Class IV Semaphorins in Disease Pathogenesis

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Abbreviations:

AD, atopic dermatitis; ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; ANCA, antineutrophil cytoplasmic antibody; AAV, ANCA-associated vasculitis; BAL, bronchoalveolar lavage; BBB, blood–brain barrier; BCR, B cell receptor; BMP, bone morphogenetic protein; CD, Crohn's disease; Cdc42, cell division cycle 42; CIA, collagen-induced arthritis; CLCP, CUB, LCCL-homology, coagulation factor V/VIII homology domains protein; CNS, central nervous system; CRALBP, cellular retinaldehyde-binding protein; CRBP1, cellular retinol binding protein-1; CTC, circulating tumor cell; DC, dendritic cell; EAE, experimental autoimmune encephalomyelitis; ECRS, eosinophilic chronic rhinosinusitis; EMT, epithelial-mesenchymal transition; ER, endoplasmic reticulum; Erk, extracellular signal-regulated kinase; FCCTX, Familial colorectal cancer type X; IBD, inflammatory bowel disease; ID, inhibitor of DNA binding; IFN, interferon; Ig, immunoglobulin; IGF, insulin-like growth factor; IL, interleukin; IL5R, interleukin-5 receptor; KD , Kawasaki disease; ILC2,

type 2 innate lymphoid cells; IRBP, interphotoreceptor retinoid-binding protein; LARG, leukemia-associated Rho GEF; LM-OVA, OVA-expressing *Listeria monocytogenes*; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; MPO, myeloperoxidase; MS, multiple sclerosis; mTOR, mechanistic/mammalian target of rapamycin; mTORC, mechanistic/mammalian target of rapamycin complex; MVB, multivesicular body; NET, neutrophil extracellular trap; Nrp, neuropilin; NSCLC, non-small cell lung cancer; OVA, ovalbumin; P, postnatal day; pDC, plasmacytoid dendritic cell; PI3K, phosphatidylinositol-3 kinase; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; PR, leukocyte proteinase; PSC, primary sclerosing cholangitis; PSD, postsynaptic density protein; PTEN, phosphatase and tensin homolog; RA, rheumatoid arthritis; RP, retinitis pigmentosa; RPE, retinal pigment epithelium; α-SMA, α-smooth muscle actin; SMAD, small mothers against decapentaplegic homolog; SNP, single-nucleotide polymorphism; SSc, systemic sclerosis; STAT, signal transducer and activator of transcription; TAM, tumor-associated macrophages; TGF, transforming growth factor; T_H1, type 1 helper T; T_H2, type 2 helper T; T_H17, T helper 17; T_{REG}, regulatory T; TRACP, tartrate-resistant acid phosphatase; TNF, tumor necrosis factor; UC, ulcerative colitis.

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ABSTRACT

Semaphorins are a large family of secreted and/or transmembrane proteins, originally identified as proteins that function in axon guidance during neuronal development. However, semaphorins play crucial roles in other physiological and pathological processes, including immune responses, angiogenesis, maintenance of tissue homeostasis, and cancer progression. Class IV semaphorins may be present as transmembrane and soluble forms and are implicated in the pathogenesis of various diseases. In this review, I discuss recent progress on the roles of class IV semaphorins determined by clinical and experimental pathology studies.

KEYWORDS

semaphorine, plexin, neuropilin, autoimmune disease, cancer

INTRODUCTION

Semaphorins are secretory and/or membrane proteins characterized by a conserved amino-terminal Sema domain. They were initially identified as axon-guidance molecules during neuronal development,[1-3] however, they also function in a wide range of other physiological processes, including immune responses,[4] angiogenesis,[5,6] and tissue homeostasis.[7,8] Invertebrate semaphorins are grouped into classes I and II, and vertebrate semaphorins into classes III–VII.[3] Semaphorins in classes IV–VII are membrane bound, whereas semaphorins in class III are secreted. Human class IV semaphorins are seven in number: Sema4A–4G. All feature Sema domains, plexin-semaphorin-integrin (PSI) domains, and immunoglobulin (Ig)-like domains (Fig. 1). The Sema domain is a highly conserved variant of the seven-blade beta-propeller fold and is the distinctive element of the semaphorins. The PSI domain is rich in cysteines and lies structurally adjacent to the Sema domain. The Ig-like domains of semaphorins are termed the C2-set domains; they resemble the antibody-constant domain. Sema4B, 4C, and 4F exhibit C-terminal PDZ-binding motifs that are known to mediate various types of signal transduction. Class IV semaphorins are implicated in the pathogenesis of, for example, autoimmune diseases, cancer, and degenerative diseases (Tables 1, 2).

RECEPTORS OF SEMAPHORINS

Two groups of transmembrane protein families, plexins and neuropilins (Nrps), are the main receptors for semaphorins (Fig. 1).[9-13] Indeed, class IV semaphorins form receptor complexes with

these molecules. These receptor complexes can encompass other transmembrane components, such as CD72 and the receptor tyrosine kinase, MET. Plexins B1, B2, and D1; neuropilin-1 (Nrp1), and TIM-2 serve as receptors for Sema4A. The CUB, LCCL-homology, coagulation factor V/VIII homology domains protein (CLCP1) serves as the receptor for Sema4B. The Plexin B2 receptor mediates Sema4C signaling. Sema4D binds to Plexins B1, B2, CD72, and MET. Plexin B2 may serve as a Sema4G receptor in the developing murine cerebellar cortex. The receptors for Sema4E and 4F have not been identified.

