Principal Investigator: Yuki Fujita
Grant Title: Analysis of regulatory mechanism of central nervous circuit formation by glycoproteins

Abstract

【 Purpose 】

The aim of this study is to elucidate the mechanism by which a nuclear protein undergoes glycosylation, transitions to the extracellular space, and regulates central nervous system (CNS) circuit formation. During the development of the CNS, neural circuits are established through the processes of neural progenitor cell proliferation and differentiation, axonal extension toward target cells, and the formation of synapses between axons and target cells. Impaired synapse formation, in particular, disrupts neural networks, hindering proper information transmission. The applicant has previously demonstrated that in mice deficient in the nuclear protein under investigation, synapse formation necessary for neural circuit formation is impaired, leading to abnormalities in higher brain functions. This nuclear protein has been identified as a glycoprotein (proteoglycan) that undergoes glycosylation at five specific amino acid residues. Proteoglycans are known to accumulate around neurons during brain development, forming a specialized extracellular matrix called the perineuronal net. The perineuronal net is essential for synapse formation and maintenance, and thus for CNS circuit formation. This study will generate mice with mutations introduced at the glycosylation sites of the nuclear protein and evaluate its function in the CNS using both in vitro and in vivo assays. By clarifying its role as a proteoglycan, the study aims to elucidate the role of glycoproteins in neural circuit formation.

【 Methods 】

Among the five Ser-Gly sites on the nuclear protein, the specific sites contributing to glycosylation and synaptic localization will be identified. Mutations (G>A) will be introduced into each of the Ser-Gly sites, as well as all five sites collectively. The mutated nuclear proteins, fused with the fluorescent protein GFP, will be expressed in neurons. The applicant has previously observed that GFP-tagged nuclear proteins are expressed not only in the nucleus but also in the cytoplasm and synapse-like regions on neuronal processes. Sites where synaptic localization is lost due to mutations will be identified, thereby pinpointing the Ser-Gly sequences involved in synaptic localization.

Mice with mutations (G>A) introduced at the glycosylation sites critical for synaptic localization, as identified above, will be generated. Following the CRISPR/Cas9 genome editing method, plasmids required for introducing the mutations were designed. The genotyping of the resulting mice confirmed the presence of mutations in the identified glycosylation sites. Using the brains of these mice, Nissl staining was performed to assess whether there were any notable abnormalities in brain size or laminar structure.

【 Results 】

Analysis using primary cultured neurons from mice revealed the specific Ser-Gly sites among the five that contribute to glycosylation and synaptic localization of the nuclear protein. Mice carrying mutations (G>A) at these glycosylation sites were successfully generated, and genotyping confirmed the introduction of the desired mutations. Brain tissue sections were analyzed in comparison to wild-type mice.