Chapter 7

Insights from Proteomics into Mild Cognitive Impairment, Likely the Earliest Stage of Alzheimer's Disease

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Abstract

Mild cognitive impairment (MCI) is arguably the earliest form of Alzheimer's disease (AD). Better understanding of brain changes in MCI may lead to the identification of therapeutic targets to slow the progression of AD. Oxidative stress has been implicated as a mechanism associated with the pathogenesis of both MCI and AD. In particular, among other markers, there is evidence for an increase in the levels of protein oxidation and lipid peroxidation in the brains of subjects with MCI. Several proteins are oxidatively modified in MCI brain, and as a result individual protein dysfunction may be directly linked to these modifications (e.g., carbonylation, nitration, modification by HNE) and may be involved in MCI pathogenesis.

Additionally, Concanavalin-A-mediated separation of brain proteins has recently led to the identification of key proteins in MCI and AD using proteomics methods. This chapter will summarize important findings from proteomics studies of MCI, which have provided insights into this cognitive disorder and have led to further understanding of potential mechanisms involved in the progression of AD.

Keywords: Mild cognitive impairment, proteomics, oxidative modifications, alzheimer's disease

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1. Introduction

Mild cognitive impairment (MCI) can be considered as the earliest form of Alzheimer's disease (AD) existing as a transitional state between normal aging and AD [1-3]. MCI exists in two forms: amnestic MCI and nonamnestic MCI [2, 3]. Amnestic MCI patients are able to perform normal daily living activities and have no signs of dementia; however, they do have cognitive complaints that include bursts of episodic memory loss [1, 4]. In some cases, amnestic MCI patients can develop AD at a rate of ~10 to 15% annually, however in other cases, the patients revert back to normal conditions [5].

Pathologic characteristics of MCI are similar to those of AD. For example, MCI patients have hippocampal, entorhinal cortex (EC), and temporal lobe atrophy based on magnetic resonance imaging studies [6-8], synapse loss, neuronal loss, low cerebrospinal fluid (CSF)-resident β amyloid levels [6], genetic risk factors including preponderance in APOE4 allele [9, 10], and increased levels of oxidative stress [11-20].

Oxidative stress is one of the underlying indices associated with MCI, AD, and other neurodegenerative disorders such as Parkinson's disease and amyotrophic lateral sclerosis. Specifically in MCI, there is substantial evidence for increased levels of oxidative stress in the brains and in plasma of MCI subjects [11-23]. Our laboratory has reported an increase in the levels of protein carbonyls (PCO) [11, 16] and 3-nitrotyrosine (3NT)-modified proteins [21], both of which are markers of protein oxidation. Additionally, we have reported an increase in the levels of 4-hydroxynonenal-(HNE) bound proteins, indicating an increase in the levels of lipid peroxidation products [13].

Others have observed decreases in the levels of antioxidant enzymes and antioxidant enzymatic activity in brain and in plasma [22-24], increased levels of oxidative stress in nuclear and mitochondrial DNA [25, 26], increased levels of isoprostanes [27], and increased lipid peroxidation as measured by free HNE levels, thiobarbituic substances, and malondialdehyde [16, 20]. It is believed that oxidative stress also is related to several vascular factors, such as heart disease, hypertension, and diabetes mellitus that conceivably contribute to the conversion of MCI into AD.

It is important to understand more about the events that lead to the progression of AD from MCI in order to develop potential therapeutics that can delay or stop AD onset. Thus, proteomics can provide considerable insight into specific pathways that are influenced by MCI and which eventually aid in the progression of disease. To this end, we and others have investigated the changes associated with the proteomes of MCI subjects relative to normal age-matched healthy controls [11, 19, 28-33].

These studies include the search for candidate biomarkers of MCI which eventually lead to AD [29, 30, 33], changes in the expression levels of proteins [28], specific levels of protein oxidation as measured by PCO [11], 3NT-modified proteins [19], and lipid peroxidation as measured by HNE-bound proteins [32]. More recently, we have also investigated other post-translational modifications that change in subjects with MCI such as glycosylation [31]. This chapter summarizes the key findings from proteomics and redox proteomics studies in MCI and their implications in AD research.