SEMAPHORINS IN PATHOGENESIS

Sema4A

Sema4A is a class IV semaphorin involved in numerous physiological processes, including immune responses, retinal homeostasis, and angiogenesis. Sema4A is expressed in multiple types of immune cells and regulates their functions. For instance, Sema4A expressed in polarized type 1 helper T (T_H1) cells is crucial in their differentiation.[14] Indeed, Sema4A-deficient mice display impaired T_H1 responses to heat-killed *Propionibacterium acnes*, a T_H1-inducing bacterium, *in vivo.* Conversely, Sema4A-deficient mice exhibit augmented type 2 helper T (T_H2) cell responses against *Nippostrongylus brasiliensis*, a T_H2-inducing intestinal nematode.[14]

Sema4A also contributes to maintenance of regulatory T (T_{REG}) cell functions.[15] Sema4A expressed by immune cells and Nrp1 by T_{REG} cells interact to potentiate T_{REG}-cell function and

survival. Ligation of Sema4A to Nrp1 abrogates Akt phosphorylation intracellularly and at the immunologic synapse by phosphatase and tensin homologue (PTEN), thereby promoting nuclear localization of the transcription factor FoxO3a.

Sema4A in retinitis pigmentosa

Retinitis pigmentosa (RP) is an inherited degenerative eye disease characterized by degeneration of photoreceptors leading to severe vision impairment. More than 100 mutations associated with RP pathogenesis have been discovered. Most of the proteins encoded by these genes are essential for maintenance of photoreceptor or retinal pigment epithelium (RPE) homeostasis.[16]

Sema4A is involved in the pathogenesis of RP. Insertion of a gene trap vector into intron 11 of the mouse *Sema4A* gene resulted in loss of the retinal photoreceptor layer.[17] In addition, Sema4A-deficient mice displayed marked photoreceptor degeneration.[18] The outer segment of photoreceptors in these mice was disrupted at postnatal day 14 (P14) and they showed complete loss of photoreceptors by P28, largely a result of increased apoptosis. Sema4A regulates two distinct endosomal-sorting pathways critical for the survival of photoreceptor cells (Fig. 2). [18] Sema4A plays a role in Rab11/FIP2-mediated endosomal sorting in RPE cells and contributes to maintenance of photoreceptor homeostasis (Fig.2a). In the absence of oxidative stress, the lysosomal precursor protein prosaposin is preferentially transported to lysosomes after binding to sortilin in late endosomes and is then processed to saposin. Some prosaposin is released via a transport mechanism of late endosomes, the

multivesicular body (MVB), and exosomes. In response to oxidative stress, Sema4A switches the endosomal sorting of prosaposin from lysosomes to exosomes (which then release the protein). Late endosomes containing prosaposin fuse with Rab11/FLIP2-dependent early endosomes expressing Sema4A, triggering MVB formation. Prosaposin binds to Sema4A in MVB, followed by exosome-dependent secretion of the complex from the RPE. The secreted prosaposin prevents light-induced photoreceptor apoptosis. Also, Sema4A plays a role in the retinoid cycle. In the absence of oxidative stress, Sema4A appropriately sorts retinoid-binding proteins with retinoids between the cell surface and endoplasmic reticulum (ER), by which 11-cis-retinal, a phototransduction chromophore, is regenerated and returned to photoreceptors (Fig. 2b). All-trans-retinol is taken up in an interphotoreceptor retinoid-binding protein (IRBP)-dependent manner. Sema4A regulates Rab11/FLIP2-dependent endosomal trafficking of all-trans-retinol bound to the cellular retinol binding protein-1 (CRBP1) from the cell membrane surface to the ER, and also the trafficking of 11-cis-retinal generated from all-trans-retinol in the ER [and then bound to cellular retinaldehyde-binding protein (CRALBP)] from the ER to the cell membrane surface.

Consistent with these experimental findings, D345H, F350C, and R713Q, mutations in *Sema4A*, have been identified in patients with retinal degenerative diseases.[19] A series of knock-in mouse lines harboring *Sema4A* with the F350C mutation showed severe retinal degeneration.[20] In the retina of knock-in mice, Sema4A^{F350C} mutant protein showed abnormal aggregation and mis-localization in RPE cells, leading to impaired endosomal sorting of factors such as prosaposin. Of note,

lentivirus-mediated transfer of *Sema4A* into RPE cells in neonatal Sema4A-deficient mice prevented retinal degeneration.

