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Review

Biological functions and signaling of a transmembrane semaphorin, CD100/Sema4D

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Abstract. The semaphorin proteins were identified originally as axonal guidance factors functioning during neuronal development. In addition to this function, several semaphorins play diverse roles outside the nervous system. The class 4 semaphorin CD100/Sema4D, which utilizes plexin-B1 and CD72 as receptors, exerts important biological effects on a variety of cells, including the neuronal, epithelial and immune cells. Here, we review recent advances exploring the molecular mechanisms governing the biological functions of CD100/Sema4D.

Key words. CD100/Sema4D; semaphorin; plexin-B1; CD72; Met; SHP-1.

Introduction

The semaphorin family comprises more than 30 phylogenetically conserved proteins [1–3]. These proteins are categorized into eight subclasses based on sequence similarity and distinctive structural features (fig. 1) [4]. The semaphorin subclasses 1, 2 and 5 contain the semaphorins identified in invertebrate species, while subclasses 3–7 contain the vertebrate semaphorins. In addition, the genomes of certain DNA viruses contain the subclass 5 semaphorin genes. Several semaphorins act as chemorepellents for axonal pathfinding during neuronal development [3, 5, 6]. Cumulative findings to date, however, suggest that semaphorins play diverse roles unrelated to axon guidance, function in organogenesis, vascularization, angiogenesis, apoptosis and neoplastic transformation [7–10]. In addition, several semaphorins, particularly the class 4 members, are crucially involved in the regulation of immune responses, performing roles as 'immune semaphorins' [11–13].

Two receptor families, plexins and neuropilins, have been implicated in mediating many semaphorin functions [14–

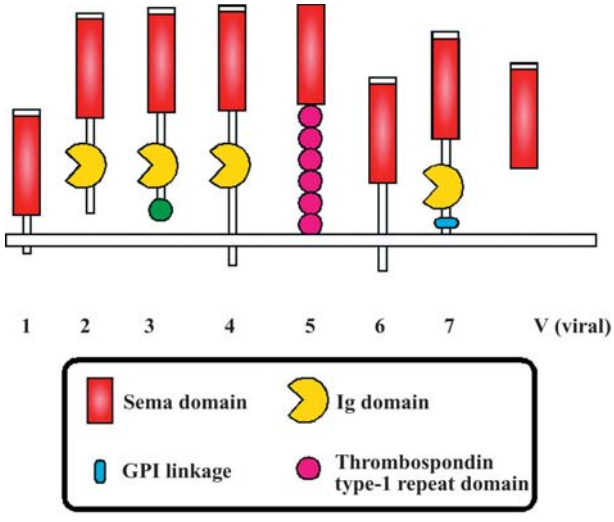

Figure 1. The semaphorin family. The semaphorin family contains a large number of phylogenetically conserved secreted and transmembrane proteins. Based on structural features, the members have been divided into eight classes, which include a unique viral class. The members of the semaphorin family all share a common sema domain.

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19]. Two neuropilins and nine plexins have been identified in the mammalian genome. A population of membrane-bound semaphorins and two viral-derived secreted semaphorins interact directly with the plexins [16–19]. The class 3 secreted semaphorins, however, utilize neuropilins as ligand-binding obligate receptors [14, 15], which form a signaling receptor complex containing a semaphorin, neuropilin and plexin [18]. Studies using truncated forms of plexins lacking the intracellular domains conclusively demonstrated the requirement of the plexin cytoplasmic domain for semaphorin signaling [17–19]. The short cytoplasmic domains of neuropilins, however, are dispensable for the repulsive functions of semaphorins [20]. In addition to plexins and neuropilins, five additional surface molecules have been implicated as either semaphorin receptors or components of a semaphorin receptor holoreceptor complex. Off-track, a protein similar to receptor tyrosine kinase lacking a catalytically active kinase domain, associates with plexin-A to mediate repulsive functions of Sema1a in *Drosophila melanogaster* [21]. L1, a cell adhesion molecule belonging to the immunoglobulin superfamily, transduces the repulsive responses induced by the class 3 semaphorin Sema3A as part of a neuropilin/plexin receptor complex [22, 23]. Met, a scatter factor/hepatocyte growth factor receptor with intrinsic kinase activity, is required to transduce CD100/Sema4D signals promoting epithelial cell invasive growth; this receptor forms a receptor complex with plexin-B1 [24]. CD72, a type 2 transmembrane protein belonging to the C-type lectin family, acts as a CD100/Sema4D receptor in the immune system [25]. Tim-2, a member of the T cell immunoglobulin domain and mucin domain (Tim) protein family, interacts with Sema4A to enhance T cell activation [26].