2. Two-dimensional (2D) Gel Electrophoresis (GE) Based Proteomics

The proteomics techniques used in the studies described herein follow the general approach outlined in Figure 1. Here proteins are extracted from brain, CSF, plasma, or other bodily tissues obtained from MCI subjects and normal age-matched controls. Extracted proteins are subjected to isoelectric focusing (IEF)/sodium dodecyl sulfate (SDS polyacrylamide gel electrophoresis (PAGE), better known as 2DGE. In this approach, proteins are separated in a first dimension based on their isoelectric point and in a second dimension based on their migration rate through the gel, which often corresponds to molecular weight. Image analysis software is used to align spots across the gels obtained from all of the samples, and protein spots that exhibit significant changes (based on Student's t-tests or analysis of variance) in expression levels between MCI and controls are excised. Excised spots are subjected to in-gel trypsin digestion and analyzed using matrix assisted laser desorption ionization (MALDI) or electrospray ionization (ESI) mass spectrometry (MS). Data from MS experiments are then submitted to appropriate databases using search engines such as MASCOT [34] for protein identification.
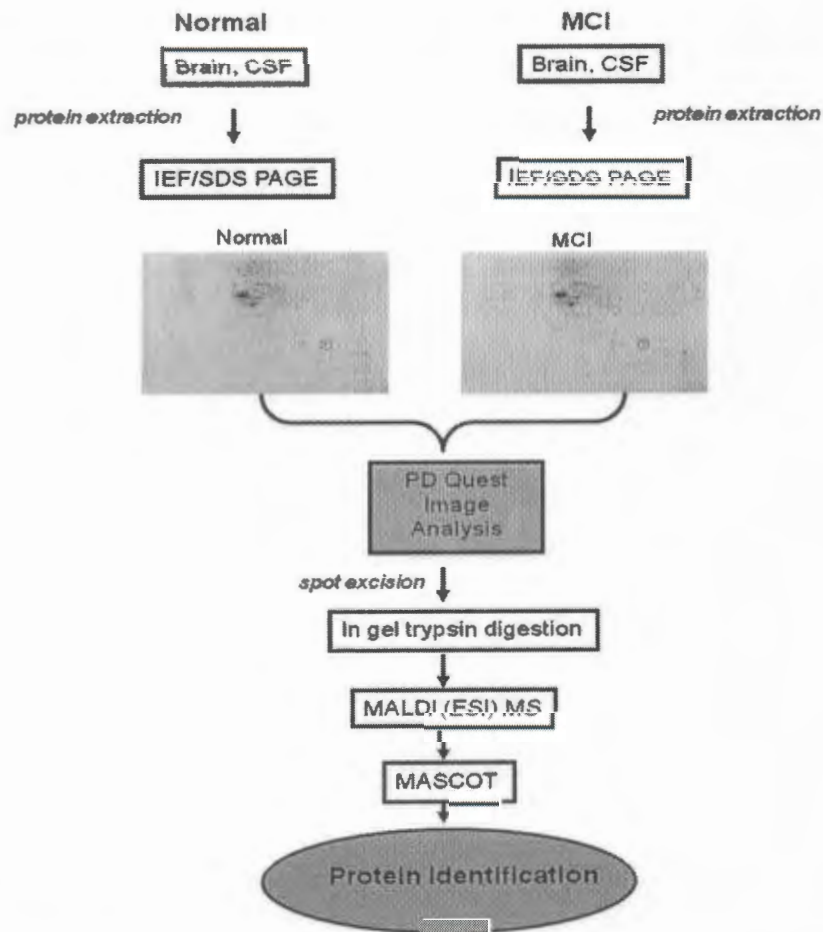

