Chondroitin Sulfate “Wobble Motifs” Modulate the Maintenance and Differentiation of Neural Stem Cells and Their Progeny*

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*Running Title: Chondroitin sulfate/Dermatan Sulfate in Central Nervous System

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Chondroitin sulfate/dermatan sulfate (CS/DS) proteoglycans, major components of the central nervous system, have the potential to interact with a wide range of growth factors and neurotrophic factors relevant to influence the neuronal migration, axon guidance pathways and neurite outgrowth. Recent studies have also revealed the role of CS/DS chains in the orchestration of the neural stem/progenitor cells micromilieu. Individual functional proteins recognize a set of multiple overlapping oligosaccharide sequences decorated to give different sulfation patterns, which are termed here “Wobble CS/DS oligosaccharide motifs”, and induce signaling pathways essential for the proliferation, self-renewal and cell lineage commitment of neural stem/progenitor cells.

The discovery of populations of multipotent self-renewing neural stem cells within the fetal and adult brain has raised the hope to develop new therapeutic strategies for central nervous system (CNS) disorders. Neural stem/progenitor cells (NSPCs) are defined as self-renewing, multipotential cells that can generate all types of neural cells including neurons and glia (astrocytes and oligodendrocytes). In an adult brain, NSPCs are present in two distinct regions; in the subgranular zone of dentate gyrus of the hippocampus and in the subventricular zone (SVZ) of the lateral ventricles (1-4). During brain development the neuroepithelial cells of the neural tube expand and self-renew by symmetric division. With increasing thickness of the neuroectoderm, radial glia cells emerge that fulfill the role of neural stem cells. In a first wave these cells self-renew by symmetrical divisions. In parallel, an asymmetric division pattern develops, where each division cycle gives rise to a radial glia cell and a neuronal progenitor. This phase of neurogenesis is followed by a phase of gliogenesis. In many regions of the CNS oligodendrocytes precede the formation of astrocytes, which constitute the final population that is formed in the developing CNS (5). The radial glia cells can transform into astrocytes and the subpopulation of astrocytes in the SVZ has been identified as...
NSPCs in the adult brain (6). Thereafter, the radial glia recedes. Solely in the cerebellum and the retina adult forms of radial glia are preserved as Bergmann glia and Müller Glia, respectively (7,8). Thus, NSPCs, which are characterized by their high proliferative potential whilst retaining self-renewal and pluripotency, encompass neuroepithelial cells, radial glial cells, and SVZ astrocytes (9,10). The self-renewal and differentiation properties of NSPCs are modulated by intrinsic factors such as transcription factors, intercellular interactions, and extrinsic factors present in the extracellular matrix (ECM). Understanding how these factors regulate the differentiation of NSPCs is essential to exploit potential therapeutic applications in treating various neurodegenerative disorders and spinal cord injuries.

Neural Stem Cell Niche

The microenvironment where the NSPCs reside and maintain their self-renewal, proliferation and differentiation is termed the “stem cell niche”. The neural stem cell niche consists of restricted sets of cell types and contains a specialized microenvironment (11-13) composed of glycoproteins, mainly tenascin-C (14-16), proteoglycans (PGs) bearing heparan sulfate (HS), chondroitin sulfate (CS), or dermatan sulfate (DS) side chains, and cell adhesion molecules such as polysialic acid (PSA) (17), stage specific embryonic antigen-1 (SSEA-1)/Lewis x (18), human natural killer-1 antigen (19), prominin (20), and gp130 (21). Studies using knockout mice have underscored the importance of ECM components in CNS development (22,23). Tenascin-C-deficient mice displayed behavioral abnormalities (24) and deficits in the stem cell compartment including a delayed acquisition of the EGF receptor (15), reduced proliferation (25) and accelerated differentiation of oligodendrocyte precursors (26-28). Studies using animals deficient in the genes involved in HS biosynthesis have provided information concerning the roles of HS in mammalian brain development (29,30). Thus, the ECM in which the NSPCs reside has a number of critical roles in the development, function, and repair after injury of the CNS, yet minimal investigation in this area has occurred. The mammalian brain is a rich source for carbohydrates, which occur in the form of PGs, glycoproteins and glycolipids. This review will focus on the role played by CS/DS-PGs in CNS development, with particular emphasis on the maintenance and differentiation of NSPCs.

