The functions of Semaphorins in the neural development

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ABSTRACT

During embryogenesis, axons reach their specific targets correctly to form the complex neural network found in the mature functional nervous system. Several groups of axon guidance molecules such as semaphorins, ephrins, netrins, and slits have been reported to repel or attract the growing axons that express their cognate receptors. Semaphorins are secreted or transmembrane proteins, and found in both vertebrates and invertebrates. Semaphorins are initially identified as repulsive axon guidance molecules. However, recent many studies suggest that semaphorin family genes perform a variety of important biological functions besides the axon guidance.

Key words : Semaphorin, Neuropilin, Axon guidance, Repellent

INTRODUCTION

During embryogenesis, axons reach their targets correctly to form the complex neural network found in the mature functional nervous system. The tip of growing axon, the growth cone, is specialized for reacting to environmental cues during navigation. Many cell-adhesion molecules and extracellular matrix molecules have been thought to play an essential role as attractive cues for such growth cone guidance. Over the past decade, however, several studies have indicated the importance of repulsive guidance cues [1]. Several groups of axon guidance molecules such as semaphorins, ephrins, netrins, and Slits have been reported to repel or attract growing axons that express their cognate receptors [2].

Semaphorins are secreted or transmembrane proteins with a conserved domain of about 500 amino acids (aa), sema domain, and found in both vertebrates and invertebrates [3]. So far, more than 20 kinds of semaphorin genes have been identified and classified into seven classes and a virus semaphorin [4] (Fig. 1). Semaphorins in classes 1 (invertebrate) and 4-7 are transmembrane protein, whereas those in classes 2 (invertebrate), 3, and 8 (virally encoded) are secreted protein. Semaphorins are initially identified as repulsive axon guidance molecules. They have diverse functions in many

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physiological processes, including cardiogenesis, angiogenesis, vasculogenesis, tumor metastasis, osteoclastogenesis, and immune regulation [5]. Here, we review the functions of class 3 semaphorins in neural development.

The in vivo functions of Semaphorin 3A

Among semaphorins, semaphorin 3A (sema 3A) is the first identified semaphorin in vertebrates on the basis of its ability to induce the collapse of axonal growth cones of the dorsal root ganglion (DRG) [6]. However, the in vivo function of Sema3A remains largely unknown. In order to determine the role of axonal guidance by Sema3A, we generated Sema3A-deficient mice. They showed a severe abnormality in the axonal projection pattern in the peripheral nervous system (PNS) during embryogenesis [7]. Analysis of peripheral nerves in deficient embryos showed significant abnormality in the trajectory of several cranial nerves, including the trigeminal, facial, glossopharyngeal, vagus and accessory nerves, but no effects on the oculomotor nerve (Fig. 2). Sema3A has been hypothesized to act as a repulsive guidance cue for axons. Our demonstration of abnormal projection in Sema3A-deficient mice verified this idea.

Individual olfactory sensory neurons express a single type of odorant receptor [8]. Sensory neurons expressing a given type of odorant receptor converge their axons onto a few topographically fixed glomeruli in the olfactory bulb. Individual glomeruli presumably represent a single type of odorant receptor. Therefore, the spatial arrangement of the glomeruli at the olfactory bulb surface provides an odorant receptor map [9]. The glomeruli are formed mainly at perinatal and early postnatal periods, and the glomerular sensory map keeps a stereotyped spatial organization despite the continuous turnover of olfactory axons throughout animal's life [10]. Molecular mechanisms for the formation and maintenance of the precise glomerular sensory map remain largely unknown.

Sema3A is expressed also in the olfactory system. In the olfactory nerve layer of the developing olfactory bulb, Sema3A is expressed in

