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Semaphorins and their receptors in lung cancer

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Abstract

Semaphorins are a large family of secreted, transmembrane and GPI-linked proteins initially characterized in the development of the nervous system and axonal guidance. Semaphorins are expressed in many tissues where they regulate normal development, organ morphogenesis, immunity and angiogenesis. They affect the cytoskeleton, actin filament organization, microtubules and cell adhesion. Semaphorin signaling is transduced by plexins, which in the case of most class-3 semaphorins requires high affinity neuropilin receptors. The neuropilins also function as receptors for VEGF and other growth factors, and their expression is often abnormal in tumors. In cancer, semaphorins have both tumor suppressor and tumor promoting functions. We review here the current status of semaphorins and their receptors in tumor development with a focus on lung cancer.

Keywords

semaphorin; neuropilin; plexin; VEGF; angiogenesis; lung cancer

1. Introduction

Shortly after semaphorins were identified as axon guidance molecules, their role in cancer development began to be understood. Knowledge about their receptors, signal transduction and functional roles has significantly evolved. Today, it is clear that several semaphorins and their receptors (neuropilins and plexins) participate in vascular development, angiogenesis, and cancer. Neuropilins, which are high-affinity receptors for class-3 semaphorins, also function as co-receptors for VEGF and other growth factors, and their expression is often abnormal in cancer. We review here the status of semaphorins and their receptors in signaling and tumor development with a focus on lung cancer. Attention will be placed on semaphorin-VEGF antagonism and semaphorin signaling as these molecular events likely account for the ability of specific semaphorins to affect tumorigenesis. Lastly, we discuss the therapeutic potential of this pathway in cancer.

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2. Lung cancer overview

Lung cancer ranks among the most common malignant diseases and currently is the leading cause of cancer-related death worldwide. In the United States, lung cancer is the most common cause of cancer-related deaths with an incidence approximating 70 per 100,000 individuals. In 2003, an estimated 171,900 Americans were diagnosed with lung cancer and approximately 152,200 succumbed to this disease. In the European Union, lung cancer accounts for one-third of all cancer-related deaths. In addition, lung cancer is rapidly emerging as a major cause of mortality in Asia and death rates are expected to substantially increase over the next decades. Unfortunately, the overall 5-year survival of patients is less than 15 % [1].

The major risk factor for lung cancer is tobacco exposure (90% of cases diagnosed). Exposure to other environmental respiratory carcinogens, such as asbestos, benzene, coal tar, and other industrial chemicals may interact with tobacco smoke to increase risk. The effect of low-energy-transfer radiation appears variable. Epidemiologic data suggest that lung cancer risk is associated with urbanization, and vehicle density is an excellent predictor of cancer mortality. It is assumed that 1% to 2% of lung cancers are directly attributable to air pollution. Although considerable attention has been given to household radon gas exposure, the risk remains controversial. In addition, several genetic and non-genetic defects are associated with this disease and have identified familial clusters and populations at increased risk for lung cancer.

Lung cancers are divided into 2 main classes depending on their histologic appearance and presumed cellular origin. Small Cell Lung Cancers (SCLCs) are of neuroendocrine origin, while Non-Small Cell Lung Cancers (NSCLC) are predominantly epithelial. NSCLCs include adenocarcinoma (now the dominant cell type), bronchioalveolar (BAC), squamous and large-cell carcinoma (which has neuroendocrine features).

