Polysialic Acid

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Abstract

Polysialic acid (polySia), particularly α2,8-linked polyNeu5Ac (DP = 8 ~ 400), is a unique polymer of sialic acid (Sia) and modifies neural cell adhesion molecule (NCAM) spatiotemporally in embryonic brains. PolySia is involved in cell migration, neural outgrowth, axonal guidance, synaptic plasticity, and the development of normal neural circuits and neurogenesis due to its anti-

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adhesive property and spatiotemporal expression. Recent improvements of polySia-detection methods have told us that polySia expression persists in certain areas of the adult brain, such as the olfactory bulb, hippocampus, subventricular zone, thalamus, prefrontal cortex, and amygdala, where neural plasticity, remodeling of neural connections, or neural generation is ongoing. Importantly, several lines of evidence have raised a new concept of polySia function that polySia works as an attractive field that associates with particular ion channels and neurologically active molecules, such as neurotrophic factor (BDNF), growth factor (FGF2), and neurotransmitter (dopamine), to regulate their involved signaling. It has been shown that polySia is involved in learning, memory, circadian rhythm, and social behaviors using polySia-impaired mice. In addition, SNPs and some deletions in genes encoding the polysialyltransferase and NCAM have been reported from patients of schizophrenia, mood disorder and autism. Such impairments of polySia structure derived from polysialyltransferase gene alterations might influence the polySia function not only as the repulsive field but also as the attractive field.

Keywords
Polysialic acid • Polysialyltransferase • ST8IA2 • ST8SIA4 • NCAM • Schizophrenia • Autism • BDNF • Dopamine • FGF

Introduction

Polysialic acid (polySia) is a linear polymer of sialic acid that exhibits structural diversity with respect to the sialic acid (Sia) components (*N*-acetylneuraminic acid, Neu5Ac; *N*-glycolylneuraminic acid, Neu5Gc; and deaminoneuraminic acid, KDN), Sia modifications (acetylation and sulfation), intersialyl linkages (α2 → 4, α2 → 5$O_{glycolyl}$, α2 → 8, α2 → 9, and α2 → 8/9), and degree of polymerization (DP = 8 ~ 400; Sato and Kitajima 2013). In nature, polySia is found in Gram-negative bacteria, echinoderms, and vertebrates. In particular, a large diversity in intersialyl linkages, components, and DP has been reported in echinoderms. In contrast, nearly all polySia that has been identified in the vertebrate brain is α2,8-linked polyNeu5Ac, although oligoSia chains are also present and exhibit some diversity in DP.

In vertebrates, polySia is a unique and important glycotope that serves as an onco-developmental antigen, as demonstrated by its spatiotemporal expression in the embryonic brain and several types of cancers. As polySia typically consists of an extremely long chain of polyanionic residues (DP = 8–400), it has hydration effects and a large excluded volume when expressed on cell surfaces. Due to these properties, the polySia structure has anti-adhesive effects when expressed on cell surfaces, where it functions as a negative regulator of cell-cell interactions. Consistent with this function, the intercellular space widens by approximately 10–15 nm when surface-expressed polySia is present. Recently, polySia has been

reported to regulate the concentrations of neurologically active molecules, such as brain-derived neurotrophic factor (BDNF), fibroblast growth factor (FGF2), and dopamine, in the extracellular space via the direct binding of these molecules. Thus, polySia is important for normal brain function by mediating cell-cell and cell-extracellular matrix interactions. However, the impairment of normal polySia expression can lead to the development of various diseases, such as cancer, and psychiatric disorders, and in animal models, disrupted polySia expression is associated with impaired learning, memory, circadian rhythm, and social behavior.

Distribution of PolySia

PolySia was first identified in 1957 as a component of polysaccharide chains from neuroinvasive Gram-negative bacteria. In vertebrates, polySia was first detected on soluble polysialoglycoprotein (PSGP) derived from salmonid fish eggs (Sato and Kitajima 2013). As PSGPs are localized in the egg cortical alveoli and are exocytosed into the perivitelline space immediately after fertilization, they are considered to have antibacterial properties and play a role in embryogenesis. PolySia (polyNeu5Ac) was later found on neural cell adhesion molecules (NCAMs) derived from chick and rat embryonic brains, where it is considered to exhibit spatiotemporal expression patterns during embryonic developmental stages. As the same exclusive expression pattern of polySia in embryonic brains was also demonstrated in fishes, birds, reptiles, and amphibians, polySia is thought to be a conserved glycotope that is involved in critical brain functions during embryonic development.

