Available online at www.sciencedirect.com
ScienceDirect
Biochemical and Biophysical Research Communications 352 (2007) 158–163
BBRC
www.elsevier.com/locate/ybbrc

# Plexin-A4 is expressed in oligodendrocyte precursor cells and acts as a mediator of semaphorin signals

Atsumasa Okada, Mitsutoshi Tominaga, Makoto Horiuchi, Yasuhiro Tomooka *

*Department of Biological Science and Technology, and Tissue Engineering Research Center, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan*

Received 30 October 2006
Available online 7 November 2006

**Abstract**

Class 3 semaphorin acts as a guidance clue for both cell migration and nerve fiber projection. The signal of class 3 semaphorin travels via a receptor complex consisting of neuropilins and Plexin-A subfamily. Although it has been reported that class 3 semaphorin acts as a repellent for oligodendrocyte precursor cells (OPCs), which migrate actively during brain development, the expression of Plexin-A subfamily has not been reported in OPCs yet. Therefore, it is currently unclear how semaphorin signals can travel in OPCs. In the present study, the expression of Plexin-A4 (PlexA4) was first demonstrated in a newly established OPC line and OPCs in developing brain. In the OPC line, repulsion for process extension was caused by both Sema3A and Sema6A, and the effect of the semaphorins was diminished in cells expressing PlexA4 lacking the cytoplasmic domain. These results strongly suggest that PlexA4 expressed in OPCs acts as a mediator of semaphorin signals.


*Keywords:* Oligodendrocyte precursor cells; Plexin-A4; Semaphorin signal

ligodendrocytes (OLs) are myelinating cells of the central nervous system (CNS). OL precursor cells (OPCs) arise from restricted regions of the neuroepithelium and migrate at a relatively long distance to reach their axonal targets [1–12]. The mechanism regulating the migration of OPCs is poorly understood, although several molecules have been reported to be involved in neural cell migration in the CNS [7,8,10]. One of the molecules, semaphorin is a large family of secreted and membrane-bound proteins [13,14], and class 3 semaphorin among them has been well documented to act as a guidance clue for cell migration and nerve fiber projection [13]. Some studies have showed that class 3 semaphorin acts as a repellent or attractant for OPCs [15–17]. For example, Sema3A repels OPCs migration from explants of embryonic mouse optic nerve, while Sema3F attracts them from the tissues [15,16]. Sema3A, 3B, 3C, and 3F coated on dish repel OPCs prepared from neonatal rat brain [17].

The signal of class 3 semaphorins travels via a receptor complex consisting of neuropilins and plexin-A subfamily (PlexAs) [13,18]. In the signaling pathway, class 3 semaphorins bind to neuropilins, not to PlexAs, and the signal can travel via the cytoplasmic segment of PlexAs [18]. The expression of neuropilins has been reported in OPCs [15–17], but the expression of PlexAs has been not reported yet in OPCs. Some of PlexAs are expressed in human malignant glioma cells [19] and in cultured rat Schwann cells [20]. Therefore, it is currently unclear how semaphorin signals can travel in OPCs.

In the present study, the expression of PlexA4 mRNA was detected in an OPC line FBD-102b newly established in our laboratory from cerebral cortical regions of $p53^{-/-}$ fetal mice [21,22]. We reported herein the results of analysis of expression and function of PlexA4 in OPCs.

* Corresponding author. Fax: +81 4 7125 1841.
*E-mail address:* tomoylab@rs.noda.tus.ac.jp (Y. Tomooka).

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.10.176

## Materials and methods

*Animals*. CD-1 mice (Charles River Japan, Yokohama, Japan) were maintained in the experimental animal facility of Tokyo University of Science. Mice were caged under a 12:12 h light:dark cycle at 22–24 °C. Food (MR standard, Nousan, Yokohama, Japan) and water were provided *ad libitum*. Care and handling conformed to the NIH guidelines for animal research, and experimental protocols were approved by the Institutional Animal Care and Use Committee.

*Cells and cell culture*. FBD-102b is an OPC line [22]. FBD-102b cells were maintained in medium (1:1 mixture of DMEM and Ham's F-12 (DMEM/F12 medium) without phenol red (Sigma, St. Louis, MO) containing heat-inactivated fetal calf serum (FCS, Sigma) at 10% supplemented with penicillin (31 μg/ml) (Sigma), streptomycin (50 μg/ml) (Sigma) (10%FCS medium) at 37 °C, 5% $CO_2$. They were also cultured on poly-D-lysine (PDL) (Sigma) coated dishes or coverslips in DMEM/F12 medium containing FCS at 0.5% (0.5%FCS medium). FBD-104 cells were astrocyte progenitor cells [23] and cultured in 10%FCS medium supplemented with insulin (10 μg/ml) (Sigma), transferrin (10 μg/ml) (Sigma), sodium selenite ($10^{-8}$ M) (Sigma), and cholera toxin (10 ng/ml) (Sigma) (10%FCS+4F medium). A clonal cell line 2Y-3t was characterized as a neural progenitor line [24]. 2Y-3t cells were maintained in 10%FCS medium at 37 °C, 5% $CO_2$. They were also cultured on PDL coated dishes in DMEM/F12 medium plus 4 factors (SF+4F medium) at 37 °C, 5% $CO_2$.

