Neurochemistry International 80 (2015) 87–98

Contents lists available at ScienceDirect

Neurochemistry International

journal homepage: www.elsevier.com/locate/nci

Temporal lobe proteins implicated in synaptic failure exhibit differential expression and deamidation in vascular dementia

Xavier Gallart-Palau [a], Aida Serra [a], Jingru Qian [a], Christopher P. Chen [b,c], Raj N. Kalaria [d], Siu Kwan Sze [a,*]

[a] School of Biological Sciences, Nanyang Technological University, Singapore
[b] Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore
[c] Memory, Aging and Cognition Centre, National University Health System, Singapore
[d] Institute for Ageing and Health, NIHR Biomedical Research Building, Newcastle University, Campus for Ageing and Vitality, Newcastle upon Tyne NE4 5PL, United Kingdom

ARTICLE INFO

Article history:
Received 2 September 2014
Received in revised form 26 November 2014
Accepted 2 December 2014
Available online 8 December 2014

Keywords:
Vascular dementia
Synaptic failure
Clathrin
Deamidation
Synapsin1
α/β-tubulins

ABSTRACT

Progressive synaptic failure precedes the loss of neurons and decline in cognitive function in neurodegenerative disorders, but the specific proteins and posttranslational modifications that promote synaptic failure in vascular dementia (VaD) remain largely unknown. We therefore used an isobaric tag for relative and absolute proteomic quantitation (iTRAQ) to profile the synapse-associated proteome of post-mortem human cortex from vascular dementia patients and age-matched controls. Brain tissue from VaD patients exhibited significant down-regulation of critical synaptic proteins including clathrin (0.29; $p < 1.0{\cdot}10^{-3}$) and GDI1 (0.51; $p = 3.0{\cdot}10^{-3}$), whereas SNAP25 (1.6; $p = 5.5{\cdot}10^{-3}$), bassoon (1.4; $p = 1.3{\cdot}10^{-3}$), excitatory amino acid transporter 2 (2.6; $p = 9.2{\cdot}10^{-3}$) and Ca^{2+}/calmodulin dependent kinase II (1.6; $p = 3.0{\cdot}10^{-2}$) were substantially up-regulated. Our analyses further revealed divergent patterns of protein modification in the dementia patient samples, including a specific deamidation of synapsin1 predicted to compromise protein structure. Our results reveal potential molecular targets for intervention in synaptic failure and prevention of cognitive decline in VaD.

1. Introduction

Vascular dementia is a potentially fatal condition that occurs following interruptions in blood supply to specific areas of the brain following a stroke or microinfarcts (Shih et al., 2013). VaD is the second most common form of dementia after Alzheimer's disease (AD) (Gorelick et al., 2011; Kalaria et al., 2008), and exhibits a pathology that is at least partially reversible (McVeigh and Passmore, 2006), indicating potential for new drug treatments if the underlying molecular mechanisms can be defined and suitable targets identified. At the cellular level, dementia syndromes are characterized by progressive synaptic failure among cortical neurons, which contributes to their common clinical manifestations; disorientation, defects in long-term memory, mood disorders and executive dysfunction (Herbert et al., 2014; Wesnes and Edgar, 2014). Synaptic degeneration begins with disrupted vesicular trafficking in presynaptic boutons, and progresses toward structural malformations in the postsynaptic dendrite arborization, preceding eventual loss of neurons in the affected cortical areas (Bolay et al., 2002; Chang et al., 2013; Mufson et al., 2012). While this process is in itself well documented, the molecular mediators of these events have yet to be identified (Muresanu et al., 2014; Yao, 2004). Synaptic failure strongly correlates with the cognitive impairments manifest in VaD and AD, even when the presence of characteristic senile plaques cannot be established (DeKosky and Scheff, 1990; Overk and Masliah, 2014; Terry et al., 1991). Moreover, synaptic failure is the pathological feature most commonly shared by VaD and AD patients (Iadecola, 2013; Kalaria, 2000, 2002), consistent with the concept that loss of synapse function is a key event in the pathogenesis of dementia syndromes.

