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Chondroitin sulfate proteoglycans inhibit oligodendrocyte myelination through PTPσ

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ABSTRACT

CNS damage often results in demyelination of spared axons due to oligodendroglial cell death and dysfunction near the injury site. Although new oligodendroglia are generated following CNS injury and disease, the process of remyelination is typically incomplete resulting in long-term functional deficits. Chondroitin sulfate proteoglycans (CSPGs) are upregulated in CNS grey and white matter following injury and disease and are a major component of the inhibitory scar that suppresses axon regeneration. CSPG inhibition of axonal regeneration is mediated, at least in part, by the protein tyrosine phosphatase sigma (PTPσ) receptor. Recent evidence demonstrates that CSPGs inhibit OL process outgrowth, however, the means by which their effects are mediated remains unclear. Here we investigate the role of PTPσ in CSPG inhibition of OL function. We found that the CSPGs, aggrecan, neurocan and NG2 all imposed an inhibitory effect on OL process outgrowth and myelination. These inhibitory effects were reversed by degradation of CSPGs with Chondroitinase ABC prior to OL exposure. RNAi-mediated down-regulation of PTPσ reversed the inhibitory effect of CSPGs on OL process outgrowth and myelination. Likewise, CSPG inhibition of process outgrowth and myelination was significantly reduced in cultures containing PTPσ$^{-/-}$ OLs. Finally, inhibition of Rho-associated kinase (ROCK) increased OL process outgrowth and myelination during exposure to CSPGs. These results suggest that in addition to their inhibitory effects on axon regeneration, CSPGs have multiple inhibitory actions on OLs that result in incomplete remyelination following CNS injury. The identification of PTPσ as a receptor for CSPGs, and the participation of ROCK downstream of CSPG exposure, reveal potential therapeutic targets to enhance white matter repair in the damaged CNS.

Introduction

Central nervous system (CNS) disease and injury result in the development of a chondroitin sulfate proteoglycan (CSPG)-rich glial scar (Fawcett and Asher, 1999; Silver and Miller, 2004). CSPGs are known to suppress axonal regeneration and plasticity following CNS injury (Bradbury et al., 2002; Fawcett and Asher, 1999; Johnson et al., 2002; Kwok et al., 2008; Lemons et al., 2003; Rhodes and Fawcett, 2004; Silver and Miller, 2004). CSPGs expressed in the CNS include the lecticans, which are comprised by aggrecan and neurocan (Yamaguchi, 2000), phosphacan (Garwood et al., 2003), and NG2 (Stallcup, 2002). The heterogeneity of CSPGs, and their particular effects, result from variations in the core protein, the number of glycosaminoglycan (GAG) chains, and the particular pattern of sulfation (Galtrey and Fawcett, 2007; Gilbert et al., 2005; Hartmann and Maurer, 2001; Properzi et al., 2003). CSPGs, either membrane bound or locally secreted, carry out a diverse set of functions during development (Bandtlow and Zimmermann, 2000; Carulli et al., 2005; Yamaguchi, 2000). However, upregulation and altered distribution of CSPGs are believed to be the major cause of regeneration failure at and around the site of CNS injury (Jones et al., 2003; Lemons et al., 2001; Moon et al., 2002; Silver and Miller, 2004; Tang et al., 2003). Accordingly, studies have sought to degrade CSPGs or to prevent CSPGs from accumulating at the site of injury. Targeting CSPGs for degradation by treatment with chondroitinase ABC (chABC), an enzyme that removes GAG side chains from the core protein, creates an environment permissive to axonal regeneration and promotes functional recovery in animal models of CNS injury (Barritt et al., 2006; Bradbury et al., 2002; Cafferty et al., 2008; Garcia-Alias et al., 2008; Massey et al., 2008; Tester and Howland, 2008).

