SHORT COMMUNICATION

Full-Length and Fragmented Netrin-1 in Multiple Sclerosis Plaques Are Inhibitors of Oligodendrocyte Precursor Cell Migration

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Oligodendrocytes exhibit a limited capacity to remyelinate in multiple sclerosis. Factors present in multiple sclerosis lesions are thought to inhibit oligodendrocyte precursor cell migration, limiting their recruitment to axons requiring remyelination; however, few inhibitors have been identified. A candidate inhibitor is netrin-1, a secreted protein that repels migrating oligodendrocyte precursor cells during neural development and is expressed by myelinating oligodendrocytes in the mature rodent central nervous system. Herein, we examined the distribution of netrin-1 in adult human white matter and multiple sclerosis lesions. We detected full-length netrin-1 protein and shorter netrin-1 fragments in samples of normal white matter and of multiple sclerosis lesions from adult human brain. We demonstrate that peptides corresponding to amino terminal domains VI and V of netrin-1 repel migrating oligodendrocyte precursor cells, but lack the chemoattractant activity of full-length netrin-1. Furthermore, recombinant domains VI-V of netrin-1 disrupt the chemoattractant activity of full-length netrin-1, consistent with a competitive mechanism of action. These findings indicate that full-length and fragmented forms of netrin-1, found in multiple sclerosis lesions, have the capacity to inhibit oligodendrocyte precursor migration, identifying netrin-1 as a potential target for therapies that promote remyelination. (*Am J Pathol* 2013; 183: 673–680; <http://dx.doi.org/10.1016/j.ajpath.2013.06.004>)

Netrin-1 is a secreted protein that regulates cell migration, process extension, and cell-cell adhesion during neural development.¹ Oligodendrocytes, the myelinating glia of the central nervous system (CNS), respond to netrin-1 during several stages of their development. In the embryonic spinal cord, netrin-1 signals through its receptors deleted in colorectal cancer (DCC) and the Unc5 homologues² to repel migrating oligodendrocyte precursor cells (OPCs) away from the ventral ventricular zone.^{3–5} As oligodendrocytes mature, netrin-1 promotes the extension and elaboration of processes⁶ via DCC-dependent activation of fyn and inhibition of RhoA.⁷ In the mature CNS, netrin-1 promotes the maintenance of axo-oligodendroglial paranodal junctions made by myelinating oligodendrocytes.^{8,9} Although the roles for netrin-1 in the rodent CNS are well established, only a few studies have addressed the functional significance of netrin-1 in the human CNS. Herein, we assess the

distribution of netrin-1 protein associated with intact white matter and multiple sclerosis (MS) lesions in the adult human brain.

MS is characterized by multifocal demyelination and axonal loss. Although remyelination does occur in MS, the extent is often limited.^{10,11} Remyelination of MS lesions is attributed to differentiation of endogenous OPCs, rather

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than previously myelinating oligodendrocytes.^{12,13} Local factors in MS lesions that inhibit OPC recruitment or their subsequent differentiation into oligodendrocytes are implicated in contributing to the inability of OPCs to remyelinate efficiently.^{14,15} Previous studies have identified inhibitors of OPC migration during remyelination, including the chemo-repellent guidance cue, Semaphorin-3A; however, the influence of inhibitors of OPC migration on remyelination is not well understood.^{16,17} The role of netrin-1 as a repellent for migrating OPCs during development^{3–5} and its expression by neurons and glia in the mature CNS⁹ suggest that it might also influence OPC migration and remyelination in MS.

Herein, we report readily detectable levels of netrin-1 protein in human adult normal control white matter (NCWM) and in chronic MS lesions. In addition to full-length netrin-1 protein, fragments of netrin-1 were detected in both types of tissue. We show that fragments containing the amino terminal domain VI of netrin-1 effectively repel migrating OPCs, but have lost chemoattractant function. These findings provide evidence that full-length and fragmented forms of netrin-1 protein present in MS lesions may impede remyelination by inhibiting OPC migration, thereby limiting the access of these cells to demyelinated axons.

