Chapter 6

Proteomic Study of Degenerative Protein Modifications in the Molecular Pathology of Neurodegeneration and Dementia

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/64693

Abstract

Dementia is a major public health burden, and the World Health Organization has identified this disorder as a major public health priority. There are limited treatment options due to poor understanding of key mechanism of dementia pathogenesis. Dementia has been regarded as a proteinopathy in which alterations of brain protein structure and function are the key features of the disorder. Proteinopathy can be triggered by degenerative protein modifications (DPMs), misfolding, aggregation, and deposition of the malformed proteins. Despite the clinical significance of alteration in protein abundances, DPMs, protein misfolding, and aggregation, the molecular mechanism that promotes these changes remains inadequately understood, mostly due to technical challenges. Proteomic is a powerful, sensitive, and advanced tool to study the progressive brain tissue damage that critically dysregulates key enzymes, accumulates modified proteins, and causes protein misfolding and aggregation, resulting in cognitive decline and dementia. The proteomic profiling of protein abundances and correlating DPMs with protein misfolding and aggregation have potential to elucidate underlying molecular mechanism of the disease. This chapter summarizes the recent proteomic developments for studying brain proteome, DPMs, and protein aggregation mechanism that may lead to dementia. We attempted to correlate DPMs and its impact on protein aggregation and deposition in brain tissues.

Keywords: dementia, Alzheimer's disease, vascular dementia, neurodegenerative disease, proteomics, degenerative protein modifications (DPMs), deamidation, citrullination, amyloid

1. Introduction

Dementia is progressively more common disease in aging population. Worldwide, in 2015 about 46.8 million people were affected by dementia and projected to increase to about 74.7 million by 2030 [1]. The increase in dementia patients is in part due to the aging society, lack of effective prevention strategies, and curative treatments. Due to this exponential increase in dementia population, the social and economic cost of this disorder is surpassing those attributed to cancer and heart diseases [1, 2]. High global prevalence, impact of this disorder on families, caregivers, and communities have posed significant public health challenge [3] forcing the global health community to recognize the need for action and to place dementia on the public health agenda. Recently, the World Health Organization (WHO) has identified dementia as a major public health priority [3]. Unfortunately, dementia research has not been given priority as well as funding share, which could be another reason for significant increase in dementia population. For example, in the UK, only 11% of research funding has been allocated for dementia research while 64% was spent on cancer research in 2012.

The most common forms of dementia are Alzheimer's disease (AD) and vascular dementia (VaD), with respective frequencies of 70 and 15% of all dementias [4]. However, the boundaries between the subtypes are sometimes not clear and mixed forms often coexist [5]. In past decades, research in different subtypes of dementia has failed to improve our understanding of dementia pathogenesis and to develop effective treatments or interventions for this disorder [6, 7]. The major mystery is the lack of information on the main causes of the disorder. This remains the main obstacle in developing a cure for the disorder. Therefore, an urgent intervention is needed to identify the key molecular mechanism that promotes dementia pathogenesis. Several theories have been put forward and only few have survived the test of time. Induction of dementia by ischemic cerebral vascular diseases or stroke was first described in clinics a century ago. However, the later discovery of aggregated β-amyloid and *tau* proteins in the brain tissues of dementia patients diverted the majority of subsequent research toward the study of these two molecules. Accordingly, it was hypothesized that this disorder is triggered by the toxicity of oligomerized protein that forms senile plaque including amyloid-beta (Aβ) and *tau* proteins [8]. However, this hypothesis failed to answer several questions regarding pathogenesis and further development in therapeutics. Although Aβ-deposition has been considered as the main cause of AD, the degree of its deposition in the brain does not correlate with dementia severity [9]. According to Arriagada et al. [10], patients without dementia have the same density of senile plaques as patients with AD. Amyloidal hypothesis could not answer questions such as why healthy elderly people have abundant senile plaques in their brains but no signs of AD [11].

The burden of senile plaques does not correlate with cognitive dysfunction in dementia indicating that protein aggregation alone is not sufficient to explain the pathology of these disorders. Accumulation of degenerative protein modifications (DPMs) triggered by nonenzymatic spontaneous posttranslational modifications, loss of protein function, protein misfolding, protein aggregation, and their depositions in brain tissues could be key features of multiple neurodegenerative diseases since protein dysfunction is likely to extend beyond

http://dx.doi.org/10.5772/64693

these Aβ and *tau* proteins alone. These deleterious protein damages can be caused by dysregulated protein repair and turnover due to hypoxia-ischemia brain injury. Recent epidemiological, clinical, and experimental studies demonstrated that cerebrovascular disease and hypoxic-ischemic brain injury are the primary causes of cognitive impairment and dementia [12–22].

