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Apolipoprotein A-I: Insights from Redox Proteomics for its Role in Neurodegeneration

Jeriel T. R. Keeney¹, Aaron M. Swomley¹, Sarah Förster^{1,2}, Jessica L. Harris¹, Rukhsana Sultana¹, and D. Allan Butterfield^{1,*}

¹Department of Chemistry, Center of Membrane Sciences, Sanders Brown Center on Aging, University of Kentucky, Lexington, KY 40506, USA

²Institute of Animal Sciences, Department of Biochemistry, University of Bonn, 53115 Bonn, Germany

Abstract

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; proteomics; 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 many of 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 blood or cerebrospinal fluid (CSF) also could lead to their use in monitoring disease

*To whom correspondence should be addressed: Prof. D. Allan Butterfield, Department of Chemistry, Center of Membrane Science, and Sanders Brown Center on Aging, 249 Chemistry-Physics Building, University of Kentucky, Lexington, KY 40506, Fax: 859-323-1464, dabcsn@uky.edu.

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 leads 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 post-translational modifications 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 oxidative/nitrosative stress. Commonly used indices of oxidative damage to protein includes 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 have 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 (FTD) [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 (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 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 3 β -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 sub-classes 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 (OFP), and reverse cholesterol transport (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 OFP 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 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] (Figure 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 which can subsequently deposit the cholesteryl esters into the scavenger receptor BI (SRB-1) 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 (NF κ B) 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 has been shown to alter both the structure and function of proteins [30–33]. Liebler and colleagues [34] found ApoA-I to be highly modified by free radical oxidation using a novel

biotin-modified linoleoylglycerolphosphatidylcholine 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 liquid chromatography-tandem mass spectrometry (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 1000uM. 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 (Figure 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_{α_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, phosphatidylcholine 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 Alzheimer disease. 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 (SODD) 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 SODD 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: NF κ B activation, mitogen-activated protein kinase (MAPK) pathway activation, and caspase-mediated apoptosis.

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

Of the three main MAPK pathways, the stress related and pro-apoptotic c-jun NH₂-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 (MAPKKK), for example, apoptosis-stimulated kinase 1 (ASK1) [64]. Stimulation of a variety of MAPKKKs 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 (FADD) to TRADD. The TNFR1/TRADD/FADD 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 NF κ B pathway both play negative regulatory roles of one another; caspase-8 and caspase-3 inhibits RIP and IKK β respectively, and the NF κ B-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, NF κ B.

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 lead to the up regulation 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 NF κ B 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. With the TNF- α -associated down regulation of EAAT2 activity, and increased generation and release of glutamate from microglia, this 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 Ca²⁺-mediated cell death by increasing the membranous expression of ionotropic glutamate receptor AMPA, through the activation of IP₃ [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 Alzheimer Disease

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 (A β) surrounded by dystrophic neurites [75]. A β 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 of AD as a multifactorial disease.

Fewer than five percent 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 A β , 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 A β 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 reverse cholesterol transport 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 lower 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/A β metabolism via different mechanisms. The findings that ApoA-I binds to A β [103], is present in senile plaques [104], and has been shown to prevent β -sheet organization and A β -induced toxicity in vitro [105] strengthens 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 A β load but did significantly increase levels of vascular amyloid deposition [107]. Cognitive decline has been correlated to soluble A β oligomers rather than to insoluble A β plaques [108, 109]. Therefore, binding of A β to ApoA-I might facilitate A β efflux from the brain, thereby preventing A β oligomerization and toxicity. In an in vitro study, ApoA-I was shown to inhibit A β aggregation, and the effective ApoA-I/A β ratio was lower than the ratio in the CSF indicating that under normal conditions with no ApoA-I decline, ApoA-I may prevent A β aggregation in vivo as well [107].

7. ApoA-I and Down Syndrome

ApoA-I is implicated in Down syndrome. 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 and colleagues 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 Parkinson disease

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 proapolipoprotein, 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 vs. 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 which 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 do 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, $\text{TNF-}\alpha$, which, as mentioned above, crosses the BBB [141, 142] via TNFR1 and TNFR2 endocytosis and activates distant receptors located in the CNS. In brain, $\text{TNF-}\alpha$ 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] (Figure 2). An activated T cell-monocyte contact interaction results in monocyte production of $\text{TNF-}\alpha$. ApoA-I inhibits $\text{TNF-}\alpha$ 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 which 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].

