Biochemical and Biophysical Research Communications 385 (2009) 6–10

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Protein tyrosine phosphatase SHP2 is involved in Semaphorin 4D-induced axon repulsion

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ARTICLE INFO

Article history:
Received 5 May 2009
Available online 9 May 2009

Keywords:
Semaphorin
Sema4D
Plexin
Plexin-B1
SHP2
Protein tyrosine phosphatase
NSC-87877

ABSTRACT

Semaphorin-4D (Sema4D), a member of class 4 membrane-bound Semaphorins, acts as a chemorepellant to the axons of retinal ganglion cells and hippocampal neurons. Plexin-B1, a neuronal Sema4D receptor, associates with either one of receptor tyrosine kinases, c-Met or ErbB2, to mediate Sema4D-signaling. In contrast to this significance, the involvement of protein tyrosine phosphatases in Semaphorin-signaling remains unknown. We here show that Src homology 2-containing protein-tyrosine phosphatase 2 (SHP2) participates in Sema4D-signaling. SHP2 was localized in the growth cones of chick embryonic retinal ganglion neurons. Phenylarsine oxide, a protein tyrosine phosphatase inhibitor, suppressed Sema4D-induced contractile response in COS-7 cells expressing Plexin-B1. Ectopic expression of a phosphatase-inactive mutant of SHP2 in the retinal ganglion cells attenuated Sema4D-induced growth cone collapse response. A SHP1/2 specific inhibitor, 8-hydroxy-7-(6-sulfonaphthalen-2-yl)diazenyl-quinoline-5-sulfonic acid (NSC-87877), also suppressed this collapse response. These results suggest that SHP2-mediated tyrosine dephosphorylation is an important step in Sema4D-induced axon repulsion.

Introduction

Semaphorin-4D (Sema4D, CD100) is a member of class 4 membrane-bound Semaphorins, which repels the axons of hippocampal and retinal ganglion neurons in the developing nervous system [1,2]. Plexin-B1 is a neuronal Sema4D receptor and commands at least two different intracellular signaling [2]. One is the regulation of small G-proteins [2]. Plexin-B1 directly binds to Rac and competes with p21 activated kinase [3]. Plexin-B1 acts as GTPase activating protein for R-Ras [4]. Another small G-protein RhoA is also activated by the complex of Plexin-B1 and Rho-GEF proteins [1,5]. The other signaling conducted by Plexin-B1 is tyrosine phosphorylation [6]. The binding of Sema4D to Plexin-B1 activates tyrosine kinases in which Plexin-B1 forms a hetero-complex with either one of receptor tyrosine kinases, c-Met or ErbB2 [7,8]. Sema4D also induces tyrosine-dephosphorylation of focal adhesion kinase in the cell lines expressing Plexin-B1 [9]. However, the molecular mechanism and biological significance of tyrosine-dephosphorylation in Sema4D-signaling have yet to be determined.

Src homology 2-containing protein-tyrosine phosphatase 2 (SHP2) is one of the cytoplasmic protein tyrosine phosphatases [10]. Recent studies suggest that SHP2 and its close homologue SHP1 are involved in the intracellular signaling of axon guidance cues. The binding of ephrin-A1 to EphA2 receptor induces transient association of SHP2 with the receptor [11]. In the immune system, CD72, an immune Sema4D receptor, interacts with SHP1 upon Sema4D-stimulation [12]. Furthermore, SHP2 associates with c-Met and ErbB2 receptor tyrosine kinases via Gab1/Grb2 adapter proteins [13,14]. These notions prompted us to investigate the role of SHP2 in Sema4D-signaling.

Here we demonstrate that SHP2 is localized in the growth cones of chick retinal neurons and dorsal root ganglion neurons. Overexpression of a phosphatase inactive mutant of SHP2 or pretreatment with a SHP1/2 specific inhibitor, 8-hydroxy-7-(6-sulfonaphthalen-2-yl)diazenyl-quinoline-5-sulfonic acid (NSC-87877) [15], attenuates Sema4D-induced collapse response of retinal neurites. These results suggest that SHP2 is involved in the Sema4D-signaling in the developing nervous system.

Materials and methods

Antibodies and reagents. A mouse anti-protein-tyrosine phosphatase 1D (PTP1D)/SHP2 antibody was purchased from BD Biosciences (San Jose, CA, US), a mouse anti-myc antibody (9E10) from Sigma–Aldrich (St. Louis, MO, US). Rhodamine–Phallodin, a control IgG, and Alexa 594-conjugated secondary antibodies were purchased from Invitrogen (Carlsbad, CA, US). Phenylarsine oxide was purchased from Sigma. NSC-87877 (SHP1/2 specific inhibitor) was purchased from Calbiochem (San Diego, CA, US).