Proteinopathy is the primary cause of dementia, but rarely attempts have been made to determine the complete composition of deposited protein aggregates, to find what promotes the protein aggregation, does proteins other than Aβ are the main culprit, what is the role of DPMs, and how the constituent proteins contribute to plaque formation. The state-of-the-art mass spectrometry-based proteomic technique has the potential to answer these questions. Proteomic techniques can be considered as an integral part of dementia research to identify biomarkers to detect the disorder at the early stage, understand mechanisms that lead to dementia pathogenesis, design new therapeutics, and monitor response of developed treatments. Proteomic discoveries in dementia and current published literature were used as a tool for review. To investigate our question regarding the mechanism of dementia pathogenesis and the role of degenerative protein modifications, we searched PubMed and Scopus for the literature using the following terms: dementia proteomics, amyloidal proteins, dementia proteomic biomarker, neurodegenerative diseases, degenerative protein modification, and posttranslational modification. We then review the returned articles to generate the summary of the mechanism of dementia pathogenesis and the role of degenerative protein modifications in dementia pathogenesis. We provide a review of the most significant findings in the field of dementia with a special focus on DPMs. This chapter aims at providing understanding on dementia pathogenesis through state-of-art proteomics technology and the impact of protein modifications. In the present chapter, we discussed the recent developments and novel proteomic approaches to study the mechanism of dementia pathogenesis, novel insights from neuroproteomic research, mechanism of protein aggregation, and the role of DPMs.

2. Proteomics studies of dementia and AD

Dementia is caused by damage to brain cells, which further interfere with the ability of brain cells to communicate with each other. The broad range of symptoms includes a decline in memory, thinking skills, and decision making. This potentially affects a person's ability to perform everyday activities. According to Alzheimer's association, dementia have several types such as AD, VaD, mixed dementia, Parkinson's Disease (PD), frontotemporal dementia, mild cognitive impairment, posterior cortical atrophy, traumatic brain injury, Down syndrome, Creutzfeldt-Jakob disease, and normal pressure hydrocephalus [23]. These subtypes are associated with damage to specific types of brain cell in particular regions of the brain. For example, hippocampus is the center of learning and memory in the brain, and damage to hippocampus cells results in memory loss, which is one of the earliest symptoms of AD. The presence of aggregated protein plaque is a common clinical manifestation of the diseases, but the specific molecular mechanisms in each type of dementia that trigger neurodegeneration remain a mystery. The main reasons are the lack of well-characterized clinical samples of brain

from particular region, suitable technology to isolate plaque and aggregated proteins, the technique that profiles quantitative composition of both soluble and aggregated proteins, and the technique that accurately identifies DPMs. Proteomic technique enables the comprehensive analysis of the protein and its work flow involves the identification of proteins following their separation, digestion by trypsin, determination of the molecular weight of the resulting peptides, and database searching to make the identification and quantification of the proteins as well as the characterization of the DPMs. In addition to label-free proteomic methods, isobaric tags for relative and absolute quantitation (iTRAQ) and tandem mass tag (TMT) protein labeling are widely accepted approaches for quantitative profiling of cell lines and clinical brain tissue samples [24–26]. Proteomics has also been used for the accurate identification of protein modifications [26–31].

2.1. Novel amyloidal protein-enrichment techniques and DPMs

The alteration in protein function and aggregation is the key feature of neurodegenerative diseases. However, what initiates the protein aggregation, and their deposition and formation of insoluble plaque are poorly defined. Due to poor solubility and self-association of these amyloidal plaque proteins, their accurate identification and quantitation in brain tissue extracts are technically challenging. Researchers [32, 33] have attempted to isolate amyloid proteins using detergents or detergent-free buffers. They adopted sequential extraction and quantification by enzyme-linked immunosorbent assay (ELISA), immunoblotting, or immunocytochemistry. But these approaches were unable to determine the aggregation state of the amyloids and complete composition of amyloidal proteins. Recently, Adav et al. [34] successfully developed ultracentrifugation-electrostatic repulsion hydrophilic interaction chromatography (UC-ERLIC)-coupled mass spectrometry-based proteomics technologies to characterize aggregated proteins in human brain tissues affected by dementia. Using a detergent buffer, they extracted soluble proteins, amyloidal proteins, and insoluble aggregated proteins to identify dementia-associated changes in amyloid protein composition, relative abundances, and the extent of DPMs such as deamidation. These authors profiled both soluble and aggregated amyloidal plaque by LC-MS/MS and found significant enrichment of proteins such as S100A9, ferritin, hemoglobin subunits, creatine kinase, and collagen among the aggregated brain proteins. According to their findings, amyloid plaque was enriched in the deamidated variant of protein S100A9. Yet, the following modified protocol (**Figure 1**) could further improve the detection and identification of amyloidal protein profile in clinical samples.

