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Proteomic profiling of brain cortex tissues in a Tau transgenic mouse model of Alzheimer's disease

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ABSTRACT

Alzheimer's disease (AD) involves regionalized neuronal death, synaptic loss, and an accumulation of intracellular neurofibrillary tangles and extracellular senile plaques. Although there have been numerous studies on tau proteins and AD in various stages of neurodegenerative disease pathology, the relationship between tau and AD is not yet fully understood. A transgenic mouse model expressing neuron-specific enolase (NSE)-controlled human wild-type tau (NSE-htau23), which displays some of the typical Alzheimer-associated pathological features, was used to analyze the brain proteome associated with tau tangle deposition. Two-dimensional electrophoresis was performed to compare the cortex proteins of transgenic mice (6- and 12-month-old) with those of control mice. Differentially expressed spots in different stages of AD were identified with ESI-Q-TOF (electrospray ionization quadruple time-of-flight) mass spectrometry and liquid chromatography/tandem mass spectrometry. Among the identified proteins, glutathione S-transferase P 1 (GSTP1) and carbonic anhydrase II (CAII) were down-regulated with the progression of AD, and secerin-1 (SCRN1) and V-type proton ATPase subunit E 1 (ATP6VE1) were up-regulated only in the early stages, and down-regulated in the later stages of AD. The proteins, which were further confirmed by RT-PCR at the mRNA level and with western blotting at the protein level, are expected to be good candidates as drug targets for AD. The study of up- and down-regulation of proteins during the progression of AD helps to explain the mechanisms associated with neuronal degeneration in AD.

1. Introduction

Alzheimer's disease (AD) is a progressive, degenerative neurological disorder, meaning that the disorder gets worse over time. AD is the most common form of dementia in the elderly, affecting more than 20% of those aged over 80 years.

Neuropathologically, AD is defined by loss of synaptic connections, regionalized neuronal death, extracellular deposition of ß-amyloid (Aß) protein (forming senile plaques), and intracellular precipitation of hyperphosphorylated tau protein (forming neurofibrillary tangles [NFTs]) within selective brain regions [1–3]. These tangles and plaques together act to disrupt brain function and are known to increase in quantity with the progression of AD.

The exact biochemical mechanism of the pathogenesis of AD is still unknown, but several hypotheses have been proposed to explain AD pathogenesis, including amyloid cascade, excitotoxicity, oxidative stress, and inflammation [4,5]. Several lines of evidence suggest an important role for oxidative stress in the pathogenesis and/or progression of AD. Protein oxidation, lipid peroxidation, and nucleic acid and carbohydrate oxidation products have been found to be elevated in the brain in AD. In addition, levels of antioxidant enzymes were found to be diminished in brains with AD, which strongly supports the role of oxidative stress in AD; the products of oxidative stress have been found in Aß-rich regions such as the cortex and hippocampus.

Previous studies of brain dysfunction in AD have only included a small number of samples from patients. In addition, studies that failed to find an enhancement effect in AD used stimuli lacking semantic coherence (e.g. lists of unrelated words, some that were emotional and others that were neutral). To circumvent these limitations, the present study examined a large number of transgenic (Tg) animal models over-expressing tau protein, followed by paired helical filament (PHF) formation, and investigated the modified protein expression levels in the cerebral cortex. The cortex is a

Abbreviations: 2-DE, 2-dimensional gel electrophoresis; AD, Alzheimer's disease; ANOVA, analysis of variance; CSF, cerebrospinal fluid; ESI-Q-TOF MS/MS, electrospray ionization quadruple time-of-flight mass spectrometry; IEF, isoelectric focusing; KFDA, Korea Food & Drug Administration; LC-MS/MS, liquid chromatography/tandem mass spectrometry; NDPK, nucleoside diphosphate kinase; NFT, neurofibrillary tangle; PHF, paired helical filament.

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region that plays an important role in memory and cognitive function. Signs of AD are first noticed in the entorhinal cortex, and then proceed to the hippocampus and other cortical regions. The level of proteins in the cortex may provide a better understanding of the pathological changes that occur.

Recently, neuroproteomics surveys provide a framework for large-scale protein research in AD, including the discovery of AD biomarkers, and a far-reaching systems biology understanding of how the brain responds to AD. The proteomic alterations in brain homogenates of two different ages of tau-Tg mice displaying PHFs, which were obtained from KFDA (Korea Food & Drug Administration), were examined.

In this study, 2-dimensional gel electrophoresis (2-DE) was used to evaluate the brain proteomes of these mice. Differentially expressed proteins in AD compared with healthy control subjects were identified using ESI-Q-TOF MS/MS and LC-MS/MS analysis.

