Cell. Mol. Life Sci. 66 (2009) 649 – 666
1420-682X/09/040649-18
DOI 10.1007/s00018-008-8518-z

Cellular and Molecular Life Sciences

Review

The many faces of semaphorins: from development to pathology

L. Roth[a,†], E. Koncina[a,†], S. Satkauskas[a,b,†], G. Crémel[a], D. Aunis[a] and D. Bagnard[a,*]

[a] INSERM U575, Physiopathologie du Système Nerveux, Centre de Neurochimie, 5 Rue Blaise Pascal, 67084 Strasbourg (France), Fax: 33-3-88-60-08-06, e-mail: Dominique.Bagnard@inserm.u-strasbg.fr
[b] Vytautas Magnus University, Department of Biology, Kaunas (Lithuania)

Received 22 August 2008; received after revision 22 September 2008; accepted 24 September 2008
Online First 27 October 2008

Abstract. The semaphorin family is a large group of proteins controlling cell migration and axonal growth cone guidance. These proteins are bi-functional signals capable of growth promotion or growth inhibition. Initially described in the nervous system, the majority of studies related to semaphorins and semaphorin signalling are nowadays performed in model systems outside the nervous system. Here, we provide an exhaustive review of the many faces of semaphorins both during developmental, regulatory and pathological processes. Indeed, because of their crucial fundamental roles, the semaphorins and their receptors represent important targets for the development of drugs directed at a variety of diseases.

Keywords. Semaphorin, development, organogenesis, neuropilin, cell signalling.

Introduction

The semaphorin family is a large group of proteins controlling cell migration and axonal growth cone guidance. These proteins are expressed in insects, rodents and human with a remarkably well-conserved structure. Based on a strategy using function blocking antibodies, the first semaphorin (Sema-1a) was described in the grasshopper as a transmembrane protein ensuring fasciculation of particular axon tracts in the limb [1]. Subsequent work identified a collapsing factor that caused rapid and transient collapse of sensory growth cones through a preparation of chick brain membranes [2, 3]. Several molecules displaying common structural features such as a 500 amino acid domain (called the "sema" domain) were then discovered and these define eight classes of semaphorins. Classes 1 and 2 have been described so far only in invertebrates. Classes 3 to 7 are found in vertebrates, whereas class 8 encodes viral semaphorins. Members of class 2 and 3 are secreted proteins while other semaphorins are GPI-linked (class 7) or transmembrane proteins (classes 1, 4, 5 and 6) [4]. Initially, the semaphorins were essentially characterized and analyzed in the developing nervous system, where they act as chemorepellent cues for various axon populations. It turned out that some members of class 3 (Sema3C and Sema3B) were able to attract growing axons [5–7]. Thus, these proteins are bi-functional signals capable of growth promotion or inhibition. Progressively, several experiments demonstrated the multiple potencies of semaphorins. Nowadays, the majority of studies related to semaphorins and semaphorin signalling are probably performed in model systems outside the nervous system. The signalling mechanisms are well understood and are nicely summarized in several recent reviews [8–11]. Several molecules have been shown to participate in the formation of the receptor complex ensuring semaphorin signalling. This includes the neuropilins (defining the ligand binding subunit of the complex for

† These authors contributed equally to this work.
* Corresponding author.

Table 1. Role of semaphorins in the nervous system.

 **Structures of the nervous system**	**Semaphorins**	**Functions**	**References**
Peripheral nerves	Sema3A	Repulses peripheral axons and contributes to proper wiring of peripheral nervous system	[19, 21]
	Sema3A, Sema3F	Can positively contribute to the observed partial regeneration of peripheral nerves	[23]
Cerebral cortex	Sema3A, Sema3F	Regulate migration of GABA neurons during cortex development	[29]
	Sema3A, Sema3C	Delineate the initial trajectory of growing cortical axons	[5, 31]
	Sema3A	Promotes outgrowth and branching of cortical dendrites	[32, 33]
Hippocampus	Sema3A, Sema3C	Govern the correct targeting of afferent and efferent fibers	[35, 36]
	Sema3A	Regulates the synaptic transmission in mature hippocampal neuronal circuits	[38, 39]
	Sema6A	Controls the projection of hippocampal mossy fibers to the correct lamina	[37]
Cerebellum	Sema6A	Is expressed by tangentially migrating neurons and controls the initiation of radial cell migration	[45]
	Sema3A	Induces the collapse of ponto-cerebellar fibbers	[40]
	Sema3C	Induces granule cells neuritogenesis *in vitro*	[41]
	Sema3F	Attracts granule cells axons during cerebellum development	[42]
Olfactory system	Sema3A	Contributes to the construction of odor maps in olfactory bulb	[46]
	Sema3B, Sema3F	Repels and attracts olfactory bulb axons respectively	[7]
Visual system	Sema3A	Controls innervation of the cornea by trigeminal axons and induces collapse in mature retinal ganglion cells axons	[48]
	Sema3E	Triggers the exclusion of retinal ganglion cell axons from specific retina layers	[49]
	Sema3D	Guides RGC axons to the contralateral optic tract	[50]
	Sema5A	May contribute to the lack of optic nerve regeneration after axotomy	[128]

soluble semaphorins), members of the plexin family[12], the receptors of Vascular Endothelial Growth factor (VEGFR1 and VEGFR2) [13, 14], the adhesion molecules L1 and NrCAM [15, 16] or receptors such as Ron, Met and OTK [17] (Fig. 1A). The diversity of the receptor complex is certainly the source of the diversity of semaphorin biological functions. This is also related to the complex signalling cascades involving RhoGTPases, protein kinases, MAP kinases and other pathways [17] (Fig. 1B). Here, we provide an exhaustive review of the many faces of semaphorins both during developmental, regulatory and pathological processes.

The role of semaphorins in the nervous system

Semaphorins are key regulators of nervous system development. They participate in the formation of major structures such as the cerebral cortex, hippocampus, olfactory bulb, the visual system, cerebellum and the spinal cord as well as in the correct patterning of peripheral nerves.