Materials and Methods

Animals

Sprague-Dawley rat pups were obtained from Charles River Canada (Senneville, QC, Canada). All procedures were performed in accordance with the Canadian Council on Animal Care guidelines.

Rodent Oligodendrocyte Culture

OPCs isolated from mixed glial cultures derived from the cerebral cortices of postnatal day 0 rat pups were grown in oligodendrocyte-defined medium, as previously described.^{3,18}

Human Adult Oligodendrocyte Culture

Tissue was obtained from surgical resections performed to treat non-tumor-related intractable epilepsy in accordance with the Biomedical Ethics Unit of McGill University (Montreal, QC, Canada) guidelines. Mature oligodendrocytes were isolated as previously described,¹⁹ and 10⁵ cells per well were plated in 16-well poly-L-lysine-coated glass-chamber slides (Nalge Nunc International, Naperville, IL) in minimal essential medium with 5% fetal calf serum, 1% penicillin-streptomycin, 1% glutamine, and 0.1% glucose. The enrichment of OPCs in these cultures has been previously characterized.²⁰

Generation of Truncated Netrin-1 VI-V and VI Protein

Truncated chicken netrin-1, containing domains VI and V with a c-terminal myc tag, was generated and purified, as

previously described.²¹ The netrin-1 domain VI was generated by PCR amplification of the N-terminal sequence of netrin-1, ending in YAVSDLQVGG, and cloning into the pFUSE-hFc1 expression vector (Invitrogen, CA), which encodes a c-terminal human Fc, to generate a netrin-1 domain VI–human Fc fusion protein. The cDNA was expressed in HEK293T cells and purified using protein A/G affinity beads (Santa Cruz Biotechnology, Santa Cruz, CA).

Antibodies and Immunocytochemistry

Primary antibodies used were rabbit polyclonal anti-platelet-derived growth factor receptor- α (C-20; Santa Cruz Biotechnology), rabbit polyclonal anti-netrin-1 PN2,⁹ mouse monoclonal anti-DCC (G97-449; BD Biosciences Pharmingen, San Diego, CA), rat monoclonal anti-netrin-1 (MAB 1109; R&D Systems, Minneapolis, MN), goat polyclonal anti-Unc5H2 (R&D Systems), rat monoclonal anti-myelin basic protein (Millipore, Billerica, MA), rabbit anti-myelin basic protein (Boehringer Mannheim, Mannheim, Germany), chicken polyclonal anti-myelin basic protein (Aves Labs, Tigard, OR), mouse anti-KiM1P (Dr. Heinz-Joachim Radzun, University of Göttingen, Göttingen, Germany), mouse anti-gial fibrillary acidic protein (Dako, Glostrup, Denmark), and mouse anti-NogoA [11c7; Dr. Martin E. Schwab (Brain Research Institute, University of Zürich, Zürich, Switzerland)]. For immunocytochemistry, cells were fixed with 2% paraformaldehyde and blocked in 3% bovine serum albumin and 0.3% Triton X-100 (Fisher Scientific, Ottawa, ON, Canada) for 1 hour at room temperature.

Netrin-1 Epitope Binding Site Mapping

Purified full-length netrin-1 and truncated netrin-1 proteins were separated using SDS-PAGE, transferred onto nitrocellulose, and then probed with netrin-1 antibodies. Truncated netrin-1 proteins are c-terminal deletions of different sizes, in some cases fused to a c-terminal Fc tag: netrin VI-V (sequence ending with QSRSPIAPCI), netrin VI-Fc (sequence ending in YAVSDLQVGG), netrin 1-188-Fc (sequence ending in YSTQCRKMYN), and netrin 1-167-Fc (sequence ending in PESMAIYKSM).

