

Semaphorin signaling in cancer cells and in cells of the tumor microenvironment – two sides of a coin

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Summary

Semaphorins are a large family of secreted and membrane-bound molecules that were initially implicated in the development of the nervous system and in axon guidance. More recently, they have been found to regulate cell adhesion and motility, angiogenesis, immune responses, and tumor progression. Semaphorin receptors, the neuropilins and the plexins, are expressed by a wide variety of cell types, including endothelial cells, bone-marrow-derived cells and cancer cells. Interestingly, a growing body of evidence indicates that semaphorins also have an important role in cancer. It is now known that cancer progression, invasion and metastasis involve not only genetic changes in the tumor cells but also crosstalk between tumor cells and their surrounding non-tumor cells.

Through the recruitment of endothelial cells, leukocytes, pericytes and fibroblasts, and the local release of growth factors and cytokines, the tumor microenvironment can mediate tumor-cell survival, tumor proliferation and regulation of the immune response. Moreover, by conferring cancer cells with an enhanced ability to migrate and invade adjacent tissues, extracellular regulatory signals can play a major role in the metastatic process. In this Commentary, we focus on the emerging role of semaphorins in mediating the crosstalk between tumor cells and multiple stromal cell types in the surrounding microenvironment.

Key words: Semaphorins, Plexins, Tumor, Tumor microenvironment

Introduction

Semaphorins were initially identified as evolutionarily conserved axon-guidance cues in the assembly of the neural circuitry (Kolodkin et al., 1993; Luo et al., 1993). However, it is now clear that they are widely expressed outside the nervous system, and consist of more than 20 family members (in vertebrates), which are implicated in a range of processes, including the regulation of cell survival, apoptosis, cell-substrate adhesion and directional cell migration (Tamagnone and Comoglio, 2004; Zhou et al., 2008).

Semaphorins are secreted or membrane-associated glycoproteins that have been grouped into eight classes on the basis of their structural elements and amino-acid-sequence similarity (Fig. 1A). Invertebrate semaphorins mainly fall into classes 1 and 2, whereas classes 3 to 7 comprise vertebrate semaphorins and the final group (class V) contains semaphorins that are encoded by viral genomes. In the current nomenclature, the abbreviation SEMA is followed by a number indicating the subclass, and a capital letter designating the individual member – for example, SEMA3A and SEMA4D (Goodman et al., 1999). Semaphorins are characterized by an N-terminal 500-amino-acid sema domain that is essential for semaphorin function. The structure of the sema domain is a seven-blade β -propeller fold that has overall structural similarity to the extracellular domain of α -integrins (Gherardi et al., 2004; Koppel et al., 1997). Next to the sema domain, semaphorins contain the plexin-semaphorin-integrin (PSI) domain, which is a cysteine-rich motif that is also referred to as a MET-related sequence (MRS) (Bork et al., 1999). Semaphorins are further distinguished by other distinct protein domains, including immunoglobulin-like, thrombospondin and basic C-terminal domains. Class-3 semaphorins are the only secreted vertebrate semaphorins and are distinguished by a conserved, basic-charged domain at the C-terminus. Class 4-7

semaphorins are cell-membrane-anchored proteins that are distinguished by their unique structural elements, such as thrombospondin repeats (in the case of class-5 semaphorins) or a glycosylphosphatidylinositol (GPI) anchor (class-7 semaphorins). Membrane-anchored semaphorins can be further processed into soluble forms through proteolytic degradation, as seen for SEMA4D (Basile et al., 2007b; Elhabazi et al., 2001).

High-affinity receptors for semaphorins include plexins and neuropilins (NPs) (He and Tessier-Lavigne, 1997; Kolodkin and Ginty, 1997; Tamagnone et al., 1999) (Fig. 1B,C). Whereas the genomes of invertebrates contain only two plexin genes, nine plexins have been identified in vertebrates, which are divided into four subfamilies: plexin-A(1-4), plexin-B(1-3), plexin-C1 and plexin-D1. Similar to their ligands, plexins contain a sema domain in the extracellular portion; in addition, they have two to three repeated PSI domains and three IPT (Ig-like fold shared by plexins and transcription factors) domains. By contrast, the cytoplasmic moiety of plexins lacks homology to known proteins or functional motifs, although it contains two amino acid stretches that are weakly similar to GTPase-activating proteins (GAPs), which catalyze the inactivation of R-Ras monomeric GTPase (Oinuma et al., 2004). NPs (NP1 and NP2), which are found only in vertebrates, are single-span transmembrane glycoproteins that share a similar domain structure and were initially characterized as co-receptors for class-3 semaphorins and for members of the vascular endothelial growth factor (VEGF) family (He and Tessier-Lavigne, 1997; Kolodkin et al., 1997; Soker et al., 1998).

Membrane-bound vertebrate semaphorins bind directly to plexins, whereas secreted semaphorins (class 3) also require NPs as obligate co-receptors; SEMA3E is one known exception (Gu et al., 2005) (Table 1). Additional transmembrane molecules are found in semaphorin-receptor complexes in association with plexins and NPs,

