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Quantitative protein profiling of hippocampus during human aging

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ABSTRACT

The hippocampus appears commonly affected by aging and various neurologic disorders in humans, whereas little is known about age-related change in overall protein expression in this brain structure. Using the 4-plex tandem mass tag labeling, we carried out a quantitative proteomic study of the hippocampus during normal aging using postmortem brains from Chinese subjects. Hippocampal samples from 16 subjects died of non-neurological/psychiatric diseases were divided into 4 age groups: 22–49, 50–69, 70–89, and >90. Among 4582 proteins analyzed, 35 proteins were significantly elevated, whereas 25 proteins were downregulated, along with increasing age. Several upregulated proteins, including transgelin, vimentin, myosin regulatory light polypeptide 9, and calcyphosin, were further verified by quantitative Western blot analysis of hippocampal tissues from additional normal subjects. Bioinformatic analysis showed that the upregulated and downregulated proteins were largely involved in several important protein-protein interaction networks. Proteins in the electron transport chain and synaptic vesicle fusion pathway were consistently downregulated with aging, whereas proteins associated with Alzheimer's disease showed little change. Our study demonstrates substantial protein profile changes in the human hippocampus during aging, which could be of relevance to age-related loss of hippocampal functions.

1. Introduction

Aging associated health, economic, and social issues are among the biggest challenges worldwide. Currently, there are approximately 177 million people aged ≥65 years in China. By 2050, it is estimated that >30% of Chinese or 390 million will be aged >65 years, and 100 million age >80 years (Liu et al., 2013). Thus aging-related diseases represent a major national burden for China (Liu et al., 2013). Aging is the major risk factor for a series of neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (Hindle, 2010). Although much research has focused on aging-related diseases, there have been few studies on the molecular biology of the aging human brain in the setting of neurodegenerative disease. Nevertheless, limited research does suggest that healthy brain aging is associated with perturbation of the protein homeostasis or deoxyribo nucleic acid damage responses that are essential for the structure and function of the nervous system (Perry et al., 2007).

The hippocampus is critical for learning and memory and is particularly vulnerable to senescence (Verret et al., 2007). Studies have shown decreases in hippocampal volume and neuronal plasticity during aging in healthy individuals, which may underlie hippocampus-dependent functional decline (Burke and Barnes, 2006). Impaired hippocampal synaptic integrity and signaling, decreased trophic factors, decreased neurogenesis, and increased neuroinflammation may contribute to the functional decline (Ojo et al., 2015), but the underlying mechanisms need to be further elucidated.

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Brain aging is accompanied by changes in protein expression in important pathways. Previous studies showed that the disruption of electron transport chain (ETC) is a major part of mitochondrial dysfunction during aging, most likely due to the perturbation of complex I and complex IV (Swerdlow, 2011). Aged brain tissue also shows evidence of increased inflammation associated with microglial activation and subsequent elevation in proinflammatory cytokines (Guest et al., 2014). Excessive proinflammatory cytokines further exacerbate oxidative stress that in turn impairs apoptosis signaling and synaptic plasticity (Dorszewska, 2013).

Proteomics is complementary to genomics. The application of quantitative proteomics using isobaric tags for relative and absolute measurements is increasingly common in neurobiological studies of aging as the field adopts more system-oriented approaches (Dayon et al., 2008; Wiese et al., 2007).

Extensive studies in animals have greatly increased our understanding of brain aging processes. However, animals generally do not develop the full spectrum of clinical or neuropathologic phenotypes of human diseases, and findings in animal models may not be replicated in human studies (Gerlach and Riederer, 1996; Nakayama et al., 2004). Furthermore, it is well recognized that genetic, environmental, and cultural factors all contribute to brain aging and disease susceptibility, and there is an extensive literature documenting Caucasian brain aging and diseases. In contrast, very limited research has been conducted with Chinese brains (Chen et al., 2003).

With the establishment of the Human Brain Bank at the Chinese Academy of Medical Sciences & Peking Union Medical College, we are now able to access fresh brain tissues with short postmortem delays from Chinese donors with well-documented clinical history and neuropathologic examinations. Here, we have used proteomic and bioinformatic strategies to investigate age-related alterations in hippocampal protein expression.

2. Materials and methods

2.1. Brains and hippocampal specimens

All brains were obtained from the Brain Bank of Chinese Academy of Medical Sciences & Peking Union Medical College, which collects brains from donors through a whole-body donation program. All donors had given informed consent for using the donated body tissue for medical research. After death, bodies were rapidly transferred to a designated autopsy facility. Brains were removed and bisected along the sagittal plane. One hemi-brain was emersion fixed in 10% phosphate-buffered formaldehyde, the other was cut into 1-cm thick coronal slices, frozen immediately and then stored in −80 °C freezer until use. The fixed hemi-brain tissue blocks were systematically sampled, paraffin-embedded, and processed for standard histologic and immunohistologic stains as recommended (Montine et al., 2012). Sections were examined from a diagnostic point of view, and neuropathologic classification and grading were made according to Montine et al. (2012).