IHC Analysis of MS Lesions and NCWM

We investigated 13 MS lesions from 11 autopsy cases (Table 1) obtained from the UK Multiple Sclerosis Tissue Bank (Imperial College, London, UK), the Montreal Neurological Institute (McGill University), and University of Göttingen. All lesions were characterized by extensive demyelination and sparse inflammation. Remyelination, when present, was confined to the lesion borders. Control tissue was derived from three patients who underwent temporal or occipital lobe surgery due to pharmacoresistant seizures, and revealed unspecific changes, such as focal microglial activation and reactive astrogliosis. Appropriate

Table 1 Characteristics of Postmortem Human Brain Tissue Analyzed

Sample ID	No. of tissue blocks	MS or control	Age (years)	Sex	Disease duration (years)	Frozen or paraffin	Time between death/surgery and sample acquisition (hours)
Tissue Used for Immunohistochemistry							
MS1	1	SPMS	78	Female	14	Frozen	18
MS2	1	SPMS	74	Female	26	Frozen	19
MS3	1	MS	71	Female	41	Frozen	10
MS4	1	SPMS	58	Female	22	Frozen	16
MS5	1	SPMS	57	Female	18	Frozen	31
MS6	2	MS	Unknown	Unknown	Unknown	Paraffin	Unknown
MS7	1	PPMS	74	Female	Unknown	Paraffin	Unknown
MS8	1	SPMS	69	Male	7	Paraffin	Unknown
MS9	2	SPMS	63	Male	18	Paraffin	Unknown
MS10	1	SPMS	54	Male	11	Paraffin	Unknown
MS11	1	PPMS	45	Female	12	Paraffin	Unknown
Control 1	1	Control	43	Female	Not applicable	Paraffin	Unknown
Control 2	1	Control	25	Male	Not applicable	Paraffin	Unknown
Control 3	1	Control	64	Male	Not applicable	Paraffin	Unknown
Tissue Used for Western Blot Analysis							
MS12		SPMS	49	Female	11	Flash frozen	7
MS13		SPMS	62	Male	16	Flash frozen	28
MS14		SPMS	55	Female	16	Flash frozen	16
Control 1		Control	10	Male	Not applicable	Flash frozen	27
Control 2		Control	34	Male	Not applicable	Flash frozen	21

SPMS, secondary progressive MS; PPMS, primary progressive MS.

consent and ethical approvals were obtained in accordance with the regional ethics guidelines of each institute.

Sections (4 μ m thick) were stained with H&E and Luxol-fast blue. Immunohistochemical (IHC) staining was performed with an avidin-biotin technique. After deparaffinization, intrinsic peroxidase activity was blocked by incubation with 5% H₂O₂ in PBS for 20 minutes. Tissue was then blocked in 10% fetal calf serum in PBS for 25 minutes. Antigen retrieval by microwave pretreatment was performed for anti-KiMIP and anti-netrin-1.

Biochemistry of MS Lesions and NCWM

Postmortem brain samples were obtained from cases with MS or no CNS disease from the NeuroResource Tissue Bank (Institute of Neurology, London, UK) (Table 1). The studies were performed in accordance with the McGill University Health Centre research ethics board. MS plaques and NCWM samples were dissected from snap-frozen tissue blocks screened with oil-red-O and hematoxylin staining. Plaques that were hypocellular, with few or no oil-red-O–positive macrophages, were identified as chronic. Approximately 250 mm³ of tissue was homogenized in ice-cold radio-immunoprecipitation assay buffer [10 mmol/L sodium phosphate (pH 7.2), 150 mmol/L NaCl, 1% NP40, 0.1% SDS, and 0.5% deoxycholate] with protease inhibitors (phenyl-methylsulfonyl fluoride, aprotinin, leupeptin, and pepstatin), using a Caframo BDC 3030 stirrer (Caframo, Warton, ON, Canada) for 10 strokes at 900 rpm. Lysed samples were analyzed by SDS-PAGE and Western blot analysis.