The distinction between SCLC and NSCLC has a major therapeutic implication. While surgical resection remains the standard of care for localized NSCLC, with ongoing international attempts to improve early diagnosis, SCLC is not routinely treated with surgery even when the disease appears localized to the chest. This is because nearly all patients subsequently relapse with metastatic disease. For patients with metastatic disease and either NSCLC or SCLC, combination chemotherapy is the mainstay of treatment, with radiation used for local problematic areas such as a brain lesion or bone metastasis that is painful or prone to fracture. For SCLC, a platinum-based combination (doublet) such as cis-platinum plus etoposide would be considered standard of care - see National Comprehensive Cancer Network (http://www.nccn.org/professionals/physician_gls/f_guidelines.asp). For advanced NSCLC, a platinum-based doublet is standard of care with bevacizumab suggested for patients without squamous histology, brain metastasis or those on anti-coagulants, all of which are associated with an unacceptable risk of fatal hemorrhage. Following disease progression, patients generally receive either a taxane (e.g., Docetaxol, which acts by stabilizing microtubules), Pemetrexed (an antifolate that specifically inhibits thymidylate synthase), or an EGFR inhibitor (Erlotinib/Tarceva). Of potential interest, class-3 semaphorins impair microtubule assembly. Whether there might be antagonism between the semaphorin and taxane effects has not been investigated. The use of EGFR inhibitors is particularly relevant in adenocarcinomas and bronchioloalveolar carcinomas, which have an increased frequency of EGFR activating mutations or increased copy number [2,3].

A number of genetic and epigenetic molecular lesions are necessary to transform normal bronchial epithelial cells to overt lung cancer. SCLCs and NSCLCs present distinct cytogenetic or expression profiles, as depicted in Table 1, adapted from [4]. These include mutations in oncogenes and tumor suppressor genes (TSG), aberrant DNA methylation, overexpression of growth factors and growth factors receptors, association with metabolic phenotypes affecting

P450 family genes, Glutathione S-transferase genes (GSTM1), Aryl hydrocarbon hydroxylase (AHH), NAD(P)H:Quinone Oxidoreductase (NQO1), genetic variation in metabolism of carcinogens, DNA damage or repair and genomic instability. Sequential events are depicted in Figure 1 and 3p allele loss is currently the earliest known change, suggesting that either a 3p TSG may act as a "gatekeeper" for lung cancer or that 3p alterations are a particularly sensitive marker of genomic instability. Interestingly, several genes involved as guidance cues are localized in 3p (Table 2), i.e. semaphorins (SEMA3B [5], SEMA3F [6,7], SEMA3G), plexin B1 (a semaphorin receptor), and Robo1 (a SLIT receptor) [8]. Because these guidance molecules act in general to regulate cell migration, adhesion (and invasion of cancer cells), we suspect they are involved in mechanisms of disease progression in lung cancer development.

3. The semaphorin family

Semaphorins, initially named collapsins, belong to a large family of about 30 proteins found in multi-cellular organisms ranging from worms to flies, fish and mammals. They are not present in unicellular eukaryotes or procaryotes, although do occur in a few viruses. Semaphorins have been divided into eight classes based on structural features, with classes 3 to 7 representing the vertebrate proteins (Fig. 2; for recent reviews see [9,10]). The hallmark of semaphorins is the sema domain, an approximately 500 amino acid, highly disulfide-linked, segment. This domain is shared with receptors of the plexin and Met/Ron families [11]. Other recurrent domains are the PSI domain ("plexin-semaphorin-integrin") and an immunoglobulin-type domain. In contrast, semaphorins diverge in their C termini, and can be either transmembranous, anchored to the plasma membrane or exclusively secreted. Proteolytic processing converts membrane-associated semaphorins to diffusible forms [12,13], and their localization to the extracellular matrix might be additionally controlled by proteoglycan binding [14,15]. Class-3 semaphorins (SEMA3s) can be further processed by furin-like cleavage with large effects on biological activity. Sema3A cleavage is essential but not sufficient for the acquisition of high repulsive activity for neuritis [16] and furin-dependent cleavage of Sema3E promotes induction of invasive growth and lung metastasis *in vivo*, and stimulates growth and motility *in vitro* [17]. This complexity has often been ignored in most studies.

