Transmembrane semaphorins: Multimodal signaling cues in development and cancer

Transmembrane Semaphorins: Multimodal Signaling Cues in Development and Cancer

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Abstract

Semaphorins constitute a large family of membrane-bound and secreted proteins that provide guidance cues for axon pathfinding and cell migration. Although initially discovered as repelling cues for axons in nervous system, they have been found to regulate cell adhesion and motility, angiogenesis, immune function and tumor progression. Notably, semaphorins are bifunctional cues and for instance can mediate both repulsive and attractive functions in different contexts. While many studies focused so far on the function of secreted family members, class 1 semaphorins in invertebrates and class 4, 5 and 6 in vertebrate species comprise around 14 transmembrane semaphorin molecules with emerging functional relevance. These can signal in

juxtacrine, paracrine and autocrine fashion, hence mediating long and short range repulsive and attractive guidance cues which have a profound impact on cellular morphology and functions. Importantly, transmembrane semaphorins are capable of bidirectional signaling, acting both in "forward" mode via plexins (sometimes in association with receptor tyrosine kinases), and in "reverse" manner through their cytoplasmic domains. In this review, we will survey known molecular mechanisms underlying the functions of transmembrane semaphorins in development and cancer.

Keywords: Semaphorins, Plexins, Development, Cancer, Signaling mechanisms.

Semaphorins and their receptors

Semaphorins are secreted, transmembrane and GPI-linked glycoproteins that have been grouped into eight classes, based on structural features and amino acid sequence similarity. There are around 20 semaphorins in humans, Drosophila has five, and two are known from viral genomes. Semaphorins found in invertebrates are grouped in classes 1-2, vertebrate ones in classes 3-7, and a final group contains those encoded by viruses. Notably, class 1, 4, 5 and 6 comprise transmembrane molecules, which include a cytoplasmic domain. All members contain a conserved extracellular domain of about 500 amino acids known as the Sema-PSI domain, located at the N-terminal of the molecule. The size of transmembrane semaphorins may range from 400 to 1000 amino acid residues. In addition, downstream to the sema domain, class 4 semaphorins include an immunoglobulin(IG)-like domain, while class 5 semaphorins contain seven thrombospondin motifs. Intracellular domains of class 4 semaphorins have a PDZ-domain

binding motif at the C-terminus. Transmembrane semaphorins of class 6 have the longest cytoplasmic domain of about 400 amino acids, which also contains proline-rich motifs.

High-affinity receptors for transmembrane semaphorins are essentially represented by plexin family members.[1-3] Neuropilins, which are important co-receptors for secreted semaphorins, do not seem to have a role in the signaling cascade of transmembrane family members (with the reported exception of an interaction between Sema4A and Neuropilin-1).[4] Invertebrates bear two plexin genes, while there are nine plexins in vertebrates. The latter are divided into four subfamilies: PlexinA(1-4), PlexinB(1-3), PlexinC1 and PlexinD1.The extracellular moiety of plexins contains one sema domain and two-three PSI motifs, similar to those of semaphorins; moreover, they include 3-4 IPT domains (shared by plexins, integrins and certain transcriptional factors). All plexins have very similar cytoplasmic structures, comprising a RasGTPase-activating protein(GAP) domain with an inserted Rho GTPase-binding domain(RBD).[5]

Different transmembrane semaphorins have been found to interact at lower affinity with additional cell surface receptors beyond plexins (see Fig.1). For example, Sema4A expressed in dendritic and B cells enhances the activation and differentiation of T cells and the generation of antigen specific T cells in vivo also via the receptor TIM-2.[6] In highly metastatic lung cancer cells, Sema4B interacts with CLCP1(CUB,LCCL-homology, coagulation factor V/VIII homology domains protein), a protein with similarity to neuropilins. Here, Sema4B acts as one of the ligands of CLCP1, and enhances its ubiquination and proteosome degradation, in turn regulating the motility of lung cancer cells.[7] A further member of the class 4, Sema4D, interacts with CD72, a negative regulator of B cell responsiveness; Sema4D stimulation induces tyrosine dephosphorylation of CD72 intracellular tail and its dissociation from the effector SHP-1, turning off CD72 inhibitory signaling.[8] Moreover, Sema5A exerts both attractive and inhibitory

65 effects on developing axons of the fasciculus retroflexus by physically interacting with
66 glycosaminoglycan chains of chondroitin sulfate proteoglycans(CSPGs) or heparin sulfate
67 proteoglycans(HSPGs), expressed by different neuronal populations. In particular, CSPGs
68 function as precisely localized extrinsic cues that convert Sema5A from an attractive to an
69 inhibitory guidance cue, whereas axonal HSPGs mediate Sema5A mediated attraction.[9]

70

71 Signaling mode paradigms used by transmembrane semaphorins

72 Transmembrane semaphorins can act by multiple signaling modes. Clearly, when exposed on the
73 cell surface, they can engage short-range cell-to-cell interactions with neighboring cells, either of
74 the same type, or belonging to a different cell population in the tissue environment. Moreover,
75 while they are synthesized as single-pass membrane-spanning molecules, in many cases their
76 extracellular moiety can be shed in soluble form, and potentially act as a secreted diffusible
77 signal. Unlike what is known for secreted class 3 semaphorins (which are processed by furin-like
78 convertases), transmembrane semaphorin cleavage is mediated by diverse metalloproteases e.g.
79 MT1-MMP mediates tumor angiogenesis through the release of Sema4D,[10] most of which have
80 not been clearly identified; moreover, the targeted cleavage sites generally need elucidation.