The case of the peripheral nerves and spinal cord. The sensory neurons in charge of the detection and conduction of sensory information are located in the dorsal root ganglia (DRG). During development, their axons are repelled by many semaphorins which are able to induce growth cone collapse. The expression of semaphorins in the surrounding of sensory neurons defines inhibitory regions repelling growing sensory axons. For example, the expression of

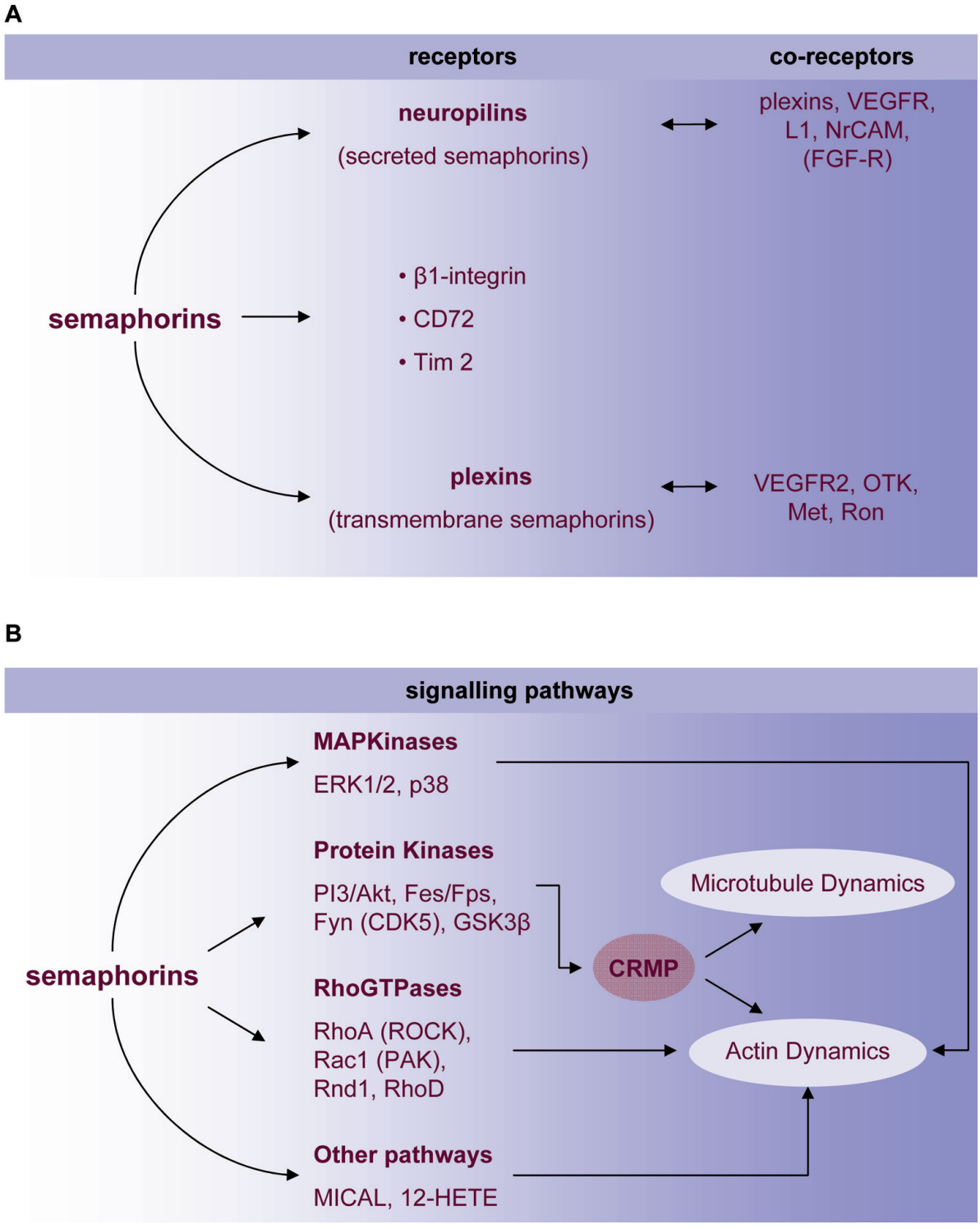


Figure 1. Receptor complex and signalling pathways of semaphorins. (*A*) Receptors and co receptors of transmembrane and diffusible semaphorins. (*B*) Diversity of the signalling pathways recruited by semaphorins.

Sema3A in the ventral horn of the spinal cord prevents proprioceptive axons from growing into this region [18]. Non-neuronal tissues, such as the limb bud, also express semaphorins to guide motor neuron axons to their appropriate target and contribute to their dorsoventral segregation. The role of Sema3A in this system is certainly more important, as many peripheral nerve projections are abnormal in Sema3A deficient trans-

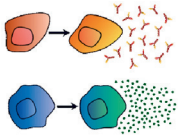
genic mice [19]. In this case, the most perceptible effect is the disorganization of nerve fascicles (defasciculation) rather than misrouting. This effect on fasciculation has been confirmed in other studies [20, 21]. Moreover, cell migration is also under the control of Sema3A. Sympathetic neurons normally arise from neural crest cells and migrate to specific loci to form compact structures. In Sema3A or neuropilin-1 (NRP1, one of the Sema3A receptors) null mutants, the sympathetic neurons are displaced, thereby suggesting that Sema3A acts as a stop signal ensuring cell compaction [21]. The positive role of semaphorins and their receptors in the peripheral nerves has also been revealed during Wallerian degeneration of the peripheral nervous system (PNS). During this degeneration of the axon distal to a site of transection, the expression of Sema3A, Sema3F and their receptors NRP1 and NRP2 is induced. It has been proposed that this expression could positively contribute to the observed partial regeneration [22, 23]. Interestingly, semaphorins are expressed in the spinal cord during development in a very precise manner. Cohen and colleagues suggested the existence of a semaphorin code defining the identity of the different pools of motor neurons within the brachial and lumbar spinal cord. While the exact function of this combinatorial expression remains elusive, the selective sensitivity of specific subpopulations of spinal motor neurons to Sema3A-induced growth cone collapse suggests this code is probably a key element for the establishment of the axonal connections ensuring one of the major functions of the nervous system: locomotion [24]. Hence, the spinal cord is located near the interface between the central and peripheral nervous systems. During development, elements from the CNS have to project or migrate to the PNS. Cells originating from the neural crest form boundary cap cell clusters which have been shown to prevent the mixing of cells devoted to each nervous system structure [25]. Two recent papers show that boundary cap cells are using semaphorin signalling to achieve their function. These papers indentified Sema6A, expressed by the boundary cap cells, as a gatekeeper, signalling through plexin-As and NRP2 [26, 27].

The case of the cerebral cortex and hippocampus. In the central nervous system, structures such as the neocortex and the hippocampus are devoted to the highest cognitive functions. Strikingly, various semaphorins play distinct roles for proper wiring of these key structures.