Chondroitin/Dermatan Sulfate in CNS

Cloning of various sulfotransferases and glycosyltransferases involved in the synthesis of sulfated glycosaminoglycan (GAG) chains of PGs has revealed crucial functions of GAGs in development and pathophysiology. CS-GAGs are detectable in the ECM and at cell surfaces in the CNS from an early stage of development (31). Immunostaining of brain sections, using antibodies CS-56 (specific for CS-A and CS-C), MO-225, 473HD (all of which recognize octasaccharide sequences containing an A-D tetrasaccharide sequence composed of A and D disaccharide units; for abbreviations of disaccharide units, see Fig. 1 and ref. (32)) and mAb 2H6 (which recognizes a C-C tetrasaccharide sequence) revealed the existence of CS chains in the neurogenic regions of embryonic and adult brain (14,16). CS-PGs have also been found deposited between Purkinje cell surfaces and the processes of Bergmann glia (33) (not a “classic” neurogenic region, however). In addition the mAb 2A12, which is specific for iD containing DS-decasaccharide(s), showed the distribution of DS chains in the hippocampus and cerebellum of P7 mice (34).

CS chains are heterogeneous molecules with repeating disaccharide units (-4GlcUAβ1-3GalNAcβ1-), where GlcUA stands for D-gluuronic acid (Fig. 1). The structural complexity of CS chains is
generated biosynthetically under the control of multiple sulfotransferases and DS-C5 epimerases, which generate a DS domain along CS chains by converting GlcUA into L-iduronic acid (IdoUA). Depending on the number and positions of sulfate groups a rich variety of CS or DS disaccharide units can be generated (for detailed information about CS biosynthesis refer to reviews (32,35-37)). Sulfate groups are transferred from 3′-phosphoadenosine 5′-phosphosulfate to the specific acceptor sites in CS/DS chains by chondroitin/dermatan sulfotransferases (C/D-STs) that are located in the Golgi apparatus (32,35). These enzymes are classified into the following four groups: chondroitin/dermatan 4-O-sulfotransferases (C4ST/D4ST), chondroitin 6-O-sulfotransferase (C6ST), uronosyl 2-O-sulfotransferase (U2ST), and GalNAc 4-sulfate 6-O-sulfotransferase (GalNAc4S-6ST). Three C4ST isoforms (38-40), two C6ST isoforms (41,42), two DS epimerase isoforms (DS-epi-1 and epi-2) (43,44), D4ST-1 (45), U2ST (46), and GalNAc4S-6ST (47) have been identified in mammals. Gene expression levels of these enzymes correlate with the amount of sulfated products that corresponded to each enzymatic activity (48), which holds the promise that studies of gene expression of C/D-STs will yield more detailed insights into the sulfation profiles of CS, DS and their hybrid chains.

The expression of CS biosynthetic enzymes in the postnatal brain is dynamically regulated during development (49). It has been demonstrated that the ratio of C4ST and C6ST activities forming the specific sulfation profile changes markedly with development in the embryonic chick brain (50). In situ hybridization of mouse brain revealed that the ST genes including C4ST1 and C4ST2, which are involved in the synthesis of A units, a precursor for B/iB units, in addition to the gene of GalNAc4S-6ST, which synthesizes E/iE units, are ubiquitously expressed in the developing brain, while the gene expression of D4ST-1 and U2ST (which synthesize iA and D/iD/B/iB units, respectively) are restricted to the cerebellum (32,44,49,51,52). Recently, Akatsu et al. (44) also found that the DS-epi2 rather than DS-epi1 is the predominant isoform that was ubiquitously expressed in the developing brain after birth and its expression correlated with the presence of high levels of IdoUA-containing iD units and iB units at every developmental stage. Based on these observations, we speculate that CS in the brain, like HS, also has structural motifs composed of oversulfated and/or IdoUA-containing disaccharide units that change markedly with embryonic development.

