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Chondroitin sulfate and neuronal disorders

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1. ABSTRACT

The brain extracellular matrix (ECM) is involved in several aspects of neuronal development, plasticity, and pathophysiology. Chondroitin sulfate proteoglycans (CSPGs), consisting of core proteins with covalently attached chondroitin sulfate (CS) chains, are essential components of the brain ECM. During late postnatal development, CSPGs condense around parvalbumin-expressing inhibitory neurons (PV-cells) and form lattice-like ECM structures called perineuronal nets (PNNs). Enzymatic or genetic manipulation of PNNs reactivates neuronal plasticity in the adult brain, probably by resetting the excitatory/inhibitory balance in neural networks. Recent studies have indicated that PNNs control PV-cell function by enhancing the accumulation of specific proteins at the cell surface and/or acting as neuroprotective shields against oxidative stress. Since dysfunction of PV-cells and remodeling of CSPGs are commonly observed in several disorders, including schizophrenia, Costello syndrome, Alzheimer's disease, and epilepsy, modulation of PV-cell function by CSPGs may provide a novel strategy for these neuronal disorders. Here we review the potential roles of CSPGs as therapeutic targets for neuronal disorders, with particular focus on structural changes of CS chains under pathological conditions.

2. INTRODUCTION

The brain extracellular space is filled with a solution of ions and extracellular matrix (ECM) molecules and occupies approximately 20% of the adult brain volume (1). The brain ECM plays important roles in neuronal development, plasticity, and pathophysiology. Chondroitin sulfate proteoglycans (CSPGs), consisting of core proteins with covalently attached chondroitin sulfate (CS) chains, are major components of the brain ECM (2, 3) (Figure 1A). One well-known function of CSPGs in the central nervous system is as a physical barrier that restricts neuronal plasticity. Enzymatic digestion of CSPGs enhances neuronal plasticity and axon regeneration after injury (4, 5). In addition, CSPGs form lattice-like structures called perineuronal nets (PNNs) around a subset of inhibitory neurons expressing parvalbumin (PV-cells) (6, 7). Recent studies have revealed that CSPGs not only act as physical barriers, but also regulate the excitatory/inhibitory balance in neural networks via their effects on PV-cell function (8, 9) (Figure 1B). Disturbances in excitatory/inhibitory balance are commonly observed in many neuronal disorders, suggesting that modulation of PV-cell function by CSPGs may offer a novel strategy for treating these disorders (10-12). We here review recent studies on the roles of CSPGs as a potential therapeutic target for neuronal disorders, including schizophrenia, Costello syndrome, Alzheimer's disease, and epilepsy.

3. CHONDROITIN SULFATE IN NEURONAL PLASTICITY AND DISORDERS

3.1. Structure of chondroitin sulfate

CS is a class of sulfated glycosaminoglycan chains composed of a repeating disaccharide unit consisting of glucuronic acid (GlcA) and *N*-acetylgalactosamine (GalNAc) (Figure 1A). Biosynthesis of CS is initiated by