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doi:10.1016/j.bbrc.2009.05.024

Plasmid construction. Human Plexin-B1 and mouse Sema4D cDNAs were kindly provided by Dr. Nagase (Kazusa DNA Inst.) and Dr. Inagaki (Osaka University), respectively. An expression vector of myc-tagged human Plexin-B1 was generated as follows. The coding region of human Plexin-B1 (33–2135 aa) was amplified with two primers (5′-atcACGTATCTGCAGCACCTGGCAAGGG-3′, 5′-tttagcggccgcCTATAGATCTGTGACCTTGTTTTCCACA-3′) and cloned into a pSec-myc vector, which is harboring a secretion signal and a N-terminal myc epitope.

An expression vector of alkaline phosphatase (AP)–Sema4D fusion protein was constructed as follows. A coding fragment of hexahistidine-tagged human placental alkaline phosphatase and a coding fragment of mouse Sema4D ectodomain (24–719 aa) amplified with two primers (5′-atcTTTGCACCTGTGCCTCGGCTC-3′, 5′-tttctcgagCTGGGGGACCGTGTTGAT-3′) were cloned into pcDNA3.1.

The coding region of mouse SHP2 was PCR-amplified from mouse embryonic brain cDNA template with two primers (5′-aag aattcaccATGACATCGCGGAGATGGTTT-3′, 5′-atcgtcgacTCTGAAACT CCTCTGCTGCTGCATGAG-3′). The fragment was cloned into EcoRI and SalI sites of pEGFP-C2 (Clontech, Mountain View, CA, US) to produce EGFP-SHP2 fusion protein. A phosphatase-inactive mutant of SHP2 (SHP2(C/S)) [16] was generated by the inverse PCR method with a primer set (5′-CACCACGACAGGGCCTGCATCCACGAT-3′, 5′-CACtccAGCGCTGGGATTGGCCGGA-3′) to substitute the catalytic Cysteine (463) residue with Serine. Recombinant Herpes-Simplex Virus of EGFP, EGFP-SHP2, and EGFP-SHP2(C/S) were prepared as previously described [17].

Production of AP-Sema4D fusion protein. The AP-Sema4D vector was transfected into HEK293T cells using Fugene-6 (Roche, Mannheim, Germany) and incubated for 3–5 days. HEK293 cells stably expressing AP-Sema4D were also prepared. AP-Sema4D conditioned medium (3–10 nM) was collected, centrifuged, and stored at −80 °C until use.

COS-7 cell contraction assay. COS-7 cells were transfected with the myc-Plexin-B1 expression vector. After 36–48 h incubation, the cells were stimulated with AP-Sema4D (1–6 nM) for 30 min at 37 °C. For tyrosine phosphatase inhibition, 2 μM phenylarsine oxide (PAO) was added 5 min prior to the AP-Sema4D treatment. The AP-Sema4D bound cells were visualized by the deposition of insoluble AP reaction product from 4-nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate. The size of stained cells was determined with the aid of digital images acquired by using a CCD camera (Spot, Sterling Heights, MI, US) equipped with a Olympus IX70 inverted microscope and an Image-Pro Plus software (Media Cybernetics, Bethesda, MD, US). Fifty to 80 cells were scored in each preparation. For EGFP-SHP2 mutant experiments, myc-Plexin-B1 was stained with anti-myc antibody and with Alexa 594-anti mouse second antibody. Dual expressed (EGFP and myc) cells were scored.

