Regulation of Oligodendrocyte Precursor Maintenance by Chondroitin Sulphate Glycosaminoglycans

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Chondroitin sulfate proteoglycans (CSPGs) have been proven to inhibit morphological maturation of oligodendrocytes as well as their myelination capabilities. Yet, it remained unclear, whether CSPGs and/or their respective chondroitin sulfate glycosaminoglycan (CS-GAG) side chains also regulate the oligodendrocyte lineage progression. Here, we initially show that CS-GAGs detected by the monoclonal antibody 473HD are expressed by primary rat NG2-positive oligodendrocyte precursor cells (OPCs) and O4-positive immature oligodendrocytes. CS-GAGs become down-regulated with ongoing oligodendrocyte differentiation. Enzymatic removal of the CS-GAG chains by the bacterial enzyme Chondroitinase ABC (ChABC) promoted spontaneous differentiation of proliferating rat OPCs toward O4-positive immature oligodendrocytes. Upon forced differentiation, the enzymatic removal of the CS-GAGs accelerated oligodendrocyte differentiation toward both MBP-positive and membrane forming oligodendrocytes. These processes were attenuated on enriched CSPG fractions, mainly consisting of Phosphacan/RPTPβ/ζ and to less extent of Brevican and NG2. To qualify CS-GAGs as universal regulators of oligodendrocyte biology, we finally tested the effect of CS-GAG removal on OPCs from different sources such as mouse cortical oligospheres, mouse spinal cord neurospheres, and most importantly human-induced pluripotent stem cell-derived radial glia-like neural precursor cells. For all culture systems used, we observed a similar inhibitory effect of CS-GAGs on oligodendrocyte differentiation. In conclusion, this study clearly suggests an important fundamental principle for complex CS-GAGs to regulate the oligodendrocyte lineage progression. Moreover, the use of ChABC in order to promote oligodendrocyte differentiation toward myelin gene expressing cells might be an applicable therapeutic option to enhance white matter repair.

GLIA 2016;64:270–286

Key words: oligodendrocyte differentiation, extracellular matrix, lectican, chondroitinase ABC

Introduction

Under physiological conditions oligodendrocytes form myelin sheaths, which wrap the axons of central nervous system (CNS) neurons. This ensures the electrical insulation of axons and is prerequisite for fast saltatory action potential propagation to occur. Within the last years, much effort has been put into the identification of new molecular players, which impact on oligodendrocyte differentiation and maturation. This is of particular importance, since several severe neurological disorders such as traumatic CNS injury or multiple sclerosis are characterized by a progressive loss of myelinated axons (Grossman et al., 2001; Lytle and Wrathall, 2007). As a consequence, unmyelinated axons become particularly vulnerable to further degeneration. Thus, a fast remyelination of these axons is necessary to prevent any secondary axonal damage (Franklin and Ffrench-Constant, 2008). Under such pathological conditions, reactive astrocytes express high levels of various extracellular matrix (ECM) molecules such as tenascins or chondroitin sulfate proteoglycans (CSPGs) of the lectican family (Barnett and Linington, 2013; Camand et al., 2004; Chen et al., 2010; Dobbertin et al., 2003; Galtrey et al., 2007; Garcia-Alias et al., 2009). Moreover, a recent study demonstrated that highly proliferative pericytes also contribute to the production of ECM molecules after spinal cord injury (Goritz

View this article online at wileyonlinelibrary.com. DOI: 10.1002/glia.22928

Published online October 10, 2015 in Wiley Online Library (wileyonlinelibrary.com). Received Jan 21, 2015, Accepted for publication Sep 16, 2015.

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et al., 2011). These molecules form a tight macromolecular meshwork, the so-called glial scar, which is considered to exert both beneficial and detrimental effects (Fitch and Silver, 2008; Rolls et al., 2009). The mainly inhibitory impact of such ECM components on axonal regeneration and neural plasticity is well established (Galtrey and Fawcett, 2007; Gervasi et al., 2008), and it has been shown that receptor protein tyrosine phosphatase σ (PTPσ), leukocyte common antigen-related phosphatase (LAR), as well as NOGO receptor 1 and 3 (NgR1, NgR3) represent cognate neuronal receptors (Dickendesher et al., 2012; Fisher et al., 2011; Shen et al., 2009).

The use of the bacterial enzyme chondroitinase ABC (ChABC) to degrade the chondroitin sulfate glycosaminoglycan (CS-GAG) side chains covalently attached to the proteoglycan core proteins proved beneficial for axonal regeneration e.g. in the case of spinal cord injury (Garcia-Alias et al., 2009; Laabs et al., 2007; Lin et al., 2011; Wang et al., 2011). These observations clearly demonstrate the importance of the CS-GAGs as regulators of the CSPG-mediated inhibition of axonal outgrowth.

While the inhibitory influence of CSPG-containing scar tissue on axonal regeneration is well established, novel data indicate that CSPGs also impair the migration and (re-) myelination capabilities of oligodendrocyte precursor cells (OPCs) in demyelinated lesions (Harlow and Macklin, 2013; Siebert et al., 2011). Along these lines, recent *in vitro* studies have shown that CSPGs inhibit oligodendrocyte process extension in a Rho-dependent manner (Pendleton et al., 2013; Siebert and Osterhout, 2011). Interestingly, several RPTPs are known to be expressed within the oligodendrocyte lineage (Canoll et al., 1996; Ranjan and Hudson, 1996), suggesting that RPTPs may also serve as a CSPG-receptor in oligodendrocytes. In fact, RPTPβ/ζ as well as RPTPσ have recently been shown to mediate the inhibitory effect of CSPGs in oligodendrocytes (Kuboyama et al., 2012; Lamprianou et al., 2011; Pendleton et al., 2013). Yet, it is not clear, whether the inhibitory effect is restricted to morphological processes or whether CSPGs act on the oligodendrocyte lineage progression as well. In this study, we show that CS-GAGs that can be detected e.g., by the monoclonal antibody 473HD, regulate the maintenance of the OPC state under proliferative conditions. Under differentiating conditions, they suppress the lineage progression toward MBP-positive mature oligodendrocytes in a ChABC-sensitive manner. Moreover, enzymatic CS-GAG removal not only stimulates a massive oligodendrocyte membrane formation from primary rodent cells but also from human-induced pluripotent stem cell (hiPSC)-derived oligodendrocytes.

Materials and Methods

Isolation and Cultivation of Rat Oligodendrocyte Precursor Cells

Mixed glial cultures were derived from postnatal rat pups (P0-P2) according to standard protocols (Czopka et al., 2009; Milner and Ffrench-Constant, 1994). Briefly, the cortices of four rat pups were carefully dissected and collected in a 15-mL reaction tube. The tissue was dissociated for 20 min in 2 mL 0.25% T/E at 37°C. The reaction was stopped by adding an equal amount of mixed glial culture medium (DMEM, 10% FCS, 1% P/S). Thereafter, the tissue was gently triturated, in order to get a single cell suspension. The suspension was filled up to 10 mL with culture medium, and the cells were spun down for 5 min at 216 rcf. The cells were then resuspended in 10-mL culture medium and finally plated in a poly-D-lysine (PDL) coated (10 μg mL^{-1}) T75 culture flask. The culture was kept at 37°C and 6% CO_2 for about 7 days, and the medium was changed every 3 days. After 7 days, OPCs and microglial cells were shaken off in a horizontal shaker over night at 37°C. Microglial cells were then removed by preplating the cell suspension for 20 min on a bacterial dish. Finally, the OPCs were pelleted by centrifugation for 5 min at 216 rcf and resuspended in OPC medium (DMEM, 1% N2 supplement, 100 μg mL^{-1} BSA, 1% P/S). For immunocytochemical analyses 25,000 cells were plated on poly-D,L-ornithine (P-Orn; 10 μg mL^{-1}) or poly-D,L-ornithine/PG coated 12-mm glass cover slips. OPCs were either expanded in the presence of 10 ng mL^{-1} PDGF-AA and 10 ng mL^{-1} FGF2 (PF) or differentiated after growth factor withdrawal in the presence of 400 ng mL^{-1} T_3 and 1% FCS (TF). In some experiments, 50 mU mL^{-1} Chondroitinase ABC (Sigma–Aldrich, Munich, Germany) were added to the culture medium, to affectively degrade CS-GAGs.

siRNA-mediated knock-down of Ptprz1

To perform *Ptprz1* knock-down experiments, 45,000 OPCs were plated on P-Orn coated 12-mm glass cover slips and expanded in OPC medium containing PDGF-AA and FGF2. After 24 h, the medium was changed to OPC medium lacking growth factors and antibiotics. Afterward, the cells were transfected with 20 μM siRNA (Sigma–Aldrich, siRNA ID: SASI_Rn01_00053280) and 20 μM siGLO Green (GE Healthcare Life Sciences, Freiburg, Germany) or only with 20 μM siGLO Green using 1% lipofectamine RNAiMAX transfection reagent (Invitrogen by Life Technologies, Darmstadt, Germany) in Opti-MEM (Life Technologies). After 6 h, the medium was changed to OPC medium containing 400 ng mL^{-1} T_3 and 1% FCS, and the cells were cultivated for further 48 h until they were fixed and used for immunocytochemical analyses.

Isolation and Cultivation of Mouse Spinal Cord Neural Precursor Cells

Except for some minor modifications E13.5 spinal cord neural precursor cells were essentially isolated and cultivated as previously described (Karus et al., 2011, 2012). Because heparin is known to interfere with Chondroitinase ABC activity, we omitted heparin from those cultures and added only 20 ng mL^{-1} EGF and 20 ng mL^{-1} FGF2. However, this did not notably affect the growth of the NPCs, since we could passage these neurosphere cultures at least 10 times and still observed a multipotent phenotype under differentiating conditions (not shown). To foster the differentiation towards glial lineages and to promote cell survival we added 1% FCS to the culture medium after growth factor withdrawal.

Isolation and Cultivation of Mouse Cortical Oligospheres

Neurospheres were prepared from E14.5 embryos from timed pregnant female wild-type mice, and oligospheres were generated from such neurospheres as previously described with some modifications (Pedraza et al., 2008). Briefly, the cortices of E14.5 mouse embryos were collected and enzymatically digested using 30 U mL^{-1} papain (Worthington, NJ) in MEM (Sigma–Aldrich, Munich, Germany) for 25 min at 37°C. The digestion was stopped by the addition of an equal amount of ovomucoid. To obtain a single cell suspension, the tissue was triturated mechanically. The cells of two cortices were cultivated in neurosphere culture medium (DMEM/F12 1:1; 2% B27 supplement; 1% P/S) containing 20 ng mL^{-1} EGF in a T12.5 flask. After 48 h, the floating neurospheres were passaged at a 1:3 ratio in neurosphere media containing EGF. For the generation of oligospheres, these secondary neurospheres were enzymatically dissociated to a single cell suspension by using 0.25% T/E. After 5 min the digestion was stopped via the addition of an equal amount of ovomucoid. The cells were resuspended at a 1:2 ratio in neurosphere medium now containing 20 ng mL^{-1} PDGF-AA (Peprotech, Rocky Hill, NJ) and 20 ng mL^{-1} FGF2 (Peprotech). This allowed the OPCs to proliferate. After 72 h, the oligospheres were passaged at a 1:2 ratio and were cultivated for another 4 days. Secondary oligospheres were enzymatically dissociated to a single cell suspension, resuspended in OPC medium (DMEM, 1% N2 supplement, 100 μg mL^{-1} BSA, 1% P/S), and the cell suspension was filtered through a 40-μm cell-strainer. For immunocytochemical analysis 25,000 cells were plated on P-Orn coated 10-mm glass coverslips. OPCs were either propagated in the presence of 20 ng mL^{-1} PDGF-AA and 10 ng mL^{-1} FGF2 or allowed to differentiate in the presence of 400 ng mL^{-1} T_3 and 1% HS.

Cultivation of Oli-Neu Cells

The oligodendrocyte precursor cell line Oli-Neu (Jung et al., 1995) was cultured in 10 μg mL^{-1} PDL-coated T75 culture flasks at 37°C, 7.5% CO_2. The cells usually reached confluence within 2–3 days and were then passaged in a 1:5 ratio. Passaging was performed by gentle trypsinization with 0.25% T/E, and the reaction was stopped by adding an equal amount of ovomucoid followed by careful mechanical trituration.

