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Sema4D-plexin-B1 implicated in regulation of dendritic spine density through RhoA/ROCK pathway

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Abstract

Plexin-B1, Sema4D receptor, mediates retraction and extension signals in axon guidance by associating with PDZ-containing Rho guanine nucleotide exchange factors (PDZ-RhoGEFs) which can activate a small Rho GTPase RhoA. RhoA is implicated in spine formation by rearranging actin cytoskeleton. Exogenous application of Sema4D to cultured neurons caused activation of RhoA, increase of spine density and changes in spine shape. Sema4D-induced changes in spine density were blocked by either Rho-kinase (a downstream of RhoA, ROCK) inhibitor Y-27632 or by overexpression of plexin-B1 mutant lacking the C-terminus which no longer associates with PDZ-RhoGEFs. This study suggests that Sema4D-plexin-B1 play a crucial role in spine formation by regulating RhoA/ROCK pathway.

Keywords: Semaphorin; Plexin; Rho; Dendritic spine; Cytoskeleton; Neuron

Semaphorins comprise a family of soluble and transmembrane proteins that play a critical role in axon guidance in the developing nervous system [16,18,32]. Semaphorins trigger dynamic rearrangements of actin cytoskeleton and induce retraction or extension of neurites [3,17,19,20,25,28]. Expressions of several semaphorins and their functional receptors, plexins, persist into adulthood after axon guidance has been completed [10,15,19–21,33,36]. However, roles of semaphorins at postnatal ages are not well understood.

Dendritic spines, actin-rich protrusions on neuronal dendrites, are the major postsynaptic sites of excitatory synapses in the brain [7,11–13]. The spine formation and morphology are regulated by reorganizing actin cytoskeleton [4,12]. Rho GTPases, RhoA, Rac and Cdc42 are important in this regulation [22,34]. They are active when bound to GTP and inactive when bound to GDP, act as intracellular molecular switches and transduce signals from extracellular stimuli to the actin cytoskeleton. However, little is known about external cues that trigger the activation of Rho GTPases in spine formation and morphogenesis. Sema4D is such a candidate, because it and its receptor plexin-B1 are highly expressed in the brain during early postnatal period the synaptic formation stage [9,36], and mediate RhoA-signaling via direct association with PDZ (postsynaptic density-95/Discs large/zona occludens-1) domain-containing Rho guanine nucleotide exchange factors (PDZ- RhoGEFs) (positive regulators of RhoA activation), p190-Rho guanine nucleotide activating protein (RhoGAP) (a negative regulator of RhoA-signaling), and a RhoGTPase Rnd1 [3,13,23,26,33]. Here, we investigate whether Sema4D-plexin-B1 is involved in spine formation and morphogenesis of cultured hippocampal neurons via RhoA/Rho-kinase (ROCK) pathway.

Hippocampal neurons were cultured as described by Banker and Goslin [2] with modifications. In brief, the hippocampal tissues were dissociated from Sprague-Dawley rat embryos of embryonic day 18 (E18) (SLC), and digested in 10 ml of 4 U/ml papain (Worthington Biochem)/0.0015% DNase (Sigma)/0.02 M phosphate buffered saline (PBS)/0.2 mg/ml DL-cysteine HCl/0.2 mg/ml bovine serum albumin (BSA) (Sigma)/5 mg/ml glucose in 50 ml tube for 15 min at 37 °C.

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Suspended neurons were plated at 150 cells/mm^2 onto coverslips (Matsunami Glass), which were coated overnight with 2 mg/ml poly-L-lysine (Sigma) in 0.15 M borate buffer (pH 8.4). Neurons were cultured in Neurobasal medium (GIBCO)/B27 (GIBCO) and 25 μM L-glutamine. Two days after plating, the medium was exchanged with fresh Neurobasal/B27 every 3 days, and after 7 day-in vitro (DIV) one half of the medium was exchanged. Hippocampal neurons were transfected at 5-DIV with green fluorescent protein (GFP, Clonetech) together with or without a deletion mutant of plexin-B1ΔC (plexin-B1 lacking C-terminal three amino acids) by the calcium phosphate method as described [2,13]. Neurons were usually analyzed at 21-DIV.

Human embryonic kidney (HEK293) cells were cultured with 10% fetal bovine serum (FBS, Race, Australia)/Dulbecco's modified Eagle's medium (DMEM), and transfected with HA-tagged-human plexin-B1 (KIAA0407) [13], human plexin-B2 (KIAA0315) [13], and mouse plexin-A1 [15], and the cells were subjected to Western blot analysis. Sema4D-Fc (Fc-tagged extracellular region of mouse Sema4D) was obtained as described previously [8,9], and used as soluble Sema4D.