4. Semaphorin receptors and signaling

4.1 A multiple receptor-ligand system

Several receptors function to transduce semaphorin stimuli (Table 3). For brevity, atypical receptors of the immune system are not presented (for reviews see [18–20]). Most semaphorins directly bind to plexins which elicit intracellular signals [21]. However, secreted SEMA3s are recruited to plexins by neuropilin (NRP) receptors [22,23] (Fig. 3). Two recent reviews describe NRP structure and function [24,25]. Due to their short cytoplasmic segment, neuropilins generally serve as binding subunits. However, their three C-terminal amino acids (SEA) are essential for endothelial cell migration [26]. NRP1 preferentially binds SEMA3A, SEMA3B and SEMA3E, whereas NRP2 has higher affinity for SEMA3F and SEMA3G [9]. The sema domain of SEMA3s binds the NRP a1a2 domain, while the PSI/Ig/basic domains bind the NRP b1b2 region [27]. Alternatively, some semaphorins and plexins may interact directly through their sema domains [28] and SEMA7A has been reported to directly bind to beta-1 integrins [29].

Neuropilins, plexins and semaphorins form complexes with other membrane-associated proteins including receptors and cell adhesion molecules. Neuropilins are co-receptors for vascular endothelial growth factor VEGF-A, VEGF-B, VEGF-C, VEGF-D and the viral ORFV2-VEGF (Fig. 3) [30–34] as they form complexes with VEGFR1 (Flt1), VEGFR2 (KDR) and VEGFR3 [24,35]. Multimerization of NRPs and VEGFRs is probably mediated

by VEGF binding to both receptors [33,36]. In this complex, NRPs may increase VEGF affinity toward VEGFRs [30], although this issue is still under investigation [37].

Multiple VEGF-A isoforms are generated by alternative splicing with $VEGF_{165}$ being the best studied [35]. Originally, only the heparin binding forms (e.g. with exons 6–7) of VEGF-A were believed to bind NRPs [30]. However, it was recently shown that $VEGF_{121}$ that does not contain exons 6–7, also binds NRPs, although it does not promote NRP-VEGFR interaction [36,38, 39]. VEGF-A binding to NRP might be due to the exon 8 C-terminus KPR motif, which inserts into a negative cleft in the NRP b1 domain (Fig. 4A) [40,41]. Consistent with this, a variant of VEGF lacking exon 8, $VEGF_{165b}$, acts as an endogenous inhibitor of angiogenesis [42]. Because the carboxyl tip of SEMA3s is basic, it was suspected that it might also bind the NRP b1 domain [43], which would support the hypothesis that SEMA3s are competitive inhibitors of VEGF binding to NRPs [44]. Interestingly, among class-3 semaphorins, SEMA3F possesses a KPR motif that overlaps a furin cleavage site (Fig. 4B). We can only speculate that this motif might be involved in SEMA3F binding to NRP b1 groove and that furin cleavage would regulate this binding. Although a recent structural report indicated that VEGF and SEMA3s may have distinct binding sites on NRPs [45], this study did not address the proteolytically processed forms of SEMA3s.

Other NRP ligands have been described including placenta growth factor 2 (PlGF-2) [46], fibroblast growth factors (FGF) [47], galectin [48], hepatocyte growth factor (HGF) [49–51] and TGF-β [52]. In addition, NRP1 interacts with c-Met [50]. There is further complexity in semaphorin receptor complexes. For instance, SEMA3E binds D1-plexin independently of NRPs [53]. More recent work indicates that NRP1 may participate in the SEMA3E-receptor complex, but when so switches its effects from repulsion to attraction [54]. Similar switches have been reported for Sema3A when the cell adhesion molecule L1-CAM is associated with NRP1 [55], and for Sema3B and Sema3F when Nr-CAM associates with NRP2 [56]. Also, CLCP1 (a NRP-related protein) is a receptor for the transmembrane SEMA4B [57]. It is thus possible that NRPs are part of larger complexes containing multiple components such as receptors and adhesion molecules. NRPs contribute to multiple relevant growth factor receptors. Whether semaphorins and other growth factors compete or have distinct binding sites is important from a potential therapeutic standpoint (see below). The interactions of semaphorins with the HGF and EGF pathways are particularly interesting with regard to lung cancer, given that both pathways are altered in this pathology.

4.2 Signaling from plexins

It is important to understand the molecular events mediated by plexins, the canonical semaphorin receptors, since these are likely responsible for the role of semaphorins in tumor development.

