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Proteomic analysis of the hippocampus in Alzheimer's disease model mice by using two-dimensional fluorescence difference in gel electrophoresis

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HIGHLIGHTS

- We perform the proteome for $APP_{E693\Delta}$-transgenic mice. Methods are two-dimensional fluorescence difference in gel electrophoresis and mass spectrometry techniques. The expression of 14 proteins are changed in the brain. Aβ oligomers contribute to the expression of proteins.

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ABSTRACT

We previously identified the E693Δ mutation in amyloid precursor protein (APP) in patients with Alzheimer's disease (AD) and then generated APP-transgenic mice expressing this mutation. As these mice possessed abundant Aβ oligomers from 8 months of age but no amyloid plaques even at 24 months of age, they are a good model to study pathological effects of amyloid β (Aβ) oligomers. The two-dimensional fluorescence difference in gel electrophoresis (2D-DIGE) technology, using a mixed-sample internal standard, is now recognized as an accurate method to determine and quantify proteins. In this study, we examined the proteins for which levels were altered in the hippocampus of 12-month-old $APP_{E693\Delta}$-transgenic mice using 2D-DIGE and liquid chromatography–tandem mass spectrometry (LC–MS/MS). Fourteen proteins were significantly changed in the hippocampus of $APP_{E693\Delta}$-transgenic mice. Actin cytoplasmic 1 (β-actin), heat shock cognate 71 kDa, γ-enolase, ATP synthase subunit β, tubulin β-2A chain, clathrin light chain B (clathrin) and dynamin-1 were increased. Heat shock-related 70 kDa protein 2, neurofilament light polypeptide (NFL), stress-induced-phosphoprotein 2, 60 kDa heat shock protein (HSP60), α-internexin, protein kinase C and casein kinase substrate in neurons protein 1 (Pacsin 1), α-enolase and β-actin were decreased. Western blotting also validated the changed levels of HSP60, NFL, clathrin and Pacsin 1 in $APP_{E693\Delta}$-transgenic mice. The identified proteins could be classified as cytoskeleton, chaperons, neurotransmission, energy supply and signal transduction. Thus, proteomics by 2D-DIGE and LC–MS/MS has provided knowledge of the levels of proteins in the early stages of AD brain.

1. Introduction

AD is neuropathologically characterized by abnormal accumulation of extracellular amyloid plaques and intracellular neurofibrillary tangles throughout cortical and limbic regions. Although the current amyloid cascade hypothesis [6] and tau hypothesis [15] provide frameworks for studying AD pathogenesis. Recently, diverse lines of evidence suggest that Aβ peptides play more important roles in AD pathogenesis [13,16,20]. Especially, soluble oligomers of Aβ could be a cause of synaptic and cognitive dysfunction in the early stages of AD. To address the relationship between Aβ oligomers and pathological features of AD, we generated APP transgenic mice expressing the E693Δ mutation, which enhanced Aβ oligomerization without fibrillization [25]. It might provide a clue for elucidating AD pathology caused by Aβ oligomers to analyze the $APP_{E693\Delta}$-transgenic mice.

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One of the most utilized approaches in proteomics to quantify and identify proteins is two dimensional gel electrophoresis (2DE) and mass spectrometry (MS) [5]. Proteomic approaches were most widely based on methods using differential expression on 2D-PAGE gels, or more recently 2D chromatography, followed by mass spectrometry protein identification. Compared to these conventional analyses, 2D-DIGE has higher reproducibility and sensitivity because of its internal standard design which minimizes gel-to-gel variation, improves spot matching, reduces number of gels needed, and permits quantitative analysis of small sample amounts.

In this study, we studied the altered expression of proteins in the hippocampus of $APP_{E693\Delta}$-transgenic mice using 2D-DIGE and LC–MS/MS approach. This approach revealed that the levels of at least 14 proteins were altered in the hippocampus of 12-month-old $APP_{E693\Delta}$-transgenic mice. These findings suggest that Aβ oligomers might cause synaptic and cognitive dysfunction by affecting the expression of these proteins in the hippocampus.

2. Experimental procedures

2.1. Materials

Sodium dodecyl sulfate, urea, thiourea, CHAPS, dithiothreitol, iodoacetamide, bromophenol blue, and RNase A and DNase I for SDS-PAGE or 2DE were all obtained from Wako Pure Chemical Industries (Osaka, Japan). Source information for all other assay reagents and materials are incorporated into their respective assay methods described below.

2.2. Animal subjects

Transgenic mice expressing human APP_{695} with the APP E693Δ mutation under the mouse prion promoter were used [25]. Heterozygous human $APP_{E693\Delta}$-transgenic mice and age-matched non-transgenic littermates were sacrificed at 12 months of age, and their hippocampi were isolated on an ice-cold plate. Animal care and handling were performed strictly in accordance with the Guidelines for Animal Experimentation at Kobe Gakuin University and Himeji Dokkyo University. Every effort was made to minimize the number of animals used and their suffering.

