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Chondroitin sulfate proteoglycans in neural development and plasticity

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1. ABSTRACT

PTPzeta and lectican family members are major chondroitin sulfate proteoglycans (CS-PGs) in the brain, which bind with many proteins via core protein and CS portions. Recent studies revealed that the oversulfated structures in CS constitute high affinity binding sites for various growth factors and axon guidance molecules, and play important roles in the proliferation of neural progenitor cells, neurite extension and neuronal migration. PTPzeta uses pleiotrophin as a ligand. The CS portion of PTPzeta constitutes a part of the pleiotrophin-binding site, and oversulfated D unit increases the binding affinity. Pleiotrophin-PTPzeta signaling regulates the morphogenesis of Purkinje cell by controlling the tyrosine phosphorylation of a Notch-related transmembrane protein, DNER. In the brain of adult animals, a subset of neurons are surrounded by CS-PG-rich extracellular matrix called perineuronal net, in which lecticans form complexes with hyaluronic acid and tenascin-R. CS-PGs in the perineuronal net regulate ocular dominance plasticity in the visual cortex by enhancing the uptake of Otx2 homeoprotein by parvalbumin-positive interneurons in a CS-dependent manner. These studies revealed unexpectedly complex mechanisms of CS-PG functions.

2. INTRODUCTION

Proteoglycans are composed of a core protein to which one or more sulfated glycosaminoglycan chains are covalently attached (1). Sulfated glycosaminoglycans are classified into chondroitin sulfate (CS)/dermatan sulfate, heparan sulfate (HS)/heparin and keratan sulfate (KS), and proteoglycans bearing CS, HS and KS chains are called CS proteoglycans, HS proteoglycans and KS proteoglycans, respectively. However, some HS proteoglycans such as syndecan family members are decorated with both HS and CS chains, and CS proteoglycans such as phosphacan and aggrecan are often modified also with KS chains. Among these proteoglycans, CS proteoglycans have attracted the attention of neuroscientists, because chondroitinase ABC, a bacterial enzyme which degrades CS, affects many aspects of the nervous system including development, plasticity and regeneration (2-5). CS proteoglycans are major components of the cell surface and extracellular matrix in the brain, and play important roles in cell-cell and cell-matrix interactions, and growth factor signaling (6-11). Both the core protein and saccharide moieties bind with various proteins such as growth factors, cell adhesion molecules and extracellular matrix components, and regulate their physiological functions. CS

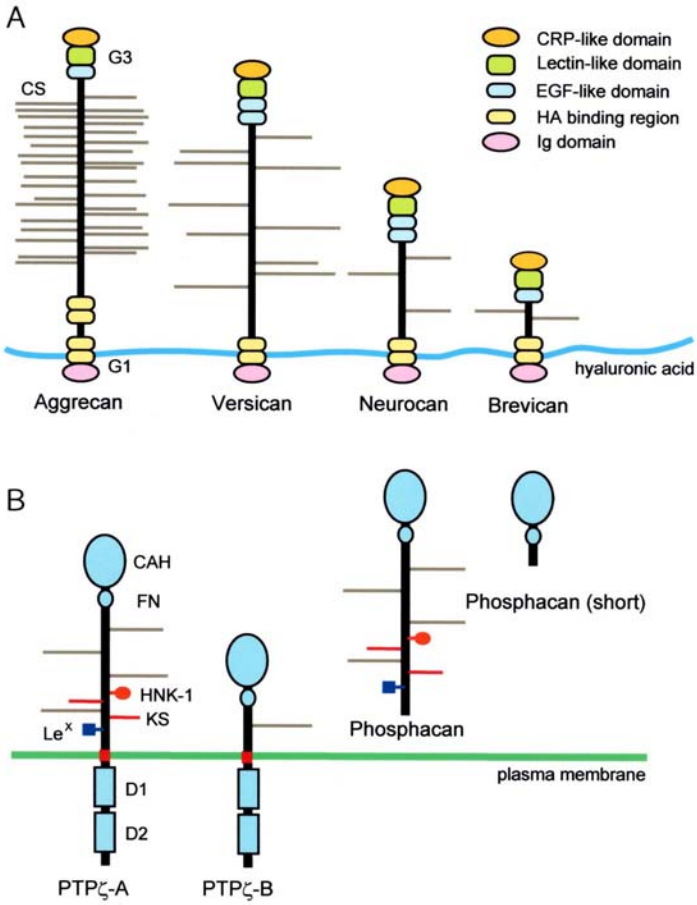

Figure 1. Structural models of chondroitin sulfate proteoglycans. (A) Lectican family members (aggrecan, versican, neurocan and brevican) consist of an N-terminal G1 domain, which binds with hyaluronic acid, a chondroitin sulfate (CS) attachment region and a C-terminal G3 domain. The G1 domain contains an immunoglobulin (Ig)-like domain (Ig domain) and a hyaluronic acid (HA)-binding region. The G3 domain is composed of epidermal growth factor (EGF)-like domains, a C-type lectin-like domain and a complement regulatory protein (CRP)-like domain. (B) PTPzeta consists of an N-terminal carbonic anhydrase-like domain (CAH), a fibronectin type III-like domain (FN), a chondroitin sulfate attachment region, a transmembrane segment and two tyrosine phosphatase domains (D1 and D2). Alternative splicing generates two transmembrane forms (PTPzeta-A and -B) and two secreted forms (phosphacan and phosphacan short form). Keratan sulfate (KS), HNK-1, and Le^X carbohydrates are attached to PTPzeta-A and phosphacan.

proteoglycans are also highly decorated with various *N*- and *O*-linked oligosaccharide chains. The structure of CS and oligosaccharide chains on CS proteoglycans changes dynamically during developmental processes, and such structural changes contribute to the regulation of many neural functions. In this review, we discuss the functional importance of saccharide chains of CS proteoglycans in neural development and plasticity.

3. CS PROTEOGLYCANS AND BIOSYNTHESIS OF CS

3.1. Structure of CS proteoglycans

Figure 1 illustrates the major CS proteoglycans found in the nervous system. CS proteoglycans are largely grouped into secreted (Figure 1A) and transmembrane (Figure 1B) forms. Lectican (hyalectan) family members are secreted extracellular matrix CS proteoglycans that bind with hyaluronic acid (9,12) (Figure 1A). Aggrecan, versican, neurocan and brevican form this family, which are composed of a highly conserved N-terminal globular G1 domain, a poorly conserved glycosaminoglycan attachment region, and a conserved C-terminal G3 domain. The G1 domain contains an immunoglobulin-like domain and tandem repeats of a hyaluronic acid-binding domain. The glycosaminoglycan attachment region is decorated with CS chains and various *N*- and *O*-linked oligosaccharides. The G3 domain is composed of one or two epidermal growth factor (EGF)-like domains, C-type lectin-like domain, and a complement regulatory protein-like domain. While the G1 domain anchors lecticans to hyaluronic acid, the binding of which is stabilized by link proteins, the C-terminal G3 domains bind with extracellular matrix proteins such as tenascin-C and tenascin-R. Thus, lecticans, hyaluronic acid and extracellular matrix proteins form large complexes that play critical roles in the various aspects of neural development and plasticity (5,7,9,12). Alternative splicing generates variants of lecticans, modulating the interactions and assembly of the extracellular matrix (13).