Most DPMs cause small shift in mass and also involve the addition of small chemical motifs to protein side-chain functional group. This causes alterations in charge and hydrophobicity of the peptide/protein. The detection of the DPM-modified peptide/protein is challenging because the DPMs containing peptides in the trypsin-digested protein sample usually exhibit very low stoichiometry; hence, it is very difficult to identify these from high abundant unmodified peptides during LC-MS/MS analysis. However, these DPMs containing peptides with different charges and hydrophilicities can be separated from unmodified peptides by using ion exchange column running in hydrophilic interaction liquid chromatography (HILIC)

http://dx.doi.org/10.5772/64693

mode that facilitates the detection and identification by LC-MS/MS [35]. Moreover, the unmodified and modified peptides elute from ion exchange column in a predictable order based on their charge densities in LC-MS/MS mobile phase. Accordingly, the modified and unmodified peptides can be separated by electrostatic-interaction-modified HILIC (emHILIC) methods using weak anion exchange (WAX)/strong anion exchange (SAX) columns in ERLIC mode for online ERLIC-MS/MS analysis, or using weak cation exchange (WCX) columns in electrostatic attraction hydrophilic interaction chromatographic mode (EALIC) for online ERLIC-MS/MS analysis.

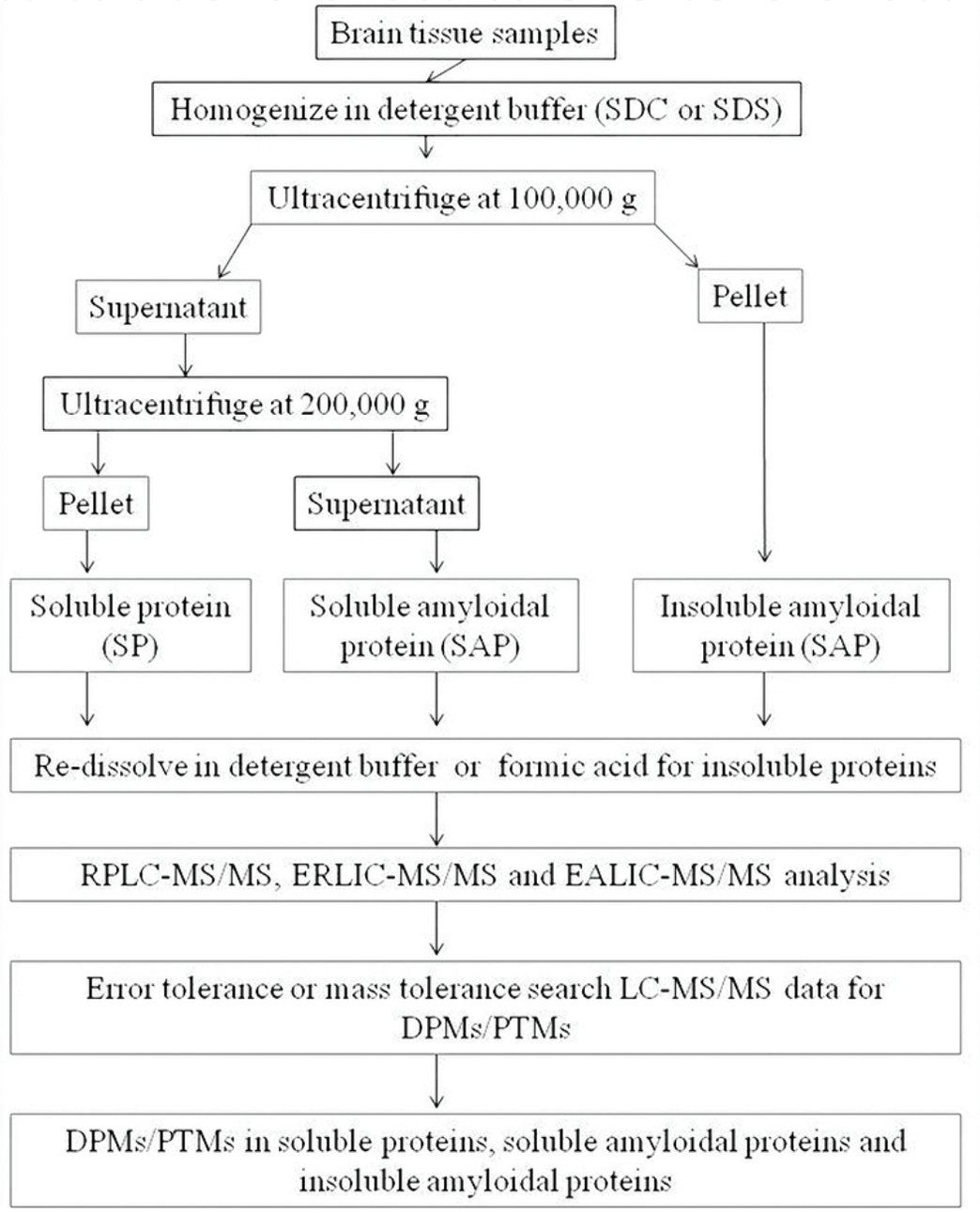

Figure 1. Isolation and identification of both soluble and insoluble amyloid proteins.

2.2. Quantitative clinical proteomics of brain tissue

Protein quantification through the incorporation of stable isotopes has become a vital technology in modern proteomics research. Applying two-dimensional (2D) liquid chromatography coupled with tandem mass spectrometry-based iTRAQ (2D-LC-MS/MS-iTRAQ) technique, Brodmann area 21 of pathologically confirmed cases of VaD and matched non-neurological controls were studied [25]. In the study, 144 differentially expressed proteins including superoxide dismutase, neural cell adhesion molecule, and ATP synthase subunit alpha were characterized to be significantly up-regulated in VaD patients, suggesting a state of hypometabolism and vascular insufficiency along with an inflammatory condition during vascular dementia. iTRAQ quantitative proteomics of brain tissue samples from VaD subjects discovered down-regulation of ion channel proteins including proteins such as V-type proton ATPase subunit D (VATD), ATP synthase, H+ transporting, mitochondrial F0 complex, subunit b-isoform (ATP5F1), Obg-like ATPase 1(OLA1), and V-type proton