2.3. Protein labeling with CyDyes

Equal amounts of total protein from 4 hippocampi of $APP_{E693\Delta}$-transgenic mice or age-matched non-transgenic littermates were separately pooled. Protein samples were labeled with CyDyes (GE Healthcare, Piscataway, NJ), as per manufacturer's instructions. In brief, 50 μg of total protein from each sample was mixed in a tube and labeled with Cy2 minimal dye, and 50 μg protein taken from the mix was used as an internal standard on each gel for the three subsequent 2DE and image analysis. In parallel, 50 μg protein from each sample was labeled with either Cy3 or Cy5, and the dyes scrambled within each group to avoid possible dye bias. As a result, one replicate was Cy3 labeled proteins and another replicate was Cy5 labeled proteins. Two replicates (Cy3 and Cy5 labeled samples) were mixed, divided and applied each three independent gels. The sample volumes were adjusted to 18 μL with labeling buffer (7M urea, 2 M thiourea, 4% CHAPS, 30 mM Tris), followed by addition of 1 μL dye (working solution) to each individual sample. The samples were left on ice for 30 min in the dark, followed by adding 1 μL of 10 mmol/L lysine to stop the reaction.

2.4. 2D electrophoresis and image analysis

One sample from each of the CyDye groups was mixed together and adjusted to final concentrations of 1% DTT, 1% IPG buffer at a total volume of 350 μL with lysis buffer (7 M urea, 2 M thiourea, 4% CHAPS) and was used to 24 cm pH 4–7 IPG strips (non-linear; GE Healthcare, Piscataway, NJ) overnight. First dimension isoelectric focusing (IEF) was carried out with IPGphor II (GE Healthcare, Piscataway, NJ). Second dimension SDS-PAGE was performed by mounting the IPG strips onto 20 × 26 cm 12.5% DIGE gels (GE Healthcare, Piscataway, NJ) using Ettan DALT six Large Electrophoresis System (GE Healthcare, Piscataway, NJ) and running the gels at 16 mA/gel for the initial hour and 25 mA/gel at 25 °C constantly until bromophenol blue reached the bottom of the gel. The lysates were labeled at the ratio of 50 μg proteins: 400 pmol Cy3 or Cy5 protein-labeling dye (GE HealthcareBiosciences) in dimethylformamide according to the manufacturer's protocol.

In summary, three analytical gels were completed in total, running 25 μg of pooled reference sample labeled with Cy2, along with two samples (25 μg each), one labeled with Cy3 and the other labeled with Cy5. Gels selected for picking were stained with Deep purple (GE Healthcare, Piscataway, NJ). Approximately 1100 spots were matched across all three analytical gels. The analytical gel was picked using an automated robotic system, Ettan Spot picker (GE Healthcare, Piscataway, NJ). The pick list was created based on the Deep purple image. 2 mm gel plugs were picked, washed, reduced and alkylated, and then digested with trypsin, and the resulting peptides were extracted. Gel trypsinization was performed as previously described [24].

2.5. LC/MS/MS identification

Trypsinized peptides were analyzed by nano LC/MS/MS on a ThermoFisher LTQ Orbitrap XL. In brief, 30 mL of hydrolysate was loaded onto a 5 mm 675 mm ID C12 (Jupiter Proteo, Phenomenex) vented column at a flow-rate of 10 mL/min. Gradient elution was conducted on a 15 cm by 75 mm ID C12 column at 300 nL/min. A 30 min gradient was employed. The mass spectrometer was operated in a data-dependent mode, and the six most abundant ions were selected for MS/MS. Mass spectrometry results were searched using Mascot (www.matrixscience.com). Samples were processed in the Scaffold algorithm using DAT files generated by Mascot. Parameters for LTQ Orbitrap XL data require a minimum of two peptide matches per protein with minimum probabilities of 90% at the protein level.

2.6. Western blotting

Approximately 25 μg of protein from mouse hippocampus was applied to a 12.5% acrylamide gel and SDS-polyacrylamide gel electrophoresis was performed at 17.5 mA/gel for 2 h in second dimension. The gels were transferred onto PVDF membranes (Pall Corporation, Pensacola, FL, USA), in a trans-blot electrophoresis transfer cell (Nihon Eido, Tokyo, Japan). Western blotting was performed by using monoclonal antibodies against β-actin (diluted 1:1000, Cell Signaling, USA) and clathrin (diluted 1:250, Abcam, USA), polyclonal antibodies HSP60, NFL, voltage-dependent anion-selective channel protein 1 (VDAC) (diluted 1:1000, Cell Signaling, USA) and Pacsin 1 (diluted 1:500, Millipore, USA). Peroxidase-conjugated antibody (diluted 1:5000, Abcam, USA) was used as secondary antibody. The reaction was detected by chemiluminescence with ECL reagents (Pierce Biotechnology, USA). A semi quantitative analysis based on optical density was performed by ImageJ software (available at http://www.rsbweb.nih.gov/ij).

3. Results and discussion

The 2D-DIGE gels of the hippocampi from wild type and $APP_{E693\Delta}$-transgenic mice pools were shown as Fig. 1. Two replicates of each pooled sample were run, labeling one replicate with