Sema4A in multiple sclerosis

Multiple sclerosis (MS) is a myelin-directed autoimmune disease, in which malfunctioning immune cells destroy the fatty substance that coats and protects nerve fibers in the brain and spinal cord.[21] In MS, myelin sheath destruction is primarily caused by multifocal inflammation resulting from T-cell and macrophage infiltration and oligodendrocyte death.[22,23] Although the pathogenesis of MS is unclear, it is multifactorial and involves genetic and environmental factors. When genetically predisposed individuals are exposed to an environmental trigger, myelin-specific T cells are activated, and MS develops.[24] Studies with animal models and/or patients with MS indicated that the involvement of T helper 17 (T_H17) cells and T_H1 cells is crucial for development of MS. Additionally, dysfunction of antigen-presenting cells is important for pathogenesis of MS—their inappropriate activation may trigger differentiation of myelin-specific T cells. The mouse model of autoimmune encephalomyelitis (EAE) is most commonly used to study autoimmune demyelinating diseases and is regarded as relevant to MS. In the EAE model, inflammatory responses and focal demyelination are observed in mice immunized with myelin proteins such as myelin basic protein, myelin oligodendrocyte glycoprotein, and proteolipid protein in combination with an adjuvant. EAE can also be reproducibly induced by the passive transfer of myelin antigen-reactive T cells. The EAE model

reproduces the histopathological features of MS, including deleterious lymphocyte infiltration, demyelination, substantial axonal loss, and reactive gliosis.

Sema4A is expressed in CD4+ T cells and is crucial for the activation and differentiation of helper T cells.[13,14] Indeed, Sema4A-deficient mice showed attenuated responses in EAE and treatment with a monoclonal antibody against Sema4A blocked the development of EAE. In addition, the serum Sema4A levels are significantly higher in patients with MS than in healthy subjects or patients with other neurological diseases.[25] Patients with MS who have high serum Sema4A levels showed significantly increased proportions of T_H17 cells compared to healthy subjects or patients with low serum Sema4A levels. Sema4A is overexpressed on dendritic cells (DCs) in patients with MS, and shed from them in a metalloproteinase-dependent manner. Furthermore, the serum level of Sema4A is correlated with unresponsiveness to interferon β (IFN-β) therapy. These results strongly support a role for Sema4A in the T_H17-mediated pathogenesis of MS.

Sema4A in protection against infectious diseases

Roles for Sema4A in T cell function and protection against bacterial infection have been reported. As described above, Sema4A is highly expressed in T_H1 cells and is involved in their antigen-specific activation and differentiation. T_H1 cells derived from Sema4A-deficient mice show attenuated responses to *Propionibacterium acne*,[14] whereas Sema4A-deficient T_H2 cells show potentiated responses against *Nippostrongylus brasiliensis*. Therefore, Sema4A is crucial for T-cell priming and

regulating T_H1/T_H2 responses to bacterial infection.

As in CD4+ T cells, Sema4A is abundantly expressed in CD8+ T cells and is crucial for their function.[26] CD8+ T cells from Sema4A-deficient mice showed impaired IFN-γ and tumor necrosis factor α (TNF-α) production and granzyme B, perforin, and FAS-L induction. In addition, Sema4A-deficient mice exhibited impaired pathogen-specific effector CD8+ T cell responses to ovalbumin (OVA)-expressing *Listeria monocytogenes* (LM-OVA) infection, an *in vivo* model of acute infection and responsive activation of CD8+ T cells. Furthermore, Sema4A-deficient CD8+ T cells showed significantly reduced mechanistic/mammalian target of rapamycin complex 1 (mTORC1) activity and, conversely, elevated mechanistic/mammalian target of rapamycin complex 2 (mTORC2) activity. This suggests that Sema4A is associated with activation of mTORC1 in CD8+ T cells.

Sema4A is also involved in virus infection and its symptoms. Depletion of plasmacytoid dendritic cells (pDCs) during primary *Pneumovirus* infection predisposed adult mice to severe inflammation in the early phase and, subsequently, asthma upon reinfection.[27] Sema4A expressed by pDCs contributed to expansion of functional Nrp1+ T_{REG} cells. Sema4A-dependent T_{REG} cell expansion induced by the microbial metabolite propionate protected against severe viral bronchiolitis and subsequent asthma in pDC-depleted neonatal mice.

Sema4A in allergic diseases

Allergic diseases including asthma, allergic dermatitis, and allergic rhinitis are prevalent chronic

immunological diseases. Allergic diseases are influenced by multiple genetic and interacting environmental factors.[28,29] These diseases have several shared clinical and histopathological features, including elevated serum levels of IgE and T_H2-type cytokines and severe infiltration of eosinophils and mast cells.[30] Fundamentally, allergy is an adaptive immune response to allergens. Various types of immune cells—including T_H2 cells, allergen-specific IgE-producing cells, eosinophils, M2 macrophages, and type 2 innate lymphoid cells (ILC2s)—are implicated in the pathogenesis of allergic diseases.[31,32]

Sema4A is involved in the pathogenesis of allergic asthma. In a model of OVA-specific experimental asthma, Sema4A-deficient mice exhibited enhanced airway hyperreactivity.[33] Functional analysis revealed increased pulmonary eosinophil infiltration in knock-out mice, as well as increased levels of IgE and T_H2 cytokines in bronchoalveolar lavage (BAL) fluid. These airway hyperreactivity symptoms and T_H2-mediated responses were reduced by *in vivo* systemic administration of Sema4A-Fc protein. Moreover, the utility of Sema4A as a novel immunotherapeutic has been reported.[34] Sema4A has also been implicated in the pathogenesis of allergic dermatitis. Sema4A-deficient mice spontaneously develop skin lesions resembling atopic dermatitis (AD) in human, and the diseased mice had severe mast cell infiltration of the skin and elevated serum levels of IgE.[33] Furthermore, roles for Sema4A in allergic rhinitis have been suggested. Sema4 is highly expressed in eosinophils infiltrating nasal polyps.[35] The serum levels of Sema4A were significantly elevated in patients with eosinophilic chronic rhinosinusitis (ECRS) and asthma. Sema4A supports

eosinophil survival and activation via an IL-5R/STAT5-dependent pathway.