OPC Migration Assays

OPC migration assays were performed as previously described.³ Briefly, 4×10^4 OPCs were plated on a 6.5-mm poly-D-lysine–coated transwell filter with 8- μ m pores (Corning, Tewksbury, MA). Recombinant proteins were added to the bottom of the chamber. After 16 hours at 37°C, cells were fixed with 4% paraformaldehyde and 0.1% glutaraldehyde. Cells that migrated to the bottom of the transfilter were counted by analysis of Hoechst-positive nuclei.

Cell Survival Assays

OPCs were treated for 6 hours, 10 hours after plating, and then fixed and stained for platelet-derived growth factor receptor- α and Hoechst. The number of surviving cells per well was counted and compared with the control condition.

Commissural Axon Outgrowth Assays

Commissural axon outgrowth was assayed from explants of embryonic day 13 rat spinal cord embedded in a three-dimensional collagen gel, as previously described.²²

DCC Dimerization Assays

HEK293T cells were seeded at 4.0×10^5 cells per well in a 6-well plate. Cells were cultured at 37°C for 24 hours and then cotransfected with 2 μ g of both DCC-HA–tagged and DCC-myc–tagged plasmids²³ using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA), according to the manufacturer's

instructions. Cells were treated with 300 ng/mL netrin-1 or 300 ng/mL netrin-1 domain VI-V protein for 5 minutes at 37°C, followed by lysis with ice-cold NP-40 buffer [50 mmol/L HEPES (pH 7.6), 150 mmol/L NaCl, 5 mmol/L EDTA, and 0.1% NP-40] with protease inhibitors (phenylmethylsulfonyl fluoride, aprotinin, and leupeptin). Co-immunoprecipitation was performed by incubating 1.5 μ g of goat anti-HA antibody (Abcam, Cambridge, MA) with whole cell lysates, and protein complexes were isolated using a 50% protein A/G agarose bead slurry (Calbiochem, Billerica, MA), followed by washing at 4°C in wash buffer [50 mmol/L HEPES (pH 7.6), 150 mmol/L NaCl, and 5 mmol/L EDTA] with protease inhibitors. Bound proteins were separated by SDS-PAGE, and the proteins of interest were detected by Western blot analysis using goat anti-HA (Abcam) and rabbit anti-myc (Abcam). Densitometry was performed using Adobe Photoshop CS4 software (San Jose, CA), as described.²⁴

Statistical Analysis

Data are presented as means $\pm$ SEM. Statistical significance was calculated using one-way analysis of variance, followed by Tukey's post hoc test ($\alpha = 0.05$).

Results

Netrin-1 in Human Adult White Matter and MS Lesions

To examine netrin-1 expression in human white matter, we first assessed whether human mature oligodendrocytes, similar to their rodent counterparts,⁹ express netrin-1 and netrin receptors. We found that adult human oligodendrocytes cultured *in vitro* express netrin-1, as well as the netrin-1 receptors, DCC and Unc5B (Figure 1, A–C). In sections of NCWM from adult human brain, IHC staining detected netrin-1 protein associated with oligodendrocyte and astrocyte cell bodies (Figure 1, D, G, and H). We then examined the distribution of netrin-1 protein in chronic demyelinated lesions from 11 postmortem MS cases (Table 1). Similar to NCWM, oligodendrocyte and astrocyte cell bodies were netrin-1 positive in MS lesions and periplaque white matter (Figure 1E). We also detected netrin-1-immunopositive macrophages (Figure 1F) and increased diffuse staining for netrin-1 associated with the extracellular matrix of lesions.

Fragments of Netrin-1 Are Detected in Adult White Matter and MS Lesions

Western blot analyses of protein lysates confirmed the presence of full-length netrin-1 (approximately 75 kDa) in both NCWM and MS lesions (Figure 2, A and B, and Table 1). In addition to the full-length protein, multiple netrin-1-immunoreactive bands ranging between approximately 30 to 50 kDa were detected. Full-length netrin-1 is

composed of two N-terminal domains homologous to the N-terminal domains VI and V of laminins, and of a distinct C-terminal domain.²⁵ Two antibodies against netrin-1, raised against different epitopes in domains VI and V (as described in *Materials and Methods*; Figure 2, C–F), detected the same bands. No staining was detected in control blots using secondary antibodies alone, indicating that the immunopositive bands were specific to the primary antibodies.

