Chapter 10
Semaphorins and Neurodegenerative Disorders

Tatsusada Okuno and Yuji Nakatsuji

Abstract Neurodegenerative disorders are characterized by progressive dysfunction or death and structural abnormalities of neurons. A number of diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS) that result from neurodegenerative processes are included in this category. Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS). However, accumulating evidence indicates that a neurodegenerative process participates in the pathogenesis, particularly in the progressive stage of the disease. Although various causes of neurodegenerative disorders, including genetic mutations, protein abnormalities, and inflammation, have been reported, changes in neural connectivity, loss of synaptic contacts and activity, and inflammatory reactions in glial cells are common features of these disorders. It has been suggested that aberrant semaphorin expression may result in altered neuronal connectivity or synaptic function and inflammation associated with a number of degenerative neuronal disorders. This role of semaphorins has been currently suggested in the pathogenesis of AD, ALS, and MS (Table 10.1).

Keywords Alzheimer's disease • Parkinson's disease • Amyotrophic lateral sclerosis • Multiple sclerosis • Experimental autoimmune encephalomyelitis

10.1 Semaphorins and Amyotrophic Lateral Sclerosis (ALS)

10.1.1 ALS

Amyotrophic lateral sclerosis (ALS) is a devastating disease characterized by progressive loss of motor neurons in the brain and spinal cord. Initial symptoms include weakness in the limbs and/or difficulties with speech and swallowing caused by weakness in the bulbar region (Rowland 1998). Patients eventually become paralyzed and die of respiratory failure if they are not maintained on a ventilator approximately 3 years after the onset of symptoms (Rowland and Shneider 2001).

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Table 10.1 The roles of semaphorins and their receptors in neurodegenerative diseases

Semaphorins	Binding partners	Related disease	Expression	Functions
Sema3A	NP-1/Plexin-As	ALS	TSC in NMJ	Repulsion of motor axons in NMJ
		AD	Neurons in CA1 and subiculum	Phosphorylation of CRMP2
		MS	Neurons, astrocytes, microglia	Inhibition of OPC recruitment toward the lesions
		EAE	EC, T cells	Migration of immune cells
Sema3F	NP-2/Plexin-As	MS	Neurons, astrocytes, microglia	Recruitment of OPCs toward the lesions
Sema4A	Plexin-Bs, TIM2	MS/EAE	DC, Th1	Th1/Th17 differentiation
Sema4D	Plexin-B1, CD72	EAE/MS	T cells	Activation of DC and microglia
Sema5A	Plexin-B3	PD	Unknown	Unknown
Sema6D	Plexin-A1	EAE	T cells	Activation of DC
Sema7A	α1β1 integrin	EAE	T cells	Activation of macrophages

NP neuropilin, *ALS* amyotrophic lateral sclerosis, *AD* Alzheimer's disease, *MS* multiple sclerosis, *EAE* experimental autoimmune encephalomyelitis, *PD* Parkinson's disease, *TSC* terminal Schwann cell, *NMJ* neuromuscular junction, *EC* endothelial cell, *DC* dendritic cell, *OPC* oligodendrocyte precursor cell

Approximately 5 % to 10 % of patients with ALS inherit the disease, which is described as familial ALS (FALS) (Beghi et al. 2006; Mitchell and Borasio 2007). Currently, riluzole, a putative glutamate receptor antagonist, is the only available drug approved for the treatment of ALS, yet its efficacy is limited (Radunović et al. 2007). Sporadic ALS (SALS) is considered as a complex multifactorial disease with an interaction of genetic and environmental factors affecting disease susceptibility and clinical expression (Siddique and Siddique 2008). Risk factors include age, sex, smoking, mechanical and electrical trauma, professional and environmental exposure to metals and herbicides, and heavy physical activity (Horner et al. 2003; Beghi et al. 2006; Sutedja et al. 2007). In addition, several pathways have been implicated in the pathogenesis of SALS, such as glutamate-mediated excitotoxicity, mitochondrial dysfunction, neuroinflammation, oxidative stress, protein aggregation, aberrant axonal transport, and abnormality in RNA-binding proteins (Wang et al. 2004; Shaw 2005; Pasinelli and Brown 2006; Van Deerlin et al. 2008; Yokoseki et al. 2008). Up to 20 % to 25 % of FALS patients exhibit mutations in the Cu/Zn superoxide dismutase-1 (SOD1) gene (Rosen et al. 1993; Cudkowicz et al. 1997). At present, more than 120 mutations in SOD1 have been reported in FALS patients. Transgenic mice overexpressing the mutant human SOD1 gene develop progressive motor neuron degeneration that resembles ALS; therefore, these mice serve as an appropriate animal model for the disease (Gurney et al. 1994). By utilizing this model, the pathogenesis of ALS has been analyzed.