ATPase subunit F (VATF) [24]. The ion channel protein Na^+–K^+–ATPase exhibits multiple functions including the maintenance of differential membrane potential in neurons, which is an essential feature of the signal transduction. Using proteomics and structural modeling of Na^+–K^+–ATPase, Sze and coworkers [24] showed that the impaired regulation and compromised activity of Na^+–K^+–ATPase contribute to the pathophysiology of VaD. Dysregulated Na^+–K^+–ATPase expression or function have been reported in both animal models and brain tissues in AD, PD, and Huntington's disease (HD) [36].

Synaptic failure is the most common feature observed in both VaD and AD. The loss of synapses and synaptic contacts is also most significant contributor to the cognitive impairment in VaD and other neurodegenerative disease [30, 37]. Similarly, a decline in synapse number in the hippocampal dentate gyrus in AD has been correlated with impairment on a variety of cognitive tests [38]. This suggests that hippocampal degeneration is central to memory loss in AD. Mitochondrial dysfunction is a vital feature of AD, but the fundamental mechanism is still unclear. Mitochondrial dysfunction in neurodegenerative disorders remains a key to the development of oxidative stress. According to Caspersen et al. [39], mitochondrial Aβ-accumulation impairs neuronal function contributing to cellular dysfunction in transgenic (Tg) mice expressing human-mutant amyloid precursor protein (mAPP). During the early stages of AD, a reduced number of mitochondria in neurons [40] and decreased brain glucose metabolism [41] have been reported. As reviewed by Butterfield et al. [42], autopsied AD brain tissue revealed a decreased pyruvate dehydrogenase activity in the parietal, temporal, and frontal cortex. Activities of cytochrome c oxidase and mitochondrial complex IV were significantly low in AD brain.

Dementia risk in women is higher than that in men. Recently, our group [43] applied discovery-based proteomics approach to evaluate gender differences in AD with cerebrovascular disease (CVD) subjects. Quantitative proteomics revealed gender-specific-altered mitochondriome. Proteomic analysis of AD-CVD brain tissues suggested hypercitrullination of arginine and deamidation of glutamine (Gln) in myelin basic protein (MBP) from female patients. It has been revealed that an increased citrullination of MBP is due to the down-regulation of

http://dx.doi.org/10.5772/64693

cathepsin D and other enzymes that degrade the damaged proteins, leading to axonal dysfunction and progressive loss of neuron function [44].