Figure 1. Schematic overview of the 2D GE experiment on brain or CSF from subjects with MCI and age-matched controls.
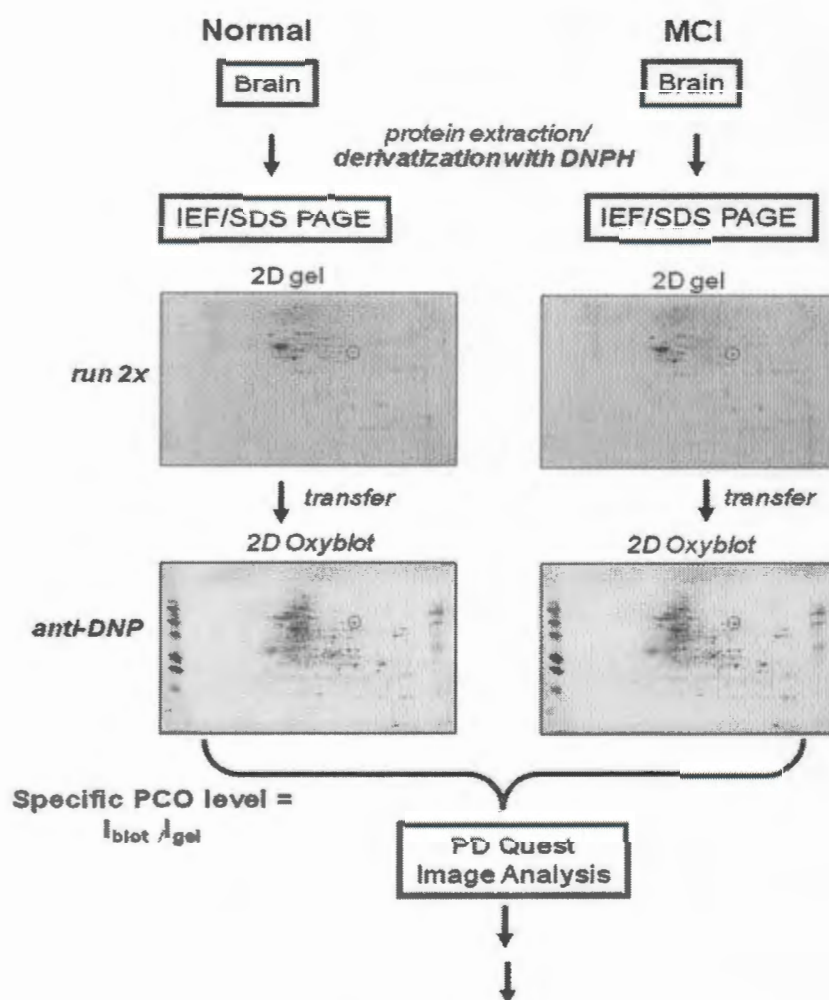
Figure 2. Schematic overview of the redox proteomics approach applied for the analysis of oxidatively modified proteins such as protein carbonyls (PCO), 3-nitrotyrosine (3NT) modified proteins and HNE-modified proteins. Extracted proteins are derivatized with 2,4-dinitrophenylhydrazine (DNPH) only for the analysis of PCO and separated with IEF/SDS PAGE. 2D gels are then transferred onto a nitrocellulose membrane and 2D Oxyblots are probed with anti-DNP (or anti-3NT, anti-HNE) antibodies and visualized using a secondary antibody linked with a colorimetric alkaline phosphatase assay. Specific oxidative levels of proteins are calculated by normalizing the intensity of spots in the 2D Oxyblot (I_{blot}) to the intensity of the corresponding spot in a 2D gel (I_{gel}). This calculation is carried out similarly for PCO, 3NT, and HNE. Protein spots exhibiting significant changes in oxidative modification are then excised, digested in-gel by trypsin, analyzed with MALDI or ESI-MS/MS, and identified with database searching as illustrated in Figure 1.

This general approach can also be adapted for the analysis of post-translational modifications. For example, changes in glycosylation of proteins can be analyzed by using affinity chromatography for purification of glycoproteins. Extracted proteins can be separated with Concanavalin-A lectin affinity columns which isolate proteins that contain asparagine (N)-linked carbohydrates. In some cases, Con-A may also have nonspecific interactions and isolate proteins based on its hydrophobic binding domain [35].

Oxidative modification of proteins can also be detected using the 2D GE approach with the incorporation of Western blotting analysis [36]. Figure 2 shows a schematic of the general approach used to detect PCO, 3NT-modified proteins, and HNE-bound proteins. As shown in

Figure 2, for the detection of PCO, extracted proteins are derivatized with 2,4-dinitrophenylhydrazine (DNPH), which forms a Schiff base with carbonyl groups on proteins. DNPH-derivatized proteins are then separated using 2D GE, and the spots on gels are transferred onto a nitrocellulose membrane forming a 2D Western blot or 2D Oxyblot. Immunochemical detection using a primary anti-DNP antibody that recognizes DNP hydrazone adducts is applied to the 2D Oxyblots, and oxidized spots are visualized with a secondary antibody linked to a colorimetric alkaline phosphatase assay. Similarly, for the detection of 3NT- and HNE-modified proteins non-derivatized extracted proteins are separated with 2D GE, transferred onto an 2D Oxyblot, and immunochemically detected with anti-3NT and anti-HNE, antibodies, respectively. Imaging analysis software and statistical approaches are applied as illustrated in Figure 1 in order to align 2D images and identify spots that have significant changes in oxidative modification. Specific carbonyl (or 3NT, HNE) levels of proteins are measured by normalizing the density of spots in the 2D Oxyblot, to the density of the same spot in a 2D gel analysis of the sample (separate experiment). Protein spots of interest (those exhibiting significant elevation or reduction in oxidative modification) are then excised from the gel, tryptically digested, analyzed by MS, and identified as described in Figure 1.