Differentiation of Oligodendrocytes from Human Radial Glia-like Neural Stem Cells

Differentiation of oligodendrocytes from hiPSC-derived radial glia-like neural precursor cells (RGL-NPCs) was performed in a three step culture paradigm according to a novel protocol (Gorris et al., 2015). Briefly, 800,000 RGL-NPCs were seeded onto matrigel coated 3.5 cm tissue culture dishes in N2-medium (DMEM/F12, 1% N2, and 1.6% D-glucose) containing 20 ng mL^{-1} FGF2 and 20 ng mL^{-1} EGF. After the cells had reached ~80% confluence, the differentiation along the oligodendrocyte lineage was started. First, OPC generation (step 1) was carried out for 2 weeks in N2-medium containing 10 ng mL^{-1} EGF, 10 ng mL^{-1} PDGF-AA, and 4 μg mL^{-1} forskolin. After that OPCs were allowed to proliferate (step 2) for another week in the presence of 10 ng mL^{-1} PDGF-AA, 30 ng mL^{-1} T_3, 20 ng mL^{-1} noggin, and 200 μM ascorbic acid. The first two steps were performed to specifically support the development and proliferation of oligodendroglial progenitors. We intentionally omitted FGF2 in the culture paradigm, because it has been shown to impair the transition of human pre-OPCs to OPCs (Hu et al., 2009). To reduce the differentiation of astroglial cells, we further added noggin to the culture medium during the second step. Finally, terminal differentiation for up to 5 weeks was carried out in the presence of 30 ng mL^{-1} T_3, 200 μM ascorbic acid, and 1 μg mL^{-1} laminin. In general the medium was changed every other day.

Immunological Reagents

In the following, all primary antibodies used in this study are listed. The monoclonal antibodies were: anti-O4 (1:50; mouse IgM) (Sommer and Schachner, 1981), anti-Nestin (1:500; mouse IgG; clone rat-401; Millipore, Hofheim, Germany), anti-MBP (1:50; mouse IgG; clone 129-138; Millipore, Hofheim, Germany); anti-CS (1:300 (immunofluorescence), 1:100 (Western-blot); rat IgM; clone mAb473HD) (Faissner et al., 1994), BrdU (1:50; mouse IgG; clone BMG6H8; Roche, Mannheim, Germany), anti-MBP (1:25; rat; Abcam, Cambridge, UK). The polyclonal antibodies were: anti-Olig2 (1:200; rabbit; Millipore, Hofheim, Germany), anti-Tnc (1:300 (immunofluorescence), 1:2,000 (Western-blot); rabbit, batch Kaf14/1) (Faissner and Kruse, 1990), anti-RPTPβ/ζ (1:300 (immunofluorescence), 1:1,000 (Western-blot); rabbit, batch Kaf13/5) (Faissner et al., 1994), anti-NG2 (1:300 (immunofluorescence and Western-blot); rabbit; Millipore, Hofheim, Germany), anti-Pdgfrα (1:300 (immunofluorescence), 1:10,000 (Western-blot); rabbit, Santa-Cruz, Hamburg, Germany).

Immunocytochemistry

After removal of the culture medium adherent cells were washed twice with PBS/A (PBS + 0.1% BSA). After that the primary antibodies for extracellular or membrane bound epitopes (473HD, O4, Kaf13/5) were incubated in PBS/A for 30 min at room temperature. Then, the cells were fixed with 4% PFA for 10 min at room temperature. In case of intracellular epitopes, the fixation was carried out prior to the incubation with primary antibodies diluted in PBT1 (PBS + 0.1% Triton X100 + 1% BSA). Next, the cells were washed two times with PBT1. The incubation with species-specific Cy2-, Cy3-, Alexa488, or Alexa555-coupled secondary antibodies (Dianova or Invitrogen) diluted in PBS/A was carried out at room temperature for further 30 min. Hoechst

or Dapi (both 1:10^5) were additionally added to visualize the cell nuclei. Finally, the cells were washed two times with PBS and mounted with either PBS/Glycerin (1:1) or mowiol.

Anion Exchange Chromatography

Sulfated proteoglycans were enriched from Oli-Neu conditioned cell culture medium using DEAE anion exchange chromatography (Sirko et al., 2010a). In brief, 250 mL conditioned medium were cleared by centrifugation for 10 min at 4,000*g* and then adjusted to 500 mL containing finally 7 M urea and 25 mM Tris pH 7.5. After that the solution was stirred overnight at 4°C. The next day, the solution was loaded on a DEAE column for 24–48 h with a constant flow rate of 0.5 mL min^{-1}. After that, the column was washed with 30 column volumes of DEAE-washing buffer with a flow rate of 2 mL min^{-1}. Bound molecules were eluted with a step gradient of 0.3 M, 0.7 M, and 2.0 M NaCl. Two column volumes were used for each NaCl concentration. During the elution fractions were collected, and the protein content of each fraction was assessed by measuring the light extinction at 280 nm. Finally, the PGs of the 0.7 M fraction were precipitated through the addition of two volumes EtOH/1.3% CH_3COOK overnight at −20°C. Precipitated proteins were spun down for 10 min at 16,000*g*, washed twice with 80% EtOH and finally resuspended in 50 µL PBS. In some cases, an aliquot of the proteoglycans was biotinylated using the EZ link biotinylation kit according to the manufacturer's instructions (Thermo Fisher Scientific, Waltham, MA).

Western-Blot

Adherent cells were homogenized and solubilized in 4°C cold cell lysis buffer (50 mM Tris-HCl pH 7.4; 150 mM NaCl; 5 mM EDTA; 5 mM EGTA; 1% Triton X100; 0.1% Na-desoxycholate; 0.1% SDS) and incubated for further 30 min on ice. After that, the lysate was cleared by centrifugation (16,000*g*) at 4°C, and the protein concentration was determined using a protein quantification kit (Pierce, Rockford, USA) according to the manufacturer's instructions. Usually, 10 µg protein were run on a 8% (v/v) SDS-gel under reducing conditions and transferred to a PVDF membrane (Roth, Karlsruhe, Germany) using a semi-dry blotting procedure. After transfer the membrane was blocked with 5% (w/v) skim milk powder in Tris-buffered-Saline (TBS) for 1 h at room temperature. The primary antibodies diluted in 5% skim milk powder in TBS + 0.05% Tween20 (TBST) were incubated over night at 4°C. Subsequently, the membrane was washed three times with TBST for 10 min and the incubation with the HRP-coupled secondary antibodies (1:5,000) diluted in 5% skim milk powder in TBST was carried out at room temperature for 1 h. The membrane was then washed again three times with TBS. Finally the signal was detected using enhanced chemiluminescence reagent (Pierce, Rockfort, USA).

Semiquantitative RT-PCR

OPCs and differentiated oligodendrocytes were lysed in RLT buffer, and total RNA was isolated according to the manufacturer's instructions (RNeasy Mini Kit, Qiagen, Hilden, Germany). Total RNA (1 µg) was reverse transcribed into cDNA using the First strand cDNA Synthesis Kit (Fermentas, St. Leon-Rot, Germany), and 1 µL cDNA was used for PCR analysis. The following primer pairs were used:

Phosphacan for: 5′-TTGACAAGTGATGAAGAGAG TGG-3′; *Phosphacan rev*: 5′-AATCAGCACATCTCG TTCTATCC-3′; *RPTPβ/ζ long for*: 5′-TCAGGAGTATCCAA CAGTTCAGAG-3′; *RPTPβ/ζ long rev*: 5′-GTGTTGGTGG TGTAGATATTACTCG-3′; *RPTPβ/ζ short for*: 5′-CCACTA GCAACATCCTCCATTC-3′; *RPTPβ/ζ short rev*: 5′-TCCA GTAGATGAGAATACCAACAAG-3′, (Dobbertin et al., 2003) *RPTPσ for*: 5′-GTCACATGGGACTCAGGCAA-3′; *RPTPσ rev*: 5′-GGTGTAGTAGACGCGGTAGC-3′; *Actin for*: 5′-TATGCCAACACAGTGCTGTCTGGTGG-3′; *Actin rev*: 5′-AGAAGCACTTGCGGTGCACGATGG-3′.

Documentation and Data Analysis

Photomicrographs of immunolabeled cells were taken at the Axioplan2 or Apotome Imager Z.1 using AxioVision 4.4 and 4.8 software, respectively. Both microscope systems were equipped with an AxioCam HRc camera (Zeiss, Jena, Germany). For quantitative analyses of immunocytochemical antigen detections, at least 200 Hoechst-positive nuclei were counted in at least three independent experiments per antibody and culture condition. The quantification of the membrane area or the area occupied by a single oligodendrocyte was performed using ImageJ software. Phase contrast images were taken at the Axiovert200M equipped with an AxioCam HRc camera using AxioVision 4.8 software (Zeiss, Jena, Germany).

All data are expressed as mean ± SD. Statistical significance was assessed using the paired and unpaired two-tailed Student's *t* test and the *P* values are given as $^*P \leq 0.05$, $^{**}P \leq 0.01$, and $^{***}P \leq 0.001$.

Results

The 473HD Epitope is Expressed in the Early Oligodendroglial Lineage

CSPGs are major constituents of the neural ECM during CNS development (Faissner and Reinhard, 2015; Maeda, 2015) and under pathological conditions, such as spinal cord injury. CSPGs bear highly sulfated GAG chains, which are believed to represent important molecular mediators of the cell biological function of CSPGs under pathological conditions (Harlow and Macklin, 2013; Iozzo and Schaefer, 2015).

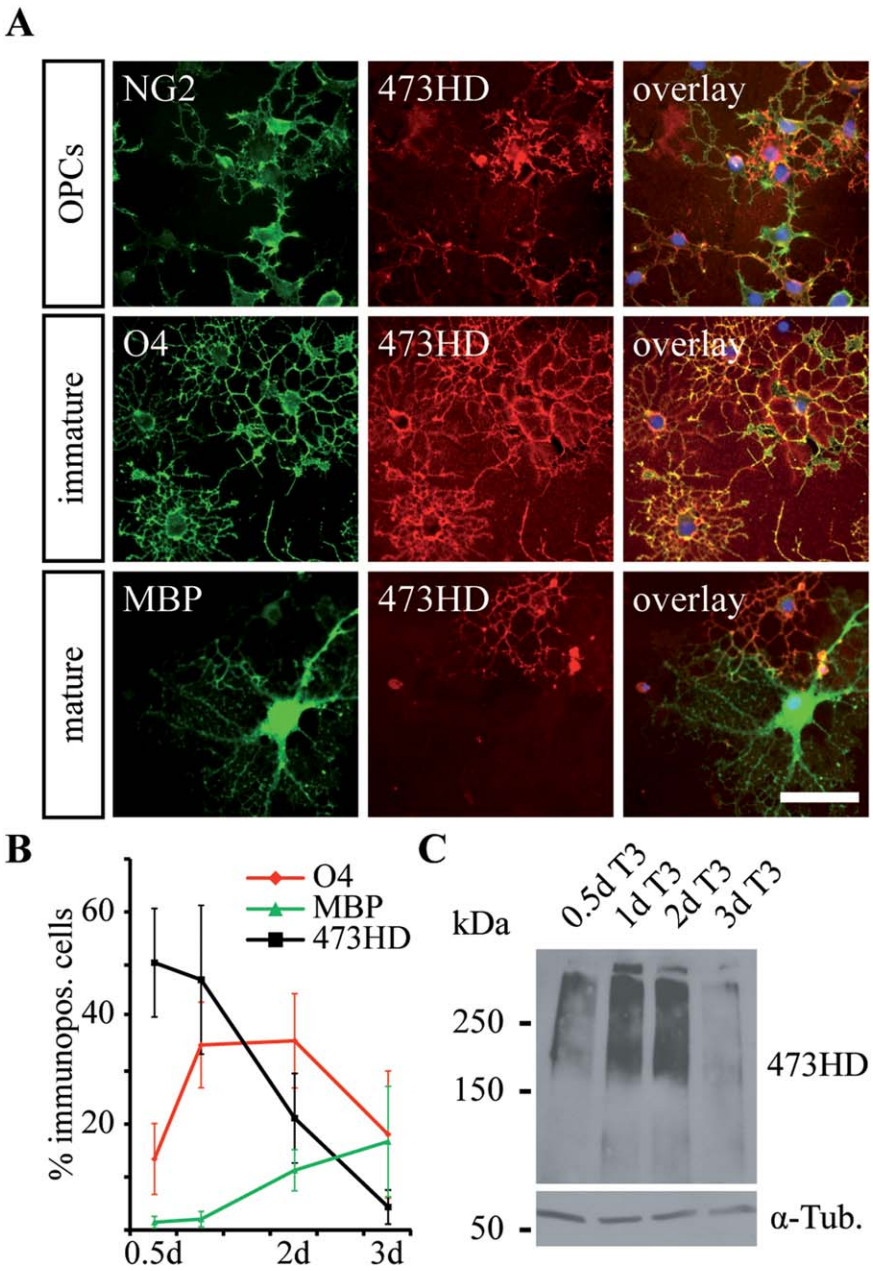

FIGURE 1: Expression of the 473HD epitope within the oligodendrocyte lineage: (A) Photomicrographs of OPCs plated under differentiating conditions labelled against the 473HD epitope in combination with stage-specific markers (NG2 for oligodendrocyte precursor cells; O4 for immature oligodendrocytes; MBP for myelin gene expressing oligodendrocytes). The 473HD epitope was present on OPCs as well as on immature O4-positive cells. MBP-positive cells did not exhibit any 473HD immunoreactivity. (B) Quantitative analysis of the 473HD-positive cell population during a 3-day differentiation period in comparison to O4- or MBP-positive cells. During differentiation the population of 473HD-positive cells declined, while especially the number of MBP-positive cells increased. (C) Western-blot analysis additionally confirmed the overall decrease of the 473HD-expression during oligodendrocyte differentiation. Scale bar: 25 μm.