81 Thus transmembrane semaphorins can function by three different signaling paradigms: in
82 juxtacrine mode (when membrane-bound), and in autocrine or paracrine mode (upon ectodomain
83 release) (see Fig.2). Sema4D is a good example of this signaling versatility, and its
84 proteolytically shed isoform has been characterized even better than its membrane-bound
85 counterpart.[11] For instance, Sema4D autocrine signals in endothelial cells promote sprouting and
86 angiogenesis;[12] however, Sema4D can also act in paracrine manner on the endothelium when

released by other cells in the microenvironment.[13] As an example of juxtacrine signaling, the ligation of Sema4D/CD100 in γδ T cells to the receptor PlexinB2 exposed by damaged keratinocytes induces cell rounding via signals through ERK kinase and cofilin, contributing to the skin wounding process.[14]

Bidirectional signaling of transmembrane semaphorins

All semaphorins are known to act through the intracellular domain of the plexins, by a so-called "forward" signaling pathway, which negatively regulates integrin-mediated adhesion and induces cytoskeletal remodeling. Moreover, exclusively transmembrane semaphorins can also mediate a "reverse" signaling mode, by acting as receptors rather than ligands, and signal through their own cytoplasmic domains.

In fruit fly *Drosophila melanogaster*, Sema1a is a repulsive ligand controlling motor axon guidance during development. Sema1a interaction *in trans* with PlexinA exposed by adjacent cells is crucial for defasciculation of nerve bundles. This forward signaling cascade is modulated by perlecan, an extracellular matrix component, which enhances semaphorin-induced downregulation of integrin adhesive function and FAK dephosphorylation, leading to motor axon defasciculation.[15]Notably, Sema1a can also mediate motor axon defasciculation through reverse signaling mechanisms, whereby its cytoplasmic domain can interact with two major antagonistic regulators of the GTPase Pebble and the inhibitor RhoGAP p190. The first activates Rho1 and promotes axon-axon repulsion and defasciculation, while p190-RhoGAP antagonizes this mechanism allowing axonal attraction;[16, 17] the extracellular Sema1a-binding molecule triggering this cascade is still unclear.

109 The signaling cascade elicited downstream of semaphorin/plexin interactions in vertebrates has
110 been studied in a variety of cell types and models. Certain forward signaling mechanisms are
111 shared by most plexins or family members of the same subclass. For instance, many plexins have
112 been found to regulate the activity of GTPases of the Ras/Rho family. In particular, plexin
113 cytoplasmic domain carries intrinsic GTPase Activating Protein (GAP) activity against R-Ras,
114 M-Ras and/or Rap-1 GTPases. In different studies, this has been shown to inhibit beta1 integrin-
115 dependent adhesion and cell detachment from the extracellular matrix; [18, 19] hinder the activity of
116 phosphoinositide 3-kinase, leading to AKT dephosphorylation and activation of GSK-3beta;[20]
117 and derepress p120-Ras-GAP activity, leading to downregulation of RAS-MAPK signaling.[21]
118 The final outcome of this signaling cascade typically is the inhibition of cell migration.
119 Moreover, Rho GTPases, such as RhoA, Rac and Cdc42, known to control cell motility by
120 regulating actin and microtubule dynamics, are considered important downstream effectors of
121 plexin receptors. For instance, it was reported that Sema4D activated PlexinB1 can regulate
122 RhoA activity via p190-RhoGAP protein,[22] or inhibit RAC-dependent PAK activation.[23] In
123 addition, PlexinB1 and PlexinB2, by means of leukaemia associated Rho-GEF(LARG) and
124 p190-PDZ-RhoGEF tethered to their C-terminus consensus sequences, can upregulate GTP-
125 bound active RhoA levels, impinging on cytoskeletal reorganization and growth cone
126 morphology.[24, 25]

127 Notably, many forward semaphorin signals are mediated by multimeric receptor complexes,
128 containing plexins in association with additional transmembrane subunits. For transmembrane
129 semaphorins, these often implicate plexin-associated tyrosine kinase receptors (RTK) (see Fig.
130 1). For example, semaphorin-dependent stimulation of PlexinB1, PlexinB2 or PlexinB3 can
131 activate and induce the phosphorylation of ERBB2, MET and RON receptor tyrosine kinases in

132 different cell types.[12, 26-29] Furthermore, Sema6D-PlexinA1 forward signaling, required for the
133 ventricular chamber morphogenesis during chick embryo heart development, depends on the
134 differential involvement of two plexin-associated RTKs. In cells of the conotruncal segment,
135 Sema6D binding to a PlexinA1-VEGF-R2 kinase complex mediates cell migration and invasive
136 growth. By contrast, Sema6D inhibits the migration of cardiac muscle cells of ventricle region,
137 which express PlexinA1 in association with another (kinase-dead) RTK, named OTK (off-track
138 kinase).[30, 31]

139 On the other side of the street, the intracellular domain of transmembrane semaphorins, including
140 Sema6D, has been found to interact with putative signaling effectors, potentially mediating
141 reverse signaling cascades. In particular, the cytoplasmic portion of Sema6D can bind to both
142 Abl kinase and Mena/Enabled. During cardiac chamber formation, upon Sema6D engagement *in*
143 *trans* with PlexinA1, Abl kinase gets activated, resulting in the phosphorylation of Mena. This
144 leads to the dissociation of Mena from Sema6D cytoplasmic tail, thereby promoting cell
145 migration and trabeculation of the myocardial layer.[31]

146 Other class 6 semaphorins have been found in association with intracellular effectors. For
147 example, Sema6A can interact with EVL (Ena/VASP-like protein) via its zyxin-like carboxy-
148 terminal domain suggesting a possible role in retrograde signaling during neuronal
149 development.[32] Furthermore, the intracellular domain of Sema6B was found to bind to the SH3
150 domain of the oncogenic tyrosine kinase c-Src (Fig.3).[33]

151 Interestingly, the cytoplasmic domain of many class 4 semaphorins terminates with a consensus
152 sequence anchoring PDZ domains.[34-36] These protein-protein interaction domains mediate
153 receptor clustering in neuronal post-synaptic membranes, and in general serve as scaffolds for

the assembly of multi-molecular signaling complexes. Indeed, three different class-4 semaphorins have been shown to co-localize and interact with PSD-95/SAP90, e.g. Sema4C in cerebral cortical neurons,[37] and Sema4B and Sema4F in hippocampal neurons.[35, 36] During muscle development, knocking down Sema4C or blocking its PDZ domain-binding motif resulted in inhibition of myogenic differentiation;[38] these data suggested a putative role of reverse signaling, though the plexin counterpart responsible for triggering this process has not been identified.