Several observations have been made concerning the molecular mechanisms by which CSPGs affect plasticity and regeneration. CSPGs block the access of growth-promoting molecules (Ohori et al., 2006;

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Sherman and Back, 2008), control the availability of calcium (Hrabetova et al., 2009), and complement the inhibitory effects of other molecules (De Wit et al., 2005; Kantor et al., 2004). Most molecular inhibitors present in the glial scar exert their effects on axonal growth and regeneration via interactions with functional membrane receptors (Liu et al., 2006; Yiu and He, 2006). It has been shown that the leukocyte common antigen-related (LAR) family of receptors, specifically LAR and protein tyrosine phosphatase sigma (PTPσ), serves as neuronal receptors mediating CSPG-induced inhibition of axon regeneration (Coles et al., 2011; Fisher et al., 2011; Fry et al., 2010; Shen et al., 2009). PTPσ$^{-/-}$ mice show enhanced axonal regeneration following spinal cord injury (SCI) compared to their wildtype littermates (Fry et al., 2010; Sapieha et al., 2005; Thompson et al., 2003). Additionally, the inhibitory effects of CSPGs on neurons are mediated, at least in part, by the Rho-associated kinase (ROCK) pathway. Axonal regeneration is promoted by inhibition of the ROCK pathway (Dergham et al., 2002; Fournier et al., 2003; Monnier et al., 2003).

While both CSPGs and regeneration have been studied extensively, there is little known about the influence that CSPGs have on other cells present at the site of injury. CNS damage results in demyelination of spared axons due to oligodendrocyte (OL) cell death in both the immediate vicinity of the injury site (Bunge et al., 1993; Guest et al., 2005; Smith and Jeffery, 2006) and at distant regions (Li et al., 1999; Liu et al., 1997; Patel and Balabanov, 2012). This loss of myelinating OLs renders spared axons dysfunctional (Goto and Hoshino, 2001; Nashmi and Fehlings, 2001) and makes them more susceptible to injury (Bunge et al., 1993; Crowe et al., 1997; Park et al., 2003), thereby exacerbating functional deficits (Li et al., 1999; Liu et al., 1997; Yu et al., 2001). Despite the presence of endogenous oligodendrocyte progenitor cells (OPCs) within the CNS lesion, remyelination of denuded axons is limited (Keirstead et al., 1998; Wolswijk, 1998). The inhibitory CNS environment, of which CSPGs are a major constituent, may affect OPCs in a manner similar to neurons. Indeed, direct exposure of differentiating OPCs to CSPGs suppresses their process outgrowth (Siebert and Osterhout, 2011). As with neurons, chABC treatment reverses the inhibitory effect of CSPGs (Siebert and Osterhout, 2011; Siebert et al., 2011). Still, the exact mechanisms through which CSPGs affect OL function are not well understood. Signaling via PTPα regulates normal OL differentiation and maturation (Ranjan and Hudson, 1996; Wang et al., 2009, 2012). However, PTPα lacks the extracellular domains necessary to bind CSPGs and is, thus, unlikely to mediate the inhibitory effects of CSPGs following their upregulation in injured CNS tissue (Johnson and Van Vactor, 2003). PTPζ acts in opposition to PTPα to negatively regulate OL differentiation and myelination (Kuboyama et al., 2012), nonetheless this regulation has not been linked to extracellular binding of CSPGs by PTPζ. Therefore, in this study we explored the role of PTPσ as a potential receptor for CSPGs in OLs. We demonstrate that CSPGs can suppress OL process outgrowth as well as the capacity of these OLs to myelinate. This inhibitory effect can be mitigated by enzymatic digestion of CSPGs by chABC and by treatment with the ROCK inhibitor Y-27632. OLs treated with PTPσ-specific shRNA and OLs isolated from PTPσ$^{-/-}$ mice were naive to the inhibitory effects of CSPGs on OL process outgrowth and myelination, suggesting a necessity for PTPσ in CSPG inhibition. Identification of PTPσ as a functional receptor for CSPGs in OLs provides a therapeutic target through which the deficits associated with demyelinating injury and disease may be limited.

Materials and methods

Primary cell culture

OPCs were derived from postnatal day 4 (P4) Sprague–Dawley rat spinal cords and expanded in a fully defined culture environment as described previously (Barres et al., 1994; Bogler et al., 1990). Briefly, intact spinal cords were digested with 2 mg/ml collagenase I (Worthington Biochemical) followed by 0.05% trypsin–EDTA for 30 min each (Thomson et al., 1999). Tissue was triturated and then filtered through a 40 μm cell strainer. Dissociated cells were plated on 10 μg/ml poly-D-lysine (PDL; Sigma) coated plastic dishes at 3×10^5 cells/cm^2 in N2 media [DMEM, 1% bovine serum albumin (BSA), 2 mM glutamax, 100 mM non-essential amino acids, 1 mM sodium pyruvate, 100 U/ml penicillin, 100 mg/ml streptomycin, 1× N2 (Invitrogen), and 1 mM N-acetyl-cystine] supplemented with 10 ng/ml PDGF (R&D Systems), 5 ng/ml NT-3 (R&D Systems), and 5 ng/ml FGF (Peprotech) to prevent differentiation (Barres et al., 1994). PTPσ$^{+/-}$ mice were donated by Michel L. Tremblay (McGill University) (Elchebly et al., 1999). PTPσ$^{+/+}$ and PTP$^{-/-}$ pups at P2 were used to generate spinal cord OPCs as described above. Genotypes were established post-culture via PCR-based genotyping (products of 781 or 1000 bp).