*Northern blot analysis*. Procedure was described previously [25].

*In situ hybridization*. Procedure for single *in situ* hybridization has been described elsewhere [25]. For double *in situ* hybridization, Olig2 riboprobe was labeled with fluorescein (Roche, Mannheim, Germany), and PlexA4 riboprobe was labeled with Digoxigenin (Roche). Both probes were hybridized simultaneously overnight. Samples were incubated with anti-Digoxigenin AP-conjugated antibody, washed, and processed for colormetric detection using NBT/BCIP stock solution (Roche) to reveal PlexA4 mRNA signal in violet. AP activity was inhibited in 0.1 M glycine (Sigma). After the washing, the slides were incubated with anti-fluorescein AP-conjugated antibody (Roche) and developed in INT/BCIP stock solution (Roche) to develop Olig2 signals in reddish brown.

*Protein preparation and Western blot analysis*. Cells were suspended in 10 volumes of Hepes (25 mM, pH 7.4), $MgCl_2$ (10 mM), sucrose (250 mM), leupeptin (5 μg/ml), phenylmethane sulfonyl fluoride (0.1 mM), aprotinin (5 μg/ml) (isotonic buffer), and homogenized. The homogenate was centrifuged at 700*g* for 10 min. The pellet was resuspended in 10 volumes of isotonic buffer, homogenized, and the suspension was centrifuged 700*g* for 10 min. The supernatants were combined and centrifuged at 100,000*g* for 60 min. The pellet was resuspended in Hepes (20 mM, pH 7.4), 1% Triton X-100. The solubilized fractions were examined by Western blot analysis. Procedure for Western blot analysis has been described elsewhere [24]. Membranes were incubated with rabbit polyclonal anti-PlexA4 antibody (a generous gift from Dr. F. Nakamura) [26].

*Immnocytochemistry*. Procedure was described previously [24]. Briefly, cells were fixed in 2% paraformaldehyde (PFA), and incubated with primary antibodies. The primary antibodies were mouse monoclonal anti-O4 (CHEMICON, Temecula, CA), rabbit polyclonal anti-NG2 (CHEMICON), Armenian hamster monoclonal anti-PlexA4 (a generous gift from Drs. H. Fujisawa and F. Suto), rabbit polyclonal anti-Neuropilin-1 (a generous gift from Drs. H. Fujisawa and F. Suto) and Armenian hamster monoclonal anti-Neuropilin-2 antibodies (a generous gift from Dr. T. Hirata). Cells were incubated with secondary antibodies.

*Primary culture*. Cells for primary culture were prepared from embryonic cerebral cortices. Cerebral cortices from 17-day-old embryos were dissociated in a mixed solution of 1× Trypsin–EDTA solution (Sigma) and 0.05% DNase I (Sigma) in phosphate-buffered saline (PBS). The dissociated cells were sieved through lens paper and cultured on PDL-coated dishes at a density of $1.5 \times 10^7$ cells/100 mm dish in 10%FCS medium supplemented with L-glutamine ($2.5 \times 10^{-3}$ M) (Sigma). After 5 days in culture, cells were passed with 1× Trypsin–EDTA solution in PBS and centrifuged. Cells were resuspended in DMEM/F12 medium supplemented with insulin (5 μg/ml), transferrin (100 μg/ml), progesterone ($2 \times 10^{-8}$ M), putrescine ($10^{-4}$ M), sodium selenite ($3 \times 10^{-8}$ M), thyroxine (T4) ($3 \times 10^{-8}$ M), tri-iodothyronine (T3) ($3 \times 10^{-8}$ M), L-glutamine ($2.5 \times 10^{-3}$ M), and incubated for 2 h at 37 °C in a $CO_2$ incubator at a density of $2.0 \times 10^6$ cells/ml. Cells were then resuspended in the same medium and cultured for 2–4 days at a density of $7.0 \times 10^4$ cells on a PDL-coated 15 mm coverslips, and then examined immunocytochemically as described above.