3. Candidate Biomarkers in CSF for the Progression of MCI to AD

CSF presents another biological fluid that can relay specific information about neurological molecular changes because the fluid encompasses the extracellular space surrounding the brain. Table 1 lists proteins that were identified as having significant changes in expression in CSF of MCI subjects relative to normalcontrols. Kim and coworkers have performed proteomics analysis on CSF from normal cognitive controls, MCI, and AD patients and identified three proteins which may be candidate markers for the diagnosis MCI and its progression into AD[29, 30]. The protein, fibrinogen γ-A chain, was detected as having a gradual elevation of expression in MCI and AD [30]. Fibrinogen γ -A chain is a part of the 340 kDahexameric soluble glycoprotein (consisting of α, β, and γ chains) that is synthesized in the liver. This protein is involved in the polymerization of fibrin, blood coagulation, signal transduction platelet activation and binding, and thrombin binding [37]. Fibrinogen has also been shown to have elevated expression levels during inflammation and in cardiovascular disease [37]; thus, its elevation in MCI may be reflective of early events of neuroinflammation.

The other two proteins, plasma retinol-binding protein (RBP) and haptoglobin precursor allele 1, were detected as having a significant decrease in expression in CSF from MCI (and AD) patients relative to normal age-matched controls by 38% and 63%, respectively [29]. RBP is a 21 kDa carrier protein that tightly binds retinol allowing for it to freely circulate through plasma. Haptoglobin is a tetrameric protein that is a part of the acute phase response and binds free hemoglobin. Through binding of hemoglobin, haptoglobin inhibits oxidative activity of hemoglobin, prevents iron loss in the kidneys, and protects the kidneys against damage that could be caused by hemoglobin [38]. The effects of decreased expression of RBP and haptoglobin in MCI is not clearly understood [29].

Table 1. Candidate Biomarker Proteins in CSF of MCI

Protein	Change in MCI	Ref
C3a des-Arg	↑	Simonsen *et al.* 2007
C4a des-Arg	↑	Simonsen *et al.* 2007
Fibrinogen γ-chain A	↑	Lee *et al.* 2007
Haptoglobin precursor allele 1	↓	Jung *et al.* 2008
Phosphorylated osteopontin C-terminal fragment	↑	Simonsen *et al.* 2007
Plasma retinol-binding protein	↓	Jung *et al.* 2008
Ubiquitin	↑	Simonsen *et al.* 2007
β_2-Microglobulin	↑	Simonsen *et al.* 2007

Using 2D GE coupled to surface-enhanced laser desorption ionization (SELDI)-MS, Simonsen *et al.* identified a panel of 17 proteins that may be potential biomarkers of patients with MCI that convert to AD and of patients with MCI who do not progress to AD [33]. Of the 17 proteins, four proteins were down-regulated and 13 proteins were up-regulated in CSF of MCI patients that converted to AD relative to stable MCI patients and normal healthy controls [33]. Five proteins were identified with MS and have elevated expression in MCI patients that progress to AD: ubiquitin, C3a anaphylatoxin des-Arg, C4a anaphylatoxin des-Arg, β_2-Microglobulin, and phosphorylated osteopontin C-terminal fragment. β-amyloid can bind to C1q and subsequently activate the complement cascade resulting in the production of C3a and C4a, as well as C5a peptides [39]. Osteopontin is a glycoprotein and proinflammatory cytokine involved in bone synthesis and various aspects of immunity such as chemotaxis [40], cell adhesion and wound healing [41], cell activation and cytokine production [41], and apoptosis [42, 43]. Elevation of the complement peptides and osteopontin in MCI patients that progress to AD suggests that innate immunity including inflammation in MCI patients may become activated and stay activated in the progression of disease. β_2-Microglobulin is a part of the class I major histocompatibility complex and mediates amyloid fibril formation *in vitro* [44] and in the presence of transition metal cations [45]. Ubiquitin is used to target proteins for degradation by the 26S proteasome [46], and has been immunhistochemically shown to be present in neurofibrillary tangles (NFT) and senile plaques (SP) [47].