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 paper reviews the key roles played by ApoA-I in cholesterol metabolism, efflux, and transport, inflammation, and neurodegeneration. Oxidative post-translational modification 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 Alzheimer disease, Parkinson disease, frontotemporal dementia, and Down syndrome 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 makes it a good candidate marker for neurodegeneration and should provide a better understanding of disease mechanisms.

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Abbreviations

Aβ	amyloid beta-peptide
ABCA1	ATP-binding cassette protein A1
AD	Alzheimer disease
AIPH	2, 2-azobis(2-(2-imidazolin-2-yl)propane)-dihydrochloride
apo	apolipoprotein
APP	amyloid precursor protein
ASK1	apoptosis-stimulated kinase 1

BBB	blood-brain barrier
C	cysteine
CSF	cerebrospinal fluid
DD	death domain
DOX	doxorubicin
DS	Down syndrome
EAAT2	excitatory amino acid transporter 2
FADD	fas-associated death domain
FTP	fuel transport pathway
H	histidine
HNE	4-hydroxy-2-trans-nonenal
IκB	inhibitor of κ B
IKKβ	inhibitor of κ B kinase β
JAK2	Janus kinase 2
JNK	c-jun NH ₂ -terminal kinase
K	lysine
LCAT	Lecithin-cholesterol acyltransferase
LDL	low-density lipoproteins
OFP	over-flow pathway
PD	Parkinson disease
PK	protein kinase
RCT	reverse cholesterol transport
MAPK	mitogen-activated protein kinase
MAPKKK	mitogen-activated protein kinase kinase kinase
NF-κB	Nuclear factor kappa-B
RIP	receptor-interacting kinase
RNS	reactive nitrogen species
SODD	Silencer of death domain
SRB-1	scavenger receptor B1
TRADD	TNF-receptor-associated death-domain
TRAF2	TNF receptor-associated factor
TNF-α	tumor necrosis factor- α
VLDL	very-low density lipoproteins

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Figure 1.**Proposed TNF- α Model**

In this proposed model, peripheral doxorubicin redox cycling between the quinone and semi-quinone states within the circulatory system produces the reactive oxygen species superoxide (O_2^-) in abundance (Box 1) which oxidatively modifies ApoA-I on HDL particles (Box 2). This modified ApoA-I 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 leads to neuronal death (Box 6). See text.

Figure 2.**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 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.

Table 1

Proteomic studies showing changes in ApoA-I expression or oxidation in neurodegenerative disorders. (* These studies also showed effects on other apolipoproteins.)

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*
AD	CSF	n = 27	2DE	-	Davidsson et al. 2002*
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*
AD	CSF	n = 6	(1DE + LC- MS/MS) 2DE (+ MS/MS)	-	Yin et al. 2009*
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*
DS	Maternal plasma from DS pregnancies	n = 24	2DE + MALDI TOF MS	-	Kolialexi et al. 2008*
DS	Maternal plasma from DS pregnancies	n = 12 (pooled samples)	iTRAQ + strong cation exchange (SCX) LC-MS/MS	ApoA-I ↓	Kolla et al. 2010*
DS	Maternal serum from DS pregnancies	n = 40 (pooled samples for 2D- CF)	2DE DIGE 2D-liquid chromatography – chromatofocusing (2D-CF) Q-TOF; MS + MS/MS	ApoA-I ↓	Nagalla et al. 2006*
DS	Amniotic fluid from DS pregnancies	n = 10	ID 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 + AMLDI 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

Disease	Sample	Sample size	Methods	Effects on ApoA-I	Reference
FTD	CSF	n = 10	2DE	proapolipoproteinAI ↑	Hansson et al. 2004[11]
PD	CSF	n = 6	2DE-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*
PD	serum	n = 22	iTRAQ (2DE LC-MS/MS)	ApoA-I ↓	Zhang et al. 2012*