2.3. Insights from hypoxia/ischemia-induced neuropathy

In mild cognitive impairment (MCI) and early phase of AD, a decrease in the cerebral blood flow has been noted and correlated with the symptoms of dementia [45]. At cellular level, a decrease in the blood flow triggers hypoxia. The conditions such as hypoxia/ischemia have been linked to the pathogenesis of AD [46]. Unbiased proteomic analysis of hypoxia-ischemia pathology in numerous disease models and clinical setting including neuronal cell lines [47], a rat model of ischemic middle cerebral artery occlusion [48], a mouse model of cardiovascular disease [49], blood or tissues samples from patients with dementia [24, 26, 30] has provided novel insight into molecular pathology of hypoxia-ischemic injury and confirmed that hypoxia induced mitochondrial dysfunction and oxidative stress, induced epigenetic changes, and dysregulated proteostasis. Thus, oxygen availability is a crucial regulator of cellular metabolism and homeostasis. Proteomic study using ischemic neuronal injury model also identified the dysregulation of proteins such as Park7 and VAP-A implicated in the chronic neurological disorders such as AD and PD [47]. When neuronal cell response to hypoxia and glucose depletion stress was studied by iTRAQ proteomics in hypoxia-ischemic penumbra model, dysregulation of housekeeping proteins, antioxidative defense, chaperone response, and protein metabolism were observed [47]. Proteomic of pathological progression from hypoxia-ischemia brain injury to clinical dementia revealed the dysregulation of energy metabolism, mitochondrial dysfunction, neuro-inflammation, synaptic failure, etc. [24–26, 50]. Further, the activity of α-ketoglutarate dehydrogenase appears to be inhibited in the cerebral cortex of AD patients, and there are substantial evidences indicating that the function of the Krebs cycle is impaired in AD brains [51, 52]. The impact of hypoxia and the γ-aminobutyric acid (GABA) shunt activation in the pathogenesis of AD has been reviewed by Salminen et al. [51]. Restated, neurodegeneration is caused by a progressive cycle of hypoxic-ischemic brain injury that induces DPMs, protein misfolding, and aggregation, leading to cognitive decline and dementia. Hypoxia-inducible transcription factor (HIF) is the key inducer of hypoxia-responsive genes that functions during general development and pathological processes in association with decreased oxygen availability. In hypoxic condition, HIF is accumulated while it is rapidly degraded in normoxic cells. HIF prolyl 4-hydroxylases (HIF-P4Hs, commonly known as PHDs and EglNs) act as oxygen sensing.

Recent studies suggest that neurodegeneration is caused by progressive cycles of hypoxia-ischemic brain injury that induces DPMs, protein misfolding, and aggregation. These processes result in cognitive decline and dementia. The molecular events that drive this proteinopathy preceding dementia symptoms have not yet been well identified. However, unbiased, global, discovery-driven approaches such as proteomics have the potential to uncover the complex molecular pathology of human proteinopathies including dementia. Our groups adopted systematic proteomic studies to investigate hypoxia effects on neuronal cell lines, animal models of ischemic brain injury, human blood plasma samples, and postmortem brain tissues from patients affected by stroke or dementia [47–50, 53–57]. We and other

investigators [53–57] have yielded a good progress in understanding how protein DPMs, and protein aggregation induced by hypoxic-ischemic brain injury can promote neurodegeneration in dementia. This "vicious cycle" of brain tissue damage is summarized in **Figure 2**.