Although recent studies have demonstrated that CSPGs regulate morphological oligodendrocyte maturation (Pendleton et al., 2013; Siebert and Osterhout, 2011), their influence on the oligodendrocyte lineage progression remained uncertain. Here, we chose the unique 473HD epitope as a typical CS epitope present on Phosphacan/RPTPβ/ζ and first analysed its expression within the oligodendrocyte lineage. For that purpose we cultivated rat OPCs and monitored the expression of the 473HD epitope under differentiating conditions in comparison to the OPC marker NG2, the immature oligodendrocyte marker O4, and the mature oligodendrocyte marker MBP. We found that the 473HD is expressed by NG2-positive OPCs as well as by O4-positive immature oligodendrocytes (Fig. 1A). In contrast MBP-positive oligodendrocytes did not express the

TABLE 1: Marker Profile of Differentiating Primary Rat Oligodendrocytes Cultivated for a Period of 3 Days

Days *in vitro*	Marker	% Immunolabeled cells	*N*
0.5	473HD	50.4 ± 10.2	3
	O4	13.4 ± 6.7	4
	MBP	1.5 ± 1.1	3
1	473HD	47.2 ± 14.0	3
	O4	34.9 ± 8.1	5
	MBP	2.1 ± 1.4	5
2	473HD	21.0 ± 8.5	4
	O4	35.7 ± 8.9	5
	MBP	11.3 ± 3.9	4
3	473HD	4.3 ± 3.2	3
	O4	18.0 ± 12.0	5
	MBP	16.7 ± 10.4	3

At least 200 cells were counted per experiment. The numbers indicate mean ± SD, N: number of independent experiments.

473HD epitope (Fig. 1A). A quantitative analysis of its expression in comparison to O4 and MBP under differentiating conditions revealed a general decrease in 473HD-positive cells within a 3 day differentiation period (473HD: 0.5 day: 50.4% ± 10.2%, 1 day: 47.2% ± 14.0%, 2 days: 21.1% ± 8.5%; 3 days: 4.3% ± 3.2%). In contrast, the number of O4-positive cells initially increased within the first day and decreased again after 3 days (O4: 0.5 days: 13.4% ± 6.7%, 1 day: 34.9% ± 8.1%, 2 days: 35.7% ± 8.9%, 3 days: 18.0% ± 12.0%). The number of MBP-positive cells continuously increased during this time period (MBP: 0.5 day: 1.5% ± 1.1%, 1 day: 2.1% ± 1.4%, 2 days: 11.3% ± 3.9%, 3 days: 16.7% ± 10.4%) (Fig. 1B and Table 1). Western-blot analysis confirmed the overall down-regulation of the 473HD epitope within the analysed differentiation period (Fig. 1C). In line with these data, we also noticed a strong expression of the 473HD epitope by O4-positive immature oligodendrocytes differentiated from mouse cortical oligosphere cultures. However, MBP-positive mature oligodendrocytes did not notably express this CS-motif (Supp. Info. Fig. S1). In summary, our expression data demonstrate that the 473HD epitope is mainly confined to immature oligodendrocyte phenotypes.

Chondroitinase ABC Effectively Degrades CS-GAGs from OPC Cultures

The bacterial enzyme ChABC is a commonly used enzyme for the degradation of CS-GAGs. To address possible functions of CS-GAGs for the oligodendrocyte lineage progression, we added 50 mU mL^{-1} ChABC to OPC cultures under proliferative and differentiating conditions. To confirm the suitability of ChABC to interfere with CS-GAG chain biology in our OPC cultures, we initially analysed the expression of the 473HD epitope and its carrier protein Phosphacan/RPTPβ/ζ in the presence and absence of ChABC under proliferating conditions. Untreated OPCs express high levels of both Phosphacan/RPTPβ/ζ and the 473HD epitope (Fig. 2A). After CS-GAG removal the immunoreactivity for Phosphacan/RPTPβ/ζ appeared to be enhanced. In contrast the 473HD immunoreactivity was strongly reduced (Fig. 2A). Western-blot analysis of OPC detergent extracts also demonstrated the effective degradation of CS-GAGs after ChABC treatment (Fig. 2B).

To analyze the influence of CS-GAGs on the oligodendrocyte lineage progression in detail, we plated OPCs on control (P-Orn) and PG-enriched substrates in the presence and absence of ChABC. The PG fractions were isolated from Oli-Neu cell line culture supernatants via DEAE anion-exchange chromatography. For that purpose, 250 mL conditioned medium were collected and loaded onto a DEAE sepharose column (Fig. 3A). After several washing cycles, the proteins were eluted in a step-wise manner using increasing NaCl concentrations (0.3, 0.7, and 2 M) (Fig. 3A). The eluate was collected in small fractions, and the light absorption of each fraction was measured at 280 nm, in order to identify the high protein fractions (Fig. 3B). While the 0.3 M fraction contained several proteins, the 0.7 M fraction was mainly composed of high molecular mass proteins (Fig. 3C). Glycoproteins such as Tenascin-C were mainly detectable in the 0.3 M fraction, whereas e.g. the 473HD epitope was clearly enriched in the 0.7 M fraction (Fig. 3C) as expected because previous studies had shown that Oli-neu releases substantial amounts of DSD-1-PG/Phosphacan (Garwood et al., 1999; Schnadelbach et al., 1998). Western-blot analysis of a biotinylated 0.7-M aliquot showed that several CSPGs were present in our preparation, since different distinct bands appeared in the western-blot after ChABC digestion, reflecting the strong glycosylation of these proteins in the absence of ChABC. A more detailed analysis of the PG fractions derived from Oli-Neu cell culture supernatants in comparison to mouse cortical neurospheres and P2 rat brain revealed that the 0.7 M fraction mainly contained Phosphacan/RPTPβ/ζ and to less extent Brevican. In contrast, Neurocan was not present in our PG enriched fractions (Supp. Info. Fig. S2A,B). NG2 represents a known CSPG expressed by OPCs and occurs in a glycosylated and non-glycosylated isoform (Fidler et al., 1999; Morgenstern et al., 2003). Thus, we additionally analysed its expression in our PG fractions. Non-glycosylated NG2 was detectable as a distinct band in the 0.3 M fraction. In contrast, a smear in the high molecular mass range was indicative of glycosylated NG2 in the 0.7 M fraction. Yet, the NG2-level in this fraction was lower than the 473HD-level (Supp. Info. Fig. S2C).

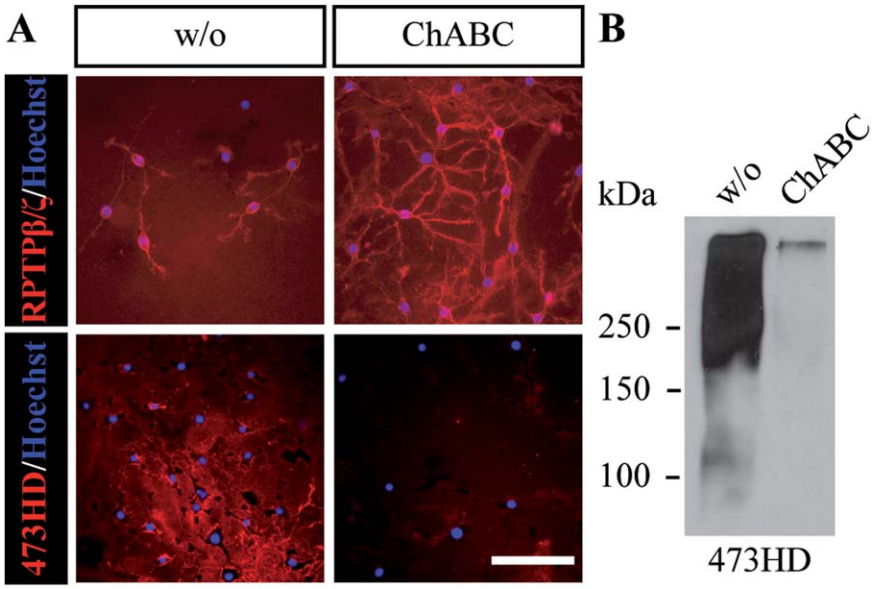

FIGURE 2: ChABC mediated degradation of the 473HD epitope. (A) Photomicrographs of OPCs labelled for the 473HD epitope and its carrier protein Phosphacan/RPTPβ/ζ in the presence and absence of the CS-GAG degrading enzyme ChABC. While OPCs expressed high levels of Phosphacan/RPTPβ/ζ and the 473HD epitope under control conditions, the addition of ChABC effectively reduced the inmmunoreactivity for the 473HD epitope. In contrast, the immunoreactivity for its carrier protein appeared to be enhanced. Hoechst (blue) was additionally added, in order to visualize the cell nuclei. (B) Western-blot analysis further demonstrated the successful degradation of the 473HD epitope in the presence of ChABC. Scale bar: 50 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

CS-GAG Removal Enhances Spontaneous Oligodendrocyte Differentiation

CS-GAGs can directly bind to growth factors such as FGF2 (Milev et al., 1998) and promote FGF2-dependent proliferation of e.g. cortical NPCs (Sirko et al., 2010b). Thus, we first investigated a possible influence of CS-GAGs on the BrdU-incorporation rate of OPCs under proliferating conditions. For that purpose we added 1 μM BrdU for 2 and 4 h to OPCs plated either on a control substrate (P-Orn) or on enriched PGs in the presence and absence of ChABC (Fig. 4A–D). The number of BrdU-positive cells, however, did not differ between all four conditions investigated (2 h: w/o P-Orn: 26.3% ± 7.9%; ChABC P-Orn: 28.7% ± 7.1%; w/o PG: 25.3% ± 7.9%; ChABC PG: 26.6% ± 5.4%; 4 h: w/o P-Orn: 30.6% ± 7.3%; ChABC P-Orn: 31.2% ± 5.9%; w/o PG: 29.7% ± 7.1%; ChABC PG: 31.0% ± 8.1%; $n = 2$ for each condition and time point) (Fig. 4E,F). Because the overall expression of CS-GAGs decreased with ongoing OPC differentiation (Fig. 1), we next analysed, whether the degradation of CS-GAGs promotes spontaneous differentiation of OPCs already under proliferating conditions. To address this issue, we determined the number of O4-positive cells in the presence of PDGF-AA and FGF2 after 2 and 3 days *in vitro*. We observed an enhanced number of O4-positive cells after CS-GAG removal on both substrates (Fig. 4G–J). The number of Pdgfrα-positive cells, however, did not notably change. Moreover, OPCs exhibited a less complex morphology on the PG substrate. Especially the number of cell processes seemed to be strongly reduced (Fig. 4G–J). The quantitative analysis of O4-positive cells revealed a significant increase of spontaneously differentiating OPCs upon ChABC treatment. In contrast, cultivation on a PG substrate did not affect the number of O4-positive cells (48 h: w/o P-Orn: 8.4% ± 0.4%; ChABC P-Orn: 12.8% ± 1.0% ($P < 0.001$); w/o PG: 9.3% ± 1.1%; ChABC PG: 13.0% ± 1.4% ($P = 0.0012$); 72 h: w/o P-Orn: 8.3% ± 1.0%; ChABC P-Orn: 13.6% ± 1.7% ($P < 0.001$); w/o PG: 8.2% ± 0.5%; ChABC PG: 14.1% ± 1.5% ($P = 0.0051$); $n = 4$ for each condition and time point) (Fig. 4K,L).