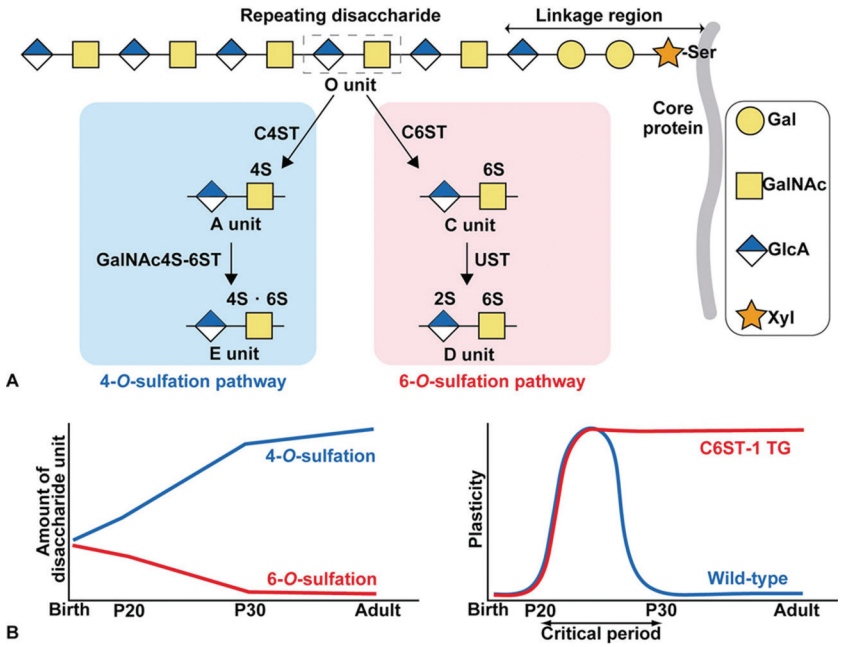

Figure 1. Sulfation patterns of CS chain and the critical period plasticity. (a) Biosynthesis of CS chain is initiated by formation of the linkage region on the Ser residue in the core protein. Alternative transfer of GlcA and GalNAc elongates chondroitin backbone consisting of the non-sulfated repeating disaccharide units called O unit. The sulfation pathways can be classified into 4-*O*-sulfation and 6-*O*-sulfation. In the 4-*O*-sulfation pathway, the C4-position of the GalNAc residue in the O unit is sulfated by C4ST, followed by conversion of the A unit to an E unit by GalNAc 4-sulfate 6-*O*-sulfotransferase (GalNAc4S-6ST). In the 6-*O*-sulfation pathway, the C6-position of the GalNAc residue in the O unit is sulfated by C6ST, followed by conversion of the C unit to a D unit by uronyl 2-*O*-sulfotransferase (UST). (B) Developmental changes of CS sulfation patterns across the critical period. In the developing mouse brain, the proportion of 6-*O*-sulfation decreases, whereas that of 4-*O*-sulfation markedly increases from birth to maturity (left). The critical period for ocular dominance plasticity in the visual cortex peaks around postnatal day (P) 25, after which visual cortical neurons become less plastic in wild-type mice. C6ST-1 TG mice, which retain high 6-*O*-sulfation level, show persistent plasticity throughout adulthood (right).

addition of the linkage tetrasaccharide sequence to a specific serine residue in a core protein (13, 14). Among the different core proteins, lectican family proteoglycans and phosphacan are highly expressed in the brain and form PNNs, as described later (2). The CS chain is elongated by the alternate transfer of GlcA and GalNAc, first to the linkage region, and then to the growing CS chain; these transfers are catalyzed by a combination of six homologous glycosyltransferases (13, 14). Chondroitin sulfotransferases then sequentially modify the chondroitin backbone with sulfates. In the first step, a non-sulfated O unit (GlcA-GalNAc) is modified by either chondroitin 4-*O*-sulfotransferase (C4ST) or chondroitin 6-*O*-sulfotransferase (C6ST), resulting in the formation of the A unit (GlcA-GalNAc(4-*O*-sulfate)) or the C unit (GlcA-GalNAc(6-*O*-sulfate)), respectively. Subsequently, a small portion of the A and C units are converted to E unit (GlcA-GalNAc(4,6-*O*-disulfate)) and D unit (GlcA(2-*O*-sulfate)-GalNAc(6-*O*-sulfate)), respectively. Therefore, the sulfation of CS can be classified into the initial "4-*O*-sulfation" or "6-*O*-sulfation" pathways (2, 3). The arrangement of these sulfated units along the CS chains creates characteristic sulfation patterns that may convey the functional information carried by CSPGs. In the central nervous system, treatment with chondroitinase ABC, which selectively degrades CS, has been shown to enhance neuronal plasticity and axon regeneration after nerve injury, indicating that the physiological functions of CSPGs are mainly attributed to their CS moieties (4, 5).

