Experimental Gerontology 111 (2018) 53–64

Contents lists available at ScienceDirect

Experimental Gerontology

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

Proteome profiling in the hippocampus, medial prefrontal cortex, and striatum of aging rat

Hamizah Shahirah Hamezah[a], Lina Wati Durani[a], Daijiro Yanagisawa[a], Nor Faeizah Ibrahim[b], Wan Mohd Aizat[c], Jean Pierre Bellier[a], Suzana Makpol[b], Wan Zurinah Wan Ngah[b], Hanafi Ahmad Damanhuri[b], Ikuo Tooyama[a,*]

[a] Molecular Neuroscience Research Center, Shiga University of Medical Science, Seta Tsukinowa-cho, Otsu 520-2192, Japan
[b] Department of Biochemistry, Faculty of Medicine, UKMMC, Universiti Kebangsaan Malaysia, Jalan Yaacob Latif, 56000 Cheras, Kuala Lumpur, Malaysia
[c] Institute of Systems Biology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia

ARTICLE INFO

Keywords:
Brain
Aging
Proteomics

ABSTRACT

Decrease in multiple functions occurs in the brain with aging, all of which can contribute to age-related cognitive and locomotor impairments. Brain atrophy specifically in hippocampus, medial prefrontal cortex (mPFC), and striatum, can contribute to this age-associated decline in function. Our recent metabolomics analysis showed age-related changes in these brain regions. To further understand the aging processes, analysis using a proteomics approach was carried out. This study was conducted to identify proteome profiles in the hippocampus, mPFC, and striatum of 14-, 18-, 23-, and 27-month-old rats. Proteomics analysis using ultrahigh performance liquid chromatography coupled with Q Exactive HF Orbitrap mass spectrometry identified 1074 proteins in the hippocampus, 871 proteins in the mPFC, and 241 proteins in the striatum. Of these proteins, 97 in the hippocampus, 25 in mPFC, and 5 in striatum were differentially expressed with age. The altered proteins were classified into three ontologies (cellular component, molecular function, and biological process) containing 44, 38, and 35 functional groups in the hippocampus, mPFC, and striatum, respectively. Most of these altered proteins participate in oxidative phosphorylation (e.g. cytochrome *c* oxidase and ATP synthase), glutathione metabolism (e.g. peroxiredoxins), or calcium signaling pathway (e.g. protein S100B and calmodulin). The most prominent changes were observed in the oldest animals. These results suggest that alterations in oxidative phosphorylation, glutathione metabolism, and calcium signaling pathway are involved in cognitive and locomotor impairments in aging.

1. Introduction

Aging is a biological process considered a significant risk factor for neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD). Recent advances in high-throughput omics technologies including transcriptomics, proteomics, and metabolomics have provided methods to further investigate at the molecular level the causes and implications of aging (Manzoni et al. 2016; Zierer et al.

Abbreviations: mPFC, medial prefrontal cortex; UHPLC, ultrahigh performance liquid chromatography; CHAPS, 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate; LC-MS, liquid chromatography-mass spectrometry; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LFQ, label-free quantification; PSM, peptide spectrum match; FDR, false discovery rate; AGC, accumulation gain control; HCD, high energy collision induced dissociation; NCE, normalized collision energy; GO, gene ontology; *E*-value, expectation value; KEGG, Kyoto Encyclopedia of Genes and Genomes; ANOVA, analysis of the variance; S.E.M., standard error of the mean; NDUFS4, NADH dehydrogenase [ubiquinone] iron-sulfur protein 4; UQCRQ, cytochrome *b*-c1 complex subunit 8; COX4I1, cytochrome *c* oxidase subunit 4 isoform 1; COX5A, cytochrome *c* oxidase subunit 5A; ATP5H, ATP synthase subunit d; ATP5I, ATP synthase subunit e; MTND4, NADH-ubiquinone oxidoreductase chain 4; MTCO1, cytochrome *c* oxidase subunit 1; PRDX1, peroxiredoxin-1; PRDX2, peroxiredoxin-2; PRDX3, thioredoxin-dependent peroxide reductase; PRDX5, peroxiredoxin-5; GLRX3, Glutaredoxin-3; GSTP1, glutathione S-transferase P; SOD1, superoxide dismutase [Cu-Zn]; GSTA3, glutathione S-transferase alpha-3; HRAS, GTPase HRas; S100B, protein S100B; NRGN, neurogranin; CALM1, calmodulin; NMDA, *N*-methyl D-aspartate; GRIN2A, glutamate receptor ionotropic, NMDA2A; GRIN2B, glutamate receptor ionotropic, NMDA 2B; GNAS, guanine nucleotide-binding protein G(s) subunit alpha isoform short; CAMK2A, calcium/calmodulin dependent protein kinase type II subunit alpha; CAMK2B, calcium/calmodulin dependent protein kinase type II subunit beta; CAMK2G, calcium/calmodulin dependent protein kinase type II subunit gamma