CS-GAGs Inhibit Morphological Maturation and Myelin Gene Expression of Differentiating OPCs

So far our data suggest that CS-GAGs are involved in OPC maintenance rather than in OPC proliferation. Therefore, we next investigated the influence of CS-GAGs on the oligodendrocyte lineage progression under differentiating conditions. Here, we used MBP as a marker for mature, myelin gene expressing oligodendrocytes. On the P-Orn control substrate OPCs readily differentiated into multi-branched MBP-expressing oligodendrocytes within 2 days. Moreover, several oligodendrocytes had already formed membrane sheets (Fig. 5A). In contrast the number of MBP-positive cells on the PG substrate was significantly reduced [w/o P-Orn: 14.3% ± 0.6%; w/o PG: 9.5% ± 0.2% ($P = 0.0011$) ($n = 4$

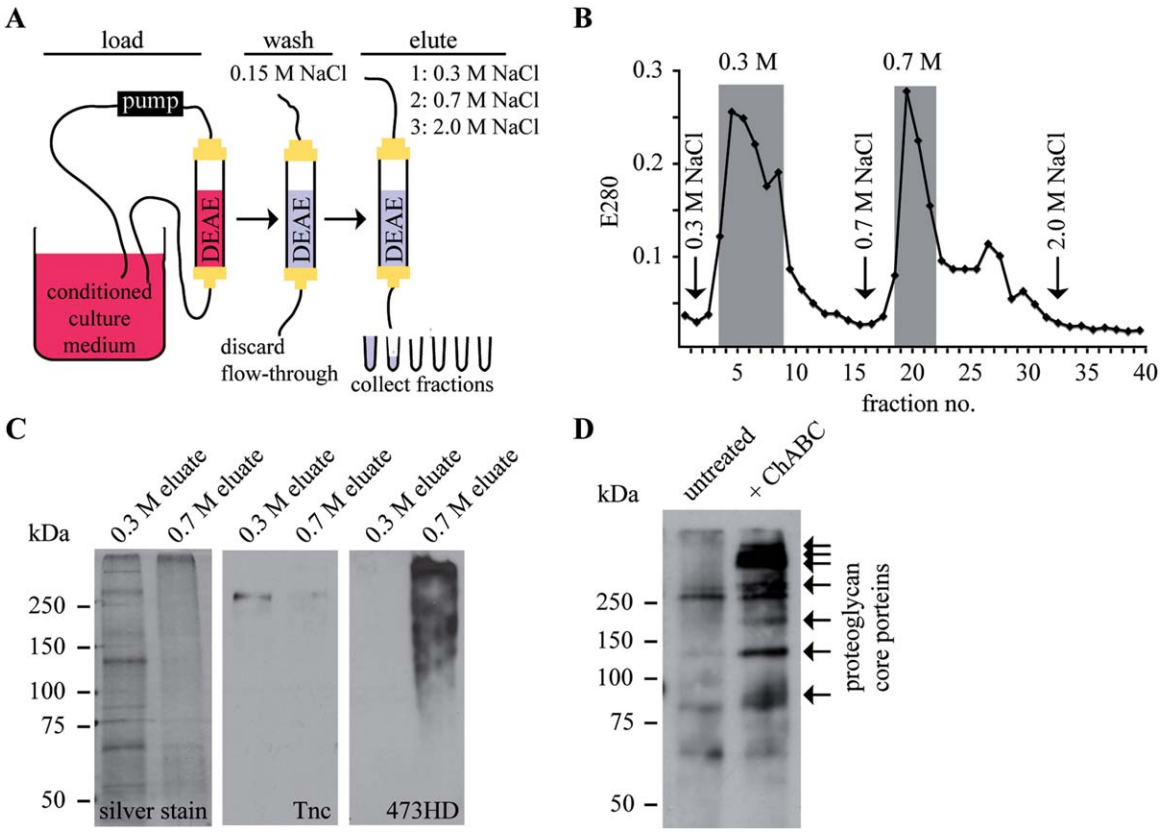

FIGURE 3: Purification of proteoglycans from Oli-Neu cell culture supernatants. (A) Schematic depiction of the purification protocol. The purification using DEAE sepharose was divided into three phases. During the first phase the column was loaded with the culture supernatant for up to 48 h. In the second phase the column was washed with a low salt solution (0.15 M). Finally, the proteins were eluted in a step wise manner using different salt concentrations. (B) The protein content of the different fractions was photometrically determined. After addition of 0.3 M and 0.7 M NaCl solutions proteins could be eluted. (C) While the 0.3 M fraction contained several proteins of varying size, the 0.7 M fraction contained high molecular mass proteins. The glycoprotein Tenascin-C was mainly present within the 0.3 M fraction. In contrast, the 0.7 M fraction contained large amounts of the 473HD epitope. (D) An aliquot of the 0.7 M fraction was additionally biotinylated and run on a SDS-gel. Western-blot analysis using streptavidin-coupled-HRP revealed the presence of different proteins within this fraction. After degradation of the CS-GAGs, however, many distinct protein signals appeared, demonstrating the high CS-GAG content within the 0.7 M fraction. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

for both conditions)] (Fig. 5B,E). In addition, the PG substrate attenuated the morphological maturation of the oligodendrocytes (Fig. 5B). The addition of ChABC did not notably affect the number of MBP-positive cells on the control substrate [ChABC P-Orn: 14.6% ± 0.5% ($n = 4$)] (Fig. 5C,E). However, ChABC treatment rescued the effect of the PG substrate on the number of MBP-positive cells [w/o PG: 9.5% ± 0.2%; ChABC PG: 15.2% ± 0.7% ($P < 0.001$); $n = 4$ for both conditions] (Fig. 5D,E). After 3 days of differentiation the number of MBP-positive cells was still reduced on the PG substrate in comparison to the control substrate [w/o P-Orn: 25.7% ± 3.0%; w/o PG: 19.1% ± 1.5% ($P = 0.0134$); $n = 4$ for both conditions] (Fig. 5F,G,J). However, the difference appeared to be less pronounced. Again, ChABC treatment did not alter the number of MBP-positive cells on the control substrate (ChABC P-Orn: 24.0% ± 2.4%; $n = 4$) (Fig. 5H,J), but could rescue the PG effect (w/o PG: 19.1% ± 1.5%; ChABC PG: 27.5% ± 1.0% ($P = 0.0012$); $n = 4$ for both conditions) (Fig. 5I,J). Strikingly, we also observed a significantly increased number of membrane forming oligodendrocytes [w/o P-Orn: 26.9% ± 7.9%; ChABC P-Orn: 33.9% ± 7.5% ($P < 0.001$); w/o PG: 25.0% ± 4.6%; ChABC PG: 33.2% ± 7.1% ($P = 0.006$); $n = 5$ for each condition] as well as larger myelin membranes [w/o P-Orn: 6600.7 ± 969.2 μm^2; ChABC P-Orn: 10481.9 ± 943.1 μm^2 ($P = 0.007$); w/o PG: 5018.8 ± 720.7 μm^2; ChABC PG: 8492.3 ± 620.3 μm^2 ($P = 0.003$); $n = 3$ for each condition] upon CS-GAG removal on both substrates (Fig. 6A–D).

Phosphacan/RPTPβ/ζ as Potential Mediator for CS-GAG-Dependent Oligodendrocyte Lineage Progression

Because the *Ptprz1* gene codes for known CS-GAG carrier proteins generated through alternative splicing, we analysed the expression of the three known isoforms Phosphacan,

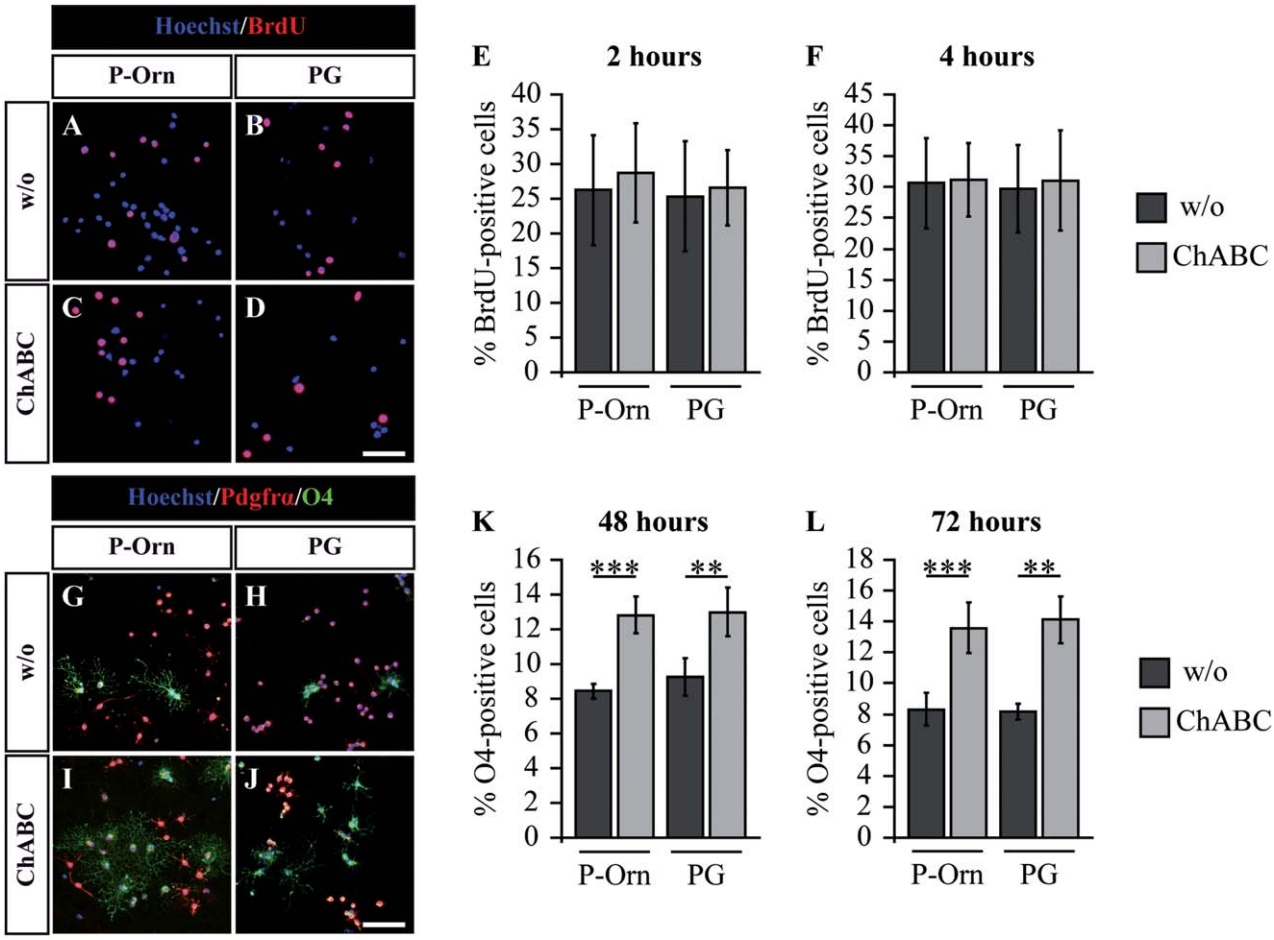

FIGURE 4: CS-GAGs are necessary for OPC maintenance: (A–D) BrdU-stainings of OPCs plated on either a control (P-Orn) or a CS-GAG enriched proteoglycan substrate (PG) in the presence and absence of ChABC. (E, F) Neither a surplus of CS-GAGs nor their removal by ChABC affected the BrdU incorporation rate of OPCs quantified after 2 and 4 h in the presence of PDGF-AA and FGF2. (G–L) Immunostainings of OPCs labelled against the OPC marker Pdgfrα and the immature oligodendrocyte marker O4. (K, L) While a surplus of CS-GAGs (PG) did not change the number of spontaneously differentiating OPCs, the ChABC-mediated degradation of CS-GAGs resulted in an enhanced differentiation towards O4-positive cells quantified after 48 and 72 h. Note that on the PG substrate OPCs (H) were much smaller and had shorter processes in comparison to the control substrate (G). This effect could be rescued after CS-GAG removal (J). Hoechst (blue) was additionally added, in order to visualize the cell nuclei. Scale bar: 50 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