*Production of recombinant semaphorin proteins*. HEK293T cells were cultured in 60 mm dishes in 10%FCS medium until reaching 70–80% confluence. Opti-MEM I Reduced-Serum Medium (Invitrogen) was mixed with Lipofectamine™2000 Reagent (Invitrogen) and with pCAGGS-alkaline phosphatase (AP), pCAGGS-AP-Sema3A, pEF-AP-Fc or pEF-AP-Sema6Aect-Fc (a generous gift from Drs. H. Fujisawa and F. Suto), respectively, and incubated. The transfection solution was added to each dish. Three or four days after the start of transfection, the culture supernatant was collected and filtered with 0.22 μm filters, and then used as recombinant semaphorin.

*Expression of Plexin-A4ΔC in FBD-102b*. PlexA4 cDNA was deleted at the sequence encoding the cytoplasmic segment of the protein, and the mutant (PlexA4ΔC, a generous gift from Drs. H. Fujisawa and F. Suto) was ligated into the *Nhe*I and *Not*I sites of a mammalian expression vector pcDNA3.1/Hygro (Invitrogen). FBD-102b cells were grown in 60 mm dishes in 10%FCS medium until reaching 60–70% confluence. Opti-MEM I Reduced-Serum Medium was mixed with Lipofectamine™2000 Reagent and with PlexA4ΔC expression vector, and then incubated. The transfection solution was added to each dish. Twenty-four hours after the start of the transfection, the medium was removed and replaced with 10%FCS medium supplemented with 1 mg/ml Hygromycin (Wako). Approximately 2 weeks after transfection, Hygromycin-resistant colonies were selected.

*Analysis of process length of FBD-102b cells*. FBD-102 cells were cultured in 12-well culture dishes ($2.0 \times 10^4$ cells/well) in 10%FCS medium for 2 days, and AP-fused recombinant proteins (AP, AP-Sema3A, AP-Fc or AP-Sema6Aect-Fc) were added to the cultures at 37 °C. The images of the cells were captured using a Phase Contrast microcopy. The process lengths of FBD-102b cells were measured with Zeiss LSM Image Browser Version 3.1 (Carl Zeiss). The process was defined as the longer process than the major axis of each cell body. All values were presented as means ± SEM from at least three independent experiments. Statistical analyses were performed by the two-tailed Student's *t*-test and ANOVA with the Bonferroni/Dun post hoc test.

## Results

### *Expression of PlexA4 mRNA and protein in OPC line*

The expression of PlexA4 was examined by Northern blot analysis in neural cell lines established from brains of $p53^{-/-}$ mice (Fig. 1A). Among the neural cell lines,

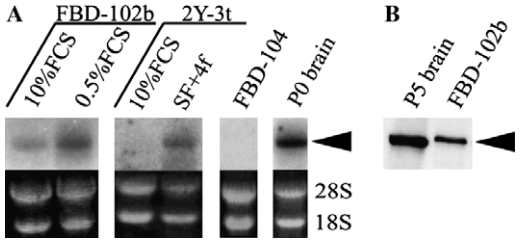


Fig. 1. Expression of PlexA4 mRNA and protein. (A) PlexA4 mRNA was expressed in neural cell lines; lanes from left to right corresponding to FBD-102b cells cultured in 10%FCS medium and 0.5%FCS medium, 2Y-3t cells cultured in 10%FCS medium and SF+4F medium, FBD-104 cells cultured in 10%FCS+4F medium and brains at P0 (positive control). (B) The expression of PlexA4 protein was detected in FBD-102b cells cultured in 10%FCS medium and brains at P5 (positive control).

PlexA4 mRNA was detected in neural progenitor cell line 2Y-3t and OPC line FBD-102b, and the expression was increased in SF+4F or 0.5%FCS medium, respectively. PlexA4 mRNA was undetected in astrocyte progenitor cell line FBD-104 (Fig. 1A). The expression of PlexA4 protein was detected in FBD-102b cells cultured in 10%FCS medium (Fig. 1B).

### *Expression of PlexA4 in cells in OL lineage in vivo*

Recent studies reported that cells in OL lineage in forebrain expressed basic-helix-loop-helix transcription factor Olig2 [27,28]. Therefore, Olig2 expressing cells were examined by *in situ* hybridization whether they co-express *PlexA4* (Fig. 2). In deep part of cerebral cortex, where cells in OL lineage are localized, signals of Olig2 mRNA were detected (Fig. 2A, C, and E), and signals of PlexA4 mRNA were also detected in the region (Fig. 2B, D, and F). In deeper layers of cerebral cortex at post-natal day 3 (P3), co-expression of *PlexA4* and *Olig2* became clearly detectable in a subset of the cells (Fig. 2G and H).

