Evaluation of the Role of Endoplasmic Reticulum Stress and Novel Neurodegeneration Inducing Factor PRMT8 in the Pathogenesis of Alzheimer's Disease Model Mouse

Summary of Doctoral Thesis

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[Introduction] Neurodegenerative diseases such as Alzheimer's disease (AD) and Amyotrophic Lateral Sclerosis (ALS) are caused by the progressive death of neurons in different regions of the nervous system. AD is the most common neurodegenerative disease, thus finding methods for diagnosis and treatment are crucial. The 'amyloid cascade hypothesis' is the most influential model for the pathogenesis of AD. It is proposed that deposition of amyloid-β (Aβ) (amyloid pathology) is the initial pathological event in AD, followed by hyperphosphorylation of tau (tau pathology), and finally neurodegeneration and cell death. However, the mechanistic link between "amyloid pathology", "tau pathology" and "neurodegeneration and cell death" remains unknown. Elucidating this molecular mechanism is extremely important in order to establish the target of AD pathogenesis and work towards fundamental therapeutic development.

Familial AD is caused by mutations in *APP, PSEN1* and *PSEN2* genes, which encode amyloid precursor protein (APP), presenilin 1 (PS1) and presenilin 2 (PS2), respectively. Several research groups generated AD model mice containing gene mutations associated with familial AD; these APP and/or PS1-overexpressing Transgenic (Tg) mice have been used widely as AD mouse models. However, the membrane proteins APP and PS1 are grossly overproduced in such model mice. It is reported that membrane protein overexpression results in chronic ER stress. Therefore, there is concern that the elevated ER stress phenotype might be an artifact of overexpression of APP and PS1. To overcome such a drawback, an App^{NL-F} mouse was recently developed in the laboratory of Takaomi Saido (RIKEN Center for Brain Science) utilizing a knock in (KI) strategy. An App^{NL-G-F} mouse carrying an additional mutation of App^{NL-F} mouse, and another mutant mouse in which the murine *Mapt* gene is humanized (*MAPT* KI mouse) were also generated. The MAPT KI mouse was crossbred to the single App^{NL-G-F} mouse to create a double knock in (dKI) mouse. However, the dKI mouse exhibited pathological and cognitive features similar to those of single App^{NL-G-F} mouse. They hypothesize that there are novel proteins which cause neurodegeneration by interacting with tau and is altered by amyloid pathology, and identified 25 candidates. I focused on Protein Arginine Methyltransferase 8 (PRMT 8) because among the 25 candidate proteins it is the only protein expressed specifically in the central nervous system.

Based on these research background, 2 projects were conducted to understand the pathogenesis of neurodegeneration in AD. The aim of project 1 was “Re-examining the relationship between AD pathophysiology and endoplasmic reticulum (ER) stress”. ER stress markers in several AD mouse models, including App^{NL-G-F} mouse, were evaluated by biochemical and molecular analysis. The aim of project 2 was “Examination of the effect of PRMT 8 on neuropathology”. To investigate the role of PRMT8 in AD pathogenesis, *PRMT8* gene was deleted or overexpressed in AD model mouse (dKI) and pathological features were investigated by biochemical and histological analysis.

Project 1: Re-examining the relationship between AD pathophysiology and endoplasmic reticulum stress.

[Material and method] The endoplasmic reticulum (ER) is both a major intracellular calcium store and the place where proteins entering the secretory pathway are synthesized, folded, modified, and delivered to their final cell surface or extracellular destination. A number of pathophysiological insults lead to accumulation of unfolded proteins in the ER and cause ER stress. To alleviate this stress, a signaling network called the Unfolded Protein Response is activated; upregulation of these proteins serve as markers of ER stress. Project 1 is divided to two parts: Firstly, to determine whether the ER stress response is heightened because of Aβ pathology, several ER stress markers (GRP78, PDI, CHOP, p-eIF2a and spliced XBP1) were investigated in wild-type (WT), $App^{NL\text{-}G\text{-}F}$, APP-single-Tg (APP23, Tg2576) and APP/PS1 double gene-modified AD mouse models (APP/PS1, 3xTg-AD). Secondly, to determine whether the ER stress response is heightened because of tau pathology, several ER stress markers were investigated in P301S-Tau-Tg mouse.