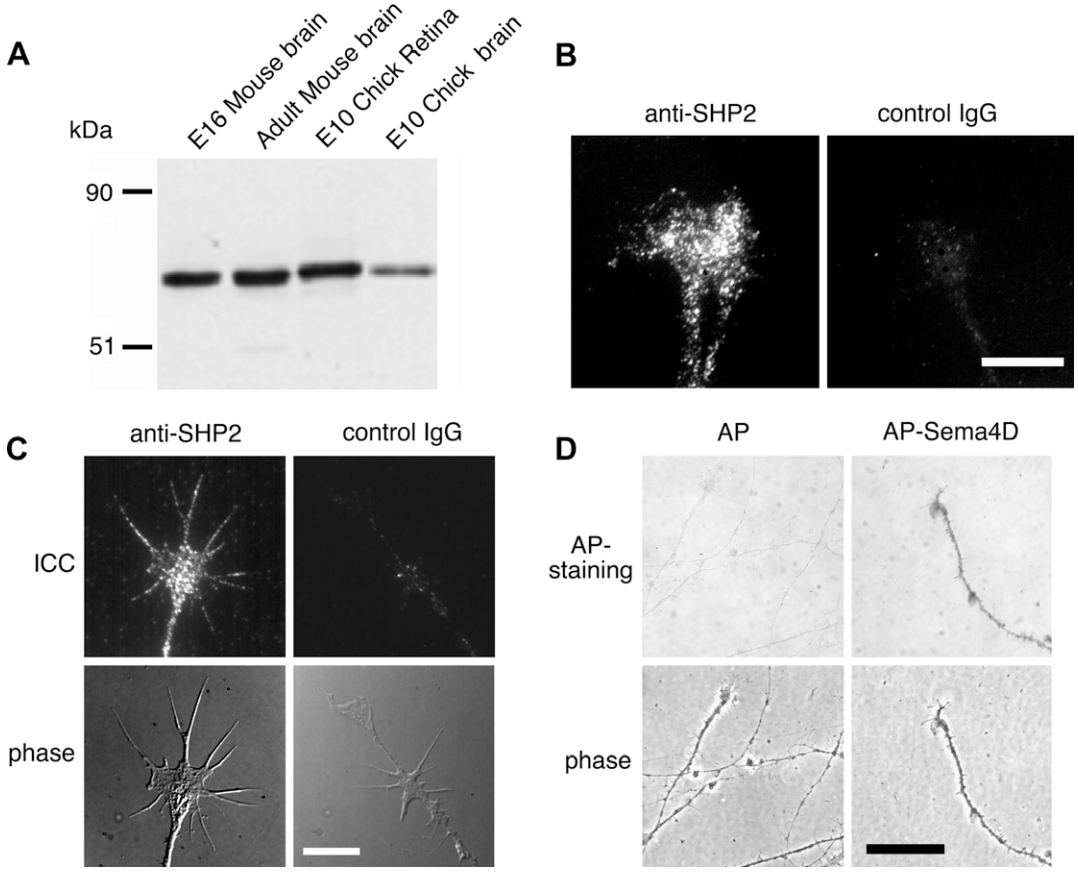

Fig. 1. SHP2 and Plexin-B1 are expressed in the chick retinal neurons. (A) Immunoblot analysis of SHP2 expression in mouse and chick nervous system. The anti-SHP2 antibody reacted with a single 72 kDa band in mouse embryonic brain, mouse adult brain, chick embryonic retina, and chick brain. (B) Immunostaining of SHP2 of cultured chick retinal ganglion cells. Immunoreactive signal within the growth cone is apparent with anti-SHP2 antibody (left) but not with control IgG (right). (C) Localization of SHP2 protein in a growth cone of chick dorsal root ganglion neurons. Upper and lower panels indicate SHP2 immunoreactive signal and phase contrast, respectively. Note that SHP2 is localized in both central domain and peripheral filopodia of the growth cone. (D) AP-Sema4D bound to chick retinal growth cones. Chick retinal explants were incubated with AP (5 nM) or AP-Sema4D (5 nM). AP-Sema4D bound to the growth cones, suggesting the expression of Plexin-B1 in chick retinal neurons. Lower panels show the phase contrast of above identical areas. Scale bars, 20 μm in B and C; 50 μm in D.

Chick retinal cell culture, immunocytochemistry, and growth cone collapse assay. All experiments using animal samples were performed according to the guidelines outlined in the Institutional Animal Care and Use Committee of the Yokohama City University School of Medicine. Throughout the experimental procedures, all efforts were made to minimize the number of animals used and their suffering. Chick retinal or dorsal root ganglion explants from E6 to E7 embryo were prepared as previously described [18]. The E6–E7 chick retinal strips were placed on plastic eight-well chambers, which were coated with poly-L-lysine (100 μg/ml) and laminin (10 μg/ml). The retinal explants were cultured for 36 h in Nutrient mixture F12 HAM (Sigma), supplemented with 10% FBS, Insulin–Transferrin–Selenium Supplement (Invitrogen) and 10 ng/ml 2.5S-Nerve Growth Factor (NGF) (WAKO, Tokyo, Japan). For immunostaining, the neurons were fixed in 4% paraformaldehyde, permeabilized with 0.3% Triton X-100 in PBS, and blocked with 10% FBS and 0.1% Triton X-100 in PBS for 1 h. The explant cultures were incubated with anti-SHP2 antibody (1:1000) at room temperature for 1 h, and then exposed to Alexa-594-labeled secondary antibody (1:1000). Fluorescence images were obtained with a CCD camera through 40× object lens. For dominant-negative experiments, the retinal explants were infected with the recombinant herpes virus of EGFP, EGFP-SHP2, or EGFP-SHP2(C/S). A specific inhibitor for SHP1/2 NSC-87877 (10–500 μM) was added 2 h before the Sema4D stimulation. The explants were treated with 1–5 nM of AP-Sema4D for 30 min, fixed with 2% paraformaldehyde, and stained with Rhodamine-Phallodin. The morphology of growth cones were scored either spread or collapsed shape.

