REVIEW

Apolipoprotein A-I: Insights from redox proteomics for its role in neurodegeneration

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Proteomics has a wide range of applications, including determination of differences in the proteome in terms of expression and post-translational protein modifications. Redox proteomics allows the identification of specific targets of protein oxidation in a biological sample. Using proteomic techniques, apolipoprotein A-I (ApoA-I) has been found at decreased levels in subjects with a variety of neurodegenerative disorders including in the serum and cerebrospinal fluid (CSF) of Alzheimer disease (AD), Parkinson disease (PD), and Down syndrome (DS) with gout subjects. ApoA-I plays roles in cholesterol transport and regulation of inflammation. Redox proteomics further showed ApoA-I to be highly oxidatively modified and particularly susceptible to modification by 4-hydroxy-2-trans-nonenal (HNE), a lipid peroxidation product. In the current review, we discuss the consequences of oxidation of ApoA-I in terms of neurodegeneration. ROS-associated chemotherapy related ApoA-I oxidation leads to elevation of peripheral levels of tumor necrosis factor-α (TNF-α) that can cross the blood-brain barrier (BBB) causing a signaling cascade that can contribute to neuronal death, likely a contributor to what patients refer to as "chemobrain." Current evidence suggests ApoA-I to be a promising diagnostic marker as well as a potential target for therapeutic strategies in these neurodegenerative disorders.

Keywords:
Alzheimer disease / Apolipoprotein A-I / Neurodegeneration / Tumor necrosis factor-α

1 Introduction

Proteomics opens new possibilities in the search for potential biomarkers as the "proteome" describes the entity of all proteins expressed at a certain time point. Since proteins are involved in many cellular functions, changes in the proteome occur in diseases, and the comparison of "healthy proteome" with "disease proteome" might lead to the discovery of diagnostic markers and also possibly point to new targets for research and therapy [1–3]. Biomarkers showing changes early in disease progression could help to start treatment even before first clinical changes occur and therefore might help delay or even prevent disease progression. In addition, the availability of biomarkers in readily accessible samples like

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Abbreviations: **Aβ**, amyloid beta-peptide; **ABCA1**, ATP-binding cassette protein A1; **AD**, Alzheimer disease; **AIPC**, 2,2-azobis(2-(2-imidazolin-2-yl)propane)-dihydrochloride; **APP**, amyloid precursor protein; **BBB**, blood-brain barrier; **CSF**, cerebrospinal fluid; **DD**, death domain; **DOX**, doxorubicin; **DS**, Down syndrome; **EAAT2**, excitatory amino acid transporter 2; **FTP**, fuel transport pathway; **HDL**, high-density lipoprotein; **HNE**, 4-hydroxy-2-trans-nonenal; **IκB**, inhibitor of κB; **IKKβ**, inhibitor of κB kinase β; **JAK2**, Janus kinase 2; **JNK**, c-jun NH_2-terminal kinase; **LCAT**, Lecithin-cholesterol acyltransferase; **LDL**, low-density lipoproteins; **MAPK**, mitogen-activated protein kinase; **MAPKKK**, mitogen-activated protein kinase kinase kinase; **NF-κB**, Nuclear factor kappa-B; **PD**, Parkinson disease; **PKA**, protein kinase A; **PKC**, protein kinase C; **RCT**, reverse cholesterol transport; **RIP**, receptor-interacting protein; **RNS**, reactive nitrogen species; **TNF-α**, tumor necrosis factor-α; **TRADD**, TNF-receptor-associated death-domain; **TRAF2**, TNF receptor-associated factor 2; **VLDL**, very-low density lipoproteins

Colour Online: See the article online to view Figs. 1 and 2 in colour.

blood or cerebrospinal fluid (CSF) also could lead to their use in monitoring disease progression or drug/treatment efficacy. A few limitations still apply to most proteomics studies, since differences in subject selection, sample preparation, and analysis techniques used could lead to conflicting results. In addition, often proteomics-based biomarker studies use only a small number of samples, which lead to preliminary results that need to be verified using different methods and confirmed with larger sample sets. Still, Taurines et al. boldly suggested that proteomics could allow personalized therapies based on each individual's proteome to be developed [4].

Proteomics allows examination and comparison of all detectable proteins in a sample of interest to determine relative amounts of proteins present. Proteins regularly undergo a variety of PTMs for activation, deactivation, and in order to perform their biological functions. Protein dysfunction can occur when reactive oxygen and reactive nitrogen species (ROS, RNS) cause aberrant oxidative and nitrosative changes to proteins altering their structure and function [5] termed as oxidative/nitrosative stress. Commonly used indices of oxidative damage to protein include carbonylation, tyrosine nitration, and binding of reactive alkenals [6]. Redox proteomics combines expression proteomics techniques with those of oxidative stress measures to allow determination of specific oxidative changes to a protein of interest and provide insight into disease mechanisms [6–10].

Using expression and redox proteomics, a number of proteins has been found to be altered in neurodegenerative disorders. In this review, we focus our discussion on apolipoprotein A-I (ApoA-I), as this protein has been found to be altered in expression or specific oxidation in the neurodegenerative disorders, Alzheimer disease (AD), Parkinson disease (PD), Down syndrome (DS), and in cancer patients post chemotherapy (Table 1) with ROS producing chemotherapeutic agents. ApoA-I may play a role in other neurodegenerative disorders as well as Hansson et al. showed an increase in proapolipoprotein A-I in frontotemporal dementia [11].

ApoA-I is a multifunctional apolipoprotein that plays a variety of roles in human physiology among which are cholesterol transport and regulation of inflammation. Through the ATP-binding cassette protein A1 (ABCA1), ApoA-I serves as the primary lipid acceptor protein for lipids such as cholesterol and phosphatidylcholine as they are effluxed from the lipid bilayer [12] as part of the high-density lipoprotein (HDL) complex taking excess cholesterol from tissues [13]. HDL is associated with a reduced risk of atherosclerosis through intervening in either lipoprotein and cholesterol metabolism or modulation of inflammatory processes [14].

In addition to its role in reverse cholesterol transport (RCT), ApoA-I has been reported to have antioxidant and anti-inflammatory properties and other studies have reported decreased serum ApoA-I with inflammation [15] and increased ApoA-I levels in CSF after brain injury [16]. As described further below, redox proteomics has identified ApoA-I as oxidatively modified in the amniotic fluid of mothers carrying a DS fetus and in plasma of persons subjected to ROS/RNS as a result of chemotherapy.

2 Cholesterol and its transport

Cholesterol is predominantly an amphipathic molecule which is dominated by its hydrophobic sterol ring that allows for the insertion of cholesterol into the bilayer, yet is able to maintain its proper orientation due to the hydrophilic 3β-hydroxyl group present in the molecule. Cholesterol has been found to play a major stabilizing role, reducing membrane lipid bilayer fluidity and increasing turgidity. Cholesterol is also well known to play a role in bile acid production and steroid hormone synthesis while much more recently cholesterol has been implicated in the formation of micro-signaling domains called lipid or membrane rafts [17–22].

Because cholesterol is centrally stored in the liver, there must be a mechanism in place for the transport of cholesterol to and from the cells that require it. Due to the fact that the majority of the surface area of cholesterol is hydrophobic in nature, transport of cholesterol through the aqueous blood supply is facilitated by a group of proteins called lipoproteins.

Lipoprotein subclasses are classified based on their hydrated density into five main groups (chylomicrons, very-low density lipoproteins (VLDL), intermediate density lipoproteins, low-density lipoproteins (LDL) and high-density lipoproteins (HDL)) that may also differ in the relative size as well as the overall lipid and apolipoprotein content [23]. Predominantly important to this discussion is the ability of apolipoproteins to direct the action of lipoproteins through direct interaction with other cell types by way of membrane lipoprotein receptors.

There are three main ways in which cholesterol and triglycerides are mobilized throughout the body: the fuel transport pathway (FTP), the over-flow pathway, and RCT. In order to move triglycerides from the liver for use in peripheral tissues, the FTP employs both chylomicrons as well as VLDL using an array of apolipoproteins such as ApoA-I, A-II, A-IV and A-V, apoB48 and B100, apoC-I, C-II, and C-III, and apoE [22–24]. Once in the periphery, lipoprotein lipase acts on chylomicrons to release fatty acids to cells while returning apolipoproteins to nearby HDL particles. VLDL particles remain partially intact after interaction with lipoprotein lipase, and the resulting VLDL remnants are returned by LDL receptors that bind apolipoprotein E (apoE). As a result of excessive remnant generation by the FTP, an increase in LDL, which has been referred to as an extracellular cholesterol depot, can accumulate in the blood supply, a process that has been linked to atherosclerosis. To combat this increase in LDL, the over-flow pathway is in place to metabolize LDL using apoB100, the predominant apolipoprotein in LDL, that interacts with the apoB/E LDL receptor and causes the internalization of the LDL particles [23].