Sema3A and Sema3F inhibit cell attachment and cell migration (for review see [58,59]. At least in part, this results from signaling changes that affect the activation or stabilization of surface integrins. Depending on the semaphorin, plexin, and the cellular context, distinct molecular cascades can be triggered with the recurrent participation of small GTPases.

One pathway, thought common to all plexins involves FARP2, R-Ras and talin in which semaphorin binding leads to a conformational change in integrins (Fig. 5A) [60,61]. Indeed, Sema3A and Sema3F cause downregulation of activated integrins due in part to inhibition of R-Ras. Integrin inhibition by SEMA3A and 3F could explain both endothelial cell and tumor cell migration blockade, leading to reduced tumor angiogenesis and metastasis.

The capacity of semaphorins to affect cell migration appears to extend beyond integrins. In the Fer/CRMP2 (collapsin response mediator protein) pathway, activation of type A-plexins by

SEMA3s induces phosphorylation of CRMP2 [62] which hinders its tubulin binding abilities and interferes with cell migration (Fig. 5B). To our knowledge, this pathway has not been studied in tumor cells.

Type B-plexins also regulate the cytoskeleton organization in a tumor context (Fig. 6). B-Plexin stimulation by SEMA4D can inhibit cell migration in the presence of MET by deactivating RhoA, while the presence of ErbB2 results in opposite effect [63]. Of note, p190RhoGAP can also bind A-plexins [64]. Thus, semaphorins exhibit diverse roles in controlling cell migration through the converging processes of regulating integrin activation and reshaping the cytoskeleton. Therefore, the regulation of migration of endothelial cells and cancer cells by semaphorins might be essential in tumor progression.

5. Semaphorins and Neuropilins at the heart of vascular development and angiogenesis

As VEGF receptors, NRPs are essential elements in cardiovascular development and tumor angiogenesis (for review see [24]). Both NRP1 and NRP2 are detected in human umbilical vein endothelial cells (HUVECs). In mice, NRP2 is restricted to veins and lymphatic vessels, while NRP1 is found in arteries and capillaries [65]. NRP1 overexpression in mice results in disproportionate blood vessels and heart defects [66]. In contrast, deficiency of NRP1 induces severe disorganization of vascular networks and agenesis [67], and absence of NRP2 leads to reduction of lymphatic vessels [68]. Targeting both NRPs results in greater vascular defects and death [69].

It was initially suspected that SEMA3s may control angiogenesis by competing for binding to NRP with VEGF. During vascular development, SEMA3s repulse vessels between somites in which they are expressed. In zebrafish, Sema3a1 and 2 function with D1-plexin [70,71]. However, increasing data indicate that the effects of semaphorins and VEGF may be independent. For example, the use of a NRP1 engineered to bind solely VEGF, and not SEMA3s, has revealed that SEMA3s have little effect on VEGF/NRP1-driven vascular development [72]. Moreover, Sema3A and Sema3F-induced ERK1/2 inhibition are unrelated to the ability of VEGF to induce VEGFR2 phosphorylation [73].Thus, a major consequence of SEMA3s in angiogenesis may derive from their ability to inhibit integrin activation [74]. In contrast, Sema3C enhances adhesion of endothelial cells (ECs) *in vitro* [75]. Thus, as often occurs with semaphorins, regulation appears to be context-dependent.

The capacity of semaphorins to regulate vessel patterning is particularly relevant to cancer since tumor neoangiogenesis is a requisite for tumor growth and metastasis. In *in vitro* and xenograft experiments, Sema4D was shown to promote experimental angiogenesis by B1-plexin-expressing endothelial cells, both in the presence or absence of Met [76]. The regulation of angiogenesis by SEMA4D was subsequently confirmed in human head and neck cancer tissues [77]. In tumor cells, Sema4D was shown to be a novel target of MT1-MMP matrix-metalloproteinase, which processes and releases soluble Sema4D thereby inducing endothelial cell chemotaxis *in vitro* and tumor-angiogenesis *in vivo* [76]. In contrast, the SEMA6A extracellular domain inhibits $VEGF_{165}$-mediated xenograft vascularization [78]. Similarly, SEMA3F impairs angiogenesis *in vivo* in various xenograft tumor models [79–82].