As this complex range of receptor usage suggests, semaphorins have diverse biological functions in various tissues. Thus, the pleiotropic functions of semaphorins may be partially explained by a differential usage of ligand binding receptors and signal-transducing components. One such example is CD100/Sema4D, which utilizes two types of receptors, plexin-B1 and CD72.

A class 4 semaphorin, CD100/Sema4D

CD100/Sema4D is a transmembrane protein containing an amino-terminal signal sequence followed by a sema domain, an immunoglobulin (Ig)-like domain, a lysine-rich stretch, a hydrophobic transmembrane region and a cytoplasmic tail (fig. 2) [27, 28]. This protein is expressed on the cell surface as a homodimer. The extracellular region of CD100/Sema4D contains both potential N-linked glycosylation sites and conserved cysteine residues within the sema domain [29, 30]. Mutational analysis of human CD100/Sema4D demonstrated that C^{679} within the sema domain is required for homodimerization [31], which is essential for the semaphorins' biological function [32]. Although CD100/Sema4D does not contain a catalytic domain within the cytoplasmic region, there are consensus sites for both tyrosine and serine phosphorylation [29, 30].

CD100/Sema4D messenger RNA (mRN)A is expressed in a broad range of human tissues, including the embry-

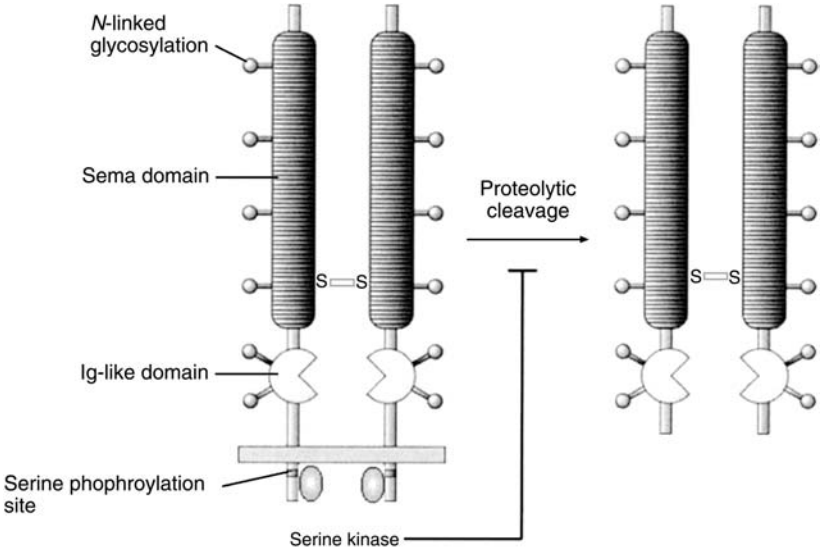

Figure 2. The structure of CD100/Sema4D. CD100/Sema4D is a member of the class 4 semaphorin subfamily. CD100/Sema4D contains an amino-terminal signal sequence, a sema domain, an Ig-like domain, a lysine-rich stretch, a transmembrane region and a cytoplasmic tail. CD100/Sema4D has several N-linked glycosylation sites. Several consensus sites for serine phosphorylation exist within the cytoplasmic domain. Although CD100/Sema4D is a transmembrane-type semaphorin, it is proteolytically cleaved from the cell surface to produce a soluble form. Serine kinase activities associated with the cytoplasmic region of CD100/Sema4D may be involved in the regulation of CD100/Sema4D proteolytic cleavage.