Sema4A and inflammatory bowel disease

Inflammatory bowel diseases (IBD), comprising Crohn's disease (CD) and ulcerative colitis (UC), is a group of chronic inflammatory diseases of the gastrointestinal tract in which autoimmunity plays a direct pathogenic role. Sema4A is also involved in the pathogenesis of these diseases. The serum levels of Sema4A are significantly lower in patients with CD or UC compared to control subjects.[36] Immunohistochemistry showed increased expression of Sema4A in infiltrating lymphocytes of the lamina propria in active CD and UC, implicating Sema4A in regulating local tissue inflammation in the bowel.

Sema4A in systemic sclerosis

Systemic sclerosis (SSc) is a rare autoimmune inflammatory disease characterized by activation of the immune system and fibrosis of the skin and major internal organs.[37,38] The resultant skin thickening and stiffness and loss of internal organ function lead to profound disability and premature death. The cause of SSc has not been elucidated, however, it is likely to involve environmental factors in a genetically primed individual.

A role for Sema4A in SSc has been suggested.[39] Serum levels of Sema4A were significantly higher in patients with SSc than in healthy subjects, and the former had elevated Sema4A expression

in circulating monocytes and CD4+ T cells. Sema4A enhanced the production of T_H17 cytokines induced by CD3/CD28. In addition, secreted interleukin-17 (IL-17) induced the production of chemokines and inflammatory mediators in dermal fibroblasts. Sema4A also plays a direct role in fibrosis by inducing the production of extracellular matrix components and the expression of the myofibroblast marker α-smooth muscle actin (α-SMA) in dermal fibroblasts.

Sema4A in cancer

Sema4A V78M germline mutation was identified by whole-exome sequencing in an Austrian kindred with familial colorectal cancer type X (FCCTX).[40] The function of $Sema4A^{V78M}$ was dependent on enhanced mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (Erk) and phosphatidylinositol-3 kinase (PI3K)/Akt signaling. Two further Sema4A mutations, G484A and S326F, and the single-nucleotide polymorphism (SNP), P682S, were also identified. The P682S SNP was significantly correlated with the FCCTX phenotype, resulting in an increased risk of cancer.

Sema4A was identified as a novel therapeutic target of myeloma.[41] Targeting Sema4A using an antibody-drug conjugate potently and selectively eradicated myeloma cells *in vitro* and *in vivo*. Sema4A conferred drug resistance on hepatocellular carcinoma by inducing the epithelial-mesenchymal transition (EMT).[42] Sema4A promoted the progression of breast cancer by preventing apoptosis induced by severe hypoxia.[43] Enhanced expression of Sema4A attenuated the progression of oral squamous cell carcinoma cells by inhibiting angiogenesis, invasion, and migration.[44]

Sema4B

The function of Sema4B in lung cancer has been investigated. Sema4B plays important roles in immune cell responses. Sema4B expressed by T and B cells negatively regulates basophil function by promoting T cell–basophil interactions.[45] Basophil is a rare type of white blood cell that accounts for less than 1% of peripheral blood leukocytes. These cells are responsible for the development of acute and chronic allergic diseases via IgE-mediated reactions. Sema4B-deficient mice have considerably elevated serum levels of IgE, although lymphocytes or DCs from these mice do not show impaired functions.[45] In addition, Sema4B-deficient mice exhibit augmented basophil-mediated memory IgE production, implying involvement of Sema4B in basophil-mediated T_H2 and humoral memory responses.

Sema4B in lung cancer

Important roles for Sema4B in non-small cell lung cancer (NSCLC) have been reported.[46-49] Sema4B interacts with CLCP1 and enhanced the ubiquitination and proteasome degradation of CLCP1.[46] RNAi-mediated knockdown of CLCP1 expression significantly reduced the motility of NSCLC cells. In the cited report, CLCP1 was expressed by NSCLC cells but endogenous Sema4B expression was not clearly evident. However, some scholars have reported Sema4B expression in NSCLC cell lines and tumor tissues,[47-49] suggesting co-expression of Sema4B and the receptor thereof (CLCP1) in

NSCLC cells. In addition, the expression of Sema4B in NSCLC cells was repressed by hypoxia-inducible factor 1 (HIF-1).[47] Ectopic expression of Sema4B abrogated hypoxia-induced invasion of NSCLC cells. Overexpression of Sema4B decreased the expression of matrix metalloproteinase-9 (MMP-9),[48] decreasing NSCLC invasiveness. Sema4B is also linked to the growth of NSCLC cells.[49] FoxO1 nuclear retention by suppression of PI3K/Akt signaling was induced by Sema4B, inhibiting NSCLC cell growth.

