論文の内容の要旨

論文題目　患者由来 iPS 細胞を用いた FTDP-17 タウ R406W 変異の病態モデル構築と病態解析

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Introduction

Mutations in the microtubule-associated protein tau (*MAPT*) gene, which encodes the tau protein, are known to cause frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17). Among the *MAPT* mutations, the R406W mutation located on exon 13 is a unique missense mutation whose patients have been reported to exhibit Alzheimer's disease (AD)-like phenotypes independent of amyloid-beta (Aβ) accumulation, rather than the more typical FTDP-17 symptoms. To date, the molecular mechanisms underlying the onset and progression of FTDP-17 remains largely unknown and there is currently no treatment effective for the disease. Therefore, the present study aimed to establish a suitable model for studying the abnormalities induced by the R406W mutant tau using patient-derived induced pluripotent stem cells (iPSCs), which are capable of providing a limitless amount of cells of any type that have the same genetic background as the actual patient. In the case of this study, the iPSCs were differentiated into neurons in order to elucidate the pathological role of tau that is critical to FTDP-17, as a basis for therapeutic development.

Results

1)　Generation of *MAPT* R406W iPSC lines and isogenic lines

iPSC lines were established from 2 Japanese FTDP-17 patients heterozygous for the *MAPT* R406W mutation ($MAPT^{R406W/+}$) using the integration-free episomal vector system. These lines were further gene-edited using CRISPR/Cas-9 to establish isogenic lines with the mutation corrected ($MAPT^{+/+}$), or homozygous for the mutation ($MAPT^{R406W/R406W}$) to assess genotype-phenotype relationship.

2) Induction of iPSCs into cortical neurons via cerebral organoid dissociation

The iPSCs were then induced into cerebral organoids, which are three-dimensional (3D) spheres that mimic human brain development and consist of a heterogenous population of neuronal cells. In order to obtain a pure population of cortical neurons, day 30 organoids were dissociated into single cells and plated onto typical 2D cultures. After 30 more days of culture, immunochemical analysis was performed, which confirmed that more than 85% of the plated cells were mature cortical neurons.

3) Biochemical characterization of R406W mutant tau

Changes in phosphorylation levels as well as fragmentation patterns of the mutant tau were investigated by western blot analysis. Results revealed that the R406W mutant tau exhibited hypophosphorylation at specific epitopes, including S409, T181, and S404, in the iPSC-derived neuronal culture. Western blot with a pan-tau antibody also revealed that the R406W mutant tau consisted of increased amounts of 48kD hypophosphorylated tau. Furthermore, the reduced tau phosphorylation levels were confirmed to be a result of less phosphorylation by multiple kinases, particularly GSK3β, as supported by *in vitro* phosphorylation assays. In terms of fragmentation, the R406W mutant tau was found to be fragmented by calpain to generate increased amounts of 35kD N-terminus tau. This increased amount of fragmentation is likely associated with the reduced phosphorylated state of the mutant tau.

4) Cellular phenotypes resulting from R406W mutant tau-induced microtubule (MT) destabilization

At the cellular level, an increased percentage of R406W tau was found to be mislocalized to MAP2-positive dendrites of the neurons. In addition, the R406W mutant neurons exhibited dystrophic axons with increased numbers of puncta and mitochondrial transport defect, as confirmed by live imaging trafficking analysis and immunochemical analysis, both of which suggest that axonal degeneration was occurring in

these neurons. These axonal phenotypes were rescued with treatment of a microtubule stabilizer, Epothilone D (EpoD), which implies that the R406W mutant tau destabilizes microtubules to trigger these phenotypes.

5) Analysis of temporal progression of phenotypes

The analyses above were performed at a single timepoint of 30 days post dissociation of the organoids. In order to assess the temporal progression of the phenotypes, the iPSC-derived neurons were also analyzed at an earlier timepoint of 10 days post dissociation. Already at this timepoint, the R406W mutant neurons exhibited reduced tau phosphorylation levels. However, the cellular phenotypes, including tau mislocalization and axonal dystrophy, were not observed yet, suggesting that changes in tau phosphorylation occurs before the emergence of the cellular abnormalities.

Discussion

The present study investigates the disease pathology caused by tau carrying the R406W mutation using iPSC-derived neurons derived from FTDP-17 patients. Several novel features that could represent some of the early hallmarks of the disease have been identified, including the hypophosphoylation of the mutant tau, increased tau fragmentation by calpain, and mitochondrial transport defect caused by microtubule destabilization. Additionally, the iPSC-derived neurons provide a physiologically relevant, human-based platform for testing drugs, such as EpoD in this case, which could ultimately translate into clinical application. In summary, the findings of this study deepen our understanding of the molecular mechanisms of FTDP-17 as well as other tauopathies, and thus, would provide a basis for novel therapeutic development.