10.1.2 ALS and the Neuromuscular Junction (NMJ)

Interestingly, recent data have suggested that pathological changes in the lower motor axons and nerve terminals precede motor neuron loss and onset of clinical symptoms (Frey et al. 2000; Fischer et al. 2004; Pun et al. 2006). These findings suggest that the pathological change may be initiated distally at the nerve terminal or NMJ and spread toward the cell body. In addition, aberrant expression of axon-guidance proteins has recently been thought to contribute to the pathological changes in motor neuron connectivity in ALS. Axon guidance proteins are important in regulating motor axon pathfinding during development, and they also influence axonal transport and synaptic function (Pasterkamp and Giger 2009). The expression of several different axon guidance molecules is changed in ALS patients and model mice (Lesnick et al. 2007, 2008; Pradat et al. 2007). Consistently, single-nucleotide polymorphisms (SNPs) in semaphorins and other genes encoding axon-guidance molecules in ALS patients are reported to be of diagnostic value for disease susceptibility, onset, and severity (Lesnick et al. 2007, 2008; Pradat et al. 2007). Therefore, semaphorins may be genetic risk factors for ALS. Together, these data suggest that abnormal axon-guidance protein expression or function may contribute to the pathological changes in motor axons and nerve terminals associated with ALS.

10.1.3 ALS and Sema3A

Progressive loss of motor neurons and subsequent muscle innervation are pathological hallmarks in the mSOD1 transgenic mouse model for ALS (Gurney et al. 1994; Wong et al. 1995). In this process, terminal Schwann cells (TSC) in NMJs come to express Sema3A NMJs in the muscles of G93A-hSOD1 mice (transgenic mice overexpressing the familial ALS-associated G93A SOD1 mutation harboring a single glycine-to-alanine substitution at codon 93) (De Winter et al. 2006). The expression of Sema3A in NMJ is not ubiquitous throughout the entire muscle sections; it is mainly located in the lateral regions of the gastrocnemius muscle. Intriguingly, most Sema3A-expressing TSCs are located on type IIb/x fibers in the gastrocnemius muscles of G93A-hSOD1 mice. When the number of Sema3A-positive TSC clusters per muscle fiber subtype was quantified, Sema3A expression was increased but restricted to IIb and IIx muscle fibers throughout the course of G93A-hSOD1 mice. The number of Sema3A-positive endplates on type IIb and IIx muscle fibers was significantly higher just before and during the onset of the disease than in the more progressive and end stages. Type IIb and IIx motor units are fast-fatigable fiber types, and fast-fatigable neuromuscular synapses are susceptible to early loss in motor neuron diseases. Type IIb and IIx muscle fibers are the first muscle subtype that is lost in ALS because nerve sprouting after injury does not occur in these subtypes of muscle fibers (Pinter et al. 1995; Frey et al. 2000;