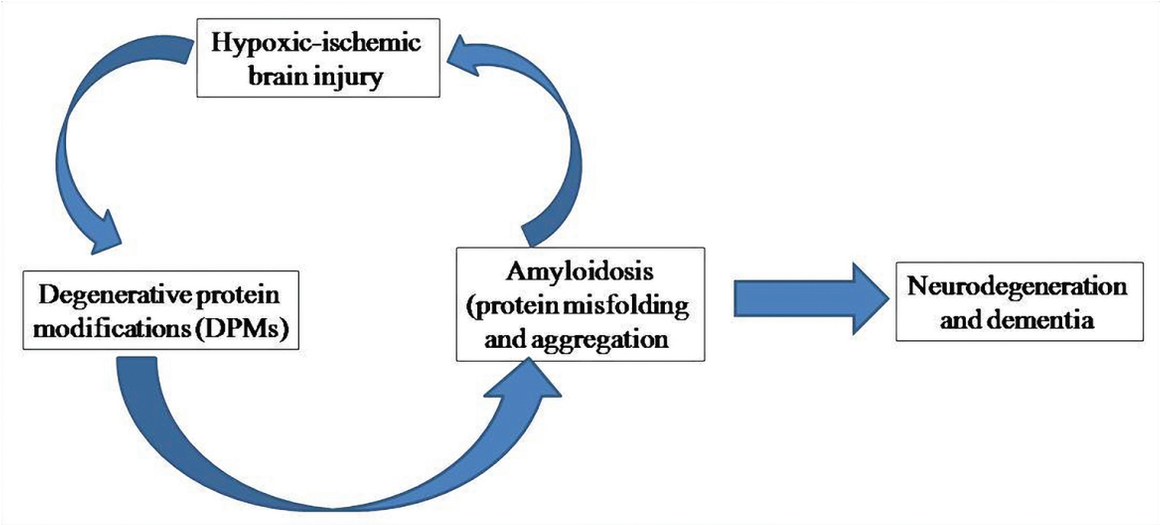

Figure 2. Vicious cycle of hypoxia-ischemic brain injury, degenerative protein modifications, and amyloidosis.

3. DPM studies in brain tissue from dementia patients

DPMs caused by spontaneous chemical reactions can radically alter protein structure and function, promoting pathological progression. Key DPMs including oxidation, deamidation, racemization, glycation, advanced glycation end products, and enzymatic modifications such as citrullination typically alter the charged state and hydrophobicity of the affected protein. This changes charge and hydrophobic nature of protein-promoting protein misfolding and aggregation. Despite the clinical importance of DPMs in neurodegenerative diseases, the mechanism and cause of modifications are poorly understood, largely due to the technical challenges. To define the role of DMPs, accurate identification of protein modification sites is important. However, it is important to avoid the introduction of artificial modification during sample preparation and improve sensitivity and confidence of identifying low-abundant modifications. According to Hao et al. [27, 31], processing proteomic samples at a mild alkaline pH and prolonged incubation at 37°C during trypsin digestion were major causes of nonenzymatic asparagines (Asn)-deamidation. Therefore, these researchers proposed an improved protocol of trypsin digestion in 50 mM ammonium acetate (pH 6) to avoid introduction of artifactual deamidation during sample preparation. Moreover, a sodium deoxycholate (SDC) and ammonium acetate-based buffer (pH 6.5) have been developed to increase protein solubility, to enhance trypsin activity, and to improve the recovery of low-abundant peptides from complex biological samples. This mildly acidic conditions and absence of urea minimized artificial asparagine deamidation and prevented artifactual carbamylation [61].

http://dx.doi.org/10.5772/64693

Under physiological conditions, deamidation of the protein residues asparagines (Asn) and glutamine (Gln) can occur spontaneously and progressively alters protein structure, function, and stability over time. Asn deamidation occurs through the formation of a succinimide ring intermediate, which quickly gets hydrolyzed to D,L-Asp and D,L-isoAsp with isoAsp predominating. Deamidation of Gln occurs much slower since it is thermodynamically less favorable to form a six-membered glutarimide ring. Deamidation causes an increase in the mass of 0.984 Da. The separation of Asp- and isoAsp remains challenging since peptides containing Asp- and isoAsp display the similar mass and hydrophobicity. However, improved ERLIC-LC-MS/MS method allows distinguishing isoAsp-containing peptide from n-Asp-containing peptide prior to their identification. Protein deamidation serves as a versatile molecular clock that can regulates many biological processes. Protein modifications and their biological impacts have been recently reviewed by Hao et al. [27]. Proteins with low turnover rates accumulate nonenzymatic modifications that cannot be repaired, and thus these modifications including deamidation cause age-related changes in biological functions and play major role in aging. Deamidation has been linked with alterations in the structure of human cortical neurons [62]. An accumulation of protein α-synuclein is a pathological characteristic of dementia with Lewy bodies (DLB), PD, AD, and multiple system atrophy (MSA), and can be linked to protein deamidation. The excessive deposition of IsoAsp residues in synapsin 1 and tubulin proteins in VaD [30] suggests that deamidation of synaptic proteins impairs its function and may cause dementia.