RPTPβ/ζ_{long}, and RPTPβ/ζ_{short}, in OPCs and oligodendrocytes. In addition, we also examined the expression of RPTPσ, which is a known receptor for CSPGs (Pendleton et al., 2013; Shen et al., 2009), mediating the inhibitory effect on oligodendrocyte process extension. For the expression analysis, we cultivated OPCs both in the presence of PDGF-AA and FGF2 and in the presence of T_3 and FCS and finally isolated the RNA after 2 days. The results of the semiquantitative RT-PCR indicate that all Phosphacan/RPTPβ/ζ isoforms were expressed under proliferating and differentiating conditions (Supp. Info. Fig. S3A). Yet, the expression of RPTPβ/ζ_{long} and RPTPβ/ζ_{short} was downregulated in differentiated oligodendrocytes, whereas the expression of Phosphacan remained unaltered in differentiated oligodendrocytes compared with the expression in OPCs. RPTPσ was also expressed by OPCs and seemed to be downregulated in differentiated oligodendrocytes reminiscent of the temporal expression of RPTPβ/ζ_{long} and RPTPβ/ζ_{short} (Supp. Info. Fig. S3B).

To more specifically address the function of Phosphacan/RPTPβ/ζ in the context of a CS-GAG-dependent oligodendrocyte lineage progression, we next performed a siRNA-mediated knock-down of the *Ptprz1* gene in rodent OPCs. For that purpose, we transfected OPC cultures with a specific *Ptprz1* siRNA and analysed the number of Phosphacan/RPTPβ/ζ-positive cells as well as MBP-positive cells 2 days after transfection. We additionally used siGLO Green, to detect transfected cells. Immunopositive cells were quantified within the siGLO-positive population. The siRNA transfected oligodendrocyte cultures showed a significant reduction in the number of Phosphacan/RPTPβ/ζ-positive cells compared with the control cultures (siGLO+siRNA: 12.94% ± 7.58%; siGLO: 65.81% ± 9.29%, $n = 3$, $P = 0.002$), demonstrating

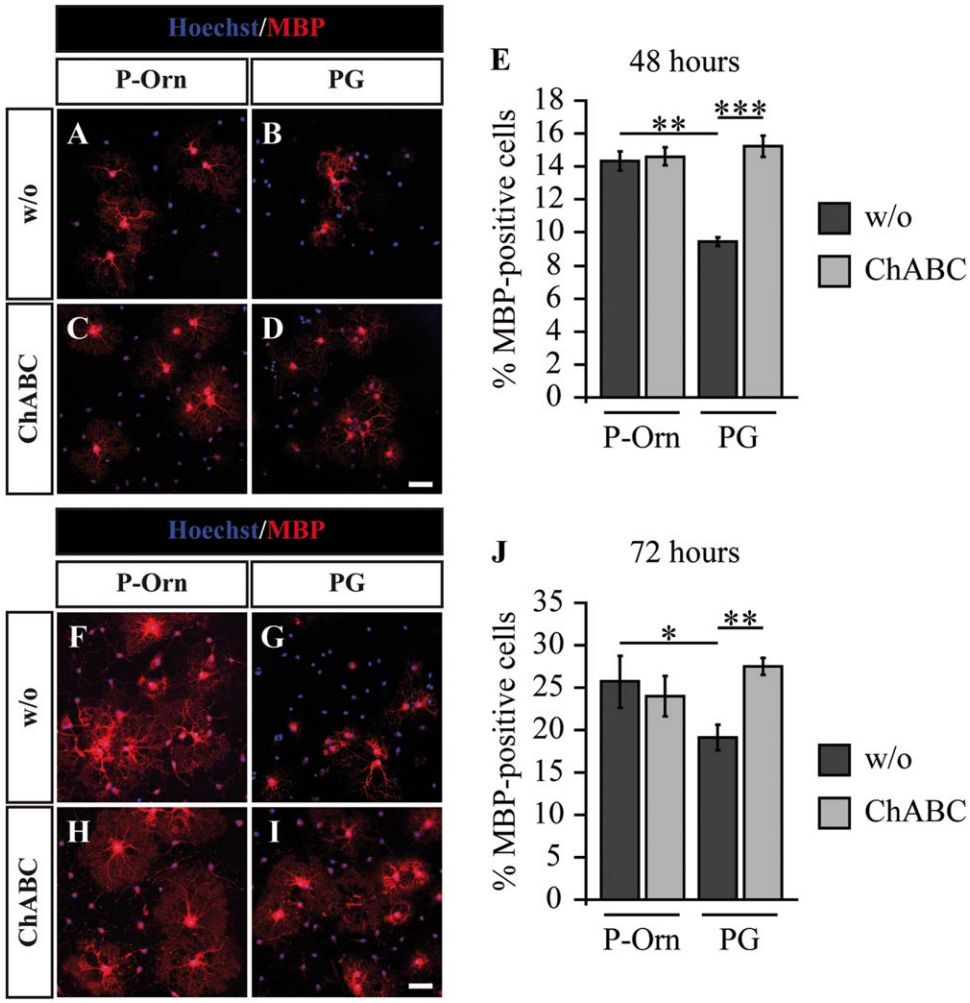

FIGURE 5: CS-GAGs attenuate myelin gene expression and membrane formation: (A–J) Differentiating OPCs plated on either a control (P-Orn) or PG-substrate in the presence and absence of ChABC were analysed after 48 (A–E) and 72 (F–J) h. The addition of ChABC on the control substrate had no effect on the number of MBP-positive cells at both time points (E, J). In contrast cultivation on the PG-substrate resulted in a significantly reduced number of MBP-positive cells in comparison to the control substrate. This effect could be reverted after CS-GAG removal (E, J). Moreover, the size of the MBP-positive cells on the PG-substrate (B, D, G, I) was much smaller in comparison to the control substrate (A, C, F, H). Hoechst (blue) was additionally added, in order to visualize the cell nuclei. Scale bar: 50 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the successful knock-down on a cellular level. However, on a global level no reduction was visible, most likely owing to the low overall transfection efficiency (~20%, data not shown). Upon knock-down of *Ptprz1* we observed a trend towards an increased number of MBP-positive cells after 2 days in culture, yet this was not statistically significant (siGLO: 28.87% ± 11.23%; siGLO+siRNA: 44.75% ± 23.48%, $n = 3$, $P = 0.3$) (Supp. Info. Fig. S4A,B). Importantly, we also found that despite knock-down the culture substrate exhibited a 473HD immunoreactivity, which was comparable to that of the control condition (Supp. Info. Fig. S4C), indicating a slow CS-GAG turnover and an ongoing substrate conditioning by non-transfected cells. Beyond that effect, the CSPGs NG2 and brevican that are not targeted by the knock-down may additionally contribute to an inhibitory environment.

CS-GAG Removal from Mouse and Human NPC-derived Oligodendrocytes Accelerates Morphological Maturation

The impact of CS-GAG degradation from rodent OPC cultures on the oligodendrocyte lineage progression prompted us to analyse, whether the ability of ChABC to stimulate oligodendrocyte differentiation could also be observed in neural precursor cell (NPC) cultures. In particular late-stage NPCs, i.e. radial glia-like NPCs, represent a versatile cellular donor source for e.g. white matter repair due to a pronounced intrinsic glial differentiation propensity. However, it is of utmost importance to identify and eventually modulate novel molecular pathways governing oligodendrocyte differentiation. To address the effect of ChABC-treatment on the differentiation of NPC-derived oligodendrocytes, we initially chose

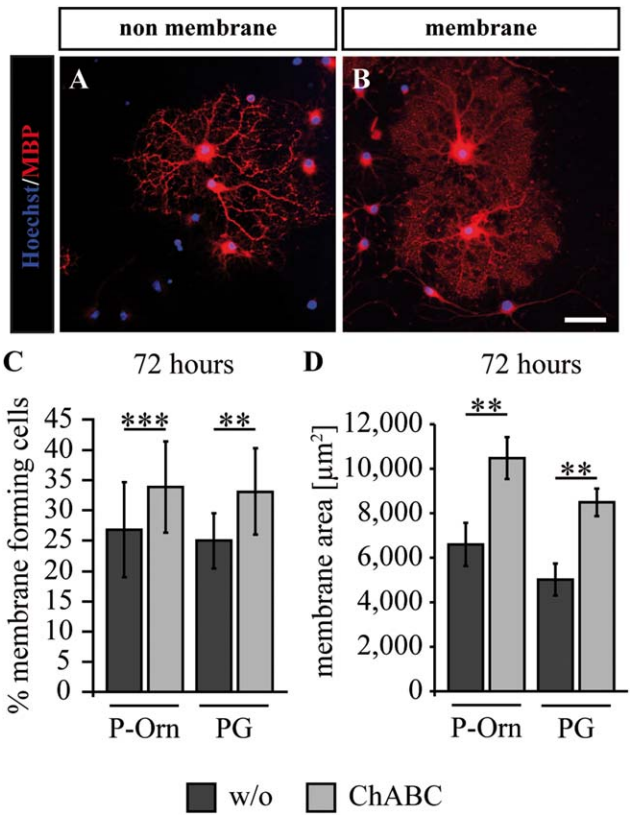

FIGURE 6: CS-GAG degradation fosters myelin membrane formation: (A, B) Representative examples of differentiating oligodendrocytes immunolabelled for MBP after 72 h. While some MBP-positive oligodendrocytes still did not form myelin membranes (A), others already exhibited a strong membrane forming phenotype (B). (C) The number of membrane forming oligodendrocytes was not affect by the PG substrate. However, enzymatic CS-GAG degradation led to a significant increase of membrane forming cells on both substrates. (D) ChABC treatment resulted in significantly larger myelin membranes independent from the substrate the oligodendrocytes were cultivated on. Hoechst (blue) was additionally added, in order to visualize the cell nuclei. Scale bar: 25 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

primary mouse spinal cord NPCs, since they exhibit a relatively low oligodendrocyte differentiation capacity under standard neurosphere culture conditions (own observation). Spinal cord NPCs grown under high density conditions readily formed neurospheres within 1 week in the presence of EGF and FGF2 (Fig. 7A). The addition of ChABC resulted in an attachment of the neurospheres and seemingly stimulated cellular migration on the bottom of the culture flask (Fig. 7B). Interestingly, many cells migrating out of the neurosphere exhibited a morphology reminiscent of OPCs or differentiating oligodendrocytes (Fig. 7B′). Thus, we analysed the expression of the OPC markers Pdgfrα, *Nkx2.2*, and *Olig2* under control and ChABC treated conditions. However, we did not observe any differences in the global expression levels of the respective genes or proteins in our NPC cultures (Fig. 7C,D), suggesting no changes of the overall oligodendroglial specification from NPCs under proliferative conditions. Thus, we subsequently analysed the effect of CS-GAG removal on the oligodendrocyte differentiation from primary NPCs after growth factor withdrawal. For that purpose we dissociated the neurospheres and plated single neurosphere cells on a laminin substrate in the presence and absence of ChABC. Under these conditions many presumptive oligodendrocytes expressed high levels of the 473HD epitope under control conditions (Fig. 7E). The relative numbers of O4-positive (Fig. 7F) or MBP-positive (Fig. 7G) cells were low under control conditions. The addition of ChABC significantly increased the number of both populations, although the increase was small (O4 w/o: 2.0% ± 0.8%; O4 ChABC: 3.4% ± 1.3%; $n = 6$; $P = 0.006$; MBP w/o: 0.93% ± 0.4%; MBP ChABC: 1.8% ± 0.5%; $n = 4$; $P = 0.050$) (Fig. 7H,I). In contrast, the amount of membrane forming oligodendrocytes (Fig. 7G) was strongly increased after CS-GAG removal (w/o: 13.5% ± 3.4%; ChABC: 35.5% ± 9.1%; $n = 4$; $P = 0.004$) (Fig. 7J). These data indicate that CS-GAG removal from differentiating mouse spinal cord NPCs also promotes the differentiation of oligodendrocytes towards more mature phenotypes.