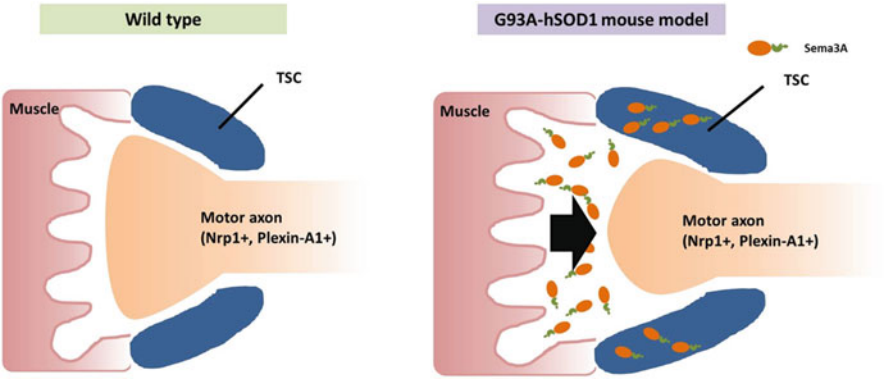

Fig. 10.1 Neuromuscular junction (*NMJ*) of ALS model mice (G93A-hSOD1 mice). Sema3A expression in terminal Schwann cells (*TSC*) is markedly increased. This increase is specifically limited to endplates on type IIb and IIx muscle fibers. Increased expression of Sema3A in TSCs may cause the dissociation or repulsion of motor axons at the NMJ, eventually resulting in axonal denervation and motor neuron degeneration

Pun et al. 2006). Therefore, it is plausible that increased expression of Sema3A in TSCs causes the dissociation or repulsion of motor axons at the NMJ, eventually resulting in axonal denervation and motor neuron degeneration (Fig. 10.1).

10.2 Semaphorins and Alzheimer's Disease (AD)

10.2.1 AD

Alzheimer's disease (AD) is the most common cause of senile dementia, affecting 35 million individuals worldwide. The most common initial manifestation is the disturbance of recent memory (Waldemar et al. 2007; Blennow et al. 2006). As the disease progresses, patients exhibit various symptoms, including deficits in language and executive function. Psychotic behavior, delusions, and hallucinations increase according to disease progression. Cholinesterase inhibitors have been approved to improve these symptoms; however, drug efficacy remains limited and is not sufficient to cure AD (Hansen et al. 2008). Accordingly, the brain function of AD patients is gradually lost, ultimately leading to death (Molsa et al. 1986, 1995).

Although the cause of AD remains incompletely understood, the pathological hallmarks of AD are amyloid plaques and neurofibrillary tangles (NFTs) in the brain (Tiraboschi et al. 2004; Wenk 2003). Amyloid plaques comprise dense, mostly insoluble deposits of beta-amyloid (Aβ), a fragment of amyloid precursor protein (APP), outside and around neurons (Hardy and Allsop 1991; Mudher and Lovestone 2002; Hsiao et al. 1996). NFTs are aggregates of the microtubule-associated

protein tau, which becomes hyperphosphorylated and accumulates inside the cells. Although Aβ and tau are the components in plaque and NFT, respectively, they have also been postulated to be the fundamental cause of AD. Mutations in genes that alter Aβ protein production, aggregation, or clearance, such as APP and presenilins, cause the early-onset forms of AD, suggesting that Aβ contributes to AD pathogenesis (Waring and Rosenberg 2008; Hsiao et al. 1996). In addition, Aβ at low doses inhibits activity-dependent synaptic transmission and is neurotoxic at higher doses. However, tau protein abnormalities supposedly initiate the disease cascade (Mudher and Lovestone 2002; Haass and Selkoe 2007). Hyperphosphorylated tau causes dysfunction of the neuronal network and neuronal death by causing disintegration of microtubules and collapse of the transport system (Goedert et al. 1991; Iqbal et al. 2005; Chun and Johnson 2007).

Presumably because of the neurotoxic effects of Aβ and hyperphosphorylated tau, there is loss of neurons and synapses in the cerebral cortex and certain subcortical regions in AD. Among various fields in the brain, the hippocampal formation is known as a selectively vulnerable subfield (Braak and Braak 1991; Price et al. 1991) in the early pathogenesis of AD. CA1 and the subiculum initially show neurodegenerative changes during the incipient phases of AD. The most vulnerable neurons of the hippocampus are the pyramidal cells of the CA1 field and subiculum, although neurons in the CA3 field are resistant to neurodegeneration. Furthermore, the dentate gyrus rarely exhibits any degenerative changes in AD. This differential vulnerability of neurons underlies the mechanisms leading to neuronal degeneration in AD.