Numerous functional studies of polySia, particularly those examining its role during neurogenesis, have been conducted. Recently, improvements in detection methods have provided new insight into the distribution of polySia. For example, polysialylated NCAM has been detected not only in vertebrate brains but also in kidney, heart, and natural killer (NK) cells. Several types of cancer cells, including neuroblastoma, Wilms' tumor, and small-cell lung carcinoma cells, also express polySia, which is considered to be involved in the detachment and metastasis of tumor cells through its anti-adhesive effects. PolySia-NCAM is also expressed in adult brains, particularly in restricted areas where neurogenesis is ongoing, such as the hippocampus and olfactory bulb. In addition to PSGP and NCAM, several other polySia-containing glycoproteins have been reported, including the sodium channel protein of electric eel, sodium channel α-subunit in rat brain, CD36 in human milk, neuropilin-2 in human lymphocytes, and SynCAM-1 in mouse brain. A novel type of polySia (α2,9-linked polyNeu5Ac), termed flagellasialin, modulates sperm motility in sea urchin via the regulation of Ca^{2+} channels. Interestingly, sea urchin sperm also contains a polySia-modified glycolipid (DP ~ 16), although its function remains unclear. (Poly)Sia structures have also been found in insects (fly and cicada).

Cellular Regulatory Functions of PolySia

Anti-adhesive Effect

The function of polySia has been best characterized for NCAM, which is mainly expressed in embryonic brains of vertebrates and consists of five immunoglobulin-like (Ig) domains with six N-glycosylation sites and two fibronectin type-III-like (FN_{III}) domains in the extracellular region (Rutishauser 2008). PolySia chains are linked to the tri- or tetra-antennary N-linked glycan chains on Ig domain V of NCAM. Homophilic and heterophilic interactions mediated by non-polysialylated NCAMs on cis and trans membranes lead to a series of signal transduction events related to neurite outgrowth and cell migration. However, NCAM-mediated interactions and downstream signaling events are negatively regulated by the presence of polySia chains, which, due to their bulky nature and large exclusion volume, have strong anti-adhesive effects (Fig. 1a). The anti-adhesive and steric properties of polySia also help to maintain the intercellular space between cell membranes, as demonstrated by the 10–15 nm reduction in the distance between cells after cleavage of polySia with endo-N, and regulate the adhesions between CAMs.

Regulation of Bioactive Molecules

Recently, cell-surface-expressed polySia has been shown to directly bind soluble bioactive molecules in intercellular spaces. Thus, polySia appears to display an attractive field for these specific bioactive factors, thereby regulating their availability and function (Fig. 1b).

Neurotrophins – Brain-derived neurotrophic factor (BDNF) promotes the growth and development of immature neurons and enhances the survival and functional maintenance of adult neurons through the high-affinity receptor TrkB and low-affinity receptor p75NTR. This neurotrophic factor also has a vital role in neural plasticity, which is integral to memory and learning. The addition of BDNF to hippocampal slices derived from $NCAM^{-/-}$ mice rescued the reduction of long-term potentiation (LTP) resulting from the disappearance of polySia-NCAM, indicating that polySia-NCAM is involved in signal transduction mediated by BDNF receptors. The direct binding between polySia (and polySia-NCAM) and BDNF dimers was first biochemically demonstrated in 2008 using gel filtration, horizontal native-PAGE, and surface plasmon resonance (SPR) methods (Kanato et al. 2008). The biological significance of the DP of polySia was first demonstrated for BDNF, as a DP of 12 or greater was shown to be required for complex formation between polySia and BDNF. Notably, the complexes formed between polySia and BDNF are extremely large (approximately 2,500 kDa). The K_D of BDNF for polySia, as estimated by SPR, is approximately 10^{-9} M, and that for TrkB and p75NTR is 10^{-12} and 10^{-10} M, respectively. Based on these affinities and the results of gel filtration experiments involving co-incubation with these receptors, BDNF-