The RCT system comprises the metabolism of HDL and the subsequent transport of cholesterol from the periphery

Table 1. Proteomic studies showing changes in ApoA-I expression or oxidation in neurodegenerative disorders

Disease	Sample	Sample size	Methods	Effects on ApoA-I	Reference
AD	CSF	$n = 86$ (pooled samples)	2DE	ApoA-I ↓	Castano et al. 2006
AD	CSF	$n = 27$	2DE (MALDI-TOF-MS + ESI-MS/MS)	Proapolipoprotein ↓	Davidsson et al. 2002[a)]
AD	CSF	$n = 27$	2DE	-	Davidsson et al. 2002[a)]
AD	CSF	$n = 19$	2DE oxyblot (protein carbonyls) + LC-MS/MS	Proapolipoprotein A1 oxidation in AD and controls	Korolainen et al. 2007
AD	CSF	$n = 14$	2DE MALDI TOF	ApoA-I ↓	Puchades et al. 2003[a)]
AD	CSF	$n = 6$	(1DE + LC-MS/MS) 2DE (+ MS/MS)	-	Yin et al. 2009[a)]
AD	Serum	$n = 20$	2DE MALDI TOF MS	ApoA-I ↓	Liu et al. 2005
DOX-treated patients	Plasma	$n = 12$ paired	2DE (MS/MS)	ApoA-I oxidation ↑	Aluise et al. 2011
DS	Maternal plasma from DS pregnancies	$n = 28$	2D DIGE MALDI- TOF MS + ESI QTOF MS/MS	-	Heywood et al. 2011
DS	Maternal serum from DS pregnancies	$n = 36$	iTRAQ + strong cation exchange (SCX) LC-MS/MS	ApoA-I ↓	Kang et al. 2012[a)]
DS	Maternal plasma from DS pregnancies	$n = 24$	2DE + MALDI TOF MS	-	Kolialexi et al. 2008[a)]
DS	Maternal plasma from DS pregnancies	$n = 12$ (pooled samples)	iTRAQ + strong cation exchange (SCX) LC-MS/MS	ApoA-I ↓	Kolla et al. 2010[a)]
DS	Maternal serum from DS pregnancies	$n = 40$ (pooled samples for 2D-CF)	2D DIGE 2D-LC–chromatofocusing (2D-CF) Q-TOF; MS + MS/MS	ApoA-I ↑	Nagalla et al. 2006[a)]
DS	Amniotic fluid from DS pregnancies	$n = 10$	1D SDS-PAGE LC-ESI-MS/MS	ApoA-I preproprotein ↑	Park et al. 2010
DS	Amniotic fluid from DS pregnancies	$n = 20$	2DE oxyblot (PC)	ApoA-I oxidation ↑	Perluigi et al. 2011
DS	Amniotic fluid from DS pregnancies	$n = 18$	2DE + MALDI-TOF MS + nano-ESI MS/MS	-	Tsangaris et al. 2006
DS	Amniotic fluid from DS pregnancies	$n = 26$	2D LC + MALDI TOF MS	ApoA-I ↓	Wang et al. 2009
DS with gout (G)	Serum	$n = 8$	2DE (+MALDI TOF MS)	ApoA-I ↓	Chen et al. 2006
FTD	CSF	$n = 10$	2DE	ProapolipoproteinA1 ↑	Hansson et al. 2004 [11]
PD	CSF	$n = 6$	2D-DIGE (MALDI-TOF + MALDI-TOF/TOF)	ApoA-I (isoform) ↓ ApoA-I (isoform) ↑ proapolipoprotein ↑	Wang et al. 2010
PD	CSF	$n = 6$	(1DE + LC-MS/MS) 2DE (+ MS/MS)	ApoA-I ↓	Yin et al. 2009[a)]
PD	Serum	$n = 22$	iTRAQ (2DE LC-MS/MS)	ApoA-I ↓	Zhang et al. 2012[a)]

a) These studies also showed effects on other apolipoproteins.

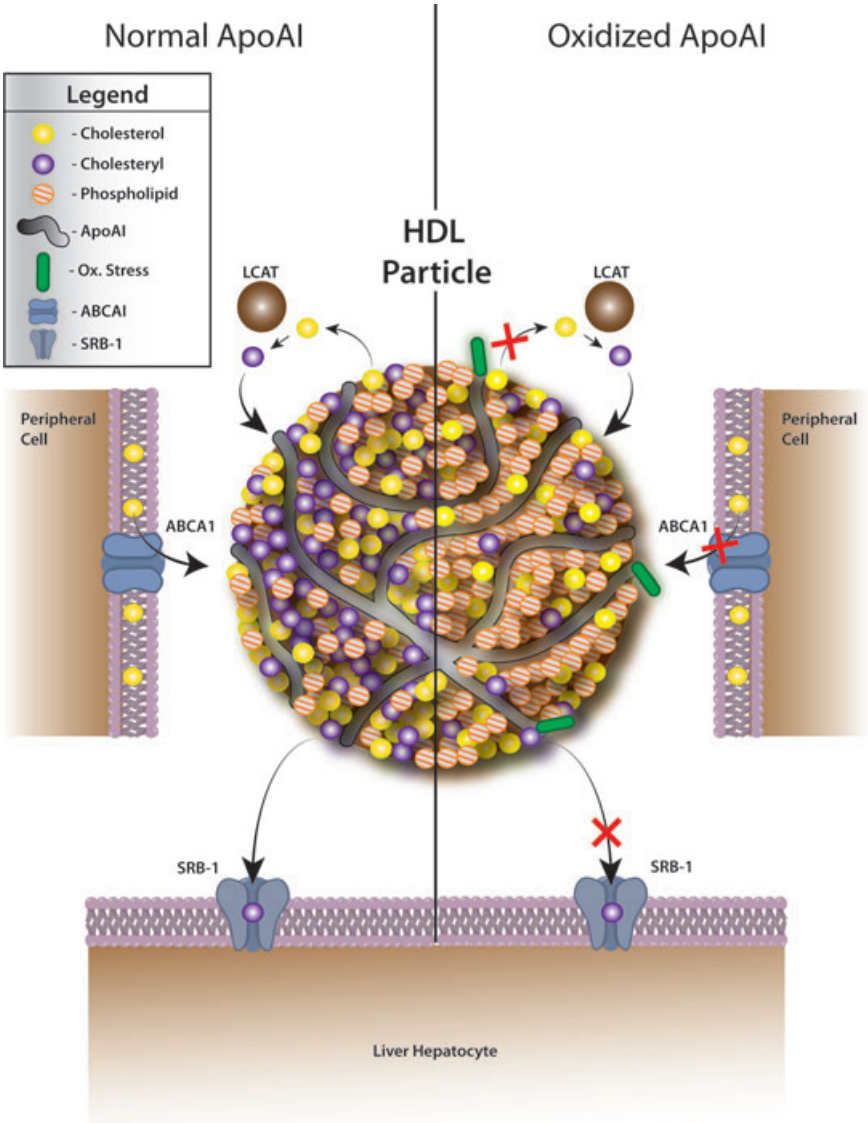

Figure 1. Oxidatively modified ApoA-I. The left-hand side of the figure depicts an HDL particle with bound ApoA-I that has not undergone oxidative damage, and as shown, is able to function normally interacting with LCAT, peripheral ABCA1 and scavenger receptor BI (SRB-1). The right-hand side of the figure displays an HDL particle that has undergone extensive oxidative modification on ApoA-I, a process that has been shown to negatively affect the ability of the HDL particle to interact peripherally with ABCA-1, LCAT, and SRB-1. This loss of function of ApoA-I has many consequences such as impaired ABCA1 signaling and cholesterol deposition to HDL, impaired cholesterol to cholesteryl conversion by LCAT, and the inability to dock with SRB-1. See text.

back to the liver, and because of this role, HDL levels have been found to have an inverse relationship with cardiovascular disease [25–27]. The primary apolipoprotein component of HDL is ApoA-I, responsible for the free cholesterol loading of HDL from cells by binding to ABCA1 as well as the secretion of apoE, which stimulates lecithin-cholesterol acyltransferase (LCAT) activity [22, 28] (Fig. 1). Once loaded, the cholesterol is esterified by LCAT, producing cholesteryl esters and transforming HDL to HDL3. HDL3 can then function to interchange the cholesteryl esters and apolipoproteins A, C, and E for triglycerides from lipoproteins, in which triglycerides are abundant, forming HDL2 that can subsequently deposit the cholesteryl esters into the scavenger receptor BI at the liver [23, 24]. Interestingly, in bovine aortic endothelial cells oxidative modification of HDL, likely on ApoA-I, by myeloperoxidase was sufficient to decrease HDL interaction with SR-B1 as well as induce a pro-inflammatory state via tumor necrosis factor-α (TNF-α) induced expression of the vascular cell adhesion molecule 1 protein as well as nuclear factor kappa-B (NFκB) activation [29].

3 HNE modification of ApoA-I

ApoA-I is particularly susceptible to modification by lipid peroxidation products such as the reactive aldehydes, 4-hydroxy-2-*trans*-nonenal (HNE) and acrolein. Electrophilic sites on these reactive aldehydes may be attacked by nucleophilic centers like those in the amino acid side chains of histidine (H), lysine (K), or cysteine (C) to form covalent adducts that have been shown to alter both the structure and function of proteins [30–33]. Liebler et al. [34] found ApoA-I to be highly modified by free radical oxidation using a novel biotin-modified linoleoyl glyceryl phosphatidyl choline probe

(PLPBSO). Free radical oxidation of human plasma containing the probe produced covalent Michael-adducts to plasma proteins. These proteins were identified and phospholipid-binding sites mapped using LC-MS/MS on streptavidin-purified proteins [31, 34].

To determine potential binding sites, human plasma was oxidized using 2,2-azobis(2-(2-imidazolin-2-yl)propane)-dihydrochloride (AIPH), a compound known to produce reactive aldehydes upon oxidation of the PLPBSO probe. In independent experiments, HNE was added in varying concentrations directly to isolated HDL. The formation of HNE adducts on ApoA-I was found at an increasing number of sites and with increasing frequency as concentrations of either AIPH in plasma or HNE in isolated HDL were increased [34]. These sites include H162, H193 at HNE concentrations of 1uM; H155 at 10uM; H135, H199, K208, K94, K96; and K107, K238, K12, K23 at 1000 uM. At low concentrations of AIPH or HNE, respectively, HNE modifications on ApoA-I were found that included H162, located in a key functional region of ApoA-I [34]. Oxidative modification of H162 on ApoA-I may disrupt the maturation of the HDL complex by structural changes in the ApoA-I domain responsible for activation of LCAT which, as discussed, converts free cholesterol to the more hydrophobic cholesteryl esters for packaging in the formation of HDL particles (Fig. 1).

4 ApoA-I and ABCA1 interaction and subsequent signaling

The binding of ApoA-I to ABCA1 has been shown to have a major role in RCT and the exporting of free cholesterol from cells for return to the liver, a process believed to be of crucial importance for anti-atherosclerosis [26, 35]. More recently, this interaction has been found to affect many areas of cellular processes, including not only direct cellular cholesterol and phospholipid efflux, but also various cell signaling pathways that may turn out to have more far reaching effects than were first thought.