Results

SHP2 is expressed in the growth cones and axons

We first examined the SHP2 protein expression in mouse and chick nervous system. Immunoblot analysis revealed that a single 72 kDa band was apparent in E16 mouse brain, adult mouse brain, E10 chick retina, and E10 chick brain (Fig. 1A). Immunocytochemistry also revealed that SHP2 was distributed in growth cones and axonal shafts of chick retinal ganglion neurons (Fig. 1B, left). Control mouse IgG antibody gave weak background signals (Fig. 1B, right). We also immunostained the growth cones from chick E7 dorsal root ganglion neurons to analyze the localization of SHP2 within the growth cones (Fig. 1C). SHP2 was distributed in central domain as well as peripheral filopodia. Since it has been shown that Sema4D induces growth cone collapse response in chick retinal ganglion cells [1], we examined the localization of neuronal Sema4D receptor in chick retinal neurons. We stained the chick retinal explants with a fusion protein of alkaline phosphatase and Sema4D-ectodomain (AP-Sema4D) (Fig. 1D). AP-Sema4D, but not AP, bound to the growth cones and axonal shafts of chick retinal neurons. These data indicate that both SHP2 and a Sema4D receptor, presumably Plexin-B1, are expressed in the chick retinal neurons.

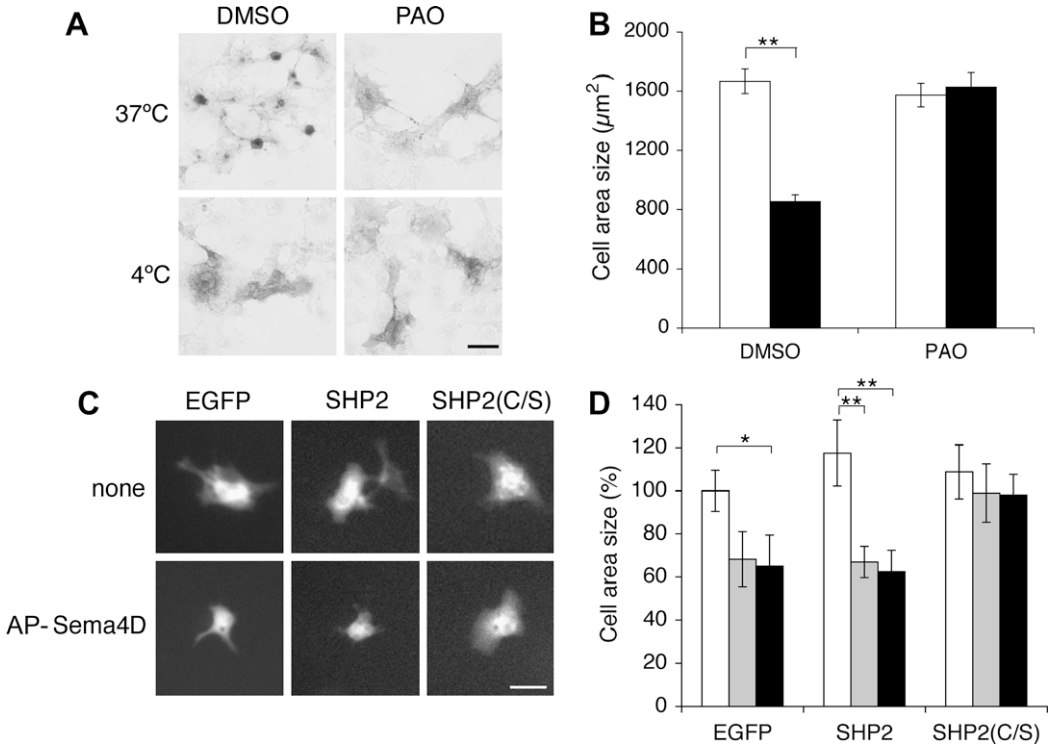

Fig. 2. Overexpression of SHP2 phosphatase-inactive mutant suppressed Sema4D-induced contraction of COS-7 cells expressing Plexin-B1. (A) COS-7 contraction assay. Plexin-B1 expression in the COS-7 cells transfected with myc-Plexin-B1 was confirmed by AP–Sema4D binding (all panels). The cells incubated with AP–Sema4D (5 nM) at 37 °C exhibited cell contraction (rounded cells in upper left panel). The cells pretreated with PAO could bind AP–Sema4D but remained in the spread morphology (upper right panel). (B) Plexin-B1-expressing cells were incubated with AP–Sema4D (5 nM) at 4 °C (open bars) or 37 °C (solid bars). AP–Sema4D bound cell area was manually scored from 70–80 cells in each preparation. The adhered area of Plexin-B1 expressing cells was reduced by 50% in control. PAO pretreatment abolished AP–Sema4D-induced cell contraction. One typical example is shown. Five independent experiments gave similar results. $^{**}p < 0.01$, *T*-test. (C) COS-7 cells were co-transfected with myc-Plexin-B1 and with EGFP, EGFP–SHP2, or EGFP–SHP2(C/S). The cells were stimulated with 1 or 6 nM AP–Sema4D. Cells were immunostained with myc antibody. EGFP and myc double positive cells are shown. Note that the cell co-expressing Plexin-B1 and EGFP–SHP2(C/S) remained in the spread shape after AP–Sema4D-stimulation. (D) Cells were incubated with medium from mock-transfected (open bars), AP–Sema4D-transfected cells (1 nM, gray bars) or AP–Sema4D-transfected cells (6 nM, black bars). Approximately 50 double stained cells were scored in each preparation. The cell area sizes were normalized against non-stimulated EGFP and Plexin-B1 co-expressing cells. The cells co-expressed Plexin-B1 with EGFP or EGFP–SHP2 but not with EGFP–SHP2(C/S) showed 30–40% reduction in attached area upon AP–Sema4D stimulation. $^{*}p < 0.05$, $^{**}p < 0.01$, *T*-test. Scale bars, 50 μm in A; 30 μm in C.

