学位論文の要旨

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学　位　論　文　名: Time-Dependent Analysis of Plasmalogens in the Hippocampus of an Alzheimer's Disease Mouse Model: A Role of Ethanolamine Plasmalogen.

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論文内容の要旨

INTRODUCTION

Plasmalogens (Pls) are important glycerophospholipids with 1-O-vinyl ether bonds at their sn-1 position. Recent studies have revealed that Pls are involved in many biological functions such as membrane formation and fusion, antioxidation, phagocytosis, neuronal and lymphatic cell survivability, and organization and stability of lipid rafts. Patients with peroxisomal diseases, neurodegenerative diseases, and cancer were shown to have altered levels of Pls. However, the role of Pls metabolism in the pathology of these diseases remained unknown.

Alzheimer's disease (AD) is a neurodegenerative disease characterized by extracellular amyloid β (Aβ) peptide deposition as plaques and intracellular neurofibrillary tangles. In AD brain, plaque-induced reactive oxygen species (ROS) increase peroxisomal dysfunction and decrease peroxisomal Pls synthesizing enzymes, like glycerone-phosphate O-acyltransferase (GNPAT) leading to reduced Pls levels. On the other hand, reduced Pls level increase amyloidogenic pathway resulting in the enhancement of Aβ plaque deposition. Hence, it is important to understand the changes of Pls during different stages of AD and its relationship with oxidative stress during the progression of the disease.

Several methods, including thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), or enzymatic measurement methods, have been employed to measure Pls in biological samples. However, these methods cannot separate and analyze different Pls species, which could be important to understand the disease pathology. Recently, we have developed an LC-targeted multiplexed SRM/MS (selected reaction monitoring tandem mass spectrometry) method where sn-1 fatty alcohols and sn-2 fatty acids of 22 Pls-PC and 55 Pls-PE species were characterized with selected reaction monitoring approach. The concentration of each Pls species was also accurately quantified in brain tissue. This method was used for the time-course analysis of Pls species in AD model mouse and correlate the changes with ROS and GNPAT levels. Furthermore, the late endosomal marker Rab7 was evaluated to determine the association of Pls change with the phagocytosis process. We found that Pls-PE levels were increased transiently in AD model mice at 9 months of age.

MATERIALS AND METHODS

Glycerophospholipids were extracted from brain tissue of AD model mouse (J20) and wild-type mice using a previously reported high-throughput method combining methyl-*tert*-butyl ether (MTBE) extraction with mechanical homogenization instead of traditional Folch method. Precursor ion and its five product ions for each Pls species were used to develop LC-targeted multiplexed SRM/MS method for the identification and quantification of different Pls species. The quantification of Pls species was performed using the corrected area with internal standard (PC (d7-33:1), PE (d7-33:1)) calibration curves. Next, the time-course analysis of Pls species were done at 3, 6, 9, 12, 15 months in the hippocampus of J20 and WT mice with this method. ROS level was evaluated using dichlorofluorescein diacetate (DCF-DA). GNPAT and Rab7 levels were quantified by Western blotting. GNPAT localization, changes of neuronal and glial cell numbers were evaluated by immunostaining. All experiments with animals in this study were approved by the Animal Care and Use Committee of Shimane University.

RESULTS AND DISCUSSIONS

At first, we identified and characterized PC (P-36:1) (commercial standard) and PE (P-36:1) (from brain tissue). Four product ions such as phosphate-choline, sn-1 ether loss, sn-1 loss – 59, sn-2 acyl loss for Pls-PC species and neutral loss of *m/z* 141.0, sn-1 ether loss, sn-1 ether + $C_2H_8NO_3P$, neutral loss of sn-1 ether + $C_2H_8NO_3P$ for Pls-PE species in the positive mode; and one product ion such as fatty acid carboxylate ion for both Pls-PC and Pls-PE species in the negative mode were selected from the database. Next, five SRM transitions were made and parameters for each transition were optimized. Analysis with five SRM transitions showed that all peaks were detected at the same retention time (RT).