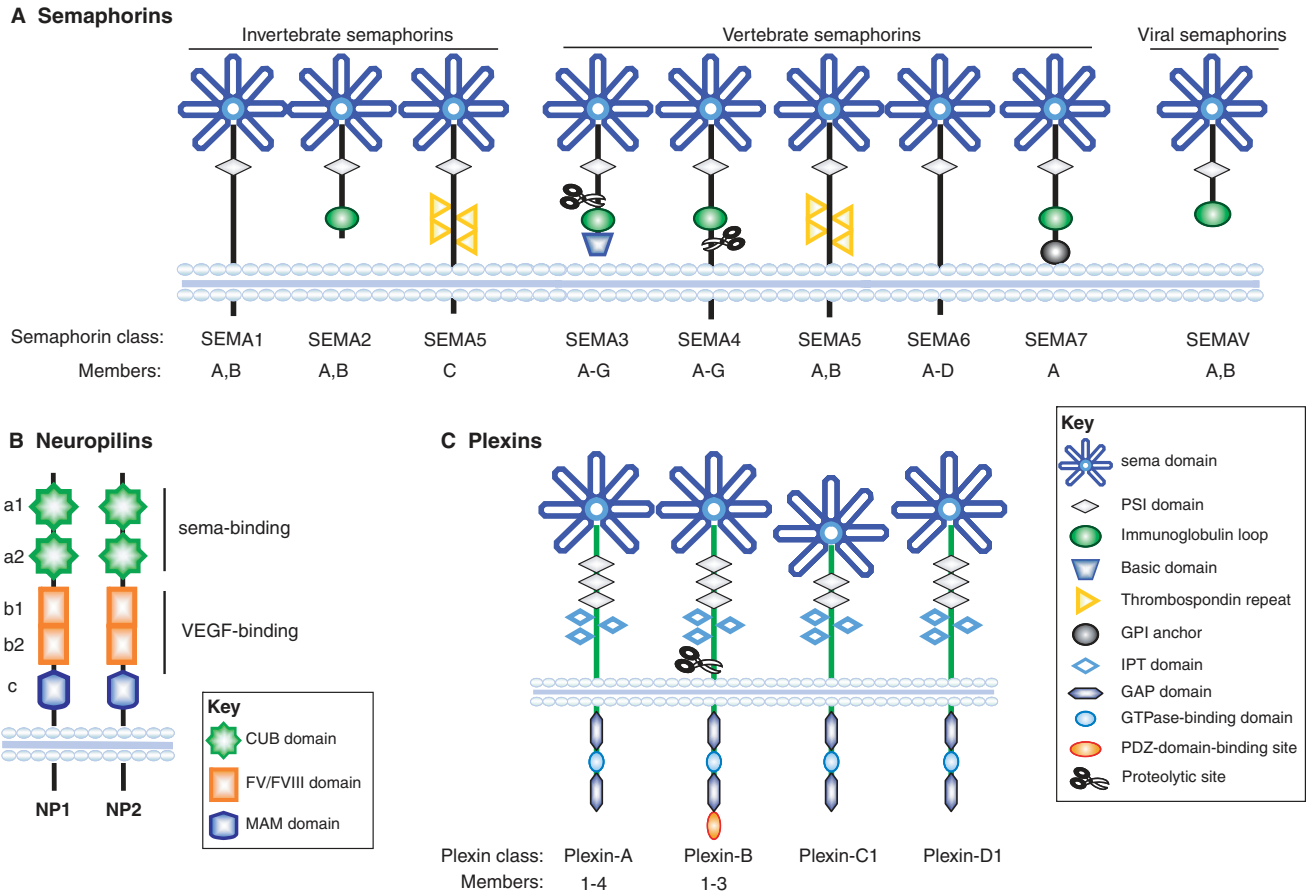


Fig. 1. Schematic representation of semaphorins and semaphorin receptors (neuropilins and plexins), divided into subfamilies on the basis of sequence similarity and structural features. (A) All semaphorins are characterized by a large sema domain and one PSI domain (a cysteine-rich motif). Of the vertebrate semaphorins, those in classes 4, 5 and 6 are transmembrane proteins, whereas those in class 7 are membrane-anchored via glycosylphosphatidylinositol (GPI). Class-3 secreted semaphorins have C-terminal basic-charged sequences, which are required for binding to neuropilins. Several class-3 semaphorins and SEMA4D have been shown to undergo regulatory cleavage by furins or metalloproteases. Class-5 semaphorins are distinguished by thrombospondin repeats. Several semaphorins also contain immunoglobulin-like domains. (B) Neuropilins are transmembrane receptors that are characterized by two complement-like (CUB) domains (also called the a1 and a2 domains), two FV/FVIII coagulation factor-like domains (also called the b1 and b2 domains) and a meprin-like MAM domain (also called the c domain). (C) Plexins contain one sema domain, two to three PSI domains and three IPT (Ig-like fold shared by plexins and transcription factors) domains. The cytoplasmic domain of plexins contains two amino acid stretches that are weakly similar to GTPase-activating proteins (GAPs). In addition, B-subfamily plexins contain a C-terminal binding site for PDZ (PSD-95/Dlg/ZO-1) domains. A cleavage site for furin-like pro-protein convertases is also present in the extracellular domain of plexin-B1 and plexin-B2, generating heterodimeric receptors (Artigiani et al., 2003).

including cell-adhesion molecule L1 (Castellani and Rougon, 2002) and the receptor tyrosine kinases (RTKs) VEGFR2 (Toyofuku et al., 2004), erythroblastic leukemia viral oncogene homolog 2 (ErbB2) (Swiercz et al., 2004), off-track kinase (OTK) (Winberg et al., 2001) and Met (also known as HGFR) (Giordano et al., 2002). In the immune system, CD72 and Tim2 (T-cell immunoglobulin and mucin domain-containing protein 2) were found to interact functionally (although with low affinity) with the transmembrane semaphorins SEMA4D and SEMA4A, respectively (Kumanogoh et al., 2002; Kumanogoh et al., 2000). Recently, other ligands for NPs have also been described, including fibroblast growth factor 2 (FGF2) (West et al., 2005), hepatocyte growth factor (HGF) (Sulpice et al., 2008) and galectin-1 (Gal-1) (Hsieh et al., 2008). In addition, NPs were recently observed to form complexes with additional cell-surface receptors, including Met (Matsushita et al., 2007), $\beta 1$ integrin (Fukasawa et al., 2007) and transforming growth factor- $\beta 1$ (TGF- $\beta 1$) (Glinka and Prud'homme, 2008) (for reviews see Neufeld and Kessler, 2008; Pellet-Many et al., 2008).

In addition to their function in a range of basic cellular processes, recent studies have shown that semaphorin-mediated signals might also play important regulatory functions in cancer (Neufeld and Kessler, 2008). On one side, tumor progression and metastatic dissemination depend on intrinsic properties of cancer cells, such as survival, self-renewal and the ability to migrate and overcome tissue barriers (invasiveness). On the other side, the tumor stroma – which includes endothelial cells, fibroblasts and cells of the immune system – is engaged in active molecular crosstalk with cancer cells. For example, blood- and lymph-vessel angiogenesis, together with inflammatory and immunosuppressive responses, further promotes cancer-cell survival, migration and invasion, as well as initiation of the metastatic cascade. In this Commentary, we review the current knowledge on the emerging role of semaphorin signals in controlling these ‘two sides of a coin’ – that is, the tumor-cell intrinsic alterations, and the crosstalk between cancer cells and other cells of the tumor microenvironment. In addition, the implications of semaphorin signaling in tumor progression will be discussed.

Table 1. The functional activity of semaphorins on tumor cells

Semaphorin	Type of tumor or tumor cells	Functional effects		References
		Anti-tumorigenic	Tumorigenic	
SEMA3A	Pancreatic cancer		Marker of poor patient survival	(Muller et al., 2007)
	Breast and prostate cancer cells	Inhibits cell migration and invasiveness		(Bachelder et al., 2003; Caunt et al., 2008; Herman and Meadows, 2007; Pan et al., 2008)
	Breast cancer cells	Inhibits in vivo tumor development; inhibits anchorage-independent growth		(Kigel et al., 2008)
	Mesothelioma cells	Negative feedback of VEGF-mediated signals		(Catalano et al., 2004)
	Myeloma	Negative control of VEGF-mediated angiogenesis		(Vacca et al., 2006)
	Leukemic T cells	Sensitizes to FAS-induced apoptosis		(Moretti et al., 2008)
SEMA3B	Glioblastoma		Marker of poor patient survival	(Rich et al., 2005)
	Melanoma and lung carcinoma cells (tumor xenografts)		Pro-metastatic effects	(Rolny et al., 2008)
	Melanoma and lung carcinoma cells	Inhibits tumor growth		(Rolny et al., 2008)
	Ovarian adenocarcinoma cells	Reduces anchorage-dependent growth; suppresses tumor formation		(Tse et al., 2002)
	Lung and breast cancer cells	Inhibits cell growth; induces apoptosis		(Castro-Rivera et al., 2004; Castro-Rivera et al., 2008; Tomizawa et al., 2001)
	Lung cancer Non-small-cell lung cancer	Gene deleted in tumors Allelic loss and promoter hypermethylation		(Potiron et al., 2009; Sekido et al., 1996) (Kuroki et al., 2003)
SEMA3C	Ovarian cancer cells		Marker of increased resistance to cytotoxic drugs	(Yamada et al., 1997)
	Lung cancer cells		Highly expressed in metastatic cells	(Martin-Satue and Blanco, 1999)
	Ovary cancer		Marker of poor patient survival	(Galani et al., 2002)
	Prostate cancer cells		Increases invasiveness	(Herman and Meadows, 2007)
SEMA3D	Breast cancer cells	Inhibits in vivo tumor development, inhibits anchorage-independent growth		(Kigel et al., 2008)
SEMA3E	Breast cancer and breast cancer cells		Expressed in metastatic cells; induces metastatic behavior	(Christensen et al., 2005; Christensen et al., 1998)
	Melanoma	Reduced expression in metastasis		(Roodink et al., 2008)
	Breast cancer cells	Inhibits in vivo tumor development		(Kigel et al., 2008)
SEMA3F	Lung cancer	Decreased expression in tumors		(Brambilla et al., 2000; Fujii et al., 2002; Lantuejoul et al., 2003)
	Lung cancer	Gene deleted in tumors		(Roche et al., 1996; Xiang et al., 1996)
	Lung cancer cells	Inhibits cell-substrate adhesion		(Kusy et al., 2005)
	Ovarian cancer cells	Induces apoptosis		(Xiang et al., 2002)
	Lung cancer cells	Loss of expression (compared with normal lung cells)		(Brambilla et al., 2000; Lantuejoul et al., 2003)
	Melanoma cells	Anti-metastatic activity		(Bielenberg et al., 2004)
	Melanoma cells	Anti-proliferative activity		(Chabbert-de Ponnat et al., 2006)
	Breast cancer cells	Inhibits cell migration		(Nasarre et al., 2003; Nasarre et al., 2005)
	Breast and melanoma cancer cells	Inhibits in vivo tumor development; inhibits anchorage-independent growth		(Kigel et al., 2008)
	SEMA3G	Melanoma cancer cells	Inhibits in vivo tumor development	
SEMA4D	Leukemia cells		Sustains proliferation and/or survival	(Deaglio et al., 2005; Granziero et al., 2003)
	Epithelial cells		Induces cell motility, invasiveness and anchorage-independent growth	(Giordano et al., 2002)
	Breast cancer cells		Induces cell migration	(Swiercz et al., 2008)
	Breast cancer cells	Inhibits cell adhesion and migration		(Barberis et al., 2004; Swiercz et al., 2008)
SEMA6D	Stomach cancer		Increased expression in tumors	(Zhao et al., 2006)

Semaphorin-dependent signaling pathways

Semaphorin receptors can elicit multiple intracellular signaling cascades (Fig. 2) (Zhou et al., 2008). Plexins are pivotal players in this scenario, whereas a putative signaling function of the NPs (beyond their essential role as binding platforms) is currently controversial. Although plexins do not carry an intrinsic kinase activity, they can trigger the activation of plexin-associated receptor-type and nonreceptor-type tyrosine kinases (for a review, see Franco and Tamagnone, 2008). By activating plexin-associated kinases and the intrinsic GAP activity of plexins (see below), semaphorin binding can regulate cytoskeletal dynamics, integrin functions, cell adhesion and migration. Remarkably, the functional responses elicited by semaphorins can vary depending on the activation of distinct signaling pathways in a cell-specific manner.