### *Expression of PlexA4 protein in OPCs in primary culture*

Expression of PlexA4 protein was immunocytochemically examined in primary culture of cerebral cortex (Fig. 3). OPCs and OLs were identified by expression of NG2 or O4 antigen and O1 antigen or myelin basic protein (MBP), respectively. The expression of PlexA4 protein was detected in NG2-positive cells (Fig. 3A–C) and in O4 antigen-positive cells (Fig. 3D–F). Expression of PlexA4 was recognized at 10–20% of NG2-positive cells and at 20–30% of O4 antigen-positive cells. No expressions of PlexA4 were detected in either O1 antigen- or MBP-positive cells (data not shown).

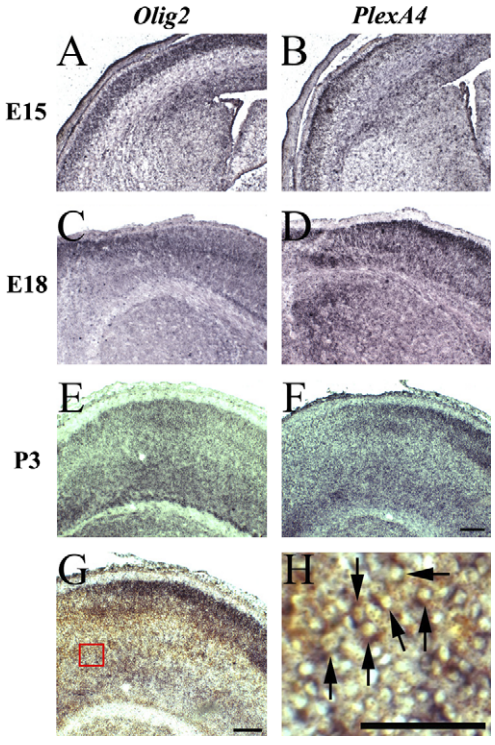


Fig. 2. *In situ* hybridization of Olig2 mRNA and PlexA4 mRNA. Coronal sections of cerebrum at embryonic day 15 (E15; A,B), E18 (C,D), and P3 (E–H) were hybridized with only Olig2 antisense probe (A,C,E), only PlexA4 antisense probe (B,D,F), and both *Olig2* (reddish brown) and *PlexA4* (violet) (G,H). Panel H showed enlargement of the boxed area in panel G, and arrows indicated cells expressing both *Olig2* and *PlexA4*. Scale bar = 200 μm.

### *Expression of neuropilins in FBD-102b cells*

Expressions of neuropilins, which are receptors for class 3 semaphorins, were immunocytochemically examined in FBD-102b cells (Fig. 3). Neuropilin 1 (Nrp1) expression was detected in the cells cultured in 10%FCS medium (Fig. 3G), and Nrp2 expression was undetected in the cells (Fig. 3H).

### *Effects of Sema3A and Sema6A on FBD-102b cells*

The effects of the semaphorins on FBD-102b cells were examined (Fig. 4). The cells were pre-incubated in 10%FCS medium, and then culture medium was switched to medium containing AP-Sema3A or AP. A rapid change was induced by AP-Sema3A; length of processes was reduced and became shortest at 1.5 h in incubation. The length gradually recovered to the original at 8 h in incubation. No significant changes in cell processes were observed in incubation with AP (Fig. 4A).

The culture medium was switched from 10%FCS medium to medium containing AP-Fc or AP-Sema6Aect-Fc. Sema6A is known to directly bind to PlexA4 and AP-Fc itself had no effects on neurons [29]. Unexpectedly, however, processes of FBD-102b cells extended till 4 h after switching to medium containing AP-Fc, and then no change was observed thereafter. On the other hand, AP-Sema6Aect-Fc significantly suppressed the extension of processes caused by AP-Fc till 6 h Finally the length of processes reached the control length at 12 h (Fig. 4B).

Next it was examined whether PlexA4 mediates semaphorin signals in FBD-102b cells (Fig. 4). FBD-102b cells were transfected with pcDNA3.1/Hygro-PlexA4ΔC vectors and a subline stably expressing PlexA4ΔC was established. Western blot analysis confirmed PlexA4ΔC expression in the subline (Fig. 4C). The culture medium was switched from 10%FCS medium to medium containing either AP-Sema3A or AP-Sema6Aect-Fc. A rapid change was induced by AP-Sema3A in cells transfected with pcDNA3.1/Hygro vectors; processes became shortest at 1.5 h in incubation, and then gradually reached the original length (Fig. 4D). As observed in Fig. 4B, AP-Fc alone stimulated process extension till 8 h and AP-Sema6Aect-Fc was inhibitory or suppressive (Fig. 4E). In cells expressing PlexA4ΔC, a rapid change was induced in cell processes by AP-Sema3A. The reduction of length, however, was lower than that observed in cells transfected with pcDNA3.1/Hygro

