006.
 156. Sultana R, Reed T, Perluigi M, Coccia R, Pierce WM, and Butterfield DA. Proteomic identification of nitrated brain proteins in amnesic mild cognitive impairment: a regional study. *J Cell Mol Med* 11: 839–851, 2007.
 157. Sultana R, Robinson RA, Bader Lange M, Fiorini A, Galvan V, Fombonne J, Baker A, Gorostiza O, Zhang J, Cai J, Pierce WM, Bredesen DE, and Butterfield DA. Do proteomics analyses provide insights into reduced oxidative stress in the brain of an Alzheimer disease transgenic mouse model with an M631L amyloid precursor protein substitution and thereby the importance of amyloid-beta-resident methionine 35 in Alzheimer disease pathogenesis? *Antioxid Redox Signal* 17: 1507–1514, 2012.
 158. Takei N, Kondo J, Nagaike K, Ohsawa K, Kato K, and Kohsaka S. Neuronal survival factor from bovine brain is identical to neuron-specific enolase. *J Neurochem* 57: 1178–1184, 1991.

159. Tremblay C, Pilote M, Phivilay A, Emond V, Bennett DA, and Calon F. Biochemical characterization of A β and tau pathologies in mild cognitive impairment and Alzheimer's disease. *J Alzheimers Dis* 12: 377–390, 2007.
160. Varadarajan S, Kanski J, Aksenova M, Lauderback C, and Butterfield DA. Different mechanisms of oxidative stress and neurotoxicity for Alzheimer's A β (1–42) and A β (25–35). *J Am Chem Soc* 123: 5625–5631, 2001.
161. Varadarajan S, Yatin S, Aksenova M, and Butterfield DA. Review: Alzheimer's amyloid β -peptide-associated free radical oxidative stress and neurotoxicity. *J Struct Biol* 130: 184–208, 2000.
162. Varadarajan S, Yatin S, Kanski J, Jahanshahi F, and Butterfield DA. Methionine residue 35 is important in amyloid β -peptide-associated free radical oxidative stress. *Brain Res Bull* 50: 133–141, 1999.
163. Vassar R, Bennett BD, Babu-Khan S, Kahn S, Mendiaz EA, Denis P, Teplow DB, Ross S, Amarante P, Loeloff R, Luo Y, Fisher S, Fuller J, Edenson S, Lile J, Jarosinski MA, Biere AL, Curran E, Burgess T, Louis JC, Collins F, Treanor J, Rogers G, and Citron M. Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. *Science* 286: 735–741, 1999.
164. Vigo-Pelfrey C, Lee D, Keim P, Lieberburg I, and Schenk DB. Characterization of beta-amyloid peptide from human cerebrospinal fluid. *J Neurochem* 61: 1965–1968, 1993.
165. Viola KL, Velasco PT, and Klein WL. Why Alzheimer's is a disease of memory: the attack on synapses by A β oligomers (ADDLs). *J Nutr Health Aging* 12: 515–575, 2008.
166. Vlassenko AG, Benzinger TL, and Morris JC. PET amyloid- β imaging in preclinical Alzheimer's disease. *Biochim Biophys Acta* 1822: 370–379, 2012.
167. Waldemar G, Phung KT, Burns A, Georges J, Hansen FR, Iliffe S, Marking C, Rikkert MO, Selmes J, Stoppe G, and Sartorius N. Access to diagnostic evaluation and treatment for dementia in Europe. *Int J Geriatr Psychiatry* 22: 47–54, 2007.
168. Walsh DM, Klyubin I, Fadeeva JV, Rowan MJ, and Selkoe DJ. Amyloid- β oligomers: their production, toxicity and therapeutic inhibition. *Biochem Soc Trans* 30: 552–557, 2002.
169. Walsh DM, and Teplow DB. Alzheimer's disease and the amyloid β -protein. *Prog Mol Biol Transl Sci* 107: 101–124, 2012.
170. Wan L, Nie G, Zhang J, Luo Y, Zhang P, Zhang Z, and Zhao B. beta-Amyloid peptide increases levels of iron content and oxidative stress in human cell and *Caenorhabditis elegans* models of Alzheimer disease. *Free Radic Biol Med* 50: 122–129, 2010.
171. Wang J, Markesbery WR, and Lovell MA. Increased oxidative damage in nuclear and mitochondrial DNA in mild cognitive impairment. *J Neurochem* 96: 825–832, 2006.
172. White AR, Du T, Laughton KM, Volitakis I, Sharples RA, Xilinas ME, Hoke DE, Holsinger RM, Evin G, Cherny RA, Hill AF, Barnham KJ, Li QX, Bush AI, and Masters CL. Degradation of the Alzheimer disease amyloid β -peptide by metal-dependent up-regulation of metalloprotease activity. *J Biol Chem* 281: 17670–17680, 2006.
173. Yao Y, Clark CM, Trojanowski JQ, Lee VM, and Pratico D. Elevation of 12/15 lipoxygenase products in AD and mild cognitive impairment. *Ann Neurol* 58: 623–626, 2005.
174. Yatin SM, Varadarajan S, and Butterfield DA. Vitamin E prevents Alzheimer's amyloid β -peptide (1–42)-induced neuronal protein oxidation and reactive oxygen species production. *J Alzheimers Dis* 2: 123–131, 2000.
175. Yatin SM, Varadarajan S, Link CD, and Butterfield DA. *In vitro* and *in vivo* oxidative stress associated with Alzheimer's amyloid β -peptide (1–42). *Neurobiol Aging* 20: 325–330; discussion 339–342, 1999.
176. Zheng H, and Koo EH. The amyloid precursor protein: beyond amyloid. *Mol Neurodegener* 1: 5, 2006.