Figure 1 Netrin-1 is expressed by human oligodendrocytes. **A–C:** Adult human oligodendrocytes grown in cell culture express netrin-1 (**A**), DCC (**B**), and Unc5 (**C**). **D** and **E:** Netrin-1 immunoreactivity is associated with oligodendrocytes (arrows) and astrocytes (arrowheads) in NCWM (**D**) and in MS lesions (MSLs; **E**). **F:** In MS lesions, macrophages (arrows) are also immunopositive for netrin-1. **G** and **H:** Costaining for NogoA (**G**) or glial fibrillary acidic protein (GFAP; **H**) in white matter identifies netrin-1-positive oligodendrocytes and astrocytes, respectively. Scale bars: 10 μ m (**A–C**, **G**, and **H**); 25 μ m (**D**); 50 μ m (**E** and **F**).

Figure 2 Full-length netrin-1 and truncated netrin-1 fragments present in NCWM and MS lesions. **A** and **B**: Western blot analyses of tissue homogenates from NCWM and MS lesions detect full-length netrin-1 (arrow), and fragmented netrin-1 protein, using antibodies directed against epitopes in netrin-1 domain VI (MAB 1109; **A**) and netrin-1 domain V (PN2; **B**). **C–F**: Mapping of the epitope binding specificity of the netrin-1 antibodies. **C**: Truncated netrin-1 constructs used to map the binding sites of the different netrin-1 antibodies using Western blot analysis (**D** and **E**). **F**: Depiction of the binding sites on netrin-1 for MAB1109 and PN2 antibodies.

Netrin-1 Fragments Containing Domain VI Repel Migrating OPCs

To determine whether fragments of netrin-1 could be functional, we purified two recombinant truncated netrin-1 proteins, the first composed of both domains VI and V (domain VI-V; approximately 48 kDa) and the second composed of only domain VI (domain VI; approximately 21 kDa). Similar to previous studies using full-length netrin-1,³ purified recombinant domain VI-V and domain VI proteins each exhibited potent repellent activity for rodent OPCs in a transfilter migration assay (Figure 3A). Application of domain VI-V or domain VI did not influence total OPC number, indicating that the reduced number of migrating cells was not an indirect result of OPC death (Figure 3B). We conclude that domain VI of netrin-1 is sufficient to activate a chemorepellent response by OPCs *in vitro*, indicating that fragments of netrin-1 *in vivo* may repel migrating cells and contribute to inhibiting the recruitment of OPCs to demyelinated MS plaques. Consistent with chronic lesions containing inhibitors of OPC migration, such as netrin-1, we confirmed that the MS plaques analyzed contained substantially fewer NogoA-positive oligodendrocytes compared with the surrounding white matter (Figure 3C).

Fragments Lacking the C-Domain of Netrin-1 Are Selectively Repellent

Although recombinant domain VI-V repelled migrating OPCs, it was unable to evoke commissural axon outgrowth of dorsal embryonic spinal cord, a standard assay of chemoattraction to netrin-1 (Figure 3D).²² Furthermore, co-application of netrin-1 with domain VI-V disrupted the

Figure 3 Full-length netrin-1 and netrin-1 fragments inhibit OPC migration. **A**: Migrating rodent OPCs are repelled by netrin-1 domain VI-V or domain VI in transfilter migration assays ($n = 3$). **B**: Netrin-1, domain VI-V, and domain VI did not influence survival of rodent OPCs ($n = 5$). **C**: Chronic MS lesions show fewer NogoA-positive oligodendrocytes compared with the surrounding white matter ($n = 10$). **D**: Full-length netrin-1 evoked significant axon outgrowth from E13 rat dorsal spinal cord explants compared with control explants cultured alone. Domain VI-V lacked chemoattractant axon outgrowth promoting activity and also blocked the chemoattractant activity of full-length netrin-1 when applied in combination ($n = 3$). **E**: Representative Western blot analysis illustrating co-immunoprecipitation analysis of HEK293T cells transfected with DCC-myc and DCC-HA constructs. Cells were stimulated for 5 minutes with either 300 ng/mL netrin-1 or domain VI-V. Increased amounts of DCC-myc co-immunoprecipitate with DCC-HA after treatment with netrin-1, compared with no treatment, consistent with DCC multimerization. In contrast, DCC multimerization did not increase after stimulation with VI-V. **F**: Quantification of multimerization data presented in **E** ($n = 4$). * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ compared with control, unless indicated.

