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review

To move or not to move?

Semaphorin signalling in cell migration

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Semaphorins were discovered 11 years ago as molecular cues for axon guidance that are conserved from invertebrates to humans. More than 20 semaphorin genes have been identified in mammals and their protein products are now known to be involved in a range of processes from the guidance of cell migration to the regulation of the immune response, angiogenesis and cancer. Plexins, either alone or in association with neuropilins, constitute high-affinity semaphorin receptors. However, other transmembrane molecules have been implicated in semaphorin receptor complexes, and interactions between plexins and a range of intracellular effectors have been reported. These data indicate that semaphorins might be able to elicit responses through more than one signalling pathway. Interestingly, according to recent findings, the semaphorin-dependent control of cell migration crucially involves integrin-based adhesive structures through which polarized cell-membrane protrusion is coupled to cytoskeletal dynamics. This review focuses on the mechanisms whereby semaphorins are thought to regulate cell migration.

Keywords: cancer; cell migration; integrins; plexin; semaphorin
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Introduction

Semaphorins are members of a large, highly conserved family of molecular signals that were identified initially through their role in axon guidance (Kolodkin *et al*, 1993; Luo *et al*, 1993), and later implicated in a range of functions from the guidance of cell migration and regulation of immune function, to angiogenesis and cancer (reviewed in Tamagnone & Comoglio, 2000). More than 20 mammalian semaphorin proteins are known and they are divided into seven subclasses according to their structural features. Vertebrate semaphorins in subclass 3 are secreted and are thought to form steep tissue gradients. Several other semaphorins are associated with the cell surface, either as transmembrane proteins (subclasses 4, 5 and 6) or through glycosylphosphatidylinositol (GPI) linkage (subclass 7). Therefore, semaphorins can mediate both long- and short-range (or contact-mediated) signals (Fig 1). Transmembrane semaphorins can also release a signalling-competent extracellular domain (Wang *et al*, 2001) or trigger 'reverse' signalling by functioning as receptors (Hall *et al*, 1996; Godenschwege *et al*, 2002). Moreover, some migrating cells and axons express both receptors and ligands on the cell surface (Winberg *et al*, 1998), or secrete semaphorins in an autocrine fashion (for example, see Serini *et al*, 2003; Catalano *et al*, 2004).

In vitro and *in vivo* experiments have implicated semaphorins in the guidance of elongating axons and dendrites, as well as in axon branching, axon pruning (Bagri *et al*, 2003) and axon degeneration (for a review of neuronal functions, see He *et al*, 2002). Furthermore, semaphorins act as guidance cues for a range of migrating cells. For example, they control oligodendrocyte migration (Spassky *et al*, 2002) and are potentially involved in the glial ensheathment of axons (Oster *et al*, 2003). The migration of neural crest cells is regulated by semaphorins (Eickholt *et al*, 1999), and defects in this process lead to the mispositioning of patterning cells in the sclerotome and in the developing cardiovascular system (Behar *et al*, 1996; Brown *et al*, 2001). Sema3A has a crucial role in regulating endothelial cell migration and angiogenesis (Miao *et al*, 1999; Serini *et al*, 2003; Shoji *et al*, 2003), as well as in the topographic congruence of nerves and blood vessels (Bates *et al*, 2003). Moreover, semaphorins regulate epithelial cell migration and morphogenesis (Fujii *et al*, 2002; Ginzburg *et al*, 2002; Giordano *et al*, 2002), and leukocyte migration (Delaire *et al*, 2001).

Semaphorins have been mainly described as inhibitory signals because they prevent cell migration and axon outgrowth, and lead to the 'collapse' of both pseudopodia and axonal growth cones. However, it has been shown that semaphorins can sometimes promote cell chemotaxis, and axon/dendrite outgrowth and attraction (for example, see Polleux *et al*, 2000; Giordano *et al*, 2002; Moreno-Flores *et al*, 2003; Pasterkamp *et al*, 2003). These opposing functional responses might entail signalling pathways that are mediated by different semaphorin receptor complexes, as discussed below. Furthermore, there is evidence that semaphorin function can be modulated by the intracellular levels of cyclic nucleotides, which convert a repellent into an attractive cue (Song *et al*, 1998; Castellani *et al*, 2002). This indicates that semaphorin signalling can be steered in different directions depending on the cross-talk between their receptors and

