主論文の要約

GlcNAc6ST3 is a keratan sulfate sulfotransferase for the protein-tyrosine phosphatase PTPRZ in the adult brain

GlcNAc6ST3 は成体脳で発現するケラタン硫酸糖鎖で修飾されたプロテインチロシンホスファターゼ PTPRZ に対する硫酸転移酵素である

名古屋大学大学院医学系研究科　総合医学専攻
生物化学講座　分子生物学分野
（指導：門松 健治　教授）
那仁图雅

< **Summary** >

Keratan sulfate (KS) is a carbohydrate side chain covalently attached to extracellular proteoglycans. KS is composed of disaccharide units of 6-sulfated *N*-acetylglucosamine (GlcNAc) and galactose. We have previously shown that GlcNAc-6-*O*-sulfotransferase (GlcNAc6ST) 1 encoded by *Chst2* is an enzyme necessary for the synthesis of GlcNAc-6-sulfated KS chains that are required for neuronal plasticity in the visual cortex of the mouse brain during the critical period, but not in adulthood. Here, we show that GlcNAc-6-sulfated KS recognized by the R-10G anti-KS antibody, of which the minimum epitope structure is Galß1-4GlcNAc(6S)ß1-3Galß1-4GlcNAc(6S), distributes diffusely in neuropils and presents densely in close proximity to the perineuronal region of the perineuronal net (PNN)-positive neurons in the adult visual cortex. Surprisingly, GlcNAc6ST3, which was discovered as an intestinal GlcNAc6ST encoded by *Chst5*, is a major brain KS sulfotransferase expressed in oligodendrocytes in adulthood. Moreover, we identified an isoform of the protein-tyrosine phosphatase PTPRZ as a R-10G-reactive KS proteoglycan**.** These results indicate that GlcNAc6ST3 may play a role in synthesis of a component of PNN in the adult brain, and that the KS-modified isoform of PTPRZ encoded by *Ptprz1* could be an extracellular molecule associated with PNNs.

<**Materials and Methods**>

C57BL/6J, GlcNAc6ST1-deficient (KO)(Chst2$^{-/-}$), GlcNAc6ST2-KO (Chst4$^{-/-}$), GlcNAc6ST3-KO (Chst5$^{-/-}$), and GlcNAc6ST4-KO (Chst7$^{-/-}$) mice were used for the experiments. Fractionation and enzymatic treatment of brain samples were performed as previously described with Endo-ß-galactosidase (10 μU/μl), keratanase (1 mU/μl), or keratanase II (50 μU/μl) at 37 °C overnight. In the case of treatment with chondroitinase ABC (1 mU/μl), the reaction time was 3 h. Immunoblots, Immunoprecipitation, Immunohistochemistry, and Identification of KS-modified proteins by LC-MS/MS analyses were performed according to standard protocols. Data pertinent to the genes of GlcNAc6STs and phosphacan were mined from a published RNA sequencing (RNA-Seq) analysis of purified neurons, oligodendrocyte precursor cells, newly formed oligodendrocytes, myelinating oligodendrocytes, astrocytes, microglia, and endothelial cells from adult mouse brains. Values were analyzed via the one-way analysis of variance with Tukey's test by using Prism software (GraphPad Software, La Jolla, CA, USA). Differences were regarded as significant when $P < 0.05$. All data are means ± SD unless otherwise noted.

<**Results**>

To confirm that the R-10G recognized molecule is indeed KS-modified, I treated the brain lysates from adult wild-type (WT) mice with KS-degrading enzymes. Pre-digestion

with endo-ß galactosidase or keratanase resulted in complete disappearance of signals. Contrary, band intensity was not altered but the apparent molecular weight of the R-10G reactive band shifted slightly downwards after treatment with keratanase II (Fig 1a). Specifically, the band shifted down to approximately 320 kDa after chondroitinase ABC treatment (Fig 1b). The susceptibility to chondroitinase ABC indicated that the R-10G-reactive protein is modified also with chondroitin sulfate. To investigate the cellular localization of the KS/CSPG, I performed immunohistochemical staining with R-10G on brain sections from adult WT mice. R-10G staining signals were punctate and seen diffusely in perineuronal space of a subset of neurons (Fig 1c).