6. Semaphorins and their receptors in normal lung

Like vessels and nerves, the lung develops by successive branching, generating a complex architecture ending in bronchioles. Correct development requires cell migration and proliferation, with high coordination between epithelial and mesenchymal cells. Notably, EGF and FGF positively regulate proliferation. The lung is also highly vascularized, and

semaphorins and their receptors have been detected in lung tissues. NRP1, NRP2 and A1-plexin are temporally and spatially regulated during mouse lung development, together with Sema3A, 3C and 3F [22,23,27,83,84]. In mouse lung explants grown *ex vivo*, Sema3A inhibits branching whereas Sema3C and Sema3F promote it [83,84]. Localized in the mesenchyme, Sema3A may maintain a low arborescence, allowing the principal branches to form. Later in development, Sema3A expression decreases while NRP1 levels rise, suggesting a switch in NRP1 function from Sema3A receptor to VEGF receptor. In the epithelium, Sema3C and Sema3F might influence the formation of terminal buds. In adult human lungs, SEMA3F mRNA is expressed [5–7] and the protein is found at the membrane of type-II pneumocytes and in endothelial cells of large vessels [85]. In contrast, SEMA3B was barely detected by northern-blot in adult normal lung [5].

7. Semaphorins in lung cancer

7.1 SEMA3B and SEMA3F

In lung cancer, one of the earliest and common genetic change is chromosome 3p deletion. The first hypothesis implicating semaphorins in lung cancer came from the cloning of two semaphorin genes, *SEMA3B* and *SEMA3F*, from a 3p21.3 homozygous deletion region in SCLC cell lines (Table 1) [5–7]. In human lung and breast cancers, this region undergoes frequent loss of heterozygosity (LOH) and both *SEMA3F* and *SEMA3B* transcripts are underrepresented in squamous cell carcinomas [86].

It was shown that *SEMA3B* transfection reduces the growth of lung cancer cells *in vitro* and in subcutaneous mouse models [87,88]. I*n vitro*, SEMA3B induces apoptosis of lung and breast cancer cells, while VEGF has opposite effects [89]. In addition, SEMA3B was lost in a metastatic variant of the NSCLC cell line, NCI-H460, compared to the less metastatic parental cell line [90]. No inactivating mutation has been detected, but a single nucleotide alteration (T415I) leads to a reduced ability to suppress tumorigenesis *in vitro* [87]. Curiously, the T415I substitution is associated with a reduced risk of lung cancer in latino-americans in the USA [91]. Moreover, SEMA3B downregulation is sustained by gene hypermethylation in lung cancer cell lines [87,92,93]. Also, *SEMA3B* is the target of the tumor suppressor, p53 [94], suggesting it could be activated during DNA damage or other stress responses. However, Rolny *et al*, [95] recently found in xenograft tumor models with MDA-MB435 melanoma and A549 lung cancer cells that SEMA3B inhibited tumor growth while simultaneously and unexpectedly triggering metastasis by activating the p38 MAPK. Although p38 activated p21, a cell cycle inhibitor, it also induced IL-8 cytokine secretion from tumor cells. The consequence was macrophage infiltration which is thought to spur metastasis by producing soluble factors such as VEGF.

The second semaphorin gene mapped in 3p21.3, *SEMA3F* (previously *SEMAIV*, [6,96] has been studied for its *in vivo* tumor suppressor function. The first evidence came from transfection of a mouse tumor cell line by a 80 Kb genomic clone containing *SEMA3F* (and additional sequences) that suppressed tumor growth [97]. In human lung cancer, while SEMA3F is expressed in normal lung, the protein is lost or delocalized in the cytoplasm of tumor cells [85]. Moreover, its loss correlates inversely with the grade and stage of lung cancer, and also with the tumor surface staining of $VEGF_{165}$ [85,98]. Similarly in ovarian cancer, an elevated VEGF/SEMA3 ratio is a poor prognostic feature [99].