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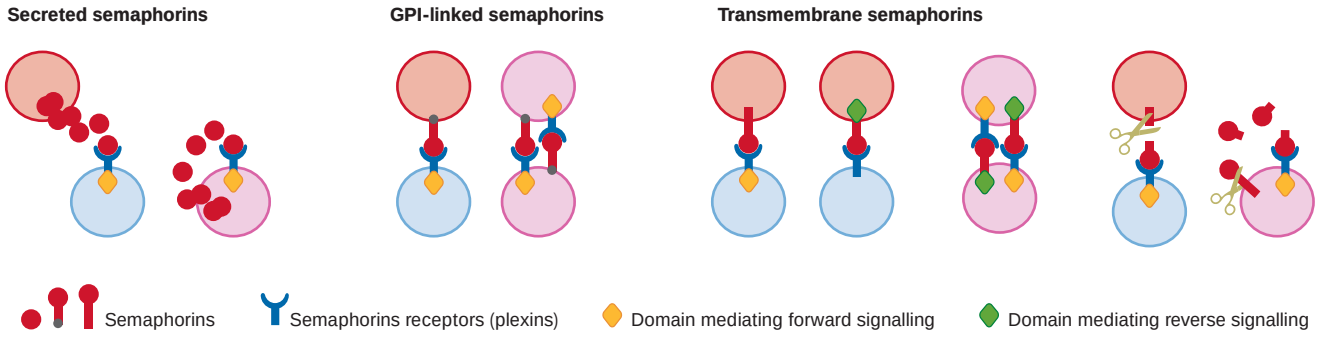

Fig 1 | Semaphorin signalling modes. Semaphorins and their receptors might be expressed on distinct cell populations (shown in red and blue, respectively) or be coexpressed (pink). Secreted semaphorins mediate either paracrine or autocrine signals. Apart from classical forward signals, transmembrane semaphorins could also trigger reverse signalling (mediated by their cytoplasmic domain) or be released into the extracellular space by proteolytic cleavage and so behave as secreted ligands.

other pathways. In fact, functional antagonism between semaphorins and neurotrophic/mitogenic factors, such as nerve growth factor (NGF; Atwal *et al*, 2003), heregulin (HRG; Barberis *et al*, 2004) and stromal-cell-derived factor 1 (SDF1; Chalasani *et al*, 2003), has been reported.

Semaphorin receptors and receptor complexes

High-affinity receptors for semaphorins have been identified. They include the plexins, which are a family of large transmembrane molecules that are conserved from invertebrates to humans, and the neuropilins (NP1 and NP2) that are found only in vertebrates (Tamagnone & Comoglio, 2000). Membrane-bound vertebrate semaphorins bind directly to plexins, whereas secreted semaphorins (class 3) also require neuropilins as obligate co-receptors. Several lines of evidence indicate that the cytoplasmic domain of plexins is required for semaphorin signalling, whereas the small cytosolic tail of neuropilins is dispensable. A recent study, however, revealed an independent functional role for the cytoplasmic tail of NP1, which is probably mediated through its PDZ (for PSD95, Discs-large and ZO1)-domain binding sequence (Wang *et al*, 2003).

Recently, two molecules that are unrelated to plexins or neuropilins, CD72 and Tim2, were found to interact functionally (although at low affinity) with transmembrane semaphorins in the immune system (Kumanogoh *et al*, 2000, 2002). Moreover, although GPI-linked Sema7A is known to bind to plexin-C1 (Tamagnone *et al*, 1999), it also has plexin-independent activity that is mediated by integrin-β1 (Pasterkamp *et al*, 2003).