The neuronal populations in the neocortex comprise pyramidal neurons and GABA neurons. The pyramidal neurons are born locally in the ventricular zone before migrating through a radial route to the cortical plate in order to form six neuronal layers displaying specific connections and interconnections. The radial migration of post mitotic cortical neurons is under the control of Sema3A [28]. The GABA neurons, on the other hand, are generated in the ganglionic eminence and have to migrate toward the neocortex through the nascent internal capsule. This migration is under the regulation of Sema3A and Sema3F [29]. Sorting of striatal and cortical interneurons is also ensured by semaphorin-neuropilin interactions [30]. Moreover, a combination of Sema3A and Sema3C is involved in the guidance of the first efferent axons exiting the cortical plate to reach the thalamus. Sema3C acts as an attractant of cortical axons and guides them toward the ventricular zone, while Sema3A prevents them from entering the ventricular zone. This functional combination of growth promotion or inhibition delineates the initial trajectory of growing axons [5, 20, 31]. Strikingly, the expression of Sema3A in the marginal zone is also implicated in cortical dendrite development, orienting them to the pial surface [32] and promoting their outgrowth and branching [33]. Interestingly, the growth promoting effect of semaphorins in the cortex relates to specific activation of matrix metalloproteinases [34]. Thus, the spatial and temporal regulation of semaphorin expression is the source of precise wiring. This is further illustrated in the hippocampus, where a tight spatiotemporal regulation of the expression of class 3 semaphorins and their receptors governs the correct targeting of afferent and efferent fibers [35, 36]. Recently, the transmembrane Sema6A, together with the receptors plexin-A4 and plexin-A2, has been shown to control the projection of hippocampal mossy fibers to the correct lamina [37], demonstrating that semaphorins not only guide axons towards appropriate structures, but also help axons to find their final destination for arborisation and synaptogenesis. The hippocampus is one of the main structures for memory. Interestingly, while the expression and functions of semaphorins are mainly developmental, there is a persistent expression and function of semaphorins in the adult hippocampus. This is illustrated in two studies revealing that Sema3A is able to regulate synaptic transmission in mature hippocampal neuronal circuits [38, 39]. Thus, semaphorins control corticogenesis from cell migration, axon and dendrite growth to synapse formation.

The case of the cerebellum, olfactory and visual systems. The cerebellum is critical for the integration of sensory perception and motor output. Many axonal projections link the cerebellum with the motor cortex and the spinocerebellar tract provides feedback on the position of the body in space. This intricate network also requires precise guidance mechanisms to build

Table 2. Role of semaphorins in the immune system.

	**Semaphorins**	**Function**	**References**
Immune system			
T cells	Sema4A	Activation, priming, regulation	[64, 65]
	Sema7A	Inhibition	[70]
	Sema3A	Proliferation	[72]
		Development, differentiation, migration	[73]
B cells	Sema4D	Activation, survival, proliferation	[53, 55, 61]
		Survival and proliferation in leukemia	[153]
Dendritic cells	Sema4D	Activation	[55]
Monocytes	Sema4D	Migration	[59]
	Sema7A	Autocrine activation	[68, 69]
Macrophages	Sema4D	Migration	[59]

appropriate connections. Indeed, several semaphorins are expressed in the cerebellum where they control axon guidance. Sema3A is expressed during cerebellar development in the Purkinje cell layer and induces the collapse of ponto-cerebellar fibbers [40]. Sema3C induces granule cells neuritogenesis *in vitro* [41] and Sema3F attracts their axons *via* a cyclic GMP-dependent mechanism [42]. Sema3G, the last discovered class 3 semaphorin at this time, shows a regulated expression during cerebellar development but the elucidation of its functional role requires additional experiments [43]. As described during cortical development, semaphorins also regulate cell migration during cerebellar ontogenesis. The cerebellar granule cells are formed in the rhombic lip and first migrate tangentially away from it, forming the external granule cell layer. The post-mitotic granule cells then migrate radially toward the inner granule cell layer [44]. Sema6A is expressed by the tangentially migrating neurons and controls the initiation of radial cell migration [45].

In any case, the optimal functioning of the brain requires efficient integration of sensory inputs. Olfactory and visual information are in this line of prime importance for individuals to behave normally. The perception and integration of these sensory inputs is ensured by sophisticated networks detecting and integrating signals. Here again, various semaphorins exert guidance effects to establish a functional connectivity. It has been shown that Sema3B repels olfactory bulb axons, whereas Sema3F acts as an attractant [7]. This functional scheme mirrors what happens in the developing cortex. The role of semaphorins in the olfactory system is certainly crucial because odour maps can be distorted in Sema3A null mutants [46]. The existence of topographic maps composed of specific neuronal networks ensuring treatment and integration of sensory inputs has also been extensively described in the visual system [47]. Most of the studies demonstrated how the spatial and temporal expression of eph/ephrins, another important family of guidance signals, govern the formation of retino-collicular projections. Nevertheless, semaphorins have also been proposed to play a role during visual system development. For example, Sema3A is expressed by the lens to control the exact timing of innervation of the cornea by trigeminal sensitive axons [48]. There is a temporal regulation of retinal ganglion cells (RGC) sensitivity to Sema3A, as early axons are insensitive to semaphorins, whereas mature axons collapse in the presence of Sema3A. In the chick, Sema3E is highly expressed when intraretinal connections are formed and triggers the exclusion of RGC axons from specific layers [49]. In the zebrafish, the guidance of RGC axons to the contralateral optic tract is mediated at the midline of the optic chiasm by Sema3D [50]. Hence, a Sema3A gradient along the optic nerve is assumed to repel migrating glial precursor cells to the retina [51].

The role of semaphorins in the immune system

Both the nervous and immune systems form complex networks with similar tasks including memory, connectivity, cell migration and stringent processes of cell selection involving programmed cell death. One of the major discoveries in the field of immunity is probably the identification of a potentially crucial role of semaphorin functions in the immune system. At least four classes have been shown to play a role in the immune system, thereby supporting an emerging view of semaphorins as a new family of immunoregulatory molecules [11].

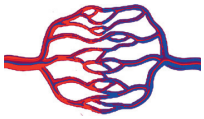
Sema4D. Sema4D, also known as CD100, was discovered by monoclonal antibody screening as a glycoprotein dimer on the surface of activated T cells [52]. It is the first known semaphorin involved in the immune system [53]. Sema4D displays high levels of expression in lymphoid organs (spleen, thymus and lymph nodes) and in non-lymphoid organs such as the heart, the brain and the kidney [53]. In lymphoid organs, it is preferentially expressed on resting T cells and weakly on resting B cells and on antigen-presenting cells, but it is upregulated after treatments with various immunological stimuli [54, 55]. Kumanogoh and collaborators identified CD72 as a Sema4D receptor, expressed on B cells and antigen-presenting cells such as dendritic cells (DCs). The binding of Sema4D turns off the negative effects of CD72 by inducing tyrosine dephosphorylation, and consequently the dissociation of the tyrosine phosphatase SHP1 from CD72. Hence, the CD72/Sema4D interaction leads to an activation of B cells and DCs. Plexin-B1 was described as another receptor for Sema4D in non-lymphoid organs [12]. Interestingly, several studies showed that Sema4D would also function as an immune receptor for plexin-B1, and thus would have bi-directional functions [52, 56, 57]. *In vitro*, Sema4D enhances B cells survival, proliferation and activation [53, 55] and enhances CD40-induced DCs maturation [58]. It also plays a role in monocytes and macrophages migration [59]. Another study showed that Sema4D is involved in the negative regulation of inflammatory responses [60]. Shi and collaborators generated and characterized Sema4D$^{-/-}$ mice [61]. Remarkably, the immunological phenotype of Sema4D$^{-/-}$ mice is almost the opposite to that of CD72$^{-/-}$ mice [62]. Sema4D$^{-/-}$ mice developed normally but exhibited severe impairments of B1 cell development and immune responses, suggesting an essential and non-redundant role of Sema4D in the immune system. No apparent phenotype was observed in non-lymphoid organs [61]. Moreover, Sema4D$^{-/-}$ mice failed to develop experimental autoimmune encephalomyelitis (EAE) induced by myelin oligodendrocyte glycoprotein (MOG) peptide, because MOG-specific T cells were not generated in the absence of Sema4D [58]. Finally, by modulating T cell/antigen presenting cells interactions, Sema4D also displays a crucial role in *in vitro* and *in vivo* allo-geneic responses [63].