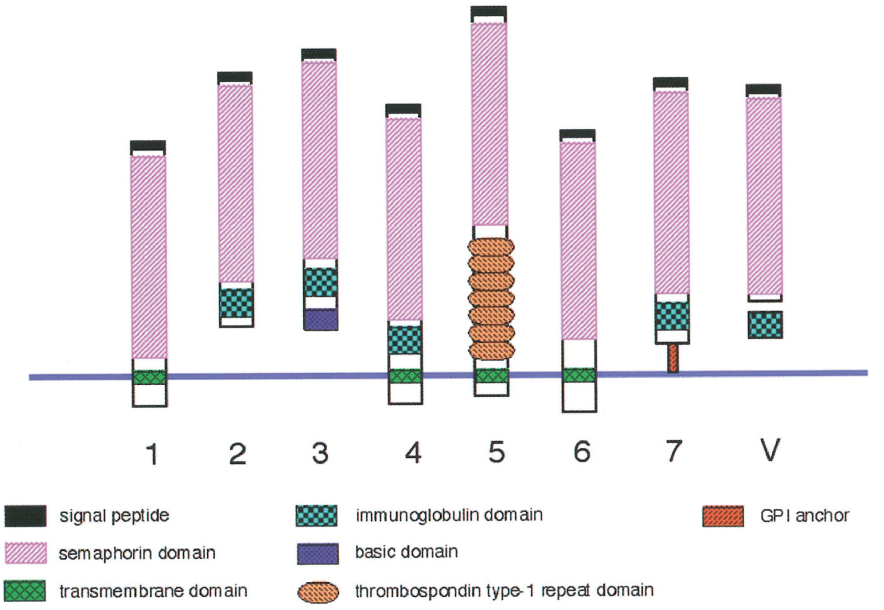

Figure 1. Semaphorins. Among the eight subclasses of semaphorins, class 1 and 2 semaphorins are found in invertebrates and class 3-7 are vertebrate semaphorins. Classes 2, 3 and viral (V) semaphorins are secreted, whereas class 4-6 are transmembrane proteins. Class 7 semaphorin represents GPI-anchored proteins.

ensheathing cells that are localized at the anteromedial and ventral regions [11]. Sema3A repels the growing olfactory axons that express Neuropilin-1, a Sema3A receptor [12, 13, 14, 15]. In adults the Neuropilin-1-expressing olfactory axons project selectively to the glomeruli within the medial and lateral bands of the olfactory bulb, and avoid the Sema3A-expressing regions[16]. In addition, Schwarting et al. (2000) showed that in the Sema3A-deficient embryos Neuropilin-1-expressing olfactory axons projected to the non-target regions [11]. These results suggest that Sema3A-mediated olfactory axon guidance plays a key role in the sensory map formation.

Sema3A repels growing olfactory axons that express Neuropilin-1, a receptor for Sema3 A. The Sema3A-mediated axon guidance seems to be essential for the formation of the glomerular sensory map in the olfactory bulb. To understand whether and how Sema3A is involved in the sensory map formation, we examined the glomerular map in the olfactory bulb of adult Sema3A-deficient mice [17]. In the wild-type mice Neuropilin-1-positive glomeruli form the lateral and medial bands and avoid the anteromedial and ventral regions of the olfactory bulb. In the Sema3A-deficient olfactory bulb Neuropilin-1-positive glomeruli spread over the entire olfactory bulb and we consistently found the ectopic arrangement of Neuropilin-1-positive glomeruli in the anteromedial and ventral regions. In addition, a specific subset of Neuropilin-1-negative and OCAM-positive glomeruli, especially those in the anteromedial region, disappeared from the mutant olfactory bulb. These results show a critical role for Sema3A in the spatial arrangement of glomeruli in the olfactory bulb. Optical