Next, we asked, whether the stimulatory effect of ChABC on oligodendrocyte differentiation from rodent radial glia-like NPCs can also be observed in cultures of hiPSC-derived oligodendrocytes. For that purpose we used radial glia-like neural precursor cells (RGL-NPCs), which we generated from hiPSCs and subsequently differentiated along the oligodendrocyte lineage according to a novel protocol (Gorris et al., 2015). Seven weeks after the initiation of the differentiation regime we added 50 mU mL^{-1} ChABC to the cultures for another week. After that, we observed many OLIG2-positive cells expressing the 473HD epitope under control conditions (Fig. 8A). ChABC treatment resulted in the loss of the 473HD immunoreactivity without notably changing the relative number of OLIG2-positive cells (Fig. 8B). Western-blot analysis additionally confirmed the effective CS-GAG degradation in these human cell cultures (Fig. 8C). In the absence of ChABC we readily found several MBP-positive cells displaying a complex morphology (Fig. 8D). ChABC treatment accelerated morphological maturation of MBP-positive oligodendrocytes resulting in a massive membrane formation (Fig. 8E). We quantified this morphological maturation in two independent experiments by assessing the territory occupied by a single oligodendrocyte (Fig. 8F). In both experiments ChABC treatment resulted in a significantly larger oligodendrocyte area given in arbitrary units [Exp1 w/o: 879.9 ± 259.5 ($n = 17$); Exp1 ChABC: 1,472.2 ± 392.9 ($n = 10$), $P < 0.001$; Exp2 w/o 827.6 ± 237.5 ($n = 11$); Exp2

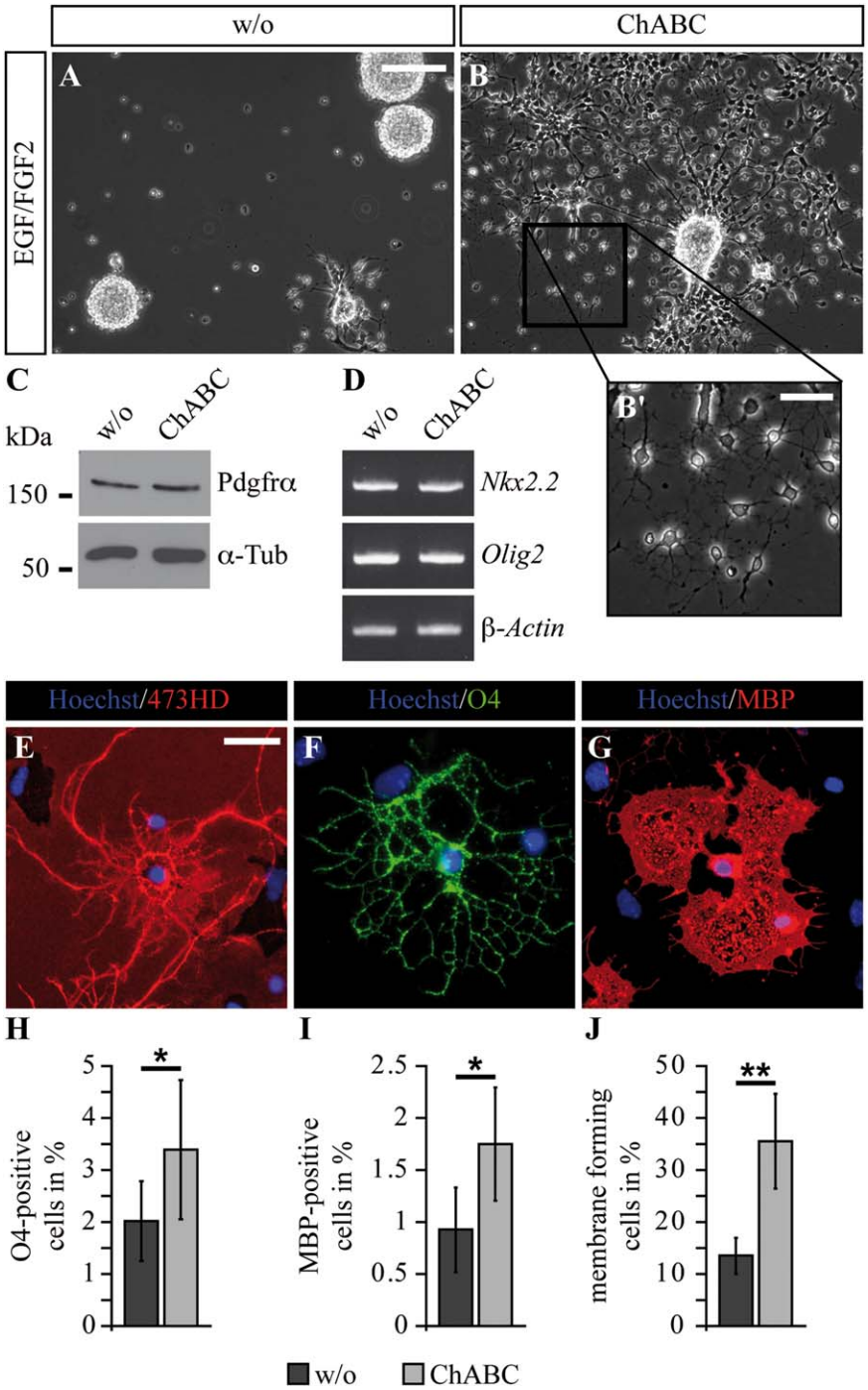

FIGURE 7: CS-GAG removal stimulates oligodendrocyte differentiation from mouse spinal cord NPCs: (A, B) Phase contrast images of mouse E13.5 spinal cord NPC cultures grown as neurospheres. Note that CS-GAG removal resulted in an adherent growth of the NPCs. Moreover, many cells reminiscent of differentiating oligodendrocytes migrated out of the neurosphere. (C, D) However, the overall expression levels of the known OPC markers Pdgfrα, *Nkx2.2*, and *Olig2* were not affected as determined by either Western-blot or RT-PCR analysis, respectively. α-Tubulin or *β-Actin* served as loading control. (E–G) Representative photomicrographs of oligodendrocytes differentiated from spinal cord NPCs after 4 days labelled for the 473HD epitope, O4, and MBP. Note, that some MBP-positive cells had already formed myelin membranes. (H–J) The enzymatic degradation of CS-GAGs resulted in a small but significant increase of both O4-positive and MBP-positive cells. Moreover, the maturation of oligodendrocytes with respect to membrane formation was strongly enhanced in the presence of ChABC. Hoechst (blue) was additionally added, in order to visualize the cell nuclei. Scale bar: 100 μm (A, B); 50 μm (B′); 25 μm (E–G). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

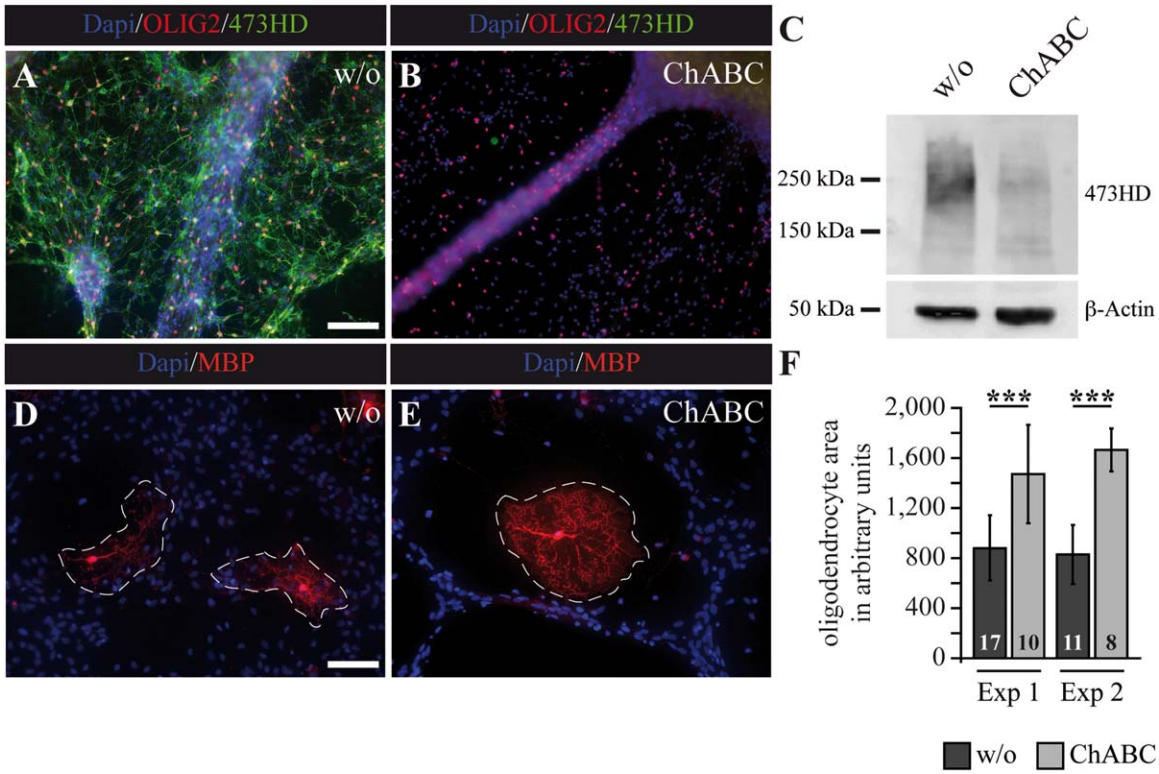

FIGURE 8: CS-GAG removal stimulates myelin membrane formation of hiPSC-derived oligodendrocytes. (A, B) Representative photomicrographs of oligodendrocytes differentiated from hiPSC-derived RGL-NPCs and stained for OLIG2 and the 473HD epitope. Under control conditions a strong 473HD immunoreactivity was detectable after 5 weeks of terminal differentiation. ChABC treatment efficiently reduced the 473HD immunoreactivity without affecting the relative number of OLIG2-positive cells. (C) Western-blot analysis confirmed the successful degradation of CS-GAGs. β-Actin served as loading control. (D) In the absence of ChABC several MBP-positive oligodendrocytes, that already displayed a complex morphology, were observed. (E) Addition of 50 mU mL^{-1} ChABC for 1 week already led to an accelerated morphological maturation. As a consequence many MBP-positive oligodendrocytes showed a massive membrane formation. (F) Quantification of the area covered by a single oligodendrocyte (white dashed lines) in two independent experiments revealed a significant increase upon ChABC treatment ($P < 0.001$ for both experiments). Numbers indicate the number of oligodendrocytes assessed in each condition. Dapi (blue) was additionally added, in order to visualize the cell nuclei. Scale bar: 100 μm (A, B); 50 μm (D, E).

ChABC: $1{,}664.3 \pm 170.2$ ($n = 8$), $P < 0.001$]. These data clearly show that the effect of CS-GAG degradation on oligodendrocyte differentiation, which we have described in detail in cultures of rodent cells, can also be recapitulated in cultures of human cells.

Discussion

CSPGs are expressed under several neuropathological conditions such as traumatic CNS injury or MS. Over many years, the role of CSPGs has been exclusively studied with regard to axon degeneration and regeneration (Busch and Silver, 2007; Galtrey and Fawcett, 2007; Harlow and Macklin, 2013; Rolls et al., 2009). However, the massive lack of intact myelin and the increasing inability of OPCs to remyelinate in the context of MS suggested, that CSPGs may also have a major impact on the biology of oligodendroglial cells. Along these lines, it has recently been shown that CSPGs affect OPC migration as well as their remyelination capability *in vivo* (Lau et al., 2012; Siebert et al., 2011). *In vitro* studies have further demonstrated that CSPGs mainly inhibit oligodendrocyte process extension and myelination in a Rho-dependent manner (Pendleton et al., 2013; Siebert and Osterhout, 2011). These data establish a prominent role of CSPGs for the morphological maturation of oligodendrocytes. However, the influence of CSPGs and their covalently attached CS-GAG side chains on the oligodendrocyte lineage progression still remained controversial. Using the monoclonal antibody 473HD, recognizing a unique CS-motif primarily present on the CSPG phosphacan/RPTPβ/ζ (Clement et al., 1998; Ito et al., 2005; Purushothaman et al., 2012), we showed that rodent OPCs as well as immature O4-positive oligodendrocytes express high levels of this epitope. Yet, the expression declines with ongoing OPC differentiation towards myelin gene expressing oligodendrocytes. In line with this, we also noticed a decreasing mRNA expression of phosphacan/RPTPβ/ζ under differentiating conditions. Along these lines, the expression of its long receptor isoform by immature glial cells has been demonstrated in earlier studies (Dobbertin et al., 2003; Faissner et al., 1994).