3.2. Chondroitin sulfate proteoglycans form perineuronal nets

In several regions of the brain, including the cerebral cortex, CSPGs preferentially condense around PV-cells to form PNNs, which are specialized ECM structures (6, 7) (Figure 2). The formation of PNNs is not exclusively restricted to PV-cells, and some pyramidal neurons are also enwrapped by PNNs (15, 16). PNNs tightly interdigitate with synaptic contacts on the soma and proximal dendrites of neurons. CSPGs belonging to the lectican family, which includes aggrecan, versican, neurocan, and brevican, are major components of PNNs. In PNNs, lecticans are cross-linked by the multimeric tenascin-R via their C-terminal lectin-like domain. In addition, the N-terminal domain of lecticans binds to hyaluronan, which is tethered to the neuronal surface by transmembrane hyaluronan synthases. Link proteins further enhance the interaction between lecticans and hyaluronan. Therefore, PNNs form massive macromolecular complexes in the pericellular space. Regulated expression of PNN components controls the formation of PNNs that begins during late development

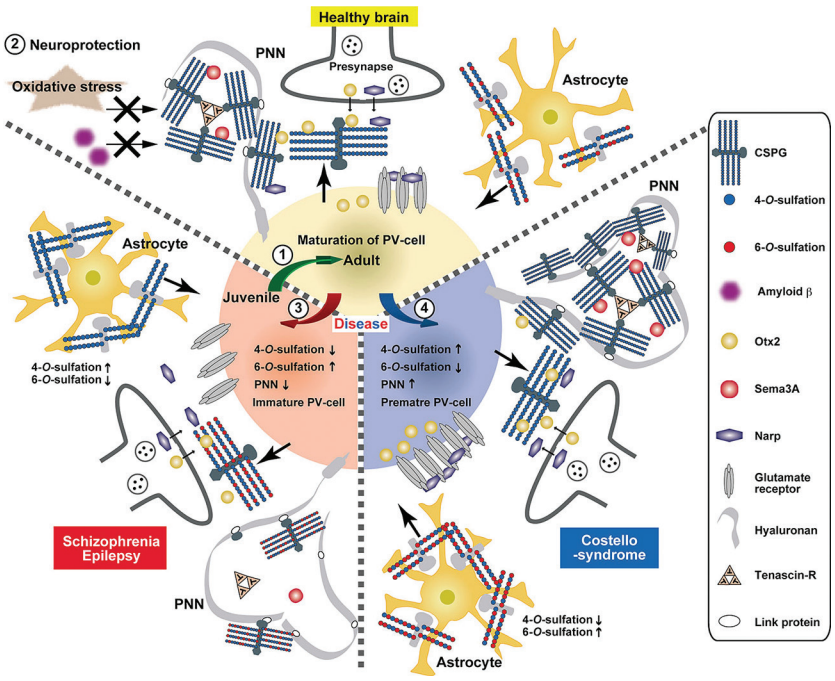

Figure 2. Roles of CS chains in PV-cell function and neuronal disorders. During late postnatal development, CSPGs, together with hyaluronan, tenascin-R, and link protein, form PNNs around PV-cells. Developmental shift in sulfation patterns from 6-*O*-sulfation to 4-*O*-sulfation regulates PNN formation. 4-*O*-sulfation produced by PV-cells is required for tightly condensed PNN structure (1). In the healthy adult brain, 6-*O*-sulfation is restricted to a subpopulation of astrocytes. CS chains in PNNs accelerate accumulation of secreted molecules, such as Otx2, Narp and Sema3A around PV-cells possibly by directly binding to these molecules. Otx2 and Narp enhance functional maturation of PV-cells. Sema3A may restrict plasticity by inhibiting new synapse formation. CS chains in PNNs also play a critical role in neuroprotection against oxidative stress and amyloid plaques formation (2). In schizophrenia and epilepsy, impairment of PNN, which is caused by reduced 4-*O*-sulfation and/or increased 6-*O*-sulfation produced in PV-cells, leads to hypofunction of PV-cells that largely contributes pathogenesis of the neurodevelopmental disorders (3). Elevated 6-*O*-sulfation may render CSPGs more susceptible to degradation by MMPs. In schizophrenia, astrocytes produce a considerable amount of WFA-positive CSPGs suggesting the up-regulation of 4-*O*-sulfation in astrocytes, which is contrast to the decreased WFA-positive PNNs around PV-cells. Abnormal balance of 4-*O*-sulfation and 6-*O*-sulfation possibly affects both PV-cell and astrocyte function in the disease. In another neurodevelopmental disorder called Costello syndrome, preconscious formation of PNNs leads to hyper maturation of PV-cells and premature closure of the critical period plasticity (4). It is possible that expression of 4-*O*-sulfation is increased in PV-cells to compensate a dysregulated production of CS chains in astrocytes.