Regulation of integrin-mediated functions

Most current information concerning semaphorin-dependent signal transduction originates from studies involving the prototype semaphorins SEMA3A and SEMA4D in neuronal and endothelial

cells; however, these pathways probably also operate in other cell types. Several reports have shown that semaphorins regulate the function of integrins, which are heterodimeric cell-surface receptors that tether cells to the extracellular matrix (ECM) (Halloran and Wolman, 2006; Serini et al., 2008) (Fig. 2). Integrin engagement is central for triggering intracellular signaling pathways that control downstream events such as cell growth and survival, apoptosis and motility (Hanahan and Weinberg, 2000). The molecular mechanisms by which semaphorin-dependent signaling regulates integrins are partly understood. Although evidence confirming that semaphorins or their receptors can directly interact with integrins is currently missing, it was found that plexins have intrinsic GAP activity that acts on the monomeric GTPase R-Ras, which is known to sustain integrin activation (Oinuma et al., 2004). R-Ras activation enhances focal-adhesion formation and cell adhesion, favors the activation of phosphoinositol-3-kinase, and might also regulate cell spreading, migration and invasion (Negishi et al., 2005). Following semaphorin binding, the GAP activity of plexins leads to R-Ras inactivation, thereby inhibiting integrin-mediated adhesion and other downstream events. In addition to acting on R-Ras, plexins can regulate the activation of the small GTPase RhoA via signal transducers that are associated with the plexin cytoplasmic domain (for reviews, see Casazza et al., 2007; Puschel, 2007). Because integrin-mediated adhesion and monomeric GTPases are pivotal players in cell motility, plexin activation frequently leads to inhibition of directional migration, which is sometimes described as cell 'repulsion' when these inhibitory signals are localized in specific areas.

Notably, many plexin-associated receptor-type and nonreceptor-type tyrosine kinases can control integrin functions and cell migration, including the SRC-family kinases FAK and PYK2, and receptor-type kinases Met and ErbB2 (Guo et al., 2006; Trusolino et al., 2001). Thus, semaphorins control integrin-mediated adhesion, cytoskeletal dynamics and cell migration both by inactivating small GTPases and by modulating tyrosine kinases.

Examples of cell-type-specific effects of semaphorins

Certain semaphorins, such as SEMA4D (the ligand of plexin-B1), have been found to trigger multiple and sometimes opposing cellular responses, depending on the receptor complex involved. For example, plexin-B1 can associate with the RTKs ErbB2 and Met and, interestingly, it was shown in breast carcinoma cells that the expression of the two different RTKs caused the opposite effect on RhoA activity in response to SEMA4D. The expression of ErbB2 led to RhoA activation and thereby promotion of chemotaxis, whereas the expression of Met caused RhoA inactivation and inhibition of directional migration (Swiercz et al., 2008). Moreover, SEMA4D stimulation was shown to inhibit PI3K and AKT signaling in neurons (Ito et al., 2006), whereas it was found to trigger AKT activation in endothelial cells (Basile et al., 2005; Basile et al., 2007a). The molecular mechanisms underlying this versatility of plexin signaling still await full elucidation.

Evidence indicates that SEMA7A might also regulate cell adhesion and migration in both a negative and positive manner via different receptors. In fact, SEMA7A promotes cell spreading and dendricity (in melanocytes) via $\beta 1$ integrin (Scott et al., 2008a), whereas it can inhibit cell-substrate adhesion (in dendritic cells) by binding to plexin-C1 (Walzer et al., 2005). Cofilin, an actin-binding protein that is implicated in cell migration, seems to be a common mediator, which is regulated in an opposing manner on activation of the two different receptors by SEMA7A (Scott et al., 2008b).

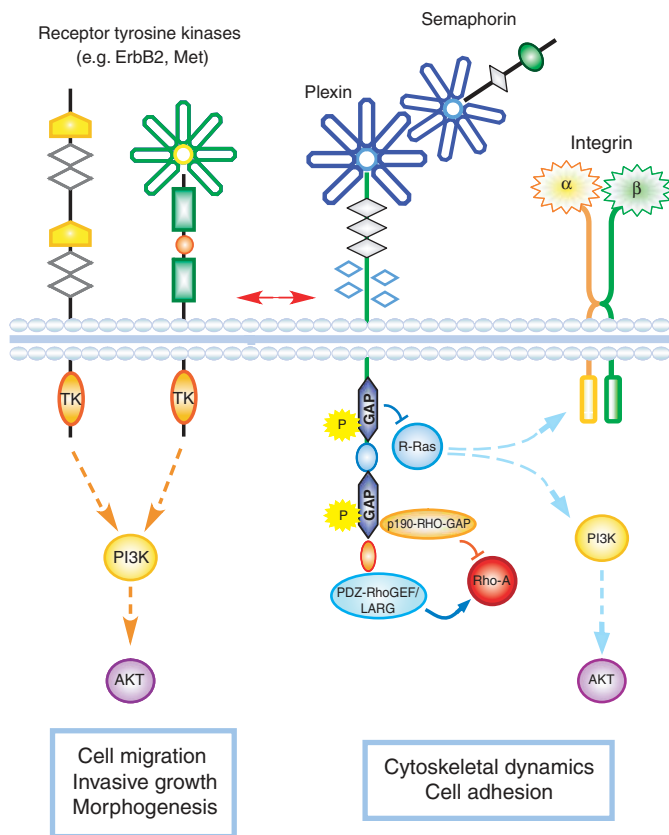


Fig. 2. Semaphorin receptors and their intracellular signaling pathways. Semaphorin receptors can elicit multiple intracellular signaling cascades (Zhou et al., 2008). The red arrow indicates that plexins can associate on the cell surface with different receptor tyrosine kinases (such as Met, ErbB2, VEGFR2 and OTK), which in turn have a range of downstream effectors. In addition, semaphorins can regulate integrin functions and cytoskeletal dynamics via the intrinsic R-Ras GAP activity of plexins and the recruitment of regulatory molecules for RhoA, and thereby can affect cell adhesion and migration. These effects can sometimes result in opposing functional responses, depending on the activation of distinct pathways in a cell-type-specific manner. LARG, leukemia-associated guanine nucleotide exchange factor; PI3K, phosphoinositide 3-kinase; TK, tyrosine kinase.

Together, these studies highlight not only the common features of semaphorin signaling pathways but also support the notion that semaphorin signaling events are cell-type specific. Therefore, it will be crucial to thoroughly assess whether the semaphorin signaling that is observed in one particular cell type or tissue also occurs in other cell types.

Expression of semaphorin receptors by tumor cells

The expression of plexins has been reported in a wide variety of tumors. For example, plexin-A expression has been observed in ovarian (Syed et al., 2005) and gastric (Zhao et al., 2007) carcinoma, as well as in tumor-derived cells of glioma (Rieger et al., 2003), melanoma, and breast (Castro-Rivera et al., 2008; Castro-Rivera et al., 2004; Kigel et al., 2008) and colon (Nguyen et al., 2006) cancer. The expression of plexin-B1 has been reported in ovarian and prostate cancers (Syed et al., 2005; Wong et al., 2007). In addition, a high frequency of somatic missense mutations in plexin-B1, and its overexpression at the protein level, seems to correlate with increased invasiveness and metastatic progression of prostate tumors (Wong et al., 2007). In contrast to these reports, a marked downregulation of plexin-B1 expression has been reported in clear-cell renal carcinoma (Gomez Roman et al., 2008), and loss of plexin-B1 gene expression was found to correlate with poor prognosis in estrogen-receptor-positive breast cancer (Rody et al., 2007). These discrepancies might be explained by the co-expression of distinctive signaling partners in the different tumors. Plexin-C1, which is expressed by melanocytes, is reduced or absent in human melanoma cells (Scott et al., 2008b); in tissue microarrays that compared nevi and melanoma samples, an inverse correlation between plexin-C1 levels and the depth of skin invasion, and a remarkable decrease in the expression of plexin-C1, was found in metastatic samples (Scott et al., 2008b). Finally, plexin-D1 is expressed in a range of tumor cells, including melanoma and breast-cancer cell lines (Kigel et al., 2008; Roodink et al., 2005; Roodink et al., 2008). A positive correlation between plexin-D1 expression and tumor grading has been reported.