PNNs selectively surround inhibitory interneurons that express parvalbumin (PV), and are also labeled with *Wisteria floribunda* agglutinin (WFA). Intriguingly, dense R-10G staining in the pericellular regions was observed (Fig 1c) in a subset of neurons that are parvalbumin (PV)-positive or WFA-positive within the cerebral cortex (Fig 2a, b). These pericellular signals were subtle in a PV-positive cell subset within motor and somatosensory cortices (1% and 3% of total PV-positive cells, respectively) (Fig 2a). In the visual cortex, the pericellular signals were seen in 20% of total PV-positive cells (Fig 2a). Similarly, these signals are less prevalent in the WFA-positive cell subset within motor and somatosensory cortices (3% and 9% of WFA-positive cells, respectively) than in the visual cortex, where 18% of WFA-positive cells were R-10G-positive (Fig 2b). Confocal microscopy analysis showed that some of the pericellular R-10G signals exist densely in close proximity to perineuronal regions (Fig 2c). These results strongly indicate that R-10G reactive KS/CSPGs are accumulated in a subset of inhibitory intercortical neurons in the adult brain cortex with the preferential localization in the visual cortex. These neurons may include subsets of the R-10G positive neurons seen in the critical period.

It is previously shown that GlcNAc6ST1 is a responsible sulfotransferase for the R-10G positive KS/CSPG in the critical period mouse brain but not in the adult brain. To identify which GlcNAc6ST is responsible for the synthesis of the R-10G-positive KS in the adult brain, I employed GlcNAc6ST1-KO, GlcNAc6ST2-KO, and GlcNAc6ST3-KO mice. I newly generated GlcNAc6ST4-KO mice, which showed no apparent gross abnormality. Surprisingly, the GlcNAc6ST3-KO mouse brain showed dramatically reduced levels of the R-10G-positive KS, while others showed levels equivalent to those of WT mice (Fig 3a). The low level (~ 2% of the WT) of the R-10G immunoreactivity seen in the GlcNAc6ST3-KO cortex was abolished in GlcNAc6ST1 and GlcNAc6ST3 doubly-deficient (DKO) mice (Fig 3b), indicating that GlcNAc6ST3 is a major sulfotransferase for the KS/CSPG in adulthood, and that GlcNAc6ST1 merely contributes to the GlcNAc-6-sulfation of KS in the adult mouse brain. I then tested which cell types express the members of the GlcNAc6ST family. mRNA expression of the GlcNAc6ST1

gene, *Chst2*, was seen broadly at a low level in microglia (Fig 3c). Expression of the GlcNAc6ST2 gene, *Chst4*, was below the threshold of detection in either cell type of the adult brain. Interestingly, mRNA of the GlcNAc6ST3 gene, *Chst5*, was selectively expressed in OPCs and newly formed oligodendrocytes (Fig 3c). Expression of the GlcNAc6ST4 gene, *Chst7*, was observed in astrocytes, OPCs, microglia, and endothelial cells in the brain (Fig 3c). These results indicate that the major source of the R-10G-reactive KS/CSPG around PV-positive and WFA-positive neurons is oligodendrocytes in the adult brain.

Next, I asked which cerebral CSPG is modified with the R-10G-reactive KS in adulthood. Brain lysates prepared from the adult mice brain were pre-treated with chondroitinase ABC and then immunoprecipitated with R-10G. The R-10G-immunoprecipitated proteins with a size of > 300 kDa were molecularly identified as phosphacan/PTPRZ with by LC-MS/MS (Fig 4a). Immunoblotting with the phosphacan/PTPRZ antibody for the R-10G-immunoprecipitated proteins confirmed that phosphacan/PTPRZ is one of the KS/CSPGs (Fig 4b). The mouse phosphacan/PTPRZ gene, *Ptprz1*, is highly expressed in both astrocytes and OPCs of the adult brain cortex (Fig 4c). It is conceivable that phosphacan and other PTPRZ isoforms (Fig 4a) expressed in astrocytes and neurons. Reactivity of the Ptprz-S antibody was negligible in the GlcNAc6ST3-KO sample (Fig 4d). These results indicate that GlcNAc6ST3 is a major KS enzyme in the adult brain. It is likely that GlcNAc6ST3 in OPCs and oligodendrocytes post-translationally modifies a portion of phosphacan that are then translocated into the vicinity of PV-positive and WFA-positive neurons.

<Conclusion>

The findings of the present study, together with our previous findings, indicates that GlcNAc6ST1 and GlcNAc6ST3 are the most important KS enzymes in the brain; the former temporally contributes to KS synthesis during the developmental stage, whereas the latter is the main KS enzyme in adulthood. Oligodendrocytes are a major source of the R-10G-positive KS in the adult brain. The possible contribution of oligodendrocyte subsets to the formation of PNNs and differentiation of PV-positive neurons through secretion of the R-10G-positive KS phosphacan and the ectopic domain of PTPRZ-FL will be investigated in the future.