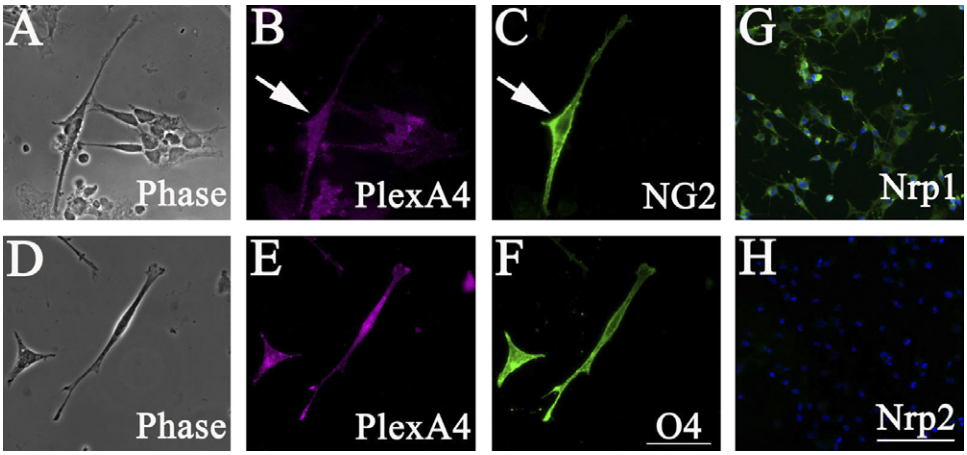


Fig. 3. Expressions of PlexA4 and neuropilins. Expression of PlexA4 was examined in cells prepared from embryonic cerebral cortices (A–F). Cells were double labeled with anti-PlexA4 (B) and anti-NG2 antibodies (C), and double labeled with anti-PlexA4 (E) and anti-O4 antibodies (F), respectively. Arrow showed cells co-expressing PlexA4 and NG2. Expression of neuropilins was examined in FBD-102b cells cultured in 10%FCS medium (G,H). FBD-102b cells were stained with anti-Nrp1 (G) and anti-Nrp2 (H) antibodies (green). Nuclei in each panel were stained with 4′,6′-diamidino-2-phenylindole hydrochloride (G,H) (blue). Scale bar = 50 μm (A–F), 200 μm (G,H).