4. Proteomics Analyses of Brain from MCI Patients

An alternative approach to 2D GE that was recently used in proteomic comparisons of brain from MCI subjects relative to normal cognitive controls, is the PowerBlot proteomic approach (BD Transduction Laboratories). This approach uses a large-scale Western Blot approach to identify 750+ proteins simultaneously in a single experiment. Using the PowerBlot approach, Ho *et al.* detected 50 candidate proteins that had >2.0 fold-change in the EC region of MCI patients relative to normal cognitive controls [28]. Of the 50 proteins detected, 23 proteins were identified and could be functionally clustered into the following categories: neurotransmitter-related, cytoskeleton/cell adhesion, cell cycle/cell proliferation

related, apopotosis related, transcription/translation related, and others. Neurotransmitter-related, apoptosis-related, and transcription/translation-related proteins were decreased in the EC of MCI patients, while cytoskeleton and cell cycle-related proteins included both increased and decreased proteins in MCI patients [28]. Several of these functional categories are similar to those observed in oxidatively modified proteins in MCI hippocampal brain regions and are discussed below.

5. Redox Proteomics Analyses of Brain from MCI Patients

Table 2 lists significantly elevated oxidatively modified proteins (i. e., PCO-, 3NT-, and HNE-modified) from the hippocampal and inferior parietal lobule (IPL) brain regions of MCI subjects relative to age-matched normal controls that were identified by our laboratory.

To-date these are the only reports of specific oxidatively modified proteins in MCI brain that may be relevant to the progression of AD [11, 19, 32]. These proteins can be grouped into several functional categories and were significantly oxidatively modified by one or more of the three oxidative parameters: PCO, 3NT, and HNE.

5.1. Energy or Mitochondrial Dysfunctions and Alterations

As listed in Table 2, several proteins involved in energy and/or mitochondrial-related pathways have significantly elevated levels of oxidative modification in the hippocampal or IPL regions of brains from subjects with MCI. These proteins are α-enolase, aldolase, pyruvate kinase (PK), malate dehydrogenase (MDH), lactate dehydrogenase (LDH), ATP synthase, phosphoglycerate kinase (PGK1), and glucose regulated protein precursor. Glycolysis plays an important role in supplying energy to the brain because glucose is the primary source of energy. Alterations in glucose metabolism and tolerance have been identified in brains of MCI and AD patients from positron emission tomography scanning, [48-50] and oxidatively modified glycolytic proteins have been identified in MCI and AD brain, and models thereof [12, 51]. α-Enolase, aldolase, PK, PGK1, and LDH are enzymes involved in or related to the glycolysis pathway. Increased oxidation of α-enolase, LDH, and PK has been shown to lead to loss of protein function measured by decreased enzymatic activity in MCI brain [11, 32]. Alterations in glycolysis could lead to less ATP production which is detrimental to cells requiring ATP to carry out normal functions, including signal transduction, maintenance of ion gradients, and protein synthesis, and detrimental to ATPases which are responsible for proper maintenance of ion pumps, lipid asymmetry, and intracellular communication. The observed impairments to glycolytic proteins in MCI brain suggest that energy metabolism is a key player in the progression of MCI to AD. This is further supported by the increased oxidation in MDH, ATP synthase α-chain, and glucose regulated protein precursor. ATP synthase α-chain is a component of complex V which plays a key role in energy production and undergoes a series of coordinated conformational changes in order to produce ATP. Oxidative modification to ATP synthase leads to reduced enzymatic activity [32]. Because ATP synthase is involved in the electron transport chain (ETC),

alterations to its activity could result in an electron leakage from ETC carrier molecules, which would lead to an increase in reactive oxygen species (ROS. These ROS could then contribute to the observed increase in oxidative stress parameters in MCI brain [11, 13, 16, 20, 21, 25-27].

An overall decrease in ATP production due to dysfunction of glycolytic enzymes, ATP synthase, and glucose regulated protein precursor (from oxidative modification) could ultimately lead to Ca2dyshomeostasis and make neurons susceptible to excitotoxicity and cell death. From these studies it is apparent that potential preventative targets for AD could be targeted to restoring energy metabolism in earlier disease stages in MCI. In contrast to the usual observation of decreased enzymatic activity of oxidatively modified proteins, oxidative modification of MDH leads to increased activity [32]. The basis of this unusual observation remains unclear.