Aside from the activation of Rho GTPases such as Cdc42, Rho, and Rac, small G-proteins that are responsible for the reorganization of cytoskeletal architecture, as well as the direct binding of calmodulin, the binding of ApoA-I and ABCA1 has been shown to cause the activation of Janus kinase 2 (JAK2). This activation has been implicated in the majority of the lipid transport conducted by ABCA1, a process that is significantly impaired when in the presence of JAK2-specific inhibitors [36–41]. Interestingly, STAT3 also has been shown to be active post ApoA-I/ABCA1 interaction to decrease the secretion of inflammatory cytokines in macrophages, a process linked to anti-inflammation [42–44].

In addition to JAK2/STAT3 activation, the ApoA-I/ABCA1 interaction has been shown to activate both protein kinase A (PKA) and protein kinase C (PKC). PKA is activated by increased cyclic-adenosine monophosphate by the activation of adenylyl cyclase through $G\alpha_s$ coupling with ABCA1. The result of PKA activation is the phosphorylation of ABCA1 and a subsequent increase in ApoA-I lipidation, a process blocked by specific PKA inhibition [26, 45–47]. PKC on the other hand is activated as a result of an initial depletion of free cholesterol, phostphatidylcholine, and sphingomyelin from the cell membrane by ApoA-I/ABCA1 interaction. This later interaction stimulates phosphatidylcholine-specific phospholipase C activity resulting in an increase of both phosphatidylcholine as well as diacylglycerol, a major activator of PKC. The activation of PKC not only leads to the phosphorylation of ABCA1, a process believed to increase the lifetime of ABCA1 via protection from degradation, but also increases cholesterol. The interaction of PKC-ζ with specificity protein-1 controls the expression level of ABCA1 [26, 48–50].

From the above, it is apparent that ApoA-I–ABCA1 interaction is a critical event that, if impaired through damage such as oxidative stress, could have much larger ramifications than simply cholesterol and fatty acid efflux impairment, i.e. may affect a large array of cellular processes directly or tangentially related to the activation or inactivation of this important intracellular signaling processes.

In the context of atherosclerosis, increased oxidative modification of ApoA-I mediated by myeloperoxidase led to decreased ABCA1-dependent cholesterol efflux from macrophages [51] as well as impaired activation of LCAT [52]. A possible, albeit weak, link has been established between a polymorphism of the ApoA-I gene negatively affecting HDL function and cognitive decline [53]. Overall, current evidence suggests ApoA-I to be a promising diagnostic marker as well as a potential target for therapeutic strategies.

5 TNF-α signaling, inflammation, and apoptosis

TNF-α is a multifunctional cytokine with involvement in pathways including pro-apoptotic, anti-apoptotic, inflammatory response, and immune function regulation and has been implicated in a large variety of diseases such as rheumatoid arthritis, Crohn's disease, and AD. Within the CNS, TNF-α has both neuroprotective and neurotoxic roles facilitating both necrosis and caspase-mediated apoptosis [54–56]. TNF-α is normally found in low levels in healthy brain, but can be found at high levels in cerebral and transient ischemic attacks, and in aged human brains [57, 58].

Extracellular TNF-α binds and activates two main receptors, TNFR1 (p55) and TNFR2 (p75) that are responsible for mediating the intracellular cytokine activity. Because the two receptors lack metabolic activity, they must recruit and bind intracellular proteins in order to transduce the signal initiated by TNF-α. TNFR1 and TNFR2 are single transmembrane glycoproteins with similar extracellular structures, yet differ in their intracellular domains [59].

TNFR1, also known as the death receptor, has an intracellular death domain (DD) consisting of 80 amino acids and is located near the carboxy-terminus [59]. Silencer of death

domain is responsible for binding and silencing the DD of inactive TNFR1 and dissociates upon receptor activation facilitated by TNF binding to its extracellular domain. Dissociation of silencer of death domain from the DD of TNFR1 allows the binding of the adaptor protein, TNF-receptor-associated death-domain (TRADD), and its subsequent recruitment of additional proteins to the receptor, as demonstrated by the interaction between TNF receptor-associated factor (TRAF2) and receptor-interacting kinase (RIP). TRADD-dependent recruitment of proteins to TNFR1 allows the initiation of the following three main pathways: NFκB activation, mitogen-activated protein kinase pathway activation, and caspase-mediated apoptosis.

NFκB is a heterodimeric transcription factor responsible for a large variety of cellular responses under stress and anti-apoptotic actions. Inactive NFκB is sequestered in the cytoplasm by inhibitor of κB, which dissociates upon its phosphorylation at two serine residues by inhibitor of κB kinase β (IKKβ). Recruitment and activation of IKKβ to TNFR1 is dependent on the presence of TRAF2 and RIP, respectively [60, 61]. Once NFκB is released from the inhibitory subunit, inhibitor of κB, it may then translocate to the nucleus and induce the gene expression of its downstream anti-apoptotic proteins. Within the CNS, this anti-apoptotic NFκB pathway, activated through TNF-α, generally has neuroprotective roles [62].

Of the three main mitogen-activated protein kinase pathways, the stress related and pro-apoptotic c-jun NH_2-terminal kinase (JNK) cascade is most strongly stimulated by the TNFR1/TRADD/TRAF2 complex [63]. The recruited TRAF2 protein is believed to be responsible for activation of a mitogen-activated protein kinase kinase kinase, for example, apoptosis-stimulated kinase 1 [64]. Stimulation of a variety of mitogen-activated protein kinase kinase kinases can lead to the activation of a cascade of kinases that ultimately result in JNK activation. Once activated, JNK translocates to the nucleus where it then may phosphorylate and subsequently activate the pro-apoptotic transcription factor c-Jun.

Stimulation of caspase-mediated apoptosis by the TNF-α-activated TNFR1 receptor involves the recruitment and binding of fas-associated death domain to TRADD. The TNFR1/TRADD/fas-associated death domain complex then recruits and activates the cysteine protease caspase-8, which leads to the activation of caspase-3, the executioner of apoptosis. The mentioned pro-apoptotic caspase cascade and anti-apoptotic NFκB pathway both play negative regulatory roles of one another; caspase-8 and caspase-3 inhibits RIP and IKKβ, respectively, and the NFκB-transcribed cellular inhibitor of apoptosis protein 1 and 2 inhibits both caspase-8 as well as caspase-3.

TNFR2, the less understood of the two TNF-α receptors, lacks a death domain and currently appears to have a stronger anti-apoptotic role than TNFR1. TRAF2 was initially recognized as a signal transduction protein of TNFR2, in which it may bind directly to the receptors intracellular domain of this receptor. In contrast to TNFR1 where TRAF2 indirectly interacts with the TNFR2 receptor by binding to the adaptor protein TRADD. Through direct interaction with TRAF2, TNFR1 is able to stimulate the anti-apoptotic transcriptional factor, NFκB.

An excitotoxic effect of excessive synaptic glutamate is promoted by the collaboration of TNF-α receptors located on the surfaces of microglia and astrocytes. Activation of microglial TNF-α receptors leads to the upregulation of the glutamate generating enzyme glutaminase and subsequent extracellular release of excess glutamate through hemichannels [65]. Extracellular TNF-α also has been found to stimulate the generation of microglial TNF-α [66], increasing its extracellular levels. In astrocytes, TNFR1-associated caspase 3 and NFkB pathways work to decrease the reuptake of synaptic glutamate by respectively inactivating and reducing the expression of the influx transporter, excitatory amino acid transporter 2 (EAAT2), from the membrane of astrocytes [67–69]. EAAT2 is an important part of the glutamine shuttle, responsible for the reuptake of glutamate from the synaptic cleft and into astrocytes, where it can be converted to its inactive form of glutamine. The TNF-α-associated downregulation of EAAT2 activity and increased generation and release of glutamate from microglia puts neurons in danger of excitotoxicity caused by high extracellular levels of glutamate.

Within neurons, TNF-α-mediated activation of TNFR1 contributes more directly to neuronal cell death through activation of the caspase-mediated apoptosis cascade described earlier [70]. Neuronal TNFR1 also has influence over Ca^{2+}-mediated cell death by increasing the membranous expression of iontropic glutamate receptor AMPA, through the activation of IP_3 [71]. The increased expression of ligand gated ion channel AMPA is yet another factor into the excitotoxic effect of high synaptic glutamate levels. Further jeopardizing neuronal survival is the TNF-α-mediated decrease of surface receptor $GABA_A$, responsible for inhibitory effects that work to counteract the excitatory effect of glutamate [71].

Through activated TNF-α receptors on microglial, astrocyte, and neuronal cell surfaces, TNF-α is able to directly and indirectly contribute to neuronal cell death. These neurotoxic effects of TNF-α help highlight the cytokine's role in a variety of neurodegenerative diseases, including Alzheimer 's disease. Such processes are likely involved in chemotherapy-induced cognitive decline [72].

6 ApoA-I and cholesterol involvement in AD

Alzheimer disease, first described in 1907 by Alois Alzheimer, is a progressive neurological disorder characterized by cognitive deficits and memory decline [73]. Histopathological hallmarks of AD are loss of neurons, intracellular neurofibrillary tangles containing hyperphosphorylated tau protein [74] as well as extracellular senile plaques consisting of a core of amyloid beta-peptide (Aβ) surrounded by dystrophic neurites [75]. Aβ peptides are derived through sequential proteolytic

processing of the amyloid precursor protein (APP) [76], a protein encoded by a single gene on chromosome 21 [77, 78]. Oxidative stress and inflammatory responses are also thought to play major roles in the pathogenesis of AD, and a number of oxidized proteins as well as activated microglia have been found in brain regions affected by AD [79–81] which lead to new insights into AD as a multifactorial disease.