SHP2 is involved in Sema4D-induced contractile response of the COS-7 cells expressing Plexin-B1

We next performed the COS-7 cell contraction assay [19], in which Plexin-B1-expressing cells reduced adhered areas upon AP-Sema4D stimulation (Fig. 2A, B). Pretreatment with phenylarsine oxide (PAO), a tyrosine phosphatase inhibitor, suppressed AP-Sema4D-induced cell contraction. Quantifying the adhered areas revealed that the Sema4D-stimulation reduced approximately 50% of attached area of Plexin-B1 expressing cells whereas PAO treatment abolished the response (Fig. 2B). The binding of AP-Sema4D to Plexin-B1 was not affected by PAO-treatment. Thus, we thought that PAO modulates the intracellular signaling of Plexin-B1 but not the expression of Plexin-B1 or Sema4D–Plexin-B1 interaction.

Since SHP2 was expressed endogenously in COS-7 cells (not shown) and it has been shown that SHP2 regulates cell adhesion [16,20], we asked if a phosphatase-inactive mutant of SHP2 perturbs Sema4D-induced cell contraction of Plexin-B1-expressing COS-7 cells. We have constructed an EGFP-tagged phosphatase inactive mutant of SHP2 (EGFP-SHP2(C/S)) and EGFP-tagged wild-type SHP2 (EGFP-SHP2). COS-7 cells were co-transfected with the expression vectors of myc-Plexin-B1 and one of EGFP, EGFP-SHP2, or EGFP-SHP2(C/S). The cells were stimulated with AP-Sema4D and the adhered areas of dual expressing cells were scored. The cells co-expressing Plexin-B1 and either EGFP or EGFP-SHP2 altered the morphology upon AP-Sema4D stimulation (Fig. 2C and D). In contrast, the cells co-expressing Plexin-B1 and EGFP-SHP2(C/S) did not respond to AP-Sema4D (Fig. 2C and D). These results suggest that SHP2 may participate in Sema4D-Plexin-B1 signaling in COS-7 cells.

A SHP2 phosphatase-inactive mutant attenuates Sema4D-induced growth cone collapse response in retinal ganglion neurons

We lastly asked whether SHP2 is involved in Sema4D repulsive signaling in neuronal cells. Chick retinal explants were ectopically expressed EGFP, EGFP-SHP2, or EGFP-SHP2(C/S) mutant with the aid of herpes simplex viral system. The expression was confirmed by the EGFP-fluorescence (not shown). AP-Sema4D-induced collapse response of the chick retinal ganglion neurons was dose-dependent (Fig. 3A and B). Although we could not determine the maximum efficacy due to the limited concentration of AP-Sema4D, we estimated the EC50 at 4 nM (Fig. 3B, open circles). Overexpression of EGFP or EGFP-SHP2 did not significantly alter the growth cone morphology or the sensitivity of AP-Sema4D collapse response. In contrast, the EGFP-SHP2(C/S) mutant significantly suppressed the AP-Sema4D induced collapse response (Fig. 3A and B). We also examined the effect of NSC-87877, a specific inhibitor of SHP1/2 [15]. While NSC-87877 did not alter the growth cone