Polyclonal antibodies to plexin-B1 were raised by immunizing rabbits with maltose binding protein (MBP)-fused-mouse plexin-B1 (amino acid, 1216–1521, NM_17725). The specificity of the antibodies was confirmed by Western blot analysis. Mouse monoclonal antibody to HA was obtained from Boehringer–Mannheim, antibody to postsynaptic density protein (PSD-95) from Upstate Biotechnology and antibody to RhoA from Santa Cruz [13,14,37]. For Western blot analysis, primary antibodies were used at a dilution of 1:2000 (anti-plexin-B1, RhoA) and 1:10,000 (anti-HA), and for immunocytochemistry, anti-PSD-95 and anti-plexin-B1 were used at 1:500.

For Western blot analysis, cells were lysed in ice-cold lysis buffer (20 mM Tris–HCl pH 7.5, 1 mM EDTA–Na, 150 mM NaCl, 1% TritionX-100, 1 mM phenyl-methylsulfonyl fluoride (PMSF)), left on ice for 30 min, and the supernatant was pro-

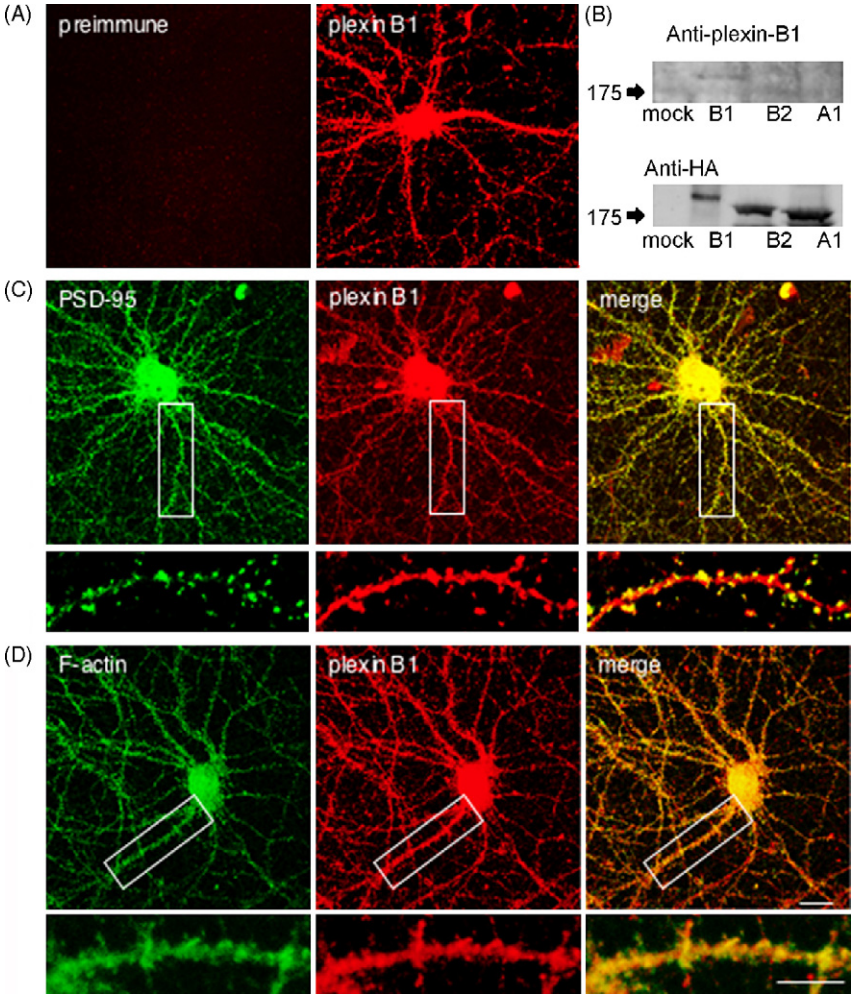

Fig. 1. Immunostaining for plexin-B1 in dendrites of cultured hippocampal neurons. (A) Cultured hippocampal neurons (21-DIV) were stained with the preimmune or the polyclonal antiserum to plexin-B1. (B) Western blot analysis shows the characteristics of the antiserum to plexin-B1. The antibodies recognize HA-tagged plexin-B1 (B1) but not plexin-B2 (B2) or plexin-A1 (A1) transfected in HEK 293 cells. (C and D) Immunofluorescent images of 21-DIV hippocampal neurons show localization of PSD-95 (green) and plexin-B1 (red) (C), and that of F-actin stained with FITC-phalloidin and plexin-B1 (red) (D). These images show colocalization of plexin-B1 with PSD-95 and with F-actin in dendritic spines. Scales: 10 μm (upper panels), 20 μm (lower panels). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)