chemoattractant activity of full-length netrin-1, consistent with a competitive interaction. We conclude that, in the absence of its C-terminal domain, truncated netrin-1 selectively retains the chemorepellent, but not chemoattractant, activity of full-length netrin-1.

It has previously been shown that domain VI-V binds both DCC and UNC5 receptors^{26,27}; however, in addition to binding to DCC, chemoattraction requires netrin-1-mediated multimerization of DCC receptors.²⁸ We hypothesized that, although domain VI-V binds DCC, it does not induce DCC multimerization, rendering it unable to activate downstream chemoattractive signaling. To test this, we co-expressed full-length DCC-myc and DCC-HA in HEK293T cells, followed by stimulation of these cells for 5 minutes with either netrin-1 or domain VI-V. Co-immunoprecipitation using antibodies against HA revealed increased amounts of DCC-myc associated with DCC-HA after application of full-length netrin-1 compared with no treatment, consistent with increased multimerization of DCC induced by netrin-1 (Figure 3, E and F). In contrast, application of domain VI-V did not increase DCC multimerization. We conclude that the netrin-1 C-domain is critical for DCC multimerization and that the inability of domain VI-V to multimerize DCC underlies the absence of chemoattractant activity.

Discussion

Our findings indicate that netrin-1 is present in human NCWM and MS lesions, associated with oligodendrocytes and astrocytes. In MS lesions, macrophages were also netrin-1 positive and the extracellular matrix exhibited an increased level of diffuse netrin-1 immunoreactivity. This distribution may result from the release of netrin-1 into the lesion environment after the destruction of myelin and the death of oligodendrocytes, where it could be phagocytosed by macrophages or accumulate in the extracellular matrix. Netrin-1 is also expressed by macrophages isolated from circulating peripheral blood mononuclear cells²⁹; thus, recruitment of these cells to MS lesions may contribute to the netrin-1 protein detected.

Many cell types, including OPCs and mature oligodendrocytes, express both DCC and Unc5 homologue receptors, which form a netrin-1 receptor complex that mediates chemorepellent responses to netrin-1.^{30–32} Previous studies indicated that OPCs are repelled by a gradient of netrin-1, migrating away from high concentrations of netrin-1 *in vitro* and away from the floor plate in the embryonic spinal cord *in vivo*.^{3,5} In addition, these *in vitro* studies demonstrated that challenging OPCs with a uniform concentration of netrin-1 inhibited OPC migration.³ This finding indicates that, in addition to the tropic effect of a gradient of netrin-1, netrin-1 also exerts a kinetic effect on OPCs, inhibiting their rate of migration in the absence of a gradient.³ Together, these findings suggest that even in the

absence of a well-organized graded distribution of netrin-1, as is likely the case in MS lesions, netrin-1 is predicted to inhibit OPC migration, thus limiting remyelination.