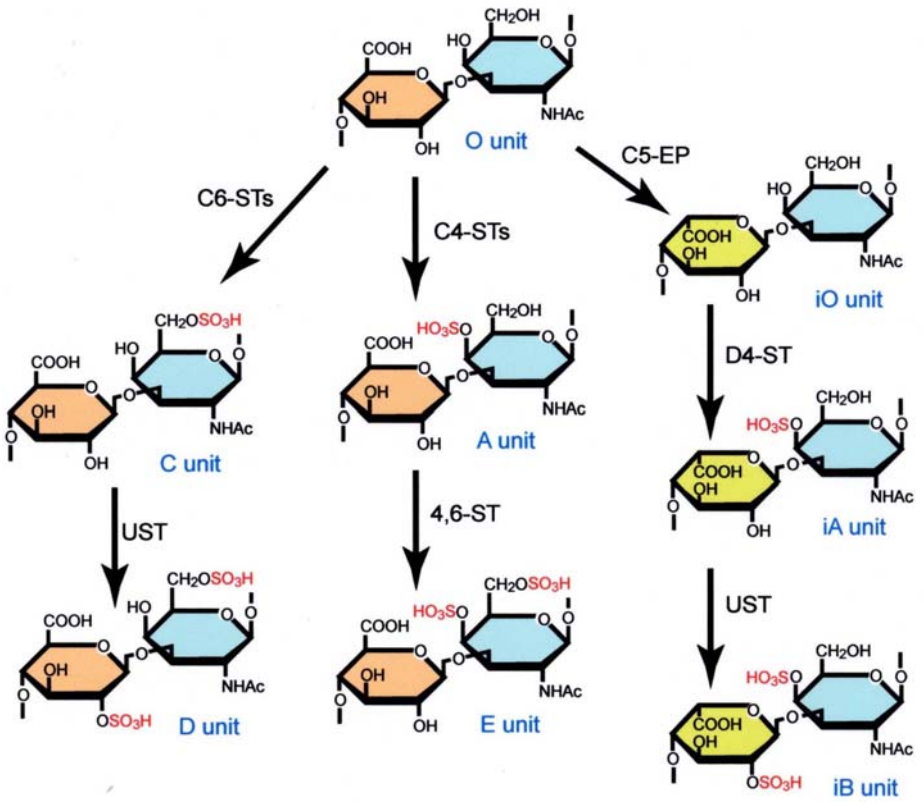

Figure 2. Biosynthesis of chondroitin sulfate. Chondroitin sulfate is synthesized in the Golgi apparatus by sequential modifications after polymerization of the repeating disaccharide unit of GlcA and GalNAc (O unit). Many of the GalNAc residues are 4-*O*-sulfated by chondroitin 4-*O*-sulfotransferases (C4-STs) or 6-*O*-sulfated by chondroitin 6-*O*-sulfotransferases (C6-STs), leading to the generation of A and C units, respectively. A portion of disaccharide units have two or three sulfate residues, which are called oversulfated structures. A unit is further sulfated by GalNAc 4-sulfate 6-*O*-sulfotransferase (4,6-ST), generating E unit. C unit is sulfated by uronyl 2-*O*-sulfotransferase (UST), generating D unit. Some of the GlcA residues are converted to IdoA by chondroitin-glucuronate C5 epimerase (C5-EP), which leads to the generation of iA and iB units by subsequent sulfation by dermatan 4-*O*-sulfotransferase (D4-ST) and UST.

Phosphacan is a major secreted CS proteoglycan in the brain, which is not related to lecticans (Figure 1B). This proteoglycan is a splicing variant of the receptor-type protein tyrosine phosphatase zeta (PTPzeta/RPTPbeta) (14-18). PTPzeta is composed of an N-terminal carbonic anhydrase-like domain, a fibronectin type III-like domain, a CS attachment region, a transmembrane segment, and the two intracellular tyrosine phosphatase domains (D1 and D2). There are four splicing variants of this molecule: (a) the full-length form (PTPzeta-A), (b) the short form (PTPzeta-B), in which a large part of the CS attachment region is deleted, (c) phosphacan, which corresponds to the extracellular region of PTPzeta-A, and (d) phosphacan short form (Figure 1B). PTPzeta-A, PTPzeta-B and phosphacan are synthesized as CS proteoglycans, although PTPzeta-B is a part-time proteoglycan (19). Phosphacan short form seems not to have CS chains (18). PTPzeta-A and phosphacan are decorated with keratan sulfate, HNK-1 and Le^X carbohydrates, but PTPzeta-B is not, suggesting that one of the function of alternative splicing is differential modification of the core proteins with carbohydrates (17,19-21). The extracellular domain of PTPzeta binds with various cell adhesion molecules (NrCAM, L1/Ng-CAM, contactin, N-CAM and TAG1), growth factors (pleiotrophin, midkine, and FGF-2) and extracellular matrix molecules (amphoterin, tenascin-C and tenascin-R) (22-29). Many of these binding partners (tenascin, N-CAM, L1, TAG-1, pleiotrophin, FGF-2 and amphoterin) also interact with neurocan, raising the possibility that PTPzeta/phosphacan and neurocan competitively bind with these proteins in the brain (25,27,28). Among the two tyrosine phosphatase domains, only the D1 domain is catalytically active (14). Beside these CS proteoglycans, there exist several CS proteoglycans in the nervous system including neuroglycan C, NG2 proteoglycan, decorin and appican, which we will not discuss because of a lack of space (1).

3.2. Biosynthesis and structure of CS

CS chains are unbranched polysaccharides that consist of repeating disaccharide units of *N*-acetylgalactosamine (GalNAc) and glucuronic acid (GlcA) (30,31) (Figure 2). CS chains are attached to specific serine residues in the core protein through a linkage tetrasaccharide (GlcAbeta1-3galactose beta1-3galactose beta1-4xylose beta1-*O*-serine). Biosynthesis of CS begins with the addition of xylose to the serine residues in the core protein by xylosyl transferase, followed by the addition of two galactose residues

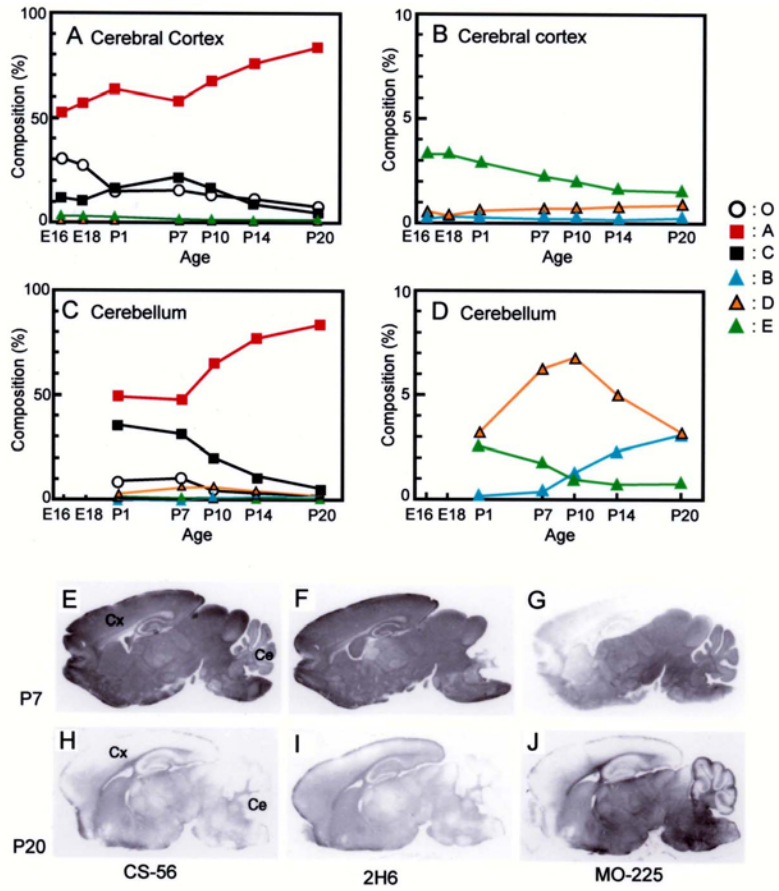

Figure 3. Structural change of chondroitin sulfate in the developing brain. (A-D) The developmental changes in the disaccharide composition of chondroitin sulfate from mouse cerebral cortex (A and B) and cerebellum (C and D) are shown (32,33). A and iA units and B and iB units were collectively quantified as A and B units, respectively. (E-J) Sagittal sections from P7 (E-G) and P20 (H-J) mouse brains were stained immunohistochemically with monoclonal antibodies against chondroitin sulfate: CS-56 (E and H), 2H6 (F and I) and MO-225 (G and J) (36). These monoclonal antibodies differentially stained cerebral cortex (Cx) and cerebellum (Ce).

and one GlcA by galactosyltransferase-I, galactosyltransferase-II and glucuronyltransferase I, respectively. Then, the repeating disaccharides (GlcAbeta1-3GalNAc) (O unit) are polymerized by chondroitin sulfate synthase complexes in the Golgi apparatus. The polymerized disaccharides are then heavily modified by C5 epimerization of GlcA and *O*-sulfation (30-33). Many of the GalNAc residues are 4-*O*-sulfated by chondroitin 4-*O*-sulfotransferases (C4-STs) or 6-*O*-sulfated by chondroitin 6-*O*-sulfotransferases (C6-STs). The resulting GlcAbeta1-3GalNAc(4S) and GlcAbeta1-3GalNAc(6S) are called A and C units, respectively. Some of the GlcA residues are converted to iduronic acid (IdoA) by chondroitin-glucuronate C5-epimerase (C5-EP), leading to the generation of iO unit (IdoAalpha1-3GalNAc). The GalNAc residues of iO units are 4-*O*-sulfated by dermatan 4-*O*-sulfotransferase (D4-ST), generating iA units (IdoAalpha1-3GalNAc(4S)). The highly iduronated CS chains are often called dermatan sulfate (DS). Monosulfated A and C units are the major components of CS chains, and DS chains are mainly composed of iA units. However, CS and DS chains contain significant amounts of di-sulfated disaccharides, which are called oversulfated structures (D, E and iB units). D units (GlcA(2S)beta1-3GalNAc(6S)) are generated from C units by uronyl 2-*O*-sulfotransferase (UST), and E units (GlcAbeta1-3GalNAc(4,6-diS)) are synthesized from A units by GalNAc 4-sulfate 6-*O*-sulfotransferase (4,6-ST). iB units (IdoA(2S)alpha1-3GalNAc(4S)) are synthesized from iA units by UST, and thus, UST is involved in the biosynthesis of both D and iB units (Figure 2). In some cases, GlcA residues of D and E units may be iduronated: iD units (IdoA(2S)alpha1-3GalNAc(6S)) and iE units (IdoAalpha1-3GalNAc(4,6-diS)), respectively. Furthermore, a small portion of disaccharide units are trisulfated: T units (GlcA(2S)beta1-3GalNAc(4,6-diS)). The combination of these various disaccharide units leads to the enormous structural heterogeneity in CS chains.