NPs are also expressed by a wide variety of human tumor cell lines and neoplasms (reviewed by Pellet-Many et al., 2008). NP1 is prevalently expressed in carcinomas, whereas NP2 is frequently expressed in non-epithelial tumors such as melanomas, leukemias and neuroblastomas (Pellet-Many et al., 2008). This expression pattern is not without exceptions and, in fact, the two receptors are often co-expressed (Marcus et al., 2005; Rieger et al., 2003). Clinical studies suggest that NPs play a role in tumor growth and disease progression because they are involved mediating semaphorin- and VEGF-mediated effects on cancer-cell proliferation, survival and migration (reviewed by Barr et al., 2005; Bielenberg et al., 2006; Ellis, 2006; Geretti et al., 2008; Guttman-Raviv et al., 2006; Pan et al., 2007). High levels of NP1 were significantly associated with a poor outcome in patients with breast cancer (Ghosh et al., 2008), and correlated with invasive behavior and metastatic potential in gastrointestinal carcinomas (Hansel et al., 2004), glioma (Osada et al., 2004) and prostate carcinoma (Latil et al., 2000). These observations are further supported by *in vitro* studies. Indeed, NP1 overexpression in prostate carcinoma cells promoted tumor growth *in vivo* (Miao et al., 2000) and, consistent with this result, knockdown of NP1 expression using RNA interference inhibited tumor-cell migration (Bachelder et al., 2003). Although most studies have indicated a tumorigenic role for NPs, other reports suggest that NP1 might have the opposite function in tumors. For example, NP1 overexpression in pancreatic

carcinoma cells has been reported to inhibit cell migration, anchorage-independent growth and tumor incidence *in vivo* (Gray et al., 2005; Kamiya et al., 2006), and elevated expression of the gene encoding NP1 is associated with a more favorable prognosis in individuals with colon cancer (Kamiya et al., 2006). These discrepancies are currently unresolved. They might be explained by the non-physiological effects that are induced by the ectopic overexpression of NP1, or they might reflect cell-type-specific responses and/or the involvement of different signaling pathways. Furthermore, an elevated expression of NP2 correlated with advanced tumor progression and poor prognosis in osteosarcomas (Handa et al., 2000) and colorectal carcinoma cells (Gray et al., 2008), and the silencing of NP2 expression by short-hairpin RNA reduced the development of tumors. Notably, treatment with an NP2-specific blocking antibody reduced metastatic dissemination of murine mammary carcinoma and rat glioma cell lines to lymph nodes and distant organs (Caunt et al., 2008).

The expression patterns of plexins and NPs in different tumor types is consistent with the putative regulatory role of semaphorins in cancer. However, current evidence does not associate any individual semaphorin receptor with a univocal function in tumor development. This is probably due to the fact that semaphorins induce multiple signaling cascades in different tumor cells and in cells of the tumor microenvironment, an aspect that deserves further investigation.

Semaphorins in the control of tumor-cell behavior

Semaphorins act as pleiotropic signals that are able to control multiple functions in tumor cells, ranging from survival, proliferation and apoptosis to cell adhesion and migration (Fig. 3; Table 1). Semaphorins might be released by cells in the tumor microenvironment (such as infiltrating leukocytes) or by cancer cells, which can establish their own autocrine regulatory loops.

Cell survival, proliferation and apoptosis

Essential alterations in cell behavior that collectively dictate cancer progression include self-sufficiency in growth signals, limitless replicative potential and evasion of apoptosis (Hanahan and Weinberg, 2000). Increasing evidence suggests that semaphorin signals regulate many of these properties. For example, it has been proposed that SEMA3B and SEMA3F act as tumor suppressors, because they undergo loss of heterozygosity, promoter hypermethylation and downregulation of expression in human tumors (reviewed by Potiron et al., 2009). Consistently, SEMA3B overexpression in tumor cell lines induced apoptosis (Castro-Rivera et al., 2004; Castro-Rivera et al., 2008), and inhibited cell proliferation and colony formation in soft agar (Tomizawa et al., 2001; Tse et al., 2002). However, data obtained from a large number of human tumor samples do not show a statistically significant correlation between SEMA3B expression and patient survival (Rolny et al., 2008). In fact, SEMA3B expression has been correlated with poor prognosis in glioblastoma cases (Rich et al., 2005), and tumor subsets that have an increased level of SEMA3B expression are associated with metastatic progression (Rolny et al., 2008). Notably, SEMA3B expression was found to inhibit tumor growth while sustaining metastatic dissemination in a number of tumorigenic models, which is consistent with p38-MAPK-dependent activation of p21, a cell-cycle inhibitor, and the induction of interleukin-8 (IL-8) cytokine secretion from tumor and stromal cells, which promotes cancer progression and metastasis (Rolny et al., 2008).