Sema4A. In lymphoid organs, Sema4A is preferentially expressed on B cells and DCs, and is upregulated on activated T cells [64]. Sema4A, unlike Sema4D, exhibits no *in vivo* binding to a plexin family member. The only known Sema4A receptor is Tim2, expressed on the surface of activated T cells [64]. Sema4A-Fc enhances T cell proliferation and interleukin-2 production following stimulation with anti-CD3 monoclonal antibody (mAb). These observations suggest that it plays a role in T cell activation by stimulating interactions between T cells and DCs. *In vivo*, administration of soluble Sema4A significantly increases the generation of antigen-specific T cells. Furthermore, the treatment of mice with anti-Sema4A mAb inhibits the development of EAE induced by MOG peptide [64]. Sema4A$^{-/-}$ mice show several functional defects in the immune system, revealing a non-redundant role of Sema4A in T cell priming and in the regulation of Th1/Th2 responses [65]. Strikingly, Sema4D and Sema4A show inverse expression, receptor expression and target cells regarding B cells, T cells and antigen-presenting cells, and appear as an entire family of immunoregulatory molecules.

Sema7A. Sema7A is expressed by myeloid and lymphoid cells in the immune system [66, 67]. Plexin-C1 has been identified as a Sema7A receptor *in vitro* [12]. Sema7A is expressed in activated lymphocytes and thymocytes, and also in monocytes for which it could act as an autocrine activator [68, 69]. T cells from Sema7A$^{-/-}$ mice are hyper responsive *in vitro*, suggesting that Sema7A is a negative regulator of T cell responses [70]. Moreover, Sema7A$^{-/-}$ knockout mice exhibit a severe EAE, an exacerbated pathology, and an enhanced delayed-type hypersensitivity (DTH) response. However, these mice show no obvious autoimmune disorder, indicating that Sema7A's inhibitory role would be specific rather than general. Hence, Suzuki and collaborators recently identified a function of Sema7A as an effector molecule in T-cell-mediated inflammation through an integrin-mediated mechanism [71].

Sema3A. Sema3A is expressed by activated T cells and DCs. The use of blocking antibody against endogenous Sema3A or antagonist peptide in DC/T cells cocultures showed a dramatic increase in T cell proliferation. This observation led Lepelletier and collaborators to propose that NRP1/Sema3A interaction would be involved in a late negative feedback loop controlling DC-induced T-cell proliferation [72]. The same group also identified a role for Sema3A during development and differentiation of T cells. In humans, Sema3A and NRP1 are expressed in the thymus, and this expression increases during thymocytes maturation and differentiation [73]. Moreover, Sema3A decreases NRP1-expressing-thymocytes adhesion, and induces their migration through a repulsive effect. Components of the Sema3A receptor complex have also been involved in the immune response. Indeed, NRP1 is expressed on T cells and DCs during primary immune response. NRP1 could

Table 3. Role of semaphorins in the vascular system.

 **Vascular system**	**Semaphorins**	**Functions**	**References**
Pro-angiogenic	Sema3E	Restricts blood vessel growth to the intersomitic boundaries	[90, 87]
	Sema4D	Enhances blood vessel formation	[92, 93]
Anti-angiogenic	Sema3A	Inhibits migration of endothelial cells Competes with VEGF (?)	[79]
		Essential for vascular remodelling	[80]
	Sema4A	Inhibits cell adhesion	[95]

mediate interactions between DCs and T cells through homotypic interactions, thus favouring the initial contact between these cells [74]. A recent work explored the role of plexin-A4, another member of the Sema3A receptor complex, in the immune system [11]. Mice lacking plexin-A4 exhibited enhanced T-cell activity, similarly to mice lacking Sema3A or expressing NRP1 without class 3 semaphorin binding site. These results suggest that plexin-A4, as a component of the receptor complex for class 3 semaphorins, negatively regulates T cell mediated immune responses [11]. Another class A plexin, plexin-A1, does also exhibit immune functions. Indeed, plexin-A1 is involved in DCs-T cells interactions [75]. Moreover, plexin-A1$^{-/-}$ mice revealed that its expression on DCs is required for the efficient generation of antigen-specific T-cells [76]. Plexin-A1 is well known as a component of Sema3A receptor complex, but it also serves as a receptor for Sema6D, a class 6 transmembrane semaphorin. The work by Takegahara and collaborators showed that Sema6D expressed on T cells might stimulate DCs *via* plexin-A1 during T cell/DC interactions, revealing the role of a novel immune semaphorin: Sema6D [76]. The involvement of class 4 semaphorins as well as Sema7A, Sema3A and Sema6D in the immune responses increases the therapeutic potential of semaphorins and their receptors. The striking similarities between the nervous and immune system is in fact partly shared by another important physiologic system: the vascular system.

The role of semaphorins in the vascular system

The vascular and the nervous systems share striking anatomical similarities, including a close juxtaposition in many territories. The similitude also resides in the high resemblance between tip cells, which are distinct and functionally specialized micro vascular endothelial cells at the origin of vascular sprouting, and axonal growth cones. Considering these elements, it is not surprising that the two systems share common guidance molecules ensuring the elaboration of intricate vascular networks or axonal connections. This is the case of the netrin, slit, and ephrin families, and of some members of the semaphorin family [77]. Semaphorins directly influence endothelial cell motility (Sema3A), repel blood vessels (Sema3E), promote blood vessel formation (Sema4D), or may indirectly modulate angiogenesis by competing with VEGF proangiogenic factor [78]. In this part of the review, we will summarize the roles of various class 3 and class 4 semaphorins during normal angiogenesis.