Sema4B in skin disease

A genome-wide meta-analysis implicated variants in *Sema4B* in the risk of severe acne vulgaris.[50] Putative causal variants disrupt the coding region of WNT10A and a P63 transcription factor binding site in intron 1 of Sema4B at 15q26.1.

Sema4C

The role of the class IV semaphorin, Sema4C, in a variety of cancers has been investigated.

Sema4C in cancers

Sema4C–Plexin B2 axis is essential for the growth of breast carcinoma cells. Sema4C is expressed in human breast cancers and its elevated expression correlates with a poor outcome.[51] Immunoblotting suggested that both Sema4C and its receptor Plexin B2 were expressed by various breast cancer cell

lines. The underlying mechanism involves the activity of leukemia-associated Rho GEF (LARG), a plexin-associated RhoA exchanger protein. Sema4C/Plexin B2/LARG-dependent RhoA signaling is required to sustain breast cancer cell proliferation. Overexpression of Sema4C bestowed on breast cancer cells a luminal-type phenotype, leading to tamoxifen resistance, estrogen-independent growth, and metastasis. Targeting Sema4C-mediated signaling resulted in cell cycle arrest in the G2/M phase and cell senescence. Reverse signaling of Sema4C elicits invasive reprogramming of breast cancer cells.[52] Although semaphorins ordinarily act as ligands via the intracellular domain of plexins, some transmembrane semaphorins act as receptors and mediate *reverse* signaling via their intracellular domains.[53,54] Sema4C reverse signaling augmented expression of the transcriptional regulators ID1 and ID3 via transforming growth factor ß (TGF-ß)/bone morphogenetic protein (BMP) receptor signaling and SMAD1/5 activation. Although this reprogramming suppressed the typical features of the EMT in invasive carcinoma cells, the resultant phenotype promoted metastatic colonization *in vivo*.

Sema4C in neural development

The Sema4C–Plexin B2 axis is reportedly involved in neural development. Sema4C-deficient mice, as well as Plexin B2-deficient mice, displayed exencephaly and distinctive defects of the cerebellar granule cell layer.[55] Sema4C-deficient mice also showed ventral skin pigmentation defects, suggesting the involvement of Sema4C in melanocyte development. In situ hybridization revealed that Sema4C

was expressed in both granule cells and Bergmann glial cells during cerebellar development. The receptor Plexin B2 was also expressed by granule cells in the developing cerebellum.

Sema4D

Sema4D, also known as CD100, is involved in multiple physiological processes including neural development, immune responses, and tissue regeneration. Sema4D expressed by oligodendrocytes, together with its receptor Plexin B1, functions in axon guidance and induces growth-cone collapse in the central nervous system.[56] Sema4D is the first shown to exert immunoregulatory effects.[57,58] The plexin B subfamily (B1, B2, and B3) and CD72 are the receptors for Sema4D in the immune system.[13,59] Sema4D is expressed by activated B cells, T cells, DCs, and mast cells. Sema4D-deficient mice show abnormal B cells and altered antibody responses.[59] Immunological assays revealed that the interaction of Plexin B1 on B cells with Sema4D enhances B-cell proliferation and lifespan.[60] Additionally, Sema4D–CD72 interactions between B cells contribute to maintenance of B-cell subsets by moderating B-cell receptor (BCR) signals. Sema4D is also expressed in germinal center B cells, and the Sema4D–CD72 axis may promote robust population expansion of these cells.[61] Sema4D is also expressed by CD4+ T cells, which is associated with B cell-mediated immunity. After formation of germinal centers in lymphatic follicles, Sema4D expressed by T cells plays an important role in the interaction between helper T cells and germinal center B cells. Sema4D signaling promotes the survival of germinal center B cells and efficient selection of high-affinity B cells. Consistent with these

functions, Sema4D-deficient mice display impaired antibody affinity maturation and poor generation of antigen-specific germinal center B cells when immunized with T cell-dependent antigens.[62] Sema4D expressed by gamma-delta T cells is crucial for healing of skin damage via interactions with Plexin B2.[63]

Sema4D in vasculitis

Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is an autoimmune disease that manifests as severe, systemic, small-vessel vasculitis.[64,65] AAV is characterized by the development of autoantibodies to myeloperoxidase (MPO-ANCA) and the neutrophil protein, leukocyte proteinase 3 (PR3)-ANCA.

Sema4D is associated with the pathogenesis of AAV. The serum levels of soluble Sema4D in patients with AAV were significantly higher than in healthy subjects and patients with other autoimmune diseases.[66] Sema4D levels in patients with AAV were correlated with disease activity scores. By contrast, cell-surface expression of Sema4D was downregulated in neutrophils from patients with AAV, suggesting that proteolytic cleavage of membrane Sema4D is augmented. A direct cellular interaction between Sema4D on neutrophils and Plexin B2 on endothelial cells negatively regulated neutrophil extracellular trap (NET) formation in neutrophils (Fig. 3a). NETs are extracellular webs of chromatin released from activated neutrophils that induce local inflammation (mainly against infection).[67] Such negative regulation of NETs by Sema4D prevents vasculitis. Indeed,

recombinant Plexin B2 suppressed neutrophil Rac1 activation and inhibited ANCA-induced oxidative burst and resultant NET formation, suggesting that Sema4A has potential as a biomarker and therapeutic target for AAV.