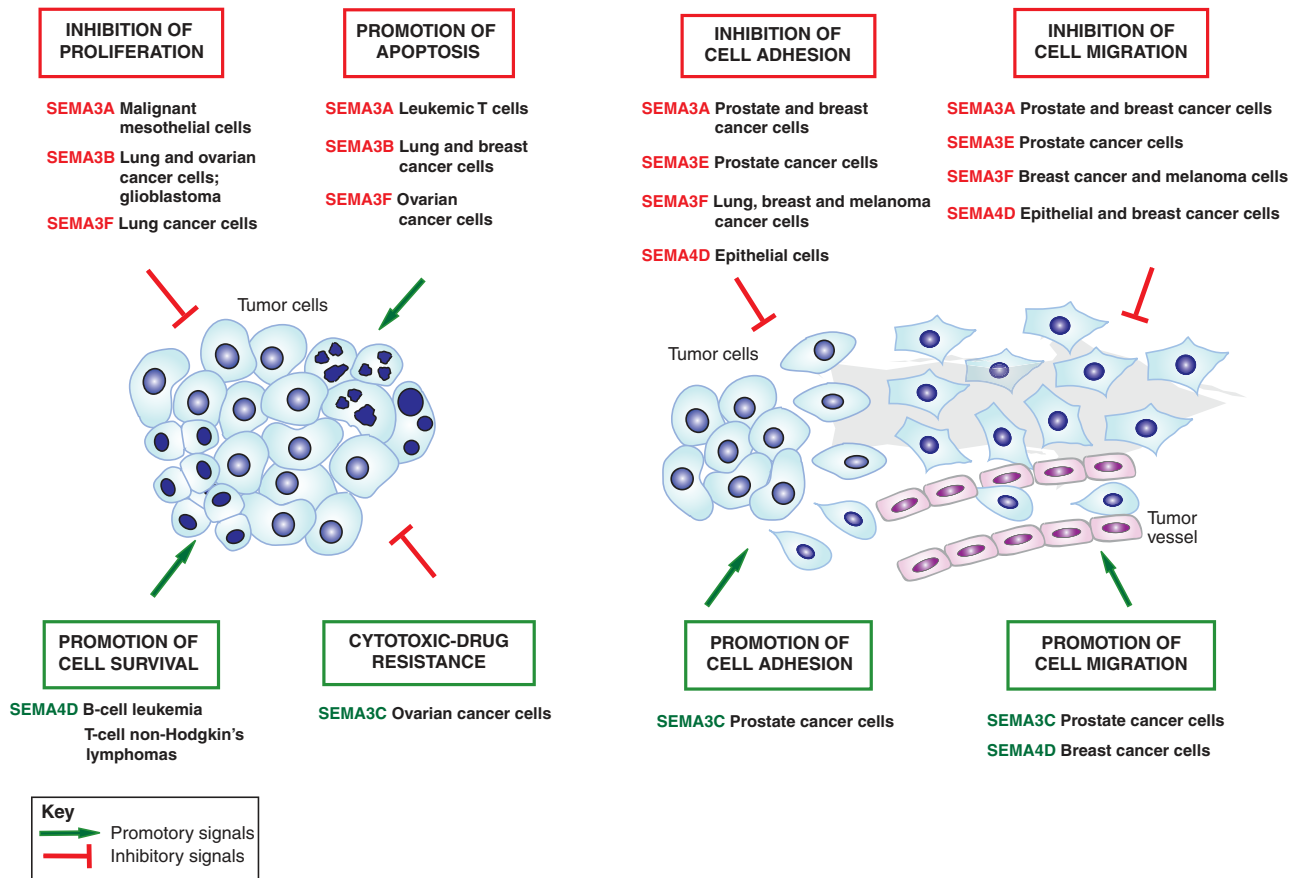


Fig. 3. Functional activities of semaphorins on tumor cells. The reported functional activities of semaphorins in the control of tumor-cell behavior are schematically shown. So far, most studies have focused on secreted class-3 semaphorins and SEMA4D. Evidence suggests that semaphorin signals can regulate (either positively or negatively, depending on the context) many intrinsic properties of tumor cells, such as self-sufficiency for growth signals, evasion of apoptosis, loss of cell polarity, and alterations in cell-cell and cell-matrix adhesion. In certain cases (e.g. SEMA4D), the same molecule has been reported to have the opposite effect in different types of tumor cells. The molecular mechanisms mediating these effects are only partly understood, and they implicate distinct and cell-specific receptor complexes and intracellular signaling cascades. Moreover, evidence suggests that semaphorins released by tumor cells not only have autocrine, cell-autonomous effects, but also regulate cells in the tumor environment in a paracrine manner (see Fig. 4).

SEMA3F expression inversely correlates with lung-cancer tumor grading and staging (Brambilla et al., 2000). In addition, increased expression of this semaphorin in tumor cells inhibits proliferation, prevents anchorage-independent growth (Futamura et al., 2007; Kusy et al., 2005; Xiang et al., 2002) and potentially inhibits tumorigenesis *in vivo* (reviewed by Pitron et al., 2009).

SEMA3A is secreted by tumor cells and has major functions in regulating the tumor microenvironment (see below). However, it was also reported to act in a negative-feedback loop that counteracts VEGF-mediated signaling and cell proliferation (Catalano et al., 2004). Moreover, SEMA3A can dramatically inhibit the proliferation of breast cancer cells that overexpress NP1 both in soft agar *in vitro* and in tumor models *in vivo* (Kigel et al., 2008). Surprisingly, however, high expression of SEMA3A seems to correlate with poor clinical outcome in pancreatic cancer (Muller et al., 2007).

Different mechanisms might explain the suppressive activity of secreted semaphorins on tumor cells. Because these ligands depend on NPs as obligate plexin-associated co-receptors, one potential mechanism might involve direct competitive binding by VEGF. In fact, several studies have reported that VEGFs and semaphorins compete for a partially overlapping binding site on the b1 domain

of NPs (Castro-Rivera et al., 2004; Castro-Rivera et al., 2008; Gu et al., 2002; Miao et al., 1999; Narazaki and Tosato, 2006). More recently, however, a growing body of evidence supports the opposite conclusion; that is, that both ligands can bind to NPs independently and trigger distinct and antagonistic signaling pathways (for example, via plexins and the VEGF receptors) (Appleton et al., 2007; Liang et al., 2007; Pan et al., 2007). In particular, it was observed that, even when SEMA3A does not inhibit VEGF-induced phosphorylation of VEGFR2, it still effectively inhibits VEGF-induced downstream activation of ERK1 and ERK2 (ERK1/2) (Guttmann-Raviv et al., 2007). Notably, as it is known that integrin-mediated signals control cell proliferation, survival and apoptosis, the tumor-suppressor activity mediated by semaphorins might also be related to the plexin-dependent regulation of integrin functions.

In contrast to the semaphorins described above, SEMA3C has been initially identified as a non-multidrug-resistance gene in human cancers (Yamada et al., 1997). In addition, the expression of SEMA3C was found to be upregulated in metastatic cells of lung adenocarcinoma (Martin-Satue and Blanco, 1999), and its expression in ovarian cancer correlates with shorter patient survival (Galani et al., 2002). Furthermore, SEMA4D has been shown to promote growth and survival of B-cell chronic lymphocytic

leukemia (B-CLL) (Deaglio et al., 2005; Granziero et al., 2003), and anchorage-independent growth of epithelial cells (Giordano et al., 2002).

Cell adhesion

A proper balance between adhesion to and dissociation from adjacent cells and the ECM is essential for morphogenesis, and plays a central role in cancer (Nishimura and Sasaki, 2008). Notably, signaling crosstalk coordinates the function of integrins with the stability of cadherin-based cell-cell-adhesion complexes. Several signals, including semaphorins, are known to control these cell-adhesion complexes through complex intracellular signaling pathways (Halloran and Wolman, 2006). Notably, it was shown that the expression of adhesion receptors in cancer cells is differentially modulated by class-3 semaphorins (Herman and Meadows, 2007; Pan et al., 2008). In particular, SEMA3F anti-tumor activity in lung carcinoma cells was associated with loss of activated $\alpha 5\beta 3$ integrin (Brambilla et al., 2000), downregulation of integrin-linked kinase (ILK) activity and decreased adhesion to the ECM (Kusy et al., 2005; Potiron et al., 2007). Consistent with these observations, the forced re-expression of SEMA3F (which is low in highly metastatic melanoma cells) inhibited adhesion, migration and the expression of $\beta 1$ integrin in melanoma cells (Bielenberg et al., 2004).

So far, experimental evidence implicating class-3 semaphorins as direct regulators of cadherin-based cell-cell adhesion is relatively weak. In fact, weakening of cell-cell junctions might also be a secondary effect of cytoskeletal remodeling and 'collapse' that is elicited by semaphorins. Nevertheless, recent findings suggesting that SEMA3A and SEMA3C regulate E-cadherin and integrin expression at the transcriptional level (Herman and Meadows, 2007) deserve further investigation owing to their potential implications in cancer progression. The downregulation of E-cadherin and the disassembly of cell-cell junctions, together with changes in integrin activity and the acquisition of a migratory phenotype, are key events in a process known as epithelial-mesenchymal transition (EMT) (Thiery and Sleeman, 2006). Dysregulated activation of EMT is typically observed in carcinomas and it is often associated with progression to malignancy (Nishimura and Sasaki, 2008; Turley et al., 2008). Notably, the gene encoding E-cadherin is one of the main targets of transcriptional regulators of EMT, and might be downregulated at both the mRNA and the protein level (as a result of changes in subcellular distribution, internalization and degradation). In this case, it is tempting to speculate that R-Ras inactivation that is mediated by plexins might repress EMT by inhibiting cell-substrate adhesion (and by increasing the expression of cell-adhesion molecules). However, it is also possible that semaphorin-mediated activation of alternative pathways (such as those that involve tyrosine kinases) leads to increased integrin-dependent adhesion, loss of cell-cell-adhesion complexes and the acquisition of a migratory and/or invasive phenotype. Thus, the reported effects of semaphorins on E-cadherin and integrins might mean that semaphorins are multi-functional regulators of EMT, which could explain some of their described anti-tumorigenic, pro-invasive and/or pro-metastatic activities.

Cell migration

During cancer progression, tumor cells can migrate and invade surrounding tissues. For this to occur, strong cell-matrix adhesions need to be dissolved, whereas transient adhesions have to be formed at the leading edge. Integrins act at the 'feet' of migrating cells by mediating adhesion to the ECM (or to other cells) and by linking

it to the intracellular actin cytoskeleton (Ridley et al., 2003). Thus, by regulating integrin-mediated adhesion (or affecting integrin expression), semaphorins can regulate tumor-cell migration. Indeed, following plexin activation by semaphorins, integrin-based focal-adhesive structures are disassembled within minutes, followed by actin depolymerization and cytoskeletal remodeling, which can result in the extreme effect of cellular collapse (Barberis et al., 2004). In addition, plexin activation can hinder the formation of new adhesive complexes and block lamellipodia formation and directional migration by uncoupling cell-substrate adhesion from the cytoskeletal dynamics that are required for cell migration (Barberis et al., 2004).

Autocrine SEMA3A was found to reduce both the migratory and invasive behavior of breast tumor cells (Bachelder et al., 2003), possibly by stimulating the expression of $\alpha 2\beta 1$ integrin, a suppressor of metastatic breast-tumor growth (Pan et al., 2008). Similarly, the tumor-suppressor gene *SEMA3F* inhibits cell-spreading and migration both in breast carcinoma (Nasarre et al., 2003) and melanoma cells (Bielenberg et al., 2004), which correlates with reduced metastatic dissemination. Conversely, SEMA3C expression increases the motility and invasion of prostate cancer cells (Herman and Meadows, 2007).