Finally, as mentioned above, the cytoplasmic domain of fly Sema1a can mediate opposite reverse signaling effects by interacting with the two major antagonistic regulators of RhoA: the GTPase exchanger Pebble and the inhibitor p190RhoGAP.[16, 17]

In cis versus *in trans* signaling functions of transmembrane semaphorins

In addition to their interaction *in trans* between adjacent cells, transmembrane semaphorins and plexins can also associate *in cis* on the surface of the same cell, resulting in the functional regulation of other signaling cascades. Notably, the association of a semaphorin with its co-expressed plexin receptor *in cis* can inhibit the signaling function of either of the two molecules *in trans* with adjacent cells. For example, *in cis* Sema6A-PlexinA4 association in dorsal root ganglion neurons hinders Plexin interactions *in trans* with Sema6A molecules expressed by adjacent cells.[39] Moreover, while Sema6A is widely expressed in the developing hippocampus, where it acts as repelling signal for extending axons (mossy fibers), its association *in cis* with PlexinA2 co-expressed in certain areas hinders Sema6A activity *in trans* there by establishing a

permissive corridor for layer-restricted axonal innervations.[40] In other settings, *in cis* interaction between a semaphorin/plexin pair can instead activate plexin signaling, as shown in *C.elegans* for transmembrane semaphorin SMP-1 and class A plexin homologue PLX-1, leading to repelling signals inhibiting moto neuron synapse formation.[41]

Transmembrane semaphorins in embryo development

The development of complex tissues and organs depends on cell proliferation, migration and differentiation. While semaphorins have been shown to regulate many of these processes, the best characterized feature of semaphorin/plexin signals is to provide repulsive or attractive cues for migrating cells and growing neurites.[42] Thus, semaphorin-deficient mouse models have been widely used to study the physiological role of these molecules in the developing nervous system. Among mutants deficient for transmembrane semaphorins, Sema4B-/-mice displayed reduced proliferation of astrocytes after CNS injury.[43] On the other hand, Sema4C and Sema4G deficient mice showed severe defects in cerebellar development: in particular, Sema4C-/- mutants show exencephaly and neonatal lethality, a phenotype less prominent in Sema4G deficient mice[44]. Sema4D-/- mutants resulted in increased oligodendrocyte number in basal conditions and upon injury[45]. Gross defects in the early development were seen in Sema5A KO mice, leading to embryonic lethality, although the implicated deficient mechanism was not elucidated.[46] Recent studies also reported aberrant projections of thalamo-cortical axons in Sema6A null mice.[47] Moreover, Sema6A is expressed by tangentially migrating granule cells in the developing cerebellum, where it controls the switch from tangential to radial migration.[48] Studies of PlexinA4 and PlexinA3/A4 double mutants have shown that these plexins regulate the patterning

197 of spinal sensory axons and cranial nerve projections.[49, 50] In a recent study, double deletion
198 mutants of PlexinB1 and PlexinB2 displayed impaired corticogenesis with cortical thinning.
199 These homologous plexins seem to play redundant/compensatory roles during forebrain
200 development, in order to ensure proper neuronal proliferation and neocortical expansion.[51] In
201 most cases the absence of dramatic neuronal phenotypes in transmembrane semaphorin mutants
202 may be explained by redundancy among family members or the existence of corrective
203 mechanisms by which early axons which are misguided are eliminated.

204 Notably, Sema4D/PlexinB1 signaling is a typical example mediating either attractive or repelling
205 cues for different neurons. In hippocampal development, Sema4D inhibits axonal extension by
206 suppressing R-Ras activity, leading to Akt dephoshorylation and activation of GSK-3β.[52]
207 Opposite effects are seen in the hypothalamus, where gonadotropin-releasing hormone
208 expressing neurons (GnRH neurons) control the release of reproductive hormones by the
209 pituitary. Indeed, failure to stimulate the pituitary with GnRH causes reproductive disorders and
210 lack of initiation of puberty, and PlexinB1 deficient mice revealed a migratory effect in GnRH-1
211 neurons, leading to smaller neuronal population in adult brains, and consequent fertility defects.
212 Notably, in this context, Sema4D promotes directional migration of GnRH-1 cells by coupling
213 PlexinB1 with MET kinase activation.[53]

214 Oligodendrocytes are a type of neuroglia found in CNS, which is responsible for the formation of
215 a myelin sheath surrounding neuronal projections. Several semaphorins, including Sema4D,
216 Sema4F, Sema5A and Sema6A are known to be major modulators of oligodendrocyte
217 development, and this is a particularly interesting model of short range cell-to-cell and
218 bidirectional semaphorin signaling. For instance, Sema4D knockout mice display an increased
219 number of oligodendrocytes in the adult cerebral cortex, which is due to reduced oligodendrocyte

apoptosis; this effect could be reversed by adding soluble Sema4D, which suggests its role as a ligand in this process.[45, 54] Another class-4 Semaphorin, Sema4F, is widely expressed by neuronal precursors, mature neurons and glial cells. Sema4F is reported to inhibit the migration of oligodendrocyte progenitor cells and promote their differentiation.[55] Sema5A expression is restricted to oligodendrocytes and their precursors, among optic nerve glial cells; and it was demonstrated that Sema5A induces growth cone collapse and inhibits axon growth of retinal ganglion cells (RGC).[56] Sema6A is also expressed at high levels during oligodendrocyte development, peaking during myelination. Sema6A knock-out mice show delayed oligodendrocyte differentiation both *in vivo* and *in vitro* and interestingly, this delayed differentiation of Sema6A-deficient oligodendrocytes is not rescued by the addition of exogenous Sema6A *ex vivo*, suggesting a possible reverse signaling mechanism, to be further elucidated.[57]