Less than 5% of AD cases are associated with specific mutations, with most of these occurring within the genes encoding APP or the *presenilin* proteins. However, the majority of AD cases are sporadic, although allele 4 of the apoE protein, which plays a role in cholesterol metabolism and has been shown to bind to Aβ, has been identified as a strong risk factor [82, 83]. Increasing evidence supports an important role of lipids in neurodegeneration. A number of studies have focused on the link between cholesterol and AD, for example, showing that high serum cholesterol levels are associated with the disease [84, 85]. Amyloidogenic APP processing has been shown to take place in lipid rafts, cholesterol-rich regions of the membrane [86], and the binding of cholesterol to APP affects its proteolytic cleavage. The various APP degradation products like Aβ in turn affect the interaction of cholesterol with LDL or apoE, respectively [87] leading to the conclusion that APP and/or its cleavage products may influence cholesterol homeostasis [82]. The statin drugs are primarily used to treat hypercholesterolemia, lowering blood cholesterol levels by inhibiting the HMGCoA reductase needed for cholesterol synthesis. However, extensive evidence supports the notion that statin treatment lowers risk of developing AD [88–91], though recent evidence shows atorvastatin has beneficial pleiotropic effects independent of cholesterol lowering [92–95].

While ApoA-I's role in RCT in the periphery is well known, its function in the nervous system and its implications in AD remain to be elucidated. Many studies have shown decreased levels of ApoA-I in the plasma or serum of AD patients as compared to healthy controls [96–98]. In a longitudinal study, decreased risk of dementia was associated with increasing ApoA-I concentrations [99] while lowered ApoA-I concentrations in AD patients were correlated with more severe cognitive impairment [97]. Early studies of CSF showed no significant decrease of ApoA-I levels between AD and control patients [100], and it was proposed that this was due to the housekeeping function of the blood brain barrier (BBB) or an increase of ApoA-I passage into the CSF [97]. However, more recent proteomics studies showed significantly reduced levels of ApoA-I in the CSF of AD patients compared to controls [101, 102]. It seems plausible that ApoA-I levels are maintained or even increased in the CSF in early disease stages but then decrease with the progression of the disease as seen in blood samples from AD patients [96–98].

More research on this possible connection of ApoA-I levels and AD is needed since ApoA-I has been implicated in APP/Aβ metabolism via different mechanisms. The findings that ApoA-I binds to Aβ [103], is present in senile plaques [104], and has been shown to prevent β-sheet organization and Aβ-iduced toxicity in vitro [105] strengthen a possible role of ApoA-I in AD pathology. Concordantly, in a mouse model of AD ApoA-I overexpression prevented learning and memory deficits, while ApoA-I deficiency increased memory deficits [106, 107]. Interestingly, ApoA-I depletion did not affect parenchymal Aβ load but did significantly increase levels of vascular amyloid deposition [107]. Cognitive decline has been correlated to soluble Aβ oligomers rather than to insoluble Aβ plaques [108, 109]. Therefore, binding of Aβ to ApoA-I might facilitate Aβ efflux from the brain, thereby preventing Aβ oligomerization and toxicity. In an in vitro study, ApoA-I was shown to inhibit Aβ aggregation, and the effective ApoA-I/Aβ ratio was lower than the ratio in the CSF indicating that under normal conditions with no ApoA-I decline, ApoA-I may prevent Aβ aggregation in vivo as well [107].

7 ApoA-I and DS

ApoA-I is implicated in DS. DS, or trisomy 21, one of the most common chromosomal disorders leading to mental retardation, is caused by a full or partial triplication of chromosome 21 as first discovered by Lejeune et al. in 1959 [110, 111]. DS patients have a high risk of developing AD [112] since the gene-encoding *APP* is localized on chromosome 21. Older DS patients develop AD-like pathology and dementia, and studies have shown the presence of senile plaques and NTFs in DS brains [113]. In addition, oxidative stress plays a major role in DS [114, 115]. Increased oxidative stress, indexed by ROS and lipid peroxidation, has been shown in DS neurons [116]. A recent study also showed a significant increase in oxidation markers in amniotic fluid of women carrying DS fetuses compared to that of women carrying chromosomally normal fetuses [117]. As mentioned above, oxidation can affect the structure and function of a protein, and oxidized proteins are often targeted for degradation [5]. However, oxidation can also lead to the aggregation and cross-linking of proteins which then might become resistant to degradation and start forming pathological deposits associated with cell damage [118].

In search of reliable diagnostic markers, ApoA-I levels have been shown to decrease in plasma and serum of women carrying a DS fetus [119–121]. In addition, decreased ApoA-I levels were found in the serum of DS patients with gout [122]. However, two recent studies showed an increase in the levels of ApoA-I preproprotein in amniotic fluid and of ApoA-I in serum of woman carrying DS fetuses compared to controls, respectively [123, 124]. These contradicting results show that ApoA-I levels are affected in DS but more studies are needed before ApoA-I could be considered a reliable marker.

Using redox proteomics, ApoA-I, among other proteins, was found to be significantly more oxidized in amniotic fluid of women carrying a DS fetus [117]. As mentioned above, oxidation affects functions of ApoA-I negatively and, being

the major HDL lipoprotein, oxidative modifications of ApoA-I would also affect functions of HDL.

8 ApoA-I and PD

Parkinson disease, like AD, is an age-related neurodegenerative disease and its pathological hallmarks include progressive loss of dopaminergic neurons in the substantia nigra, pars compacta, and formation of Lewy body inclusions in the cytoplasm of neurons [125]. Additionally, oxidative stress has been implicated in the disease, and increased levels of DNA, lipid, and protein oxidation markers were found in brain of PD subjects [126–131].

In a recent proteomics study of CSF samples from PD patients and healthy controls, ApoA-I was shown to be differentially expressed. Using 2D-DIGE an ApoA-I isoform was shown to be downregulated while a different ApoA-I isoform was upregulated in PD [2]. In addition, Wang et al. 2010 found pro-apolipoprotein, the immature precursor of ApoA-I, was upregulated in PD as well. Western blot analysis performed to confirm these results showed significantly upregulated ApoA-I levels in CSF [2].

These conflicting results point out one of the strengths of 2D approaches versus Western blotting: A protein could show a certain change in expression, for instance an increase, when probing with a specific antibody when actually this overall increase might be due to changes in the expression of different isoforms that might even include decreased expression of a certain isoform. However, other proteomics studies have shown decreased ApoA-I levels in CSF and serum from PD patients compared to healthy controls [132, 133]. While ApoA-I expression in severely affected PD patients was still lower than that in the control group, ApoA-I levels were considerably lower in early disease stages [133]. Samples from patients with different PD severities could possibly explain the inconsistency of the results of the different studies. Additionally, the small number of samples per group does not allow for generalization and require larger sample analysis. Nonetheless, the finding of ApoA-I as a possible disease marker for PD proposes a possible link between PD and cholesterol [2].

9 Apo A-I and chemotherapy-induced cognitive dysfunction

Redox proteomics [9] provided new insights into mechanisms involved in chemotherapy-induced cognitive dysfunction [72], is found in up to 75% of breast cancer survivors [134]. Given that nearly 50 percent of FDA-approved chemotherapeutic agents are associated with ROS, a significant proportion of the current 12 million cancer survivors in the United States likely show symptoms of cognitive dysfunction.

Oxidative modification of ApoA-I has been implicated in a cascade of events involving elevation of inflammatory species leading to chemotherapy-induced cognitive dysfunction [72]. Chemotherapy patients often complain of noticeable memory impairment as well as cognitive decline following chemotherapy, an ailment described by patients with the term "chemobrain", with symptoms that persist sometimes for years post treatment. The anthracycline, doxorubicin (DOX), used to treat a variety of solid tumors and leukemias often as part of multidrug chemotherapy regimens, leads to the production of ROS and RNS [72, 135, 136] that can damage biomolecules including proteins, lipids, and DNA [137–139]. A quinone in the DOX structure undergoes redox cycling to the semiquinone, accepting a single electron from an oxidant potentially involving NADPH oxidases, Fe^{2+}, Cu^{+}, or cytochrome P450. In the presence of molecular oxygen, the quinone is reformed by transferring the extra electron to oxygen producing the superoxide radical anion. Superoxide can damage biomolecules directly or indirectly through formation of other ROS/RNS [140].

Oxidative stress analysis of plasma samples from human patients treated with DOX showed global elevation of plasma-protein carbonylation compared to plasma samples from the same persons prior to DOX administration. A subset of these patients also showed elevated plasma-protein-bound HNE, a lipid peroxidation byproduct of arachidonic acid [72, 139, 140].

Our group hypothesized that the oxidative damage to plasma resident ApoA-I, as a result of ROS/RNS induced by DOX and other chemotherapeutic agents, leads to peripheral elevation of the inflammatory cytokine, TNF-α, which, as mentioned above, crosses the BBB [141, 142] via TNFR1 and TNFR2 endocytosis and activates distant receptors located in the CNS. In brain, TNF-α was shown to lead to biomolecule and mitochondrial damage, and neuronal death, and cognitive decline in these cancer patients is proposed to result from such changes [72, 143, 144] (Fig. 2). An activated T-cell-monocyte contact interaction results in monocyte production of TNF-α. ApoA-I inhibits TNF-α release either by inhibition of the monocyte–macrophage contact interaction or by interaction with ABCA1 [13, 44]. We showed, based on redox proteomic evidence, elevated oxidative alterations of ApoA-I induced by protein carbonylation and protein-bound HNE in the plasma of chemotherapy patients, results that were confirmed in DOX-treated mouse models compared to saline-treated controls [72]. Once oxidized, ApoA-I loses the ability to inhibit the monocyte–macrophage interaction thereby allowing increased TNF-α release [72]. Despite the inability of DOX or its metabolites to cross the BBB, through ApoA-I oxidation, DOX increases levels of TNF-α in the periphery that can migrate in the bloodstream to the brain and other tissues and stimulate local inflammation and oxidative stress leading to apoptosis and, potentially, the deleterious cognitive effects experienced by chemotherapy patients [72, 143]. Once in the CNS, TNF-α activates astrocytes, specifically microglia, elevation of iNOS is induced causing damage to mitochondrial MnSOD, leading to a cascade involving cytochrome C, caspase 3, and cell execution [144]. Anti-TNF-α antibody prevents these effects [143]. Sustained activation of microglia can lead to long-term elevation of TNF-α in the CNS [145, 146].