4. FUNCTIONAL SIGNIFICANCE OF STRUCTURAL VARIATION OF CS CHAINS

4.1. Structural change of CS chains during development of the brain

The structure of CS changes during development, aging and pathological processes (32-35). Figure 3 reveals the developmental change of the CS structures in the mouse

cerebral cortex and cerebellum (32,33). A, C and O units are the major components of the CS in cerebral cortex (Figure 3A). While the amounts of A unit gradually increased during development, those of O unit decreased. The amounts of C unit peaked at postnatal day 7 (P7), and decreased thereafter. Similar developmental changes were observed in the postnatal cerebellum (Figure 3C). In contrast, the expression of oversulfated structures differed quite markedly between the two regions (Figure 3B and D). In the cerebral cortex, significant amounts of E unit were observed during development with almost no B unit (Figure 3B). Although the expression of D unit was low, it gradually increased after E18. On the other hand, the oversulfated structures showed a quite characteristic pattern of expression in the cerebellum (Figure 3D). D unit was highly expressed, its level peaking at P10 and then rapidly decreasing. While the expression of E unit gradually decreased, that of B unit increased during postnatal development. So, it seems that the expression of E, D and B units correlated with the early, middle and later stages of postnatal development of the cerebellum, respectively. The expression of E and D/B units was highly correlated with the expression of 4,6-ST and UST mRNAs, respectively, suggesting the expression of these structures to be transcriptionally regulated by these enzymes.

The developmental and regional changes in the structure of CS were also revealed by immunohistochemical analyses using anti-CS monoclonal antibodies (36). Figure 3E-J shows the immunohistochemical staining of P7 and P20 mouse brains with three kinds of monoclonal antibodies against CS epitopes: CS-56, 2H6 and MO-225. At P7, the staining patterns of these antibodies were quite different especially in the cerebrum and cerebellum. Both the cerebrum and cerebellum were stained strongly with CS-56 (Figure 3E), confirming that CS chains were expressed in both regions. However, the staining with 2H6 was weak in the cerebellum, although it was strong in the cerebrum (Figure 3F). In contrast, the cerebellum was strongly stained with MO-225 with little staining in cerebrum (Figure 3G). MO-225 specifically recognizes D unit-rich CS (37), and this immunohistochemical result is consistent with the above disaccharide composition analyses, in which cerebellum was enriched with D unit but its content was low in the cerebrum (Figure 3B and D). The staining pattern with these antibodies apparently changed during development (compare Figure 3E-G with Figure 3H-J). Although the epitopes of CS-56 and 2H6 are not fully characterized (38-40), it is apparent that CS structures change regionally and developmentally in the brain.

4.2. CS binds with various proteins in a structure-dependent manner

It has been revealed that CS binds with various growth factors, chemokines, axon guidance molecules and extracellular matrix proteins (41-46). Among them, the interaction between CS and pleiotrophin/HB-GAM/HARP, an 18-kDa heparin-binding growth factor (47-49), was intensively studied. Pleiotrophin promotes neurite outgrowth and migration of various types of cells including neurons (47,50-52). It also stimulates the proliferation of various cells and is deeply involved in angiogenesis and tumor growth (53). It is known that syndecan-3 and PTPzeta work as receptors for pleiotrophin (24,54,55). Recently, anaplastic lymphoma kinase (ALK) has also been proposed to be a pleiotrophin receptor (56), although this notion is highly controversial (57). This kinase may not be a direct receptor for pleiotrophin but a substrate of PTPzeta. That is, ALK may be a downstream molecule of PTPzeta and activated by pleiotrophin through a PTPzeta signaling pathway (58). While pleiotrophin binds to the HS portion of syndecan-3 (54), the CS portion of PTPzeta constitutes a part of the pleiotrophin-binding site (24) (see below).

Figure 4A-D shows the interaction between pleiotrophin and commercially available CS preparations revealed using a surface plasmon resonance biosensor (36). Binding assays were performed for whale cartilage CS-A (O unit : A unit : C unit : D unit : E unit = 1.6 : 76.2 : 19.3 : 2.7 : 0.3), pig skin CS-B (O unit : A/iA unit : iB unit : C unit : D unit = 0.7 : 90.0 : 6.5 : 1.9 : 0.6) , shark cartilage CS-D (O unit : A unit : C unit : D unit : E unit = 1.5 : 25.9 : 50.4 : 18.8 : 3.4) and squid cartilage CS-E (O unit : A unit : C unit : E unit = 5.9 : 22.9 : 9.6 : 61.5) (59). CS-A showed no binding to pleiotrophin at the concentrations tested (Figure 4A). The other CS preparations bound with pleiotrophin with distinct affinities. CS-E showed the strongest affinity for pleiotrophin with a Kd of 0.76 nM (Figure 4D), and CS-D had moderate affinity for pleiotrophin with a Kd of 2.7 nM (Figure 4C). CS-B showed much less affinity (Kd = 34 nM) in comparison with CS-D and -E (Figure 4B). These results indicated that the binding between CS and pleiotrophin is highly dependent on the CS structure. The contents of oversulfated structures in CS-E, CS-D, CS-B and CS-A were ~62, ~22, ~7, and 3%, respectively, and seems to correlate with the affinity for pleiotrophin. In fact, using oligosaccharides prepared from CS-D, we observed that the affinity between pleiotrophin and these oligosaccharides was critically dependent on the amount of D unit (60).

Using the same CS preparations, Ship *et al.* revealed that CS interacted with FGFs and various axon guidance molecules (45). CS-E strongly bound to FGF2, slit2, netrin1, semaphorin5B, ephrinA1 and ephrinA5, and weakly interacted with FGF1. The other CS preparations showed weak affinity for these proteins. From the finding that CS-E interacted strongly with these proteins, they suggested that the E structure plays a critical role in the formation of high affinity binding sites for various proteins. Furthermore, Mikami *et al.* indicated that a cell adhesion molecule, contactin-1, bound strongly with CS-E but not with CS-A and CS-C (61). CS-E coated on substrates promoted neurite extension of hippocampal neurons *in vitro*, in which contactin-1 on neurons acted as a CS-E receptor. However, CS-E has an extremely high oversulfated structure content compared with the other commercial CS preparations and the CS chains in the brain. Thus, the data obtained using CS-E should be carefully interpreted, because the extremely high negative charge density of CS-E may explain such specific activity.

Sugahara's group purified CS chains form adult and embryonic pig brains, and examined their interactions with various proteins (62). While the CS preparations from embryonic brain (e-CS) contained 8~9% IdoA-containing disaccharides, those from adult brain (a-CS) did not (<1%). Though both preparations showed similar amounts of