A further mechanism through which class-3 semaphorins can regulate cell migration is by interfering with VEGF-mediated signaling. In human tumor cells, it was shown that cell migration is finely regulated by a balance between autocrine loops of SEMA3A and VEGF expression (Bachelder et al., 2003). As discussed above, it is still controversial whether both factors compete for a partly overlapping binding site on NP1 (Gu et al., 2002; Miao et al., 1999) or whether they trigger antagonistic downstream signaling pathways in tumor cells (Appleton et al., 2007; Liang et al., 2007; Pan et al., 2007).

Experimental evidence indicates that SEMA4D can inhibit or promote cell migration, depending on the receptor complexes that are expressed by the tumor cells. For instance, whereas plexin-B1 activation by SEMA4D can inhibit cell migration (Barberis et al., 2004; Swiercz et al., 2008), it was shown that the same receptor-ligand interaction can promote chemotaxis and sustain invasive growth, depending on whether plexin-B1 was associated with the oncogenic RTKs Met and ErbB2 (Conrotto et al., 2004; Giordano et al., 2002; Swiercz et al., 2008).

In summary, the behavior of cancer cells might be regulated by multiple semaphorins that are either produced by tumor cells in an autocrine manner or provided by non-cancerous cells that are recruited to the tumor microenvironment. Some semaphorin-mediated signals might inhibit rather than promote tumor growth and invasion, as many studies have shown that their expression is often lost during advanced cancer. Other semaphorins might promote tumor-cell survival, invasion and metastasis, and their expression might be upregulated to sustain tumor progression.

Semaphorins as regulators of cells in the tumor microenvironment

Tumors are a complex network of different cell types, and the full manifestation of their malignant potential depends on supporting signals from adjacent non-cancerous cells. In fact, tumor cells secrete many factors to regulate their own microenvironment, including multiple semaphorins that have the potential to act on different stromal cells. Importantly, the tumor microenvironment is complex and dynamic, and varies depending on the type of cancer. It can comprise resident fibroblasts, blood vessels and lymphatic

vessels, and can be infiltrated by bone-marrow-derived cells that are recruited from the circulation. Recent studies have shown that these cell types influence tumor progression to various degrees, depending on the tumor type. In the following section, we summarize the proposed role of tumor-cell-derived semaphorins in regulating different cell types of the tumor microenvironment (Fig. 4; Table 2).

Hematopoietic cells

Bone-marrow-derived cells that are recruited from the circulation represent a crucial component of the tumor microenvironment, in which they play complex roles in both inhibiting and promoting tumorigenesis (Dolcetti et al., 2008). The population of infiltrating

leukocytes can range from sparse myeloid cells to dense populations of neutrophils, monocytes and/or macrophages, dendritic cells and lymphocytes. T-cell infiltration of the tumor microenvironment might be beneficial for cancer patients (Leffers et al., 2008; Wakabayashi et al., 2003), yet it often fails to correlate with good prognosis. Conversely, an abundance of innate immune cells, including mast cells and macrophages, often correlates with angiogenesis and poor prognosis (Nonomura et al., 2007; Taskinen et al., 2008). On the basis of experimental evidence, it is currently thought that inflammation can either stimulate or inhibit cancer, depending on the composition of the infiltrating cell populations, the context and the stage of tumor development (Le Bitoux and Stamenkovic, 2008).

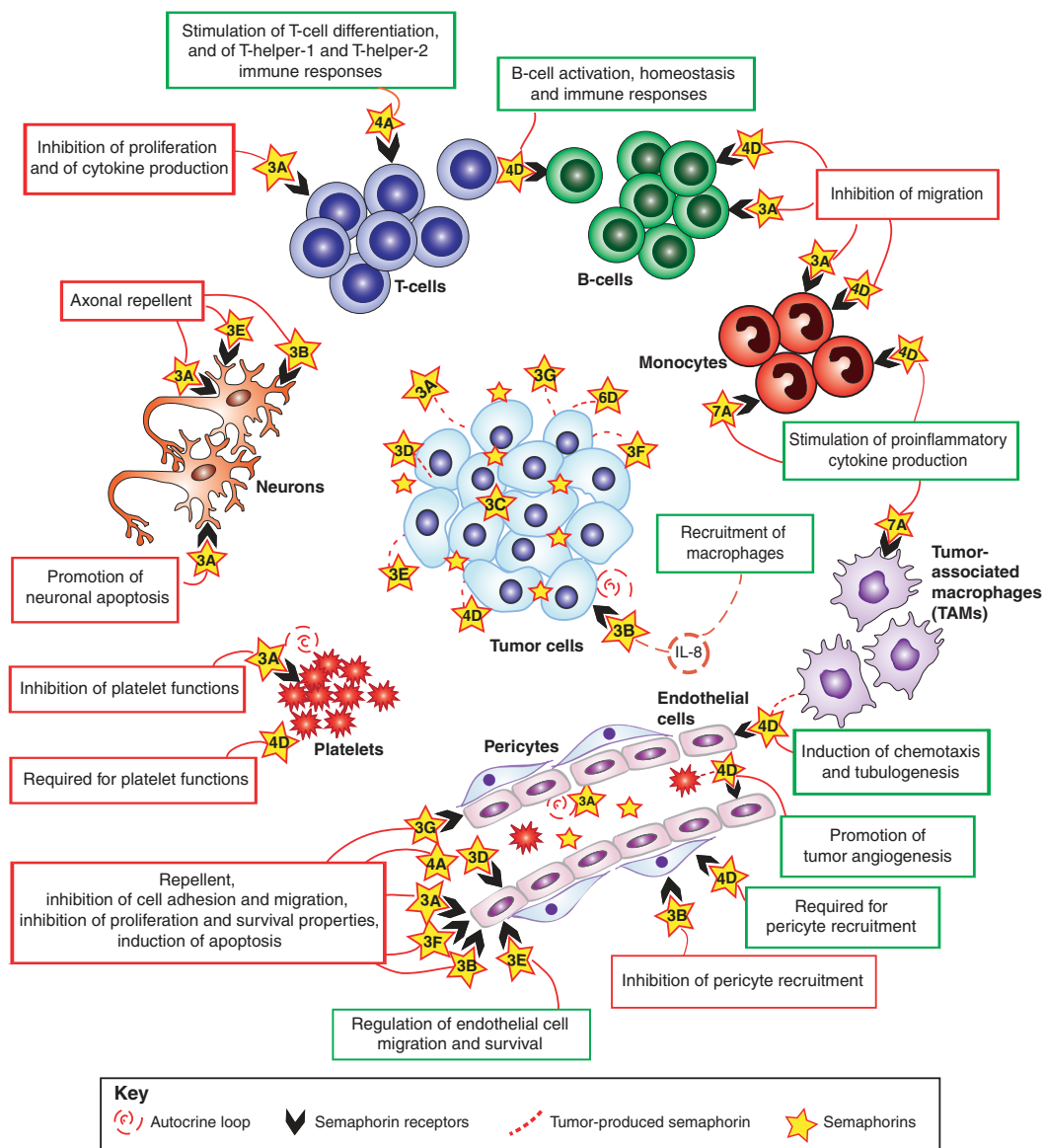


Fig. 4. Functional activity of semaphorins on cells of the tumor microenvironment. The diagram shows the reported functions of semaphorin signals that are secreted by tumor cells on different cell types found in the tumor microenvironment, including the effects on immune cells and endothelial cells. Consequently, semaphorins can positively or negatively regulate multiple important mechanisms underlying cancer progression, such as neo-angiogenesis, regulation of the immune response or neurogenesis. Accumulating data strongly suggest that stromal cells influence tumor progression to a varying degree, depending on tumor type. See main text for details.

