外 国 語 要 旨

学位論文題目　Studies of brain specific *O*-mannosyl glycan in demyelination

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In vertebrates, myelin sheaths surround neuronal axons, providing rapid saltatory conduction and axonal integrity. Multiple sclerosis (MS) is the most frequent demyelination disease in the central nervous system. Its etiology remains unclear and the current therapies for MS are not completely effective. Remyelination is a spontaneous myelin regeneration process, but in a diseased state such as MS, remyelination is inadequate. For treatment of demyelination disease, promoting remyelination is important.

The protein tyrosine phosphatase receptor-type zeta (PTPRZ) is reported to be involved in the demyelination process. In our previous study, we found that PTPRZ has a branched *O*-mannosyl glycan, which is specifically expressed in the brain. We also found that mice deficient in the *O*-mannose branching enzyme, GnT-IX, showed enhanced remyelination in the cuprizone-induced demyelination model. Furthermore, PTPRZ with branched *O*-mannosyl glycan is expressed in astrocytes, and astrogliosis is reduced in GnT-IX deficient mice as compared to wild type mice. These results suggest that this glycan could be a therapeutic target for demyelinating diseases. In the present study, we aimed to investigate in detail the expression of branched *O*-mannosyl glycan during cuprizone-induced demyelination.

First, we tried to explore the probe of this glycan. Currently, there are no antibodies or lectins that specifically recognize branched *O*-mannosyl glycans. Since this glycan has the human natural killer-1 (HNK-1) glycan at its non-reducing terminal, we synthesized the branched *O*-mannosyl-serine with HNK-1 epitope to obtain a specific antibody against the branched *O*-mannosyl glycan. Additionally, using a series of synthesized *O*-mannosyl glycans, we characterized the HNK-1 related antibodies. We selected the chemoenzymatic strategy and succeeded in synthesizing a sufficient amount of a series of HNK-1 capped branched *O*-mannosyl glycan as antigen. HNK-1 terminated branched *O*-mannosyl serine-Keyhole limpet hemocyanin conjugated immunized rabbit serum showed upregulated reactivity with branched *O*-mannosyl glycan regardless of the terminal glycan structure. However, the antibody titer was not sufficiently high. We found that HNK-1 related antibodies including Cat-315, HNK-1, and M6749 antibodies were reactive with branched *O*-mannosyl glycan with slightly different binding specificity. Using these antibodies, we analyzed the brains of cuprizone-induced demyelination mice and found that the Cat-315 antibody specifically detects reactive astrocytes.

Second, we characterized the Cat-315 positive astrocytes in demyelination model

mice. We conducted the immunohistochemical analysis of mouse brains at 0, 2, 3, 4, and 6 weeks after cuprizone feeding. Although activated astrocytes were observed after 2 weeks of cuprizone treatment, Cat-315–positive astrocytes emerged after 6 weeks. Cuprizone feeding for 6 weeks causes irreversible and severe demyelination, suggesting that Cat-315 positive astrocytes are involved in the chronic stage of demyelination. Notably, Cat-315 positive astrocytes were restricted to the corpus callosum in which demyelination mainly occurred, and showed unique morphologies with less processes and thick shapes compared to Cat-315 negative reactive astrocytes in the cortex.

Thirdly, to clarify the origin of the Cat-315 positive cells, cuprizone was administered to Olig2-CreER;ROSA-GFP and Aldh1l1-EGFP mice to specifically visualize oligodendrocyte precursor cells and astrocytes, respectively. Cell lineage analysis of Cat-315 positive cells showed that Cat-315 positive cells originated from astrocytes.

Finally, we found that in MS brain parenchyma, more Cat-315 positive astrocytes were observed in demyelinated regions.

This study suggests that branched *O*-mannosyl glycan expressing astrocytes are involved in the progressive phase of demyelination. These astrocytes and GnT-IX could be the therapeutic target for demyelinating disorders.