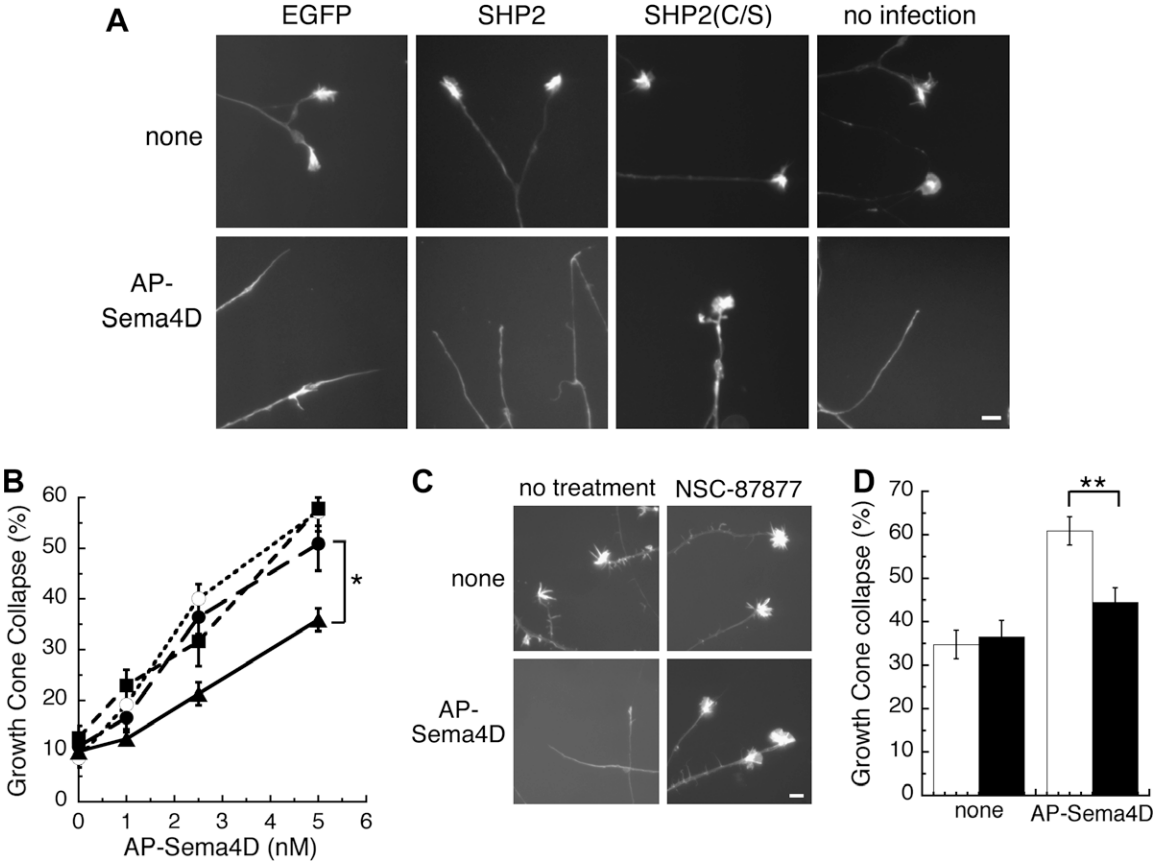

Fig. 3. Inhibition of SHP2 suppressed Sema4D-induced growth cone collapse of retinal ganglion neurons. (A) EGFP–SHP2(C/S) attenuates Sema4D-collapse response. Chick retinal explants were infected with recombinant herpes virus harboring EGFP, EGFP–SHP2, or EGFP–SHP2(C/S). Upper panels, no stimulation; lower panels, AP–Sema4D (5 nM) stimulation. The growth cones infected with the virus harboring EGFP–SHP2(C/S) were less sensitive to AP–Sema4D than with other preparations. (B) Dose–response curve. Dotted line and open circles, no infection; Small dashed line and closed squares, EGFP; Large dashed line and closed circles, EGFP–SHP2; solid line and triangles, EGFP–SHP2(C/S). Overexpression of EGFP–SHP2(C/S) suppressed Sema4D-induced collapse response. $^{*}p < 0.05$, *T*-test. (C) A SHP1/2 specific inhibitor NSC-87877 suppressed Sema4D-induced growth cone collapse. NSC-87877 did not alter the growth cone morphology even at 500 μM concentration (upper right). After incubating in NSC-87877 for 2 h, AP–Sema4D (3.1 nM) was added to cultured chick retinal explants and incubated for 30 min. NSC-87877 (50 μM) inhibited the AP–Sema4D-induced collapse response (lower right). (D) The bar graph represents the average ± standard error of three independent experiments. White and black bars represent no pretreatment and NSC-87877 (100 μM) treatment, respectively. The inhibitor significantly suppressed the collapse response induced by AP–Sema4D (10 nM). $^{**}p < 0.01$, *T*-test. Scale bars, 10 μm.

morphology with 10–500 μM range (Fig. 3C, upper right), the inhibitor (100 μM) significantly suppressed AP-Sema4D-induced growth cone collapse response of retinal ganglion neurons for about 20% (Fig. 3C and D). These results suggest that SHP2 mediates Sema4D-induced repulsive signaling in neurons.