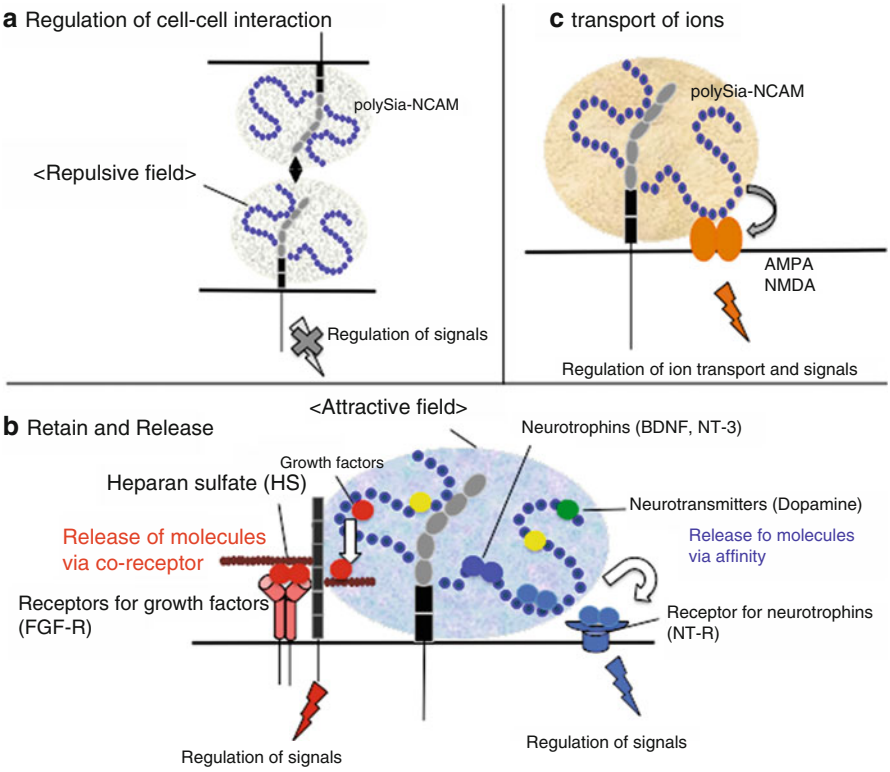

Fig. 1 Current views of polySia functions. (**a**) Anti-adhesive effect. PolySia-NCAM gives repulsive fields on the cell surface to regulate cell-cell interaction negatively by a large excluded volume of polySia, as shown in gray. (**b**) Retention or reservoir of bioactive molecules. As an attractive field, polySia on NCAM directly binds to the neurologically active molecules, such as neurotrophins, neurotransmitters, and growth factors. PolySia regulates their concentrations outside the cells and their signalings. (**c**) Regulation of ion transport. PolySia on NCAM has effects on AMPA and NMDA receptor and regulates their opening time span, through which polySia controls ion transport

polySia complexes easily migrate toward TrkB and p75NTR receptors, thereby regulating the concentration of available BDNF.

Growth Factors – FGF2 is a prototypical FGF that was first identified in the bovine pituitary gland as a factor with the potential to induce the proliferation of fibroblast cells but was later shown to stimulate the growth of various cell types, including tumor cells, and is also important for brain development and neural cell proliferation. FGF2 mediates cellular signal transduction through the binding of FGF tyrosine kinase receptors (FGFRs). It is well known that FGF2-FGFR signals are enhanced following the formation of ternary complexes with heparan sulfate (HS), which is a component of the extracellular matrix. Biochemical evidence suggests that polySia binds FGF2 with a similar affinity as that of HS ($K_d = 10^{-9}$ M)

(Ono et al. 2012). However, FGF2-polySia and FGF2-HS complexes are different in size and have distinct biochemical properties. For example, FGF2 complexed with polySia does not migrate toward receptors, even if the receptors are located. In addition, FGF2 can only migrate from polySia-FGF2 complexes to HS. Studies in NIH-3T3 cells have revealed that polysialylated NCAM regulates FGF-2-dependent cell growth, but not cell survival, through the regulation of phosphorylation of Erk in Ras/MAP kinase. Taken together, these results suggest that the polySia present on NCAM directly mediates a FGF2 signaling pathway that is distinct from that mediated by NCAM and FGFR interaction, which has a K_d of 10^{-6} M.

