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Sugar-dependent modulation of neuronal development, regeneration, and plasticity by chondroitin sulfate proteoglycans

Gregory M. Miller, Linda C. Hsieh-Wilson *

Division of Chemistry and Chemical Engineering, California Institute of Technology, 1200 E. California Blvd., Pasadena, CA 91125, USA

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ABSTRACT

Chondroitin sulfate proteoglycans (CSPGs) play important roles in the developing and mature nervous system, where they guide axons, maintain stable connections, restrict synaptic plasticity, and prevent axon regeneration following CNS injury. The chondroitin sulfate glycosaminoglycan (CS GAG) chains that decorate CSPGs are essential for their functions. Through these sugar chains, CSPGs are able to bind and regulate the activity of a diverse range of proteins. CSPGs have been found both to promote and inhibit neuronal growth. They can promote neurite outgrowth by binding to various growth factors such as midkine (MK), pleiotrophin (PTN), brain-derived neurotrophic factor (BDNF) and other neurotrophin family members. CSPGs can also inhibit neuronal growth and limit plasticity by interacting with transmembrane receptors such as protein tyrosine phosphatase σ (PTPσ), leukocyte common antigen-related (LAR) receptor protein tyrosine phosphatase, and the Nogo receptors 1 and 3 (NgR1 and NgR3). These CS–protein interactions depend on specific sulfation patterns within the CS GAG chains, and accordingly, particular CS sulfation motifs are upregulated during development, in the mature nervous system, and in response to CNS injury. Thus, spatiotemporal regulation of CS GAG biosynthesis may provide an important mechanism to control the functions of CSPGs and to modulate intracellular signaling pathways. Here, we will discuss these sulfation-dependent processes and highlight how the CS sugars on CSPGs contribute to neuronal growth, axon guidance, and plasticity in the nervous system.

1. Introduction

Chondroitin sulfate proteoglycans (CSPGs) play critical roles in the developing central nervous system (CNS) and in response to adult CNS injury. During embryonic development, axons must elongate, navigate specific paths, and form synapses with their target neurons. To establish precise patterns of connectivity, a range of attractive or repulsive cues guide axons to their proper targets. Several families of extracellular receptors and their ligands are known to attract or repel growth cones, including netrins, ephrins, semaphorins, and slits (Bashaw and Klein, 2010; Dickson, 2002; Tessier-Lavigne and Goodman, 1996). In addition to these prototypical axon guidance molecules, increasing evidence suggests that the chondroitin sulfate (CS) sugars on CSPGs can serve as guidance cues for growth cones and contribute to the formation of neuronal boundaries in the developing CNS (Brittis et al., 1992; Carulli et al., 2005; Snow et al., 1990). CSPGs are also major components of perineuronal nets (PNNs), where they play crucial roles in the maturation of synapses and the closure of critical periods by limiting synaptic plasticity (Berardi et al., 2003; Dityatev et al., 2010; Kwok et al., 2011; Miyata and Kitagawa, 2015). In the adult CNS, CSPGs are dramatically upregulated in the glial scar around injury sites, where they restrict synaptic and anatomical plasticity, neuronal regeneration, and repair (Bartus et al., 2012; Galtrey and Fawcett, 2007; Silver and Miller, 2004; Yiu and He, 2006). Enzymatic digestion of the CS GAG chains on CSPGs can promote axon regeneration, sprouting, and functional recovery in *in vivo* models of CNS injury, underscoring again critical roles for CS sugars. In this review, we will highlight the various functions of the CS sugars on CSPGs and how they contribute to neuronal growth, axon guidance, and plasticity in the nervous system. We will also discuss strong evidence that specific sulfated motifs within CS chains can serve as ligands for extracellular receptors, thereby enabling CSPGs to activate key signaling pathways important for neuronal development and function.

1.1. Structure of chondroitin sulfate proteoglycans

CSPGs are composed of a core protein with one or more covalently attached CS GAG chains (Galtrey and Fawcett, 2007; Kjellén and Lindahl, 1991). They are major components of the extracellular matrix (ECM), where they provide structural support and modulate neuronal activity (Busch and Silver, 2007; Galtrey and Fawcett, 2007; Kwok et al., 2011). In addition, some CSPGs are inserted into the membrane via a single membrane-spanning domain or a glycosylphosphatidylinositol (GPI) anchor, or are localized to secretory granules. The most abundant CSPGs in the CNS are members of the lectican family, which is comprised of

* Corresponding author.
E-mail address: lhw@caltech.edu (L.C. Hsieh-Wilson).