Discussion

This study demonstrates the expression of SHP2 in the embryonic nervous system of both chick and mouse and the localization in the growth cones of chick retinal neurons (Fig. 1). Sema4D-induced contractile response of COS-7 cells expressing Plexin-B1 was suppressed either by pretreatment of a protein tyrosine phosphatase inhibitor (PAO) or by the overexpression of SHP2 phosphatase-inactive mutant (Fig. 2). Ectopic expression of the SHP2 mutant in chick retinal ganglion cells attenuated the Sema4D-induced growth cone collapse response (Fig. 3A and B). A SHP1/2 specific inhibitor also suppressed this response (Fig. 3C and D). Taken together, these data suggest that SHP2 is involved in the intracellular signaling of Sema4D/Plexin-B1 system.

Since we have not been able to demonstrate stable interaction between Plexin-B1 and SHP2 by immunoprecipitation (not shown), SHP2 may weakly associate with the heterocomplexes of Plexin-B1 and c-Met/ErbB2. It has been shown several tyrosine residues of c-Met and ErbB2 are phosphorylated upon Sema4D-stimulation [7,8]. These phosphotyrosine residues may serve as binding sites for SHP2 and/or Gab1, which interacts multiple SH2 containing proteins including SHP2 [13,21]. Sema4D-stimulation might induce transient association of SHP2 to the signaling complexes of Plexin-B1. Such transient interaction has been reported in the ephrin-A1 signaling [11].

The recruitment of SHP2 to ephrin-A1 receptor EphA2 is followed by the dephosphorylation of focal adhesion kinase and paxillin [11]. Barberis et al. demonstrated that Sema4D-stimulation leads to the dephosphorylation of focal adhesion kinase in Plexin-B1 expressing NIH3T3 fibroblasts [9]. It is thus likely that focal adhesion kinase is one of the substrates of SHP2 in Sema4D-signaling. This dephosphorylation may bring the inactivation of integrin-mediated cell adhesion, cell rounding and/or the growth cone collapse response. The contribution of SHP2 to repulsive response could also be explained by the regulation of small G-protein RhoA [22]. It has been shown that SHP2 dephosphorylates and inactivates p190B-RhoGAP [23]. Since Sema4D activates RhoA through the interaction of Plexin-B1 and Rho-GEFs [1,5], SHP2-mediated inactivation of p190B-RhoGAP may also augment the activation of RhoA, which leads to the reorganization of cytoskeleton in the neurons. Alternatively, Sema4D-activated SHP2 may dephosphorylate Rho-kinase II to induce deadhesion of growth cones as suggested in non-neuronal cells [24]. Identification of neuronal substrates of SHP2 in Sema4D-repulsive signaling will provide new insights into the molecular mechanism of axon guidance.

Acknowledgments

This work was supported by Grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan (13670128 and 15590250 to F.N. and 17082006 to Y.G.). The authors thank Ms. Sandy Chen for critical reading of the manuscript.