5.2. Neuritic Abnormalities/Structural Dysfunction

Oxidatively modified proteins in MCI related with neuritic and structural functions are dihydropyrimidinase like-2 (DRP2), β-actin, and fascin 1. DRP-2 is involved in axonal outgrowth and transmission and modulation of extracellular signals through the proteincollapsin [52, 53]. In AD, DRP-2 also has increased oxidation [54, 55], which may be reflective of increasing neuritic degeneration, shortened dendritic length, and synapse loss as MCI progresses to AD. β-actin is crucial for proper maintenance of cellular and cytoskeletal integrity and morphology. High levels of actin can be found in growth cones, presynaptic terminals and dendritic spines, and thus its oxidation could lead to elongation of growth cones and synapse loss. Alterations in cellular integrity could be detrimental to cellular trafficking of key proteins involved in neurotransmission. Fascin 1, also known as p55, is a structural protein involved in cell adhesion and motility [56-58] and is used as a marker of normal dendritic function [59]. Overall, oxidation of structural proteins which could result in altered functionality ultimately can lead to impaired structural integrity, shortened dendritic lengths and faulty axonal growth, loss of interneuronal connections and poor neurotransmission. Neuritic abnormalities and structural dysfunction are documented in AD brain and thus may be key events in the loss of neurotransmission in MCI to AD.

5.3. Excitotoxicity

Overstimulation of neurons can result from an increase in the levels of extracellular glutamate. Glutamine synthetase, which converts glutamate to glutamine, was oxidatively modified in MCI brain and has been shown to have decreased activity [11]. Thus, decreased glutamine synthetase activity directly leads to a buildup in glutamate, which can lead to excitotoxicity. This phenomenon also affects Ca^{2+}homeostasis and eventually leads to neuronal death. Similar changes in glutamine synthetase oxidation and activity were observed in AD brain [60-62], and suggest that synapse loss observed in AD brain occurs early on in MCI with a contribution by excitotoxicity.

Table 2. Functional Categorization of Oxidatively Modified Proteins Identified in Brains of MCI Patients

Functional Categories	Protein	Oxidative Modifications
Energy/mitochondrial dysfunction	α-enolase	PCO, 3NT, HNE
	aldolase	3NT
	malate dehydrogenase	3NT
	glucose-regulated protein precursor	3NT
	lactate dehydrogenase	HNE
	phosphoglycerate kinase	HNE
	pyruvate kinase	PCO, HNE
	ATP synthase α-chain	HNE
Lipid abnormalities & cholinergic dysfunction	neuropolypeptide h3	HNE
Excitotoxicity	glutamine synthetase	PCO
Cell cycle, tau phosphorylation, A β production	PIN1	PCO
Neuritic abnormalities & structural dysfunction	DRP2	3NT
	fascin 1	3NT
	β actin	HNE
Antioxidant defense/Detoxification system dysfunction	GSTM3	3NT
	MRP3	3NT
	peroxiredoxin 6	3NT
	HSP70	3NT, HNE
	carbonyl reductase 1	HNE
Cell signaling dysfunction	14-3-3-γ	3NT
Protein synthesis alterations	Initiation factor α	HNE
	Elongation factor Tu	HNE

5.4. Lipid Abnormalities and Cholinergic Dysfunction

Neuropolypeptide h3 [(also known as phosphatidylethanolamine binding protein (PEBP)] is an enzyme involved in acetylcholine production and may play roles in phospholipid asymmetry. Oxidation of neuropolypeptide h3 in MCI brain and possible loss of function correlates well with the already known cholinergic loss observed in AD brain [63-66]. Also, loss of phospholipid asymmetry has been reported in MCI and AD brain [67-69], and thus oxidation of PEBP could play a role in lipid peroxidation events which lead to cellular apoptosis.

5.5. Antioxidant Defense/Detoxification System Dysfunction

Proteins involved in the antioxidant defense and detoxification system work to remove harmful species such as free radicals and toxic compounds from the cell. Peroxiredoxin 6 (PR6), multidrug resistance protein 3 (MRP3), glutathione-S-transferase Mu 3 (GSTM3), heat shock protein 70 (HSP70), and carbonyl reductase are oxidatively modified brain proteins in