Sema4D plays an important role in the pathogenesis of Kawasaki disease (KD).[68] KD is a systemic medium-vessel vasculitis and is the most common type of childhood systemic vasculitis in which coronary arteries are most frequently affected.[69,70] Serum levels of soluble Sema4D were elevated in KD patients, especially in those with coronary artery lesions. The Sema4D levels correlated positively with disease severity and the serum concentrations of IL-1β, IL-6, and IL-8. Sema4D released from neutrophils promotes inflammatory cytokine production in endothelial cells via binding with Plexin B1 and B2.

Sema4D in rheumatoid arthritis

Rheumatoid arthritis (RA) is an autoimmune disease characterized by synovial inflammation. The pathogenesis of RA involves chronic inflammation of the synovial membrane, which also destroys articular cartilage and bone. Both genetic and environmental factors contribute to the pathogenesis of RA, in which several immune pathways involving various types of immune cells (T cells, B cells, macrophages, neutrophils, and DCs) and non-immune cells (fibroblasts and chondrocytes), play crucial roles. Sema4D is also involved in the pathogenesis of RA.[71] Serum and synovial-fluid levels of soluble Sema4D were elevated in patients with RA and were correlated with disease activity. Soluble

Sema4D is proteolytically shed by a disintegrin and metalloproteinase with thrombospondin motifs 4 (ADAMTS4). The resultant soluble Sema4D enhanced the production of TNFα and IL-6 by monocytes, thereby upregulating ADAMTS4 expression in synovial cells. Therefore, Sema4D-mediated RA pathogenesis involves a positive-feedback loop. Treatment with an anti-Sema4D antibody reduced inflammation in a collagen-induced arthritis (CIA) mouse model. This indicates that Sema4D could be a potential therapeutic target for RA. An anti-Sema4D antibody (VX15/2503) showed promise in phase I clinical trials involving patients with advanced solid tumor (NCT01313065)[72] and multiple sclerosis (NCT01764737).[73] VX15/2503 inhibits the interaction between Sema4D and Plexin B1,[74,75] and is expected to exert a therapeutic effect by preventing tumor angiogenesis and the recruitment of tumor associated macrophages (TAMs) in antitumor immunity[76,77] or by impairing inflammatory responses and demyelination in multiple sclerosis.[73]

Sema4D in eosinophilic chronic rhinosinusitis

Eosinophilic chronic rhinosinusitis (ECRS) is a type of chronic rhinosinusitis characterized by severe eosinophilic infiltration. Although the causes of ECRS are unclear, stimulation of T_H2 cells and resultant IgE production are thought to be key factors. Sema4D is involved in the pathogenesis of ECRS.[78] Serum levels of soluble Sema4D were elevated in patients with ECRS and positively correlated with disease severity. Expression of Sema4D in tissue-infiltrating eosinophils in nasal polyps was higher in patients with ERS than in healthy subjects. Sema4D expression on the surface

of eosinophils was decreased in an MMP-9-mediated manner. Sema4D increases the permeability of vascular endothelial cells by binding to Plexin B1 and activating RhoA.[76,79] Therefore, the increased number of eosinophils in ERS is a result of the Sema4D–Plexin B1 axis-mediated increased vascular permeability (Fig.3b). Indeed, blockade of Sema4D by a therapeutic antibody ameliorated eosinophilic infiltration in sinus tissues and nasal lavage fluid in an ECRS animal model.

Sema4D in primary sclerosing cholangitis

Whole-exome sequencing analysis demonstrated the involvement of Sema4D in primary sclerosing cholangitis (PSC).[80] PSC is a severe hepatobiliary disease characterized by inflammatory and fibrotic bile duct lesions, in which multiple genetic and environmental factors are implicated.[81] There are neither early diagnostic markers nor effective therapies for PSC. PSC causes considerable morbidity and mortality via the subsequent development of liver cirrhosis and high risk of cholangiocarcinoma.[82,83] A large number of PSC susceptibility genes have been identified. Whole-exome sequencing in a family with autosomal dominant inheritance of PSC revealed a heterozygous germline missense mutation in *Sema4D* encoding a K849T variant.[80] This mutation was associated with impairment of T-cell functions and INF-γ secretion. Notably, knock-in mice harboring the *Sema4D* K849T mutation developed severe cholangitis phenotypes in an *in vivo* model of cholestatic disease induced by a diet rich in 3,5-diethoxycarbonyl-1,4-dihydrocollidine.