Although the presence of netrin-1 in lesions may have a negative impact on remyelination, it might play a positive anti-inflammatory role in patients with MS. Previous studies have shown netrin-1 to have anti-inflammatory functions in several conditions, including acute kidney injury, acute experimental colitis, corneal injury, peritonitis, and acute lung injury.^{29,33–40} Although the mechanism by which netrin-1 regulates inflammation remains largely unknown, roles in inhibiting immune cell infiltration^{34,36,38,39,41} and macrophage polarization³⁵ and reducing the levels of proinflammatory cytokines and chemokines^{29,42} have been reported. On the other hand, netrin-1 has also been shown to be proinflammatory in atherosclerotic plaques, where it inhibits emigration of macrophages from the lesions and promotes macrophage survival.^{43,44}

In addition to full-length netrin-1, we also detected fragments of netrin-1 in human NCWM and MS lesions. These fragments are not disease specific, because they were present in samples from both control individuals and patients with MS. We have previously documented a gradual increase in the expression of an approximately 50-kDa netrin-1 fragment during the postnatal maturation of the rat spinal cord.⁹ Notably, 50 kDa is approximately the predicted size of the N-terminal laminin-related domains VI and V of netrin-1 (428 amino acids, approximately 48 kDa). Consistent with this possibility, the two netrin-1 antibodies used bind to different epitopes in domains VI and V of netrin-1 (Figure 2D). An alternatively spliced isoform of netrin-1 that lacks part of the amino terminus of domain VI has recently been described in a small subset of tumor cell types⁴⁵; however, alternative splicing of netrin-1 mRNA that would result in the production of shorter netrin-1 isoforms has not been reported in the CNS or any other normal tissue. Based on our findings, we speculate that the smaller netrin-1 peptides detected are likely proteolytic fragments of full-length netrin-1 that include portions of domains VI and V.

To characterize the functionality of fragmented netrin-1, we generated and purified recombinant netrin-1 peptides corresponding to either domains VI and V or only domain VI. Both truncated proteins retained the capacity of full-length netrin-1 to repel migrating OPCs *in vitro*. We acknowledge that the truncated netrin-1 variants tested herein may not be identical to those present *in vivo*; however, our findings provide evidence that peptides present *in vivo* that include domain VI may repel migrating OPCs. This suggests that the accumulation of full-length and fragmented netrin-1 in the extracellular matrix of MS lesions will inhibit OPCs from migrating into regions of demyelination.

Previous studies have shown that recombinant protein composed of domains VI and V of netrin-1 is sufficient to bind the ectodomains of both DCC and UNC5.^{26,27} However, despite binding both receptors, we demonstrate that, although domain VI-V is sufficient to activate a chemorepellent

response, the chemoattractant activity of full-length netrin-1 has been lost. Multimerization of DCC is required for DCC-mediated chemoattraction.²⁸ We have shown that in the absence of the C-terminal domain, netrin-1 loses its ability to induce DCC multimerization. The C-domain has been shown to mediate dimerization of netrin-4, a netrin-1 paralogue that shares the same overall domain structure.⁴⁶ We hypothesize that the lack of the netrin-1 C-domain prevents netrin-1 dimerization and, consequently, efficient DCC multimerization, which underlies the loss of chemoattractant activity. In support of this, a previous study has shown that chemoattraction is unaffected when truncated netrin-1 domain VI-V protein is dimerized via fusion to a human Fc.²⁶ In addition to the loss of chemoattractant activity, domain VI-V protein blocked the capacity of netrin-1 to promote DCC-dependent chemoattractive axon outgrowth, suggesting that it competes with full-length netrin-1 for DCC binding sites. More important, this suggests that, in addition to repelling OPCs, netrin-1 fragments detected *in vivo* may also disrupt the function of intact full-length netrin-1.

Our findings show that full-length and fragmented netrin-1, an extracellular cue that directs myelin formation and maintenance,^{3,5,6,8} are present in adult human white matter, as well as in demyelinated MS lesions, where they are positioned to inhibit OPC migration. Notably, this inhibitory effect of netrin-1 may negatively affect remyelination in patients with MS because of the accumulation of netrin-1 associated with the extracellular matrix in lesions. Although netrin-1 and netrin-1 fragments may play a positive role in the mature CNS by restricting cell migration, axon growth, and sprouting,^{47–49} our findings indicate that they will inhibit the capacity of OPCs to access and repair demyelinated plaques in pathological circumstances. Thus, the development of strategies to block netrin-1 function holds promise for future treatment of demyelinating diseases, such as MS.

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