Table 2. Semaphorin functional activities on cells in the tumor microenvironment

Semaphorin	Cell type	Functional effects	References
SEMA3A	Endothelial cells and blood vessels (in vivo)	Inhibits cell adhesion and migration; induces apoptosis; inhibits angiogenesis	(Acevedo et al., 2008; Guttman-Raviv et al., 2007; Kigel et al., 2008; Miao et al., 1999; Serini et al., 2003)
	Blood vessels (in vivo)	Increases vessel permeability	(Acevedo et al., 2008)
	T cells	Inhibits immune function	(Catalano et al., 2006)
	B cells	Inhibits cell migration	(Delaire et al., 2001)
	Platelets	Inhibits aggregation and adhesion	(Kashiwagi et al., 2005)
	Neuronal axons	Chemorepellent for axonal outgrowth in tumors; induces neuronal apoptosis	(Ben-Zvi et al., 2008; Luo et al., 1993; Vachkov et al., 2007)
SEMA3B	Endothelial cells	Anti-proliferative; pro-apoptotic; inhibits angiogenesis	(Castro-Rivera et al., 2004; Castro-Rivera et al., 2008; Varshavsky et al., 2008)
	Monocytes and macrophages	Stimulates recruitment (via IL-8)	(Rolny et al., 2008)
SEMA3C	Endothelial cells	Induces integrin-mediated adhesion	(Banu et al., 2006)
SEMA3D	Endothelial cells	Repellent; inhibits tumor angiogenesis	(Kigel et al., 2008)
SEMA3E	Endothelial cells	Induces chemotaxis	(Christensen et al., 2005)
	Endothelial cells	Repellent; inhibits tumor angiogenesis	(Gu et al., 2005; Kigel et al., 2008)
	Neuronal axons (different populations)	Chemoattractant or chemorepellent	(Chauvet et al., 2007)
SEMA3F	Endothelial cells	Repellent; induces apoptosis; inhibits angiogenesis	(Bielenberg et al., 2004; Guttman-Raviv et al., 2007; Kessler et al., 2004)
SEMA3G	Endothelial cells	Inhibits tumor angiogenesis in vivo	(Kigel et al., 2008)
SEMA4A	Endothelial cells	Inhibits migration, proliferation and angiogenesis	(Toyofuku et al., 2007)
SEMA4D	B cells	Mediates differentiation and antibody production	(Hall et al., 1996; Shi et al., 2000)
	Monocytes and macrophages	Stimulates production of pro-inflammatory cytokines; chemotaxis	(Kikutani and Kumanogoh, 2003; Sierra et al., 2008)
	Monocytes, B cells	Inhibits chemotaxis	(Delaire et al., 2001)
	Endothelial cells	Stimulates chemotaxis and angiogenesis	(Basile et al., 2004; Basile et al., 2005; Conrotto et al., 2005)
	Platelets	Required for aggregation and thrombus formation	(Zhu et al., 2007)
SEMA6A	Endothelial cells	Inhibits migration (SEMA6 recombinant extracellular domain)	(Dhanabal et al., 2005)
SEMA7A	Monocytes	Induces chemotaxis	(Holmes et al., 2002)
	T cells	Initiates T-cell pro-inflammatory responses	(Suzuki et al., 2007)

Tumor-associated macrophages

The presence of tumor-associated macrophages (TAMs) at the tumor site is one of the hallmarks of cancer-associated inflammation (Allavena et al., 2008; Biswas et al., 2008; Sica et al., 2008), and most experimental and clinical studies have associated these cells with cancer progression. TAMs originate from circulating monocytes and are key orchestrators of the smoldering inflammation of the tumor microenvironment. They produce several growth factors that stimulate epithelial and endothelial cell growth, as well as inflammatory cytokines and chemokines that contribute to tumor survival, proliferation and invasion. In addition, immunosuppressive mediators that are released by local inflammatory or tumor cells extinguish host-mediated anti-tumor responses and facilitate tumor progression (Sica et al., 2008).

Interestingly, the recruitment of TAMs into the tumor microenvironment has recently been associated with semaphorin signals. In fact, the expression of SEMA3B by cancer cells activates a NP-mediated signaling pathway that leads to an autocrine release of IL-8, which, in turn, recruits TAMs into the tumor microenvironment (Rolny et al., 2008). Thus, although it has been proposed to be a putative tumor suppressor, SEMA3B seems to trigger an escape program from growth inhibition, which is mediated by p38-dependent IL-8 production by tumor cells and the ensuing stromal response that promotes cancer progression and metastatic dissemination to the lungs (Rolny et al., 2008). Notably, it was also found that TAMs are a major source of SEMA4D in the tumor stroma, and their ability to produce SEMA4D proved to be crucial for tumor angiogenesis and vessel maturation (Sierra et al., 2008). Furthermore, SEMA4D has been shown to induce the production

of pro-inflammatory cytokines by monocytes, in support of the idea that SEMA4D has a multifaceted role in the inflammatory response (Kikutani and Kumanogoh, 2003). Finally, it has also been shown that the expression of SEMA7A on the surface of activated T cells can stimulate cytokine production by monocytes and macrophages by activating $\alpha 1\beta 1$ integrin (Suzuki et al., 2007).

Lymphocytes and dendritic cells

Immune cells represent a double-edged sword during tumorigenesis (Mantovani et al., 2008). Although many tumors are potentially immunogenic, they are known to 'escape' immunosurveillance by various mechanisms, and can 'educate' immune cells to support tumor-cell survival and proliferation (Chaput et al., 2008). Apparently, this tumor-induced immune 'privilege' not only allows primary-tumor outgrowth but also metastasis (Mantovani et al., 2008). Accumulating evidence indicates a role for semaphorins and plexins in the immune response, and at least five semaphorins (SEMA3A, SEMA4A, SEMA4D, SEMA6D and SEMA7A) and four plexins (plexins-A1, -A4, -B1 and -C1) have been found to be expressed by dendritic cells and lymphocytes (O'Connor and Ting, 2008; Suzuki et al., 2008).

It was shown that tumor-derived SEMA3A negatively modulates T-cell functions by inhibiting T-cell receptor (TCR)-mediated proliferation and cytokine production (Catalano et al., 2006; Lepelletier et al., 2006). Moreover, SEMA3A can trigger a pro-apoptotic program in T cells (which is often lost in leukemias) by sensitizing them to Fas (CD95)-induced apoptosis (Moretti et al., 2008). In agreement with this finding, plexin-A4 was detected in the receptor complex for class-3 semaphorins in T cells, dendritic

cells and macrophages, and was found to negatively regulate T-cell-mediated immune responses (Yamamoto et al., 2008). Moreover, SEMA3A and SEMA4D inhibit spontaneous and chemokine-induced migration of monocytes and B cells (Delaire et al., 2001). Conversely, the transmembrane semaphorin SEMA4A, through interactions with Tim2, appears to be involved both in T-cell priming (Kumanogoh et al., 2002), and T-helper-1 and T-helper-2 immune responses (Kumanogoh et al., 2005). Finally, the immune regulatory activity of GPI-anchored SEMA7A is still debated (Czopik et al., 2006; Holmes et al., 2002). As noted above, SEMA7A expression by activated T cells stimulates monocytes and/or macrophages to produce proinflammatory cytokines, thereby suggesting that SEMA7A is an important cue for the effector phase of the inflammatory immune response (Suzuki et al., 2007).

Collectively, these observations indicate that there are important roles for semaphorin signaling in modulating the activity of these immune-cell types during cancer progression. By inhibiting T-cell-mediated responses, semaphorins might help cancer cells to escape immune detection and clearance; moreover, by sustaining innate inflammatory responses, they might favor immune dysfunction in the tumor microenvironment and support cancer progression.

Platelets

Platelets are highly reactive components of the blood-circulatory system that exert not only hemostatic activity but also contribute to inflammation, cancer progression and metastasis, through several different mechanisms (Gupta and Massague, 2004). Notably, an increasing amount of evidence suggests that platelets play important roles in tumor angiogenesis; they are a rich source both of pro-angiogenic factors and of inhibitors of angiogenesis, and, by adhering to the endothelium, they can also facilitate the transmigration of pro-angiogenic cells to the extracellular space (Sierko and Wojtukiewicz, 2007). Furthermore, platelets tend to aggregate with circulating tumor cells, thereby facilitating immune evasion and the binding of tumor cells to microvascular endothelium (followed by extravasation and metastasis) (Borsig, 2008). Platelets might also contribute to the formation of fibrin clots that surround tumor cells, supporting the survival of these cells (Boccaccio and Comoglio, 2005).

Interestingly, semaphorins might be able to modulate platelet function. It has been shown that SEMA3A inhibits platelet aggregation, allowing speculation that, by keeping platelets in the resting state, endothelial-derived SEMA3A may contribute to maintaining blood flow in newly synthesized vessels (Kashiwagi et al., 2005). Conversely, SEMA4D is expressed by platelets and is required for their function (Zhu et al., 2007). Interestingly, following platelet aggregation, membrane-bound SEMA4D is proteolytically cleaved and shed (Zhu et al., 2007); it could then contribute to tumor-cell survival and tumor angiogenesis in the microenvironment by acting on adjacent endothelial cells.