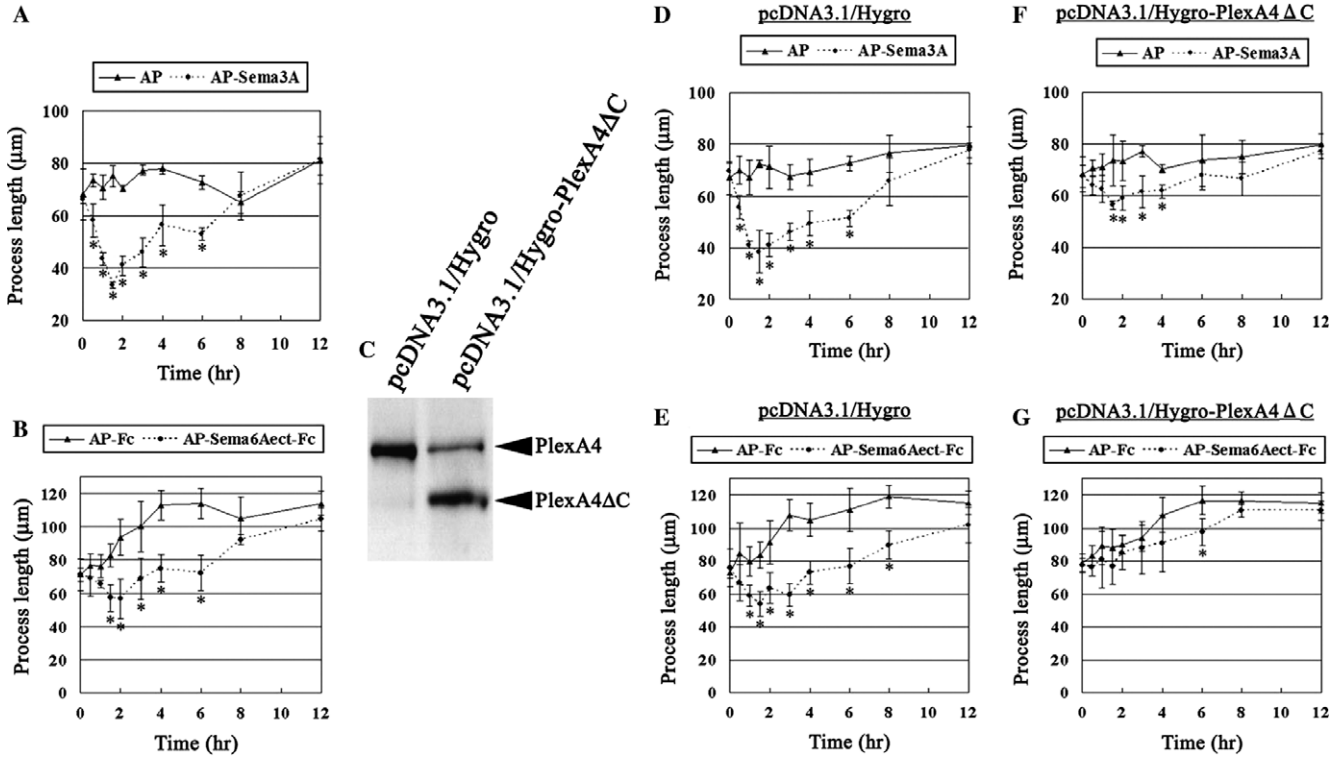


Fig. 4. Effects of Sema3A and Sema6A on FBD-102b cells. Length of cell processes was measured at times indicated after incubation with conditioned medium of HEK293T cells transfected with pCAGGS-alkaline phosphatase (AP) or pCAGGS-AP-Sema3A (AP-Sema3A) (A,D,E), and after incubation with conditioned medium of HEK293T cells transfected with pEF-AP-Fc (AP-Fc) or pEF-AP-Sema6Aect-Fc (AP-Sema6Aect-Fc) (B,F,G). Expression of PlexA4ΔC was confirmed by Western blot analysis in cells of an FBD-102b subline transfected with pcDNA3.1/Hyro-PlexA4ΔC vectors (C). Effects of semaphorins were examined on FBD-102b cells transfected with pcDNA3.1/Hygro vector (D,F) and pcDNA3.1/Hygro vector encoding PlexA4ΔC (E,G). The values represented means ± SEM from at least three independent experiments. Asterisk marks indicated statistically significant difference (two-tailed Student's $t$-test; $p < 0.05$).

vectors. The length gradually recovered to the original length (Fig. 4F). In cells expressing PlexA4ΔC, an inhibitory or suppressive effect of AP-Sema6Aect-Fc on process extension was markedly diminished. In both treatments, processes gradually increased in length till 6–8 h (Fig. 4G).

## Discussion

To date, expression of Plexin-As has not been reported in OPCs, and it is currently unclear how signals of class 3 semaphorin can travel in OPCs. In the present study, the

expression of PlexA4 mRNA was demonstrated in an OPC line and a subset of *Olig2* expressing cells in fetal cerebral cortex. Immunocytochemistry showed that PlexA4 protein was expressed in a subset of both NG2- and O4-positive cells in primary culture. Therefore, the present study firstly raised the possibility that PlexA4 acts as a mediator of the semaphorin signals in OPCs.

Immunocytochemistry revealed that PlexA4 expression was detected in small populations of both NG2- and O4 antigen-positive cells (Fig. 3A–F), and the expression was undetected in O1 antigen- and MBP-positive cells (data not shown), suggesting that PlexA4 may act as a mediator of semaphorin signals only in a subset of OPCs. *PlexA4* expression in FBD-102b cells was increased when cultured in 0.5%FCS medium which stimulated differentiation of FBD-102b cells (Fig. 1A) [22]. Taken together, the expression of the protein may be transiently up-regulated during the differentiation from OPCs to OLs. Such a delicate coordinated expression might be critical for cells in OL lineage in migration and final positioning in the developing CNS.

The culture supernatant of HEK293T cells transfected with the expression vector (AP, AP-Sema3A, AP-Fc, AP-Sema6Aect-Fc) was used to examine the effect of semaphorin on OPCs in the present study. AP-fused protein in culture supernatant was confirmed with anti-human placental alkaline phosphatase antibody by Western blot analysis (data not shown). A repulsive or suppressive effect on process extension was apparently observed in incubation with both AP-Sema3A and AP-Sema6Aect-Fc, and no significant repulsion or suppression was observed in incubation with both AP and AP-Fc (Fig. 4A and B), indicating that the semaphorins are involved in regulation of process extension of cells in OL lineage.

Expression of mutated PlexA4 diminished the repulsive or suppressive effects of both Sema3A and Sema6A on cell processes (Fig. 4F and G), showing that PlexnA4 acts as a mediator of semaphorin signals in OPCs, and that the mutated molecules compete with endogenous PlexA4 molecules in the signal induction.