Neurotransmitters – Dopamine is a neurotransmitter in the catecholamine family that plays important roles in the brain, where it is deeply involved in reward-motivated behavior by signaling through dopamine receptors. Therefore, dopamine is considered to be an important bioactive molecule for animal behavior and psychiatric diseases of human. The interactions between neurotransmitters and acid glycans were first demonstrated in 2010 by frontal affinity chromatography (FAC) analysis (Sato et al. 2010; Isomura et al. 2011). PolySia, but not monoSia, was shown to specifically bind dopamine and other catecholamine neurotransmitters, including epinephrine and norepinephrine ($K_d \sim 10^{-6}$ M). As the K_D of dopamine toward polySia changes depending on the pH of the solution, the specific interaction between these molecules might be fine-tuned by subtle changes in the extracellular pH. As polySia-deficient neuroblastoma cells show impaired Akt signaling in the presence of added dopamine, polySia appears to be involved in Akt signaling via dopamine receptors (Isomura et al. 2011).

Regulation of Ion Transport

The polySia modification of NCAM regulates the function of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA) receptors located in synapses involved in learning and memory. Using reconstituted membranes containing glutamate receptors, polySia-NCAM was found to potentiate AMPA receptor currents. PolySia-NCAM has also been reported to inhibit the function of NR2B-containing receptors (NMDA) and prevent glutamate-induced cell death. These findings suggest that polySia-NCAM is involved in the differential regulation of several types of channels that mediate synaptic function in the hippocampus (Fig. 1c; Senkov et al. 2012).

Function of PolySia at the Animal Level

Transgenic Mice

The functions of polySia and its carrier protein NCAM at the animal level were examined in $NCAM^{-/-}$ mice (Cremer et al. 1994). Almost no polySia was detectable in $NCAM^{-/-}$ mice, indicating that the majority of polySia is linked to NCAM.

NCAM$^{-/-}$ mice exhibit several interesting morphological characteristics, including reduced olfactory bulb (OB) size due to disturbed migration from the subventricular zone (SVZ), disrupted mossy fiber architecture, and reduced amygdalo-hippocampal theta synchronization during fear memory retrieval. *NCAM*$^{-/-}$ mice also show several behavioral changes, such impaired spatial learning, locomotion, and social interactions. In addition, *NCAM*$^{-/-}$ and conditional NCAM-deficient mice (forebrain specific) display impaired cognitive functions, as contextual and cued fear conditioning, particularly under stress. D2R expression and dopamine sensitivity are upregulated in cells derived from *NCAM*$^{-/-}$ mice.

The identification and cloning of two polysialyltransferases, *ST8SIA2* and *ST8SAI4*, involved in the polysialylation of NCAM allowed for the single polysialyltransferase-deficient mouse lines *ST8SIA2*$^{-/-}$ and *ST8SIA4*$^{-/-}$ to be established. Although these mice contain a large amount of polySia (50–95 %) in the brain compared to wild-type mice, each transgenic mouse line displays a unique phenotype (Hildebrandt et al. 2007). PolySia staining in *ST8SIA2*$^{-/-}$ mice is markedly decreased in the OB and cerebral cortex. In the hippocampus, reduced polySia levels are observed in the inner rim of the granular layer of the dentate gyrus (DG), where polySia staining of precursors from the subgranular layer is first detected. *ST8SIA2*$^{-/-}$ mice also have misguided infrapyramidal mossy fibers and improperly formed ectopic synapses in the hippocampus (HY) CA3 region. In addition, *ST8SIA2*$^{-/-}$ mice exhibited higher exploratory drive, reduced behavioral responses to Pavlovian fear conditioning, and impaired social interaction (Angata et al. 2004). In contrast, precursor migration and mossy fiber organization were normal in *ST8SIA4*$^{-/-}$ mice. However, polySia expression in the CA1 region of Ammon's horn decreased in this mouse line, and LTP and LTD were also impaired in the CA1 region. *ST8SIA4*$^{-/-}$ mice also displayed decreased motivation in social interaction (Eckhardt et al. 2000).