onic and adult brain, kidney and heart [29]. In mice, CD100/Sema4D mRNA is detectable throughout embryonic neuronal tissues, with strong expression in the cortical plate and dorsal root ganglia [30]. In developing embryos, thymus also exhibits marked expression, while the lung and kidney demonstrate only moderate expression. Expression of CD100/Sema4D is detected on the majority of hematopoietic cells, with the exception of immature bone marrow cells, red blood cells and platelets [28]. In particular, CD100/Sema4D is expressed abundantly on resting T cells, but only weakly on resting B cells and antigen-presenting cells (APCs), such as dendritic cells (DCs). Upon cellular activation, CD100/Sema4D expression is significantly upregulated at the cell surface [11, 25, 33].

Receptors of CD100/Sema4D: plexin-B1 and CD72

Plexin-B1

Plexin-B1, a receptor prominently expressed in the fetal brain and kidney, demonstrates a high binding affinity (K_d = ~1×10^{-9} M) for CD100/Sema4D (fig. 3) [19, 34, 35]. Plexin-B1 is a member of the plexin family of transmembrane proteins. The extracellular domain of plexin-B1, with 28% similarity to Met, contains a sema domain and a cleavage site for subtilisin-like proprotein convertases (PCs), which are proximal to the transmembrane domain [35, 36].

Proprotein Convertases

In cells and tissues, plexin-B1 exists in a heterodimeric form, following proteolytic cleavage by PCs, of α and β subunits [36]. The β transmembrane subunit contains a short extracellular sequence and the cytoplasmic domains. The α subunit, which includes the majority of the extracellular domain, remains associated with the cell surface through weak bonding interactions with the β subunit. These events appear to occur in a post-Golgi compartment, likely at the cell surface. The proteolytic conversion of plexin-B1 into a heterodimeric receptor significantly enhances the binding and functional responses to the ligand CD100/Sema4D; thus, the proteolytic processing of plexin-B1 by PCs is a crucial regulatory step in CD100/Sema4D-mediated functions.

Small GTPases in CD100/Sema4D signaling

Small GTPases have been implicated as mediators of semaphorin function. Plexin-B1 associates with active Rac [37–39]. CD100/Sema4D binding to plexin-B1 stimulates the recruitment of active Rac to the cytoplasmic region of plexin-B1, which competitively inhibits the binding of active Rac to p21-activated kinase (PAK), a downstream effector of Rac, although this competition has not been clarified in vivo.