Abbreviations: iTRAQ, isobaric tag for relative and absolute quantitation; VaD, vascular dementia; AD, Alzheimer's disease; BA21, Brodmann area 21; PTM, post-translational modification; SV, synaptic vesicle; SNARE, soluble NSF attachment protein receptors family; RMSD, root mean square deviation; SEM, standard error of the mean; PDB, protein database; LTP, long-term potentiation; CBF, cerebral blood flow; CNS, central nervous system; PTM, protein posttranslational modification; IsoAsp, isoaspartic acid; Asp, aspartic acid; Asn, asparagine; GLN, glutamine; ERLIC, electrostatic repulsion–hydrophilic interaction chromatography; TEAB, triethylammonium bicarbonate; HPLC, high-performance liquid chromatography; LC–MS/MS, liquid chromathography–tandem mass spectrometry; MDLC, multidimensional liquid chromatography; TM-score, template modeling score; FDR, false discovery rate; CME, clathrin-mediated endocytosis.

* Corresponding author. School of Biological Sciences, Division of Chemical Biology & BioTechnology, Nanyang Technological University, 60 Nanyang Drive, Singapore 637551, Singapore. Tel.: +65 6514 1006; fax: +65 6791 3856.
E-mail address: sksze@ntu.edu.sg (S.K. Sze).

http://dx.doi.org/10.1016/j.neuint.2014.12.002
0197-0186/

Synaptic failure is promoted by a combination of oxidative stress, mitochondrial wreckage, neuroinflammation and excessive accumulation of misfolded proteins (Overk and Masliah, 2014). Recently, the role of the intracellular microenvironments and pH in particular has received much attention as a potential contributor to the pathology of synaptic failure. Indeed, just small changes in pH can have profound consequences for the normal function of enzymes that mediate protein posttranslational modifications (PTMs) (Chaumeil et al., 2012). Abnormal patterns of PTMs can significantly alter protein structure and function, and accumulation of isoaspartic acid (IsoAsp) residues due to dysregulated deamidation has already been implicated in the pathogenesis of neurodegenerative diseases and dementia (Desrosiers and Fanélus, 2011; Dunkelberger et al., 2012; Gaza-Bulseco et al., 2008).

Numerous critical synaptic and neuronal structural proteins including synapsins, dynamins and α/β-tubulins are highly susceptible to aspartic acid (Asp) isomerization and asparagine (Asn) deamidation events, while the Asn deamidation repair enzyme protein L-isoaspartyl methyltransferase (PIMT) is silenced in brain tissue (Qin et al., 2013). The clinical significance of these processes is illustrated by the prominent dementia-like symptoms seen in PIMT knock-out mice (Yang and Zubarev, 2010; Zhu et al., 2006). Synapsins play a critical role in neurotransmitter release via the unfastening of synaptic vesicles (SVs) from the actin cytoskeleton, thus enabling their incorporation into reserve pools in close proximity to the active zone of axon terminals (Bolay et al., 2002; Ferreira and Rapoport, 2002; Gitler et al., 2004a; Hilfiker et al., 1999). In addition, synapsins are thought to be important regulators of SV fusion with presynaptic plasma membranes (Hilfiker et al., 1999; Humeau et al., 2001), hence the normal functioning of synapsin proteins is likely to be crucial for effective synaptic transmission. Modifications to other synaptic structural proteins have also been implicated in neuron dysfunction, including the isomerization of α/β-tubulins in human temporal lobe epilepsy and in mouse models of neurodegenerative disease (Lanthier et al., 2002; Zhu et al., 2006). The excess accumulation of IsoAsp residues in α/β-tubulins has also been linked with marked alterations in the structure of human cortical neurons (Lanthier et al., 2002), and may contribute to the aberrant cerebral elongations apparent in PIMT null mice (Shimizu et al., 2002).

In the present study, we used an isobaric tag for relative and absolute quantification (iTRAQ) to profile the synaptic proteome in the temporal cortex of VaD patients. Our initial investigations revealed that the expression of PIMT enzyme is significantly dysregulated in VaD patients, hence we also performed an in-depth analysis of the deamidation profiles of synapsin1 and tubulin proteins in these samples. Using this approach, we identified numerous synaptic proteins that were differentially expressed and/or modified in VaD brain tissue compared with samples from age-matched control patients. These data shed new light on the molecular basis of VaD and may lead to the development of novel interventions that preserve synaptic function and prevent cognitive decline in human dementia patients.