Receptors on the plasma membrane often oligomerize in complexes, which allows for cross-talk between different signalling pathways. Semaphorin receptor complexes seem to be a good example of these interaction centres (Fig 2). In fact, as well as plexins and neuropilins, other transmembrane molecules are functionally coupled to semaphorin receptors, including cell-adhesion molecule L1 (Castellani *et al*, 2002), and the receptor-type tyrosine kinases off-track kinase (OTK; Winberg *et al*, 2001) and the hepatocyte growth-factor/scatter-factor receptor Met (Giordano *et al*, 2002). For instance, in cells that express a complex of plexin-B1 and Met, Sema4D can trigger Met activation and intracellular signalling (Giordano *et al*, 2002). This leads to a programme known as 'invasive growth', which is implicated in a range of morphogenetic processes from neurite outgrowth to branched tubulogenesis of epithelia, as well as in cancer invasion and metastasis (Trusolino & Comoglio, 2002). Furthermore, recent data show that other plexins of the B subfamily specifically associate with the scatter-factor receptors Met and Ron (Conrotto *et al*, 2004). Importantly, evidence indicates that semaphorins can induce different functional responses, depending on the signalling molecules that are found in the receptor complex. For example, Sema4D can mediate attraction through Met activation, whereas it inhibits cell adhesion and cell migration through plexin-specific and Met-independent signalling (Barberis *et al*, 2004). By analogy, Kikutani and co-workers have recently shown that, in response to its newly identified ligand Sema6D, plexin-A1 can alternatively mediate attractive or repellent cues in different cell populations, depending on its association with tyrosine kinase receptors of vascular endothelial growth factors (VEGFs) or with OTK (Toshihiko *et al*, 2004).

Neuropilins, in addition to binding secreted semaphorins, are also VEGF co-receptors (Soker *et al*, 1998; Gluzman-Poltorak *et al*, 2001) and are crucially required for vascular development (Kawasaki *et al*, 1999; Takashima *et al*, 2002). However, the mechanisms by which neuropilins switch between semaphorin and VEGF signalling are unclear. It has been shown that Sema3A competes with VEGF165 for binding to NP1 and that it inhibits VEGF-mediated function in endothelial cells (Miao *et al*, 1999), although recent data challenge the relevance of this regulation *in vivo* (Gu *et al*, 2003). Conversely, several experiments indicate that plexins have an important role in the functional response to secreted semaphorins in endothelial, epithelial and mesothelial cells (Bachelder *et al*, 2003; Serini *et al*, 2003; Catalano *et al*, 2004), which suggests that secreted semaphorins are more likely to regulate cell migration and angiogenesis through plexin-specific signalling than by inhibiting VEGF-receptor activation.

Mechanisms of semaphorin-mediated cell guidance

Semaphorins guide both axonal extension and cell migration. There are notable similarities (and some peculiarities) between the leading edge of a migrating cell and that of an extending axon, or the 'growth cone' (Dent & Gertler, 2003). We focus on the molecular mechanisms that are thought to mediate plexin function in cell migration. This is a complex process that is regulated at many levels (Ridley *et al*, 2003). To migrate, a cell must free its tethers to