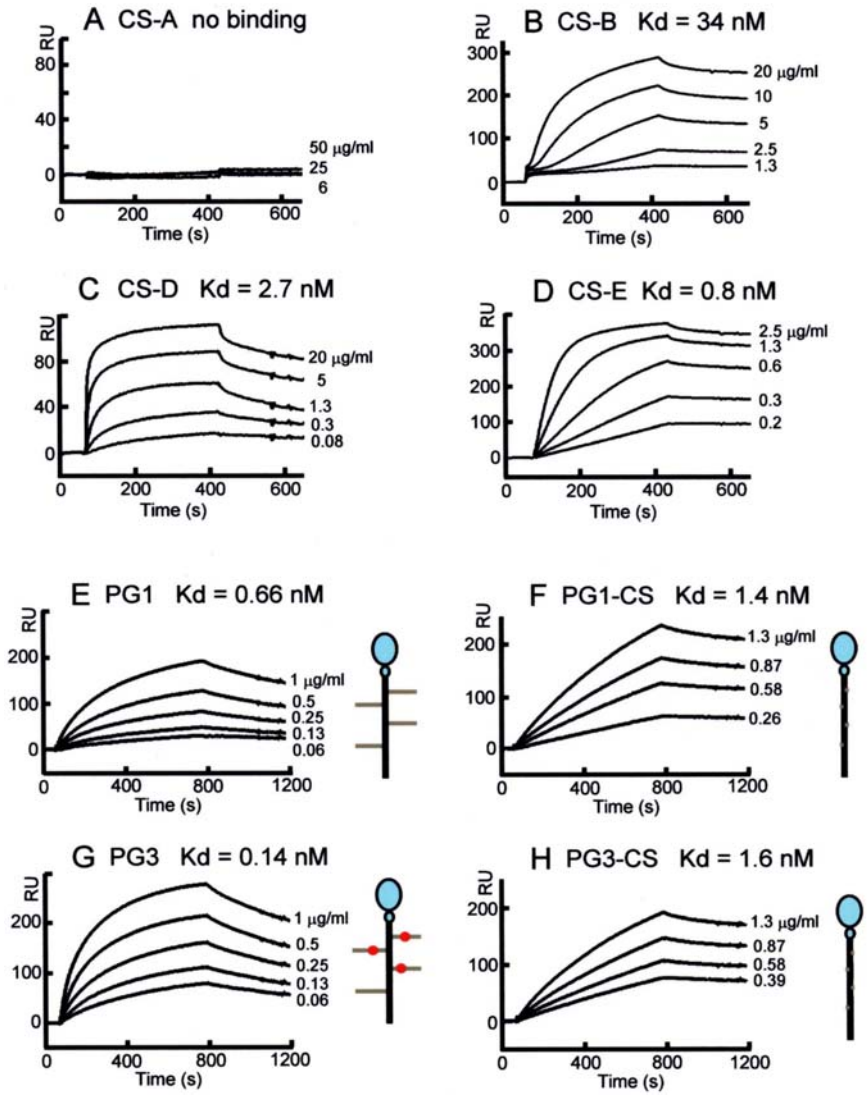

Figure 4. Binding of various chondroitin sulfate and phosphacan preparations with pleiotrophin. (A-D) Various concentrations of whale cartilage CS-A (A), pig skin CS-B (B), shark cartilage CS-D (C) and squid cartilage CS-E (D) were applied onto a pleiotrophin-immobilized biosensor using the BIAcore system (36). The samples were injected at 75 s and the dissociation phase began at 425 s. (E-H) Various concentrations of phosphacan bearing chondroitin sulfate without D unit (E, PG1) and phosphacan containing D unit (G, PG3) were applied onto a pleiotrophin-immobilized biosensor using the BIAcore system (36). PG3 showed higher affinity for pleiotrophin than PG1. However, both phosphacan preparations showed similar low affinity binding to pleiotrophin after chondroitinase ABC treatment (F and H). The samples were injected at 80 s, and the dissociation phase began at 800 s.

oversulfated disaccharides (2.5~3.8%), e-CS showed higher affinity for FGF-2, FGF-10, FGF-18, pleiotrophin and midkine than a-CS did. In addition, e-CS coated on substrates promoted neurite extension of hippocampal neurons, but such activity was not observed for a-CS. They further analyzed the interaction of pleiotrophin with oligosaccharides isolated from the chondroitinase B-digested e-CS (63). They indicated that decasaccharides containing E/iE, B/iB and D/iD units showed strong affinity for pleiotrophin. Thus, it seems that critical oversulfated structures, especially iduronated ones, were clustered in e-CS, and contributed to the high affinity binding with pleiotrophin.

4.3. Structural variation of CS regulates the binding affinity of phosphacan for pleiotrophin

Since CS chains are present in the form of proteoglycans, the binding assays using free CS chains may not reflect the physiological interactions. In fact, Herndon *et al.* indicated that core proteins can drastically strengthen the affinity of glycosaminoglycans for various proteins (43). Thus, we analyzed the binding of pleiotrophin with phosphacan preparations bearing CS of different structures (36). As described previously, the structures of CS chains differs depending on the region and developmental stage of the brain (Figure 3). So, we anticipated that phosphacan with different

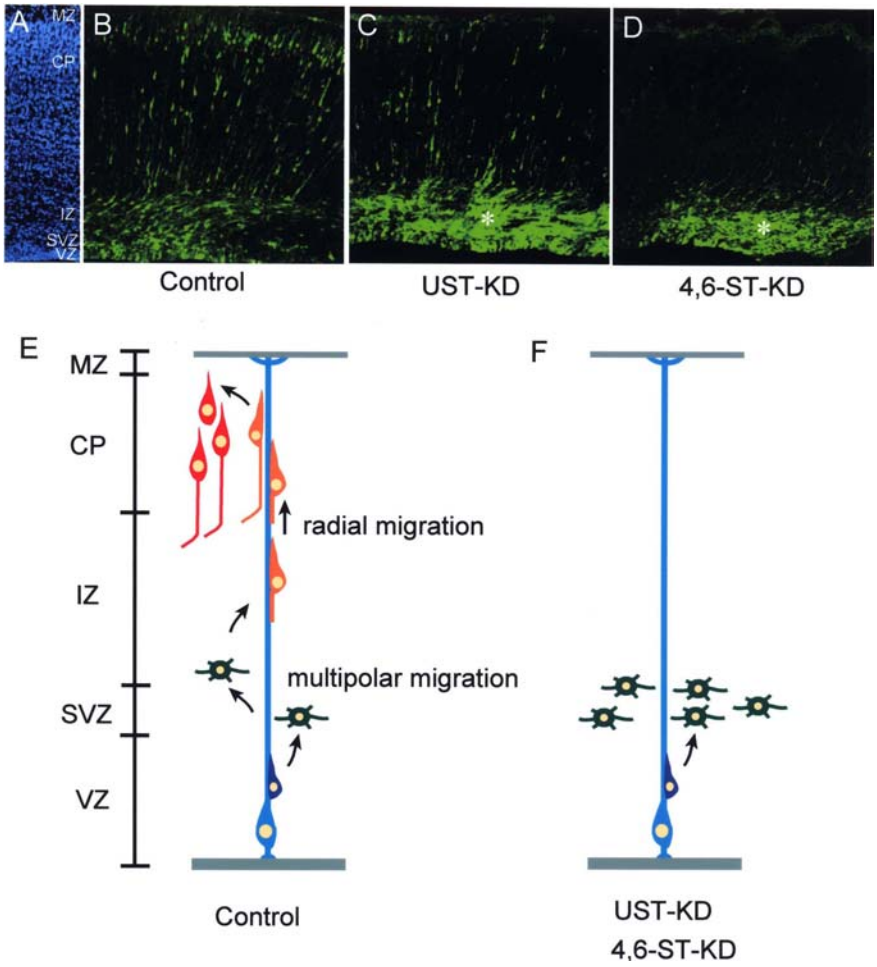

Figure 5. Oversulfated CS structures are required for neuronal migration. The cortices of mouse embryos were electroporated *in utero* with shRNA constructs at E14 and harvested 4 days later. Control GFP-positive neurons actively migrated from the ventricular zone (VZ) to the cortical plate (CP) (B and E). In contrast, GFP-positive neurons electroporated with shRNA constructs of UST (C) and 4,6-ST (D) accumulated in the lower intermediate zone (IZ) and subventricular zone (SVZ) (asterisks) showing multipolar morphology (F) (33). The nuclei were stained with TO-PRO-3 (A). MZ: marginal zone.

CS chains can be purified from appropriate regions of the brains of various developmental stages. Immunohistochemical analyses using MO-225, which recognizes D unit-rich CS, indicated that D unit was poorly expressed in P7 cerebral cortex (Figure 3G). Thus, we purified phosphacan from P7 rat cerebral cortex and found that this preparation (PG1) contained no D unit as expected (O unit : A unit : C unit : D unit = 4.3 : 63.6 : 32.1 : 0). We also purified phosphacan from P12 cerebral cortex (PG2). Compared with PG1, PG2 showed higher and lower contents of A and C units, respectively, with no D unit (O unit : A unit : C unit : D unit = 2.7 : 83.2 : 14.1 : 0). On the other hand, phosphacan purified from P20 whole brain (PG3) contained 1.3% D unit (O unit : A unit : C unit : D unit = 6.9 : 85.8 : 6.0 : 1.3). The chain lengths of CS and CS contents of these preparations did not differ significantly.

Analyses using a surface plasmon resonance biosensor indicated that PG3 had ~5-fold stronger affinity for pleiotrophin (Kd = 0.14 nM) than PG1(Kd = 0.66 nM) and PG2 (Kd = 0.57 nM), although these preparations showed similar low affinity binding to pleiotrophin after chondroitinase ABC treatment (Kd = ~1.5 nM) (Figure 4E-H). PG1 and PG2 showed similar affinity for pleiotrophin, although they differed significantly in the amounts of A and C units they contained. On the other hand, PG3 showed higher affinity for pleiotrophin than PG2, in spite of similar levels of A and C units. Considering the importance of oversulfated structures to the binding of pleiotrophin, we concluded that the presence of 1.3% D unit in the CS of PG3 strengthened the affinity for pleiotrophin. These experiments clearly indicated that structural variation of CS chains, even if subtle, can play critical roles in the determination of binding affinities of CS proteoglycans for their ligands.