The increase in length of processes was observed in incubation with both AP-Fc and AP-Sema6Aect-Fc (Fig. 4B, E, and G). The proteins had no effects on neurons [29]. No significant effects on cell processes were observed in incubation with the culture supernatants of both non-transfectant and cells transfected with pEF-Fc control vectors (data not shown). The significant increase in length of processes was not observed in incubation with AP (Fig. 4A, D, and F). These results indicated that human immunoglobulin G Fc fragment, which is a component of the recombinant proteins, may be involved in the extension of processes. Recently it was demonstrated that receptors for immunoglobulin G Fc fragment (FcRs) were expressed in OPCs and stimulation of FcRs with immunoglobulin G induced morphologic differentiation such as increase in number and length of processes in OPCs [30]. RT-PCR analysis revealed that FBD-102b cells expressed *FcRγ*, *FcγRIα*, *FcγRIIB*, and *FcγRIV* (data not shown). Furthermore, binding of AP-Fc with FBD-102b cells was detected, and binding of AP with FBD-102b cells was not detected (data not shown). Taken together, the increase in length of processes might be caused by binding of Fc fragment to FcRs expressed in FBD-102b cells.

In conclusion, our findings indicate that PlexA4 expressed in OPCs acts as a mediator of semaphorin signals. However, the question what is the role of the semaphorin signals via PlexA4 in OPCs *in vivo* is unanswered. The answer will shed light on the molecular mechanisms of migration and positioning of OPCs in developing CNS.

## Acknowledgments

Financial assistance was provided by Special Coordination Funds (SPSBS) of the Japanese Ministry of Education, Sports, Science, and Technology, and by MEXT. HAITEKU (2003). We thank Drs. H. Fujisawa (Nagoya University) and F. Suto (National Institute of Genetics) for providing the expression vectors (AP, AP-Sema3A, AP-Fc, AP-Sema6Aect-Fc, PlexA4, and PlexA4ΔC), anti-PlexA4, and anti-Nrp1 antibodies, Dr. T. Hirata (National Institute of Genetics) for providing anti-Nrp2 antibody, and Dr. F. Nakamura (Yokohama City University) for anti-PlexA4 antibody.

## References

[1] W.D. Richardson, Oligodendrocyte development, in: K.R. Jessen, W.D. Richardson (Eds.), Glial cell development, second ed., Oxford University Press, New York, 2001, pp. 21–54.
[2] D.M. Orentas, R.H. Miller, Regulation of oligodendrocyte development, Mol. Neurobiol. 18 (1998) 247–259.
[3] N. Baumann, D. Pham-Dinh, Biology of oligodendrocyte and myelin in the mammalian central nervous system, Physiol. Rev. 81 (2001) 871–927.
[4] R.H. Woodruff, N. Tekki-Kessaris, C.D. Stiles, D.H. Rowitch, W.D. Richardson, Oligodendrocyte development in the spinal cord and telencephalon: common themes and new perspectives, Int. J. Dev. Neurosci. 19 (2001) 379–385.
[5] R.H. Miller, Regulation of oligodendrocyte development in the vertebrate CNS, Prog. Neurobiol. 67 (2002) 451–467.
[6] D.H. Rowitch, Glial specification in the vertebrate neural tube, Nat. Rev. Neurosci. 5 (2004) 409–419.
[7] A.A. Jarjour, T.E. Kennedy, Oligodendrocyte precursors on the move: mechanisms directing migration, Neuroscientist 10 (2004) 99–105.
[8] R.I. Cohen, Exploring oligodendrocyte guidance: 'to boldly go where no cell has gone before', Cell. Mol. Life Sci. 62 (2005) 505–510.
[9] B.B. Le, E. Chatzopoulou, K. Heydon, S. Martinez, K. Ikenaka, L. Prestoz, N. Spassky, B. Zalc, J.L. Thomas, Oligodendrocyte development in the embryonic brain: the contribution of the plp lineage, Int. J. Dev. Biol. 49 (2005) 209–220.
[10] F.C. De, A. Bribian, The molecular orchestra of the migration of oligodendrocyte precursors during development, Brain Res. Brain Res. Rev. 49 (2005) 227–241.
[11] N. Kessaris, M. Forgarty, P. Iannarelli, M. Grist, M. Wegner, W.D. Richardson, Competing waves of oligodendrocytes in the forebrain and postnatal elimination of an embryonic lineage, Nat. Neurosci. 9 (2006) 173–179.
[12] W.D. Richardson, N. Kessaris, N. Piringle, Oligodendrocyte war, Nat. Rev. Neurosci. 7 (2006) 11–18.