SEMA3F potently inhibits tumorigenesis in several xenograft cancer models induced by ovarian cancer [100], melanoma [79], lung cancer [81,82,101], murine fibrosarcoma [100] and HEK293 cells [80]. One recurrent observation has been that tumors formed by SEMA3F-expressing cells display reduced vascularization [79–82]. *In vitro*, SEMA3F secretion by transfected tumor cells repels endothelial cells (ECs) [79]. SEMA3F also inhibits VEGF and

FGF-induced ERK1/2 activation and EC proliferation [80]. Similarly, SEMA3F repels breast cancer cells [102] and has an antagonistic effect on breast cancer cells spreading induced by VEGF [103].

Consistent with the inhibition of integrin by plexin signaling, reduced beta-1 or beta-3 integrin activation was found in both melanoma cells and H157 lung cancer cells, along with reduced adhesion to fibronectin and vitronectin [79,82,101]. Additional signaling changes in H157 lung cancer cells included loss of activated ERK1/2, AKT and STAT3 with downstream inhibition of HIF1α translation and VEGF mRNA expression [82,101]. Mechanistically, SEMA3F inhibited the activity of integrin-linked kinase (ILK) although this appeared to account only for the loss of phospho-ERK1/2. Thus, SEMA3F has emerged as a potent tumor suppressor and antagonist of VEGF-driven tumor neovascularization. Interestingly, reduction of VEGF transcription was also observed after treatment of myeloma cells by Sema3A, suggesting that this effect might not be restricted to SEMA3F [104].

To date, no inactivating mutation of *SEMA3F* has been reported, although its promoter is the target of DNA hypermethylation in lung and breast cancer cell lines. Treatment with a histone deacetylase inhibitor alone can restore SEMA3F expression [105]. *SEMA3F* can be turned off by direct binding of the transcriptional repressor Zeb-1 (J. Clarhaut, personal communication), a factor involved in the epithelial-mesenchymal transition in lung cancer cells and loss of E-cadherin [106]. Like SEMA3B, SEMA3F is also a transcriptional target of p53 in lung cancer cells [81].

7.2 SEMA6A

There are few data concerning SEMA6A in lung cancer, except that *SEMA6A* is located in 5q23.1 (Table 2), a region that can be deleted in lung cancer [107]. Interestingly, SEMA6A is moderately expressed in the lung, but not expressed in the lung adenocarcinoma cell line A549 [78,108]. In kidney cancers, SEMA6A is more expressed in tumor tissues than in adjacent normal tissues and might contribute to tumor angiogenesis. However, treatment of ECs with the extracellular domain (ECD) of SEMA6A reduces VEGF-promoted migration *in vitro*, and inhibited ERK1/2, Src and focal adhesion kinase (FAK) phosphorylation [78]. This was independent of VEGFR2 phosphorylation status, and SEMA6A-ECD did not bind NRP1/2. *In vivo*, the SEMA6A-ECD inhibited tumor formation induced by 786-0 kidney cancer cells and VEGF-induced xenograft vascularization.

7.3 Other semaphorins

In lung adenocarcinoma, SEMA3C was reported to be upregulated in metastatic cells [109]. SEMA4B has been described as a migration and metastasis-promoting factor in the H460 NSCLC cell line [57] and can bind a homolog of NRPs, CLCP1, which was overexpressed in a metastatic variant of H460 cells [110]. SEMA4D, and its receptor B1-plexin, are highly expressed in head and neck tumors and lung carcinomas [77]. Although B1-plexin expression was detected in non-malignant bronchial epithelium of adult lungs, SEMA4D was not [111]. When released from the tumor cell membrane by a protease, SEMA4D stimulates EC chemotaxis and may therefore promote tumor angiogenesis [77]. Semaphorins implicated in other cancers are listed in Table 4.