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aggrecan, brevican, neurocan, and versican (Yamaguchi, 2000). Lecticans contain an N-terminal G1 domain, C-terminal G3 domain, and a central region decorated with varying numbers of CS chains (ranging from 1 to >100). Unlike other lecticans, aggrecan also contains a G2 domain following the N-terminal G1 domain (Yamaguchi, 2000). The phosphacan or receptor-type protein-tyrosine phosphatase zeta (PTPζ/RPTPβ) family, which consists of both transmembrane and soluble secreted forms, is expressed predominantly in the CNS and is found in neurons and astrocytes throughout development and adulthood (Hayashi et al., 2005; Maurel et al., 1994). The small leucine-rich proteoglycans (SLRPs) such as decorin and biglycan have N-terminal binding sites for 1 or 2 CS chains and leucine-rich repeats flanked by cysteine residues in their central domain (Hocking et al., 1998). Another prominent CSPG in the nervous system is NG2, a transmembrane proteoglycan with a CS chain attached to its large extracellular domain (Stallcup, 2002).

1.2. Structure of chondroitin sulfate sugars

Proteoglycan core proteins are decorated at certain serine residues with CS GAG chains via a tetrasaccharide linker. CS GAGs are linear polysaccharides comprised of a repeating disaccharide unit containing *N*-acetyl-D-galactosamine (GalNAc) and D-glucuronic acid (GlcA) (Gama and Hsieh-Wilson, 2005; Sugaharaet al., 2003). Each chain contains up to 100 disaccharide units and undergoes extensive sulfation in the Golgi by chondroitin sulfotransferases (Kusche-Gullberg and Kjellen, 2003; Mikami and Kitagawa, 2013). The commonly occurring CS disaccharide units are characterized by the number and position of their sulfate modifications (Fig. 1A). For instance, the monosulfated CS-A and CS-C motifs contain sulfate groups at the 4-*O* and 6-*O* positions of the GalNAc residue, respectively, and are generated by the sulfotransferases chondroitin 4-*O*-sulfotransferase (C4ST) and chondroitin 6-*O*-sulfotransferase (C6ST), respectively (Fig. 1B). The disulfated CS-D unit is synthesized from the CS-C precursor via 2-*O* sulfation of the GlcA residue by uronyl 2-*O*-sulfotransferase (UST), while the CS-E unit is generated from a CS-A unit by 6-*O* sulfation of the GalNAc residue by the sulfotransferase *N*-acetylgalactosamine 4-sulfate 6-*O*-sulfotransferase (GalNAc4S-6ST). Thus, a suite of sulfotransferase enzymes works in concert to produce a structurally complex, heterogeneously sulfated polysaccharide. This non-template driven process results in diverse patterns of sulfation that allow CS GAGs to interact with a wide range of proteins, including different growth factors, cytokines, and transmembrane receptors.

1.3. CSPG receptors

Many of the diverse functions of CSPGs arise from their ability to bind a large number of protein partners. CSPGs interact with various proteins in the ECM, including fibronectin, laminin, neural cell adhesion molecule (NCAM), and neural glial cell adhesion molecule (NgCAM) (Friedlander et al., 1994; Grumet et al., 1993; Wu et al., 2005b). Through these interactions, CSPGs can block laminin-mediated integrin activation, as well as cell adhesion molecules important for promoting neuronal migration and growth (Muir et al., 1989; Tan et al., 2011; Zuo et al., 1998). CSPGs are also known to interact with a variety of growth factors, such as midkine (MK), pleiotrophin (PTN), nerve growth factor (NGF), and brain-derived neurotrophic factor (BDNF), and in some cases, can help assemble growth factor–receptor complexes (Deepa et al., 2002; Gama et al., 2006; Maeda et al., 2003; Rogers et al., 2011; Zou et al., 2003). In this way, CSPGs can modulate growth factor signaling pathways by presenting soluble factors to their cell surface receptors and/or potentially sequestering them from their cell surface receptors. It is becoming increasing clear that CSPGs can also interact with and modulate the activity of many membrane-associated proteins, including the protein tyrosine phosphatases PTPσ and leukocyte common antigen-related (LAR) and the Nogo receptors NgR1 and NgR3 (Brown et al., 2012; Dickendesher et al., 2012; Fisher et al., 2011; Shen et al., 2009). For example, the interaction of CS and heparan sulfate (HS) GAGs with the thrombospondin repeats of semaphorin 5A (Sema5A) guides neurons in the developing diencephalon fiber tract, with each interaction resulting in different functional outcomes. While HS is required for Sema5A-mediated attraction, the interaction with CS converts Sema5A to a repulsive guidance cue, suggesting that neuronal responses to axon guidance cues can depend on their GAG binding status (Kantor et al., 2004). We will highlight additional examples below where the CS sugar chains mediate the interactions and activity of CSPGs toward their protein partners.