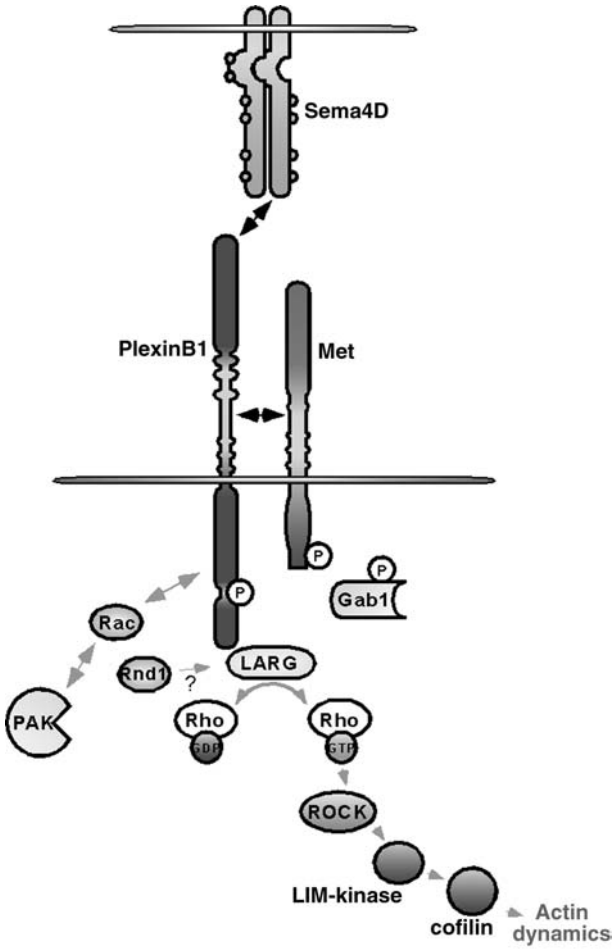

Figure 3. Models of CD100/Sema4D signaling through plexin-B1. Plexin-B1 mediates CD100/Sema4D-induced axon repulsion by coordinately regulating the activity of the GTPases Rac and Rho; plexin-B1 binds to Rac-GTP to downregulate its activity by blocking access to PAK, while binding to the RhoGEF/PDZ-RhoGEF and LARG increasing the activity of RhoA. During regulation of epithelial cell invasive growth, CD100/Sema4D signals through a plexin-B1/Met receptor complex. Binding of CD100/Sema4D to plexin-B1 activates Met, resulting in the phosphorylation of Met itself, plexin-B1 and the Met target Gab1.

Guanine exchange factors (GEFs), which facilitate the exchange of GDP for GTP, participate in vertebrate class B plexin (plexin-B1-3) signaling [37, 40–43]. The RhoGEFs, postsynaptic density protein 95-kDa/Discs Large/zona occludens-1 (PDZ)-RhoGEF and leukemia-associated RhoGEF (LARG), associate with the extreme C-terminal portion of plexin-Bs through interactions of the PDZ domains. Both binding of CD100/Sema4D to plexin-B1 and activation of the chimeric plexin-B2 proteins regulate PDZ-RhoGEF/LARG activity, leading to subsequent RhoA activation [40, 42, 43]. Dominant-negative forms of both PDZ-RhoGEF and LARG block CD100/Sema4D-induced growth cone collapse and neurite retraction [42, 43], demonstrating a requirement for Rho guanine nuclear exchange factors (RhoGEFs) and

RhoA activation in CD100/Sema4D-mediated cytoskeletal changes.

Coreceptor and tyrosine phosphorylation

In epithelial cells, plexin-B1 forms a functional receptor complex with Met (the scatter factor-1/hepatocyte growth factor receptor) [24]. Met is structurally similar to the plexins and semaphorins, also containing a Sema domain. The binding of CD100/Sema4D to plexin-B1 stimulates the intrinsic tyrosine kinase activity of Met, leading to the phosphorylation of both the receptors and a Met substrate, Gab1. Activation of Met through plexin-B1 requires the extracellular domains of both receptors. The phosphorylation of the plexin-B1/Met complex induced by CD100/Sema4D, which is significantly increased when both CD100/Sema4D and the Met ligand scatter factor-1 are present, is crucial for epithelial cell invasive growth. This evidence suggests that these two ligands function cooperatively.

CD72

CD100/Sema4D utilizes CD72 as a functional receptor (K_d = ~ 3×10^{-7} M) in lymphoid tissues (fig. 4) [25]. CD72, also known as Lyb-2, is a 45-kDa type II transmembrane protein belonging to the C-type lectin family [44, 45]. CD72 expression is detectable throughout B cell differentiation from the earliest B cell progenitors to ma-