As mentioned above, during chick embryo heart development, knockdown of Sema6D or its receptor PlexinA1 results in lesser expansion of the primitive ventricle and poor trabeculation of the muscular layer. In this context, the interaction between endocardial and myocardial cells (expressing both Sema6D and PlexinA1) can trigger both forward and reverse signaling cascades controlling cell migration, morphogenic patterning of the cardiac chambers and muscle layer trabeculation. In particular, (endocardial-expressed) Sema6D forward signals to myocardial cells of the conotruncal segment expressing PlexinA1-VEGFR2 receptor complexes to promote cell migration and invasive growth. By contrast, Sema6D inhibits the migration of cardiac muscle cells of ventricle region, which express PlexinA1 in association with the catalytic inactive off-track kinase.[30, 31] On the other hand, trabecular formation is promoted by Sema6D reverse signaling into myocardial cells of the compact layer.[31]

Transmembrane semaphorins implicated in cancer

Accumulating evidence indicates that semaphorin signals can play a major role in the tumor context, beyond their established role in development. Various cancer cells express both semaphorins and their receptor, and experimental evidence shows that these signals can either promote or impede the various hallmarks of cancer, like tumor cell proliferation and survival, tumor angiogenesis and evasion from immune response, to name a few. Notably, the expression of various semaphorins and their receptors has been found to be either up-regulated or down-regulated compared to normal tissues, consistent with their potential role as tumor promoters or suppressors.[58]

Also in the cancer context, while considerably more attention has been devoted to the role of semaphorins of the secreted type, scattered reports started to highlight the potential relevant role of transmembrane semaphorins, and their peculiar signaling modes. Especially semaphorins belonging to class 4 have been found to regulate the behavior of cancer cells, as well as tumor angiogenesis. Germline variants of Sema4A have been associated with increased risk for a type of familial non-polyposis colorectal cancer; Sema4A-V78M mutation in particular caused increased MAPK/Erk and PI3K/Akt signaling in HCT-116 colorectal cancer cells *in vitro*[59] and more studies are required to validate its tumorigenic activity *in vivo*.

In lung cancer, the role of Sema4B seems rather controversial. Sema4B expression is suppressed by hypoxia[60]and it may inhibit growth of non-small lung cancer cells by suppressing PI3K/Akt signaling pathway[61] and metastasis by down regulating expression of MMP9.[62] Other data

264 showed that Sema4B interacts with CLCP1 and may drive its degradation and enhance cell
265 motility; CLCP1 is a protein similar to neuropilins overexpressed in lung cancer metastatic
266 cells.[7]

267 Aberrant expression of Sema4C has been reported in esophageal, gastric and colorectal cancer.[63]
268 In paclitaxel-resistant lung and breast cancer cells Sema4C levels is regulated by miR-125b, and
269 its overexpression not only resensitizes these cells to the drug, but also reverts a mesenchymal to
270 epithelial phenotype.[64, 65] In glioblastoma, the activation of PlexinB2 receptor by the ligand
271 Sema4C, induces actin-based cytoskeletal dynamics and cell migration by RhoA and Rac1
272 activity.[66] The expression of Sema4C was up regulated both at the transcriptional and the
273 translational levels in lymphatic endothelial cells of breast cancer tissues.[67]

274 Sema4D is widely expressed in cancer cells and it is the most studied transmembrane
275 semaphorin in cancer. High expression of Sema4D was associated with poor survival in
276 pancreatic ductal adenocarcinoma, where it enhances tumor cell motility[68], and its higher
277 expression was correlated with poorer overall and disease free survival in soft tissue sarcoma.[69]
278 In breast carcinoma cells, PlexinB1 and PlexinB2 form complexes with ErbB2 tyrosine kinase,
279 which elicits a pro-migratory effect in response to Sema4D. In these cells, Sema4D-PlexinB1
280 signaling can instead mediate an anti-migratory effect when associated with MET receptor.[26, 70]
281 In addition,Sema4D production by head and neck carcinoma cells elicits the expression of
282 Platelet Derived Growth Factor-B and Angiopoietin-like-protein-4 by endothelial cells (in a
283 PlexinB1/RhoA dependent manner) inducing proliferation and differentiation of pericytes, and
284 vascular permeability. These data suggest that targeting Sema4D along with VEGF could be a
285 better therapeutic option for the treatment of solid tumors.[71] Recent studies have identified

286 Sema4D as an oncogene in osteosarcoma by forward genetic screening, where by Sema4D was
287 demonstrated to be highly expressed in large fraction of human osteosarcoma tumors and cell
288 lines associated, and overexpression of Sema4D is these cells lines activated AKT and/or MAPK
289 pathways.[72] In addition to cancer cells, Tumor Associated Macrophages (TAM) may be a major
290 source of Sema4D in the tumor microenvironment;[13] this was found to enhance angiogenesis and
291 tumor cell invasiveness by transactivating oncogenic receptor tyrosine kinase MET, associated
292 with PlexinB1.[28, 73] In general, effective silencing of Sema4D in cancer cells inhibits tumor
293 vasculature and tumor burden.[10, 74-80] Moreover, Sema4D activity in cancer can be targeted with
294 monoclonal antibodies, such as VX15/2503,[81-84] currently in clinical trials for treating solid
295 tumors. Notably, blocking Sema4D with monoclonal antibodies in tumors may promote immune
296 cell infiltration and enhance response to immunomodulatory drugs such as anti-CTLA-4.[85]
297 Another member of this subclass, Sema4F, is a critical regulator of neuroepithelial interactions
298 and considered as a biomarker in prostate cancer, as its cytoplasmic expression also correlates
299 with nerve density and perineural invasion.[86]