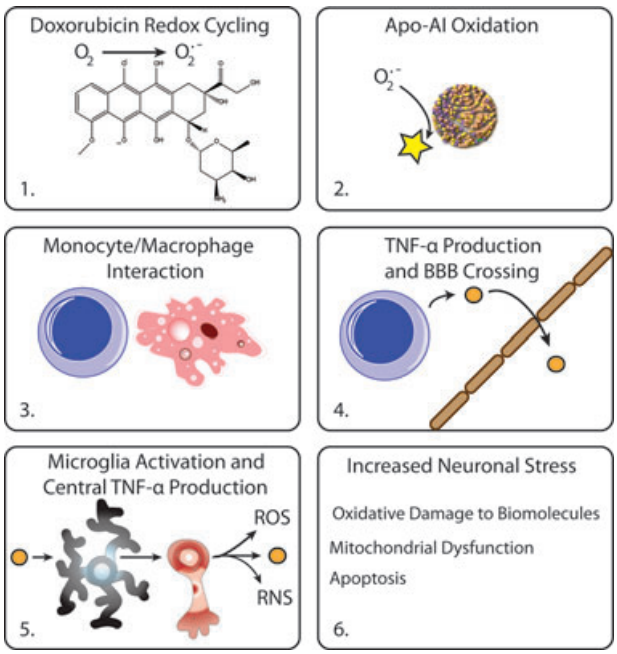

Figure 2. Proposed TNF-α model in this proposed model, peripheral doxorubicin redox cycling between the quinone and semiquinone states within the circulatory system produces the reactive oxygen species superoxide ($O_2^{\cdot-}$) in abundance (Box 1) that oxidatively modifies ApoA-I on HDL particles (Box 2). This modified ApoAI is no longer able to inhibit the monocyte/macrophage interaction (Box 3) and because of this, TNF-α is produced. TNF-α is subsequently able to cross the BBB (Box 4) and activate microglia initiating the production of a host of pro-inflammatory mediators ultimately leading to central TNF-α production as well as increased ROS/RNS (Box 5), which damage neurons and ultimately lead to neuronal death (Box 6). See text.

10 Conclusions and discussion

Redox proteomics identified ApoA-I as a key protein involved in DS and in potential mechanisms of "chemobrain." This, in turn, led to more biochemical studies to interrogate the role of ApoA-I in TNF signaling. This article reviews the key roles played by ApoA-I in cholesterol metabolism, efflux, and transport, inflammation, and neurodegeneration. Oxidative PTM of ApoA-I results in dysfunctional cholesterol processing, dysregulation of inflammatory processes, and neurodegeneration, ultimately, learning and cognitive decline. ApoA-I has been found at altered levels and oxidatively damaged in such neurodegenerative disorders as AD, PD, frontotemporal dementia, and DS as well as in the plasma of cancer patients post chemotherapy.

DOX-related ApoA-I inhibition results in the elevation of peripheral TNF-α levels. Unlike DOX, peripherally generated TNF-α can cross the BBB via TNFR1 and TNFR2 endocytosis and activate distant receptors located in the CNS. Within the CNS, both TNF-α receptors, TNFR1, and TNFR2, are located in the membranes of neuronal and glial cell populations [147, 148], and can contribute to neuronal death. Biomarkers for detection of neurodegeneration in its early stages may allow intervention early enough to prevent further damage and preserve cognitive function. The expression and oxidative changes in ApoA-I seen in these studies make it a good candidate marker for neurodegeneration and should provide a better understanding of disease mechanisms.

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11 References

[1] Davidsson, P., Sjogren, M., The use of proteomics in biomarker discovery in neurodegenerative diseases. *Dis. Markers* 2005, *21*, 81–92.

[2] Wang, E. S., Sun, Y., Guo, J. G., Gao, X. et al., Tetranectin and apolipoprotein A-I in cerebrospinal fluid as potential biomarkers for Parkinson's disease. *Acta Neurol. Scand.* 2010, *122*, 350–359.

[3] Aluise, C. D., Sowell, R. A., Butterfield, D. A., Peptides and proteins in plasma and cerebrospinal fluid as biomarkers for the prediction, diagnosis, and monitoring of therapeutic efficacy of Alzheimer's disease. *Biochim. Biophys. Acta* 2008, *1782*, 549–558.

[4] Taurines, R., Dudley, E., Grassl, J., Warnke A. et al., Proteomic research in psychiatry. *J. Psychopharmacol.* 2011, *25*, 151–196.

[5] Butterfield, D. A., Stadtman, E. R., Protein oxidation processes in aging brain. *Cell Aging Gerontol.* 1997, *2*, 161–191.

[6] Dalle-Donne, I., Scaloni, A., Giustarini, D., Cavarra, E. et al., Proteins as biomarkers of oxidative/nitrosative stress in diseases: the contribution of redox proteomics. *Mass Spectrom. Rev.* 2005, *24*, 55–99.

[7] Butterfield, D. A., Castegna, A., Proteomics for the identification of specifically oxidized proteins in brain: technology and application to the study of neurodegenerative disorders. *Amino Acids* 2003, *25*, 419–425.

[8] Butterfield, D. A., Boyd-Kimball, D., Castegna, A., Proteomics in Alzheimer's disease: insights into potential mechanisms of neurodegeneration. *J. Neurochem.* 2003, *86*, 1313–1327.

[9] Butterfield, D. A., Dalle-Donne, I., Redox proteomics. *Antioxid. Redox. Signal.* 2012, *17*, 1487–1489.

[10] Dalle-Donne, I., Scaloni, A., Butterfield, D. A., *Redox Proteomics: From Protein Modifications to Cellular Dysfunction and Diseases*, John Wiley & Sons, Hoboken, NJ, 2006.

[11] Hansson, S. F., Puchades, M., Blennow, K., Sjögren, M. et al., Validation of a prefractionation method followed by two-dimensional electrophoresis–Applied to cerebrospinal fluid proteins from frontotemporal dementia patients. *Proteome Science* 2004, *2*, 7.

[12] Quazi, F., Molday, R. S., Lipid transport by mammalian ABC proteins. *Essays Biochem.* 2011, *50*, 265–290.

[13] Hyka, N., Dayer, J. M., Modoux, C., Kohno, T. et al.,

Apolipoprotein A-I inhibits the production of interleukin-1beta and tumor necrosis factor-alpha by blocking contact-mediated activation of monocytes by T lymphocytes. *Blood* 2001, *97*, 2381–2389.

[14] Rader, D. J., Daugherty, A., Translating molecular discoveries into new therapies for atherosclerosis. *Nature* 2008, *451*, 904–913.

[15] Tape, C., Kisilevsky, R., Apolipoprotein A-I and apolipoprotein SAA half-lives during acute inflammation and amyloidogenesis. *Biochim. Biophys. Acta* 1990, *1043*, 295–300.

[16] Saito, K., Seishima, M., Heyes, M. P., Song, H. et al., Marked increases in concentrations of apolipoprotein in the cerebrospinal fluid of poliovirus-infected macaques: relations between apolipoprotein concentrations and severity of brain injury. *Biochem. J.* 1997, *321* (Pt 1), 145–149.

[17] Liscum, L., Underwood, K. W., Intracellular cholesterol transport and compartmentation. *J. Biol. Chem.* 1995, *270*, 15443–15446.

[18] Waarts, B. L., Bittman, R., Wilschut, J., Sphingolipid and cholesterol dependence of alphavirus membrane fusion. Lack of correlation with lipid raft formation in target liposomes. *J. Biol. Chem.* 2002, *277*, 38141–38147.

[19] Shadan, S., James, P. S., Howes, E. A., Jones, R., Cholesterol efflux alters lipid raft stability and distribution during capacitation of boar spermatozoa. *Biol. Reprod.* 2004, *71*, 253–265.

[20] Oh, H. Y., Lee, E. J., Yoon, S., Chung, B. H. et al., Cholesterol level of lipid raft microdomains regulates apoptotic cell death in prostate cancer cells through EGFR-mediated Akt and ERK signal transduction. *The Prostate* 2007, *67*, 1061–1069.

[21] Chaudhuri, A., Chattopadhyay, A., Transbilayer organization of membrane cholesterol at low concentrations: implications in health and disease. *Biochim. Biophys. Acta* 2011, *1808*, 19–25.

[22] Dominiczak, M. H., Risk factors for coronary disease: the time for a paradigm shift? *Clin. Chem. Lab. Med.: CCLM/FESCC* 2001, *39*, 907–919.

[23] Dominiczak, M. H., Caslake, M. J., Apolipoproteins: metabolic role and clinical biochemistry applications. *Annals of Clin. Biochem.* 2011, *48*, 498–515.

[24] Hill, S. A., McQueen, M. J., Reverse cholesterol transport–a review of the process and its clinical implications. *Clin. Biochem.* 1997, *30*, 517–525.

[25] Yang, R., Li, L., Seidelmann, S. B., Shen, G. Q. et al., A genome-wide linkage scan identifies multiple quantitative trait loci for HDL-cholesterol levels in families with premature CAD and MI. *J. Lipid Res.* 2010, *51*, 1442–1451.

[26] Zhao, G. J., Yin, K., Fu, Y. C., Tang, C. K., The interaction of ApoA-I and ABCA1 triggers signal transduction pathways to mediate efflux of cellular lipids. *Mol. Med.* 2012, *18*, 149–158.

[27] Cucuianu, M., Coca, M., Hancu, N., Reverse cholesterol transport and atherosclerosis. A mini review. *Romanian J. Int. Med. Revue Roumaine De Medecine Interne* 2007, *45*, 17–27.

[28] Bencharif, K., Hoareau, L., Murumalla, R. K., Tarnus, E. et al., Effect of ApoA-I on cholesterol release and apoE secretion in human mature adipocytes. *Lipids in Health and Disease* 2010, *9*, 75.

[29] Undurti, A., Huang, Y., Lupica, J. A., Smith, J. D. et al., Modification of high density lipoprotein by myeloperoxidase generates a pro-inflammatory particle. *J. Biol. Chem.* 2009, *284*, 30825–30835.

[30] Butterfield, D. A., Bader Lange, M. L., Sultana, R., Involvements of the lipid peroxidation product, HNE, in the pathogenesis and progression of Alzheimer's disease. *Biochim. Biophys. Acta* 2010, *1801*, 924–929.