Endothelial cells

Neo-angiogenesis, defined as the growth and development of new blood vessels, is a crucial step in cancer progression, as tumors have to establish a sufficient blood supply in order to grow and metastasize. New blood vessels in tumors can sprout from pre-existing vessels or grow by recruitment of rare, circulating bone-marrow-derived endothelial progenitor cells (Avraamides et al., 2008). Accumulating reports suggest that tumor cells, as well as macrophages and fibroblasts in the microenvironment, can secrete a wide range of semaphorins that can modulate blood-vessel development.

Many secreted semaphorins act as inhibitory signals for tumor angiogenesis. SEMA3A suppresses the adhesion and migration of endothelial cells (Miao et al., 1999; Serini et al., 2003), induces the collapse of the actin cytoskeleton, promotes apoptosis and inhibits angiogenesis *in vitro* (Guttman-Raviv et al., 2007). Furthermore, SEMA3A can inhibit angiogenesis *in vivo* (Acevedo et al., 2008; Guttman-Raviv et al., 2006; Guttman-Raviv et al., 2007; Varshavsky et al., 2008) and induce microvascular permeability (Acevedo et al., 2008). Similarly, SEMA3F repels endothelial cells and inhibits their adhesion, migration, proliferation and survival; moreover, the SEMA3F expression pattern correlates with large areas of diminished vascularity and apoptosis in experimental tumors (Bielenberg et al., 2008; Guttman-Raviv et al., 2007; Kessler et al., 2004). Moreover, melanoma-cell-derived SEMA3F has chemorepulsive activity for lymphatic endothelial cells and inhibits lymphangiogenesis and lymphatic metastasization *in vivo* (Bielenberg et al., 2004).

An emerging concept suggests that VEGF might physiologically induce the autocrine secretion of anti-angiogenic semaphorins in endothelial cells, as a negative-feedback loop, to restrain its own signaling and prevent unwarranted angiogenesis. For instance, it has been shown that the increased aggressiveness of multiple myeloma is associated with an angiogenic switch driven by an imbalanced secretion of VEGF165 and SEMA3A by endothelial cells (Vacca et al., 2006).

Similarly to SEMA3A and SEMA3F, three other class-3 semaphorins – SEMA3B, SEMA3D and SEMA3G – were also reported to repel endothelial cells and/or inhibit tumor angiogenesis (Kigel et al., 2008). Moreover, notable defects in the recruitment of pericytes to blood vessels have been observed in SEMA3B-expressing tumors (Charlotte Rolny and L.T., unpublished data). The anti-angiogenic function of SEMA3B might be partly balanced in tumors by its ability to increase the production of the angiogenic factor IL-8 (Rolny et al., 2008). Interestingly, it was also demonstrated that the production of SEMA3B by tumor cells fails to repel endothelial cells if they are subjected to the proteolytic activity of furin-like convertases (pro-protein convertases; PPCs) (Varshavsky et al., 2008). Thus, it seems that cleavage by PPCs that are produced by cancer cells might be an additional mechanism by which to curb the anti-angiogenic effects of SEMA3B.

Other class-3 semaphorins have been reported to regulate tumor angiogenesis in a different manner. For instance, SEMA3C was found to induce endothelial-cell growth and migration (Banu et al., 2006). Moreover, highly metastatic cancer cells often secrete SEMA3E, and its specific receptor, plexin-D1, is well expressed in vascular endothelium both during development and in tumor vessels (Christensen et al., 2005; Gu et al., 2005; Roodink et al., 2008). However, the function of SEMA3E in regulating angiogenesis is not clear, as different studies have reported either pro-migratory or inhibitory activities for this semaphorin in different types of endothelial cells (Christensen et al., 2005; Gu et al., 2005; Kigel et al., 2008). These discrepancies might be explained by the involvement of different receptor complexes. Interestingly, the generation of a smaller isoform of SEMA3E by furin-dependent proteolytic processing is required to reveal its chemotactic activity for endothelial cells (Christensen et al., 2005). Therefore, cancer cells might be able to convert this secreted inhibitory signal into a pro-angiogenic and pro-metastatic factor.

Of the membrane-bound semaphorins, SEMA4D has been reported to exert potent pro-angiogenic activity *in vitro* and in

vivo by binding to the high-affinity receptors plexin-B1 (Basile et al., 2004; Basile et al., 2005; Conrotto et al., 2005) and possibly plexin-B2 (Fazzari et al., 2007), which are expressed by endothelial cells. Notably, SEMA4D is not crucial for developmental angiogenesis. However, many human cancers express this semaphorin, which can be processed and released in a soluble form by the activity of membrane type 1 matrix metalloproteinase (MMP-1) and thereby induce endothelial-cell chemotaxis and blood-vessel growth in vivo (Sierra et al., 2008). Moreover, because recruited macrophages are a main source of SEMA4D in the microenvironment, experimental tumors grown in *sema4D*^{-/-} mice were found to contain smaller and aberrant vessels that were poorly lined with pericytes, and both cancer growth and metastases were strikingly reduced (Toyofuku et al., 2007). Conversely, SEMA4A was shown to suppress VEGF-dependent endothelial-cell migration, proliferation and angiogenesis by binding to the plexin-D1 receptor (Toyofuku et al., 2007); in addition, the recombinant extracellular domain of SEMA6A could inhibit tumor-induced angiogenesis in vivo (Dhanabal et al., 2005).

Nerve fibers

Semaphorins are well-known axon-guidance molecules that have been further implicated in axon branching, axon pruning and axon degeneration (He et al., 2002; Tran et al., 2007). In fact, although they have mainly been described as inhibitory signals, they can also promote neurite outgrowth and/or attraction. Examples are the soluble SEMA4D, a neurotrophic molecule that can enhance neurite outgrowth (Masuda et al., 2004), and SEMA3E, which can mediate attractant or repellent signals through the plexin-D1 receptor, depending on the 'gating' function played by the extracellular domain of NP1 (Chauvet et al., 2007). Interestingly, plexin-A3 has been recently described to mediate semaphorin-induced neuronal cell death (Ben-Zvi et al., 2008), supporting the idea that naturally occurring neuronal cell death might involve pro-apoptotic signaling mediated by semaphorins.

Interestingly, new evidence supports a crucial role for the neuroendocrine system in cancer progression; innervation of the tumor mass and the local release of neurotransmitters and hormones can potentially act as regulatory signals for multiple cell types in the tumor microenvironment (Entschladen et al., 2006). This process is bidirectional, as tumor cells can also release attracting, repelling and neurotrophic factors. Notably, whereas many class-3 semaphorins seem to inhibit axonal growth in the tumor microenvironment (Vachkov et al., 2007), SEMA4F has recently been implicated in prostate-cancer neurogenesis (Ayala et al., 2008). Thus, during tumor progression, the balance between the neurotrophic and restrictive cues that are mediated by semaphorins could impinge on tumor innervation. In this scenario, trophic signals that accompany neurogenesis might represent a metastatic impetus to tumor cells. It will be intriguing to explore further the suggested role of semaphorins in the process known as 'perineural invasion' (Entschladen et al., 2007). This is a common occurrence in some tumors (such as those in the prostate and pancreas) whereby cancer cells seem to migrate along the route of nerve fibers; this is putatively linked with invasion and cancer progression.

Concluding remarks and future perspectives

We have summarized here the current evidence indicating that semaphorins, plexins and NPs are important regulators of tumor

progression. Semaphorins are multifaceted signals that can be secreted by tumor cells and by other cell types in the tumor microenvironment, and that control the behavior of a broad range of cell types and thereby might affect tumor progression through multiple mechanisms. Therefore, further studies on the semaphorin-mediated crosstalk between tumor cells and tumor stroma are required for a better understanding of this scenario. It will be important to obtain further insights regarding the molecular mechanisms through which semaphorins regulate cancer-cell survival, proliferation and migration, and – on the other side – instruct the function of stromal cells in the tumor microenvironment. Moreover, the potential role of semaphorins in the regulation of EMT is an intriguing hypothesis that could be addressed in future studies. Finally, because our knowledge of semaphorin signaling pathways is still far from complete, further studies on the different transducers that are involved downstream of plexin activation in tumor cells will be necessary. This might allow the identification of molecular targets to develop pathway-selective inhibitors, and provide further support for the concept that interfering with semaphorin-mediated signals might be a promising anti-cancer strategy.

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