* Corresponding author.
E-mail addresses: hamiza49@belle.shiga-med.ac.jp (H.S. Hamezah), lina@belle.shiga-med.ac.jp (L.W. Durani), daijiroy@belle.shiga-med.ac.jp (D. Yanagisawa), nor.faeizah.ibrahim@ppukm.ukm.edu.my (N.F. Ibrahim), wma@ukm.edu.my (W.M. Aizat), bellier@belle.shiga-med.ac.jp (J.P. Bellier), suzanamakpol@ppukm.ukm.edu.my (S. Makpol), wwanzurinah@yahoo.com (W.Z.W. Ngah), hanafi.damanhuri@ppukm.ukm.edu.my (H.A. Damanhuri), kinchan@belle.shiga-med.ac.jp (I. Tooyama).

https://doi.org/10.1016/j.exger.2018.07.002
Received 9 May 2018; Received in revised form 28 June 2018; Accepted 1 July 2018
Available online 05 July 2018

2015). Recently, transcriptomic studies on aging have reported links between age-related RNA and gene expression changes with significant biological processes and cellular responses (Brink et al. 2009; Lee et al. 2000; Yang et al. 2015). Investigating the effect of aging at the proteome level may enhance our understanding of the cause and mechanisms of aging, particularly in the brain.

Proteomics study investigates the distribution, abundance, modifications, interactions, and function for sets of proteins (Aebersold and Mann 2003; Yarmush and Jayaraman 2002). A number of studies have attempted to identify age-related protein alterations in the brain across various species, including mouse (Walther and Mann 2011), rat (Ori et al. 2015), and human (Manavalan et al. 2013). Abnormalities in the proteome profile throughout aging were previously reported to be related with different processes, including glutamate regulation, protein synthesis, mitochondrial function, molecular transport, synaptic plasticity, and energy metabolism (Freeman et al. 2009; Manavalan et al. 2013; Poon et al. 2006; Stauch et al. 2015). Considering that each brain region differs in its function, the identification of proteins changing with age in different rat brain regions will provide essential data to show interactions of these proteins in aging.

Our recent study reported that increased brain atrophy in the hippocampus, medial prefrontal cortex (mPFC), and striatum occurred between middle- to late-aged in rats, which was accompanied by impairment in learning, memory, and locomotor activity (Hamezah et al. 2017). Our metabolomics analysis of middle- to late-aged rat brains identified age-related changes in 38, 29, and 14 metabolites in the hippocampus, mPFC, and striatum, respectively, which were involved in multiple pathways including glutathione and purine metabolism (Durani et al. 2017). To accompany our previous studies, we sought to identify age-related protein changes that would help us to understand molecular events of aging in the hippocampus, mPFC, and striatum, brain regions associated with learning, memory, or locomotor activity.

To date, the emergence of liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based proteomics has provided new opportunities for the large-scale comparison of the proteins between samples, with faster scanning mode, greater robustness, sensitivity, and accuracy (Scheltema et al. 2014). Hence, the current study was conducted to identify potential proteins related biomarkers in the aging brain using this platform.

2. Materials and methods

2.1. Proteomes analysis in rat brain

2.1.1. Animals

Male Sprague-Dawley rats were purchased from Clea Japan, Inc. (Tokyo, Japan). The animals were maintained at room temperature under a 12-h light/dark cycle (lights on at 8 AM). Rats were housed two per cage with ad libitum access to food and water throughout the study period. The rats were kept in transparent cages (40 cm × 25 cm × 20 cm) with non-enriched environments, therefore the possibility for the rats to exercise and explore was limited. However, animals were free to explore the cage and interact with one another. The same housing protocol was applied in a previous study where locomotor activity and exploration behavior were evaluated using open field test; the result demonstrated an age-dependent impairment in the exploration behavior of the rats (Hamezah et al. 2017).