Sema3A. Work from Miao and collaborators underlined the direct and indirect effects of Sema3A in angiogenesis. Sema3A binds to endothelial cells, inhibits their migration, retracts lamellipodia and inhibits capillary sprouting in an aortic ring assay. Besides, Sema3A competes with $VEGF_{165}$ in binding endothelial cell in motility test and in DRG collapse assay [79]. Sema3A produced by angiogenic endothelial cells would also inhibit extracellular matrix ligand recognition by integrins, allowing the de-adhesion necessary for vascular remodelling [80]. Moreover, embryonic Sema3A$^{-/-}$ mice (E9.5) show vascular defects in the head, have abnormal trunk blood vessels and the primitive capillary plexus appearance does not undergo appropriate remodelling [80]. These observations resulted in a widely accepted model of vascular patterning in which the balance of $VEGF_{165}$ and Sema3A, which shares NRP1 as a common receptor, determines endothelial cell behaviour. According to this model, neurovascular congruency would be defined by a shared molecular patterning mechanism that involves both Sema3A and NRP1 [81]. However, other studies deny the Sema3A/VEGF competition. Rather, the authors showed that Sema3A inhibits VEGF activity downstream to the VEGFR-2 [82]. Elegant work using NRP1 knock-in mice deleted from the Sema3A binding domain but

still containing the VEGF-binding domain demonstrated that VEGF/NRP1 binding in endothelial cells is essential for angiogenesis, but not Sema3A/NRP1 binding [83]. Moreover, recent work based on Sema3A/VEGF165 double-mutants demonstrates that Sema3A is not required for angiogenesis in mouse, which is instead controlled by VEGF. This work illustrated the existence of cooperation rather than competition between Sema3A and VEGF165 [84]. Consistently, a crystallization study based on antibodies directed against different subdomains of NRP1 revealed that Sema3A and VEGF do not directly compete for NRP1 binding [85]. Hence, Sema3A also exerts an antiangiogenic activity in adult organisms while acting as a strong chemoattractant for bone marrow cells ($CD11b^+$ cells known to participate in the formation of new blood vessels). The exact mechanism of this complex cellular and molecular interaction remains to be clarified. [86].

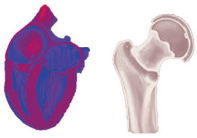
Sema3E. Another class 3 semaphorin, Sema3E, has been involved in angiogenesis. Indeed, Sema3E restricts blood vessel growth to the intersomitic boundaries. Gu and collaborators identified plexin-D1 as Sema3E receptor and showed that Sema3E signalling is neuropilin-independent [87]. This confirmed the role of plexin-D1 in embryonic vasculogenesis previously suggested upon its expression in the endothelial cells of the developing blood vessels [88]. Moreover, plexin-$D1^{-/-}$ mice definitely identified plexin-D1 as an essential receptor for normal vascular patterning [89]. $Sema3E^{-/-}$ mice exhibit disrupted vascular patterning as well, and show a vascular phenotype similar to plexin-$D1^{-/-}$ embryos. Several studies demonstrated that Sema3E binding to plexin-D1 controls endothelial cells positioning and the patterning of the developing vasculature in the mouse. Sema3E expressed by somites acts as a repulsive cue for plexin-D1-expressing endothelial cells of adjacent intersomitic vessels to restrict vessel growth and branching to intersomitic regions during embryogenesis [87, 90]. As discussed in the section on cancer, other class 3 semaphorin such as Sema3B [10] and Sema3F [82] also display antiangiogenic properties.

Sema4A and Sema4D. Finally, some of the class 4 semaphorins known to be involved in the immune system may also play a role in angiogenesis. Sema4D triggers epithelial cells invasive growth through binding to plexin-B1 [91]. This protein potently induces chemotaxis and tubulogenesis in endothelial cells and enhances blood vessel formation in an *in vivo* mouse model [92]. Sema4D proangiogenic activity requires coupling and activation of the Met tyrosine kinase, implicated in endothelial cells migration, proliferation, and organization into new blood vessels during the angiogenic process [93]. Sema4D/plexin-B1 signalling controls cell migration by modulating the activity of β1 integrins [94]. On the other hand, the Sema4A expressed in endothelial cells acts through plexin-D1 to suppress VEGF-mediated Rac activation and integrin-dependent cell adhesion [95].
Unlike Sema4D, Sema4A acts as an inhibitor of angiogenesis. Thus, the same semaphorins exert different growth promoting or growth inhibiting action in multiple biological systems. This can be illustrated further in the following part detailing the role of semaphorins in many other organs.

The role of semaphorins in organogenesis

Heart. There are a number of studies showing an important role of semaphorins in the development of the cardiovascular system. $Sema3A^{-/-}$ mice, in addition to nervous system defects, exhibit cardiac defects, characterized by right ventricular hypertrophy and a grossly dilated right atrium [96]. In addition, Sema3A is important in establishing cardiac sympathetic innervation patterning that is critical for heart rate control [97]. $Sema3C^{-/-}$ mice exhibit even stronger dysfunctions of cardiovascular system. They die within hours after birth from congenital cardiovascular defects consisting of interruption of the aortic arch and improper septation of the cardiac outflow tract [98]. The study also revealed that these defects were reminiscent to those resulting from ablation of the cardiac neural crest in chick embryos. Consequently, it was suggested that Sema3C is implicated in the regulation of crest cell migration into the proximal cardiac outflow tract [98]. The specific deletion of semaphorin binding site on NRP1 results in developmental cardiovascular defects, suggesting that neuropilin-mediated semaphorin signalling is critical for heart development [83]. Further studies showed that disruption of a signalling pathway, involving secreted class 3 semaphorins, neuropilins, and a plexin receptor, plexin-D1, results in cardiovascular defects [89]. In addition, this work shows that this form of congenital heart defects can be caused by cell-autonomous endothelial defects, demonstrating that endothelial cells might orchestrate critical aspects of cardiac morphogenesis [89]. The involvement of semaphorin signalling in cardiac morphogenesis was further demonstrated in a series of elegant studies utilizing chick and mice embryos [99,100]. It was shown that Sema6D is expressed in the developing tubular heart and in the neural fold of both mice and chicks. Suppression or over expression of Sema6D in chick embryos causes morphological abnormalities of

Table 4. Summary of the semaphorins implicated in organogenesis.