As large amounts of polySia remain in *ST8SIA2*$^{-/-}$ (55 %) and *ST8SIA4*$^{-/-}$ (95 %) mice, *ST8SIA2*$^{-/-}$/*ST8SIA4*$^{-/-}$ mice were established to completely suppress the expression of polySia. *ST8SIA2*$^{-/-}$/*ST8SIA4*$^{-/-}$ mice show a severe phenotype that culminates in death within 8 weeks of birth. The major phenotypes of these mice are hypoplasia of the corticospinal tract, reduced size of the internal capsule, hypoplasia of the mammillothalamic tract, high incidence of hydrocephalus, growth retardation, and precocious death. All of the functions underlying these phenotypes are considered to be NCAM specific. The other phenotypes displayed by these mice, which include small OB, rostral migratory stream expansion, and delamination of mossy fibers, are considered to be related to the impairment of polySia-specific functions. Interestingly, in *NCAM*$^{-/-}$/*ST8SIA2*$^{-/-}$/*ST8SIA4*$^{-/-}$ mice, the severe phenotype of the double knockout mice is rescued, suggesting that an uncontrolled type of NCAM-mediated cell adhesion leads to increased signal transduction events (Hildebrandt et al. 2007). The improved signaling via increased cell-cell interactions in the polySia-deficient brain is likely due to the reduced levels of cell adhesion molecules resulting from NCAM deficiency. Thus, the reduction of NCAM leads to the recovery of normal physiological interactions and the rescue of the severe phenotype of polySia-deficient mice.

Human Disease

The relationship between polySia and diseases related to brain function, particularly psychiatric disorders, has been intensively studied because polySia expression is almost exclusively restricted to the brain (Sato and Kitajima 2013). Accumulating evidence suggests that polySia is involved in schizophrenia, which is a psychiatric disorder with a complex pathophysiology that is influenced by multiple factors. In schizophrenic brains, the number of polySia-NCAM immunostained cells derived from the hippocampus is decreased compared with that of normal brains. Supporting the proposed involvement of polySia in schizophrenia, the gene encoding the polysialyltransferase ST8SIA2 localizes to chromosome 15q26, which has been identified as the genomic region related to schizophrenia and bipolar disorders among the population of Eastern Quebec.

Recently, a relationship between single nucleotide polymorphisms (SNPs) in the promoter region of ST8SIA2 and schizophrenia was revealed by genome-wide studies among Japanese and Chinese-Han populations (Arai et al. 2006). The ST8SIA2 gene is also reported to be a generalized susceptibility marker for psychotic and mood disorders on chromosome 15q25-26 and is associated with an increased risk of mental illness, such as autism. Biochemical-based studies of ST8SIA2 containing SNP-7 (Glu141Lys) in the coding region from a schizophrenic patient have shown that the in vitro and in vivo enzymatic activities of this point-mutated polysialyltransferase are dramatically decreased and that the produced polySia is impaired with respect to quantity and quality (Isomura et al. 2011, Hane et al. 2012). Considering that polySia functions as a regulator of biologically active molecules, such as BDNF, FGF2, and dopamine, which are intimately involved in brain function and psychiatric disorders, polySia-NCAM synthesized by mutated ST8SIA2 likely plays a role in the development of schizophrenia. Anatomically, schizophrenic brains contain a reduced volume of OB derived, a phenotype that is similar to that of *NCAM*$^{-/-}$ mice. The functional impairment and disturbed organization of the hippocampus are also involved in the etiology of schizophrenia. In this regard, it is interesting that the loss of ST8SIA2 or NCAM results in the misguidance of infrapyramidal mossy fibers and formation of ectopic synapses in the hippocampus. In addition, several characteristic properties, such as altered brain structure and neural plasticity, and various morphological, cognitive, and emotional deficits related to schizophrenia have been observed in *ST8SIA2*$^{-/-}$ and *ST8SIA4*$^{-/-}$ mice. For this reason, *ST8SIA2*$^{-/-}$ mice are considered to be a useful model for studying schizophrenia. Interestingly, a number of reports have indicated that in addition to schizophrenia, polySia might be involved in several other brain disorders, including autism, Alzheimer's disease, and Parkinson's disease. Recently, a patient suffering from severe autism was shown to have a 520-kbp deletion on chromosome 15q26.1 that included the *ST8SIA2*. Although polySia expression is decreased in schizophrenia, increased polySia levels have been observed in the brains of patients suffering from Alzheimer's and Parkinson's diseases, suggesting that polySia expression in normal brain is finely tuned.