**Functions of CS/DS Chains in Developing Brain**

Genetically engineered mice deficient in PTP-z/RPTP-b CS-PG, which is a receptor protein tyrosine phosphatase with one transmembrane domain and two intracellular tyrosine phosphatase modules (an isoform of this gene that comprises the complete ectodomain is released as CS-PG and known as phosphacan/DSD-1-PG in rat and mouse, respectively), exhibit an age-dependent impairment of spatial learning and enhancement of long-term potentiation (LTP) in the hippocampus (53). This is suggested to be due to impairment in the signaling of pleiotrophin (PTN)/midkine (MK), since PTP-z/RPTP-b is a receptor for these cytokines. Similarly knockdown of other CS-PGs like neurocan and brevican in mice also showed no obvious abnormalities in the brain, but the maintenance of LTP was disrupted, supporting the function of CS-PG in memory and learning (35). The reported functions of brain CS/DS chains in neuritogenesis are controversial, since it can act as a promoter as well as an inhibitor (32,35,54-59). Such apparently contradictory functions are probably attributable to the structural diversity of CS/DS chains. CS/DS chains bind and present neurotrophic factors such as PTN, MK...
and hepatocyte growth factor (HGF) to neuronal cells to promote neurite outgrowth (32,56,60,61). PTN and MK serve as a ligand for PTP-z/RPTP-b and the affinity binding of these cytokines is dependent upon the D and E disaccharide units of the CS chains in PTP-z/RPTP-b (62-64). The preferred HGF-binding sites on neuronal cell surface CS are composed of oversulfated iB and E disaccharide units. However, in vitro experiments for studying the neurite outgrowth promoting activity of CS/DS chains are dependent on cell types used. For example, CS-E, which promotes neurite outgrowth of embryonic mouse hippocampal neurons in vitro (60), is a potent inhibitor towards dorsal root ganglion explants from chick embryos (65). Similarly, phosphacan/DSD-1-PG also has opposing effects on neuritogenesis depending upon the neuronal lineages (66). In view of these findings, further investigations are needed for a better understanding of the neurite outgrowth promoting activity of CS/DS chains in vitro.

CS in the brain also functions as an axon guidance molecule. Injection of chondroitinase ABC (ChaseABC), a bacterial enzyme that degrades CS and hyaluronic acid, into developing nervous system structures leads to deviations in axon guidance pathways (67,68). In addition, oversulfated CS has been shown to influence the migration of cortical neurons, which is mediated by a PTP-z/RPTP-b-PTN/MK signaling complex (69). Knockdown of U2ST (the enzyme involved in the synthesis of B and D units, both of which contain GlcUA 2-O-sulfate) and GalNAc4S-6ST in the neural progenitor cells of embryonic cortex resulted in severe defects in the radial migration of cortical neurons, suggesting that the expression of oversulfated CS structures changes the behavior of neurons, possibly by modifying their modes of interaction with ECM components (70). In addition, oversulfated CS has been shown to reinforce integrin signaling, leading to the selective growth of CS-positive nascent axons (71). Removal of CS by ChaseABC induces the formation of unstable axons in hippocampal neurons that undergo multiple extensions and growth retardation (71). More recently, Nakanishi et al. (72) has shown that PTN, in addition to its interaction with CS chains, can also interact with the core protein of the brain-specific CS-PG neuroglycan. Another study by Mikami et al. (73) has shown that CS can function via a cell surface receptor contactin-1. Considering all these findings it is tempting to suggest that CS/DS-PGs are Master Regulators in CNS development.

CS/DS-PGs Expressing Functional “Wobble Oligosaccharide Motifs” Are Localized in the Niche of NSPCs

CS-PGs are major components of the neural stem cell niche and using the mAb CS56, brain specific CS-PGs consisting of neurocan, phosphacan and neuroglycan have been detected in the VZ of E14 fetal rat telencephalon, where the NSPCs are abundant (74,75). NSPCs by themselves participate in the construction of their own milieu by synthesizing CS-PGs including lectican PG family members (aggrecan, versican, neurocan and brevican) (76) and depositing them in their surroundings. Consistent with these observations several CS-PGs were detected in neurospheres, which are cellular aggregates that grow in suspension and composed of NSPCs and differentiating progeny. Using the monoclonal antibody 473HD, specific CS-structural motifs could be clearly attributed to cells positive for the NSPC and radial glia markers including Nestin, BLBP (Brain Lipid Binding Protein) and GLAST (Glutamate Aspartate Transporter) (77-79). Phosphacan/DSD-1-PG is another component present in the postnatal and adult NSPCs niche and as well as in the neurospheres (55,66,80). This CS-PG is selectively recognized by the mAb 473HD and its particular CS epitope, enriched
with the D, A and B disaccharide units (32), is functionally active and promotes neurite outgrowth of several types of CNS neurons (55).