To identify different species from brain tissues, at first, precursor ion scan between *m/z* 400.0 and *m/z* 900.0 for the product of *m/z* 184.0 and neutral loss of *m/z* 141.0 were performed. Most of the expected precursor ions from these scans were not found. Thus, the precursor ions of Pls-PC and Pls-PE species containing sn-1 C16:0, C18:0 and C20:0 were selected from the database. Pls-PE species containing C18:1, C20:1 were chosen from previous reports. The precursor ions for target candidates of identification were selected from the transitions $[M+H]^+ > 184.0$ and $[M+H]^+ >$ neutral loss of 141.0 which produced good peak intensity. Five product ions, like PC (P-36:1) and PE (P-36:1), for identification were chosen from database and theoretical calculation. After analysis, 22 Pls-PC species was identified by matching the RT of at least two of five SRM transitions and 55 Pls-PE species was identified by matching the RT of all five SRM transitions. All identified species with same carbon number showed that the RTs were shorter with a higher degree of unsaturation. After identification, we quantified them by a single SRM/MS method using the transitions $[M+H]^+ > 184.0$ and $[M+H]^+ >$ neutral loss of 141.0. Importantly, this method does

not require full scan-scan MS/MS analysis, pretreatment with acid hydrolysis or use of alkali metal solvent to confirm Pls species, thus reducing the identification steps.

Next, the time-course analysis of Pls species showed Pls-PE, but not Pls-PC levels, were increased at 9 months and subsequently decreased at 15 month in J20 mice. In addition, principal component analysis of Pls-PE species could separate J20 and WT mice both at 9 and 15 months confirming the change of Pls-PE metabolism. These results suggested that the difference of Pls-PE metabolism might started at early and late stages of Aβ plaque deposition.

Peroxisomal dysfunction and ROS-mediated degradation are known to be the main reasons of Pls-PE alteration in AD brain. In this study, elevated GNPAT expression at 9 months was subsequently decreased at 15 months in J20 mice. These results suggests that GNPAT expression might elevated Pls-PE levels at 9 months. ROS levels was also increased in J20 mice except for 9 months, indicating that increased Pls-PE at 9 months might induce a protective response against early Aβ plaque deposition in J20 mice through ROS mitigation.

Further, we checked which cells might contribute to Pls-PE increase at 9 months. We found the number of microglia and astrocytes were increased, and the number of neurons was decreased at 9 months in J20 mice. Both glial cells revealed to express GNPAT. These results suggest that increased glial cells might contribute to the transient increase of GNPAT and Pls-PE at 9 months in J20 mice.

Analysis at species level showed that Pls-PE species having polyunsaturated fatty acids (PUFA) (22:4, 22:5, 22:6, 20:2, 20:3, 20:4) were increased at 9 months. These PUFA containing Pls-PE species facilitate the membrane fusion process to form phagosome and regulate phagocytosis in macrophage cell. We also found Rab7 (essential to form phagolysosome) level was increased in J20 mice at 9 months. Thus, increase Pls-PE might be involved in phagocytosis process at 9 months in J20 mice.

Finally, ROS levels were increased extensively, and GNPAT and total Pls-PE levels were decreased at 15 months when Aβ plaque deposition increased remarkably in J20 mice. These results suggest that elevated Aβ plaque deposits increase ROS levels which decrease GNPAT and Pls-PE levels in J20 mice.

CONCLUSION

Our result showed that LC-targeted multiplexed SRM/MS is very sensitive identification and quantification method for Pls Analysis. In the second study, our time-course analysis of Pls, ROS and GNPAT demonstrated that in J20 mice, Pls-PE changes at early Aβ plaque deposition might be important for counteracting ROS levels and contributing to the phagocytosis process. On the other hand, at the late stage, progressive Aβ deposition decreases Pls-PE levels, which might contribute to the increase of Aβ plaque deposition in J20 mice.

別紙

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