and is completed at adulthood (17, 18). PNN formation is significantly diminished in aggrecan-deficient neurons, indicating that aggrecan is an essential component of PNNs (19). Mice lacking link proteins or tenascin-R have attenuated PNN formation (20-22). Furthermore, there are drastic changes in the sulfation patterns of CS chains during the formation of PNN. Specifically, 6-*O*-sulfation is dominant in the juvenile brain, whereas 4-*O*-sulfation becomes dominant in the adult brain (8, 23) (Figure 1B). This shift in sulfation patterns is essential for PNN formation: transgenic (TG) mice overexpressing C6ST-1 retain juvenile-like CS sulfation and show impaired PNN formation (8).

3.3. Chondroitin sulfate and neuronal plasticity

Recent studies have proposed that PNNs regulate experience-dependent neuronal plasticity by enhancing PV-cell maturation. Plasticity is most robust during a limited time window early in life, the so-called critical period (24). For example, monocular deprivation during the critical period (age 5-8 for human and postnatal days 20-30 for mouse) leads to reduced response of visual cortical neurons to the deprived eye and subsequent persistent loss of visual acuity (amblyopia) (25). Importantly, amblyopia can be recovered by patching the stronger eye if this treatment is performed during the critical period. However, the ability to recover from amblyopia declines with age, and this treatment has little effect in adulthood. This phenomenon is called ocular dominance plasticity and has been studied extensively as a classic example of the effects of experience on cortical circuits.

Functional changes in the balance between excitation and inhibition may control ocular dominance plasticity. The onset and termination of the critical period can be accelerated or delayed by enhancing or preventing inhibitory tone, respectively (26-29). Although “gamma”-aminobutyric acid (GABA) ergic inhibitory neurons show great diversity in their morphological and physiological properties (30), recent evidence has demonstrated that maturation of a single class of inhibitory neurons, PV-cells, plays a crucial role in controlling the critical period plasticity (24). Unlike other inhibitory neurons, PV-cells

emerge in the late postnatal stage just before onset of the critical period (31). Maturation of PV-cells, which precedes the termination of the critical period, occurs in parallel with the appearance of PNNs. Strikingly, removal of PNNs with chondroitinase ABC has been shown to reactivate ocular dominance plasticity after the closure of the critical period in adult animals (4). Thus, CSPGs in PNNs may act as molecular "brakes" for termination of the critical period (32).

CSPGs have been classically thought to restrict plasticity by acting as a non-specific physical barrier that prevents rearrangement of synaptic connections (33). However, recent studies have demonstrated that PNNs actively regulate PV-cell function by enhancing the accumulation of secreted proteins at the cell surface (Figure 2). One striking example is Otx2 homeoprotein, which is produced in the retina and choroid plexus and is then transported to PV-cells in the cerebral cortex (31, 34). Incorporation of Otx2 accelerates PV-cell maturation and the timing of the critical period, whereas genetic deletion of Otx2 prevents the onset of the critical period. The accumulation of Otx2 in C6ST-1 TG mice is dependent on the sulfation patterns of CS chains in PNNs (8). 6-*O*-Sulfation-enriched PNNs show a less condensed morphology in these mice, which may lead to diffusion and reduced accumulation of Otx2. It was also reported that Otx2 directly binds to CS chains rich in D and E units, but not in A and C units (31, 35). A 15 amino acid peptide containing an arginine-lysine sequence within Otx2 was identified as a CS binding sequence.