[31] Kim, H. Y., Tallman, K. A., Liebler, D. C., Porter, N. A., An azido-biotin reagent for use in the isolation of protein adducts of lipid-derived electrophiles by streptavidin catch and photorelease. *Mol. Cell Proteomics* 2009, *8*, 2080–2089.

[32] Liebler, D. C., Protein damage by reactive electrophiles: targets and consequences. *Chem. Res. Toxicol* 2008, *21*, 117–128.

[33] Subramaniam, R., Roediger, F., Jordan, B., Mattson, M. P. et al., The lipid peroxidation product, 4-hydroxy-2-transnonenal, alters the conformation of cortical synaptosomal membrane proteins. *J. Neurochem.* 1997, *69*, 1161–1169.

[34] Szapacs, M. E., Kim, H. Y., Porter, N. A., Liebler, D. C., Identification of proteins adducted by lipid peroxidation products in plasma and modifications of apolipoprotein A1 with a novel biotinylated phospholipid probe. *J. Proteome Res.* 2008, *7*, 4237–4246.

[35] Vergeer, M., Holleboom, A. G., Kastelein, J. J., Kuivenhoven, J. A., The HDL hypothesis: does high-density lipoprotein protect from atherosclerosis? *J. Lipid Res.* 2010, *51*, 2058–2073.

[36] Vaughan, A. M., Tang, C., Oram, J. F., ABCA1 mutants reveal an interdependency between lipid export function, ApoA-I binding activity, and Janus kinase 2 activation. *J. Lipid Res.* 2009, *50*, 285–292.

[37] Tang, C., Vaughan, A. M., Oram, J. F., Janus kinase 2 modulates the apolipoprotein interactions with ABCA1 required for removing cellular cholesterol. *J. Biolog. Chem.* 2004, *279*, 7622–7628.

[38] Tang, C., Vaughan, A. M., Anantharamaiah, G. M., Oram, J. F., Janus kinase 2 modulates the lipid-removing but not protein-stabilizing interactions of amphipathic helices with ABCA1. *J. Lipid Res.* 2006, *47*, 107–114.

[39] Tsukamoto, K., Hirano, K., Tsujii, K., Ikegami, C. et al., ATP-binding cassette transporter-1 induces rearrangement of actin cytoskeletons possibly through Cdc42/N-WASP. *Biochem. Biophys. Res. Comm.* 2001, *287*, 757–765.

[40] Iwamoto, N., Lu, R., Tanaka, N., Abe-Dohmae, S. et al., Calmodulin interacts with ATP binding cassette transporter A1 to protect from calpain-mediated degradation and upregulates high-density lipoprotein generation. *Arterioscler. Thromb. Vasc. Biol.* 2010, *30*, 1446–1452.

[41] Matsunaga, T., Hiasa, Y., Yanagi, H., Maeda, T. et al., Apolipoprotein A-I deficiency due to a codon 84 nonsense

mutation of the apolipoprotein A-I gene. *Proc. Nat. Acad. Sci. USA* 1991, *88*, 2793–2797.

[42] Yin, K., Deng, X., Mo, Z. C., Zhao, G. J. et al., Tristetraprolin-dependent post-transcriptional regulation of inflammatory cytokine mRNA expression by apolipoprotein A-I: role of ATP-binding membrane cassette transporter A1 and signal transducer and activator of transcription 3. *J. Biol. Chem.* 2011, *286*, 13834–13845.

[43] Murray, P. J., Understanding and exploiting the endogenous interleukin-10/STAT3-mediated anti-inflammatory response. *Curr. Op. Pharmacol.* 2006, *6*, 379–386.

[44] Tang, C., Liu, Y., Kessler, P. S., Vaughan, A. M. et al., The macrophage cholesterol exporter ABCA1 functions as an anti-inflammatory receptor. *J. Biol. Chem.* 2009, *284*, 32336–32343.

[45] Jeon, K. I., Jono, H., Miller, C. L., Cai, Y. et al., Ca2+/calmodulin-stimulated PDE1 regulates the beta-catenin/TCF signaling through PP2A B56 gamma subunit in proliferating vascular smooth muscle cells. *FEBS J.* 2010, *277*, 5026–5039.

[46] Yang, J. H., Dai, X. Y., Ou, X., Hao, X. R. et al., Effect of apolipoprotein A-I on expression and function of ATP-binding cassette transporter A1 through PKA signaling. *Prog. Biochem. Biophys.* 2007, *34*, 611–619.

[47] Hu, Y. W., Ma, X., Li, X. X., Liu, X. H. et al., Eicosapentaenoic acid reduces ABCA1 serine phosphorylation and impairs ABCA1-dependent cholesterol efflux through cyclic AMP/protein kinase A signaling pathway in THP-1 macrophage-derived foam cells. *Atherosclerosis* 2009, *204*, e35–e43.

[48] Yamauchi, Y., Hayashi, M., Abe-Dohmae, S., Yokoyama, S., Apolipoprotein A-I activates protein kinase C alpha signaling to phosphorylate and stabilize ATP binding cassette transporter A1 for the high density lipoprotein assembly. *J. Biol. Chem.* 2003, *278*, 47890–47897.

[49] Yamauchi, Y., Chang, C. C., Hayashi, M., Abe-Dohmae, S. et al., Intracellular cholesterol mobilization involved in the ABCA1/apolipoprotein-mediated assembly of high density lipoprotein in fibroblasts. *J. Lipid Res.* 2004, *45*, 1943–1951.

[50] Chen, X., Zhao, Y., Guo, Z., Zhou, L. et al., Transcriptional regulation of ATP-binding cassette transporter A1 expression by a novel signaling pathway. *J. Biol. Chem.* 2011, *286*, 8917–8923.

[51] Zheng, L., Nukuna, B., Brennan, M. L., Sun, M. et al., Apolipoprotein A-I is a selective target for myeloperoxidase-catalyzed oxidation and functional impairment in subjects with cardiovascular disease. *J. Clin. Invest.* 2004, *114*, 529–541.

[52] Wu, Z., Wagner, M. A., Zheng, L., Parks, J. S. et al., The refined structure of nascent HDL reveals a key functional domain for particle maturation and dysfunction. *Nat. Struct. Mol. Biol.* 2007, *14*, 861–868.

[53] Helbecque, N., Codron, V., Cottel, D., Amouyel, P., An apolipoprotein A-I gene promoter polymorphism associated with cognitive decline, but not with Alzheimer's disease. *Dement. Geriatr. Cogn. Disord.* 2008, *25*, 97–102.

[54] Schneider-Brachert, W., Tchikov, V., Neumeyer, J., Jakob, M. et al., Compartmentalization of TNF receptor 1 signaling: internalized TNF receptosomes as death signaling vesicles. *Immunity* 2004, *21*, 415–428.

[55] Badiola, N., Malagelada, C., Llecha, N., Hidalgo, J. et al., Activation of caspase-8 by tumour necrosis factor receptor 1 is necessary for caspase-3 activation and apoptosis in oxygen-glucose deprived cultured cortical cells. *Neurobiol. Dis.* 2009, *35*, 438–447.

[56] Selmaj, K. W., Farooq, M., Norton, W. T., Raine, C. S. et al., Proliferation of astrocytes in vitro in response to cytokines. A primary role for tumor necrosis factor. *J. Immunol.* 1990, *144*, 129–135.

[57] Gupta, S., Bi, R., Kim, C., Chiplunkar, S. et al., Role of NF-kappaB signaling pathway in increased tumor necrosis factor-alpha-induced apoptosis of lymphocytes in aged humans. *Cell Death Diff.* 2005, *12*, 177–183.

[58] Rubio-Perez, J. M., Morillas-Ruiz, J. M., A review: inflammatory process in Alzheimer's disease, role of cytokines. *Sci. World J.* 2012, *2012*, 756357.

[59] MacEwan, D. J., TNF receptor subtype signalling: differences and cellular consequences. *Cell. Signal* 2002, *14*, 477–492.

[60] Wajant, H., Pfizenmaier, K., Scheurich, P., Tumor necrosis factor signaling. *Cell Death Diff.* 2003, *10*, 45–65.

[61] Song, H. Y., Regnier, C. H., Kirschning, C. J., Goeddel, D. V. et al., Tumor necrosis factor (TNF)-mediated kinase cascades: bifurcation of nuclear factor-kappaB and c-jun *N*-terminal kinase (JNK/SAPK) pathways at TNF receptor-associated factor 2. *Proc. Nat. Acad. Sci. USA* 1997, *94*, 9792–9796.

[62] Liu, Z. G., Hsu, H., Goeddel, D. V., Karin, M., Dissection of TNF receptor 1 effector functions: JNK activation is not linked to apoptosis while NF-kappaB activation prevents cell death. *Cell* 1996, *87*, 565–576.

[63] Chen, G., Goeddel, D. V., TNF-R1 signaling: a beautiful pathway. *Science* 2002, *296*, 1634–1635.

[64] Nishitoh, H., Saitoh, M., Mochida, Y., Takeda, K. et al., ASK1 is essential for JNK/SAPK activation by TRAF2. *Molec. Cell* 1998, *2*, 389–395.

[65] Takeuchi, H., Jin, S., Wang, J., Zhang, G. et al., Tumor necrosis factor-alpha induces neurotoxicity via glutamate release from hemichannels of activated microglia in an autocrine manner. *J. Biol. Chem.* 2006, *281*, 21362–21368.

[66] Tangpong, J., Sompol, P., Vore, M., St Clair, W. et al., Tumor necrosis factor alpha-mediated nitric oxide production enhances manganese superoxide dismutase nitration and mitochondrial dysfunction in primary neurons: an insight into the role of glial cells. *Neuroscience* 2008, *151*, 622–629.

[67] Boston-Howes, W., Gibb, S. L., Williams, E. O., Pasinelli, P. et al., Caspase-3 cleaves and inactivates the glutamate transporter EAAT2. *J. Biol. Chem.* 2006, *281*, 14076–14084.

[68] Sitcheran, R., Gupta, P., Fisher, P. B., Baldwin, A. S., Positive and negative regulation of EAAT2 by NF-kappaB: a

role for *N*-myc in TNFalpha-controlled repression. *EMBO J.* 2005, *24*, 510–520.