 **Organs**	**Semaphorins**	**Functions**	**References**
Heart	Sema3A, Sema3C	Crucial role in the development of the heart	[96, 98]
	Sema6D	Normal expression is critical for cardiac tube formation	[99]
Lung	Sema3A	Inhibits foetal lung branching morphogenesis	[101]
	Sema3C, Sema3F	Stimulate foetal lung branching morphogenesis	[102]
Kidney	Sema3A	Acts as negative regulator in the development of renal collecting system	[104]
	Sema3A, Sema3F	Regulate pattern of endothelial cell migration during kidney morphogenesis	[103]
Bone	Sema3A	Important during normal bone development and remodelling	[96]
	Sema7A	Triggers osteoblast migration and osteoclast differentiation *in vitro*	[110]
	Sema3B, Sema6D	Important in bone homeostasis	[111, 76]
Tooth	Sema3A	Regulates timing of tooth innervation and dental axon navigation and patterning	[113]
	Sema4D	Negatively regulates odontoblast differentiation and dental mineralization	[115]
	Sema3A, 3B, 3C and 3F	Exhibit distinct developmentally regulated expression patterns during tooth organogenesis in the embryonic mouse	[112]

the cardiac tube, indicating that a tight regulation of Sema6D expression is critical for cardiac tube formation [100]. In addition to appearance of morphological defects, Sema6D exerts inhibitory and promoting effects on cell migration of ventricular and conotruncal segments, respectively. Since the disruption of plexin-A1 receptor blocked the effects of Sema6D, it was proposed that Sema6D mediates its effect *via* plexin-A1 [100]. Interestingly, in heart, expression of the plexin-A1 extracellular domain alone can rescue the defective trabeculation induced by suppression of plexin-A1, but not that resulting from defective Sema6D expression. This indicates a possible Sema6D reverse signalling within the myocardium. Thus, Sema6D can function as both a ligand and a receptor for plexin-A1 [99].

Lung. Class 3 semaphorins modulate lung development. It was first described that Sema3A inhibits foetal lung branching morphogenesis. NRP1 mediates this inhibitory effect, because blocking NRP1 binding sites on Sema3A with a soluble form of NRP1 prevents Sema3A-induced inhibition of branching morphogenesis [101]. Later studies showed that the semaphorins act as both positive and negative regulators of branching morphogenesis in the developing lung. While Sema3A inhibits the branching morphogenesis of the E11.5 lungs, Sema3C and Sema3F stimulate branches formation *in vitro* [102]. Thus, in the developing lung as in the nervous system, semaphorins act as both positive and negative regulators.

Kidney. Semaphorins are thought to be important in development of kidney. It was shown that expression of semaphorins 3A and 3F and their corresponding receptors NRP1 and NRP2 are regulated during kidney ontogeny [103]. This study proposes that semaphorins 3A and 3F together with VEGF may be important determinants of the pattern of endothelial cell migration during kidney morphogenesis. *In vitro* loss and gain-of-function experiments revealed increased ureteric bud branching upon Sema3A knockdown and decreased branching in the presence of recombinant Sema3A [104]. Thus, Sema3A acts as negative regulator in the development of renal collecting system. Guan and colleagues showed that Sema3A regulates the expression of plexins (plexin-A1, A2, A3 and D1) in immortalized podocytes [105]. Glomerular podocytes are highly specialized cells forming part of the glomerular filtration barrier. Sema3A is probably able to induce podocyte apoptosis *via* an autocrine loop. Moreover, Sema3A decreases the expression of crucial molecular components of the glomerular physiology: podocin, CD2AP and nephrin [105]. This may have therapeutic implication considering the role of podocytes in glomerular pathobiology [106].

Bone. Sema3A seems to be implicated in bone development. First, all three bone cell lineages (chondrocytes, osteoblasts, and osteoclasts) express Sema3A, NRP1, plexin-A1 and plexin-A2 [107, 108]. The spatial and temporal expression of these molecules suggest the implication of Sema3A signalling to modulate the vascularization of bone and the innervations of osteoblasts and osteoclasts during bone development and remodelling. Indeed, Sema3A$^{-/-}$ mice display fusion of cervical bones, partial duplication of ribs and poor alignment of the rib-sternum junction [96]. Notably, recent data on single nucleotide polymorphisms of plexin-A2 show that it may be a useful marker for osteoporosis and its related fracture [109]. Sema7A, a glycosylphosphatidylinositol-anchored membrane semaphorin is expressed in all stages of osteoblast differentiation [110]. *In vitro* data showing that Sema7A triggers osteoblast migration and osteoclast differentiation clearly indicate the importance of this semaphorin in osteogenesis [110]. Sema3B also appears to be important in skeletal homeostasis. Sema3B expression in osteoblast is induced by 1,25-dihydroxyvitamin D3, a bioactive metabolite of vitamin D, and results in reduced bone mineral density. Sema3B transgenic mice exhibit decreased body weight, shorter tibiae, and display a deficit in trabecular and cortical bone mineralization [111].

Tooth. Together with involvement in bone development, semaphorins are also important in tooth development. Sema3A, 3B, 3C and 3F as well as their receptors NRP1 and NRP2 exhibit distinct developmentally regulated expression patterns during tooth organogenesis in the embryonic mouse [112]. Sema3A regulates timing of tooth innervation and dental axon navigation and patterning [113]. Another RT-PCR study extended these results by demonstrating the existence of distinct expression patterns of plexin receptors in several adult periodontal mesenchymal cell types as well as in dermal fibroblasts [114]. A most recent study reveals Sema4D as another semaphorin implicated in dental development. This work shows that epithelial and mesenchymal cells of the tooth germs both express Sema4D [115]. Sema4D represses collagen synthesis of pulp-derived cells, which indicates a possible role of Sema4D in negative regulation of odontoblast differentiation and dental mineralization. Altogether, these studies reveal that semaphorins, neuropilins and plexins are potential regulators during periodontal development, regeneration and dental innervation.