DRG neurons were prepared from P4 Sprague–Dawley rats by digestion in collagenase and 0.05% trypsin–EDTA as described (Mehta et al., 2007). Dissociated cells were plated on a 10 μg/ml laminin-coated dishes at 37 °C at 4×10^4 cells/cm^2 in DRG media [Neurobasal, 1× B27 (Invitrogen), and 5% FBS] supplemented with 50 ng/ml NGF (Sigma) and 5 μM Ara-C (Sigma) to enhance DRG survival and reduce non-neuronal populations, respectively.

Antibodies and reagents

At indicated times, cells were fixed in 4% paraformaldehyde (PFA) and incubated overnight at 4 °C with primary antibodies: mouse anti-A2B5 (R&D Systems, MAB1416), rat anti-platelet derived growth factor receptor alpha (PDGFRα; BD Biosciences, 558774), rabbit anti-oligodendrocyte transcription factor 2 (Olig2; Millipore, AB9610), mouse anti-neuron-glial antigen 2 (NG2; Chemicon, MAB5384), mouse anti-O4 antigen (Developmental studies hybridoma bank, Dr. Patrick Wood), mouse anti-galactocerebroside (GalC; Chemicon, MAB342), mouse anti-O1 antigen (Developmental studies hybridoma bank, Dr. Patrick Wood), rabbit anti-myelin basic protein (MBP; Millipore, AB980), mouse anti-MBP (Calbiochem, NE1018), chicken anti-neurofilament (NF; Millipore, AB5539), rabbit anti-glial fibrillary acid protein (GFAP; ImmunoStar, 22522), or mouse anti-protein tyrosine phosphate sigma (PTPσ; Santa Cruz, 100419). Secondary antibodies were applied for 2 h at RT and include goat anti-chicken Alexa Fluor 488 (Jackson ImmunoReasearch, 103-485-155), goat anti-mouse Cyanine Cy3 (Jackson ImmunoReasearch, 115-165-003), and goat anti-rabbit Cyanine Cy3 (Jackson ImmunoReasearch, 111-165-003). Nuclei were counterstained with 1 μg/ml Hoechst 33342 (Molecular Probes, H-3570). The ROCK inhibitor (Sigma, Y-27632) was used at 10 μM and the non-competitive pan-PTP inhibitor (Tocaris, BVT 948) was used at 2 μM.

OL process outgrowth assays

Glass coverslips were placed within 24-well plates and coated with PDL. Subsequently, coverslips were washed twice in PBS and coated with 10 μg/ml laminin (Invitrogen, 23017-015), 10 μg/ml laminin + 10 μg/ml aggrecan (Sigma, A1960), 10 μg/ml laminin + 5 μg/ml neurocan (Millipore, AG270), or 10 μg/ml laminin + 10 μg/ml NG2 (William B. Stallcup, Sanford-Burnham Medical Research Institute) for 3 h at 37 °C. OPCs were plated at a density of 1×10^4 cells/well (5×10^3 cells/cm^2) and maintained in differentiation media [N2 media supplemented with 40 ng/ml triiodothyronine (T3, Sigma-Aldrich) (Ibarrola et al., 1996)] without media change. We identify this method of CSPG treatment as co-exposure. Adherent cells were counted, 6 h after plating, from 5 randomly selected fields in each of 3 replica wells by phase microscopy to determine plating efficiency ($n = 3$). After 4 days in culture, cells were fixed and stained as described above. Images of MBP + OLs were obtained from 3 to 12 replica wells as indicated, 10 randomly selected images/well ($n = 3$-12). OL process outgrowth was measured using ImageJ software by determining the minimal area of