In situ hybridization of E13 mouse brain showed a prominent expression of various sulfotransferase genes such as C4ST-1, C6ST-1, D4ST and U2ST in the ventricular zones of the dorsal and the ventral telencephalon (51). It has to be remembered that this is the same region where the 473HD epitope consisting of A, B and D CS-disaccharides resides and is presumably synthesized in a pathway involving the sulfotransferases for these disaccharide units. NSPCs cultured as neurospheres also maintain the expression of these enzymes (51). However, the expression of these C/D-STs changes during the lineage-specific differentiation of NSPCs. Yamauchi et al. (81) has recently reported that the expression of C4ST1, C4ST2 and C6ST1 decrease while that of U2ST and DS C5-epimerase mRNAs increase during the differentiation of NSPCs to neurons and astrocytes. They also showed that the expression of GalNAc4S-6ST is lower only in astrocytes, while the expression of D4ST-1 is lower in neurons and higher in astrocytes. This provides insights into the role of CS/DS hybrid chains (characterized by disulfated disaccharide units such as B/iB, D/iD and E/iE) in critically modulating cytokine signaling involved in the lineage-specific differentiation of NSPCs. Further inhibition of sulfation of CS/DS chains using sodium chlorate, in cells from secondary neurospheres of E13 mouse cerebral cortex resulted in a significant, dose-dependent decrease in the number of neurospheres. This decrease in neurosphere population could not be rescued by the addition of individual purified GAG chains, including heparin, CS-B, CS-D or CS-E (51). The possible explanation for this is the difference in the sulfation pattern of GAGs from CNS and non-CNS sources. Thus, neural stem cell maintenance might require the information of a “sulfation code”, as has been proposed for neurite branching influenced by HS chains in the nematode (82). Such a hypothetical code would differ for neural stem cell self-renewal versus growth and proliferation behavior since the latter could be rescued by defined CS and heparin (82). Thus, the patterned level of sulfation in CS/DS of the neural stem cell niche may allow or even instruct NSPCs behavior by modulating the activities of growth factors and cytokines.

It should be noted, however, that the term “sulfation code” (73,82) may not be appropriate. The structural information contained by specific sulfation patterns of GAG oligosaccharide motifs is not rigid but flexible, and functional proteins such as growth factors recognize the overall organization of functional GAG domains or motifs (83) but not just one set of a combination of peculiar sulfate groups at specific positions (83,84). Rather, the common features of the conformation of the overlapping multiple oligosaccharide molecules and of the electrostatic potential distribution over the surface of the sugar molecules are crucial for recognition as has been revealed by structural investigation for the CS and DS oligosaccharides that bind monoclonal antibodies (85) or PTN/HGF using biochemical and computational approaches (86,87). Hence, we propose here that such structural entities are termed “wobble GAG oligosaccharide motifs” (Table 1 shows the wobble CS motifs for PTN binding).

CS/DS Chains Promotes the Proliferation and Self-renewal of NSPCs

Stem cell maintenance and differentiation are governed by local cues found in the microenvironment (11,88). Proliferation capacities and motility of NSPCs are retained even in the adult brain and therefore understanding the mechanism of
NSPCs proliferation and differentiation is of great therapeutic significance for a wide variety of clinical disorders, including cell replacement strategies. NSPCs display multipotentiality and adopt a wide range of phenotypes in response to various stimulating factors from the microenvironment. These responses are partially mediated by the CS/DS chains that are found on the surface of NSPCs and in the niche. Degradation of CS chains by ChaseABC, both in vivo and in vitro, reduces NSPCs proliferation and the differentiation of radial glia to neurons, favoring the maturation of the gliogenic subtype of radial glia and the formation of astrocytes in the telencephalon (77, 78). Deglycanation of CS reduced the number of neurospheres and of proliferating NSPCs. Utilizing the clonal density assay it was demonstrated that CS can also promote self-renewal of early telencephalic NSPCs grown in a neurosphere culture. Twice as many secondary neurospheres originated from cell suspensions derived from untreated primary cortical and striatal neurospheres, than from neurospheres that had been exposed to ChaseABC. The findings described provide the first experimental evidence for CS/DS chains in regulating the self-renewal and proliferation of NSPCs. This is reminiscent of the functions of HS, which contributes to the self-renewal and proliferation of embryonic stem cells (89, 90). Recently, Tham et al. reported that the soluble phosphacan/DSD-1-PG can stimulate the survival of neural stem cells by preferential signaling through EGF receptor, JAK and PI3K pathways (91). The same study also revealed that the CS-PGs can enhance the survival of neural stem cells derived from ES cells and thus can be used as a tool to generate ES cells-derived neural stem cells (91).