10.2.2 AD and Sema3A

Evidence of the involvement of semaphorins in AD is provided by the fact that an isolated multiprotein complex from the brain tissue of AD patients contains the phosphorylated microtubule-associated protein (MAP) 1B, Sema3A, CRMP-2, plexin-A1, and plexin-A2 (Good et al. 2004). Interestingly, AD brain tissue includes a hyperphosphorylated form of CRMP-2 that shows increased phosphorylation on both Ser522 and Thr509 residues (Uchida et al. 2005). Phosphorylation of CRMP-2 at Ser522 and Thr509 by cyclin-dependent kinase-5 (Cdk-5) and glycogen synthase kinase-3 beta (GSK-3b), respectively, decreases its interaction with tubulin and is required for repulsion of neurites by Sema3A (Uchida et al. 2005; Yoshimura et al. 2005; Good et al. 2004). Because Sema3A is aberrantly produced and released in the subiculum, and then taken up and transported to CA1 in the early stage of AD (Good et al. 2004) (Fig. 10.2), Sema3A may contribute to the neurodegeneration of AD by inducing neural collapse or enhancing phosphorylation of CRMP2. Interestingly, Aβ, the most important component of senile plaques in the AD brain, decreases neurite length in vitro and regulates the phosphorylation of CRMP-2 through a RhoA GTPase-dependent mechanism (Petratos et al. 2008). Therefore, there may

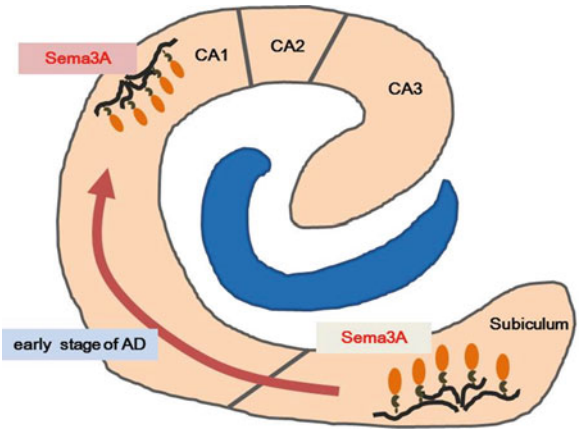

Fig. 10.2 Aberrant expression of Sema3A in AD hippocampal formation. In the early stage of Alzheimer's disease (AD), Sema3A is aberrantly produced and released in the subiculum, and then taken up and transported to CA1. As a result, Sema3A accumulates in the soma of CA1 neurons. Sema3A may contribute to the neurodegeneration of AD by inducing neural collapse or enhancing phosphorylation of CRMP2

be crosstalk between Sema3A and Aβ in the context of neural repulsion. In AD patients, Aβ aggregation induces hyperphosphorylation of CRMP-2, which may then lead to changes in Sema3A-induced modulation of microtubule dynamics (Fukata et al. 2002).

10.3 Semaphorins and Parkinson's Disease (PD)

10.3.1 PD

Parkinson's disease (PD) is a common neurodegenerative disorder, and its prevalence is approximately 160 per 100,000. PD is biochemically characterized by the degeneration of the nigrostriatal dopamine system, which results in a marked loss of striatal dopamine content, and is pathologically characterized by the loss of dopaminergic neurons in the substantia nigra and the presence of neuronal intracellular Lewy bodies. The clinical features of PD are manifested by resting tremors, rigidity, bradykinesia, and postural instability (Calne et al. 1992). Both environmental and genetic factors contribute to the development of PD, but the exact mechanism has not yet been identified. Mutations of several genes such as α-synuclein, parkin, LRRK2, and PINK1 have been identified as causes of familial PD (Polymeropoulos et al. 1997; Valente et al. 2001; Nichols et al. 2005).

10.3.2 PD and Sema5A

Semaphorin 5A is a transmembrane protein belonging to the semaphorin protein family. The extracellular domain of Sema5A contains seven thrombospondin (TSP)

type-1 repeats in addition to the sema domain (Adams et al. 1996). It is essential for the development of extra-embryonic tissues and the cardiovascular system, and it can elicit multiple differentiation, cell–cell signaling, and nervous system development through its functional receptor plexin-B3 (Oster et al. 2003; Pineda et al. 2005; Artigiani et al. 2004). In the nervous system, SEMA5A is expressed by oligodendrocytes and inhibits axonal growth (Goldberg et al. 2004).

