Tubulin polyglutamylation by TTLL1 and TTLL7 regulate glutamate concentration in the mice brain

博士（医学）Ping Yashuang

論文題目

Tubulin polyglutamylation by TTLL1 and TTLL7 regulate glutamate concentration in the mice brain

(TTLL1 および TTLL7 を介したチューブリンポリグルタミル化によるマウス脳内グルタミン酸濃度制御)

論文の内容の要旨

[Introduction]

Glutamate is the most abundant excitatory neurotransmitter in the brain. It acts in over 90% of excitatory synapses. Excess glutamate causes neurological disease. Its metabolic pathway is complicated, and the glutamate pool in neurons has not been fully elucidated.

Tubulin in neurons undergoes various post-translational modifications (PTMs). Polyglutamylation is one of the most abundant PTMs in the brain, where a polymer of different numbers of glutamates is added at the C-terminal tail of α- and β-tubulins. Polyglutamylation of α- and β-tubulins has distinct effects and is done by specific tubulin tyrosine ligase-like (TTLL) proteins. TTLL1 and TTLL7 play major roles in catalyzing polyglutamylation in the brain. In this study, we constructed pure lines of *Ttll1* and *Ttll7* knockout mice to reveal that glutamate concentration in mice brains was regulated by tubulin polyglutamylation.

[Materials and Methods]

Wild type (WT), *Ttll1*, *Ttll7,* and *Ttll1/Ttll7* double knockout mice were euthanized by cervical dislocation. Subsequently, the brain tissues were rapidly removed and frozen in dry ice powder. The frozen brain tissues were cut at a thickness of 10 μm using a cryostat microtome. Then the brain sections were thawed and mounted on indium tin oxide-coated slides at −20 °C. Transferred the frozen sections to a vacuum pump and left therein for 20 min to keep brain sections from condensation. The sections warm to room temperature during drying. The derivatization solution containing 2,4-diphenyl-pyranylium tetrafluoroborate in methanol (1.33 mg/mL), was sprayed manually to each section. After that, an automatic sprayer was used to spray 40 mg/mL of 2,5-dihydroxybenzoic acid in 50% methanol onto the samples. Matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry (IMS) was performed by Fourier-transform ion cyclotron resonance mass spectrometer (Solarix XR, Bruker Daltonics). The experimental protocols were approved by the Animal Care and Use Committee of Laboratory Animals of Kyoto University (approved number: MedKyo 08165, MedKyo 09539) and of the Hamamatsu University School of Medicine

(approved number: 2-20).

[Results]

Ttll1 and *Ttll7* knockout decreased polyglutamylated α- and β-tubulins, respectively, which change the electric properties of tubulin. The *Ttll7* gene was associated with anti-epileptic function and plays an important role in basis of mood and locomotor activity. MALDI IMS analyses of these brains showed *Ttll1* and *Ttll7* knockout increase in not only glutamate but also γ-aminobutyric acid and some other amino acids related to glutamate. These results suggest that tubulin polyglutamylation by these TTLLs acts as a pool of glutamate in neurons, and some other amino acids were modulated by the glutamate.

As morphological concerns, *Ttll1/Ttll7* double knockout reduced the number of neurofilaments in the apical dendrites of cortical neurons, resulting in the reduction of the diameter of dendritic shafts. Several microtubule-associated proteins (MAPs) and motor proteins were reduced in tubulin fraction by loss of TTLL1 and TTLL7.

Liquid chromatography-coupled tandem mass spectrometry (LC-MS/MS) quantitative analyses of glutamate indicated no difference between *Ttll7* Knockout and WT Mice. The LC-MS/MS and MALDI IMS results are inconsistent.

[Discussion]

Summarizing these findings, we speculated that polyglutamylation on tubulin mediated by TTLL1 and TTLL7 changed the binding affinity of several MAPs and motor proteins, which then regulated neurofilament alignment in dendrites. It is surprising that when we used LC-MS/MS to quantitatively analyze the glutamate in the mouse brain, there was no obvious difference between WT and *Ttll7* knockout mice. LC-MS/MS did not correspond to the results obtained by MALDI IMS. This may be due to the different detection modes of MALDI IMS and LC-MS/MS. MALDI IMS can detect *in situ* and image the distribution of the molecules. LC-MS/MS detects all of the molecules in the sample. We assumed that when *Ttll1* and *Ttll7* knockout removes the glutamate from the tubulin C-terminal tail. The glutamate is released into the cytoplasm. Then mostly stored in glutamate synaptic vesicles. The detection efficiency of MALDI IMS for glutamate was influenced by pH. When we used MALDI IMS to detect mice brains, most signals came from the lower pH of synaptic versicle. Another reason is that MALDI IMS and LC-MS/MS might detect glutamate from different intracellular and extracellular sites or differentially pooled glutamates, such as polymers or monomers.

[Conclusion]

In conclusion, our study demonstrated for the first time that *Ttll1* and *Ttll7* knockout altered the concentration of glutamate in the mouse brain. Tubulin polyglutamylation by these TTLLs acts as a pool of glutamate in neurons. *Ttll1* and *Ttll7* can be potential

therapeutic target genes for neurological diseases through the investigation of drugs modulating tubulin polyglutamylation levels.