The capacity of CS/DS chains to bind to various growth factors might impinge on the proliferation rate of NSPCs. CS has recently been documented for its ability to induce stem cell proliferation in the nematode, and the involvement of chondroitin and CS in controlling embryonic cell division is mediated by regulating proper embryonic cytokinesis (35, 92, 93). Studies have also shown that CS serve as docking sites for growth factors and thereby modulate responsiveness to FGF-2 in embryonic NSPCs (94). In experiments by Sirko et al. (78), NSPCs were grown as freely floating neurospheres in defined media containing the growth factors EGF and FGF-2 and therefore the secreted or cell surface CS-PGs can intervene in the FGF-2- or EGF-dependent signaling pathways and thereby foster NSPCs proliferation and self-renewal. This could be effected either through the manner by which CS-GAGs bind and store factors in the pericellular environment (48), or by their serving as cis-acting cofactors for growth factor receptors, analogous to the role played by HS-PGs with respect to the FGF receptor (54). Indeed, the growth factors PTN and MK, which have been associated with the proliferation of NSPCs, are also secreted into the neurosphere conditioned media and strongly interact with the CS chains of phosphacan/DSD-1-PG (94, 95). Taken together, the profound effect of elimination of CS/DS chains in inhibiting the proliferation and self-renewal of NSPCs is likely due to impacts on a multitude of signaling pathways. This corroborates with the studies of tenascin-C, a glycoprotein that interacts with the phosphacan/DSD-1-PG (96, 97). Tenascin-C facilitates NSPCs development by altering the response of cells to mitogenic growth factors, and one possibility is that this could be due to the interaction of tenascin-C with CS-PGs. This assumption is further supported by the finding that tenascin-C stimulates contactin-dependent neurite outgrowth (98), and recently it has been shown that contactin also serves as a receptor for CS-E (73).
CS/DS Chains Participate in the Decision of Fates of NSPCs

The subject of cell fate also referred to as the ultimate differentiated state, to which a cell has become committed, is governed by a set of intrinsic transcriptional regulators, but also by the unique local microenvironment (99). Towards the end of embryogenesis and during early postnatal life, astrocytes and oligodendrocytes are mainly generated from NSPCs while neurogenesis has largely ceased (1,5). This timely differentiation of NSPCs is dependent on the presence of CS and the elimination of CS/DS from NSPCs inhibits neurogenesis and increases gliogenesis (78). In these studies telencephalic neurospheres were used as a model for NSPCs because these cellular aggregates self-renew in response to FGF-2 and EGF, and give rise to neurons, astrocytes and oligodendrocytes upon differentiation. Elimination of CS chains from neurospheres reduced the number of BLBP-positive neurogenic neural progenitor cells and increased the GLAST-positive radial glial cells that preferentially generate astroglia (78). In vivo removal of CS/DS chains from the cerebral ventricles of E13-14 embryos reduced the number of phospho-histone H3-positive and BrdU-positive proliferating cells (phospho-histone H3 and BrdU are markers for mitosis and DNA-synthesis, respectively) in the area close to the ventricular surface, indicating that CS/DS regulates the proliferation of precursor cells residing in the VZ of developing brain. Further removal of CS chains is shown to decrease the expression of neural precursor markers nestin and BLBP, and to increase the number of GLAST-positive precursor cells in the cortical regions (78). These studies show the essentiality of CS/DS chains for the timely differentiation of neurogenic-positive radial glia to neurons. The authors in this paper interpret that CS/DS might be part of the control machinery that delays the onset of gliogenesis and promotes self-renewal of stem cells and neurogenic precursors (78).