Analysis of SNPs in semaphorins and other genes encoding axon-guidance molecules in PD patients was reported to be of diagnostic value for disease susceptibility, onset, and severity. These data suggest that mechanisms involved in axonal maintenance and repair can participate in the pathogenesis of PD. Therefore, semaphorins have been suggested to be associated with PD. In this context, one study recently showed that an SNP, rs7702187, within Sema5A was associated with the disease susceptibility of PD (Maraganore et al. 2005). Other groups evaluated rs7702187 and other SNPs of Sema5A in two independent case-control series from Finland and Taiwan and found that rs7702187 was associated with a decreased risk whereas rs3798097 was associated with an increased risk of PD in the Taiwanese population, but not in the Finland population (Clarimon et al. 2006). However, conflicting results were obtained from a case-control study of Polish Caucasians and Asians from Singapore. They concluded that rs7702187 was not a marker of PD risk (Bialecka et al. 2006). Another genome-wide association study, wherein most participants were of Caucasian ethnicity, did not support the data of Maraganore et al. (2005) and Elbaz et al. (2006). Further investigations are required to clarify the significance of Sema5A as a disease-susceptible gene in PD.

10.4 Semaphorins and Multiple Sclerosis (MS)

10.4.1 Multiple Sclerosis (MS)

Multiple sclerosis (MS) is a demyelinating autoimmune disease and a leading cause of neurological disabilities in young adults (Noseworthy et al. 2000; Compston and Coles 2002). Both genetic and environmental factors are supposed to participate in the pathogenesis of MS, and it is developed when genetically predisposed individuals are exposed to an environmental trigger that stimulates myelin-specific T cells (McFarland and Martin 2007). Genome-wide association studies revealed that the IL-2 receptor, IL-7 receptor, and HLA class II are associated with disease susceptibility (Lincoln et al. 2005; Hafler et al. 2007; Fugger et al. 2009). Recent evidence suggests that Th17 lymphocytes play crucial roles in MS in addition to Th1 cells (Bettelli et al. 2007; Tzartos et al. 2008; Sospedra and Martin 2005). Therefore, antigen presentation and subsequent $CD4^+$ T-cell activation and differentiation are essential steps for the development of MS, and it has been characteristically classified as an immune-mediated disorder. However, it also has characteristics of a neurodegenerative disease. MS is a heterogeneous disease and is

pathologically classified into four patterns (Lucchinetti et al. 2000, 2004). Although patterns I and II show T-cell-mediated or T-cell plus antibody-mediated autoimmune pathology, patterns III and IV are suggestive of oligodendrocyte apoptosis or primary oligodendrocyte dystrophy rather than autoimmunity. Axonal damage is observed in a relatively early phase of the disease course (Trapp et al. 1998; Trapp and Nave 2008). In the chronic phase of the disease, the disability persistently progresses without recovery. These features are observed in secondary progressive MS and are thought to arise primarily from neuronal or axonal damage (Bjartmar et al. 2000). Therefore, the neurodegenerative process seems to participate in MS pathology, and its involvement increases in accordance with disease duration.

10.4.2 Experimental Autoimmune Encephalomyelitis (EAE)

Experimental autoimmune encephalomyelitis (EAE) is a representative animal model for MS. It is believed to reproduce many of the clinical and histopathological features of the human disease and is induced by immunizing myelin proteins such as myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), and proteolipid protein (PLP) in susceptible animals together with an adjuvant. EAE can also be induced by the passive transfer of myelin antigen-reactive T cells (Mix et al. 2008).

10.4.2.1 Sema3A

Sema3A directly binds to neuropilin-1, which induces the activation of plexin-A proteins and the transduction of axon-guidance signals. In the immune system, plexin-A1 is expressed in dendritic cells (DCs), and Sema3A/neuropilin-1 (NP-1)/plexin-A1 interactions promote DC migration to the draining lymph nodes after immunization. Because of impaired migration of DC, antigen-specific T-cell priming is inhibited in plexin-A1-deficient mice (Takamatsu et al. 2010). Accordingly, plexin-A1-deficient mice exhibit less severe EAE induced by MOG peptide, with impaired MOG peptide-specific CD4+ T-cell responses (Takegahara et al. 2006).