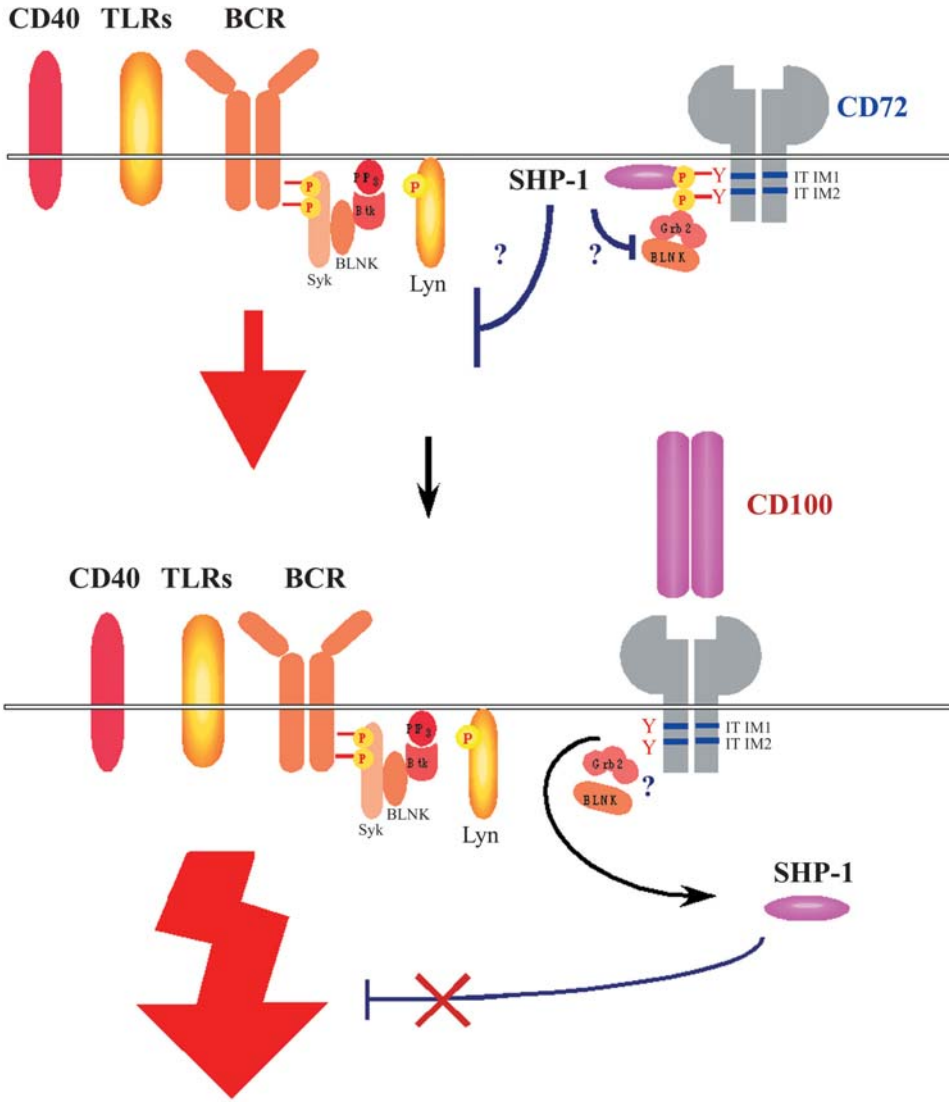

Figure 4. Models of CD100/Sema4D signaling through CD72. CD100/Sema4D turns off the negative signaling of CD72. Signals from BCR, CD40 and TLR4 are homeostatically regulated by Sema4D-CD72 interactions. In the absence of Sema4D, SHP-1 is associated with the ITIMs of CD72. SHP-1 induces tyrosine dephosphorylation and inactivation of several signaling proteins, including syk and lyn. Binding of Sema4D to CD72 dephosphorylates CD72 ITIMs, resulting in the dissociation of SHP-1 from CD72.

ture B cells, but is downregulated upon terminal differentiation into plasma cells. CD72 is also expressed by APCs, such as macrophages and DCs [46, 47].

Crosslinking of anti-CD72 monoclonal antibodies (mAbs) on B cells can transform a subset of small resting B cells into blast cells and can induce the proliferation of activated B cells [48–51]. Anti-CD72 mAbs have also been shown to block B cell receptor (BCR)-mediated cell death, promote B cell survival and proliferation, increase major histocompatibility complex (MHC) class II expression, and enhance the production and shedding of CD23 from B cells [48, 52, 53]. Anti-CD72 mAbs also induce interleukin (IL)-12 production by DCs [33]. Soluble CD100/Sema4D and CD100/Sema4D-expressing transfectants exhibit similar biological functions; CD100 synergistically enhances CD40-induced B cell and DC responses [33, 54, 55], while human CD100/Sema4D stimulation enhances the shedding of CD23 from B cell plasma membranes [29]