Sema4D in osteoporosis/osteopetrosis

The Sema4D signaling axis plays an important role in bone homeostasis. Sema4D-deficient mice, as well as its receptor Plexin B1-deficient mice, show impaired osteoclast differentiation and the phenotype of osteopetrosis.[7] Binding of Sema4D on osteoclasts to Plexin B1 on osteoblasts maintained osteoblast motility by activating the small GTPase, RhoA, and suppressing insulin-like growth factor 1 (IGF-1) signaling; the dysregulation of this homeostasis led to osteopetrosis (Fig. 3c). A Sema4D-specific antibody markedly suppressed bone loss in animal models of postmenopausal osteoporosis. In addition, the bone resorption phenotype in Sema4D-deficient mice was dependent on ovarian function; ovariectomy significantly abrogated the osteopetrotic phenotype.[84] Spatial segregation of osteoblasts and osteoclasts is essential for bone homeostasis. The Sema4D–Plexin-B1 signaling axis is essential in contact inhibition of locomotion between osteoblasts and osteoclasts.[85] Osteoclasts express Sema4D and induce contact inhibition of locomotion in osteoblasts, the morphological changes of which are associated with reorganization of myosin II, phosphatidylinositol (3,4,5)-trisphosphate (PIP3), and adhesion and active cell division cycle 42 (Cdc42). The serum Sema4D levels were significantly higher in postmenopausal patients with osteoporosis, compared to healthy subjects.[86] In these patients, the serum Sema4D levels were positively correlated with those of bone resorption markers such as tartrate-resistant acid phosphatase 5b (TRACP-5b) and N-terminal telopeptide (NTX) and negatively correlated with those of bone formation markers such as bone alkaline phosphatase and osteocalcin. Additionally, Sema4D might be involved in the pathogenesis of other

skeletal diseases. Copy number loss of the *Sema4D* region that confers a risk of acetabular dysplasia, a major and common cause of secondary hip osteoarthritis, was identified by whole-genome screening.[87]

Sema4D in cancer

Sema4D is associated with the etiology of various types of cancer.[88,89] Roles for Sema4D have been demonstrated in breast cancer,[90-101] colorectal carcer,[102-108] gastric cancer,[109] esophageal cancer,[110,111] lung cancer,[112-114] pancreatic cancer,[107,115-117] cholangiocarcinoma,[118] prostatic cancer,[119-123] bladder cancer,[124,125] kidney cancer,[125] cervical cancer,[126] ovarian cancer,[127-130] malignant melanoma,[131] osteosarcoma,[132] head-and-neck cancer,[133-137] medulloblastoma,[138] leukemia,[139-143] malignant lymphoma,[144] and multiple myeloma (Fig. 4).[145] Cancer cells express Sema4D or its receptor, which are typically up- or down-regulated in tumors compared to normal tissues. These Sema4D-mediated signals can promote or inhibit the proliferation, survival, migration, and chemotherapy resistance of cancer cells. The effects can switch from positive to negative and *vice versa*, depending on the situation. For instance, in breast carcinoma cells, switching of the expression of the receptor tyrosine kinases, ErbB2 and Met, results in a shift from activation to inactivation of RhoA, leading to conversion from chemotaxis to inhibition of migration.[90] The significance of Sema4D in breast cancer cells was confirmed by genome-wide sequencing. Metastatic variants of circulating tumor cells (CTCs) from breast cancer patients suggested that Sema4D regulates tumor-cell transmigration through the

blood–brain barrier.[91] Sema4D mediates brain metastasis by promoting CTC transmigration through the blood–brain barrier (BBB), in initial step of which Sema4D is crucial. Amplification of the oncoprotein MYC may cooperate with Sema4D to promote brain metastasis. Sema4D and MYC expression levels were significantly associated in breast cancer cells with the brain metastatic phenotype.

Sema4D is a critical factor in the tumor microenvironment, particularly for angiogenesis. The development of new blood vessels is a crucial step in cancer progression because tumors require a sufficient supply of oxygen and nutrients for growth. Thus, tumors release pro-angiogenic stimuli and depend on angiogenesis for their growth and survival, which explains the tumor shrinkage induced by anti-angiogenics. These drugs are associated with tissue hypoxia, which induces neo-angiogenesis–sustaining signals and may enhance the invasive/metastatic behavior of cancer cells.[146,147] Sema4D exerts a potent proangiogenic effect *in vitro* and *in vivo* by binding to the high-affinity receptors Plexin B1 and B2 on endothelial cells.[148] A study in Sema4D-deficient mice indicated that Sema4D is not essential for developmental angiogenesis. However, many human cancers express Sema4D, which can be released in soluble form and induce endothelial cell chemotaxis and blood vessel growth *in vivo*.[149] In addition, experimental tumors grown in Sema4D-deficient mice contained smaller and aberrant vessels poorly lined with pericytes, and both cancer growth and metastases were strikingly reduced.[76] Notably, in addition to being produced by cancer cells, Sema4D may be released into the tumor microenvironment by inflammatory cells, tumor-associated macrophages,[76]

and, potentially, platelets.[150]

Sema4E

The physiological and pathological roles of Sema4E in mammals are unclear. However, in a zebrafish model, Sema4E functioned as a repellant factor for facial and gill motor axons within the pharyngeal arches.[151]

Sema4F

The importance of Sema4F in neural development and cancer biology has been reported. Sema4F interacts with scaffolding proteins such as postsynaptic density protein 95 (PSD-95) in the CNS.[152] The participation of Sema4F in oligodendrocyte precursor migration was also reported.[153]

Sema4F in cancer

Sema4F is implicated in breast cancer[99] and prostate cancer.[154] Sema4F overexpression induces the proliferation and migration of, and may be involved in perineural invasion by, prostate cancer cells.[154] Sema4F might be a critical regulator of the interaction between cancer and nerves and contribute to cancer-induced neurogenesis. Patients with prostate cancer with high Sema4F expression are at significantly higher risk of biochemical recurrence, suggesting the utility of Sema4F as a biomarker. Moreover, Sema4F suppressed the proliferation of differentiated Schwann cells ensheathing axonal

processes in peripheral nerves.[155] Loss of neurofibromin in Schwann cells leads to disruption of Schwann cell/axonal interactions by upregulating the Ras/Raf/ERK signaling pathway. Thus, Sema4F is involved in the mechanism by which heterotypic cell-cell contacts control cell proliferation and suppress tumorigenesis.