[Result] No difference in any of the stress markers was observed between WT, $App^{NL\text{-}G\text{-}F}$, APP23, and Tg2576. In Addition, no elevation of ER stress markers was observed between 3- and 15-month-old P301S-Tau-Tg mice. On the other hand, APP/PS1, 3xTg-AD mouse showed upregulation of some ER stress markers. These results indicate that neither Aβ deposition, APP overexpression, nor tau pathology result in detectable ER stress. It is assumed that the genetic modification of PS1 induces ER stress through a mechanism that is not related to the Aβ pathology.

Project 2: Examination of the effect of PRMT 8 on neuropathology.

[Material and method] It is important to investigate the mechanistic link(s) between amyloid and Tau pathology with neurodegeneration. PRMT8 was identified as protein that and tau interaction altered by amyloid pathology. PRMT8 is a central nervous system specific member of the PRMT family and acts as a phospholipase. Thus, PRMT8 is expected to connect amyloid and Tau pathology with neurodegeneration. However, whether PRMT8 plays a role in AD pathogenesis has not been characterized. There are three parts to Project 2. In the first part, the dKI mouse is used as AD model mouse, and *PRMT8* gene deleted dKI ($dKI^{PRMT8\text{-}/\text{-}}$ mouse) were generated by CRISPR/Cas9-mediated genome editing. In this dKI mouse, total tau and phosphorylated tau level were investigated by western blot. Astrocytosis, gliosis, neurodegeneration and cell death were analysed by immunohistochemistry. Secondly, *PRMT8* gene was introduced into dKI mouse brain using Adeno Associated Virus (AAV) vector. AAV-GFP was used as the control for the AAV injection. Resulting pathological features were

examined using similar methods. Finally, to investigate whether PRMT8-induced pathological effects depend on tau pathology, AAV-PRMT8 was injected into Tau-KO mouse and WT mouse. Again, all pathological features were similarly examined.

[Result] There was no detectable difference between dKI$^{PRMT8-/-}$ mouse and dKIPRMT8+/+ mouse. On the other hand, introducing PRMT8 into dKI mouse via the AAV vector resulted in upregulated phosphorylated tau level, activated microglia and apoptotic cell-death pathways on the brain. In addition, it also induced severe vacuole-like structure. Therefore, in order to investigate the possibility that PRMT 8 induces neurodegeneration via tau pathology, AAV-PRMT 8 was injected into Tau-KO and WT mouse brain. As a result, neurodegeneration was observed in both the Tau-KO mouse and WT mouse. Against expectations, this suggests that PRMT 8 directly induction of inflammation , ~~and~~ activation apoptotic cell-death pathways and vacuole-like structure independent of tau pathology. However, in this study, the molecular mechanism by which PRMT8 induces neurodegeneration is not revealed and further analysis is needed. PRMT8 has been reported to interact with fused in sarcoma (FUS), related to another neurodegenerative disease ALS, and methylation of FUS increases aggregation. Moreover, PRMT8 induced pathological features are recognized in ALS patients and ALS model mouse. Thus, it is necessary to analyze PRMT8 expression and function in ALS pathology as well.

[Discussion] The aim of this research was to understand the pathogenesis of neurodegeneration in AD. The first project focused on ER stress on AD model mouse. It is suggested that ER stress-induced cell death plays a key role in some neurodegenerative diseases. However, my study indicated that "amyloid pathology and tau pathology do not necessarily induce ER stress on AD model mouse". This suggests that ER stress may not be suitable as an effective therapeutic target for AD. On the other hand, mutations in the *SOD1* gene, one of the causes of familial ALS, has been shown to cause ER stress. Thus, upregulation of ER stress is considered to be strongly disease specific.

The project 2 focused on PRMT8 which was assumed to connect the pathological features found in AD. Contrary to expectations, “PRMT8 is a factor that induces neurodegeneration independent of tau pathology”. This also suggests that PRMT8-induced pathology are not specific to AD pathology, elucidation of the molecular mechanisms of PRMT8-induced neuropathology may lead to novel mechanisms of neurodegeneration.

This research provides new information on the study of neurodegenerative diseases including AD, and further work based on these findings is desirable.