iTRAQ Quantitative Clinical Proteomics Revealed Role of Na⁺K⁺-ATPase and Its Correlation with Deamidation in Vascular Dementia

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Supporting Information

ABSTRACT: Dementia is a major public health burden characterized by impaired cognition and loss of function. There are limited treatment options due to inadequate understanding of its pathophysiology and underlying causative mechanisms. Discovery-driven iTRAQ-based quantitative proteomics techniques were applied on frozen brain samples to profile the proteome from vascular dementia (VaD) and age-matched nondementia controls to elucidate the perturbed pathways contributing to pathophysiology of VaD. The iTRAQ quantitative data revealed significant up-regulation of protein-L-isoaspartate O-methyltransferase and sodium–potassium transporting ATPase, while post-translational modification analysis suggested deamidation of catalytic and regulatory subunits of sodium–potassium transporting ATPase. Spontaneous protein deamidation of labile asparagines, generating abnormal L-isoaspartyl residues, is associated with cell aging and dementia due to Alzheimer’s disease and may be a cause of neurodegeneration. As ion channel proteins play important roles in cellular signaling processes, alterations in their function by deamidation may lead to perturbations in membrane excitability and neuronal function. Structural modeling of sodium–potassium transporting ATPase revealed the close proximity of these deamidated residues to the catalytic site during E2P confirmation. The deamidated residues may disrupt electrostatic interaction during E2P phosphorylation, which may affect ion transport and signal transduction. Our findings suggest impaired regulation and compromised activity of ion channel proteins contribute to the pathophysiology of VaD.

KEYWORDS: dementia, Na⁺/K⁺-ATPase, ion channel proteins, iTRAQ, mass spectrometry

INTRODUCTION

In addition to Alzheimer’s disease (AD), cerebrovascular disease is also a principal cause of age-related cognitive impairment.¹ AD and vascular dementia (VaD) are major contributors to disability, dependence, and mortality among older adults; by recent estimates, 36 million peoples are suffering with dementia worldwide with an addition of 4.6 million new cases annually.² Thus, further research is needed to understand the molecular events in the brain leading to dementia, and, in particular, the possible interlink between protein deamidation and dementia. With age, proteins undergo several spontaneous modifications that alter their structure and influence stability, folding, and biological function. Studies by Geiger and Clarke³ on aspartyl (Asp) and asparaginyl (Asn) deamidation, isomerization, and racemization revealed that L-Asp and L-Asn residues can be converted to four different isoforms (L-Asp, L-isoAsp, D-Asp, and D-isoAsp) via cyclic intermediates. Such transformations or isomerization denatures the protein, induces an autoimmune response to self-proteins, and also leads to the accumulation of potential abnormal L-isoaspartyl residues that alters their 3-D structure, stability, folding, and, finally, loss of function.⁴–⁶ The accumulation of isomerized proteins has been linked to metabolic dysfunction in neuronal cells and neurodegenerative diseases.⁷,⁸ The occurrence or accumulation of such abnormal residues has been demonstrated in development and aging⁹ and has been shown to accelerate amyloid formation and alter amylin fiber structure, which may lead to AD.¹⁰

Isoaspartate (isoAsp) formation and its accumulation is a major source of protein damage. However, methyltransferases can recognize these changes and correct them through the