Studies of CD72 signaling pathways demonstrated that anti-CD72 mAbs induce the tyrosine phosphorylation of phospholipase C-γ and CD19 [56, 57]. These antibodies activate Lyn, Blk and Btk kinases, providing positive signals for B cell activation. Cumulative evidence suggests a potential role for CD72 as a negative regulator of B cell responses. The cytoplasmic domain of CD72 contains two immunoreceptor tyrosine-based inhibitory motifs (ITIMs) [58, 59]. Crosslinking of the BCR induces tyrosine phosphorylation of CD72, inducing the association of CD72 with SHP-1, an SH2-containing protein tyrosine phosphatase. SHP-1 induces tyrosine dephosphorylation and inactivation of multiple signaling proteins. SHP-1 associates with many inhibitory receptors, such as CD22 and killer inhibitory receptors, through these ITIM motifs [60–62]. Not surprisingly, B cells from CD72-deficient mice are hyperproliferative in response to various stimuli, exhibiting more rapid Ca^{2+} responses following BCR stimulation [63].

The mechanisms by which CD100/Sema4D regulates B cell responses through CD72, a negative regulator, have remained elusive. Intriguingly, both agonistic anti-CD72 mAbs and CD100/Sema4D block tyrosine phosphorylation and SHP-1 association of CD72, both of which are normally induced by anti-BCR stimulation [25, 64, 65]. These findings suggest that CD100/Sema4D enhances B cell responses by shutting off CD72-mediated negative signaling; thus, agonistic anti-CD72 mAbs likely mimic the effects of CD100/Sema4D. CD72 is constitutively tyrosine phosphorylated and associated with SHP-1 in CD100/Sema4D-deficient mice [65], supporting our model of B cell activation. A current paradigm in immune regulation states that positive outputs are generated from positive receptors, while negative outputs originate from negative receptors. The CD100/Sema4D-CD72 interaction, therefore, is a unique example of ligand binding to a negative regulator yielding a positive output [11, 12]. It is therefore logical that the immunological phenotype of CD100/Sema4D-deficient mice is the opposite of that of CD72-deficient mice; CD100/Sema4D-deficient B cells and CD72-deficient B cells are hyporesponsive and hyperresponsive to various stimuli, respectively.

Although cumulative evidence indicates strongly that CD72 is involved in B cell activation, several questions remain: (i) Is CD72 the exclusive receptor for CD100/Sema4D in the immune system? (ii) Does CD100/Sema4D require additional proteins to generate immune responses? (iii) Can CD72 function also as a positive regulator? Further comprehensive studies are required to answer these questions.

Biological functions of CD100/Sema4D

As a ligand

CD100/Sema4D exerts a wide variety of biological activities on neuronal cells, epithelial cells and immune cells (B cells, DCs and monocytes) as a ligand through the receptors plexin-B1 and CD72 [24, 25, 31, 33, 43, 54, 55, 65]. This next section reviews the additional functions of CD100/Sema4D throughout these different systems.

Neuronal cells

Immunohistochemical analysis indicates that plexin-B1 is expressed in the brain, distributed over neuronal cell bodies as well as in the neuropil. In the axonal growth cone of chick retinal ganglion neurons, plexin-B1 colocalizes with LARG [43]. As with retinal ganglion neurons, both somata and the growth cones of hippocampal neurons express plexin-B1, to which the binding of CD100/Sema4D can be detected. CD100/Sema4D induces growth cone collapse in hippocampal neurons, which can be completely blocked by Rho-kinase inhibitors. This evidence suggests the involvement of Rho or other Rho family kinases in growth cone collapse. In PC12 cells, which display neuritis, contacts with CD100/Sema4D-expressing cells induce cell rounding and the retraction of neuritis [42]. These results suggest that CD100/Sema4D-plexin-B1 interactions help to guide developing neuronal cells.

Epithelial cells

Liver progenitor cells (MLP29 cells) endogenously express plexin-B1 and Met, but not CD72. These cells form tight epithelial monolayers with junctional complexes. Giordano et al. recently demonstrated that purified soluble CD100/Sema4D, as well as scatter factor, triggers MLP29 cell ‘invasive growth’ [24]. This phenomenon is a complex program, including cell-cell dissociation, anchorage-independent growth and branching morphogenesis, in which cells acquire polarity and form tubules

arranged like the branches of a tree. These findings suggest that CD100/Sema4D functions as a ligand via the plexin-B1/Met receptor complex in the control of invasive growth. As both scatter factor and Met have been implicated in cancer and metastasis, this observation also suggests the involvement of CD100/Sema4D in these processes.

