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Temporal lobe in human aging: A quantitative protein profiling study of samples from Chinese Human Brain Bank

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ABSTRACT

The temporal lobe is a portion of the cerebral cortex with critical functionality. The age-related protein profile changes in the human temporal lobe have not been previously studied. This 4-plex tandem mass tag labeled proteomic study was performed on samples of temporal lobe from Chinese donors. Tissue samples were assigned to four age groups: Group A (the young, age: 34 ± 13 years); Group B (the elderly, 62 ± 5 years); Group C (the aged, 84 ± 4 years) and Group D (the old, 95 ± 1 years). Pooled samples from the different groups were subjected to proteomics and bioinformatics analysis to identify age-related changes in protein expression and associated pathways. We isolated 5072 proteins, and found that 67 proteins were downregulated and 109 proteins were upregulated in one or more groups during the aging process. Western blotting assays were performed to verify the proteomic results. Bioinformatic analysis identified proteins involved in neuronal degeneration, including proteins involved in neuronal firing, myelin sheath damage, and cell structure stability. We also observed the accumulation of extracellular matrix and lysosomal proteins which imply the occurrence of fibrosis and autophagy. Our results suggest a series of changes across a wide range of proteins in the human temporal lobe that may relate to aging and age-related neurodegenerative disorders.

1. Introduction

The brain is one of the most age-resilient systems in the human body. Aging of the brain is a progressive process, and chronological changes occur throughout life, making the brain more vulnerable to impairments and diseases (Jové et al., 2014). Many studies have focused on the development of diseases, but insights into the pre-disease aging process have seldom been reported (Abutalebi et al., 2014; Sala-Llonch et al., 2014). The results of such studies may be useful in the advancement of investigations into the underlying mechanisms of aging and aging related disease.

The temporal lobe is involved in establishing long-term memory and everyday memory, and is affected by aging and diseases (Squire and Zola-Morgan, 1991; Bailey et al., 2013). Accepted radiological signs of brain aging include convolutional atrophy and deepening of the cerebral sulci. These morphological changes are accompanied by discernable deficits in object recognition (Kochunov et al., 2012), which are also characteristic of neurodegenerative disorders, such as dementia. In both AD (Alzheimer's disease) and MCI (mild cognitive impairment), the greatest microstructural degradation in white matter occurs in the temporal lobe (Huang et al., 2007; Sturm et al., 2013). However, the underlying molecular basis of these changes is unknown, and further research is needed to identify the mechanisms responsible for age-related changes in the temporal lobe (Lesné et al., 2013; Scheff et al., 2011).

It is now generally accepted that human brain aging is accompanied by changes in protein profiles, which lead to impairments in key biological pathways (Perluigi et al., 2014). Proteomes represent the entire set of proteins produced in specific cell types and tissues. The term 'proteomics', first coined in 1997, refers to the large-scale study of proteins, and particularly their structures and functions (Cho, 2014; Dove, 1999). Proteomics is complementary to the transcriptional analyses which are widely used in genetic analyses, and has been used successfully for the identification and quantification of both single post-translational modification or their combinational patterns. The development of tandem mass tags (TMTs) has facilitated the use of proteomic

Abbreviations: TMT(s), tandem mass tag(s); CNP, 2′,3′-cyclic-nucleotide 3′-phosphodiesterase; PLP1, proteolipid protein 1; MBP, myelin basic protein; FN1, fibronectin 1.

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approaches in research including in neurological studies of aging (Licker and Burkhard, 2014).

Here, we describe a 4-plex TMT labeled proteomic study using tissue samples from the Chinese Human Brain Bank, and identify changes in protein expression and in associated signaling pathways in aged temporal lobe in a Chinese population, with the goal of identifying potential therapeutic targets for treatment and prevention of neurodegenerative disorders.

2. Materials and methods

2.1. Temporal lobe specimens

The temporal lobe autopsy specimens used in this study were supplied by the Chinese Human Brain Bank of Peking Union Medical College. None of the donors used in the study had any evidence of neurodegenerative disease (Table S1). Written informed consent was obtained either from the donor or a close relative. The 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 interval between the donor's death and brain autopsy and specimen collection was ≤26 h. Temporal lobe specimens were stored at −80 °C. The age of death ranged from 22 to 96 years. Temporal lobe specimens were divided into four age groups: Group A (the young, age: 34 ± 13 years); Group B (the elderly, 62 ± 5 years); Group C (the aged, 84 ± 4 years) and Group D (the old, 95 ± 1 years; see Fig. 1 for more details). The Alzheimer's disease "ABC" scoring system was used for neuropathological assessment of the postmortem human brains (Montine et al., 2012). Donor histories showed no indication of known neurological disease during their lifetimes, and the corresponding samples used in this study were diagnosed as none or "Low" level of AD neuropathology according to the "ABC" scoring system. Detailed information for each temporal lobe specimen is given on Table S1 and Fig. S1.