For the present study, fresh frozen hippocampal specimens were dissected out from cases in which neuropathologic examinations of the fixed hemi-brains revealed no sufficient neuropathologic change meeting the diagnostic criteria for a certain neurologic disease (Supplementary Figure S3 and Table S1). The present study was approved by the ethics committee of The Institute of Basic Medical Sciences of the Chinese Academy of Medical Sciences (Approval Number: 009-2014).

The postmortem intervals (from the time of death to brain tissue emersion fixation or freezing) were less than 16.5 hours except for case B6 (Table S1). The age of death of the donors ranged from 22 to 98 years, and the hippocampal specimens were grouped into 4 age groups: group A (A1–A4, age = 20–49 years, age = 34 ± 13 years), group B (B1–B6, age = 50–69 years, age = 60 ± 7 years), group C (C1–C6, age: 70–89 years, age = 84 ± 5 years), and group D (D1–D5, age: >90 years, age = 94 ± 2 years).

2.2. Reagents

Idoacetamide, dithiothreitol (DTT), and urea were purchased from GE Healthcare (LC, UK). Proteinase inhibitor cocktail and sequencing-grade trypsin were purchased from Roche (BS, CH). The TMT (Tandem Mass Tag) Mass Tagging Kits were purchased from Thermo Scientific (NJ). Sequencing-grade endoproteinase Lys-C was purchased from Promega (WI). Anti-calcyphosin (ab186740), anti-transgelin (ab14106), anti-vimentin (ab92547), and anti-myosin regulatory light polypeptide 9 (MYL9, ab191393) were from Abcam, Cambridge, UK. Anti-GAPDH (M171-3) were from MBL, MA, and anti-β-actin (GTX124123) were from GeneTeX, CA. The Enhanced Chemiluminescence Kit was purchased from Millipore (MA). All the rest of the reagents were obtained from Sigma (MO).

2.3. Protein sample preparation

Protein extracts were prepared as described elsewhere with modifications (Crabb et al., 2002). Briefly, the hippocampal tissue from each case was homogenized in ice-cold homogenization buffer (8 M urea in PBS, pH 8.0, 1× cocktail, 1-mM phenylmethanesulfonyl fluoride [PMSF]). Cell debris was removed by pelleting via centrifugation at 12,000 rpm for 15 minutes at 4 °C. The supernatant was transferred to a fresh 1.5-mL tube, and protein concentration was determined using a Nanodrop 2000 (Thermo Scientific, NJ) according to the manufacturer's instructions.

2.4. Tandem mass Tag labeling

Each age group consisted of 4 cases, and 25 μg of proteins from each case assayed in parallel. Thus, for the 4 age groups A to D, 4-pooled protein extracts each containing 100 μg of proteins were obtained. Protein extracts were treated with 10-mM dithiothreitol for 1 hour at 55 °C, followed by 25-mM idoacetamide for 30 minutes in the dark at room temperature. The protein extracts were then digested with endopeptidase Lys-C (1:100 w/w) overnight at 37 °C. The protein extracts were diluted with PBS (pH 8.0) to a final urea concentration of 1.0 M and digested with trypsin (1:50 w/w) overnight. On the following day, the protein extracts were acidified with 1% formic acid (FA) and desalted with a reverse phase column (Oasis HLB; Waters, MC). The protein extracts were then dried with a vacuum concentrator and dissolved in 200-mM triethylammonium bicarbonate buffer (TEAB, pH = 8.5).

After protein digestion, peptides were labeled with the TMT 4-plex reagents (Thermo Scientific). Briefly, TMT reagent (0.8-mg TMT dissolved in 40 μL of 99.9% acetonitrile) was added to the peptide solution and reacted for 1 hour at room temperature. The labeling reaction was terminated by adding 5 μL of 5% hydroxylamine for 5 minutes. Peptides of each age group were labeled with different TMT labels: group A labeled with TMT-126, group B with TMT-127, group C with TMT-128, and group D with TMT-129. The labeled peptides from the 4 groups were then mixed, desalted, dried as before, and finally dissolved in 100 μL of 0.1% FA.

2.5. High-performance liquid chromatography separation

Labeled peptides were fractionated as described previously (Gokce et al., 2011). Briefly, TMT labeled peptides (100 μL in 0.1% FA) were transferred to MS tubes for high-performance liquid