Immune cells

Exogenous expression of CD100/Sema4D or treatment with recombinant soluble CD100/Sema4D proteins both promote B cell activation, as measured by both proliferation and immunoglobulin production [25, 54, 55]. Administration of soluble recombinant mouse CD100/Sema4D in vivo accelerates the production of antigen-specific antibodies [25]. Soluble recombinant CD100/Sema4D enhances CD40-induced DC maturation, measured by the upregulation of CD40 and CD80 expression and enhanced IL-12 production [33]. The immunological activities of CD100/Sema4D on B cells and DCs have been confirmed by the phenotypes of CD100/Sema4D-deficient mice, in which both antibody production and T cell priming against specific antigens are impaired [65]. CD100/Sema4D also appears to play a role in monocyte and macrophage activation. Either recombinant soluble human CD100/Sema4D or agonistic anti-human CD72 monoclonal antibody (mAb) can induce monocyte production of proinflammatory cytokines, such as IL-6 and IL-8 [66]. Boumsell et al. have also reported that soluble human CD100/Sema4D inhibits both the spontaneous and MCP-3-induced migration of either freshly isolated monocytes or a monocytic cell line [31]. It remains unclear, however, whether CD72 or plexin-B1 is involved in the inhibitory activity of CD100/Sema4D in immune cell migration.

Collectively, these findings indicate that CD100/Sema4D plays crucial roles as a ligand functioning via plexin-B1 and CD72 in nonlymphoid and lymphoid tissues, respectively. Although the abnormalities in deficient mice have only been observed in lymphoid, but not nonlymphoid tissues, it remains possible that compensatory mechanisms lacking in lymphocytes may function outside lymphoid tissues [65]. Further studies will be required to evaluate the roles of CD100/Sema4D as a ligand in both physiological and pathological conditions.

As a receptor

Several findings also suggest a role of CD100/Sema4D as a cell surface receptor. In early studies using mAbs specific for human CD100/Sema4D, antibody crosslinking of human CD100/Sema4D enhanced T cell proliferation in the presence of submitogenic doses of anti-CD3 or anti-CD2 mAbs [27]. Thus, CD100/Sema4D was thought to mediate signals through its cytoplasmic domain. Furthermore, in human T cells, CD100/Sema4D is associated with serine/threonine kinase and protein tyrosine phosphatase (PTP) activity [67]. In addition, it has been reported that PTPs are differentially associated with human CD100/Sema4D during the terminal stages of B cell differentiation [68–70], suggesting that CD100/Sema4D may function as a receptor transmitting signals to lymphocytes. Granziero et al. recently reported that human plexin-B1-expressing transfectants sustain the proliferation of normal and leukemic $CD5^+$ cells, both of which express human CD100/Sema4D [71]. This evidence suggests that human CD100/Sema4D functions as a receptor for human plexin-B1. Although the physiological significance of CD100/Sema4D as a receptor remains to be determined, it is plausible that CD100/Sema4D possesses bidirectional functions in cognate cell-cell contacts.

Which is the active form of CD100/Sema4D, a soluble form or transmembrane-form?