References

[1] J.M. Swiercz, R. Kuner, J. Behrens, S. Offermanns, Plexin-B1 directly interacts with PDZ-RhoGEF/LARG to regulate RhoA and growth cone morphology, Neuron 35 (2002) 51–63.
[2] R.P. Kruger, J. Aurandt, K.L. Guan, Semaphorins command cells to move, Nat. Rev. Mol. Cell Biol. 6 (2005) 789–800.
[3] H.G. Vikis, W. Li, K.L. Guan, The Plexin-B1/Rac interaction inhibits PAK activation and enhances Sema4D ligand binding, Genes Dev. 16 (2002) 836–845.
[4] I. Oinuma, Y. Ishikawa, H. Katoh, M. Negishi, The Semaphorin 4D receptor plexin-B1 is a GTPase activating protein for R-Ras, Science 305 (2004) 862–865.
[5] J. Aurandt, H.G. Vikis, J.S. Gutkind, N. Ahn, K.L. Guan, The Semaphorin receptor plexin-B1 signals through a direct interaction with the Rho-specific nucleotide exchange factor, LARG, Proc. Natl. Acad. Sci. USA 99 (2002) 12085–12090.
[6] M. Franco, L. Tamagnone, Tyrosine phosphorylation in Semaphorin signalling: shifting into overdrive, EMBO Rep. 9 (2008) 865–871.
[7] S. Giordano, S. Corso, P. Conrotto, S. Artigiani, G. Gilestro, D. Barberis, L. Tamagnone, P.M. Comoglio, The Semaphorin 4D receptor controls invasive growth by coupling with Met, Nat. Cell Biol. 4 (2002) 720–724.
[8] J.M. Swiercz, R. Kuner, S. Offermanns, Plexin-B1/RhoGEF-mediated RhoA activation involves the receptor tyrosine kinase ErbB-2, J. Cell Biol. 165 (2004) 869–880.
[9] D. Barberis, S. Artigiani, A. Casazza, S. Corso, S. Giordano, C.A. Love, E.Y. Jones, P.M. Comoglio, L. Tamagnone, Plexin signaling hampers integrin-based adhesion, leading to Rho-kinase independent cell rounding, and inhibiting lamellipodia extension and cell motility, FASEB J. 18 (2004) 592–594.
[10] M. Dance, A. Montagner, J.P. Salles, A. Yart, P. Raynal, The molecular functions of Shp2 in the Ras/Mitogen-activated protein kinase (ERK1/2) pathway, Cell. Signal. 20 (2008) 453–459.
[11] H. Miao, E. Burnett, M. Kinch, E. Simon, B. Wang, Activation of EphA2 kinase suppresses integrin function and causes focal-adhesion-kinase dephosphorylation, Nat. Cell Biol. 2 (2000) 62–69.
[12] A. Kumanogoh, C. Watanabe, I. Lee, X. Wang, W. Shi, H. Araki, H. Hirata, K. Iwahori, J. Uchida, T. Yasui, M. Matsumoto, K. Yoshida, H. Yakura, C. Pan, J.R. Parnes, H. Kikutani, Identification of CD72 as a lymphocyte receptor for the class IV Semaphorin CD100: a novel mechanism for regulating B cell signaling, Immunity 13 (2000) 621–631.
[13] U. Schaeper, N.H. Gehring, K.P. Fuchs, M. Sachs, B. Kempkes, W. Birchmeier, Coupling of Gab1 to c-Met, Grb2, and Shp2 mediates biological responses, J. Cell Biol. 149 (2000) 1419–1432.
[14] W. Vogel, R. Lammers, J. Huang, A. Ullrich, Activation of a phosphotyrosine phosphatase by tyrosine phosphorylation, Science 259 (1993) 1611–1614.
[15] L. Chen, S.S. Sung, M.L. Yip, H.R. Lawrence, Y. Ren, W.C. Guida, S.M. Sebti, N.J. Lawrence, J. Wu, Discovery of a novel shp2 protein tyrosine phosphatase inhibitor, Mol. Pharmacol. 70 (2006) 562–570.
[16] S. Manes, E. Mira, C. Gomez-Mouton, Z.J. Zhao, R.A. Lacalle, A.C. Martinez, Concerted activity of tyrosine phosphatase SHP-2 and focal adhesion kinase in regulation of cell motility, Mol. Cell. Biol. 19 (1999) 3125–3135.
[17] F. Nakamura, M. Tanaka, T. Takahashi, R.G. Kalb, S.M. Strittmatter, Neuropilin-1 extracellular domains mediate Semaphorin D/III-induced growth cone collapse, Neuron 21 (1998) 1093–1100.
[18] Q. Cheng, Y. Sasaki, M. Shoji, Y. Sugiyama, H. Tanaka, T. Nakayama, N. Mizuki, F. Nakamura, K. Takei, Y. Goshima, Cdk5/p35 and Rho-kinase mediate ephrin-A5-induced signaling in retinal ganglion cells, Mol. Cell. Neurosci. 24 (2003) 632–645.
[19] 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.
[20] D.H. Yu, C.K. Qu, O. Henegariu, X. Lu, G.S. Feng, Protein-tyrosine phosphatase Shp-2 regulates cell spreading, migration, and focal adhesion, J. Biol. Chem. 273 (1998) 21125–21131.
[21] M. Tanowitz, J. Si, D.H. Yu, G.S. Feng, L. Mei, Regulation of neuregulin-mediated acetylcholine receptor synthesis by protein tyrosine phosphatase SHP2, J. Neurosci. 19 (1999) 9426–9435.
[22] S.M. Schoenwaelder, L.A. Petch, D. Williamson, R. Shen, G.S. Feng, K. Burridge, The protein tyrosine phosphatase Shp-2 regulates RhoA activity, Curr. Biol. 10 (2000) 1523–1526.
[23] M.I. Kontaridis, S. Eminaga, M. Fornaro, C.I. Zito, R. Sordella, J. Settleman, A.M. Bennett, SHP-2 positively regulates myogenesis by coupling to the Rho GTPase signaling pathway, Mol. Cell. Biol. 24 (2004) 5340–5352.
[24] H.H. Lee, Z.F. Chang, Regulation of RhoA-dependent ROCKII activation by Shp2, J. Cell Biol. 181 (2008) 999–1012.