8. Neuropilins and other semaphorin-related proteins in lung cancer

The importance of NRPs in cancer has been presented in recent excellent reviews [24,25,35, 112–114]. In brief, NRPs are frequently overexpressed and often associated with poor prognosis or advanced disease. In ECs, NRP1 and VEGFR2 stimulate PI3K activation [115]. Additionally, VEGF appears to be an autocrine survival factor for NRP-positive tumor cells

[116,117]. In lung cancer, high levels of NRP1 were correlated with shorter disease-free and overall survival [118]. In another study, co-expression of NRP1 and NRP2 were associated with increased tumor vascularization and poor prognosis [119]. Compared to benign bronchial hyperplasia and squamous metaplasia, progressive upregulation of NRP levels was observed in pre-malignant lesions ranging from dysplasia to microinvasive carcinoma [98].

In lung cancer, loss of the semaphorin effector, CRMP1, has been associated with a more aggressive phenotype [120]. Recent evidence indicates that plexins may participate in tumor progression as well [77,121–123]. Another important molecule in cell migration and oncogenesis is MET which functions with B-plexins in invasive growth [124] while its ligand HGF binds NRPs [51].

9. The semaphorin pathway as a target for cancer treatment

Semaphorins and their receptors have now emerged as key components in tumor development or progression. NRPs have been viewed as VEGF co-receptors and this has led to different VEGF-inhibiting strategies, such as VEGF or NRP-blocking antibodies, NRP-blocking peptides, and NRP soluble forms [24]. A signaling inhibitor of both VEGF and SEMA3A has even been made [125].

In an animal model, injection of anti-NRP antibodies resulted in inhibition of tumor angiogenesis, which showed increased activity when combined with an anti-VEGF antibody [126,127]. In breast cancer cells, a peptide matching the VEGF binding site of NRPs induced apoptosis [128]. Two other peptides that block VEGF-mediated NRP1 signaling have shown tumor inhibitory properties [118]. A peptide (ATWLPPR) specifically blocking the VEGF exon 8 binding to the NRP1 b1 domain has been tested with promising results [40,41,129, 130]. Peptides blocking NRP1 dimerization and function have also been used in the nervous system [131].

Another strategy to block NRP1 is to induce its internalization. As recently shown [132], sulfated polysaccharides like dextran sulfate and fucoidan reduce endothelial cell surface levels of NRP1, NRP2 and, to a lesser extent, VEGFR-1 and VEGFR-2, and block the binding and function of SEMA3A and $VEGF_{165}$. These compounds appear to bridge the extracellular domain of NRP1 to the scavenger receptor SREC-I and promote their internalization to lysosomes.

Most therapeutic strategies have focused on NRP1 as less is known about the biological properties of NRP2. Recently, it was reported that NRP2 plays a critical role in colorectal cancer development and may represent a potential therapeutic target [133]. Indeed, NRP2 silencing rendered colon carcinoma cells less tumorigenic in nude mice. In the future, semaphorin-based therapies could be used by themselves or in combination. *In vitro*, the synchronized use of SEMA3A and SEMA3F demonstrated synergy in repelling and inducing EC apoptosis [73]. A strategy employing the extracellular domain of SEMA6A has been developed with success; injection of this protein fragment in mice reduced tumor and VEGF-dependent vascularization [78].

10. Conclusion

Members of class-3 semaphorins, especially SEMA3F, have emerged as important factors in the development/progression of lung cancer. While SEMA3F and SEMA3B are downregulated in many tumors, their NRP receptors are conversely upregulated, most likely due to their role as receptors for other growth factors. Recent data have suggested that SEMA3s might not compete with VEGF for NRP binding thus strengthening the argument for a combined semaphorin/anti-VEGF approach.