MCI. PR6 is an antioxidant enzyme that reduces the reactive nitrogen species, peroxynitrite, and was detected as having elevated nitration levels in MCI. PR6 also reduces reactive phospholipids and hydroperoxides [70] and has other roles which include cell differentiation, apoptosis, and detoxification [71]. Nitration of PR6, which could lead to loss of function, may result in increased levels of nitrated proteins, such as those detected in MCI brain [19, 21] . PR6 and GSTM3, a detoxification enzyme, form a complex that alters individual enzymatic activities [71] but which works to protect cells from toxic species such as HNE. GST catalyzes the conjugation of the low molecular weight intracellular thiol, glutathione, with toxins, and these toxins are transported out of the cell by MRP [72-74]. Oxidation and potential loss of function of PR6, MRP3, and GSTM3 could impair the cell's ability to remove toxicants leading to an increase in toxic species that subsequently attack cellular molecules (e. g., increased PCO, 3NT, or HNE) and lead to cell death. These observations in MCI brain are consistent with changes to MRP, GST, and PR6 which are observed in AD brain [73, 75], and demonstrate that proper antioxidant and detoxification defense systems may help to delay the progression of MCI to AD.

HSP70 is a molecular chapcronc protein that repairs misfolded proteins and helps in the transportation of misfolded proteins to the proteasome. HSP70 belongs to the class of HSPs that also protect proteins from various stresses, such as oxidative damage [76]. Nitration of HSP70, leading to loss of function, could result in buildup of misfolded proteins and hence protein aggregates and "clogging" of the proteasome. Carbonyl reductase is an enzyme that reduces carbonyl compounds to thcir corresponding alcohols. HNE-modification of carbonyl reductase is rather interesting considering that it has been shown to reduce HNE levels [77], and thus its oxidative modification by HNE would lead to an increase in HNE available for attack on proteins such as those HNE-modified proteins identified in subjects with MCI [32].

5.6. Cell Signaling Dysfunction

14-3-3 γ belongs to a family of scaffolding proteins that normally bridges glycogen synthase kinase 3β (GSK-3β) and tau by forming a multiprotein tau phosphorylation complex [78]. Other functions include signal transduction, protein trafficking, and metabolism [79, 80]. 14-3-3 γ was observed as nitrated in MCI brain and has previously been observed to have elevated expression levels in AD brain [81, 82] and in AD CSF [83]. Nitration of 14-3-3 γ may contribute to tau hyperphosphorylation and thus NFT formation and dysfunction in cell signaling, events which are consistent with changes observed in AD brain.

5.7. Cell Cycle; Tau Phosphorylation; Aβ Production

Peptidyl-prolyl*cis/trans*isomerase 1 (Pin1) is a multifunctional protein involved in the cell cycle, tau phosphorylation, and Aβ production and regulates cellular processes such as protein foldingtranscription, intracellular transport, and apoptosis [84-86]. Pin1 is oxidized in MCI brain [11] and has been previously reported as oxidized in AD brain [55]. Pin1, through its interactions with kinases and phosphatases such as GSK-3β, can directly regulate the phosphorylation of the tau protein [84, 87]. Thus, inactivation of Pin1 as a result of oxidative modification directly leads to hyperphosphorylation of tau and an increase in NFT. Pin1 has

also been shown to bind to APP and influence the production of Aβ [84, 88]. Altered regulation of cell cycle processes by oxidized Pin1 may be related to elevation of cell cycle proteins in brain of MCI subjects [89]. Therefore, alterations to Pin1 activity also may lead to an increase in Aβ and SP. Oxidative impairment of Pin1 in early stages of AD, such as MCI, is consistent with and likely contributes to the major pathological hallmarks of AD: SP, NFT, and synapse loss.

5.8. Protein Synthesis Alterations

Initiation factor α (eIF-α) and elongation factor Tu (EF-Tu) are proteins involved in protein synthesis. eIF-α has been reported to have roles in cell proliferation and senescence [90], cytoskeletal organization [91], and apoptosis [92]. EF-Tu is a nuclear-encoded protein that assists in the translation of proteins in the mitochondria [93]. Specifically, EF-Tu binds aminoacylatedtRNA in the cytoplasm and hydrolyzes GTP in order to allow the aminoacylatedtRNA to enter the A site of the ribosome. Nitration of these proteins can directly influence protein synthesis. Decreased protein synthesis has been reported in MCI and AD [94-96], and thus these alterations are consistent with these reports. Alterations to protein synthesis in MCI may result in a reduction of key proteins necessary to combat many of the cellular insults observed in AD brain, which not only could result in compromised neuronal functions, but also contribute to progression of MCI to AD.