All experimental procedures were carried out in accordance with the regulations of the Animal Care and Use Committee of Shiga University of Medical Science. The rats were divided into four age groups: 14, 18, 23, and 27 months old, and three rats were employed in each group. All of the rats used in the study were subjected to magnetic resonance imaging measurements and behavioral tests (open field, object recognition, and Morris water maze) as we reported (Hamezah et al. 2017).

2.1.2. Brain tissue preparation

The rats were euthanized with inhaled isoflurane. Each brain was rapidly removed from the skull and rinsed in ice-cold 10 mM phosphate buffered saline. The brain was then sectioned into 2.0 to 3.0 mm coronal slices using a Rat Brain Slicer Matrix (Ted Pella, Inc., CA, USA), and further dissected into three different regions, hippocampus, mPFC, and striatum, on an ice-cold plate. Each sample was transferred into individual tubes, immediately weighed, frozen on dry ice, and stored at −80 °C until analysis. The schematic overview of sample preparation workflow is summarized in Supplementary Fig. 1.

2.1.3. Protein extraction

Proteins from the rat brain tissue were extracted according to a previous study with some modifications (Drew et al. 2005). Each brain tissue was homogenized in 40 mM Tris/HCl pH 7.4, containing 3% dithiothreitol (Sigma Aldrich, St. Louis, MO, USA) and protease inhibitor cocktail (Roche, Mannheim, Germany) using an ultrasonicator (QSonica, Newtown, CT, USA). Subsequently, the homogenates were centrifuged at 20,600 ×*g*, 4 °C, for 15 min and the resulting supernatants, which contains soluble proteins, were transferred to a new tube. The pellets were solubilized in thiourea rehydration buffer (a mixture of: 1 M urea (Sigma Aldrich, St. Louis, MO, USA), 2M thiourea (Wako, Tokyo, Japan), 4% 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS) (Nacalai Tesque Inc., Kyoto, Japan), and 2% immobilized pH gradient (IPG) buffer (pH 3–10) (GE Healthcare Bui-Science, Uppsala, Sweden), containing 0.28% dithiothreitol and protease inhibitor cocktail), followed by 15 min centrifugation at 20,600 ×*g*, 4 °C. The use of IPG buffer in this mixture was to increase protein solubility (Rabilloud et al. 2007). The supernatant was then pooled with the corresponding soluble fraction. A Bio-Rad Bradford protein assay kit (Bio-Rad, California, USA) was used to quantify protein concentrations in each sample. This extraction method was also applied for preparing protein samples for western blotting analysis.

2.1.4. Protein separation

Extracted protein (50 μg) from each sample was mixed with 5× sample buffer (0.1 M Tris-HCl pH 6.8, 1% sodium dodecyl sulfate (SDS), 20% glycerol, bromophenol blue) in ultrapure water (Merck Millipore, Billerica MA, USA), denatured for 5 min at 90 °C in a thermomixer (Eppendorf, Hamburg, Germany) and resolved by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) at 120 V constant current. Each gel was washed three times with ultrapure water (Merck Millipore) for 5 min each time and stained for 60 min in Simply Blue Safe Stain (Invitrogen, USA). The gel was then washed twice with ultrapure water, 60 min for each wash.

2.1.5. In-gel digestion

In-gel digestion was conducted according to a published protocol with some modifications (Shevchenko et al. 2007). First, protein bands in the lanes for samples from the four age groups of each region and each rat were excised from the gel and fractionated into six fractions. Each fraction was diced into 1 to 2 mm pieces and transferred into microcentrifuge tubes. The gel plugs were destained in 100 μl of 50% acetonitrile in 50 mM ammonium bicarbonate, 15 min each time with shaking, repeated until the gel plugs were clear. The gel plugs were then incubated in 300 μl of 10 mM dithiothreitol in 100 mM ammonium bicarbonate for 30 min at 60 °C followed by transfer to 300 μl of 55 mM iodoacetamide in 100 mM ammonium bicarbonate for 20 min in the dark. The gel plugs were again washed three times with 1 ml of 50% acetonitrile in 100 mM ammonium bicarbonate for 20 min each time and dehydrated with 100 μl of acetonitrile. The gel plugs were dried in a vacuum concentrator (Eppendorf) for 15 min at ambient temperature. Finally, 6 ng of trypsin in 50 mM ammonium bicarbonate was added to the dried gel plugs, which were incubated overnight at 37 °C to allow for protein digestion.

After overnight digestion (approximately 18 h), the gel plugs were