[69] Zou, J. Y., Crews, F. T., TNF alpha potentiates glutamate neurotoxicity by inhibiting glutamate uptake in organotypic brain slice cultures: neuroprotection by NF kappa B inhibition. *Brain Res.* 2005, *1034*, 11–24.

[70] Kogo, J., Takeba, Y., Kumai, T., Kitaoka, Y. et al., Involvement of TNF-alpha in glutamate-induced apoptosis in a differentiated neuronal cell line. *Brain Res.* 2006, *1122*, 201–208.

[71] Stellwagen, D., Beattie, E. C., Seo, J. Y., Malenka, R. C., Differential regulation of AMPA receptor and GABA receptor trafficking by tumor necrosis factor-alpha. *J. Neurosci.* 2005, *25*, 3219–3228.

[72] Aluise, C. D., Miriyala, S., Noel, T., Sultana, R. et al., 2-mercaptoethane sulfonate prevents doxorubicin-induced plasma protein oxidation and TNF-alpha release: implications for the reactive oxygen species-mediated mechanisms of chemobrain. *Free Radic. Biol. Med.* 2011, *50*, 1630–1638.

[73] Alzheimer, A., Stelzmann, R. A., Schnitzlein, H. N., Murtagh, F. R., An English translation of Alzheimer's 1907 paper, "Uber eine eigenartige Erkankung der Hirnrinde". *Clin. Anat.* 1995, *8*, 429–431.

[74] Grundke-Iqbal, I., Iqbal, K., Quinlan, M., Tung, Y. C. et al., Microtubule-associated protein tau. A component of Alzheimer paired helical filaments. *J. Biol. Chem.* 1986, *261*, 6084–6089.

[75] Masters, C. L., Simms, G., Weinman, N. A., Multhaup, G. et al., Amyloid plaque core protein in Alzheimer disease and Down syndrome. *Proc. Natl. Acad. Sci. USA* 1985, *82*, 4245–4249.

[76] Kang, J., Lemaire, H. G., Unterbeck, A., Salbaum, J. M. et al., The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature* 1987, *325*, 733–736.

[77] Goldgaber, D., Lerman, M. I., McBride, O. W., Saffiotti, U. et al., Characterization and chromosomal localization of a cDNA encoding brain amyloid of Alzheimer's disease. *Science* 1987, *235*, 877–880.

[78] Tanzi, R. E., Gusella, J. F., Watkins, P. C., Bruns, G. A. et al., Amyloid beta protein gene: cDNA, mRNA distribution, and genetic linkage near the Alzheimer locus. *Science* 1987, *235*, 880–884.

[79] Butterfield, D. A., Reed, T., Newman, S. F., Sultana, R., Roles of amyloid beta-peptide-associated oxidative stress and brain protein modifications in the pathogenesis of Alzheimer's disease and mild cognitive impairment. *Free Radic. Biol. Med.* 2007, *43*, 658–677.

[80] Sultana, R., Boyd-Kimball, D., Poon, H. F., Cai, J. et al., 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* 2006, *27*, 1564–1576.

[81] Itagaki, S., McGeer, P. L., Akiyama, H., Zhu, S. et al., Relationship of microglia and astrocytes to amyloid deposits of Alzheimer disease. *J. Neuroimmunol.* 1989, *24*, 173–182.

[82] Carter, C. J., Convergence of genes implicated in Alzheimer's disease on the cerebral cholesterol shuttle: APP, cholesterol, lipoproteins, and atherosclerosis. *Neurochem. Int.* 2007, *50*, 12–38.

[83] Strittmatter, W. J., Saunders, A. M., Schmechel, D., Pericak-Vance, M. et al., Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc. Natl. Acad. Sci. USA* 1993, *90*, 1977–1981.

[84] Scarmeas, N., Invited commentary: lipoproteins and dementia – is it the apolipoprotein A-I? *Am. J. Epidemiol.* 2007, *165*, 993–997.

[85] Evans, R. M., Emsley, C. L., Gao, S., Sahota, A. et al., Serum cholesterol, APOE genotype, and the risk of Alzheimer's disease: a population-based study of African Americans. *Neurology* 2000, *54*, 240–242.

[86] Vetrivel, K. S., Cheng, H., Lin, W., Sakurai, T. et al., Association of gamma-secretase with lipid rafts in post-Golgi and endosome membranes. *J. Biol. Chem.* 2004, *279*, 44945–44954.

[87] Yao, Z. X., Papadopoulos, V., Function of beta-amyloid in cholesterol transport: a lead to neurotoxicity. *FASEB J.* 2002, *16*, 1677–1679.

[88] Kurata, T., Miyazaki, K., Kozuki, M., Panin, V. L. et al., Atorvastatin and pitavastatin improve cognitive function and reduce senile plaque and phosphorylated tau in aged APP mice. *Brain Res.* 2011, *1371*, 161–170.

[89] Haag, M. D., Hofman, A., Koudstaal, P. J., Stricker, B. H. et al., Statins are associated with a reduced risk of Alzheimer disease regardless of lipophilicity. The Rotterdam Study. *J. Neurol. Neurosurg. Psychiat.* 2009, *80*, 13–17.

[90] Sparks, D. L., Connor, D. J., Sabbagh, M. N., Petersen, R. B. et al., Circulating cholesterol levels, apolipoprotein E genotype and dementia severity influence the benefit of atorvastatin treatment in Alzheimer's disease: results of the Alzheimer's disease cholesterol-lowering treatment (ADCLT) trial. *Acta Neurol. Scand.* 2006, *185*, 3–7.

[91] Hajjar, I., Schumpert, J., Hirth, V., Wieland, D. et al., The impact of the use of statins on the prevalence of dementia and the progression of cognitive impairment. *J. Gerontol. A Biol. Sci. Med. Sci.* 2002, *57*, M414–M418.

[92] Butterfield, D. A., Atorvastatin and Abeta(1–40): not as simple as cholesterol reduction in brain and relevance to Alzheimer disease. *Exp. Neurol.* 2011, *228*, 15–18.

[93] Butterfield, D. A., Barone, E., Di Domenico, F., Cenini, G. et al., Atorvastatin treatment in a dog preclinical model of Alzheimer's disease leads to up-regulation of haem oxygenase-1 and is associated with reduced oxidative stress in brain. *Int. J. Neuropsychopharmacol.* 2012, *15*, 981–987.

[94] Butterfield, D. A., Barone, E., Mancuso, C., Cholesterol-independent neuroprotective and neurotoxic activities of statins: perspectives for statin use in Alzheimer disease

and other age-related neurodegenerative disorders. *Pharmacol. Res.* 2011, *64*, 180–186.

[95] Barone, E., Cenini, G., Di Domenico, F., Martin, S. et al., Long-term high-dose atorvastatin decreases brain oxidative and nitrosative stress in a preclinical model of Alzheimer disease: a novel mechanism of action. *Pharmacol. Res.* 2011, *63*, 172–180.

[96] Kawano, M., Kawakami, M., Otsuka, M., Yashima, H. et al., Marked decrease of plasma apolipoprotein AI and AII in Japanese patients with late-onset non-familial Alzheimer's disease. *Clin. Chim. Acta* 1995, *239*, 209–211.

[97] Merched, A., Xia, Y., Visvikis, S., Serot, J. M. et al., Decreased high-density lipoprotein cholesterol and serum apolipoprotein AI concentrations are highly correlated with the severity of Alzheimer's disease. *Neurobiol. Aging.* 2000, *21*, 27–30.

[98] Liu, H. C., Hu, C. J., Chang, J. G., Sung, S. M. et al., Proteomic identification of lower apolipoprotein A-I in Alzheimer's disease. *Dement. Geriatr. Cogn. Disord.* 2006, *21*, 155–161.

[99] Saczynski, J. S., White, L., Peila, R. L., Rodriguez, B. L. et al., The relation between apolipoprotein A-I and dementia: the Honolulu-Asia aging study. *Am. J. Epidemiol.* 2007, *165*, 985–992.

[100] Song, H., Saito, K., Seishima, M., Noma, A. et al., Cerebrospinal fluid apo E and apo A-I concentrations in early- and late-onset Alzheimer's disease. *Neurosci. Lett.* 1997, *231*, 175–178.

[101] Puchades, M., Hansson, S. F., Nilsson, C. L., Andreasen, N. et al., Proteomic studies of potential cerebrospinal fluid protein markers for Alzheimer's disease. *Brain Res. Mol. Brain Res.* 2003, *118*, 140–146.

[102] Castano, E. M., Roher, A. E., Esh, C. L., Kokjohn, T. A. et al., Comparative proteomics of cerebrospinal fluid in neuropathologically-confirmed Alzheimer's disease and non-demented elderly subjects. *Neurol. Res.* 2006, *28*, 155–163.

[103] Golabek, A., Marques, M. A., Lalowski, M., Wisniewski, T., Amyloid beta binding proteins in vitro and in normal human cerebrospinal fluid. *Neurosci. Lett.* 1995, *191*, 79–82.

[104] Wisniewski, T., Golabek, A. A., Kida, E., Wisniewski, K. E. et al., Conformational mimicry in Alzheimer's disease. Role of apolipoproteins in amyloidogenesis. *Am. J. Pathol.* 1995, *147*, 238–244.

[105] Koldamova, R. P., Lefterov, I. M., Lefterova, M. I., Lazo, J. S., Apolipoprotein A-I directly interacts with amyloid precursor protein and inhibits A beta aggregation and toxicity. *Biochemistry* 2001, *40*, 3553–3560.

[106] Lewis, T. L., Cao, D., Lu, H., Mans, R. A. et al., Overexpression of human apolipoprotein A-I preserves cognitive function and attenuates neuroinflammation and cerebral amyloid angiopathy in a mouse model of Alzheimer disease. *J. Biol. Chem.* 2010, *285*, 36958–36968.

[107] Lefterov, I., Fitz, N. F., Cronican, A. A., Fogg, A. et al., Apolipoprotein A-I deficiency increases cerebral amyloid angiopathy and cognitive deficits in APP/PS1DeltaE9 mice. *J. Biol. Chem.* 2010, *285*, 36945–36957.