10.4.2.2 Sema4D

Sema4D is highly expressed in T cells, playing an important role in the activation of T cells by causing maturation of DCs. Regarding the Sema4D receptors, plexin-B1 and CD72 have been identified in the nervous and immune systems (Tamagnone et al. 1999; Kumanogoh et al. 2000). Sema4D-deficient mice exhibit attenuated EAE with impaired antigen-specific T-cell responses (Kumanogoh et al. 2002b). In addition to the priming phase, Sema4D on encephalitogenic T cells also directly

activates microglia through plexin-B1. When MOG-reactive CD4+ T cells prepared from wild-type mice were adoptively transferred into plexin-B1-deficient mice or bone marrow chimera mice with plexin-B1-deficient central nervous system (CNS) resident cells, the development of EAE was considerably improved (Okuno et al. 2010). Consistent with this, anti-Sema4D blocking antibodies were effective after the onset of EAE (Okuno et al. 2010). In addition, Sema4D in T cells causes the collapse of process extensions in immature oligodendrocytes and the death of immature neural cells (Giraudon et al. 2004). Collectively, these findings indicate that T cell-derived Sema4D is important in both the priming and effector phases of EAE.

10.4.2.3 Sema4A

Sema4A is expressed in dendritic and Th1 cells and is important in the activation of Th cells and the differentiation of Th1 and Th17 cells (Kumanogoh et al. 2002a, 2005). Consistently, the development of MOG-induced EAE in wild-type mice can be improved by intravenous injection of an anti-Sema4A monoclonal antibody concurrently with MOG immunization. The infiltration of mononuclear inflammatory cells into the spinal cord is diminished in anti-Sema4A antibody-treated mice, in which CD4+ T cells isolated from the draining lymph nodes have markedly decreased responses to the MOG peptide (Kumanogoh et al. 2002a).

10.4.2.4 Sema6D

Sema6D directly interacts with plexin-A1 independently of NP-1 and activates the plexin-A1–DAP12–TREM2 complex (Toyofuku et al. 2004; Steinman 2004; Takegahara et al. 2006). Plexin-A1-deficient mice exhibit milder severity when EAE is induced (Takegahara et al. 2006). Similarly, DAP12-deficient mice exhibit attenuated development of MOG-induced EAE and impaired generation of MOG-specific T cells (Bakker et al. 2000). Therefore, Sema6D is suggested to contribute to the resistance to EAE in plexin-A1-deficient mice by activating signals downstream of the DAP12/TREM2 complex.

10.4.2.5 Sema7A

Sema7A is a membrane-associated glycosyl phosphatidylinositol (GPI)-linked protein. In the nervous system, Sema7A has been shown to promote olfactory bulb axon outgrowth and is required for the appropriate formation of the lateral olfactory tract during embryonic development. Although plexin-C1 was initially identified as a receptor for Sema7A (Tamagnone et al. 1999), Sema7A has an arginine-glycine-aspartate sequence in its Sema domain, which is a well-conserved integrin-binding

motif, and Sema7A attracts axons through the β1-integrin receptor, not through plexin-C1, by activating the downstream mitogen-activated protein kinase pathway (Pasterkamp et al. 2003).

T cell-derived Sema7A is involved in inflammation by activating macrophages in the inflammatory lesion (Suzuki et al. 2007). Sema7A on antigen-primed effector T cells plays a role in inducing inflammation in EAE through interactions with α1β1 integrin and contributes to the exacerbation of EAE. These data indicate that Sema7A is involved in the pathogenesis of EAE in the effector phase.

10.4.3 Sema3A and Sema3F in MS

The presence of demyelinated plaques in the CNS is the central pathology of MS. One of the most important causes of permanent damage is the disturbance of remyelination. A possible reason is the lack of migration of oligodendrocyte precursor cells (OPCs) to the lesion. Sema3A and Sema3F transcripts are upregulated in grey matter neurons, astrocytes, and the microglial cells around active inflammatory lesions in patients with MS (Williams et al. 2007). Piaton et al. reported the role of Sema3A and Sema3F in remyelination in the adult CNS, showing that they exert opposite effects in the recruitment of adult OPCs to CNS lesions in a toxin-induced demyelination model (Piaton et al. 2011). The expression of Sema3F and its receptor Np-2 is upregulated following demyelination, guiding the recruitment of OPCs toward the lesion. In this context, the overexpression of Sema3F enhances early remyelination and accelerates myelin repair. In contrast, the expression of Sema3A and Np-1 is delayed and inhibits remyelinating precursor cell recruitment (Piaton et al. 2011). These data suggest that Sema3A may act as a stop signal for OPC recruitment after an adequate number of precursor cells have been attracted to the lesion by Sema3F. Accordingly, an inhibitory role for Sema3A in remyelinating processes is identified (Syed et al. 2011), indicating a block of OPC differentiation in the presence of Sema3A. These observations support the idea that aberrant expression of Sema3A and Sema3F in the CNS may underlie impaired OPC recruitment and differentiation in MS lesions, ultimately limiting myelin repair.