CS chains in PNNs may facilitate the sequestration of other secreted molecules to regulate PV-cell function. Trans-synaptic transport of neuronal activity-regulated pentraxin (Narp) can be supported by PNNs (36). Narp expression in excitatory neurons is dependent on neuronal activity. Narp is released from the presynaptic terminals, then binds to GluR4-containing glutamate receptors at postsynaptic sites on PV-cells to promote clustering of the receptors, which results in enhanced excitatory inputs to PV-cells. Narp-deficient mice show decreased excitatory synapse density on PV-cells and abnormal critical period plasticity in the visual cortex (37). It should be noted that disruption of PNNs with chondroitinase ABC prevents the localization of Narp on PV-cells (36). However, it is currently unknown whether Narp directly interacts with CS chains in PNNs. Semaphorin3A (Sema3A), a chemorepulsive axon guidance protein, is another candidate that regulates PV-cell function and plasticity. In the adult cerebral cortex, Sema3A is co-localized with PNNs surrounding PV-cells (38). The accumulation of Sema3A is supported by its interaction with CS chains rich in E units (39). Although it remains unclear whether Sema3A is involved in PV-cell function, Sema3A may act as an inhibitory cue for synapse rearrangements and restricts critical period plasticity. Notably, dysregulation of PV-cell function not only affects developmental plasticity but is also involved in several neuronal disorders, as we will discuss in the next sections.

3.4. Chondroitin sulfate and neurodevelopmental disorders

Schizophrenia is a neurodevelopmental disorder affecting approximately 1% of the world's population (10, 40). Although psychosis (e.g., hallucinations and delusions) is the most striking clinical aspect of schizophrenia, impairments in cognitive function such as perception, memory, and learning are now recognized as the core features of the disorder. Several lines of evidence propose that deficient GABAergic transmission contributes to impaired cognition in schizophrenia (10, 40). Reduced levels of glutamic acid decarboxylase(GAD) 67, a GABA-synthetic enzyme, appear to be a conserved feature of schizophrenia. Specifically, *GAD67* mRNA levels in PV-cells, but not other neurons, are markedly reduced in the prefrontal cortex of schizophrenia patients. Recent studies have implicated that decreased inhibitory tone in schizophrenia is not a primary cause of the disease but rather is a result of compensation for an upstream deficit in excitatory neurons. Although this compensation is thought to reset the excitation/inhibition balance, this new level of excitation/inhibition balance may be insufficient to support cognitive function.

Importantly, postmortem studies of human brains have shown that the number of PNNs is markedly reduced in specific regions of brains with schizophrenia (41) (Figure 2). In the prefrontal cortex, the number of PNNs increases during postnatal development through the peripubertal period until late adolescence, and this period overlaps with the onset and emergence of schizophrenia symptoms (42). The number of PNNs was decreased by approximately 70% in layers 3 and 5 of the prefrontal cortex in schizophrenia as compared with controls. Thus, the aberrant formation of PNNs presumably results in dysfunction of PV-cells, one of the hallmark characteristics of schizophrenia. In schizophrenia, insufficient PNN formation may affect PV-cell function by mechanisms similar to those observed in developmental plasticity. In this regard, it should be noted that the Sema3A signaling pathway is a candidate for susceptibility to schizophrenia (43, 44). It was recently reported that *NARP* mRNA levels are significantly decreased in the prefrontal cortex of schizophrenia subjects (45). A reduced amount of Narp may lower the excitatory inputs onto PV-cells, resulting in deficits in cognitive function characteristic of schizophrenia. As described above, the aberrant formation of PNN possibly decreases the effect of Narp on PV-cells and contributes to symptoms of schizophrenia.

A similar reduction in PNN formation has been reported in the amygdala and entorhinal cortex, regions

known to be affected in schizophrenia (46). *Wisteria floribunda agglutinin* (WFA) lectin is widely used as a broad maker for PNNs. WFA is proposed to recognize a 4-*O*-sulfated CS structure on aggrecan, based on the following observations: 1) WFA-staining is abolished by either chondroitinase ABC digestion of CS chains or by genetic deletion of aggrecan (4, 19); 2) There is a drastic structural shift in CS chains, from 6-*O*-sulfation to 4-*O*-sulfation, during formation of PNN in late postnatal development (8, 23); 3) Overexpression of C6ST-1 in PV-cells produces abnormal PNNs, which are negative for WFA staining but positive for CS56 antibody recognizing 6-*O*-sulfation (8). It should be noted that in normal brain development, 6-*O*-sulfation is abundant in early stages but its expression is restricted to a subpopulation of astrocytes in adult brains (47, 48). Thus, sulfation patterns of CS chains are probably differentially regulated in PV-cells and astrocytes in healthy brains. Remarkably, drastic changes in WFA- and CS56-reactivity have been reported in the amygdala of schizophrenia patients (46, 49). In schizophrenia, the number of WFA-positive PNNs surrounding PV-cells is significantly decreased. In sharp contrast, WFA-labeled astrocytes are increased 5- to 10-fold and CS56-labeled astrocytes are decreased. These data may indicate that in schizophrenia, 4-*O*-sulfation is down-regulated in PV-cells but is up-regulated in astrocytes, and this abnormal balance of 4-*O*-sulfation and 6-*O*-sulfation may affect both PV-cell and astrocyte function. Furthermore, C6ST-1 TG mice may provide a novel animal model to elucidate how the sulfation patterns of CS chains are involved in the pathophysiology of the disorders.