4.4. Functions of oversulfated CS structures in proliferation of neural progenitor cells

In the developing cerebral cortex, precursors of pyramidal neurons are generated from neural progenitor cells (radial glia) in the ventricular zone (VZ) (64) (Figure 5E). The neural progenitor cells in the VZ express various CS

sulfotransferases including UST and 4,6-ST (33,65). Faissner's group indicated that these progenitor cells expressed CS epitopes recognized by a monoclonal antibody, 473HD (66). When the neural progenitor cells were cultured in the presence of 473HD, the generation of neurospheres was significantly reduced. A similar suppression of neurosphere formation and proliferation was observed after the treatment of progenitor cells with chondroitinase ABC (67). Furthermore, injection of chondroitinase ABC into the telencephalic ventricles of mouse embryos *in utero* resulted in a reduction of cell proliferation in VZ and a diminution of self-renewing radial glia. Since the 473HD monoclonal antibody has been reported to react with some D unit-containing sequences in CS (39), this oversulfated CS structure seems to be involved in the proliferation of neural progenitor cells.

Oohira's group indicated that neural progenitor cells in the VZ were surrounded by various CS proteoglycans including neurocan, phosphacan and neuroglycan C (68). The CS preparations purified from early embryonic rat cortex contained relatively high levels of iA and E units, and promoted FGF-2-mediated proliferation of neural progenitor cells. Commercially available iA unit-rich CS-B and CS-E preparations also exerted similar effects on progenitor cells, suggesting that iA and E units are involved in the proliferation of neural progenitor cells. On the other hand, in the presence of EGF and FGF-2, standard conditions for neurosphere culture, CS-D and CS-E but not CS-B promoted cell proliferation. Thus, it seems that neural progenitor cells require distinct CS structures for cell proliferation in a context-dependent manner.

4.5. Function of oversulfated CS structures in neuronal migration

To directly reveal the functions of oversulfated structures in the development of the cerebral cortex, we tried to downregulate the expression of oversulfated structures in the cerebral cortex *in vivo* (33). Since the oversulfated structures in the CS chains are generated by the two CS sulfotransferases, UST and 4,6-ST (Figure 2), we knocked down these enzymes in the developing cerebral cortex using *in utero* electroporation. The uterine horns of pregnant mice were exposed, and shRNA constructs of UST and 4,6-ST were injected into the lateral ventricles of the embryos (E14). The application of electrical pulses across the heads of embryos resulted in the electroporation of plasmid DNAs into the neural progenitor cells in the ventricular zone. The uterine horns were returned into the abdominal cavity, and the embryos were allowed to develop until E18. Then, the brains were removed from the embryos, and the electroporated GFP-positive neurons were observed (Figure 5A-D).

The postmitotic neurons generated in VZ firstly move to the subventricular zone (SVZ), where they enter a multipolar stage (69) (Figure 5E). These multipolar cells show meandering behavior in the SVZ and intermediate zone (IZ) for various periods, and then transform into a bipolar shape and migrate radially from the IZ to cortical plate (CP) along radial glial fibers, processes of radial glia spanning all the cortical layers. In the CP, neurons detach from radial glial fibers, and early and later born neurons occupy the deep and superficial layers, respectively, leading to an inside-out lamination of the cerebral cortex. The neurons electroporated with the control plasmids migrated radially along radial glial fibers from the VZ toward the CP, and many post-migratory neurons were accumulated in the CP (Figure 5B). In contrast, the neurons electroporated with the shRNA constructs of UST and 4,6-ST accumulated in the SVZ and lower IZ (33) (Figure 5C and D). It is remarkable that the knockdown effects of 4,6-ST were severer than those of UST. While a significant portion of UST-knocked down cells reached the CP, most of the neurons expressing shRNAs of 4,6-ST accumulated in the SVZ and few were found in the CP. Because the level of E unit generated by 4,6-ST was much higher than that of D unit in the embryonic cortex, the knockdown of 4,6-ST should decrease the oversulfated structures more effectively than that of UST (33). This may explain the difference in the knockdown effects.

The knocked down cells accumulated in the SVZ and the deep IZ showed a multipolar morphology (Figure 5C and D), suggesting that knockdown of these enzymes disturbed the transition of neurons from the multipolar migration stage to the radial migration stage (33) (Figure 5F). This transition could be induced by cytoskeletal changes stimulated by some extracellular factors or by the changes in the adhesive properties of neurons. Pleiotrophin induces the migration of cortical neurons by binding with PTPzeta *in vitro*, and has been found to distribute along radial glial fibers (52,70) As described above, pleiotrophin binds strongly with PTPzeta bearing oversulfated CS chains, and thus, knockdown of 4,6-ST and UST might suppress the signaling of this growth factor in the cortical neurons. Furthermore, it has been revealed that CS proteoglycans associate with several integrin family members, which are critically involved in neuronal migration (71). Melanoma CS proteoglycan (MCSP)/NG2 proteoglycan interacts with $alpha_4beta_1$ integrin, and PTPzeta also associates with $alpha_4beta_1$, $alpha_6beta_1$ and $alpha_vbeta_3$ integrins (72-74). It has been revealed that CS binds with $alpha_4beta_1$ integrin via a basic amino acid-rich sequence in the $alpha_4$ subunit (72). CS oversulfated structures might strengthen the integrin-CS proteoglycan interaction, leading to the enhanced signaling of integrins.

5. SIGNAL TRANSDUCTION MECHANISM OF PTPzeta

5.1. Tyrosine phosphatases

The tyrosine phosphorylation levels of cytoplasmic proteins are determined by the coordinated activities of protein tyrosine kinases and protein tyrosine phosphatases (PTPs). PTPs are a large family of enzymes that are defined by an active-site signature motif, HCX_5R, in which the cysteine residue attacks the substrate phosphate as a nucleophile (75). Like protein tyrosine kinases, PTPs are grouped into receptor-type PTPs and cytoplasmic nonreceptor PTPs. Receptor-type PTPs are composed of an extracellular region, a transmembrane segment, and one or two tyrosine phosphatase domains (D1 and D2). The extracellular regions show marked structural diversity with immunoglobulin-, fibronectin type III-, meprin-, and carbonic anhydrase-like domains.

Receptor-type PTPs have high catalytic activity in an isolated form, and therefore, the signal transduction of these

receptors is considered to be based on a stimulus-induced inactivation of enzymatic activity (75). Although many homophilic interactions and heterologous binding partners have been reported for receptor-type PTPs, a little is known about the effects of these interactions upon the phosphatase activities. However, it is generally believed that receptor-type PTPs are inactivated by ligand-induced dimerization. Recently, it has been established that PTPs are inactivated by reversible oxidation of the catalytic cysteine residue by reactive oxygen species (ROS). The level of ROS is increased in response to various growth factors, cytokines and calcium signals by the activation of NADPH oxidases. Therefore, the signaling of receptor-type PTPs may be initiated by indirect inactivation after the generation of ROS as well as by direct inactivation after ligand-induced dimerization.

5.2. Signaling mechanism of PTPzeta

Among the receptor-type PTPs, PTPzeta is quite unique, because it is synthesized as a CS proteoglycan (17, 19). This receptor uses pleiotrophin as a ligand, which is the first soluble ligand found to trigger the signaling of a receptor-type PTP (24,55). Pleiotrophin binds to the extracellular region of PTPzeta and induces its dimerization, leading to inactivation of the tyrosine phosphatase activity (24,29,55,76). As described above, PTPzeta also binds with many other proteins, however, it is unclear whether these binding partners inactivate the catalytic activity of PTPzeta. As revealed by the binding assay using phosphacan (Figure 4), the affinity of PTPzeta for pleiotrophin is regulated by the CS portion of this receptor. Notably, the oversulfated D structure greatly increases the binding affinity (36,60). Pleiotrophin stimulation increases the tyrosine phosphorylation levels of PTPzeta substrates because of inactivation of the tyrosine phosphatase activity. beta-catenin, GIT1/Cat-1, fyn, beta-adducin, p190RhoGAP, ALK and DNER have been identified as substrates of PTPzeta (55,58,77-81). It seems that pleiotrophin-PTPzeta signaling regulates diverse cellular processes by changing the tyrosine phosphorylation levels of these substrates. These processes include neurite extension, cell-cell adhesion, migration of normal and tumor cells, learning and tumor growth (24,50-52,55,78-82).