6. Concanavalin-A Associated Glycoproteins in Brain Regions from MCI Patients

DRP-2, glucose-regulated protein 78 (GRP78), protein phosphatase-related protein Sds-22, glial fibrillary acidic protein (GFAP, and β-synuclein were isolated in the ConA associated protein fraction using lectin affinity chromatography coupled to 2D GE and identified as having altered levels in the brains of subjects with MCI relative to age-matched controls [31]. DRP-2 and GRP78 were detected as significantly decreased and GFAP and protein phosphatase –related protein Sds22 as significantly increased in the hippocampal brain region of MCI patients, while β-synuclein was significantly decreased in the inferior parietal lobule region of MCI patients relative to age-matched controls [31]. DRP-2 as (discussed above) is a structural protein involved in axonal outgrowth and neuronal communication thus, its decreased expression may be indicative of neuritic dysfunction that occurs early in MCI and continues with disease progression into AD. GRP78 is an endoplasmic reticulum (ER) associated protein that belongs to the HSP70 protein family and is involved with the unfolfed protein response (UPR). HSP70 is significantly oxidatively modified in MCI brain (see Table 2). Because GRP78 normally reduces the levels of amyloid precursor protein (APP) and Aβ40 and 42 secretion [97], decreased expression of GRP78 in MCI brain could possibly play roles in the elevation of APP and Aβ levels found in AD brain. Also, alteration to GRP78 expression may disrupt Ca2+ homeostasis [98]. Conflicting reports of GRP78 expression in AD have been reported [99, 100], and thus its role in MCI progression to AD is not completely clear. Activation of the UPR in the ER might also mean

that GRP78 is less available for refolding other damaged proteins or shuttling them to the 26S proteasome for degradation.

Protein phosphatase-related protein Sds22 is involved in the cell cycle and was detected as increased in MCI brain, the significance of this increase in the progression of MCI to AD is yet to be determined but as noted above, cell cycle proteins are elevated in brains of subjects with MCI [89]. β-synuclein is involved in synaptic functions, similar to the functions of α-synuclein in Parkinson's disease. β-synuclein also binds to Aβ [101] and has previously been shown by our laboratory to be oxidized *in vivo* following injection of Aβ(1-42) [102[. Decreased expression of β-synuclein in MCI brain could be related with altered synaptic functions which occur also due to the oxidatively modified proteins involved in synaptic functions mentioned above. GFAP, a glial specific intermediate filament protein is significantly increased in MCI brain. GFAP is involved in cytoskeletal integrity and maintenance of cellular shape and movement in astrocytes. Increased expression of GFAP in MCI brain is consistent with increased expression levels in AD [103] and with inflammation related to NFT and SP [104]. This finding provides further evidence supporting the notion that neuroinflammation is an event that occurs in the early stages of AD (MCI) and continues with disease progression.

Conclusion

This chapter has summarized some of the key findings from proteomics studies involving comparisons of brain and CSF in MCI subjects relative to normal age-matched controls. Candidate biomarkers of MCI that may help in early AD diagnosis were identified in CSF and may be useful as additional markers for AD diagnosis to the traditional tau (τ and p), and Aβ40 and 42 markers. Expression and redox proteomics analyses of various brain regions (e. g., EC, IPL, and hippocampus) revealed that a number of processes are altered in MCI including, neurotransmitter-related, apoptosis-related, energy/mitochondrial dysfunction, neuritic abnormalities/structural dysfunction, excitotoxicity, lipid abnormalities and cholinergic dysfunction, antioxidant defense/detoxification systems, cell signaling dysfunction, cell cycle/tau phosphorylation/Aβ production, and transcription/translation (protein synthesis) alterations. It is apparent that MCI to AD progression is a multifactorial process in which many pathways may be potential targets for intervening therapeutics. A large number of energy-related proteins were oxidatively modified in MCI further supporting the concept that normal energy maintenance is crucial and lacking in MCI and AD brain. In addition to oxidative modifications, concanavalin-A associated proteins have altered expression levels in IPL and hippocampal regions of MCI patients. These proteins are involved in structural integrity and molecular chaperoning, and altered levels of these proteins are congruent with the observed oxidative modifications and hence alterations of several structural and antioxidant defense/detoxification proteins. Proteomics has provided much insight into pathways that are related to MCI and with its progression to AD. Each of these pathways should be further investigated for their potential as therapeutic targets for early AD diagnosis, treatment, and/or prevention.

Acknowledgments

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