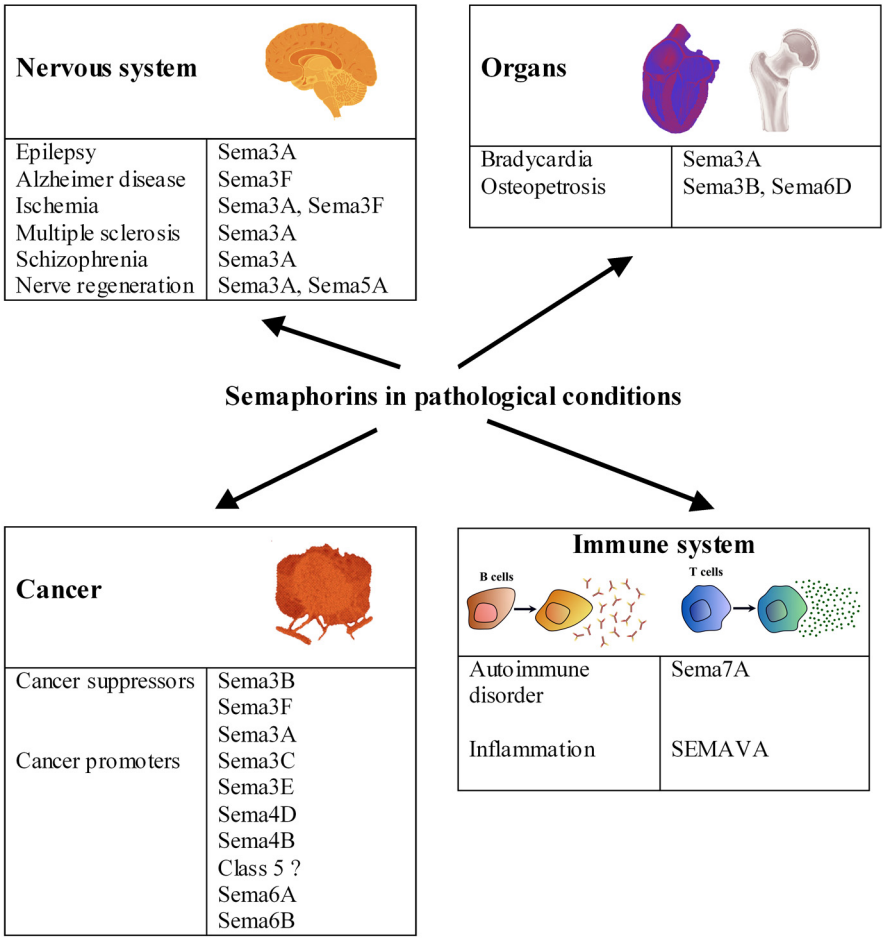
At this point, it becomes clear that semaphorins are almost ubiquitous signals controlling development and functioning of the most important organs and systems. Thus, it is not surprising that abnormal expression or signalling of these factors has profound impacts in physiopathology.

Semaphorins in pathologies

Pathologies of the nervous system. The documentation of deregulation of semaphorin expression in many pathological conditions such as ischemia, degenerative diseases, multiple sclerosis, etc. together with the development of specific inhibitors may represent a challenging future therapeutic avenue. For example, the hyperexcitability of the hippocampus in patients suffering from temporal lobe epilepsy is partly due to abnormal mossy fibber sprouting. In a rat model of temporal lobe epilepsy, it has been shown that Sema3A is downregulated and may allow mossy fibber sprouting, thereby contributing to hippocampal circuitry remodelling in favour of epilepsy [116]. This is further supported by the detailed analysis of semaphorin gene expression in rat brain after kaïnic acid-induced status epilepticus [117, 118]. Another intriguing issue is certainly the accumulation of Sema3A in the hippocampus during Alzheimer disease [119]. This accumulation is consistent with other studies showing the abnormal expression of Sema3F in the brain of patients with Alzheimer's disease [120] or the significant increase of hCRMP2 (a key regulator of Sema3A signalling) in neurofibrillary tangles [121, 122]. Williams and co-workers suggested that Sema3A and Sema3F can be key players in myelin repair in multiple sclerosis (MS). Indeed, authors have demonstrated that Sema3A and Sema3F transcripts are up-regulated in MS brain, as well as in experimental models of demyelinization [123]. What the exact role of these semaphorins is during the course of MS remains to be elucidated.

In the same way, it has been shown that Sema3A and NRP1 expression is increased and sustained in ischemic brain territories [124]. The inhibition of Sema3A/NRP1 signalling would be beneficial for patients because this abnormal expression is supposed to prevent new neurons from entering the infarct area. Similarly to ischemic brain, the abnormal expression of Sema3A is observed following spinal cord injury [125, 126]. The inhibitory Sema3A signals delivered by the glial scar are considered to contribute to the establishment of a non-permissive environment preventing nerve regeneration. Strikingly, the therapeutic potential of semaphorin inhibitors in this context has been recently proved in a study showing a functional recovery in rats treated with a Sema3A inhibitor after a spinal cord transection [127]. As in the spinal cord,

Figure 2.



the presence of semaphorins in the visual system may have significant implication during optic nerve regeneration. Sema5A, which is expressed by oligodendrocytes, is also able to repel RGC axons and it has been proposed that this expression may contribute to the lack of optic nerve regeneration after axotomy [128]. Sema3A is over expressed in the cerebellum of schizophrenic patients [129]. This abnormal over expression is associated with down regulation of genes involved in synaptic formation and maintenance. While these results are suggestive of a Sema3A induced impairment of synaptic connectivity in schizophrenia, the exact mechanism and role of the semaphorin signalling must be clarified in this complex syndrome triggered by multiple genetic and epigenetic factors.

Hence, a recent study showed that Sema3A expression in the heart contributes to correct sympathetic innervation. The immediate consequence of an altered projection of these neurons is the apparition of bradycardia, as demonstrated in Sema3A-deficient mice [97].

The role of semaphorins in cancer

Consistent with their importance during fundamental cell behaviours including proliferation, differentiation or migration, several semaphorins are involved in cancers and tumorigenesis. As usual for these bifunctional signals, they can act in this case either as tumour suppressors (Sema3B, Sema3F) or as tumour promoters (Sema3C, Sema3A, Sema4).

Semaphorins as tumour suppressors. SEMA3B and SEMA3F are both localized in chromosome 3p21, a region often associated with human lung and ovarian cancers [130–132]. SEMA3F expression is down-regulated in several cancer cell lines and tumours but over expressed in migrating lung cancer cells [133]. Likewise, loss of expression of SEMA3B mRNA occurs in 80% of lung cancers, and allelic loss together with hypermethylation of SEMA3B promoter is observed in non-small cell lung cancers [134].

Several studies have shown that Sema3B functions as a tumour suppressor gene. Applied as a soluble ligand

or transfected in tumour cells, Sema3B inhibits lung cancer cell growth and induces apoptosis [135]. It also acts as a tumour suppressor in an ovarian cancer cell line [136]. Besides, Sema3B induces apoptosis in lung and breast cancer and mediates its tumour-suppressing effects, at least in part, by blocking the VEGF autocrine activity [137]. An emerging model would be that, in premalignant cells, activation of p53 pathway induces an inhibition of Sema3B expression, or/and increases VEGF expression, which allows cancer cells to survive and proliferate.