2. Materials and methods

2.1. Subjects

Frozen post-mortem samples of the temporal cortex (Brodmann area 21, BA21) were obtained from VaD subjects and aged-matched controls via the Newcastle Brain Tissue Resource (NBTR) Institute for Ageing and Health (demographic details in Appendix: Supplementary Table S1). Informed consent was obtained from all study participants prior to donation of brain tissues after death. The average post-mortem delay was 24.5 and 36.5 hours for control and VaD subjects respectively.

VaD was diagnosed pathologically by the presence of features including lacunar infarcts, cortical infarcts, border-zone infarcts, micro-infarcts and small vessel disease in sub-cortical regions (Kalaria et al., 2004). None of the VaD samples displayed sufficient neuritic plaque or neurofibrillary pathology to meet the diagnostic criteria for AD. Age-matched controls had no evidence of neurological or psychiatric disease at the time of death, and samples showed either limited or no age-related Alzheimer's-type pathology. Neither VaD nor age-matched samples exhibited visible infarcts or any other lesions. Further details of the samples can also be found in Datta et al. (2014b).

2.2. Tissue preparation

All procedures were approved and performed in accordance with the ethical guidelines of the Nanyang Technological University ethics board. Ten VaD patients (mean age 84.0 ± 8.5 years) and ten age-matched controls (mean 80.3 ± 8.9 years) were used for the analyses, with all experiments performed in triplicate. In each group approx. 10 mg of brain tissue from each subject was pooled and homogenized before the proteins were purified by acetone precipitation: this is a common approach in quantitative proteomics due to the expense of iTRAQ reagents, the limited amount of sample available, and the excessive use of instrument time (Bostanci et al., 2013; Gan et al., 2007). The sample pooling strategy also reduces the impact of biological variation by reporting the averaged quantity of proteins associated with disease-specific changes (Datta et al., 2014a; Weinkauf et al., 2006).

Two hundred micrograms of protein from each replicate from each experimental group were resolved by SDS-PAGE and visualized by Coomassie Brilliant Blue staining. Total protein was excised from the gel, cut into 1 mm^2 pieces and washed with 75% acetonitrile containing 25 mM triethylammonium bicarbonate (TEAB). Following de-staining, the gel pieces were reduced with Tris 2-carboxyethyl phosphine hydrochloride (5 mM) and then alkylated with methyl methanethiosulfonate (10 mM). Gel pieces were dehydrated using acetonitrile and subjected to protein digestion at 37 °C in sequencing-grade modified trypsin (Promega, Madison, WI, USA). The peptides in gel were extracted using 50% acetonitrile, 5% acetic acid three times under ultrasound sonication. The extracted peptides were concentrated using vacuum concentrators (Eppendorf AG, Hamburg, Germany) before labeling with iTRAQ reagents.

2.3. iTRAQ labeling and LC–MS/MS analysis

The iTRAQ labeling of dried peptides from the control and disease groups was performed using 4-plex iTRAQ reagent Multiplex kits (Applied Biosystems, Foster City, CA) following the manufacturer's protocol. The control and VaD samples in each triplicate were labeled with 114 and 115 isobaric tags, respectively. Three independent iTRAQ replicates were performed to generate three sets of samples for each of the VaD and control groups. The labeled samples in each replicate were pooled together and concentrated using a vacuum centrifuge for subsequent electrostatic repulsion–hydrophilic interaction chromatography (ERLIC) fractionation and LC–MS/MS analysis. The concentrated iTRAQ-labeled peptides from the three sets were then desalted separately using Sep-Pak C18 cartridges and then ERLIC fractionated by high-performance liquid chromatography (HPLC). The iTRAQ-labeled peptides were reconstituted in buffer A (10 mM ammonium acetate, 85% acetonitrile, 0.1% formic acid) and fractionated on a PolyWAX LP column (4.6 × 200 mm, 5 μm, 300 Å) (PolyLC, Columbia, MD, USA) adopting the HPLC system (Shimadzu, Kyoto, Japan) at a flow rate of 1 ml/min (Hao et al., 2010). The 60 min gradient HPLC mobile phases consisted of buffer A (10 mM ammonium acetate, 85% acetonitrile, 0.1% acetic acid) and