5.3. PTPzeta and morphogenesis of Purkinje cells

The cerebellar cortex expresses CS chains highly enriched with D unit, which surrounds Purkinje cells (32,35,83) (Figure 3). Thus, we examined the roles of CS in the development of Purkinje cells using an organotypic slice culture system of rat cerebellum (84). Most control Purkinje cells had a single primary dendrite, which was directed toward the pial surface and branched extraordinarily (84,85) (Figure 6B). When cerebellar slices were cultured in the presence of chondroitinase ABC, the number of Purkinje cells with multiple primary dendrites increased (84). Furthermore, many of the primary dendrites were disoriented toward the granule cell layer. A similar malformation of Purkinje cells was observed when slices were treated with CS preparations. The effects of CS were structure-dependent, and CS-D and CS-E but not CS-A induced malformation of Purkinje cells. Since this selectivity was similar to that of the inhibition of pleiotrophin-phosphacan binding (24,29), we next examined the effects of function-blocking antibodies against PTPzeta and pleiotrophin. Both treatments induced malformation of Purkinje cells, indicating that CS-mediated pleiotrophin-PTPzeta signaling plays important roles in dendrite formation of Purkinje cells.

Immunohistochemical and biochemical analyses indicated that Purkinje cells were surrounded with phosphacan bearing D unit-rich CS and pleiotrophin, and chondroitinase ABC and CS-D treatments of the cerebellar slices resulted in a marked decrease in the pleiotrophin content of the tissue (83). This suggested that phosphacan with D unit-rich CS chains served as a reservoir of pleiotrophin around Purkinje cells. On the other hand, PTPzeta was expressed in Purkinje cells and Bergmann glia (81, 84). Bergmann glia closely associated with the dendrites and cell bodies of Purkinje cells, and CS was deposited between the Purkinje cell surface and the processes of Bergmann glia (83). These observations suggested that pleiotrophin-PTPzeta signaling contributes to the morphogenesis of Purkinje cells by regulating Purkinje cell-Bergmann glia interaction.

5.4. DNER as a substrate of PTPzeta

To reveal the signaling mechanism of PTPzeta in the morphogenesis of Purkinje cells, we tried to identify the PTPzeta substrate in the Purkinje cells (81). We found that Delta/Notch-like epidermal growth factor (EGF)-related receptor (DNER) associated with PTPzeta. DNER is a single-pass transmembrane protein with 10 EGF-like repeats in the extracellular domain (86) (Figure 6A). DNER is highly expressed in Purkinje cells and acts as a ligand of Notch expressed by Bergmann glia. It has been revealed that DNER-Notch signaling regulates the morphogenesis and maturation of Bergmann glia (87). We found that DNER was co-localized with PTPzeta in the cytoplasmic vesicles present in the dendrites of Purkinje cells, suggesting that PTPzeta is involved in the intracellular transport of DNER (81). The cytoplasmic region of DNER contains a tyrosine-based sorting motif, and the C-terminal tail with a dileucine-type sorting motif, which are involved in the intracellular transport of this protein (86) (Figure 6A). When a DNER mutant lacking the cytoplasmic region was expressed in Purkinje cells, they extended multiple primary dendrites, the morphology of which was similar to that induced after inhibition of PTPzeta-pleiotrophin signaling (Figure 6C). This DNER mutant was accumulated on the plasma membrane of Purkinje cells, suggesting that endocytosis of DNER is required for normal morphogenesis (81).

We next examined the dynamics of DNER transport by using Neuro-2a neuroblastoma cells. While normal DNER protein was actively internalized by these cells (Figure 6D), various DNER mutants lacking sorting motifs were accumulated on the cell surface (Figure 6E). The Neuro-2a cells expressing these DNER mutants extended several processes, although the cells expressing normal DNER had a rounded shape and no processes (81) (Figure 6D). This suggested that the sorting motifs in the cytoplasmic region of DNER regulate the endocytosis of this protein and the blocking of this process leads to neurite extension. Our biochemical studies indicated that the tyrosine residue in the tyrosine-based sorting motif (Tyr-677) was phosphorylated, and dephosphorylated by PTPzeta (Figure 6A). Furthermore, we found that pleiotrophin increased the tyrosine

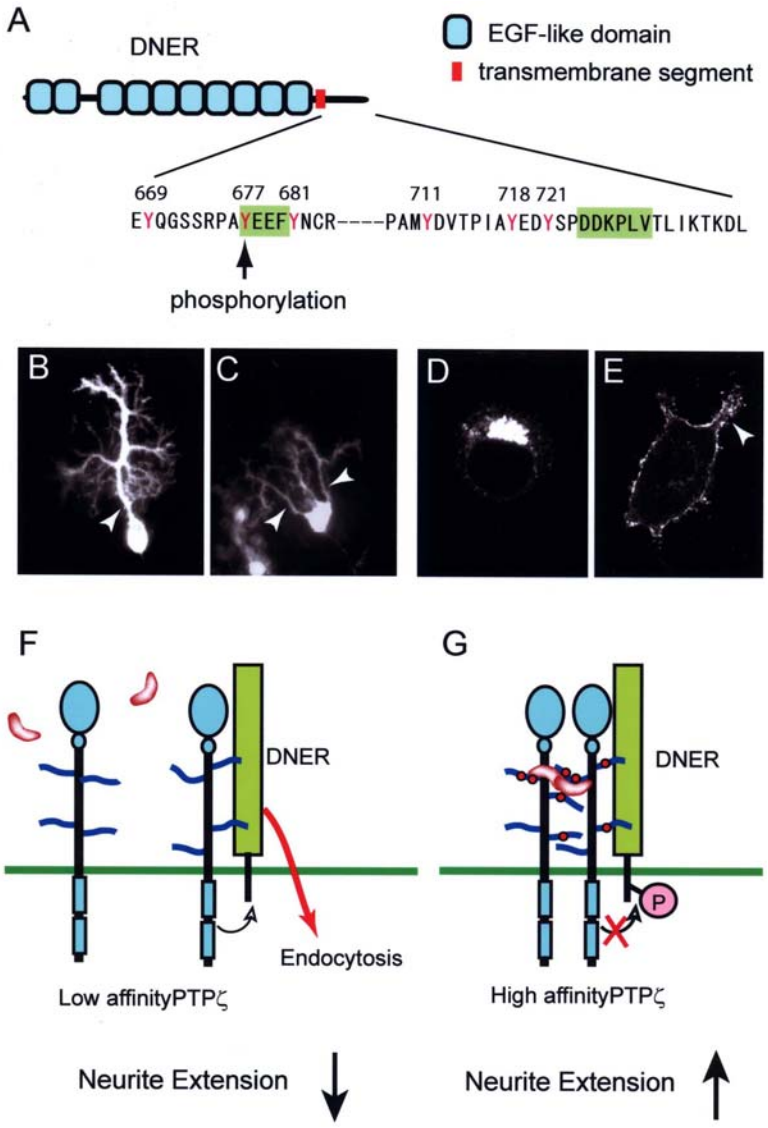

Figure 6. Pleiotrophin-PTPzeta signaling regulates endocytosis of DNER. (A) DNER is a Notch-related transmembrane protein with 10 EGF-like domains in the extracellular region. The cytoplasmic region contains a tyrosine-based sorting motif and a dileucine-type sorting motif (green box), which are involved in endocytosis and intracellular transport of this protein. The tyrosine residue in the tyrosine-based sorting motif is phosphorylated, leading to an inhibition of endocytosis. (B-E) When DNER mutants lacking the sorting motifs were overexpressed in the Purkinje cells (C) and Neuro-2a cells (E), odd neurites were extended (C and E, arrowheads) compared with control cells (B and D) (81). While normal DNER was actively endocytosed and accumulated in the cytoplasm (D), DNER mutants lacking sorting motifs were detected on the plasma membrane (E). (F and G) Schematic models of pleiotrophin-PTPzeta signaling. PTPzeta associates with DNER and dephosphorylates this protein (F). Pleiotrophin dimerizes PTPzeta and inactivates its phosphatase activity, leading to an increase in the tyrosine phosphorylation of DNER (G). Tyrosine phosphorylated DNER accumulates on the plasma membrane, leading to neurite extension. D unit in the CS chains (red circle) stimulates pleiotrophin signaling (G).

phosphorylation of DNER and suppressed the endocytosis of this protein (81). Thus, it is considered that PTPzeta-pleiotrophin signaling inactivated the tyrosine-based sorting motif of DNER by promoting the phosphorylation of Tyr-677.