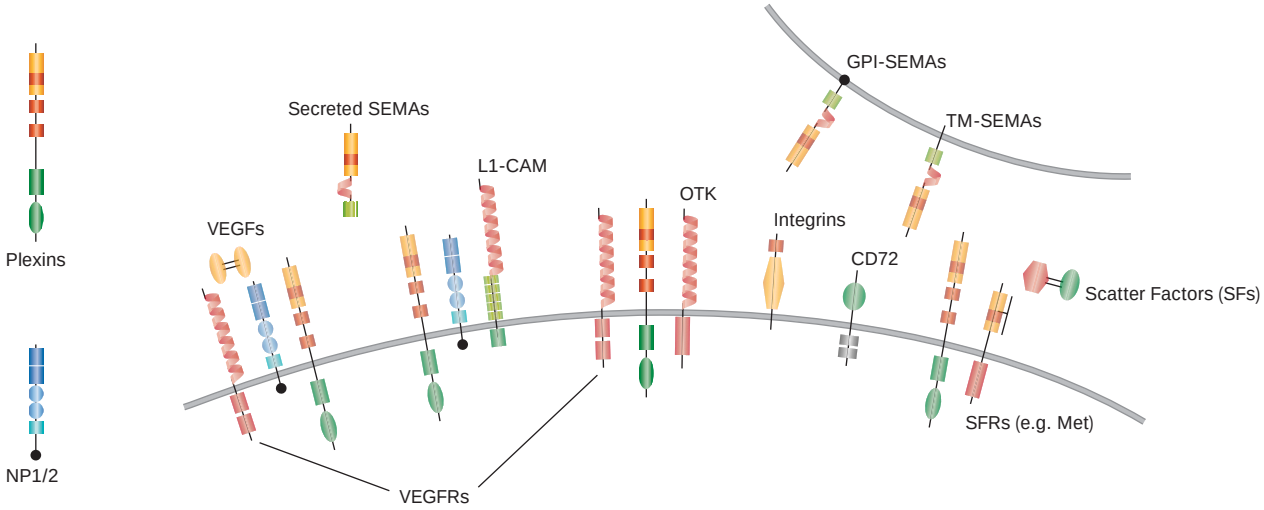

Fig 2 | Semaphorin receptor complexes. Plexins bind semaphorins (SEMAs) and can form receptor complexes with neuropilins 1 and 2 (NP1/2), with cell-adhesion molecule L1 (L1-CAM) and with receptor tyrosine kinases, such as off-track kinase (OTK), scatter-factor receptors (SFRs) and vascular endothelial growth factor receptors (VEGFRs). Neuropilins act as co-receptors for both secreted semaphorins and VEGFs. Integrins are receptors of extracellular matrix components, but integrin-β1 also mediates Sema7A activity. CD72 is a low affinity receptor for Sema4D. GPI, glycosylphosphatidylinositol; TM, transmembrane.

the extracellular matrix and sometimes to neighbouring cells (such as in epithelia). It must then form polarized cellular protrusions (filopodia and lamellipodia), which requires actin polymerization and new transient adhesive structures on the leading edge. According to the prevailing view, these focal complexes are privileged sites for Rac signalling and the polymerization of an actin meshwork, which pushes forward the leading edge so that it invades the surrounding tissue (Fig 3). Conversely, rear-edge retraction and cell-body translocation require myosin-mediated pulling on F-actin cables, which are anchored to stabilized focal adhesions behind the leading edge. This mechanism is triggered by Rho and Rho-dependent kinase (ROCK). Importantly, during cell migration, leading protrusions might retract owing to repelling signals or to the absence of permissive adhesive substrates and chemoattractant cues. Moreover, the absence of survival factors can abort cell migration via apoptosis. Intriguingly, semaphorins have been shown to mediate cell-to-cell repulsion, regulate cell–substrate adhesion and actin polymerization, induce retraction of cellular processes (a process often termed 'cellular collapse') and elicit cell apoptosis.

Although plexins must have a role in mediating these effects, the signalling mechanisms that are triggered by the large conserved cytoplasmic domain of these receptors are poorly understood. In fact, this sequence is not notably related to any other found in the databases and, although it bears limited similarity to GTPase-activating proteins (Rohm *et al*, 2000), there has been no report of any catalytic activity that is intrinsic to plexins. During the past two years, several potential semaphorin signal transducers have been identified on the basis of their association with plexins (for a review, see Pasterkamp & Kolodkin, 2003). However, the specific role of these molecules in semaphorin-mediated functions is still unclear.

The small GTPases of the Rho family are well-known regulators of cytoskeletal dynamics, cell migration and axon guidance, and several reports indicate that they have a role in semaphorin function. For example, plexins of the B subfamily can associate with GTP/GDP exchange molecules or Rho-GEFs, and induce Rho activation (for example, see Perrot *et al*, 2002; Swiercz *et al*, 2002). However, the functional role of the effector molecule ROCK in semaphorin signalling is debated (for example, see Jin & Strittmatter, 1997; Swiercz *et al*, 2002; Oinuma *et al*, 2003; Barberis *et al*, 2004).

Moreover, human plexin-B1 and fly plexin B, but not other family members, interact with activated Rac (Vikis *et al*, 2000; Driessens *et al*, 2001). It was suggested that these plexins sequester activated Rac and antagonize its signalling pathway (Hu *et al*, 2001; Vikis *et al*, 2002). However, other evidence indicates that Rac activity is required for semaphorin function, and possibly mediates actin rearrangement, membrane transport and endocytosis (Jin & Strittmatter, 1997; Fournier *et al*, 2000; Jurney *et al*, 2002; Vikis *et al*, 2002). Taken together, it seems that the available evidence does not reach a consensus on the mechanisms whereby Rho GTPases could mediate plexin signalling, and further experiments are required to more fully determine their role as regulators of semaphorin functions.