[13] H. Fujisawa, Discovery of semaphorin receptors, neuropilin and plexin, and their functions in neural development, J. Neurobiol. 59 (2004) 24–33.
[14] J.A. Raper, Semaphorins and their receptors in vertebrates and invertebrates, Curr. Opin. Neurobiol. 10 (2000) 88–94.
[15] N. Spassky, F.C. De, B.B. Le, K. Heydon, F. Queraud-LeSaux, E. Bloch-Gallego, A. Chedotal, B. Zalc, J.L. Thomas, Directional guidance of oligodendroglial migration by class 3 semaphorins and netrin-1, J. Neurosci. 22 (2002) 5992–6004.
[16] Y. Sugimoto, M. Taniguchi, T. Yagi, Y. Akagi, Y. Nojyo, N. Tamamaki, Guidance of glial precursor cell migration by secreted cues in the developing optic nerve, Development 128 (2001) 3321–3330.
[17] R.I. Cohen, D.M. Rottkamp, D. Maric, J.L. Barker, L.D. Hudson, A role for semaphorins and neuropilins in oligodendrocyte guidance, J. Neurochem. 85 (2003) 1262–1278.
[18] T. Takahashi, A. Fournier, F. Nakamura, L.H. Wang, Y. Murakami, R.G. Kalb, H. Fujisawa, S.M. Strittmatter, Plexin-neuropilin-1 complexes form functional semaphorin-3A receptors, Cell 99 (1999) 59–69.
[19] J. Rieger, W. Wick, M. Weller, Human malignant glioma cells express semaphorins and their receptors, neuropilins and plexins, Glia 42 (2003) 379–389.
[20] J. Ara, P. Bannerman, F. Shaheen, D.E. Pleasure, Schwann cell-autonomous role of neuropilin-2, J. Neurosci. Res. 79 (2005) 468–475.
[21] Y. Tomooka, S. Aizawa, Cell lines established from fetal brains of p53-deficient mice, Cell Struct. Funct. 23 (1998) 101–107.
[22] M. Horiuchi, Y. Tomooka, An oligodendroglial progenitor cell line FBD-102b possibly secretes a radial glial-inducing factor, Neurosci. Res. 56 (2006) 213–219.
[23] M. Horiuchi, Y. Tomooka, An attempt to generate neurons from an astrocyte progenitor cell line FBD-104, Neurosci. Res. 53 (2005) 104–115.
[24] M. Tominaga, S. Honda, A. Okada, A. Ikeda, S. Kinoshita, Y. Tomooka, A bipotent neural progenitor cell line cloned from a cerebellum of an adult p53-deficient mouse generates both neurons and oligodendrocytes, Eur. J. Neurosci. 21 (2005) 2903–2911.
[25] M. Tominaga, Y. Tomooka, Novel genes cloned from a neuronal cell line newly established from a cerebellum of an adult $p53^{-/-}$ mouse, Biochem. Biophys. Res. Commum. 297 (2002) 473–479.
[26] O. Nakajima, F. Nakamura, N. Yamashita, Y. Tomita, F. Suto, T. Okada, A. Iwamatsu, E. Kondo, H. Fujisawa, K. Takei, Y. Goshima, FKBP133: a novel mouse FK506-binding protein homolog alters growth cone morphology, Biochem. Biophys. Res. Commum. 346 (2006) 140–149.
[27] A. Ivanova, E. Nakahira, T. Kagawa, A. Oba, T. Wada, H. Takebayashi, N. Spassky, J. Levine, B. Zalc, K. Ikenaka, Evidence for a second wave of oligodendrogenesis in the postnatal cerebral cortex of the mouse, J. Neurosci. Res. 73 (2003) 581–592.
[28] T. Shimizu, E. Nakahira, T. Kagawa, K. Ikenaka, Regulation of development of oligodendrocyte, Nihon Shinkei Seishin Yakurigaku Zasshi 23 (2003) 129–134.
[29] F. Suto, K. Ito, M. Uemura, M. Shimizu, Y. Shinkawa, M. Sanbo, T. Shinoda, M. Tsuboi, S. Takashima, T. Yagi, H. Fujisawa, Plexin-A4 mediates axon-repulsive activities of both secreted and transmembrane semaphorins and plays roles in nerve fiber guidance, J. Neurosci. 25 (2005) 3628–3637.
[30] J. Nakahara, K. Tan-Takeuchi, C. Seiwa, M. Gotoh, T. Kaifu, A. Ujike, M. Inui, T. Yagi, M. Ogawa, S. Aiso, T. Takai, H. Asou, Signaling via immunoglobulin Fc receptors induces oligodendrocyte precursor cell differentiation, Dev. Cell 4 (2003) 841–852.