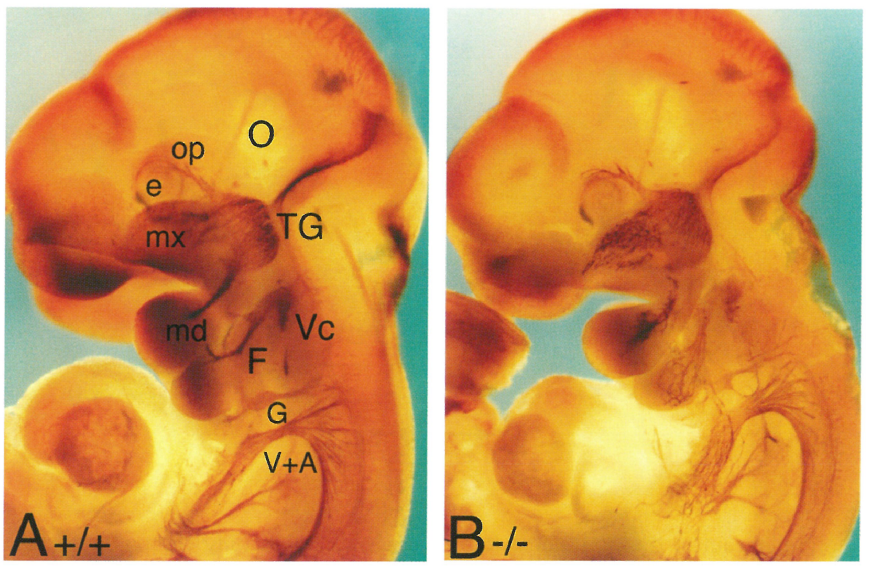

Figure 2. Abnormal nerve projections in cranial nerve tracts for Sema3A-deficient nutants at E10.5. Wild type (A) and homozygous embryos of null mutants (B) at E10.5, showing whole mount staining with anti-neurofilament antibody. (A) Ophthalmic (op), maxillary (mx), mandibular (md), facial (F) and glossopharyngeal (G) nerves in wild type embryo is indicated. (B) In homozygous mutants, these nerves extend in correct direction to their targets. However, on the way to the target, these nerves defasciculate and occupy wider area. Vestibulocochlear nerve (Vc) looks like normal in homozygous embryos, although it seems to be slightly thick and spread. Aberrant projections are occasionally seen further beyond the otic vesicle. On the other hand, no abnormality is seen in oculomotor nerve (O). (A) Vagus (V) and accessory nerves (A) in wild type embryo is indicated. (B) In homozygous embryos terminals of vagus and accessory nerves spread out. e, eye ; TG, trigeminal ganglion.

imaging from the dorsal olfactory bulb showed that the distorted glomerular map conserved molecular-feature domains. However, the positions of the domains were shifted, which suggests a secondary rearrangement of the glomerular map in the Sema3A-deficient olfactory bulb (Fig. 3).

Semaphorins and their receptors

We produced Neuropilin-1-deficient mice and found that the PNS efferents of the mutants exhibited abnormalities similar to the Sema3A mutants [14]. The similarity in the phenotype between the Neuropilin-1 and Sema3A mutants suggests that Neuropilin-1 function is correlated with the chemorepellent Sema3A. To test this possibility, we cultured the explants of DRG of embryos at E12.5 in the presence of nerve growth factor (NGF). When the culture supernatant contained Sema3A was added to the DRG cultures of either the wild-type or heterozygous embryos, 93%-95% of the growth cones collapsed, while no collapse was induced on growth cones of the neuropilin-1 mutant embryos (Fig. 4). Sema3A binds Neuropilin-1 [12, 13]. These results suggest that Neuropilin-1 is a functional Sema3A receptor.

Neuropilins (NPs) are functional receptors for class 3 semaphorins and plexin-As are co-receptors for class 3 semaphorins [18, 19]. Plexins are also known as receptors for other types of semaphorins [19]. Most membrane-bound semaphorins directly bind plexins. However, increasing evidence has shown that semaphorin receptor usage is more complex than previously thought. For example, Sema3E signals independently of Neuropilins through Plexin-D1 [20] while Sema7A uses integrins to exert its functions in

