Graduate School of Advanced Science and Engineering
Waseda University

Screening Results Reports
博士論文審査報告書

Thesis Theme
論　文　題　目

Mitochondrial Dynamics and Autophagy in the Hippocampus of Animal Model of Cognitive Aging

加齢性記憶障害モデル動物の海馬における ミトコンドリアダイナミクスおよび ミトコンドリアオートファジー

Applicant Name
申　請　者

Kosuke	KATAOKA
片岡	孝介

Department of Life Science and Medical Bioscience
Research on Solid State Bioscience

July, 2020

1. Synopsis of the thesis

The endocannabinoid system contributes to the homeostatic defense against aging and thus may counteract the progression of brain aging. The type 1 cannabinoid receptor (CB1) is mainly expressed in neuron, and one of the most abundant G-protein coupled receptors in brain. Its activity is known to decline along the aging in the brain, which impairs neuronal network integrity and cognitive functions. However, the underlying mechanisms which link to CB1 activities and memory decline are still not well known.

Mitochondrial quality control is an essential system for maintaining their integrity and profoundly influences neuronal functions. Age-dependent change of mitochondrial activities is one of the hallmarks of aging. Recent studies have suggested that CB1 regulates mitochondrial bioenergetics in tissues including hippocampus. The aim of this study was to investigate whether CB1 influenced mitochondria in hippocampal neurons using CB1 knock out mice (CB1-KO), which show rapid decline in cognitive function with advancing age.

First, it was investigated whether immunohistochemistry against phosphorylated ubiquitin (pUb) was available for measuring *in vivo* mitophagy marker in the mouse hippocampus. In the mouse hippocampus, some cells exhibiting strong signal of pUb were distributed, whereas the remainder of the cells expressed no or weak pUb signal. *PTEN-induced kinase 1* (*PINK1*)-deficient mice reduced the density of pUb-positive cells, especially in CA3 pyramidal and DG granule cell layers. pUb signal was predominantly expressed in neuron, rather than astrocyte. In addition, lipofuscin autofluorescence signal was distinguishable from pUb signal in the mouse hippocampus. Overall, pUb can be a marker for the PINK1 activity in specific hippocampal regions, but the result should be interpreted with cautions as a substantial number of pUb-positive cells was observed even in the absence of *PINK1*.

Next, the effects of CB1 deletion on mitochondrial dynamics and autophagy were examined in adult (6-7 months old) and young (1-2 months old) animals. The immunohistochemistry against pUb suggested that CB1-KO showed a reduction in mitophagy activity in CA1 pyramidal cell layer with advancing age, whereas in wild-type (WT) controls, pUb expression did not change across ages. Electron microscopic (EM) studies also indicated that mitophagy-like events declined with age in CB1-KO hippocampus, while those of WT controls were unchanged. Moreover, 2D and 3D EM analyses also revealed that the hippocampal mitochondrial morphology in CB1-KO showed elongation and interconnection with age, while adult WT animals showed fragmented and spherical mitochondria.

Collectively, these findings indicate that CB1 signaling regulates mitochondrial dynamics and autophagy in hippocampal neurons. It was hypothesized that the impairments in mitochondrial quality control could be a mechanism, which links between age-related decline in CB1 activity and loss of neuronal integrity in hippocampus.

2. Screening results

Followings are questions (Q) and answers (A) in the public hearing held on 4th June 2020:

Q1. Is there a relationship between mitochondrial morphology and their functions?

A1. It depends on the conditions surrounding mitochondria. Next study should focus on the

mitochondrial function among young and adult CB1-KO and WT controls. Now I am planning to investigate mitochondrial membrane potential as a readout of their function in acute hippocampal slices.

Q2. Are there any changes in mitochondrial morphology in aged human?

A2. Patients with Alzheimer's disease, the most common aging-related neurodegenerative disease, show mitochondria with round shape.

Q3. PINK1 mutation is associated with Parkinson's disease. Did you check dopaminergic neurons in PINK1-KO?

A3. I would like to emphasize on hippocampal neuron in this study. But, as dopaminergic neurons are sensitive to oxidative stress, mitochondrial morphology and their functions in dopaminergic neuron may be also affected in the course of aging.

Q4. If you would like to measure PINK1 activity in immunohistochemical studies, why don't you use the antibody which can directly recognize the complex of Parkin and pUb, or PINK1 itself?

A4. Commercial antibody against PINK1 is not suitable for PINK1 in mouse tissue. pUb is shown to be important for recruiting components required for mitophagy. I think that measuring pUb should be a good method for measuring PINK1 activity in the mouse tissue.

Q5. How exogenous cannabinoids can directly activate mitochondrial CB1?

A5. Endocannabinoids are lipid-based molecules, which enable them to pass through cellular membrane.

Q6. Are there any idea as to how you prevent cognitive aging from your research?

A6. Studies on cannabinoids including this study suggests that there are obviously bimodal effects (beneficial or adverse) of cannabinoids on mitochondria and thus physiological functions in brain. Understanding how cannabinoid regulates these opposite effects might lead to development of the drug delivery design of cannabinoid-derived compounds with lower risk for unwanted side effects.

Q7. Physical exercise is known to suppress cognitive aging. Can you speculate the connection among the effects of physical exercise on body metabolism, mitochondria and cognitive function?

A7. Some studies suggest that physical exercise enhances mitochondrial biogenesis. Improved mitochondrial turnover might lead to suppression of cognitive aging.

From the presentation and the subsequent question and answer session in the public hearing, the referees judge that the candidate understands the aim and the significance of the study, and meets required scholarly standards in the academic discipline. The study demonstrates that endocannabinoid signaling through CB1 receptor regulates mitochondrial dynamics and autophagy in the course of aging. This study is expected to be a benchmark study that will contribute to development of therapeutic strategies against cognitive aging. The doctoral thesis is therefore in fulfillment of the requirements for the Doctor of Science degree.

June 2020

The referees certify that:

1. We attend the public hearing for Mr. Kosuke Kataoka's doctoral dissertation held on 4th June 2020.
2. We have confirmed the Screening Results Report.

Principal referee

Toru Asahi, Dr. Sci. Waseda University,
Professor, Faculty of Science and Engineering, Waseda University

Referees

Andreas Zimmer, Dr. rer. nat. Justus-Liebig-Universität Gießen,
Deputy Rector for Research and Innovation, Rheinische Friedrich-Wilhelms-Universität Bonn

Haruko Takeyama, Dr. Eng. Tokyo University of Agriculture and Technology,
Professor, Faculty of Science and Engineering, Waseda University

Shinji Takeoka, Dr. Eng. Waseda University,
Professor, Faculty of Science and Engineering, Waseda University