In addition to Phosphacan/RPTPβ/ζ immature oligodendrocytes express the NG2 proteoglycan *in vivo* and *in*

vitro (Levine et al., 2001; Nishiyama et al., 2009; Sakry et al., 2011; Stallcup and Beasley, 1987; Trotter et al., 2010). The fact that such CSPGs are exclusively expressed in the early oligodendrocyte lineage suggests that they may restrict OPC differentiation. To address this hypothesis, we designed a combinatorial experimental setup in which rat OPCs were plated either on a control substrate or on a PG-enriched substrate in the presence and absence of ChABC. We demonstrate that CS-GAG removal accelerates rat OPC differentiation, while an overrepresentation of affinity purified proteoglycans attenuates differentiation towards MBP-positive oligodendrocytes. The latter effect could be rescued by ChABC. This indicates that CSPGs act on OPCs primarily via their CS-GAG side chains rather than via their core proteins. Moreover, it appears as if CS-GAGs are necessary for maintaining OPCs in an immature state, since ChABC treatment also results in an enhanced spontaneous differentiation towards O4-positive cells already under proliferating conditions. We also observed an enhanced oligodendrocyte differentiation from mouse spinal cord NPCs, which is consistent with a previous report demonstrating an increased oligodendrocyte differentiation from rat forebrain NPCs upon CS-GAG removal (Gu et al., 2009). Therefore, our data strongly support the idea that CSPGs are important regulators of the oligodendrocyte lineage progression. Yet, a recent study reported that the CSPG Aggrecan does not interfere with the oligodendrocyte lineage progression (Pendleton et al., 2013). Importantly, our PG-substrate was mainly composed of Phosphacan/RPTPβ/ζ, Brevican, and NG2, and we documented an inhibitory effect of such PGs on the number of MBP-positive cells under differentiating conditions. Thus, it is tempting to speculate that different CSPGs may differentially affect oligodendrocyte differentiation. In this context, we observed a trend towards an increased number of MBP-positive oligodendrocytes upon siRNA-mediated *Ptprz1* knock-down in rodent OPCs. Although this observation still needs more experimental support, it already indicates that Phosphacan/RPTPβ/ζ contributes to a CS-GAG-dependent oligodendrocyte lineage progression. Moreover, a similar effect has already been documented for primary human OPCs (Sim et al., 2006). In addition, the fact that functional regeneration after demyelinating lesions is affected in Ptprz1 knock-out mice (Harroch et al., 2002) demonstrates the importance of Phosphacan/RPTPβ/ζ for oligodendrocyte biology.

Yet, a key question is: How do CSPGs in general and Phosphacan/RPTPβ/ζ in particular regulate OPC maintenance? CSPGs are known to bind different growth factors or cytokines such as FGF2, PDGF-AA, and pleiotrophin (Goretzki et al., 1999; Iozzo and Schaefer, 2015; Maeda et al., 1999; Milev et al., 1998; Purushothaman et al., 2012; Sugahara and Mikami, 2007). Signalling via the pleiotrophin-RPTPβ/ζ axis regulates self-renewal of human fetal OPCs (McClain et al., 2012). It is conceivable that the enzymatic degradation of the CS-GAGs might have altered the mitogenic activity of growth factors in our culture system, since all are potent mitogens for OPCs (Bogler et al., 1990; Noble et al., 1990; Pringle et al., 1989). Along these lines, we have recently documented a reduced FGF2 signalling accompanied by an impaired proliferation of telencephalic NPCs after CS-GAG removal (Sirko et al., 2010b). Nevertheless, CS-GAG removal in primary rat OPC cultures did not affect the proliferation rates based on BrdU incorporation analysis. But we cannot formally exclude that the overrepresentation of growth factors (i.e., FGF2 and PDGF-AA) in our primary oligodendrocyte cultures might have masked any effect on proliferation.

In line with the current view that CSPGs inhibit the morphological maturation of oligodendrocytes, we observed a massive increase of myelin membrane formation upon ChABC treatment. Although this effect was clearly visible in all culture systems used, it was particularly pronounced in cultures of hiPSC-derived oligodendrocytes. It is known, that CSPGs signal through RPTPσ in oligodendrocytes and act on their morphology in a Rho-dependent manner (Pendleton et al., 2013; Siebert and Osterhout, 2011). Interestingly, we recently identified the Rho-GEF Vav3 as an intracellular downstream component of CS-GAG signalling (unpublished data). Vav3 regulates the activity of small GTPases such as RhoA and RhoG (Movilla and Bustelo, 1999). Therefore, Vav3 might be an interesting candidate for CSPG mediated signaling in oligodendrocytes. Vav3 has been shown to be expressed by NPCs (Pollard et al., 2008), suggesting key functions of Vav3 for neural development. Along these lines recent studies demonstrated a crucial role of Vav3 for cerebellar (Quevedo et al., 2010) and retinal (Luft et al., 2015) development.

Our data not only support recent observations concerning the inhibitory effect of CSPGs on the morphological maturation, but also qualify CSPGs as important regulators of the oligodendrocyte lineage progression. Moreover, we propose that CSPGs orchestrate oligodendrocyte differentiation in a universal manner, because we did neither observe species-specific (rat, mouse, human) nor region-specific (forebrain, spinal cord) differences. In the future, it would be interesting to analyse, whether the beneficial effect of ChABC-injection observed in various animal models (Galtrey et al., 2007; Garcia-Alias et al., 2009; Laabs et al., 2007) is accompanied by an increased oligodendrocyte differentiation and myelination. An initial study has already demonstrated that ChABC-injection into the damaged area after a spinal cord contusion injury not only ameliorated axonal regeneration but also stimulated the migration of endogenous OPCs towards the

lesion site (Siebert et al., 2011). Moreover, pharmacological interference with CS-GAG synthesis fosters remyelination after lysolecithin-induced demyelination (Lau et al., 2012). In conjunction with our observation in cultures of hiPSC-derived oligodendrocytes, these studies suggest that CSPGs may represent promising pharmacological targets for enhancing white matter repair.

Acknowledgment

Grant sponsor: Research School at the Ruhr-University (DFG); Grant number: GSC 98/1; Grant sponsor: German Research Foundation (DFG); Grant numbers: SPP-1757, Fa 159/20-1; Grant sponsor: German Ministry of Education, Research and Technology; Grant number: BMBF 01GN0503; Grant sponsor: California Institute of Regenerative Medicine and the BMBF; Grant numbers: 0316020, 1316020; Grant sponsors: PhD program of the International Graduate School of Neuroscience (IGSN); Wilhelm and Günther Esser Foundation; the Hertie Foundation; the Ruhr-University.

The authors thank Prof. Jörn Oliver Sass (Childrens' Hospital, Zurich) for kindly providing Canavan Disease patient fibroblasts for the generation of the respective hiPSC lines and their neural derivatives.

References

Barnett SC, Linington C. 2013. Myelination: do astrocytes play a role? Neuroscientist 19:442–450.

Bogler O, Wren D, Barnett SC, Land H, Noble M. 1990. Cooperation between two growth factors promotes extended self-renewal and inhibits differentiation of oligodendrocyte-type-2 astrocyte (O-2A) progenitor cells. Proc Natl Acad Sci USA 87:6368–6372.

Busch SA, Silver J. 2007. The role of extracellular matrix in CNS regeneration. Curr Opin Neurobiol 17:120–127.

Camand E, Morel MP, Faissner A, Sotelo C, Dusart I. 2004. Long-term changes in the molecular composition of the glial scar and progressive increase of serotoninergic fibre sprouting after hemisection of the mouse spinal cord. Eur J Neurosci 20:1161–1176.

Canoll PD, Petanceska S, Schlessinger J, Musacchio JM. 1996. Three forms of RPTP-beta are differentially expressed during gliogenesis in the developing rat brain and during glial cell differentiation in culture. J Neurosci Res 44: 199–215.

Chen J, Joon Lee H, Jakovcevski I, Shah R, Bhagat N, Loers G, Liu HY, Meiners S, Taschenberger G, Kugler S, Irintchev A, Schachner M. 2010. The extracellular matrix glycoprotein Tenascin-C is beneficial for spinal cord regeneration. Mol Ther 18:1769–1777.

Clement AM, Nadanaka S, Masayama K, Mandl C, Sugahara K, Faissner A. 1998. The DSD-1 carbohydrate epitope depends on sulfation, correlates with chondroitin sulfate D motifs, and is sufficient to promote neurite outgrowth. J Biol Chem 273:28444–28453.

Czopka T, Von Holst A, Schmidt G, Ffrench-Constant C, Faissner A. 2009. Tenascin C and tenascin R similarly prevent the formation of myelin membranes in a RhoA-dependent manner, but antagonistically regulate the expression of myelin basic protein via a separate pathway. Glia 57:1790–1801.

Dickendesher TL, Baldwin KT, Mironova YA, Koriyama Y, Raiker SJ, Askew KL, Wood A, Geoffroy CG, Zheng B, Liepmann CD, Katagiri Y, Benowitz H, Geller HM, Giger RJ. 2012. NgR1 and NgR3 are receptors for chondroitin sulfate proteoglycans. Nat Neurosci 15:703–712.

Dobbertin A, Rhodes KE, Garwood J, Properzi F, Heck N, Rogers JH, Fawcett JW, Faissner A. 2003. Regulation of RPTPbeta/phosphacan expression and glycosaminoglycan epitopes in injured brain and cytokine-treated glia. Mol Cell Neurosci 24:951–971.

Faissner A, Clement A, Lochter A, Streit A, Mandl C, Schachner M. 1994. Isolation of a neural chondroitin sulfate proteoglycan with neurite outgrowth promoting properties. J Cell Biol 126:783–799.

Faissner A, Kruse J. 1990. J1/tenascin is a repulsive substrate for central nervous system neurons. Neuron 5:627–637.

Faissner A, Reinhard J. 2015. The extracellular matrix compartment of neural stem and glial progenitor cells. Glia 63:1330–1349.

Fidler PS, Schuette K, Asher RA, Dobbertin A, Thornton SR, Calle-Patino Y, Muir E, Levine JM, Geller HM, Rogers JH, Faissner A, Fawcett J. 1999. Comparing astrocytic cell lines that are inhibitory or permissive for axon growth: The major axon-inhibitory proteoglycan is NG2. J Neurosci 19:8778–8788.

Fisher D, Xing B, Dill J, Li H, Hoang HH, Zhao Z, Yang XL, Bachoo R, Cannon S, Longo FM, Sheng M, Silver J, Li S. 2011. Leukocyte common antigen-related phosphatase is a functional receptor for chondroitin sulfate proteoglycan axon growth inhibitors. J Neurosci 31:14051–14066.

Fitch MT, Silver J. 2008. CNS injury, glial scars, and inflammation: Inhibitory extracellular matrices and regeneration failure. Exp Neurol 209:294–301.

Franklin RJ, Ffrench-Constant C. 2008. Remyelination in the CNS: From biology to therapy. Nat Rev Neurosci 9:839–855.

Galtrey CM, Asher RA, Nothias F, Fawcett JW. 2007. Promoting plasticity in the spinal cord with chondroitinase improves functional recovery after peripheral nerve repair. Brain 130:926–939.

Galtrey CM, Fawcett JW. 2007. The role of chondroitin sulfate proteoglycans in regeneration and plasticity in the central nervous system. Brain Res Rev 54: 1–18.

Garcia-Alias G, Barkhuysen S, Buckle M, Fawcett JW. 2009. Chondroitinase ABC treatment opens a window of opportunity for task-specific rehabilitation. Nat Neurosci 12:1145–1151.