The sulfation patterns of CS chains are dependent on the expression of chondroitin sulfotransferases such as C6ST and C4ST and their expression is regulated by cytokines, morphogens, and growth factors (50, 51). For example, the expression of *C4ST-1* mRNA in astrocytes is rapidly increased by transforming growth factor “beta” after brain injury (52). A recent finding suggests that aberrant production of CSPGs may contribute to another neurodevelopmental disorder called Costello syndrome, which is caused by mutant Harvey rat sarcoma viral oncogene homolog (HRAS) and is characterized by delayed development, craniofacial and heart problems, and cognitive impairment (53) (Figure 2). Astrocytes expressing mutant HRAS are hyperproliferative, hypertrophic, and secrete more CSPGs than control cells. Interestingly, a mouse model for the disease that expresses mutant HRAS selectively in astrocytes shows a precocious formation of WFA-positive PNNs around PV-cells. PNNs observed in model mice may be composed of CSPGs secreted from mutant astrocytes, or alternatively, mutant astrocytes may stimulate PV-cells to form PNN by an unknown factor(s). The former seems less likely, given that *C4ST-1* mRNA and 4-*O*-sulfation are rather down-regulated, whereas 6-*O*-sulfation is accumulated in fibroblasts derived from patients (54, 55). It is also reported that sulfation patterns of CS chains produced in PV-cells, but not in astrocytes, mainly contributed to PNN formation (8). It is possible that 4-*O*-sulfation is up-regulated in PV-cells to compensate for the dysfunction of mutant astrocytes. Further studies focusing on the sulfation patterns of CS chains produced by PV-cells and astrocytes may help to understand the disease process and lead to therapeutic strategies.

3.5. Neuroprotective effects of chondroitin sulfate

CS chains in PNNs have a critical role in neuroprotection against oxidative stress, which can be a risk factor of many neurodegenerative disorders (56) (Figure 2). Alzheimer’s disease is the most common form of neurodegenerative disorder that results in dementia associated with cognitive decline and altered network activity (57). The pathological hallmarks are protein aggregates called amyloid plaques containing amyloid “beta” peptide and neurofibrillary tangles containing hyperphosphorylated Tau protein, which disrupt normal cell physiology and cause neurotoxicity. It has been proposed that abnormal metabolic oxidative reactions in the brain contribute to the pathogenesis of Alzheimer’s disease. Oxidative stress may enhance amyloid plaque formation by increasing the amyloid precursor protein level or modulating the activity of amyloid precursor protein processing enzymes. Furthermore, amyloid “beta” peptide itself acts as an oxidant and induces more oxidative stress, which creates positive feedback on amyloid plaque formation that leads to neurofibrillary degeneration (57).

It is well known that protein aggregates are not uniformly distributed but occur in selective regions of brains with Alzheimer’s disease. Strikingly, it has been shown that regions rich in PNNs tend to be protected from the deposition of protein aggregates as compared with regions with sparse PNNs (58). Even in severely affected regions, neurons surrounded by PNNs are less affected by neurofibrillary degeneration than are neurons lacking PNNs. CS chains in PNNs may exert a neuroprotective effect by acting as antioxidants, because depositions of lipofuscin pigment, which are generated by iron-catalyzed oxidative processes, are rarely observed in neurons with PNNs (59). A recent study using transgenic animal models further supported a causative effect of PNNs in neuroprotection from iron-induced neurodegeneration (60). The neuroprotective properties of PNNs largely disappeared in mice heterozygous null for aggrecan, but not in mice homozygous null for brevican, suggesting that CS chains on aggrecan contribute to the neuroprotective function of PNNs. The polyanionic nature of CS chains possibly reduces the local oxidative potential by scavenging reactive oxygen species. In addition, PNNs in mice lacking link proteins or tenascin-R, which have attenuated PNN formation,

lose the neuroprotective effect, indicating that not only the polyanionic nature of CS chains but also the tightly condensed structure of PNNs around neurons are required for the protective function of PNNs.