300 Also Sema5A-receptor PlexinB3 was found to interact with MET and promote tumor cell
301 invasiveness.[29] Sema5A regulates cell motility and morphology of human glioma cells via
302 RhoGDIalpha-mediated inactivation of Rac1 GTPase and the functional regulation of fascin-1
303 actin-binding protein.[87, 88] In renal cell carcinoma cells, Sema5A downregulation significantly
304 reduced viability.[89] On the other hand, lower expression of Sema5A was associated with poor
305 survival among non-smoking women bearing non-small cell lung carcinomas (NSCLC).[90]

306 A recent report pointed to the requirement of Sema6A for the survival of BRAF V600E human
307 melanoma cells, whereby depletion of Sema6A causes loss of anchorage-independent growth

308 and inhibition of migration and invasion.[91] Sema6B could have a pro-proliferative effect on
309 U87MG cells as silencing it inhibited tumor formation.[92]

310

311 Conclusion and future perspectives

312 Consistent evidence indicates that transmembrane semaphorins are major guidance cues for axon
313 pathfinding and the wiring of the neural network, and emerging regulators of angiogenesis and
314 tumor progression. They can act as versatile, short or long range signals, in either membrane
315 bound or secreted form, respectively. Moreover, they can mediate downstream "forward" and
316 "reverse" signaling cascades, which implicate a variety of potential effector molecules, beyond
317 plexin receptors. In sum, our knowledge of transmembrane semaphorin functions and signaling
318 pathways is still far from complete and further studies will be required to understand their
319 relevance in development and cancer.

320

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837 **Figure legends**

838 **Figure 1. Representative transmembrane semaphorins and their receptor**
839 **complexes.**

840 A number of transmembrane semaphorins signal through diverse receptor complexes. Notable
841 examples are illustrated in this figure. Sema4A can bind to Tim-2, a protein expressed on T cells,
842 in addition to plexins. In lymphocytes, Sema4D can associate with CD72, a member of the C-
843 type lectin family. In cancer cells, Sema4D can signal through complexes including PlexinB1
844 and ErbB2 or Met depending on the cell type. Sema5A can signal through PlexinB3 and Met in
845 epithelial cancer cells. However, in neurons, proteoglycans such as HSPG and CSPG modulate
846 Sema5A signaling, independent of PlexinB3. PlexinA1 is alternatively associated with OTK or
847 VEGFR2 receptor tyrosine kinases in different cells of the developing heart, and these signaling
848 complexes have distinct functions in cardiac development.

849 **Figure 2. Various signaling mode paradigms used by Sema4D transmembrane**
850 **semaphorin.**

851 Sema4D is taken as an example of diverse signaling paradigms of transmembrane semaphorins.
852 In particular, Sema4D produced by endothelial cells can function in autocrine manner on its
853 surface receptor such a PlexinB1. In addition, Sema4D released by other cells in the tumor
854 microenvironment (e.g., Tumor Associated Macrophages) can signal in paracrine fashion to
855 endothelial cells. Moreover, during wound healing, Sema4D expressed by dendritic epidermal T
856 cells can bind to PlexinB2 expressed on the surface of damaged keratinocytes, acting in
857 juxtacrine mode.

858 **Figure3. Forward and reverse signaling effectors of transmembrane**
859 **semaphorins.**

860 The general paradigm of forward and reverse signaling of transmembrane semaphorins is
861 depicted on the left. On the right, a table summarizes various effectors implicated in these
862 distinctive signaling modes for different family members.

863 **Table 1.**

864 **Transmembrane Semaphorin functions in development and pathophysiology**

Semaphorin	Reported role in embryo development or adult pathophysiology
Sema4A	Disruption of Sema4A associated with retinal degeneration[93] Deficient mice for Sema4A has defective T cell priming[94] Induces growth cone collapse of hippocampal neurons in a Rho/Rho-kinase dependent manner[95] Mutation associated with retinal degenerative disease[96] Associated with experimental autoimmune myocarditis[97] Downregulation reduces severity of allergic response[98] Supports photoreceptor survival in retinal pigment epithelium[99] Maintains stability of regulatory T cells[4] Inhibitory role in allergic asthma[100] Required for optimal activation and differentiation of CD8+ T cells[101] Involved in rheumatoid arthritis[102]
Sema4B	Negative regulator of basophil-mediated immune response[103] Associates with brain injury induces astrogliosis[104]
Sema4C	Required in myogenic differentiation[38] Required in cerebellar development[44] Expressed in neuronal stem cells[105-107] Modulates morphogenesis of ureteric epithelium[108, 109] Induces EMT in renal tubular epithelial cells[110]
Sema4D	Regulates B cell signaling[8] Deficiency of Sema4D leads to defective B and T cells activation[111] Released by activated lymphocytes[112] Sustains proliferation and survival of normal and leukemic CD5+B lymphocytes[113] Expressed by oligodendrocytes and upregulated after CNS lesion[114] Stimulates outgrowth of embryonic DRG sensory neurones[115] Induces growth cone collapse by R-Ras GAP activity[20] Involved in induction of immune allo-response[116] Regulates dendritic spine density through RhoA/ROCK pathway[117] Released by platelet in response to vascular injury[118]

	Inhibits collagen synthesis of rat pulp derived cells[119] Regulates gonadotropin hormone releasing hormone-1 neuronal migration[53] Controls epithelial branching morphogenesis[120] Regulates SHP-2 to induce axon repulsion[121] Remodels dendrite morphology by inactivating M-Ras[122] Deficiency results in increased number of oligodendrocytes in mouse brains[45] Controls microglia activation[123] Deficiency associates with superior mouse motor behavior[124] Stimulates PTEN activity to induce growth cone collapse[52] Lack of Sema4D impairs thrombus growth[125] Reduces intimal neovascularization and plaque growth[126] Inhibitory regulator of oligodentrocyte development[54] Promotes rapid assembly of GABAergic synapses in rodent hippocampus[127] Required for optimal lung allergic inflammation[128] Required for development of the hindbrain boundary and skeletal muscle in zebrafish[129]
Sema4E	Guides branchiomotor axons to their targets in zebrafish[130]
Sema4F	Involved in Schwann cell axonal interactions[131] Regulates oligodendrocyte precursor migration in the optic nerve[55]
Sema4G	Required in cerebellar development[44]
Sema5A	Inhibition serves as ensheathing function during optic nerve development[132] Inhibits axon growth by retinal ganglion cells[56] Bifunctional guidance cue for axons of fasciculus retroflexus[9] Inactivation leads to embryonic lethality [46] Bifunctional axon guidance cue for axial motoneurons in vivo[133] Controls selective mammalian retinal lamination and function[134] Involved in mammalian retinal development[135] Inhibits synaptogenesis in early postnatal and adult born hippocampal dentate granule cells[136] Modulates attraction of dorsal root ganglion axons in vertebrates[137] Mutation associates with risk of Parkinson disease[138]
Sema5B	Mediates synapse elimation in hippocampal neurons[139] Control selective mammalian retinal lamination and function[134] Proteolytically processed into a repulsive neural guidance cue[140] Repellent cue for sensory afferents projection in developing spinal cord[141]