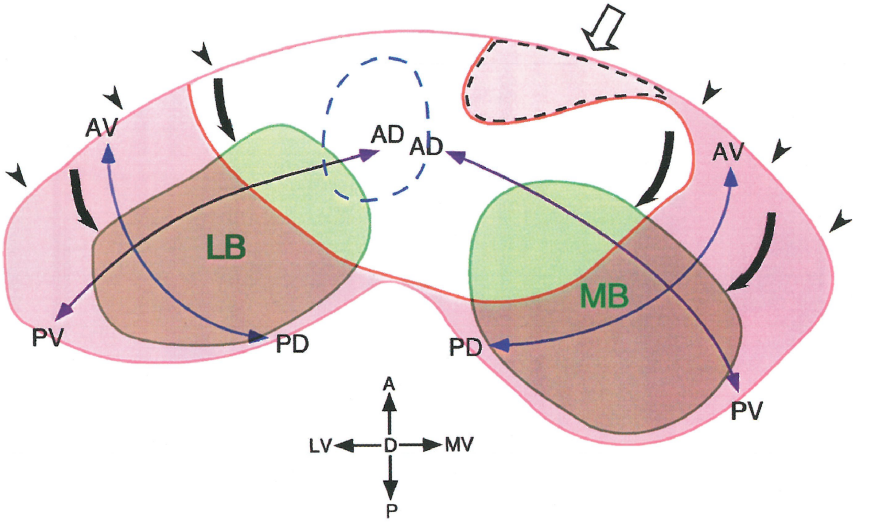

Figure 3. A schematic diagram of the flattened left olfactory bulb illustrating the possible role of Sema3A in the formation of the olfactory sensory maps. Blue broken line indicates the region from which we recorded the optical imaging of intrinsic signals. The OCAM-positive ($OCAM^+$) zones are colored by pink, while $NP\text{-}1^+$ bands are shown green. In the wild-type mice, $NP\text{-}1^+$ glomeruli are excluded from the ventral region (filled arrow heads) and the anteromedial region (an open arrow) where Sema3A is expressed. In the Sema3A-deficient olfactory bulb, $NP\text{-}1^+$ glomeruli spread over the entire olfactory bulb including the ventral and anteromedial regions. The alteration suggests that Sema3A functions in determining the position of $NP\text{-}1^+$ glomeruli along the posterodorsal (PD)-anteroventral (AV) axis pushing them away from the ventral region (filled arrows). In addition, Sema3A-deficient mice lacked a specific subset of $OCAM^+$/ $NP\text{-}1^-$ glomeruli including those of the tongue-like area (surrounded by a black broken line and indicated by an open arrow). A, anterior; P, posterior; D, dorsal; LV, lateroventral; MV, medioventral; AD, anterodorsal; PV, posteroventral; LB, lateral band containing $NP\text{-}1^+$ glomeruli; MB, medial band containing $NP\text{-}1^+$ glomeruli.

both the nervous and immune systems [21,22]. In addition, two molecules unrelated to plexins and neuropilins, CD72 [23] and T-cell immunoglobulin and mucin domain containing protein 2 (TIM-2)[24], functionally interact with Sema4D and Sema4A, respectively, in the immune system. Plexins are canonical semaphorin receptors that have large cytoplasmic domains. In the nervous system, plexin-mediated signals have been shown to exert diverse neural functions by regulating GTPase activities and cytoplasmic/receptor-type protein kinases [25]. These signals are also involved in integrin- mediated attachment. Of note, Plexins can associate with different co-receptors in distinct tissues to allow semaphorins to exert pleiotropic functions. For instance, plexin-A1 is associated with the tyrosine kinase receptors off-track (OTK) and vascular endothelial growth factor receptor 2 (VEGFR2) in heart morphogenesis [26]. Furthermore, plexin-B1 has been shown to associate with the receptor tyrosine kinases Met and ErbB2, inducing invasive growth of epithelial cells. These observations provide insight into the diversity of semaphorin functions.

The function of Sema3A in axonal regeneration

Several factors inhibit axonal regeneration after central nervous system (CNS) traumas such as spinal cord injury (SCI). Myelin-associated proteins, such as Nogo-A, myelin-associated glycoprotein (MAG) and oligodendrocyte-myelin glycoprotein (OMgp), have a central role in the inhibition of axonal regeneration. Neutralization of Nogo receptor (NgR) signaling leads to marked axonal regeneration and functional recovery after SCI. In contrast, studies using Nogo knockout mice, NgR knockout mice and other models suggest that the blockade of axonal growth inhibitors other than myelin-associated proteins may also be important for axonal regeneration [27,28].