Although the semaphorin family contains secreted-type and transmembrane-type member proteins, most of the functions, such as axonal guidance cues, have been demonstrated to be mediated by the secreted-type members. CD100/Sema4D, a class 4 semaphorin, is a transmembrane-type semaphorin. Both human and mouse transmembrane-type CD100/Sema4D are proteolytically cleaved into a 120-kDa soluble form (fig. 2) [31, 54, 55, 68, 72, 73]. It is thus critical to determine the active form of CD100/Sema4D, either the transmembrane or soluble form. The generation of soluble CD100/Sema4D appears to be well regulated; its release from primary T and B cells is strictly dependent on a proteolytic cascade that follows cellular activation [54]. Interestingly, significant soluble CD100/Sema4D is detectable in the sera of both mice immunized with a T-cell-dependent antigen and autoimmunity-prone MRL/lpr mice. In these animals, the levels of soluble CD100/Sema4D correlate well with antigen-specific antibody or autoantibody titers, although soluble CD100/Sema4D is below detection levels in the sera of unimmunized normal mice [54]. The in vivo profiles of soluble CD100/Sema4D suggest the involvement of soluble CD100/Sema4D in physiological and pathological immune responses. Indeed, the majority of CD100/Sema4D biological functions on various cells as a ligand have been revealed using recombinant or naturally cleaved soluble CD100/Sema4D, suggesting that soluble CD100/Sema4D released from the cell surface may be responsible for the observed activities. Although it remains unclear whether the transmembrane-type CD100/Sema4D requires conversion into a soluble form to exert its functions, it is reasonable to state that soluble CD100/Sema4D is active. In this context, the transmembrane-type CD100 may thus exist as a reservoir for soluble CD100/Sema4D, facilitating a broader spectrum of

biological activities. If so, the proteolytic cleavage of CD100/Sema4D may be a critical step for regulating the functions of CD100/Sema4D throughout a broad range of tissues. However, it has been demonstrated that CD100/Sema4D-expressing transfectants have activities on B cells and neuronal cells [29, 42], indicating their functional importance. Further studies will be required to know whether the biological activity of the transmembrane-type CD100/Sema4D is qualitatively different from that of soluble CD100/Sema4D.

Although the cleavage site has not been determined, the cleavage of transmembrane-type CD100/Sema4D on the cell surface relies on an enzymatic process, as it is inhibited by either azide or incubation at 4 °C [72]. In addition, the light metal chelators EDTA and EGTA inhibit up to 50% of CD100/Sema4D shedding, suggesting that metalloprotease activity may facilitate shedding. However, several metalloprotease inhibitors cannot inhibit this process, indicating that the mechanism governing CD100/Sema4D cleavage is likely to be different from that employed for other surface molecules. The cytoplasmic region of CD100/Sema4D also appears to plays a role in the regulation of soluble CD100/Sema4D production. Staurosporine (a cell-permeable, broad-range inhibitor of serine kinases) enhances the release of soluble CD100/Sema4D, suggesting that serine phosphorylation regulates CD100/Sema4D cleavage [72]. Interestingly, large quantities of soluble CD100/Sema4D are detectable in the sera of transgenic mice expressing a truncated form of CD100/Sema4D that lacks the cytoplasmic region, despite weak cell surface expression of the transgene product [55]. These findings collectively implicate the cytoplasmic region of CD100/Sema4D in the regulation of soluble form generation.

Perspectives

While in this article we focus on the current advances concerning a class 4 semaphorin, CD100/Sema4D, several other semaphorins also appear to function outside the nervous system. In the immune system, Sema4A, a class 4 semaphorin, is crucially involved in in vitro and in vivo T cell activation through interactions with Tim-2 [13, 26]. The viral semaphorins, A39R (encoded by vaccinia virus) and AHVsema (encoded by alcelaphine herpes virus), bind to their cellular receptor, virus-encoded semaphorin protein receptor (VESPR)/CD232/plexin-C1, to induce proinflammatory cytokine production in human monocytes [16, 74]. The glycosylphosphatidylinisitol (GPI)-anchored class 7 semaphorin CD108/ Sema7A/Sema-K1, a mammalian counterpart of AVHsema, also binds VESPR/CD232/plexin-C1 to induce proinflammatory cytokine production [19]. Even Sema3A, the most well characterized semaphorin identified as an axonal guidance factor, appears to inhibit monocyte migration [31]. Several semaphorins also play crucial roles outside the nervous and immune systems, functioning in organogenesis, vascularization and tumorgenesis. We have reviewed here that CD100/Sema4D induces neuronal growth cone collapse through interactions with plexin-B1, invasive growth of epithelial cells via the plexin-B1/Met receptor complex and immune responses through CD72. These findings naturally suggest the directions necessary to clarify the diverse activities of semaphorins. More comprehensive studies to determine receptor usage, including the presence or absence of additional receptor components in a broad range of tissues, will help delineate the pleiotropic effects of the semaphorin-receptor system.

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