Like Sema3B, the role of Sema3F as a tumour suppressor has been well documented. Its involvement in cell adhesion and migration has been demonstrated [138]. Furthermore, Sema3F transfected A9 fibrosarcoma cells result in complete loss of tumorigenicity in nude mice and block apoptosis. The same effects have been observed in human ovarian adenocarcinoma cell line [136]. Moreover, Sema3F inhibits cell attachment and spreading in the breast cancer cell line MCF7, whereas VEGF has opposite effects [139]. The role of Sema3F as a tumour angiogenesis inhibitor has been further explored in melanoma cell lines expressing Sema3F. These transfected tumours repel vascular and lymphatic endothelial cells expressing NRP2, the Sema3F receptor. In subcutaneous tumours, Sema3F forms a barrier that prevents blood vessel invasion from the skin [140]. Sema3F-expressing tumours develop at a much slower rate and have a significantly lower concentration of tumour-associated blood vessels, indicating that Sema3F is a chemorepellent that inhibits tumour angiogenesis [141]. In an *in vivo* lung cancer model, the Sema3F potent antitumour effect may be mediated by inhibition of integrin adhesion [142]. Besides, Sema3F inhibits VEGF-A and C-induced migration and survival by competing for NRP2 binding [143]. Finally, a very recent work showed that p53, implicated in Sema3B effect, would negatively regulate tumour vessel formation and cell growth via the NRP2/Sema3F pathway [144].

Sema3A has also been proposed as a tumour inhibitor because of competition with the angiogenic factor $VEGF_{165}$ (see "Semaphorin in the vascular system"). Sema3A is a chemorepellent for human medulloblastoma [14]. This study also pointed out the importance of the spatial and temporal distribution of Sema3A in the tumour microenvironment. Long-term exposure and/or saturation of Sema3A in the medium trigger medulloblastoma cells apoptosis. The different mechanisms of cell repulsion or apoptosis are mediated by differential recruitment of NRP1 and VEGFR1 and selective activation of MAP kinases ERK1/2 or p38, respectively [145]. By interacting with NRP1 and plexin-A1, Sema3A induces an autocrine loop that inhibits breast carcinoma cells migration [146]. The same inhibition loop, involving p38, has been involved in malignant mesothelial cells [147]. The balance between VEGF and Sema3A has once more been underlined in a recent study showing that loss of endothelial SEMA3A in favour of VEGF165 could be responsible for the angiogenic switch occurring in multiple myeloma [148]. Hence, Sema3A could also favour tumour growth by modulating primary T-cell activation, thereby contributing to the T-cell dysfunction in the tumour microenvironment [149].

Semaphorins as tumour promoters. Sema3C is expressed by several human glioma cell lines, but its function remains unclear. It could control the vascularization of the tumours, have a protective action on tumour cells, or stimulate migration [150]. Sema3E is the third class 3 semaphorin involved in tumour progression. Its expression correlates with tumour progression, and Sema3E mRNA is overexpressed in carcinoma cell lines [151, 152]. It is frequently expressed in human cancer cell lines and solid tumours from breast cancer patients. *In vivo*, Sema3E promotes lung metastasis and displays migration and growth promoting activity *in vitro*. Both these activities are associated with a p61-Sema3E isoform known to down-regulate the repellent activity [151]. Sema4D, from class 4 semaphorins, binds to plexin-B1 that interacts with the scatter factor receptor Met. Upon ligand binding, Met induces "invasive growth", a complex biological response involved in tissue morphogenesis and, when deregulated, in tumour progression and metastasis [91,93]. Hence, Sema4D/plexin-B1 interaction activates a survival or proliferation signal in CD5+ B cells that may favour the expansion of leukemic clones [153]. Overexpression of Sema4D often occurs in squamous cell carcinomas, as well as in breast, colon, lung and prostate cancers. Tumour knockdown for Sema4D exhibits dramatic decrease in tumour vascularity and tumour growth. Sema4D, besides its role in angiogenesis, increases growth, survival and metastatic potential of tumours [92, 154]. Another class 4 semaphorin, Sema4B, promotes cell motility in a highly metastatic lung cancer cells line by interacting with CLCP1, a gene encoding a protein with high structural similarities with neuropilins [155].

Class 5 semaphorins seem to be widely expressed in cancers and cancer cell lines and need further investigations to determine their exact role [156–159]. Among class 6 semaphorins, Sema6A and Sema6B may also play a role in tumour progression [160–162].

Pathologies of the immune system

Virus-encoded semaphorins define a specific class of secreted proteins and are the smallest semaphorins. A39R (SEMAVA) is encoded by the vaccinia virus, a member of the poxvirus family [163]. AHVSema (SEMAVB) is encoded by the alcelaphine herpesvirus [164]. Plexin-C1 binds both SEMAVA and SEMAVB [165]. SEMAVA has been shown to induce cytokine production from human monocytes [165]. Moreover, *in vivo* analyses demonstrated that SEMAVA expression affects the outcome of dermal infection by vaccinia virus. The late expression of SEMAVA during infection is correlated to pro-inflammatory properties but is non-essential for virus-replication. Therefore, SEMAVA has a role of host immune modulator [166]. A work by Walzer and collaborators showed the importance of plexin-C1/ SEMAVA interaction on actin reorganization and on integrin-mediated adhesion. Activation of plexin-C1 by SEMAVA triggered chemokine-induced migration on neutrophils and DCs. Finally, SEMAVA inhibits phagocytosis by DCs and neutrophils [167, 168].
Sema7A was discovered in a search for vertebrate homologues of virally encoded semaphorins [66, 67]. It also defines the John-Milten-Hagen human blood group antigen on erythrocytes, which has been implicated in a clinically benign autoimmune disorder [169]. The analogy between viral semaphorins and Sema7A raises the possibility that virus-encoded semaphorins could mimic Sema7A.

Conclusion

The discovery and characterization of semaphorins opened an extraordinary wide field of investigation. They act as crucial modulators during development of the whole body, being implicated in the establishment of highly structured and complex organs and networks. Consistently, abnormal semaphorin expression or regulation is associated with numerous diseases ranging from nerve lesion and degenerative diseases, autoimmune disease or cancer. The recent demonstration of the role of Sema3B and Sema6D in bone homeostasis [111] also pushes semaphorin signalling to enter the dance of rare diseases such as osteopetrosis (Albers-Schönberg disease), a very rare inherited disorder leading to an excess of bone formation due to lack of bone resorption by osteoclasts [170]. While the signalling mechanisms need to be further investigated, there is still an important gap to fill on the exact functions and mechanisms of action of semaphorins in pathological processes. One key question is certainly to evaluate the relative importance of semaphorin signalling compared to that of other guidance cues such as the netrins or the eph/ephrins. In the nervous system, the combinatorial expression and activity of multiple guidance cues is already described as the key determinant of brain wiring. This is for instance the case for thalamocortical axons using multiple cues to reach their final laminar position [11]. Taking into account the existence of compensatory and redundancy mechanisms illustrated for example by the lack of abnormal phenotype of Sema3B knock-out mice [171], the next challenge will be to define the molecular hierarchy between semaphorins and between semaphorins and other guidance cues. However, the colossal efforts accomplished over the last fifteen years have already opened a new avenue toward a novel family of therapeutic targets: the semaphorins and their receptors.

Acknowledgments. This work was supported by INSERM, ACI JC (#5327), APETREIMC and ARC to Dominique Bagnard.

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