2. Materials and methods

2.1. Animals

A Tau transgenic mouse expressing neuron-specific enolase (NSE)-controlled human wild-type tau (NSE/htau23) was obtained from the Division of Laboratory Animal Resources, Korea FDA (Food and Drug Administration), National Institute of Toxicological Research. According to their procedures [6], the pNSE/htau23 gene was constructed by inserting the human wild type tau cDNA (htau23) (GenBank accession No. J03778) linked to the NSE promoter and microinjecting the gene into the male pronucleus of a fertilized mouse egg. The egg was obtained by mating a female BDF1 mouse with a male BDF1 mouse. The injected eggs were then transferred into the oviducts of the pseudopregnant ICR recipient females. Then, the transgenic founder mice were crossed with the parental strain of the BDF1 background to establish the transgenic lines. 9-week-old transgenic and non-transgenic littermate mice were brought and acclimatized to the new laboratory conditions for 3 weeks following their arrival. The animals were fed a standard laboratory chow and water was freely available. The mice were maintained under the standard condition of a 12-h light–dark cycle, temperature at 23 ± 1 °C and humidity at 50 ± 10%. All animal experiments were approved by the Committee for the Care and Use of Laboratory Animals of Korea University.

2.2. Sample preparation

Mice were sacrificed through euthanasia using carbon dioxide gas at 6 and 12 months, respectively. The cortex samples were taken from the brain of the mice at 6 and 12 months. We use PlusOne Sample Grinding Kit (GE Healthcare) designed for the grinding of small tissue or cell sample for protein extraction. Extraction solution consisted of 8 M urea, 2% CHAPS, 40 mM DTT, 0.5% IPF buffer, and 2% protease inhibitor. After vortexing to resuspend the grinding resin, add up to 100 mg of the cerebral cortex to the tube. A disposable grinding pestle is used to grind the sample for 1 min. Cellular debris and grinding resin are by centrifugation for 10 min at maximum speed, carefully removed transfer the clear supernatant to another tube. The protein concentration of the homogenates was measured by the modified Bradford method with BSA as standard and then stored at 70 °C until further use [7].

2.3. PCR analaysis

The transgenes were identified by DNA-PCR analysis of the genomic DNA isolated from the tails of 4-week-old mice [8]. The quantity of isolated genomic DNA was measured by NanoDrop (Dea Myung Science Co. Ltd.) at 260 nm. After 25 cycles of amplification, the levels of the htau23 products (1112-bp) were quantified on 1% agarose gels. The total RNA from the brain cortex was extracted by Trizol (Life Technologies). In order to eliminate any contamination with genomic DNA, DNase I (Invitrogen) was utilized in accordance with the manufacturer's protocols. *Gstp1*, *CaII*, *Atp6v1e*, and *Scrn1* primers used in this study were obtained from COSMO (Korea). Initial denaturation at 94 °C for 2 min, followed by denaturation at 94 °C for 1 min, annealing at primer specific temperatures for 45 s (Table 1), and extension at 72 °C for 1 min; followed by a final extension of 72 °C for 1 min. PCR products were separated via electrophoresis in 1% agarose gel.

2.4. Isoelectric focusing

Ready-to-use Immobiline DryStrips (24 cm, pH 3–10 NL) were used for IEF. The DryStrips were rehydrated for 5 h in 450 mL of solubilization solution containing 8 M urea, 2% CHAPS, 40 mM DTT, 0.5% IPG buffer, a trace of bromphenol blue, together with the sample (100 μg). IEF was conducted using the IPGphor IEF system system (GE Healthcare) at 200 V for 30 min by step-n-hold, 500 V for 1 min by gradient, 8000 V for 1 h by gradient and constant of 8000 V until approximately 146,000 Vh were reached [9].

2.5. SDS–PAGE

The gel strips were equilibrated twice for 10 min with gentle shaking. The first equilibration solution was 50 mM Tris–HCl buffer (pH 8.8) containing 6 M urea, 20% glycerol, 2% SDS and 1% DTT in order to diminish electroendosmotic effects which result in a reduced protein transfer from the first to the second dimension. In the second equilibration buffer, DTT was replaced with 2.5% iodoacetamide (IAA). After equilibration, the IPG strips were slightly rinsed, then applied to 12.5% homogeneous SDS–PAGE gels (26×20 cm^2) and overlaid with a solution of 0.5% agarose with a trace of bromophenol blue. Second-dimensional SDS–PAGE was conducted using the Ettan DALT II system (GE Healthcare) at 55 V for 1 h, 160 V for 1 h and 330 V for 4 h.

2.6. Silver staining

Proteins were visualized using the silver staining method as described previously [9,10] with some modifications.

2.7. Image analysis

The spot detection, volume calculation and comparasion analysis were performed using Image Master 2D Platinum Software Ver. 6.0 (Amersham Pharmacia Biotech). Expression levels of the spots were determined by the percentage volume of each spot. For each spot, the relative volume intensity was averaged and expressed as a mean ± standard error of the mean (SEM). The spots differently expressed were selected by one-way analysis of variance (ANOVA) followed by Bonferroni's test ($P < 0.05$).

2.8. LC–MS/MS

Proteins were subjected to in-gel trypsin digestion [11], and excised gel spots were destained with 100 μL of destaining solution (1:1 = 30 mM potassium ferricyanide: 100 mM sodium thiosulfate, v/v) with shaking for 10 min. After removal of the solution, gel spots were incubated with 200 mM ammonium bicarbonate for 20 min. The gel pieces were dehydrated with 100 μL of acetonitrile and dried in a vacuum centrifuge for 20 min. The dried gel pieces were kept in 20 μL of 50 mM ammonium bicarbonate containing 0.2 μg modified trypsin (Promega Corp.) overnight at 37 °C for

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