3.1. Deamidation of ion channel and other proteins in dementia

The ion channel protein Na^+–K^+–ATPase exhibits multiple functions including the maintenance of differential membrane potential in neurons, which is an essential feature of the signal transduction processes. Dysregulation of Na^+–K^+–ATPase expression or function has been reported in both animal model and human brain tissues affected by AD, PD, and HD. In the study of human brain tissues from patients with VaD, Adav et al. [24] noted deamidation of Na^+–K^+–ATPase subunits in the evolutionary-conserved regions. Using structural model, they located the modification sites and proposed that the disruption of Mg^{2+}– and Cu^{2+}–binding sites impaired electrostatic interactions and function of ion channel proteins in VaD (**Figure 3**). Modification of residues 210 and 220 has been proposed to cause defects in protein phosphorylation and dephosphorylation mechanisms, leading to altered ATP hydrolysis. Deamidation-induced changes in Na^+–K^+–ATPase subunit proteins may lead to defects in membrane excitability and neuronal function. Moreover, the enzyme "protein L-isoaspartate (D-aspartate) O-methyltransferase" (PIMT) functions as a protein repair enzyme and has the potential to recognize these abnormal residues (isoAsp) and convert them to the normal L-Asp form. Thus, deamidation can be repaired. However, according to proteomic analysis of VaD brain tissues, PIMT was also deamidated. Deamidation of PIMT could manipulate its potential to recognize abnormal residues or impair its potential to convert isoaspartyl to the normal L-aspartyl form [24]. In mammalian cells and mouse models lacking repair enzyme PIMT, isoASP accumulation causes hyperactivation of key cell-signaling pathways, weakening animal growth and even fatal seizures [63].

During the characterization of the human brain amyloidal plaque from dementia patients, deamidation of aggregated proteins was noted. The extensively deamidated proteins were S100A9, ferritine, and hemoglobin. In addition to these proteins, proteins such as S100 calcium-binding protein B (S100-B), α2(IV), and α2(I) chains of human collagen, extracellular matrix such as laminin subunit β-2 was found to be deamidated. Further, these authors found deamidated adhesion junction plaque protein dystonin (isoform 3) and many others [34]. Proteins coronin-1A and syntaxin-binding protein 2, which were previously been implicated in the neurodegeneration of the hippocampus, were also found deamidated detected in brain tissue sample of demented patients. Deamidation introduces negative charge at sites of modification. This change in charge promotes protein aggregation and remains as a pathological hallmark of age-related disorders and neurodegenerative diseases. Thus, the multiple deamidated residues of S100A9 (**Figure 3C** and **D**) could introduce a negative charge to form pathological aggregates in the brain. Hence, an accurate identification of DMPs and modification sites is important to understand the role of DPMs in human diseases. A comprehensive investigation including method development for accurate identification of DPMs has been performed for biomedical research [24, 26, 27, 30, 31, 34, 35].