Two recent reports have shown that semaphorins and plexins regulate integrin function in cell–substrate adhesion and cell migration (Serini *et al*, 2003; Barberis *et al*, 2004). Serini and colleagues showed that Sema3A inhibits the adhesion of endothelial cells to the extracellular matrix (ECM) and impedes their directional motility, which could explain the aberrant vascularization that is observed in Sema3A-deficient mice. Moreover, we have shown that plexin signalling negatively regulates integrin-based adhesive complexes, which leads to the inhibition of substrate adhesion, lamellipodia extension and cell migration (Barberis *et al*, 2004). This study also indicates that the plexin-mediated disassembly of adhesive structures is responsible for the typical collapsing response that is observed *in vitro*.

Semaphorins, plexins and scatter-factor receptors all contain a sema domain, which is a conserved sequence of approximately 500 amino acids. Intriguingly, this domain and the extracellular domain of α-integrins have a similar structural motif, the

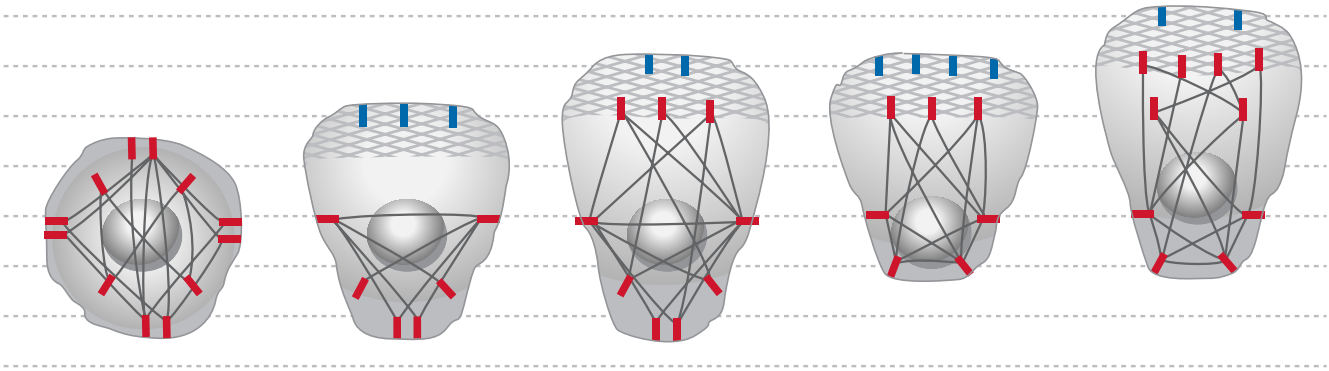

Fig 3 | Actin cytoskeleton and focal adhesive structures in lamellipodia extension and cell locomotion. F-actin cables are shown in black. Stabilized focal adhesions are indicated by red bars. The patterned areas at the leading edge indicate the membrane-pushing actin meshwork. Blue bars indicate newly formed transient focal complexes. The advancement of the leading edge correlates with the maturation of focal complexes into stabilized structures that are connected to F-actin cables.

β-propeller module, which is thought to act as a homo- and heterodimerization motif (Antipenko *et al*, 2003; Gherardi *et al*, 2003; Love *et al*, 2003). In addition, all sema domains are flanked by short, conserved cysteine-rich motifs (the Met-related sequence (MRS), also known as the plexin–semaphorin–integrin (PSI) domain) that are similar to sequences found in the extracellular domain of β-integrins (Bork *et al*, 1999). Until now, a direct interaction between integrins and plexins has not been reported, although their structural similarity could reflect the phylogenetic conservation of functional domains. For example, the extracellular domain of integrins is flexed in the inhibited conformation and straight in the active conformation. By analogy, it is proposed that the sema domain of plexins acts as an inhibitory moiety by steric hindrance, which is displaced on ligand binding (Takahashi & Strittmatter, 2001; Antipenko *et al*, 2003).