These observations can be interpreted as follows. In the absence of pleiotrophin stimulation, PTPzeta constitutively dephosphorylates DNER (Figure 6F). In this situation, DNER is actively endocytosed, and neurite extension is suppressed. When pleiotrophin inactivates the phosphatase activity of PTPzeta, the tyrosine phosphorylation level of DNER increases and the endocytosis is suppressed (Figure 6G). In this situation, neurite extension is promoted. Oversulfated D structures in the CS chains on PTPzeta may strengthen the pleiotrophin signaling (Figure 6G). It is likely that such regulated tyrosine phosphorylation of DNER contributes to the

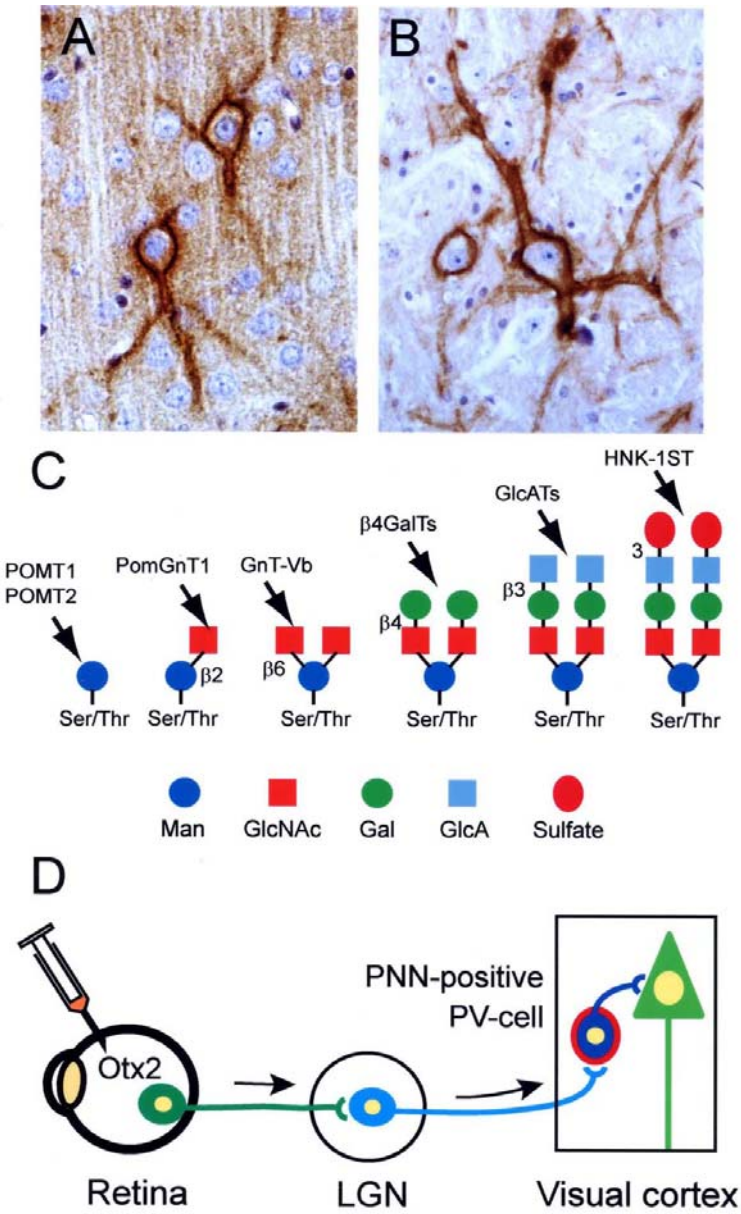

Figure 7. Perineuronal net and neural plasticity. (A and B) Perineuronal nets in the cerebral cortex (A) and parvocellular reticular nucleus (B) were immunohistochemically stained with MAb6B4. (C) Pathway for the production of the *O*-mannosyl-linked HNK-1 carbohydrate proposed by Abott *et al.* (105). POMT1/POMT2 catalyzes the transfer of mannose (Man) to a serine/threonine residue, followed by PomGnT1 adding a GlcNAc residue in beta1-2-linkage. Next, GnT-Vb adds GlcNAc in beta1-6-linkage. After the addition of galactose (Gal) residues by beta4-galactosyltransferases (beta4GalTs), GlcATs and HNK-1 sulfotransferase (HNK-1ST) generate the HNK-1 epitope. (D) After the intraocular injection of biotinylated Otx2, this protein was transported to the visual cortex, where it was internalized by perineuronal net (PNN)- and parvalbumin (PV)-positive interneurons.

morphogenesis of Purkinje cells. The controlled endocytosis of DNER may also contribute to the regulation of Notch signaling in the Bergmann glia. We observed that inhibition of pleiotrophin-PTPzeta signaling suppressed the expression of GLAST, a glial glutamate transporter, in Bergmann glia (84). There is a possibility that pleiotrophin-PTPzeta signaling regulates the differentiation of both Purkinje cells and Bergmann glia by modulating the DNER-Notch signaling pathway.

6. NEURAL PLASTICITY AND CS PROTEOGLYCANS

6.1. Perineuronal net and CS proteoglycans

In the adult central nervous system, the cell bodies and proximal dendrites of a subset of neurons are surrounded by a CS-rich extracellular matrix that is called the perineuronal net (88-90) (Figure 7A and B). This structure is excluded from the synaptic sites, forming a reticular extracellular matrix enveloping neurons. The perineuronal net is composed of

various CS proteoglycans including aggrecan, neurocan, versican, brevican, and phosphacan as well as hyaluronic acid, tenascin-R and link proteins (91). Treatment with *Streptomyces* hyaluronidase, which specifically degrades hyaluronic acid, released CS proteoglycans from the perineuronal net, indicating that these proteoglycans are anchored to hyaluronic acid. Furthermore, tenascin-R-deficient mice had a less developed perineuronal net, suggesting that CS proteoglycans, hyaluronic acid and tenascin-R form ternary complexes in the perineuronal net (92).

It is remarkable that different monoclonal antibodies against CS such as Cat-316, MAb 473 and MAb 376 immunohistochemically stained distinct but partially overlapping neuronal subsets, suggesting that the structure of CS accumulated in the perineuronal net varies depending on neuronal type (89,93,94). This means that different neurons may be surrounded by CS of different structures (sugar code). These CS might accumulate different proteins around neurons in a cell type-specific manner. Furthermore, CS binds with calcium ions in a structure-dependent manner, and such different calcium chelating activities of CS might modify the voltage-dependent calcium channels on neurons in a cell type-specific manner (95).

6.2. Perineuronal net and HNK-1 epitope

In addition to CS, some sulfated oligosaccharides are also concentrated in the perineuronal net. Cat-315 is a monoclonal antibody that immunohistochemically stains the perineuronal net of various neurons in the central nervous system (90,96). It has been revealed that Cat-315 recognized a glycoform of aggrecan in the adult brain (94). However, a recent study by Dino *et al.* revealed that PTPzeta/phosphacan was decorated with Cat-315-reactive carbohydrates in the developing central nervous system (97). This Cat-315-positive PTPzeta/phosphacan was synthesized by neurons and expressed at extrasynaptic sites prior to synapse formation, suggesting that this glycoform of PTPzeta/phosphacan prepatterns the neuronal surface before innervation. Cat-315-reactive carbohydrates might regulate both the formation and maintenace of synapses on specific neurons by changing the core proteins to which they are attached. Detailed biochemical analyses by Dino *et al.* (97) indicated that Cat-315 recognized a subset of *O*-mannosyl-linked HNK-1 epitopes on PTPzeta/phosphacan in the developing brain and on aggrecan in the adult brain (Figure 7C). *O*-mannosyl-linked glycosylation is rare in mammals and only observed in the nervous system and muscle (98). In the brain, however, ~30% of *O*-linked glycans are *O*-mannosyl-linked glycans (99). It is well known that defects in the *O*-mannosyl glycosylation of alpha-dystroglycan in muscle cause congenital muscular dystrophy (98). On the other hand, the HNK-1 epitope, a 3-sulfated GlcA linked to a *N*-acetyllactosamine structure (Figure 7C), is expressed in lymphoid and nervous systems, and is considered to play important roles in cell-cell and cell-matrix interactions (100). We previously prepared a monoclonal antibody, MAb 6B4, that recognizes PTPzeta/phosphacan purified from early postnatal animals (101). This antibody immunohistochemically stained the perineuronal net of a subset of neurons in the adult brain, the staining pattern of which was similar to that of Cat-315 (Figure 7A and B). We recently revealed that MAb 6B4 recognized carbohydrate epitopes closely related or identical to those of Cat-315 (102). Thus, the experimental results obtained using Cat-315 and MAb6B4, both of which are commercially available, should be interpreted carefully, because these antibodies may recognize PTPzeta/phosphacan and/or aggrecan depending on the age of animals.