Garwood J, Schnadelbach O, Clement A, Schutte K, Bach A, Faissner A. 1999. DSD-1-proteoglycan is the mouse homolog of phosphacan and displays opposing effects on neurite outgrowth dependent on neuronal lineage. J Neurosci 19:3888–3899.

Gervasi NM, Kwok JC, Fawcett JW. 2008. Role of extracellular factors in axon regeneration in the CNS: Implications for therapy. Regen Med 3:907–923.

Goretzki L, Burg MA, Grako KA, Stallcup WB. 1999. High-affinity binding of basic fibroblast growth factor and platelet-derived growth factor-AA to the core protein of the NG2 proteoglycan. J Biol Chem 274:16831–16837.

Goritz C, Dias DO, Tomilin N, Barbacid M, Shupliakov O, Frisen J. 2011. A pericyte origin of spinal cord scar tissue. Science 333:238–242.

Gorris R, Fischer J, Erwes KL, Kesavan J, Peterson DA, Alexander M, Nothen MM, Peitz M, Quandel T, Karus M, Brustle O. 2015. Pluripotent stem cell-derived radial glia-like cells as stable intermediate for efficient generation of human oligodendrocytes. Glia. doi: 10.1002/glia.22882. [Epub ahead of print].

Grossman SD, Rosenberg LJ, Wrathall JR. 2001. Temporal-spatial pattern of acute neuronal and glial loss after spinal cord contusion. Exp Neurol 168: 273–282.

Gu WL, Fu SL, Wang YX, Li Y, Lu HZ, Xu XM, Lu PH. 2009. Chondroitin sulfate proteoglycans regulate the growth, differentiation and migration of multipotent neural precursor cells through the integrin signaling pathway. BMC Neurosci 10:128.

Harlow DE, Macklin WB. 2013. Inhibitors of myelination: ECM changes, CSPGs and PTPs. Exp Neurol 251:39–46.

Harroch S, Furtado GC, Brueck W, Rosenbluth J, Lafaille J, Chao M, Buxbaum JD, Schlessinger J. 2002. A critical role for the protein tyrosine phosphatase receptor type Z in functional recovery from demyelinating lesions. Nat Genet 32:411–414.

Hu BY, Du ZW, Li XJ, Ayala M, Zhang SC. 2009. Human oligodendrocytes from embryonic stem cells: Conserved SHH signaling networks and divergent FGF effects. Development 136:1443–1452.

Iozzo RV, Schaefer L. 2015. Proteoglycan form and function: A comprehensive nomenclature of proteoglycans. Matrix Biol 42:11–55.

Ito Y, Hikino M, Yajima Y, Mikami T, Sirko S, von Holst A, Faissner A, Fukui S, Sugahara K. 2005. Structural characterization of the epitopes of the monoclonal antibodies 473HD, CS-56, and MO-225 specific for chondroitin sulfate D-type using the oligosaccharide library. Glycobiology 15:593–603.

Jung M, Kramer E, Grzenkowski M, Tang K, Blakemore W, Aguzzi A, Khazaie K, Chlichlia K, von Blankenfeld G, Kettenmann H, Trotter J. 1995. Lines of murine oligodendroglial precursor cells immortalized by an activated neu tyrosine kinase show distinct degrees of interaction with axons in vitro and in vivo. Eur J Neurosci 7:1245–1265.

Karus M, Denecke B, ffrench-Constant C, Wiese S, Faissner A. 2011. The extracellular matrix molecule tenascin C modulates expression levels and territories of key patterning genes during spinal cord astrocyte specification. Development 138:5321–5331.

Karus M, Samtleben S, Busse C, Tsai T, Dietzel ID, Faissner A, Wiese S. 2012. Normal sulfation levels regulate spinal cord neural precursor cell proliferation and differentiation. Neural Dev 7:20.

Kuboyama K, Fujikawa A, Masumura M, Suzuki R, Matsumoto M, Noda M. 2012. Protein tyrosine phosphatase receptor type z negatively regulates oligodendrocyte differentiation and myelination. PLoS One 7:e48797.

Laabs TL, Wang H, Katagiri Y, McCann T, Fawcett JW, Geller HM. 2007. Inhibiting glycosaminoglycan chain polymerization decreases the inhibitory activity of astrocyte-derived chondroitin sulfate proteoglycans. J Neurosci 27: 14494–14501.

Lamprianou S, Chatzopoulou E, Thomas JL, Bouyain S, Harroch S. 2011. A complex between contactin-1 and the protein tyrosine phosphatase PTPRZ controls the development of oligodendrocyte precursor cells. Proc Natl Acad Sci USA 108:17498–17503.

Lau LW, Keough MB, Haylock-Jacobs S, Cua R, Doring A, Sloka S, Stirling DP, Rivest S, Yong VW. 2012. Chondroitin sulfate proteoglycans in demyelinated lesions impair remyelination. Ann Neurol 72:419–432.

Levine JM, Reynolds R, Fawcett JW. 2001. The oligodendrocyte precursor cell in health and disease. Trends Neurosci 24:39–47.

Lin R, Rosahl TW, Whiting PJ, Fawcett JW, Kwok JC. 2011. 6-sulphated chondroitins have a positive influence on axonal regeneration. PLoS One 6: e21499.

Luft V, Reinhard J, Shibuya M, Fischer KD, Faissner A. 2015. The guanine nucleotide exchange factor Vav3 regulates differentiation of progenitor cells in the developing mouse retina. Cell Tissue Res 359:423–440.

Lytle JM, Wrathall JR. 2007. Glial cell loss, proliferation and replacement in the contused murine spinal cord. Eur J Neurosci 25:1711–1724.

Maeda N. 2015. Proteoglycans and neuronal migration in the cerebral cortex during development and disease. Front Neurosci 9:98.

Maeda N, Ichihara-Tanaka K, Kimura T, Kadomatsu K, Muramatsu T, Noda M. 1999. A receptor-like protein-tyrosine phosphatase PTPzeta/RPTPbeta binds a heparin-binding growth factor midkine. Involvement of arginine 78 of midkine in the high affinity binding to PTPzeta. J Biol Chem 274:12474–12479.

McClain CR, Sim FJ, Goldman SA. 2012. Pleiotrophin suppression of receptor protein tyrosine phosphatase-beta/zeta maintains the self-renewal competence of fetal human oligodendrocyte progenitor cells. J Neurosci 32:15066–15075.

Milev P, Monnerie H, Popp S, Margolis RK, Margolis RU. 1998. The core protein of the chondroitin sulfate proteoglycan phosphacan is a high-affinity ligand of fibroblast growth factor-2 and potentiates its mitogenic activity. J Biol Chem 273:21439–21442.

Milner R, Ffrench-Constant C. 1994. A developmental analysis of oligodendroglial integrins in primary cells: Changes in alpha v-associated beta subunits during differentiation. Development 120:3497–3506.

Morgenstern DA, Asher RA, Naidu M, Carlstedt T, Levine JM, Fawcett JW. 2003. Expression and glycanation of the NG2 proteoglycan in developing, adult, and damaged peripheral nerve. Mol Cell Neurosci 24:787–802.

Movilla N, Bustelo XR. 1999. Biological and regulatory properties of Vav-3, a new member of the Vav family of oncoproteins. Mol Cell Biol 19:7870–7885.

Nishiyama A, Komitova M, Suzuki R, Zhu X. 2009. Polydendrocytes (NG2 cells): Multifunctional cells with lineage plasticity. Nat Rev Neurosci 10:9–22.

Noble M, Barnett SC, Bogler O, Land H, Wolswijk G, Wren D. 1990. Control of division and differentiation in oligodendrocyte-type-2 astrocyte progenitor cells. Ciba Found Symp 150:227−243; discussion 244–249.

Pedraza CE, Monk R, Lei J, Hao Q, Macklin WB. 2008. Production, characterization, and efficient transfection of highly pure oligodendrocyte precursor cultures from mouse embryonic neural progenitors. Glia 56:1339–1352.

Pendleton JC, Shamblott MJ, Gary DS, Belegu V, Hurtado A, Malone ML, McDonald JW. 2013. Chondroitin sulfate proteoglycans inhibit oligodendrocyte myelination through PTPsigma. Exp Neurol 247:113–121.

Pollard SM, Wallbank R, Tomlinson S, Grotewold L, Smith A. 2008. Fibroblast growth factor induces a neural stem cell phenotype in foetal forebrain progenitors and during embryonic stem cell differentiation. Mol Cell Neurosci 38:393–403.

Pringle N, Collarini EJ, Mosley MJ, Heldin CH, Westermark B, Richardson WD. 1989. PDGF A chain homodimers drive proliferation of bipotential (O-2A) glial progenitor cells in the developing rat optic nerve. EMBO J 8:1049–1056.

Purushothaman A, Sugahara K, Faissner A. 2012. Chondroitin sulfate "wobble motifs" modulate maintenance and differentiation of neural stem cells and their progeny. J Biol Chem 287:2935–2942.

Quevedo C, Sauzeau V, Menacho-Marquez M, Castro-Castro A, Bustelo XR. 2010. Vav3-deficient mice exhibit a transient delay in cerebellar development. Mol Biol Cell 21:1125–1139.

Ranjan M, Hudson LD. 1996. Regulation of tyrosine phosphorylation and protein tyrosine phosphatases during oligodendrocyte differentiation. Mol Cell Neurosci 7:404–418.

Rolls A, Shechter R, Schwartz M. 2009. The bright side of the glial scar in CNS repair. Nat Rev Neurosci 10:235–241.

Sakry D, Karram K, Trotter J. 2011. Synapses between NG2 glia and Neurons. J Anat 219:2–7.

Schnadelbach O, Mandl C, Faissner A. 1998. Expression of DSD-1-PG in primary neural and glial-derived cell line cultures, upregulation by TGF-beta, and implications for cell-substrate interactions of the glial cell line Oli-neu. Glia 23:99–119.

Shen Y, Tenney AP, Busch SA, Horn KP, Cuascut FX, Liu K, He Z, Silver J, Flanagan JG. 2009. PTPsigma is a receptor for chondroitin sulfate proteoglycan, an inhibitor of neural regeneration. Science 326:592–596.

Siebert JR, Osterhout DJ. 2011. The inhibitory effects of chondroitin sulfate proteoglycans on oligodendrocytes. J Neurochem 119:176–188.

Siebert JR, Stelzner DJ, Osterhout DJ. 2011. Chondroitinase treatment following spinal contusion injury increases migration of oligodendrocyte progenitor cells. Exp Neurol 231:19–29.

Sim FJ, Lang JK, Waldau B, Roy NS, Schwartz TE, Pilcher WH, Chandross KJ, Natesan S, Merrill JE, Goldman SA. 2006. Complementary patterns of gene expression by human oligodendrocyte progenitors and their environment predict determinants of progenitor maintenance and differentiation. Ann Neurol 59:763–779.

Sirko S, Akita K, Von Holst A, Faissner A. 2010a. Structural and functional analysis of chondroitin sulfate proteoglycans in the neural stem cell niche. Methods Enzymol 479:37–71.

Sirko S, von Holst A, Weber A, Wizenmann A, Theocharidis U, Gotz M, Faissner A. 2010b. Chondroitin sulfates are required for fibroblast growth factor-2-dependent proliferation and maintenance in neural stem cells and

for epidermal growth factor-dependent migration of their progeny. Stem Cells 28:775–787.

Sommer I, Schachner M. 1981. Monoclonal antibodies (O1 to O4) to oligodendrocyte cell surfaces: An immunocytological study in the central nervous system. Dev Biol 83:311–327.

Stallcup WB, Beasley L. 1987. Bipotential glial precursor cells of the optic nerve express the NG2 proteoglycan. J Neurosci 7:2737–2744.

Sugahara K, Mikami T. 2007. Chondroitin/dermatan sulfate in the central nervous system. Curr Opin Struct Biol 17:536–545.

Trotter J, Karram K, Nishiyama A. 2010. NG2 cells: Properties, progeny and origin. Brain Res Rev 63:72–82.

Wang D, Ichiyama RM, Zhao R, Andrews MR, Fawcett JW. 2011. Chondroitinase combined with rehabilitation promotes recovery of forelimb function in rats with chronic spinal cord injury. J Neurosci 31:9332–9344.