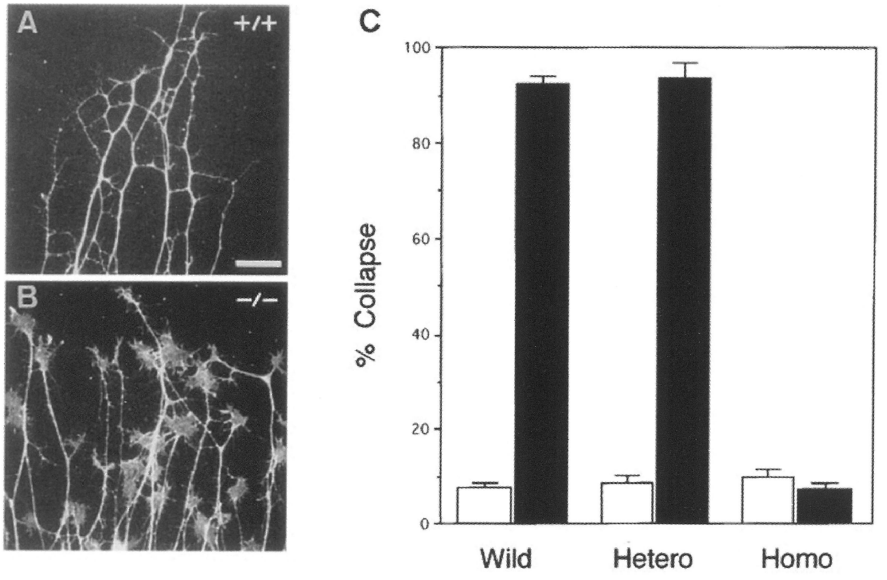

Figure 4. Comparison of effects of recombinant Sema3A on normal and Neuropilin-1-deficient DRG growth cones. (A and B) Morphology of DRG growth cones of the wild-type and homozygous neuropilin-1 mutant embryos in vitro, treated with a sufficient amount of recombinant Sema3A (the culture supernatant of COS7 cells transfected with sema3A cDNA). The cultures were labeled with fluorescein-conjugated Con A. (C) Average percentages of collapsed DRG growth cones treated with a sufficient amount of recombinant Sema3A (filled columns) of the wild-type embryo (Wild), heterozygous embryos (Hetero), and homozygous mutants (Homo). Open columns indicate the percentage of collapsed DRG growth cones without recombinant Sema3A of the wild-type embryos, heterozygous embryos, and homozygous mutants. Vertical bars indicate the SEM.

Among these factors, extracellular matrix molecules are likely to be important for the inhibition of axonal regrowth after SCI. CNS injury results in scar tissue formation at the injury site, which contains extracellular matrix molecules such as chondroitin sulphate proteoglycans (CSPGs). CSPGs are crucial as axonal growth inhibitors: enzymatic degradation of CSPGs results in axonal regeneration and functional recovery [29]. Sema3A is another extracellular matrix molecule that may contribute to the inhibition of axonal regeneration by acting on microtubules and the actin cytoskeleton. In models of conditioning peripheral nerve injury, regenerating DRG axons halt selectively at Sema3A-enriched regions in the lesion site. Thus, Sema3A may be a crucial extracellular factor that inhibits axonal regeneration after CNS injury. Neutralization of this molecule may hence lead to axonal regeneration and functional recovery after SCI. The high lethality of Sema3 A-deficient mice, however, has prevented close and large-scale genetic analyses of the contribution of Sema3A to the inhibitory environment of the injured spinal cord. An alternative method for large-scale analysis is to use a pharmacological approach.

Recently, we identified a small molecular agent, SM-216289, from a fungal extract, which can strongly inhibit Sema3A functions in vitro, including growth cone collapse and chemorepulsion of neurite extension [30]. SM-216289 strongly inhibits Sema3A functions in vitro and SM-216289 acts directly on Sema3A to inhibit the binding of Sema3A to neuropilin-1, putatively by changing its steric structure.

The current study has two major findings: first, that Sema3A contributes considerably to the inadequate axonal regeneration at the spinal cord lesion site after transection injury; and second, that SM-216289 strongly inhibits the Sema 3A signal both in vivo and in vitro, and is an effective promoter of regenerative responses including axonal regeneration and/or preservation, Schwann cell-mediated myelination and axonal regeneration, angiogenesis, and the inhibition of apoptosis after spinal cord transection [31].

To achieve further axonal regrowth, including the regeneration of long fiber tracts, the concomitant use of SM-216289 with other therapeutic modalities such as neurotrophic factors, soluble Nogo receptors, chondroitinase ABC, or tissue or cell transplantation, could potentially be beneficial. As a model for SCI, the transection protocol is appropriate for assessing axonal regeneration, but it does not fully parallel SCI in the clinical setting. In contrast, the contusion model may be more representative of human SCI because it reveals features similar to those seen in the clinical context. Therefore, future studies should focus on the concurrent use of SM-216289 with other treatments, its application to contusion lesions, or its application to nonhuman primates. In conclusion, this study demonstrates that Sema3A plays an essential role in regenerative failure (including the inhibition of axonal regeneration) after SCI and that SM-216289 may be a possible therapeutic agent for human SCI.

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