The timely differentiation of NSPCs into neurons and glia is differentially controlled by CS via regulation of the responsiveness to FGF-2 and EGF (79). Using the neurosphere culture model it was observed that the selective removal of CS/DS chains preferentially affected neurosphere formation of NSPCs in response to FGF-2, rather than in response to EGF. Quantification of BrdU positive cells on ChaseABC treated neurospheres revealed that FGF-2-sensitive NSPCs require intact CS chains for proliferation. Further, the enzymatic removal of CS-GAGs suppressed the expansion of FGF-2-responsive, BLBP-positive, and preferentially neurogenic NSPCs. Removing CS chains favored the increase of GLAST-positive and EGF-responsive progenitors that preferentially generate astroglia. The preferential expansion of GLAST-positive radial glia can reflect an enhanced propensity of dividing cortical progenitors to generate glial-restricted progenitors, with a concomitant decrease in the generation of BLBP-expressing neurogenic progenitors. This switch in phenotype of neural progenitor cells most likely corresponds to radial glia cell subtypes that appear at later developmental stages (100). In this way, a selective expression of CS-PGs in subsets of cells may account for the generation of NSPC diversity. The gradually declining or changing disaccharide composition of CS-GAGs during forebrain development results in CS/DS chains with functional “wobble motifs” which can direct the NSPCs to neurogenic and/or gliogenic lineage commitment. But still there is no extensive in vivo evidence for the roles of CS-PGs in the control of neural stem cells. Further studies based on gene knockout for CS-PGs will help to fully understand the precise role of this sugar molecule in CNS development.
Conclusions

The discovery of NSPCs and their ability to differentiate in the adult brain can be viewed as one of the major breakthroughs in the field of neuroscience. Yet, the application of stem cells for cell replacement therapies is still in its infancy. In an optimistic perspective much work remains to be done to attain a safe and secure state of empirical research and application. The studies discussed herein for the first time demonstrated that CS/DS-carbohydrates play a pivotal role in the orchestration of the NSPC micromilieu. CS-PGs are the major components of the neural stem cell niche, and fine structures of CS/DS chains are dynamically and spatiotemporally regulated during CNS development. CS-PGs fine tune the neural stem cell microenvironment and mediate various biological processes including the proliferation, self-renewal, cell lineage commitment and cytokinesis (Fig.2).

Encouraged by these findings, we should exploit and manipulate the powerful influence that the microenvironment holds for stem cell fate decision. The capacity of CS/DS chains containing functional “wobble GAG oligosaccharide motifs” to possibly instruct NSPCs and direct their lineage commitment opens a new avenue for the use of stem cells in regenerative medicine. The ease of isolation of CS fragments from biological sources including marine organisms may yield slow acting yet safer sugar based drugs (36), which can harness the processes of neural stem cell based therapies. Chemical synthesis of active CS/DS fragments is also a powerful approach once various wobble GAG oligosaccharide motifs are elucidated.

REFERENCES


FOOTNOTES
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The abbreviations used are: NSPCs, neural stem/progenitor cells; CS/DS, chondroitin sulfate/dermatan sulfate; C/D-STs, chondroitin/dermatan sulfotransferases; ECM, extracellular matrix; GAG, glycosaminoglycans; PG, proteoglycan; GlcUA, D-glucuronic acid; IdoUA, L-iduronic acid; ChaseABC, chondroitinase ABC; CNS, central nervous system; PTN, pleiotrophin; MK, midkine; HGF, hepatocyte growth factor; SVZ, subventricular zone; GLAST, glutamate aspartate transporter; BLBP, brain lipid-binding protein; LTP, long term potentiation; PTP-z/RPTP-b and phosphacan/DSD-1-PG (are doublets).

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Figure Legends

FIGURE 1. Structure of CS/DS disaccharide unit. A, The repeating disaccharide unit of CS are composed of GlcUA and GalNAc residues. GlcUA often undergoes epimerization to form L-iduronic acid (IdoUA) and generates the DS disaccharide units along CS chains. CS units are named traditionally and the corresponding DS units are indicated by “i”, which stands for IdoUA. Sulfation of the sugar residues, indicated by “S”, occurs at the 2nd, 4th and 6th carbon positions in the ring (32). Depending on the position of sulfation, there are 6 major CS/DS disaccharide units that generate numerous polymer sequences. The enormous diversity thus generated by the various disaccharide units can result in the formation of various sets of “wobble motifs” along the CS/DS hybrid chains. B, Biosynthetic pathways of CS/DS chains. C4ST, chondroitin 4-O-sulfotransferase; C6ST, chondroitin 6-O-sulfotransferase; D4ST, dermatan 4-O-sulfotransferase; epimerase, glucuronyl C5-epimerase; GalNAc4S-6ST, GalNAc 4-sulfate 6-O-sulfotransferase; UST, uronyl 2-O-sulfotransferase.
FIGURE 2. Proposed mechanism of CS-PGs in modulating the neural stem cell and their progeny. CS-PGs, the major components of the neural stem cell niche in the subventricular zone (SVZ) interact with various growth factors and neurotrophic factors and promote the proliferation/self-renewal and differentiation of neural stem/progenitor cells (NSPCs) and neurite outgrowth of terminally differentiated neurons. CS/DS-PGs interact with growth factors like FGF and EGF, and promote the proliferation and self-renewal of NSPCs. The timely differentiation of NSPCs to neurons is controlled by CS/DS chains, which specifically bind and favor the responsiveness to FGF. Thus, CS-PGs could instruct the NSPCs and direct their lineage commitment. CS/DS-PGs also bind and present neurotrophic factors such as PTN, MK and HGF to neuronal cells to promote neurite outgrowth. The high affinity binding sites for these factors appear to contain D, E and/or B disaccharide units and are also enriched in IdoUA. Hence, the ligand binding sites on the CS/DS hybrid chains are not rigid but flexible, and individual functional proteins recognize a set of multiple oligosaccharide sequences decorated by wobble sulfate groups to give different sulfation patterns. They are termed here “Wobble CS/DS motifs” and influence various cellular events including proliferation, self-renewal and differentiation of NSPCs, and neuritogenesis of terminally differentiated neurons.
Table 1: PTN-binding ‘Wobble GAG Motifs” expressed by CS/DS hybrid chains during CNS development