Sema5C	Contributes to olfactory behavior in adult drosophila[142]
Sema6A	Repels embryonic sympathetic axons[143] Regulates cerebellar granule cell migration[48] Induced by interferon-gamma in Langerhans cells [144] Acts as a gate keeper between central and peripheral nervous system[145] Controls lamina-restricted projection of hippocampal mossy fibers[40] Controls nucleus centrosome coupling in migrating granule cells[146] Controls guidance of corticospinal tract axons[147] Promotes dentritic growth of spinal motor neuron[148] Improves functional recovery after cerebral ischemia[149] Mutation disrupts limbic and cortical connections during neurodevelopment[150] Regulates oligodendrocyte differentiation and myelination[57] Promotes eye vesicle cohesion[151]
Sema6B	Regulates lamina restricted projections of hippocampal mossy fibers[152] Acts as a receptor in post crossing commissural axon guidance[153]
Sema6C	Leads to GSK-3-dependent growth cone collapse[154] Expressed in innervated and denervated skeletal muscle[155]
Sema6D	Plays dual role in cardiac morphogenesis[30] Regulates myocardial patterning in cardiac development by reverse signaling[31] Altered signaling inhibits synapse formation[156] Promotes retinal axon midline crossing[157]

875 **Table.2**

876 **Transmembrane semaphorins implicated in cancer development**

Target protein	Functions potentially relevant in cancer
Sema4A	Suppresses angiogenesis via PlexinD1[158] Germline variant is associated with increased risk for colorectal cancer[59]
Sema4B	Interacts with CLCP1,a protein with high sequence similarity to neuropilins and regulates motility of lung cancer cells[7] Repressed by HIF-1 alpha to promote non-small cell lung cancer invasion[60] Inhibits MMP9 to prevent metastasis and inhibits growth invitro and invivo of non-small cell lung cancer[61, 62]
Sema4C	Elevatedexpression in esophageal, gastric and rectal carcinomas[63] Mutated in some colorectal cancer cell lines[159] Promotes invasive growth in malignant gliomas[66] Regulated by MiR-138 and involved in cell proliferation and epithelial mesenchymal transition in non-small cell lung cancer cells[160] Regulated by MiR-125b and involved in paclitaxel-resistance of breast cancer cells and epithelial to mesenchymal transition in lung cancer[64]in breast cancer[65]
Sema4D	Promotes angiogenesis by stimulating Rho pathways[74] Associated with poor clinical outcome in cervical cancer[161] Promotes tumor angiogenesis and progression,as TAMs are a major source of Sema4D[13] Induces angiogenesis by Met recruitment to Plexin B1[12] Promotes tumor associated macrophage dependent metastatic behavior in colon cancer[162] Regulated by HIF-1which affects tumor growth and vascularity[163] Increases tumor cell motility via Plexin B1 in pancreatic cancer cells[68] Activates NF-KappaB and IL-8 to promote a pro-angiogenic response in endothelial cells[77] Promotes growth and invasion in HeLa cells[164] Promotes perineural invasion in a RhoA/ROK-dependent manner[80] Overexpression is related to poor prognosis in ovarian cancer[165] Suppresses c-Met activation and migration and promotes melanocyte survival[166] Cooperates with VEGF to promote angiogenesis and tumor progression[79] Over expression as a poor prognosis marker in ovarian cancer and promotes monocyte differentiation towards M2

	macrophage[167] Promotes proliferation, migration and invasion in lung cancer cells[168] Recruits pericyte and regulates vascular permeability through endothelial production of PDGF-B and ANGPLT4[71] Promotes osteosarcoma development and metastasis[72] Blocking Sema4D with monoclonal anti Sema4D antibody promotes immune infiltration into tumor and enhances response to various other immunomodulatory therapies[85] Induction of expansion of myeloid derived suppressor cells by Sema4D derived from Head and Neck Squamous Cell Carcinoma[169]
Sema4F	Biomarker of aggressive prostate cancer and critical regulator of neuroepithelial interactions[86, 170]
Sema4G	Significantly downregulated in colorectal cancer[171]
Sema5A	Identified as a functional cell adhesion molecule with potential role in metastasis[172] Inhibits glioma cell motility through RhoGDIalpha-mediates inactivation of Rac1-GTPase[88] Identified as a novel biomarker for non-small lung carcinoma in non smoking women[90] Promotes angiogenesis by increasing endothelial cell proliferation, migration and decreasing apoptosis[173] Highly expressed in pancreatic cancer and associated with tumor growth, invasion and metastasis[174] Soluble Sema5A suppresses pancreatic tumor burden but increases metastasis and endothelial cell proliferation[175]
Sema5B	Promotes cell viability of Clear cell renal carcinoma[89] Repressed by FoxP1 in endothelial cells [176]
Sema5C	Required for I(2)gl cancer metastatic phenotype in drosophila model system[177]
Sema6A	Promotes tumor progression and angiogenesis by enhancing VEGF and bFGF signaling[92, 178] Controls cell growth and survival of BRAFV600E human melanoma cells[91] Prognostic biomarker in glioblastoma[179]
Sema6B	Expression is downregulated by all-trans-retinoic acid in glioblastoma[180] and by PPAR and RXR ligands in breast cancer cells[181] Expression is strongly downregulated in breast cancer and a new isoform of Sema6B is identified[182]
Sema6D	Activates VEGF-2 and NF-KappaB to mediate survival of malignant mesothelioma cells[183] Co-predictor in breast cancer survival[184] Putative driver of osteosarcoma development and metastasis[72]