Sema4G

Although the roles of Sema4G are unclear, a study in a mouse model showed that *Sema4G* gene deletion causes no overt phenotype, but combined deletion of Sema4C and Sema4G results in an enhanced cerebellar phenotype.[55] The work suggested that Plexin B2 served as a receptor for Sema4G. In addition, the Sema4G gene is a component of the signatures of cancers,[156-158] schizophrenia,[159] and diabetes mellitus.[160]

SUMMARY AND PERSPECTIVES

Semaphorins and their receptors, axon guidance factors, are implicated in the pathogenesis of several diseases. A deeper understanding of semaphorin-dependent pathogenesis could lead to development of therapies and prognostic methods for various diseases. Inhibitory semaphorin signals that restrict disease phenotypes in preclinical mouse models might be therapeutic targets. Indeed, drugs targeting certain semaphorins or their receptors, including Sema4D and Plexin A1, are under development. Because the semaphorin signaling network is complex and their functions are cell-context dependent,

further studies are needed to establish their value members as prognostic predictors and putative therapeutic targets. Because semaphorins function in diverse physiological processes, drugs based on them might have unexpected side effects, particularly in the central nervous and vascular systems. To develop safer drugs, further data are needed. Finally, deciphering semaphorin-mediated pathogenesis will facilitate the development of novel therapeutics for a variety of diseases.

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CONFLICT OF INTERESTS

None declared.

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FIGURE LEGENDS

Figure 1. Structure of class Ⅳ semaphorins and their receptors. Class Ⅳ semaphorins (Sema4A-G) are membrane-bound proteins composed of a signal peptide (SP), Sema domain (Sema), plexin-semaphorin-integrin domain (PSI), immunoglobulin-like domain (Ig), and transmembrane domain (TM). Semaphorins bind to class B and D plexins. Associations between Plexin B1 and Sema4A and 4D; Plexin B2 and Sema4A, 4C, and 4D; and Plexin D1 and Sema4A have been reported. In addition, SemaA binds to neuropilin-1 and TIM-2 (T-cell, immunoglobulin, and mucin domain protein 2). Sema4D binds to MET and CD72. Sema4B binds to CLCP1 (CUB,LCCL-homology, coagulation factor V/VIII homology domains protein).

Figure 2. Roles of Sema4A in retinal homeostasis. (**a**) Schematic of Sema4A-mediated endosomal sorting. Sema4A contributes to the appropriate secretion of prosaposin via the Rab11/FIP2 endosomal-sorting pathway. Upon exposure to light, prosaposin preferentially binds to Sema4A in the multivesicular body (MVB), from which exosomes containing prosaposin are secreted. Secreted prosaposin functions as an antiapoptotic factor for photoreceptors. (**b**) Schematic of Sema4A-mediated intracellular sorting of retinoid-binding proteins in the retinoid cycle. Sorting of CRALBP and CRBP1, which are associated with all-trans-retinol and 11-cis-retinal transport, depends on Sema4A and the Rab11/FIP2-mediated endosomal-sorting machinery.

Figure 3. Roles of Sema4D in the pathogenesis of autoimmune diseases. (**a**) Schematic of Sema4D-mediated pathogenesis of antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV). In health, Sema4D on neutrophils binds Plexin B2 on endothelial cells and inhibits excessive Rac1 activation of neutrophils. In AAV, Sema4D on the neutrophil surface is shed, resulting in aberrant activation of neutrophils with generation of reactive oxygen species (ROS) and neutrophil extracellular trap (NET) formation. (**b**) Schematic of Sema4D-mediated pathogenesis of eosinophilic chronic rhinosinusitis (ECRS). In nasal polyps from patients with ECRS, Sema4D on eosinophils is shed by the action of matrix metalloproteinase-9 (MMP-9) and secreted as a soluble form. Soluble Sema4D enhances eosinophil transendothelial migration by increasing endothelial permeability. (**c**) Schematic of Sema4D-mediated pathogenesis of osteopetrosis. Sema4D binds to Plexin B1 on osteoblasts and activates RhoA, inhibiting bone formation by suppressing insulin-like growth factor-1 (IGF-1) signaling and by modulating osteoblast motility. Inappropriate activation or inactivation of these Sema4D/Plexin B1-mediated signals could be associated with the pathogenesis of osteoporosis and/or osteopetrosis.

Figure 4. Roles of class IV semaphorins in cancer. Class IV semaphorins are associated not only with various processes in cancer cells but also in the tumor microenvironment, including tumor-infiltrating immune cells, angiogenesis, and neurogenesis.