In addition to brain and psychiatric disorders, polySia is associated with several types of cancer, including neuroblastomas, Wilms' tumors, non-small cell lung carcinomas, medulloblastomas, pheochromocytomas, medullary thyroid carcinomas, pituitary adenomas, and breast cancer. In contrast to normal tissue cells, cancer cells express polySia on the surface. As polysialylation has an anti-adhesive effect on cell-cell interactions, polySia is likely involved in the detachment and metastasis of cancer cells. In the case of non-small cell lung carcinoma cells, tumor progression was shown to be correlated with the levels of polySia and its biosynthesizing enzyme, ST8SIA2.

Future Perspectives

As the expression and functional structure of polySia-NCAM are highly regulated, imbalances in polySia-NCAM levels lead to the development of several human diseases, including psychiatric disorders and cancer. It is therefore important to understand the chemical structural changes of polySia-NCAM and the mechanisms underlying its expression in normal and diseased brains.

References

Angata K, Long JM, Bukalo O, Lee W, Dityatev A, Wynshaw-Boris A, Schachner M, Fukuda M, Marth JD (2004) Sialyltransferase ST8Sia-II assembles a subset of polysialic acid that directs hippocampal axonal targeting and promotes fear behavior. J Biol Chem 279:32603–32613

Arai M, Yamada K, Toyota T, Obata N, Haga S, Yoshida Y, Nakamura K, Minabe Y, Ujike H, Sora I, Ikeda K, Mori N, Yoshikawa T, Itokawa M (2006) Association between polymorphisms in the promoter region of the sialyltransferase 8B (SIAT8B) gene and schizophrenia. Biol Psychiatry 59:652–659

Cremer H, Lange R, Christoph A, Plomann M, Vopper G, Roes J, Brown R, Baldwin S, Kraemer P, Scheff S (1994) Inactivation of the N-CAM gene in mice results in size reduction of the olfactory bulb and deficits in spatial learning. Nature 367:455–459

Eckhardt M, Bukalo O, Chazal G, Wang L, Goridis C, Schachner M, Gerardy-Schahn R, Cremer H, Dityatev A (2000) Mice deficient in the polysialyltransferase ST8SiaIV/PST-1 allow discrimination of the roles of neural cell adhesion molecule protein and polysialic acid in neural development and synaptic plasticity. J Neurosci 20:5234–5244

Hane M, Sumida M, Kitajima K, Sato C (2012) Structural and functional impairments of polySia-NCAM synthesized by a mutated polysialyltransferase of a schizophrenic patient. Pure Appl Chem 84:1895–1906

Hildebrandt H, Mühlenhoff M, Weinhold B, Gerardy-Schahn R (2007) Dissecting polysialic acid and NCAM functions in brain development. J Neurochem 103(Suppl 1):56–64

Isomura R, Kitajima K, Sato C (2011) Structural and functional impairments of polysialic acid by a mutated polysialyltransferase found in schizophrenia. J Biol Chem 286:21535–21545

Kanato Y, Kitajima K, Sato C (2008) Direct binding of polysialic acid to a brain-derived neurotrophic factor depends on the degree of polymerization. Glycobiology 12:1044–1053

Ono S, Hane M, Kitajima K, Sato C (2012) Novel regulation of fibroblast growth factor 2 (FGF2)-mediated cell growth by polysialic acid. J Biol Chem 287:3710–3722

Rutishauser U (2008) Polysialic acid in the plasticity of the developing and adult vertebrate nervous system. Nat Rev Neurosci 9:26–35

Sato C, Kitajima K (2013) Disialic, oligosialic, and polysialic acids: distribution, functions, and related disease. J Biochem 154:115–136

Sato C, Yamakawa N, Kitajima K (2010) Analysis of glycan-protein interaction by frontal affinity chromatography and Biacore. Methods Enzymol 478:219–232

Senkov O, Tikhobrazova O, Dityatev A (2012) PSA–NCAM: synaptic functions mediated by its interactions with proteoglycans and glutamate receptors. Int J Biochem Cell Biol 44:591–595