Inactivating mutations of the glycosyltransferases that act early in the *O*-mannosyl glycosylation pathway, POMT1 and PomGnT1, cause congenital diseases with severe neurological defects (103,104) (Figure 7C), suggesting that *O*-mannosyl-linked glycans play critical roles in the nervous system. Recently, it was found that the signaling of PTPzeta is regulated by *O*-mannosylation revealed by manipulating a glycosyltransferase, GnT-Vb (105). After addition of *N*-acetylglucosamine (GlcNAc) to the mannose (Man) at position 2 by PomGnT1, GnT-Vb adds GlcNAc to Man at position 6, leading to the synthesis of 2,6-disubstituted *O*-mannosyl glycans. They are further modified by beta4-galactosyltransferases, glucuronyltransferases and HNK-1 sulfotransferase, forming the HNK-1 epitope (Figure 7C). Overexpression of GnT-Vb in SH-SY5Y neuroblastoma cells resulted in a remarkable increase of the *O*-mannosyl-linked HNK-1 carbohydrate on PTPzeta accompanied by decreased cell-cell adhesion and increased cell migration on laminin. The PTPzeta highly decorated with the HNK-1 epitope was stably retained on the cell surface, and showed increased signaling after pleiotrophin stimulation compared with PTPzeta bearing little HNK-1 epitope. It has been proposed that *O*-mannosyl-linked HNK-1 on PTPzeta bound with galectin-1, which suppressed the endocytosis of PTPzeta and enhanced its dimerization on the plasma membrane (105). Thus, HNK-1 epitope as well as CS on PTPzeta plays critical roles in the signaling of this receptor. Functional roles of the *O*-mannosyl-linked HNK-1 epitope on aggrecan is a future important problem, because this carbohydrate seems to be attached to aggrecan in the perineuronal net (94).

6.3. CS proteoglycans and neural plasticity

The formation of the perineuronal net is neuronal activity-dependent both *in vitro* and *in vivo* (106,107). Destruction of perineuronal net by chondroitinase ABC treatment *in vitro* increased the excitability of hippocampal parvalbumin-positive interneurons, suggesting that the perineuronal net is involved in regulation of the activity of GABAergic interneurons (106). The perineuronal net is detected late in development, when synaptic connections mature and are stabilized (107). From these properties, perineuronal net has been considered to be involved in the critical period plasticity, because this type of plasticity occurs in young animals in a neuronal activity-dependent manner with a major contribution of GABAergic interneurons (see below). Many neuronal circuits are intensively remodeled by experience during critical periods in early postnatal life (108). After the critical period, neural plasticity is remarkably reduced, and little remodeling of neuronal circuits occurs. A typical critical period is observed in ocular dominance plasticity. Monocular deprivation (MD), the occlusion of one eye, during the critical period leads to a shift of ocular dominance of cortical neurons in the primary visual cortex in favor of the non-deprived eye. Loss of visual acuity through

the deprived eye persists into adulthood, but MD causes no shift of ocular dominance in adult animals. The appearance of perineuronal net in the visual cortex occurs at the close of the critical period of visual plasticity (109). Dark rearing of animals from birth, which prolongs the critical period in the visual cortex, suppresses the formation of the perineuronal nets. Reintroducing such animals into a normal light-dark cycle rapidly terminates the visual plasticity, and the perineuronal net soon develops (107). These observations suggested that the perineuronal net is involved in the regulation of ocular dominance plasticity. This was confirmed by Pizzorusso *et al.* (107), who injected adult rat visual cortex with chondroitinase ABC. Since chondroitinase ABC degrades both CS and hyaluronic acid, it is considered to destroy hyaluronic acid-lecticans-tenascin complexes as well as remove CS chains from CS proteoglycans (110). After the injection of chondroitinase ABC into adult rats, MD caused an ocular dominance shift toward the non-deprived eye, as in the young animals. This showed that chondroitinase ABC reactivated ocular dominance plasticity in the adult visual cortex probably through destruction of the perineuronal net.

CS proteoglycans have been considered to inhibit the extension of axons (2,3), and therefore, chondroitinase ABC might have removed the non-permissive environment for the neuronal activity-dependent generation and/or rearrangement of synaptic connections. In other words, the perineuronal net may stabilize the synaptic connections through the CS proteoglycan-rich extracellular matrix at the end of critical periods. Removal of CS may lead to the destabilization of neuronal circuits and to the formation of new synaptic connections in an experience-dependent manner. Recently, a new molecular mechanism of ocular dominance plasticity has been proposed by Hensch's group (111, 112). They found that ocular dominance plasticity after MD failed to occur in mice lacking a GABA-synthesizing enzyme, GAD65 (111). In these knockout mice, synaptic release of GABA was significantly reduced, however, infusion of benzodiazepines, which enhance GABA signaling, restored ocular dominance plasticity. Furthermore, treatments with benzodiazepines prematurely activated this type of plasticity in normal mice. These observations indicated that the development of GABAergic interneurons plays important roles in the critical period plasticity. They further revealed that the maturation of parvalbumin-positive GABAergic interneurons in the primary visual cortex was regulated by Otx2 homeoprotein (108,112). Although Otx2 protein was strongly expressed in these interneurons after the critical period, its mRNA was not detected in the cortex. Rather, Otx2 mRNA was expressed in the retina and lateral geniculate nucleus (LGN), a thalamic relay center in the visual pathway. Surprisingly, after the injection of biotynylated Otx2 protein into the eyes, labeled protein was detected in the visual cortex, where parvalbumin-positive interneurons selectively accumulated this protein (Figure 7D). These observations suggested that Otx2 protein translated in the retina and LGN was transferred along the visual pathway and internalized by parvalbumin-positive interneurons in the visual cortex. Since homeoprotein family members have signals for secretion and internalization in the homeodomain, they may be transferred anterogradely along axons, secreted at synapses and internalized by postsynaptic neurons. The transfer of Otx2 protein seemed to be experience-dependent, leading to the activity-dependent maturation of parvalbumin-positive GABAergic interneurons. The parvalbumin-positive interneurons were surrounded by the perineuronal net, and chondroitinase ABC treatment of adult brain depleted these cells of Otx2 protein. This suggested that negatively charged CS in the perineuronal net entrapped positively charged Otx2 and promoted uptake of this protein by parvalbumin-positive interneurons. Interestingly, another homeoprotein, En2, was not internalized by these cells. The sugar code, that is, structural variability of CS in the perineuronal net, may contribute to the selective binding and subsequent uptake of specific transcription factors (108).

7. PERSPECTIVE

Besides neural development and plasticity, CS proteoglycans play critical roles in various pathological processes. Among them, the regeneration of axons is receiving much attention (3,4). In adult animals, the expression of CS proteoglycans is upregulated around scar tissues after damage to the central nervous system. Axon regeneration fails at scar tissues, leading to severe paralysis and a loss of sensation. However, the injection of chondroitinase ABC into the injured sites resulted in a promotion of axon regeneration (113,114). From this finding, CS proteoglycans are now regarded as the major inhibitor of axon regeneration. It is generally believed that CS chains synthesized by reactive astrocytes inhibit the extension of injured axons at scar tissue (3). However, the mechanism of action of chondroitinase ABC is highly controversial (115-117). As we discussed in this review, mechanisms of the action of CS proteoglycans are not simple, and little is known about the roles of molecular interactions mediated by them. Further basic studies on CS proteoglycans should reveal the unexpected mechanism of their activities and lead to the development of new treatments for many neurological disorders.

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Abbreviations: ALK: anaplastic lymphoma kinase, CS: chondroitin sulfate, C4-ST: chondroitin 4-*O*-sulfotransferase, C6-ST: chondroitin 6-*O*-sulfotransferase, C5-EP: chondroitin-glucuronate C5 epimerase, CP: cortical plate, D4-ST: dermatan 4-*O*-sulfotransferase, DNER: Delta/Notch-like epidermal growth factor-related receptor, DS: dermatan sulfate, E: embryonic day, EGF: epidermal growth factor, Gal: galactose, A unit: GlcAbeta1-3GalNAc(4S), B unit: GlcA(2S)beta1-3GalNAc(4S), C unit: GlcAbeta1-3GalNAc(6S), D unit: GlcA(2S)beta1-3GalNAc(6S), E unit: GlcAbeta1-3GalNAc(4, 6-diS), T unit: GlcA(2S)beta1-3GalNAc(4,6-diS), O unit: GlcAbeta1-3GalNAc, GlcA: glucuronic acid, HS: heparan sulfate, IdoA: iduronic acid, iA unit: IdoAalpha1-3GalNAc(4S), iB unit: IdoA(2S)alpha1-3GalNAc(4S), iD unit: IdoA(2S)alpha1-3GalNAc(6S), iE unit: IdoAalpha1-3GalNAc(4,6-diS), iO unit: IdoAalpha1-3GalNAc, IZ: intermediate zone, KS: keratan sulfate, LGN: lateral geniculate nucleus, Man: mannose, MCSP: melanoma chondroitin sulfate proteoglycan, MD: monocular deprivation, GalNAc: *N*-acetylgalactosamine, GlcNAc: *N*-acetylglucosamine, 4,6-ST: *N*-acetylgalactosamine 4-sulfate 6-O-sulfotransferase, P: postnatal day, PTP: protein tyrosine phosphatase, ROS: reactive oxygen species, S: sulfate, shRNA: short hairpin RNA, SZ: subventricular zone, UST: uronyl 2-O-sulfotransferase, VZ: ventricular zone.

Key Words: Chondroitin sulfate, MAb6B4, Neural plasticity, Neuronal migration, Perineuronal net, pleiotrophin, Proteoglycan, Purkinje cell, Receptor-type protein tyrosine phosphatase zeta, Review

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