Address correspondence to:
 Prof. D. Allan Butterfield
 Department of Chemistry
 Center of Membrane Sciences
 Sanders-Brown Center on Aging
 University of Kentucky
 249 Chem-Phys Bldg.
 Lexington, KY 40506-0055
 E-mail: dabcsn@uky.edu

Date of first submission to ARS Central, October 22, 2012; date of final revised submission, December 05, 2012; date of acceptance, December 17, 2012.

Abbreviations Used

A β	= amyloid- β peptide
α -sAPP	= α -secretase-cleaved soluble APP
β -sAPP	= β -secretase-cleaved soluble APP
AD	= Alzheimer disease
AICD	= APP intracellular domain
aMCI	= amnesic MCI
APP	= amyloid precursor protein
CQ	= 5-chloro-7-iodoquinolin-8-ol
CSF	= cerebral spinal fluid
CTF	= C-terminal fragment
DS	= Down syndrome
EAD	= early AD
F ₂ isoP	= isoprostane
FAD	= familial AD
H ₂ O ₂	= hydrogen peroxide
HNE	= 4-hydroxy-nonenal
IPL	= inferior parietal lobule
LAD	= late-stage AD
MCI	= mild cognitive impairment
MetSOx	= methionine sulfoxide
MMSE	= Mini Mental State Evaluation
MRI	= magnetic resonance imaging
MSR	= methionine sulfoxide reductase
NFTs	= neurofibrillary tangles
NO \cdot	= nitric oxide
NOS	= nitric oxide synthase
O ₂ \cdot^-	= superoxide radical anion
\cdot OH	= hydroxyl radical
ONOO $^-$	= peroxynitrite anion
PCAD	= preclinical AD
PET	= positron emission tomography
Pin1	= peptidyl-prolyl <i>cis-trans</i> isomerase 1
PS	= presenilin
RNS	= reactive nitrogen species
ROS	= reactive oxygen species
SOD	= superoxide dismutase
SP	= senile plaques

This article has been cited by:

1. Magdalena Sastre, Loukia Katsouri, Amy Birch, Alexander Renziehausen, David T. Dexter, Robert R. Crichton, Roberta J. Ward. Neuroinflammation in Alzheimer's, Parkinson's and Huntington's Diseases 111-150. [[CrossRef](#)]
2. Sreeram Krishnan, P. Rani. 2014. Evaluation of Selenium, Redox Status and Their Association with Plasma Amyloid/Tau in Alzheimer's Disease. *Biological Trace Element Research* **158**:2, 158-165. [[CrossRef](#)]
3. Federica Belluti, Angela De Simone, Andrea Tarozzi, Manuela Bartolini, Alice Djemil, Alessandra Bisi, Silvia Gobbi, Serena Montanari, Andrea Cavalli, Vincenza Andrisano, Giovanni Bottegoni, Angela Rampa. 2014. Fluorinated benzophenone derivatives: Balanced multipotent agents for Alzheimer's disease. *European Journal of Medicinal Chemistry* **78**, 157-166. [[CrossRef](#)]
4. Michela Rosini, Elena Simoni, Andrea Milelli, Anna Minarini, Carlo Melchiorre. 2014. Oxidative Stress in Alzheimer's Disease: Are We Connecting the Dots?. *Journal of Medicinal Chemistry* **57**:7, 2821-2831. [[CrossRef](#)]
5. Marzia Perluigi, Gilda Pupo, Antonella Tramutola, Chiara Cini, Raffaella Coccia, Eugenio Barone, Elizabeth Head, D. Allan Butterfield, Fabio Di Domenico. 2014. Neuropathological role of PI3K/Akt/mTOR axis in Down syndrome brain. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* . [[CrossRef](#)]
6. Fabio Di Domenico, Gilda Pupo, Antonella Tramutola, Alessandra Giorgi, Maria Eugenia Schininà, Raffaella Coccia, Elizabeth Head, D. Allan Butterfield, Marzia Perluigi. 2014. Redox proteomics analysis of HNE-modified proteins in Down syndrome brain: clues for understanding the development of Alzheimer disease. *Free Radical Biology and Medicine* . [[CrossRef](#)]
7. Eugenio Barone, Fabio Di Domenico, Cesare Mancuso, D. Allan Butterfield. 2014. The Janus face of the heme oxygenase/biliverdin reductase system in Alzheimer disease: It's time for reconciliation. *Neurobiology of Disease* **62**, 144-159. [[CrossRef](#)]
8. Su-Yi Li, Xiao-Bing Wang, Ling-Yi Kong. 2014. Design, synthesis and biological evaluation of imine resveratrol derivatives as multi-targeted agents against Alzheimer's disease. *European Journal of Medicinal Chemistry* **71**, 36-45. [[CrossRef](#)]
9. Sarita S. Hardas, Rukhsana Sultana, Govind Warriar, Mo Dan, Peng Wu, Eric A. Grulke, Michael T. Tseng, Jason M. Unrine, Uschi M. Graham, Robert A. Yokel, D. Allan Butterfield. 2013. Rat hippocampal responses up to 90 days after a single nanoceria dose extends a hierarchical oxidative stress model for nanoparticle toxicity. *Nanotoxicology* 1-12. [[CrossRef](#)]
10. Agnese Picco, M. Cristina Polidori, Michela Ferrara, Roberta Cecchetti, Dario Arnaldi, Mauro Baglioni, Silvia Morbelli, Patrizia Bastiani, Irene Bossert, Giuliana Fiorucci, Andrea Brugnolo, Massimo Eugenio Dottorini, Flavio Nobili, Patrizia Mecocci. 2013. Plasma antioxidants and brain glucose metabolism in elderly subjects with cognitive complaints. *European Journal of Nuclear Medicine and Molecular Imaging* . [[CrossRef](#)]
11. S Dalleau, M Baradat, F Guéraud, L Huc. 2013. Cell death and diseases related to oxidative stress: 4-hydroxynonenal (HNE) in the balance. *Cell Death and Differentiation* **20**:12, 1615-1630. [[CrossRef](#)]
12. Aaron M. Swomley, Sarah Förster, Jierel T. Keeney, Judy Triplett, Zhaoshu Zhang, Rukhsana Sultana, D. Allan Butterfield. 2013. Abeta, oxidative stress in Alzheimer disease: Evidence based on proteomics studies. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* . [[CrossRef](#)]
13. Oana Cioanca, Lucian Hritcu, Marius Mihasan, Monica Hancianu. 2013. Cognitive-enhancing and antioxidant activities of inhaled coriander volatile oil in amyloid β (1-42) rat model of Alzheimer's disease. *Physiology & Behavior* . [[CrossRef](#)]
14. Said AbdAlla, Andreas Langer, Xuebin Fu, Ursula Qwitterer. 2013. ACE Inhibition with Captopril Retards the Development of Signs of Neurodegeneration in an Animal Model of Alzheimer's Disease. *International Journal of Molecular Sciences* **14**:8, 16917-16942. [[CrossRef](#)]
15. Massimo Stefani, Stefania Rigacci. 2013. Protein Folding and Aggregation into Amyloid: The Interference by Natural Phenolic Compounds. *International Journal of Molecular Sciences* **14**:6, 12411-12457. [[CrossRef](#)]