As described above, PNNs are selectively formed around PV-cells, which are identified electrophysiologically as fast-spiking cells generating high-frequency trains of action potentials (24). The high metabolic demands of PV-cells may render them vulnerable to oxidative stress and could explain the requirement for a neuroprotective shield around the cells (61). Although PV-cells seem to be protected from neurodegenerative cell death, their electrophysiological properties are severely impaired in Alzheimer's disease, which possibly contributes to abnormalities in network synchrony and memory (62). Using mice deficient for glutamate cysteine ligase, a rate-limiting enzyme in the production of glutathione, a major antioxidant, it was demonstrated that the formation of PNN cells autonomously protects PV-cell function (63, 64). In this model, degradation of PNNs by chondroitinase ABC renders PV-cells more susceptible to oxidative stress (63). Conversely, genetic dysregulation of redox homeostasis selectively in PV-cells results in a marked loss of PNNs and persistent visual cortical plasticity in adulthood (64).

The beneficial effect of PNNs is not limited to Alzheimer's disease, as oxidative stress and decreased levels of antioxidant are reported in other neurodevelopmental disorders such as schizophrenia and bipolar disorder (61). Furthermore, mounting evidence indicates the involvement of PNNs in the pathogenesis of epilepsy, which is characterized by repeated seizures caused by a disturbance in the electrical activity of the brain (65-67). This is not surprising because the disruption of PNNs potentially leads to dysregulation of the excitation/inhibition balance, a characteristic of epilepsy (12). The number of WFA-positive PNNs is known to be markedly decreased in animal models for seizures and epilepsy (65-67). This may be, at least partially, due to changes in the sulfation patterns of the CS chains, because recent findings indicate that there is a marked increase in 6-*O*-sulfation that is mirrored by a decrease in 4-*O*-sulfation following kainic acid (KA)-induced seizures (68). Contrary to the decrease in WFA-positive PNNs, KA treatment increases CS56-positive PNNs and astrocytes rich in 6-*O*-sulfation via upregulation of the *C6ST-1* mRNA level. Notably, C6ST-1 TG mice are more susceptible to KA-induced seizures than controls, indicating that overproduction of 6-*O*-sulfation leads to dysfunction of PV-cells that may contribute to the epileptogenesis process. Additionally, seizures may induce degradation of aggrecan, a critical component of PNNs, by matrix metalloproteinase (MMP) activity (69). It is reported that MMP-cleaved aggrecan fragments rapidly emerge around PV-cells after induction of seizures (65). In this regard, it is possible that upregulation of 6-*O*-sulfation on aggrecan renders it more susceptible to degradation by MMPs, since the aggrecan level is significantly decreased in adult C6ST-1 TG mice (70). Furthermore, administration of MMP inhibitor prevents the disruption of PNNs and epileptogenesis (69). Taken together, the neuroprotective properties of PNNs may have high therapeutic potential for neurodegenerative diseases. Further studies to elucidate the precise molecular mechanisms underlying the neuroprotective effect of CS chains in PNNs may offer a novel strategy for treating these disorders.

4. CONCLUSIONS

CSPGs have long been known to act as non-specific physical barriers for axonal growth and neural plasticity. However, recent studies have revealed that CSPGs can actively regulate neuronal function depending on the sulfation modification of CS chains. Structural changes of CS chains may cause aberrant formation of PNNs, which resulted in dysfunction of PV-cells and a disturbed excitatory/inhibitory balance under pathological conditions including schizophrenia, Costello syndrome, Alzheimer's disease, and epilepsy. Therefore, further studies should address whether genetic and enzymatic control of CS structures can correct brain function to shed light on novel therapeutic strategies for alleviating neuronal disorders.

5. ACKNOWLEDGEMENT

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