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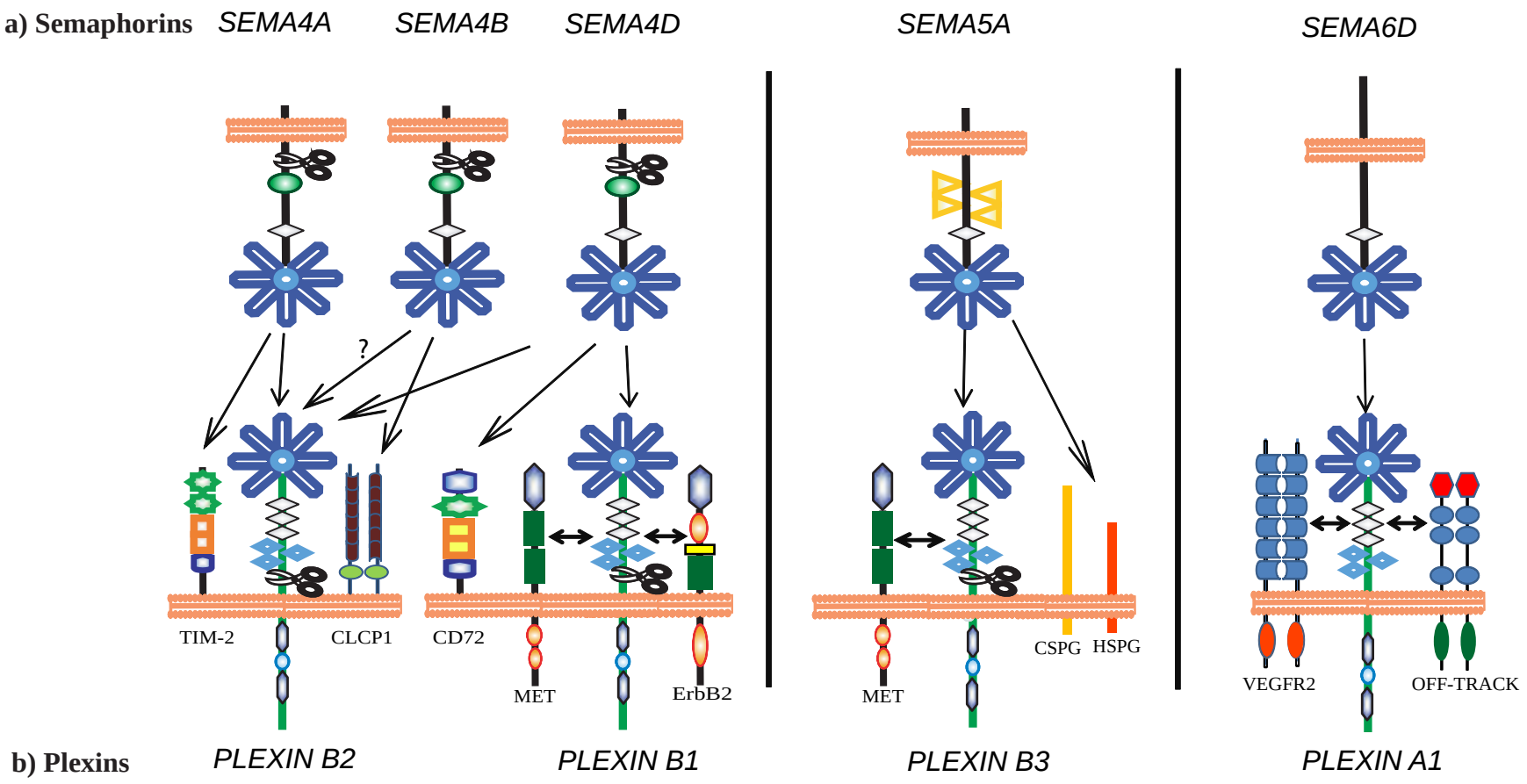
a) Semaphorins
SEMA4A
SEMA4B
SEMA4D
SEMA5A
SEMA6D
?
TIM-2
CLCP1
CD72
MET
ErbB2
MET
CSPG
HSPG
VEGFR2
OFF-TRACK
b) Plexins
PLEXIN B2
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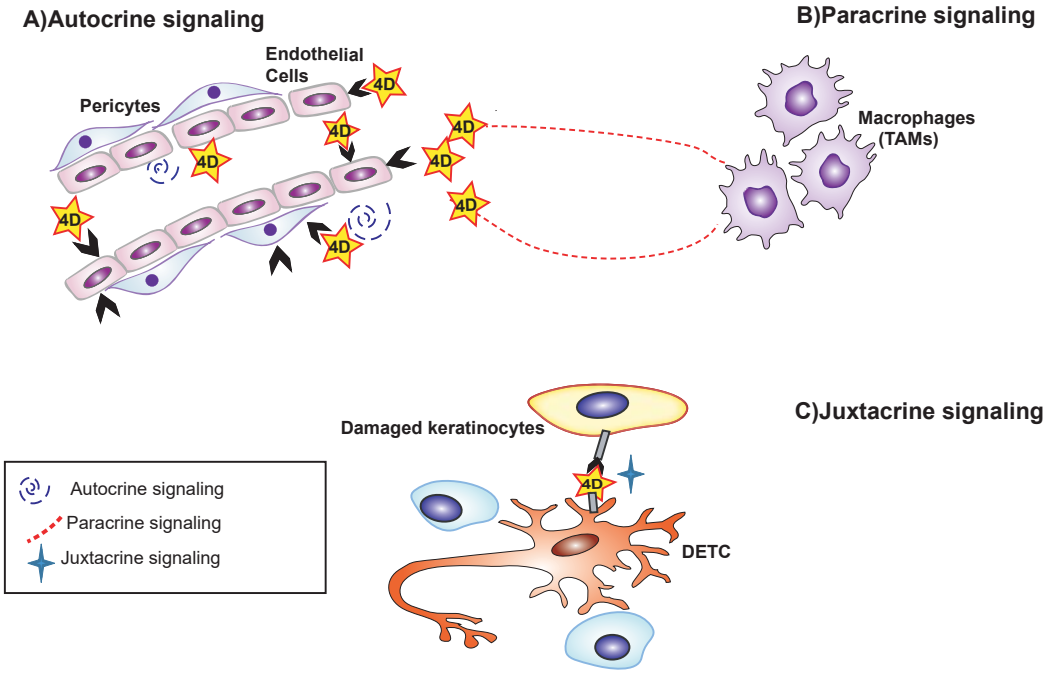
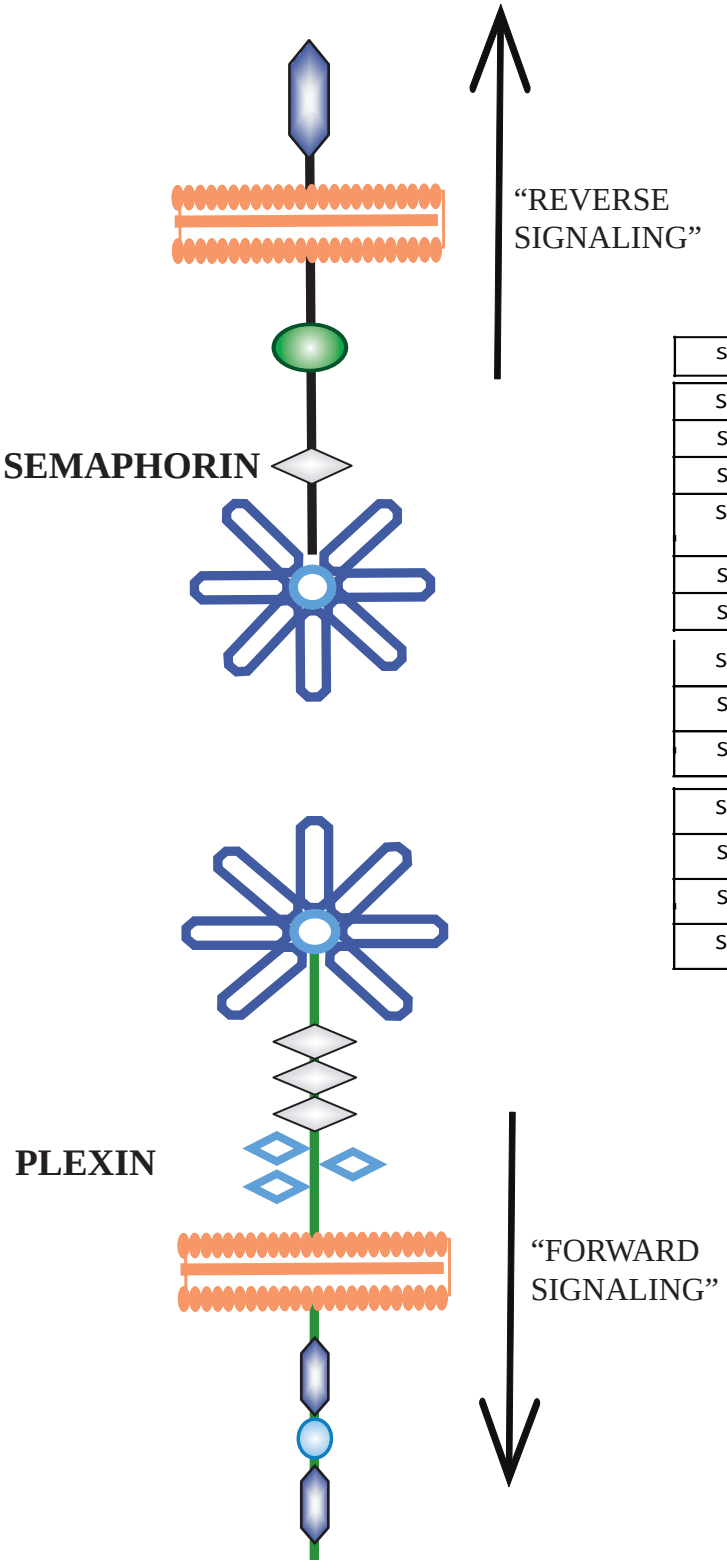
PLEXIN B3
PLEXIN A1


A)Autocrine signaling
B)Paracrine signaling
Endothelial Cells
Pericytes
4D
Macrophages (TAMs)
C)Juxtacrine signaling
Damaged keratinocytes
DETC
Autocrine signaling
Paracrine signaling
Juxtacrine signaling



	FORWARD SIGNALING PARTNERS	REVERSE SIGNALING PARTNERS
SEMA1A	PLEXNA,OTK	ENA
SEMA4A	PLXNB2,PLXND1,TIM-2	?
SEMA4B	CLCP1,PLXN ?	PSD-95
SEMA4C	PLXNB1, PLXNB2	PSD-95,GIPC,NORBIN
SEMA4D	PLXNB1,PLXNB2,CD72, MET,ERBB2	CD45
SEMA4E	PLXN ?	?
SEMA4F	PLXN ?	PSD-95
SEMA5A	PLXNB3,HSPG,CSPG,MET	?
SEMA5B	PLXN ?	?
SEMA5C	PLXN ?	?
SEMA6A	PLXNA4	EVL
SEMA6B	PLXNA4	SRC
SEMA6C	PLXN ?	?
SEMA6D	PLXNA1,OTK,VEGFR2	ABL