10.4.4 Sema4A in MS

It is not surprising that Sema4A deeply participates in MS development in addition to EAE because both Th1 and Th17 cells are suggested to be involved in human MS pathogenesis (Stromnes et al. 2008).

Serum Sema4A levels are significantly higher in patients with MS than in those with other neurological diseases (ONDs) and healthy volunteers when assayed by enzyme-linked immunosorbent assay (ELISA) (Nakatsuji et al. 2012). Approximately one fourth of patients with relapsing-remitting (RR) MS have extremely

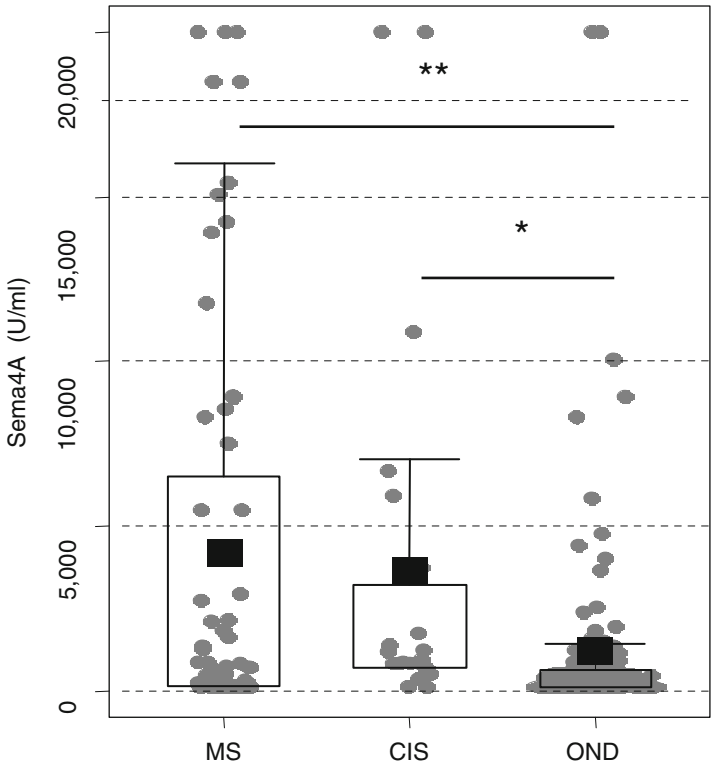

Fig. 10.3 Elevated serum Sema4A levels in multiple sclerosis (MS) patients. Serum Sema4A levels were significantly increased in MS and clinically isolated syndrome (CIS) patients compared to OND patients. The levels of serum Sema4A were assayed by ELISA in relapsing-remitting MS (*RRMS*) patients in the remitting phase, CIS patients, and age- and gender-matched other neurological disease (OND) patients. The *black squares* show the means. The *top* and *bottom* of the box in the box-and-whisker plot indicate the 25th and 75th percentiles, respectively, and the end of the whisker represents 1.5 times the interquartile range from the *top* of the box or the maximum point of all the data. $*p < 0.05$; $**p < 0.01$

high Sema4A levels. Furthermore, serum Sema4A levels of patients with clinically isolated syndrome (CIS), which is considered to be an early stage of MS, are as high as those in patients with MS and significantly higher than those in patients with ONDs. Therefore, Sema4A levels are increased in the early stage of MS (Fig. 10.3).

With regard to the source of serum Sema4A, T and B cells show very low expression. $CD11c^+$, $HLADR^+$ monocytes, and dendritic cells (DCs) from healthy donors express moderate amounts of Sema4A, and significantly increased expression is observed on these cells from patients with MS.