<table>
<thead>
<tr>
<th>Sequence</th>
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<tbody>
<tr>
<td>iC-C-D-C-iX</td>
</tr>
<tr>
<td>iA-C-D-C-iX</td>
</tr>
<tr>
<td>iC-A-D-C-iX</td>
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<tr>
<td>iC-D-D-C-iX</td>
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<td>iE-D-A-D-iX</td>
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<tr>
<td>iE-D-iA-D-iX</td>
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</table>

CS/DS hybrid chains isolated from embryonic pig brain were fractionated using a PTN affinity column and the PTN-bound oligosaccharide fractions were sequenced by HPLC after digestion with ChaseB that cleaves GalNAc-IdoUA but not GalNAc-GlcUA linkages followed by fluorescent labeling (84). The table shows the PTN-binding motifs in the CS/DS chains containing IdoUA, which therefore are not rigid but rather flexible. PTN thus binds not just one set of a particular sequence, rather an overall functional domain structure in the GAG chain. Based on these findings, we propose a ‘Wobble Hypothesis” and states that the growth factor binding-moieties on GAGs are a set of multiple overlapping oligosaccharide sequences with similar conformation and distributed electrostatic potential but not just one specific sequence. ‘i’ represents IdoUA and ‘X’ represents any disaccharide unit including A, C, D, E, B or T (tri-sulfated) unit.
Figure 1

A

Chondroitin Sulfate (CS)

Dermatan Sulfate (DS)

<table>
<thead>
<tr>
<th>CS Disaccharide Unit</th>
<th>DS Disaccharide Unit</th>
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<tbody>
<tr>
<td>O unit: GlcUA-GalNAc</td>
<td>iO unit: IdoUA-GlnAc</td>
</tr>
<tr>
<td>A unit: GlcUA-GalNAc(4S)</td>
<td>iA unit: IdoUA-GlnAc(4S)</td>
</tr>
<tr>
<td>C unit: GlcUA-GalNAc(6S)</td>
<td>iC unit: IdoUA-GlnAc(6S)</td>
</tr>
<tr>
<td>D unit: GlcUA(2S)-GlnAc(6S)</td>
<td>iD unit: IdoUA(2S)-GlnAc(6S)</td>
</tr>
<tr>
<td>B unit: GlcUA(2S)-GlnAc(4S)</td>
<td>iB unit: IdoUA(2S)-GlnAc(4S)</td>
</tr>
<tr>
<td>E unit: GlcUA-GalNAc(4S, 6S)</td>
<td>iE unit: IdoUA-GalNAc(4S, 6S)</td>
</tr>
</tbody>
</table>

B

Epimerase

Epimerase

Epimerase

O unit → iO unit

C6ST

C4ST

D4ST

C unit

A unit

D unit

E unit

GalNAc4S-6ST

UST

UST

UST

iA unit

iE unit

iB unit
Figure 2

Subventricular zone

CS/DS-PG

Neural stem/progenitor cells

Proliferation/Self-Renewal

Differentiation

Neurotropic factors/HGF

Neurite outgrowth

CS/DS-PG

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Chondroitin Sulfate Wobble Motifs Modulate the Maintenance and Differentiation of Neural Stem Cells and Their Progeny

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