2.2. Reagents

Reagents and kits were from commercial sources. Iodoacetamide, dithiothreitol and urea were from GE Healthcare, LC, UK. BCA protein assay kit and TMT Mass Tagging Kits were from Thermo Scientific, NJ, USA, and protease inhibitor cocktail and sequencing-grade trypsin were from Roche, Basel, Switzerland. Sequencing-grade endoproteinase Lys-C was from Promega, WI, USA. Anti-Filamin A (ab76829), anti-Myosin 11(ab133567), anti-Desmin (ab32362), anti-Calcyphosin (ab186740), anti-PPT1 (Palmitoyl-protein thioesterase 1, ab89022), anti-Transgelin (ab14106), anti-Profilin 2 (ab55611), anti-CNP (2′,3′-cyclic-nucleotide 3′-phosphodiesterase, ab6319), anti-MBP (myelin basic protein, ab62631) and anti-Vimentin (ab92547) were from Abcam, Cambridge, UK. Anti-GAPDH (M171-3) was from MBL, MA, USA. Anti-β-actin (GTX124123) was from GeneTeX, CA, USA. Enhanced

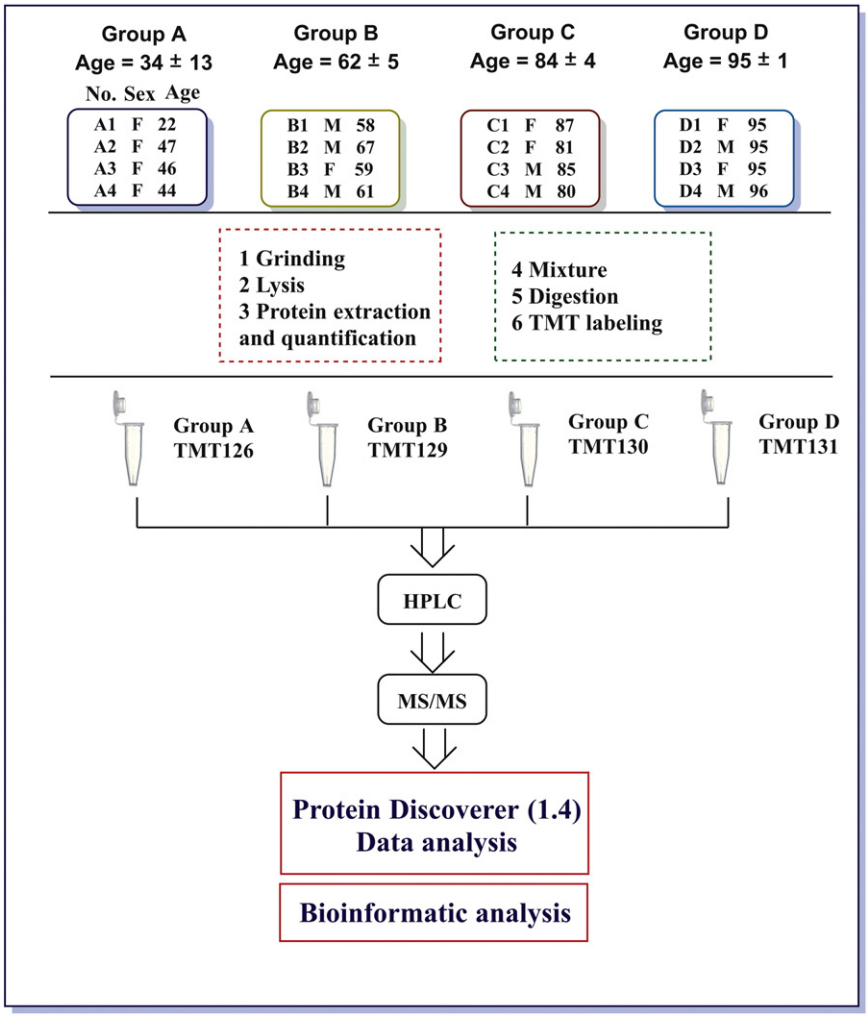

Fig. 1. Experimental workflow for protein profiling of the human temporal lobe.