Sema4A is suggested to be released from the cell surface by metalloproteinases because its release can be inhibited by protease inhibitors such as the metalloproteinases ADAM and matrix metalloproteinases (MMPs). An MMP inhibitor,

phosphoramidon, and light metal chelators also inhibit Sema4A shedding (Nakatsuji et al. 2012). The mRNAs for metalloproteinases, including ADAM 10 and MMPs, are increased in peripheral blood mononuclear cells (PBMCs) from MS patients with high serum Sema4A levels compared with those in MS patients with low serum Sema4A levels and healthy controls. These facts collectively suggest that Sema4A, which is abundantly expressed on monocytes and DCs from patients with MS, is enzymatically shed in a subpopulation of these patients.

With regard to the characteristics of MS patients with high Sema4A levels, these are very important findings. One important feature is that MS patients with high serum Sema4A levels have a significantly higher proportion of IL-17-positive cells among their $CD4^+$ T cells compared with those with low serum Sema4A levels or healthy controls (Nakatsuji et al. 2012). With regard to the serum cytokine levels of patients with high Sema4A levels, their IL-2 levels are higher, which is compatible with the observation that DC-derived Sema4A activates T cells. Serum IL-10 levels are lower in patients with high Sema4A levels, which is compatible

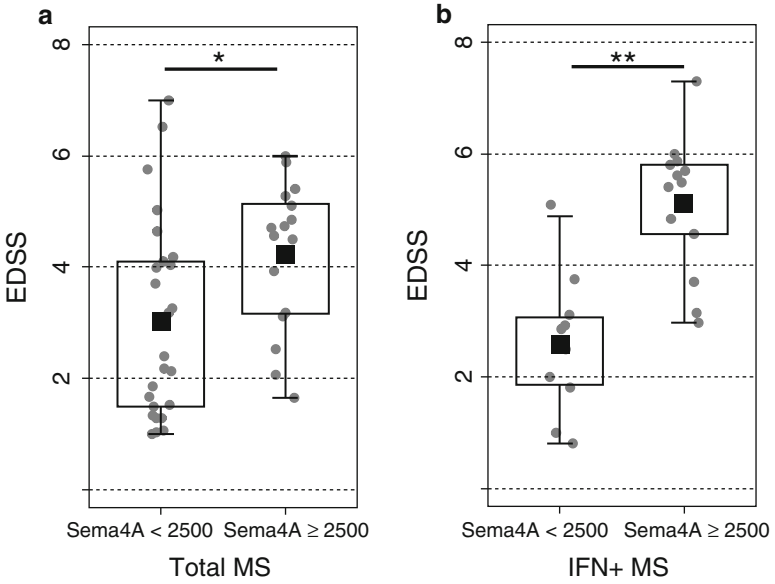

Fig. 10.4 Unresponsiveness of MS patients with high Sema4A to IFN-β treatment. (**a**) Correlation between Sema4A levels and neurological disabilities (EDSS score). RRMS patients were divided into two groups based on Sema4A titer. MS patients with higher Sema4A levels showed worse EDSS scores than patients with lower Sema4A levels. (**b**) Correlation between serum Sema4A levels and the EDSS scores of MS patients treated with IFN-β. MS patients with higher Sema4A levels had worse EDSS scores than patients with lower Sema4A levels. The *top* and *bottom* of the box in the box-and-whisker plot indicate the 25th and 75th percentiles, respectively; the end of the whisker represents 1.5 times the interquartile range from the box or the most extreme points of all the data. $*p < 0.05$; $**p < 0.01$

with the observation that Sema4A inhibits Th2 differentiation (Makino et al. 2008). Therefore, Sema4A levels in patients with MS seem to reflect an underlying Th17-mediated MS pathogenesis.

Another important feature is that MS patients with high Sema4A levels exhibit a significantly more severe disease course compared with those with low Sema4A levels as evaluated by the expanded disability status scale (EDSS) (Kurtzke 1983). More importantly, MS patients with high Sema4A levels are refractory to the first-line drug interferon (IFN)-β (Fig. 10.4). Therefore, MS patients with high Sema4A levels have some undesirable characteristics such as high disease activity and refractoriness to treatment. These facts suggest that serum Sema4A may be a biomarker of refractoriness to IFN-β in addition to being a reliable aid for arriving at an early diagnosis.

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