Furthermore, as mentioned earlier, the GPI-anchored semaphorin Sema7A activates integrin-β1 and mitogen-activated protein kinase (MAPK) signalling in a plexin-independent manner (Pasterkamp *et al*, 2003). This indicates that semaphorins can regulate integrin-mediated adhesion by at least two distinct mechanisms.

It is known that cell migration is inhibited by both lack of adhesion and the presence of stiff non-dynamic adhesive structures (Webb *et al*, 2002). In fact, signals that release cell-substrate adhesion are normally required to start cell migration, whereas sustained inhibition of integrin function blocks cell motility and eventually leads to the passive retraction of pseudopodia. By impinging on this delicate balance, semaphorins could potentially act both as permissive and as inhibitory cues for lamellipodia extension and cell migration. Moreover, as integrin signalling is required for cell survival and proliferation (Stupack & Cheresh, 2002), its sustained inhibition might account for the reduced growth and apoptotic events that are observed in semaphorin-treated cells (for example, see Tomizawa *et al*, 2001).

Semaphorins and cancer

The involvement of semaphorins in cancer progression is suggested by several reports. For example, the overexpression of secreted semaphorins Sema3E and Sema3C is associated with the invasive and metastatic behaviour of tumour cells (Yamada *et al*, 1997; Christensen *et al*, 1998). However, Sema3B and Sema3F are putative oncosuppressor genes that undergo gene deletion or promoter hypermethylation in human tumours (Tomizawa *et al*, 2001; Xiang *et al*, 2002; Kuroki *et al*, 2003). The mechanisms that mediate these opposing effects are largely unknown at present. They could depend on both cell-autonomous effects on tumour cell motility and cell survival, and on the paracrine regulation of the tumour environment, for example, neo-angiogenesis and leukocyte chemotaxis. As discussed above, secreted semaphorins might negatively regulate VEGF-receptor-mediated signalling by sequestering the shared neuropilin co-receptors or they could trigger plexin signalling to regulate cell adhesion and cell migration, and potentially induce apoptosis. For example, Sema3A inhibits endothelial cell migration and tumour cell growth *in vitro* in a neuropilin- and plexin-dependent manner (Serini *et al*, 2003; Catalano *et al*, 2004). In addition, recent studies have shown that VEGF can act as a survival and chemotactic factor for cancer cells in a VEGF-receptor-independent manner, probably by antagonizing the activity of semaphorins that is mediated by neuropilin/plexin complexes (Bachelder *et al*, 2003).

Conversely, membrane-bound semaphorin Sema4D (which is unable to bind neuropilins) can trigger the activation of the oncogenic receptor Met, which is associated with plexin-B1 on the cell surface. This confirms that semaphorins can regulate cancer progression both positively and negatively through distinctive pathways. Future studies will be required to elucidate the network of these molecular mechanisms, and to define whether specific semaphorins and semaphorin receptors should be regarded as promoters or suppressors of cancer progression.

Conclusions and future perspectives

Our understanding of the signalling pathways that are elicited by semaphorins is still incomplete. GTPases of the Rho family are candidate signal transducers of the plexins; however, evidence of the direct mechanisms through which they are involved is lacking. Recent findings indicate that plexin signalling regulates integrin-based adhesion, although the molecular mechanisms still need to be defined.

A few years ago, we proposed that semaphorins could guide cell migration through 'stop or go' signals in addition to their role in axon guidance. So far, we know that, by modulating integrin function and cytoskeletal dynamics in a site-specific manner, plexins can guide directional lamellipodia extension and cell motility. Moreover, recent evidence shows that plexins can couple to many other cell-surface signalling molecules. A better understanding of the mechanisms that regulate these interactions could be key to explaining the spectrum of functional responses that are mediated by semaphorins in different cells and tissues, including the control of such complex processes as tubular morphogenesis, angiogenesis, the immune response and the invasive growth of cancers.

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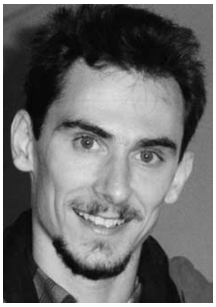

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