[108] Wang, J., Dickson, D. W., Trojanowski, J. Q., Lee, V. M., The levels of soluble versus insoluble brain Abeta distinguish Alzheimer's disease from normal and pathologic aging. *Exp. Neurol.* 1999, *158*, 328–337.

[109] McLean, C. A., Cherny, R. A., Fraser, F. W., Fuller, S. J. et al., Soluble pool of Abeta amyloid as a determinant of severity of neurodegeneration in Alzheimer's disease. *Ann. Neurol.* 1999, *46*, 860–866.

[110] Perluigi, M., Butterfield, D. A., Oxidative stress and Down syndrome: a route toward Alzheimer-like dementia. *Curr. Gerontol. Geriatr. Res.* 2012, *2012*, 724904.

[111] Lejeune, J., Gautier, M., Turpin, R., (Study of somatic chromosomes from 9 mongoloid children). *C R Hebd Seances Acad. Sci.* 1959, *248*, 1721–1722.

[112] Schupf, N., Sergievsky, G. H., Genetic and host factors for dementia in Down's syndrome. *Br. J. Psychiat.* 2002, *180*, 405–410.

[113] Schochet, S. S., Jr., Lampert, P. W., McCormick, W. F., Neurofibrillary tangles in patients with Down's syndrome: a light and electron microscopic study. *Acta Neuropathol.* 1973, *23*, 342–346.

[114] Zana, M., Janka, Z., Kalman, J., Oxidative stress: a bridge between Down's syndrome and Alzheimer's disease. *Neurobiol. Aging* 2007, *28*, 648–676.

[115] Cenini, G., Dowling, A. L., Beckett, T. L., Barone, E. et al., Association between frontal cortex oxidative damage and beta-amyloid as a function of age in Down syndrome. *Biochim. Biophys. Acta* 2012, *1822*, 130–138.

[116] Busciglio, J., Yankner, B. A., Apoptosis and increased generation of reactive oxygen species in Down's syndrome neurons in vitro. *Nature* 1995, *378*, 776–779.

[117] Perluigi, M., Di Domenico, F., Fiorini, A., Cocciolo, A. et al., Oxidative stress occurs early in Down syndrome pregnancy: a redox proteomics analysis of amniotic fluid. *Proteomics Clin. Appl.* 2011, *5*, 167–178.

[118] Feeney, M. B., Schoneich, C., Tyrosine modifications in aging. *Antioxid. Redox Signal.* 2012, *17*, 1571–1579.

[119] Kolla, V., Jeno, P., Moes, S., Tercanli, S. et al., Quantitative proteomics analysis of maternal plasma in Down syndrome pregnancies using isobaric tagging reagent (iTRAQ). *J. Biomed. Biotechnol.* 2010, *2010*, 952047.

[120] Kang, Y., Dong, X., Zhou, Q., Zhang, Y. et al., Identification of novel candidate maternal serum protein markers for Down syndrome by integrated proteomic and bioinformatic analysis. *Prenat. Diagn.* 2012, *32*, 284–292.

[121] Koster, M. P., Pennings, J. L., Imholz, S., Rodenburg, W. et al., Bead-based multiplexed immunoassays to identify new biomarkers in maternal serum to improve first trimester Down syndrome screening. *Prenat. Diagn.* 2009, *29*, 857–862.

[122] Chen, Y. C., Wang, P. W., Pan, T. L., Wallace, C. G. et al., Proteomic analysis of Down's syndrome patients with gout. *Clin. Chim. Acta* 2006, *369*, 89–94.

[123] Park, J., Cha, D. H., Jung, J. W., Kim, Y. H. et al., Comparative proteomic analysis of human amniotic fluid supernatants with Down syndrome using mass spectrometry. *J. Microbiol. Biotechnol.* 2010, *20*, 959–967.

[124] Nagalla, S. R., Canick, J. A., Jacob, T., Schneider, K. A. et al., Proteomic analysis of maternal serum in Down syndrome: identification of novel protein biomarkers. *J. Proteome Res.* 2007, *6*, 1245–1257.

[125] Olanow, C. W., Tatton, W. G., Etiology and pathogenesis of Parkinson's disease. *Annu. Rev. Neurosci.* 1999, *22*, 123–144.

[126] Yoritaka, A., Hattori, N., Uchida, K., Tanaka, M. et al., Immunohistochemical detection of 4-hydroxynonenal protein adducts in Parkinson disease. *Proc. Natl. Acad. Sci. USA* 1996, *93*, 2696–2701.

[127] Alam, Z. I., Daniel, S. E., Lees, A. J., Marsden, D. C. et al., A generalised increase in protein carbonyls in the brain in Parkinson's but not incidental Lewy body disease. *J. Neurochem.* 1997, *69*, 1326–1329.

[128] Kikuchi, A., Takeda, A., Onodera, H., Kimpara, T. et al., Systemic increase of oxidative nucleic acid damage in Parkinson's disease and multiple system atrophy. *Neurobiol. Dis.* 2002, *9*, 244–248.

[129] Seet, R. C., Lee, C. Y., Lim, E. C., Tan, J. J. et al., Oxidative damage in Parkinson disease: measurement using accurate biomarkers. *Free Radic. Biol. Med.* 2010, *48*, 560–566.

[130] Lee, C. Y., Seet, R. C., Huang, S. H., Long, L. H. et al., Different patterns of oxidized lipid products in plasma and urine of dengue fever, stroke, and Parkinson's disease patients: cautions in the use of biomarkers of oxidative stress. *Antioxid. Redox Signal* 2009, *11*, 407–420.

[131] Jenner, P., Oxidative stress in Parkinson's disease. *Ann. Neurol.* 2003, *53* Suppl 3, S26–S36; discussion S36–28.

[132] Yin, G. N., Lee, H. W., Cho, J. Y., Suk, K., Neuronal pentraxin receptor in cerebrospinal fluid as a potential biomarker for neurodegenerative diseases. *Brain Res.* 2009, *1265*, 158–170.

[133] Zhang, X., Yin, X., Yu, H., Liu, X. et al., Quantitative proteomic analysis of serum proteins in patients with Parkinson's disease using an isobaric tag for relative and absolute quantification labeling, two-dimensional liquid chromatography, and tandem mass spectrometry. *Analyst.* 2012, *137*, 490–495.

[134] Raffa, R. B., Duong, P. V., Finney, J., Garber, D. A. et al., Is 'chemo-fog'/'chemo-brain' caused by cancer chemotherapy? *J. Clin. Pharm. Ther.* 2006, *31*, 129–138.

[135] Bachur, N. R., Gordon, S. L., Gee, M. V., Anthracycline antibiotic augmentation of microsomal electron transport and free radical formation. *Mol. Pharmacol.* 1977, *13*, 901–910.

[136] Deres, P., Halmosi, R., Toth, A., Kovacs, K. et al., Prevention of doxorubicin-induced acute cardiotoxicity by an experimental antioxidant compound. *J. Cardiovasc Pharmacol.* 2005, *45*, 36–43.

[137] Fornari, F. A., Randolph, J. K., Yalowich, J. C., Ritke, M. K. et al., Interference by doxorubicin with DNA unwinding in MCF-7 breast tumor cells. *Mol. Pharmacol.* 1994, *45*, 649–656.

[138] Chen, Y., Jungsuwadee, P., Vore, M., Butterfield, D. A. et al., Collateral damage in cancer chemotherapy: oxidative stress in nontargeted tissues. *Mol. Interv.* 2007, *7*, 147–156.

[139] Jungsuwadee, P., Cole, M. P., Sultana, R., Joshi, G. et al., Increase in Mrp1 expression and 4-hydroxy-2-nonenal adduction in heart tissue of Adriamycin-treated C57BL/6 mice. *Mol. Cancer Ther.* 2006, *5*, 2851–2860.

[140] Aluise, C. D., Sultana, R., Tangpong, J., Vore, M. et al., Chemo brain (chemo fog) as a potential side effect of doxorubicin administration: role of cytokine-induced, oxidative/nitrosative stress in cognitive dysfunction. *Adv. Exp. Med. Biol.* 2010, *678*, 147–156.

[141] Gutierrez, E. G., Banks, W. A., Kastin, A. J., Murine tumor necrosis factor alpha is transported from blood to brain in the mouse. *J. Neuroimmunol.* 1993, *47*, 169–176.

[142] Osburg, B., Peiser, C., Domling, D., Schomburg, L. et al., Effect of endotoxin on expression of TNF receptors and transport of TNF-alpha at the blood-brain barrier of the rat. *Am. J. Physiol. Endocrinol. Metab.* 2002, *283*, E899–E908.

[143] Tangpong, J., Cole, M. P., Sultana, R., Joshi, G. et al., Adriamycin-induced, TNF-alpha-mediated central nervous system toxicity. *Neurobiol. Dis.* 2006, *23*, 127–139.

[144] Tangpong, J., Cole, M. P., Sultana, R., Estus, S. et al., Adriamycin-mediated nitration of manganese superoxide dismutase in the central nervous system: insight into the mechanism of chemobrain. *J. Neurochem.* 2007, *100*, 191–201.

[145] Dammann, O., Durum, S., Leviton, A., Do white cells matter in white matter damage? *Trends Neurosci.* 2001, *24*, 320–324.

[146] Wang, L. W., Tu, Y. F., Huang, C. C., Ho, C. J., JNK signaling is the shared pathway linking neuroinflammation, blood-brain barrier disruption, and oligodendroglial apoptosis in the white matter injury of the immature brain. *J. Neuroinflammation* 2012, *9*, 175.

[147] Figiel, I., Dzwonek, K., TNFalpha and TNF receptor 1 expression in the mixed neuronal-glial cultures of hippocampal dentate gyrus exposed to glutamate or trimethyltin. *Brain Res.* 2007, *1131*, 17–28.

[148] Dopp, J. M., Mackenzie-Graham, A., Otero, G. C., Merrill, J. E., Differential expression, cytokine modulation, and specific functions of type-1 and type-2 tumor necrosis factor receptors in rat glia. *J. Neuroimmunol.* 1997, *75*, 104–112.