1.4. Regulation of the 'sulfation code'

The sulfation profiles of CS are spatially and temporally regulated in a tissue-specific and cell type-specific manner. In the nervous system, particular CS sulfation motifs are upregulated during early and postnatal development (Fernaud-Espinosa et al., 1996; Ishii and Maeda, 2008a, 2008b; Kitagawa et al., 1997; Maeda et al., 2003; Mitsunaga et al., 2006). For example, distinct immunohistochemical patterns are exhibited by monoclonal antibodies that recognize sulfated CS chains (antibodies CS-56, 2H6 and MO-225). Although these antibodies recognized a varied set of partially overlapping epitopes, each revealed distinct CS expression patterns in the brain (Sugahara and Mikami, 2007). For example the CS-56 and 2H6 epitopes were highly expressed in the postnatal day 7 (P7) mouse cortex and showed decreased expression in P12 and P20 cortical tissue (Maeda et al., 2003; Sugahara et al., 2003). In the cerebellum, CS-56 immunoreactivity was detected at P7 and P12 but was absent at P20. Interestingly, the MO-225 epitope was not observed in the cortex but showed strong expression in the cerebellum at P7, P12, and P22 (Maeda et al., 2003; Sugahara and Mikami, 2007).

Additional insights into the expression dynamics of CS motifs was obtained from high-performance liquid chromatography (HPLC) analysis of CS disaccharides following digestion of the GAG chains. The monosulfated CS-A motif was the most abundant motif in the embryonic mouse cortex and cerebellum, and its levels increased as development progressed (Ishii and Maeda, 2008a, 2008b; Mitsunaga et al., 2006). Expression of the CS-C motif was highest during embryonic development and steadily decreased through development, but it remained the second most abundant CS motif (Ishii and Maeda, 2008a, 2008b; Mitsunaga et al., 2006). Interestingly, the disulfated CS-E motif exhibited its highest expression in the embryonic mouse cortex, and its levels decreased into adulthood but remained higher in the cortex than in the cerebellum (Ishii and Maeda, 2008a, 2008b; Mitsunaga et al., 2006). In contrast, expression of the disulfated CS-D motif was highest in the cerebellum and peaked around P10 (Ishii and Maeda, 2008a, 2008b). Notably, deletion of these highly sulfated motifs by genetic knockdown of the sulfotransferases UST and GalNAc4S-6ST via *in utero* electroporation resulted in impaired migration of cortical neurons *in vivo* (Ishii and Maeda, 2008a). Electroporated neurons accumulated in the lower intermediate zone and the subventricular zone and did not migrate radially in the neocortex.

In addition to dynamic changes in CS sulfation, the expression of specific CS sulfotransferases is spatially and temporally regulated (Ishii and Maeda, 2008a, 2008b; Kitagawa et al., 1997; Maeda, 2010; Purushothaman et al., 2007; Sugahara and Mikami, 2007). Widespread mRNA expression of *C4ST* and *GalNAc4S-6ST* was observed in the cortex, hippocampus, cerebellum, striatum, and the olfactory bulb during postnatal development and into adulthood, while *UST* was preferentially expressed in the developing cerebellum (Mitsunaga et al., 2006; Purushothaman et al., 2007). In the cerebellum, expression of *GalNAc4S-6ST* shifted during postnatal development from the external to the internal granular layer (Ishii and Maeda, 2008b; Purushothaman et al., 2007). This change in sulfotransferase mRNA expression profile matched the levels of CS-E expression observed by immunostaining and coincided with the migration and maturation of granular cells (Purushothaman et al., 2007).