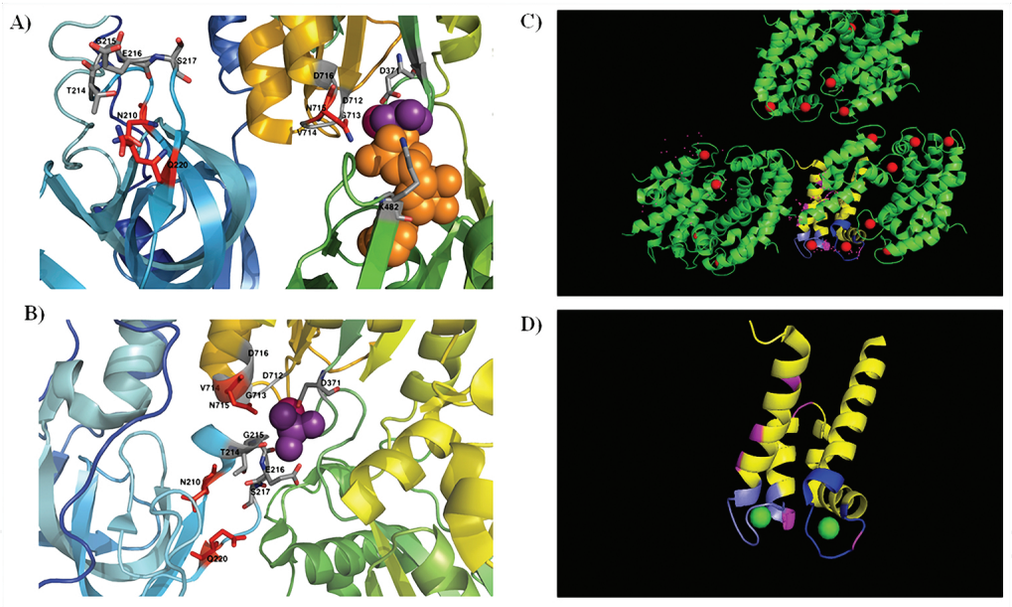

Figure 3. Structural models of Na^+/K^+-ATPase catalytic site in (A) E_1P (PDB ID 4HQJ) and (B) E_2P (2ZXE). Domain A is shown in blue and cyan color, domain P is shown in yellow, while domain N is shown in green. The deamidation sites (N210, D220, and N715) are shown in red color (adapted with permission from Adav et al. [24]). The deamidation sites of protein S100A9 (RCSB Protein Data Bank accession code: 1XK4) are shown in (C). EF hands have been displayed in yellow color and deamidation sites in magenta and blue. EF hands alone are shown in (D).

Loss of synapses is one of the most significant contributors to the cognitive impairment manifest in VaD and other neurodegenerative diseases. Following synapses loss, the remaining synapses alter their shape. According to recent literature [26], synaptic immunoglobulins were perturbed proteins in VaD temporal cortices, while SNAP25 was substantially up-regulated. Further, deamidation studies revealed that the protein synapsin 1 displayed

http://dx.doi.org/10.5772/64693

significant accumulation of deamidated asparagine and glutamine residues when compared with age-matched control [30]. The location of the modification site using structural model demonstrated that the deamidation sites in synapsin 1 were likely to induce pathological changes in protein conformation.

4. Proteomic biomarkers of dementia

Mass spectrometry-based proteomics has been widely used for biomarkers of dementia and AD [64]. Proteins such as Aβ40, Aβ42, and their ratio Aβ42:Aβ40 have been linked with AD and dementia [65]. Proteins such as Apolipoprotein E (ApoE) level in serum of AD patients [66], interleukins (IL-1α, IL-6) [67], clusterin [68], and α-1-antichymotrypsin (α-ACT) [69] have been considered as biomarkers of AD. Other than blood, cerebrospinal fluid (CSF), which directly interacts with the space of the brain and reflects biochemical changes that occurs in the brain, has also been used for the biomarker of dementia and AD. Proteins such as phospholipases A2, visinin-like 1, microtubule-associated protein *tau*, neurofilament proteins, and many more that were reviewed by Liu et al. [70] have been considered as CSF biomarkers of AD. The increase in the generation of 2,4-dihydroxybutyrate with the progression of MCI was noted and considered as a promising biomarker of AD [51, 71]. Using human CSF samples and adopting targeted approach, Shi et al [72] proposed a panel consisting of five peptides/proteins such as osteopontin (SPP1), prolow-density lipoprotein receptor-related protein 1 (LRP1), macrophage colony-stimulating factor 1 receptor (CSF1R), ephrin type-A receptor 4 (EPHA4), and metalloproteinase inhibitor 1 (TIMP1) are biomarkers of PD or AD. Alzheimer's Disease Neuroimaging Initiative (ADNI) biomarker core progress has been reviewed by Kang et al. [73].

5. Future outlook and conclusions

Dementia is a global public health challenge that requires urgent action to discover underlying molecular mechanism and to develop cure. Classical biological methods involving analyses of one or several genes have been adopted in the study of the pathogenesis of neurodegenerative disorders. However, it has become clear that neurodegenerative disorders exhibit complex interactions involving wide range of proteins. Proteomics technologies have ushered in a new era in the fields of clinical research by enabling us in identifying and quantifying disease-related protein profiles. Unbiased, global, discovery-driven approaches such as proteomics are well suited to uncover the complex pathology of human proteinopathies such as dementia. Therefore, in this chapter, we exploited state-of-the-art quantitative proteomic profiling of brain proteome, and discussed recent developments in neuroproteomics including DPMs, its impact on protein aggregation that alters protein function and causes deposition, which are key features of dementia and neurodegenerative disorders. To further understand the pathology in depth, along with discovery proteomic approach, targeted proteomics need to be applied to develop cure. In addition, commitments are needed to generate strategies,

government policies, programs, and research funding for neurodegenerative diseases. However, obtaining well-characterized clinical samples of specific brain areas remains a major limitation.

Acknowledgements

This work is in part supported by the Singapore Ministry of Education (Tier 2: ARC9/15), NTU-NHG Ageing Research Grant (ARG/14017), and the Singapore National Research Foundation under its CBRG (NMRC/CBRG/0004/2012) administered by the Singapore Ministry of Health's National Medical Research Council.

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