Relatively little is known about which semaphorin signaling pathways significantly contribute to preventing tumorigenesis, except that integrin regulation is recurrently observed. There is a need in future studies to determine which signaling pathways or components predominate in the semaphorin anti-tumor effects. This might allow small molecules to recapitulate the effects of semaphorins, which are more cumbersome and expensive to produce. Recently, antibodies targeting the VEGF-A/C binding sites on NRP1 and NRP2 have been engineered and show promising results on blocking tumor angiogenesis, lymphangiogenesis and metastasis in rodents [127,134]. It is likely that such strategies will see their ways to clinical trials in the near future

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Abbreviations

CRMP
collapsin response mediator protein

EC
endothelial cell

ECD
extracellular domain

EGFR
epidermal growth factor receptor

FHIT
fragile histidine triad

GAP
GTPase activating protein

GEF
guanine nucleotide exchange factor

GRP
gastrin-releasing peptide

HGF
hepatocyte growth factor

LKB1
serine/threonine kinase 11

NRP
neuropilin

NSCLC
non-small cell lung cancer

PTEN
phosphatase and tensin homolog

RAR

retinoic acid receptor

Rb

retinoblastoma protein

SCF

stem cell factor

SCLC

small cell lung cancer

TGF

transforming growth factor

VEGF

Vascular Endothelial Growth Factor

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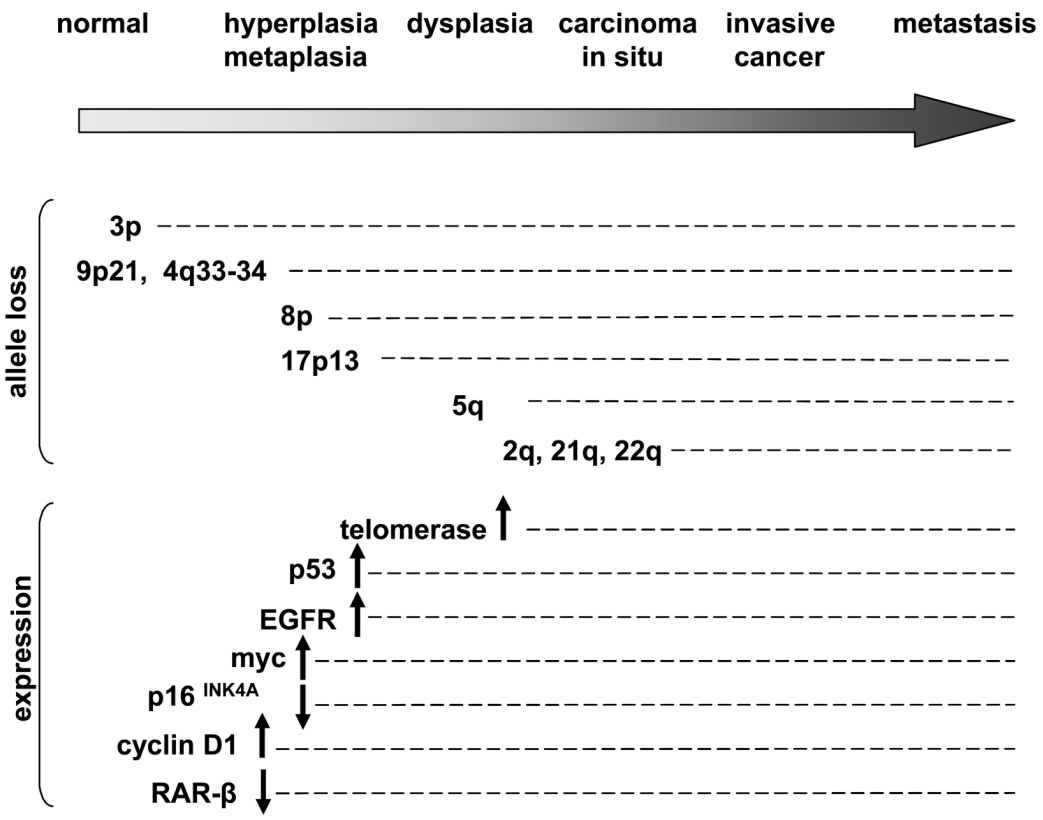


Fig. 1. Sequential events in lung cancer studied in squamous cell lung carcinoma

Not every change is necessary and the timing may be subjected to variation. Adapted from [4].

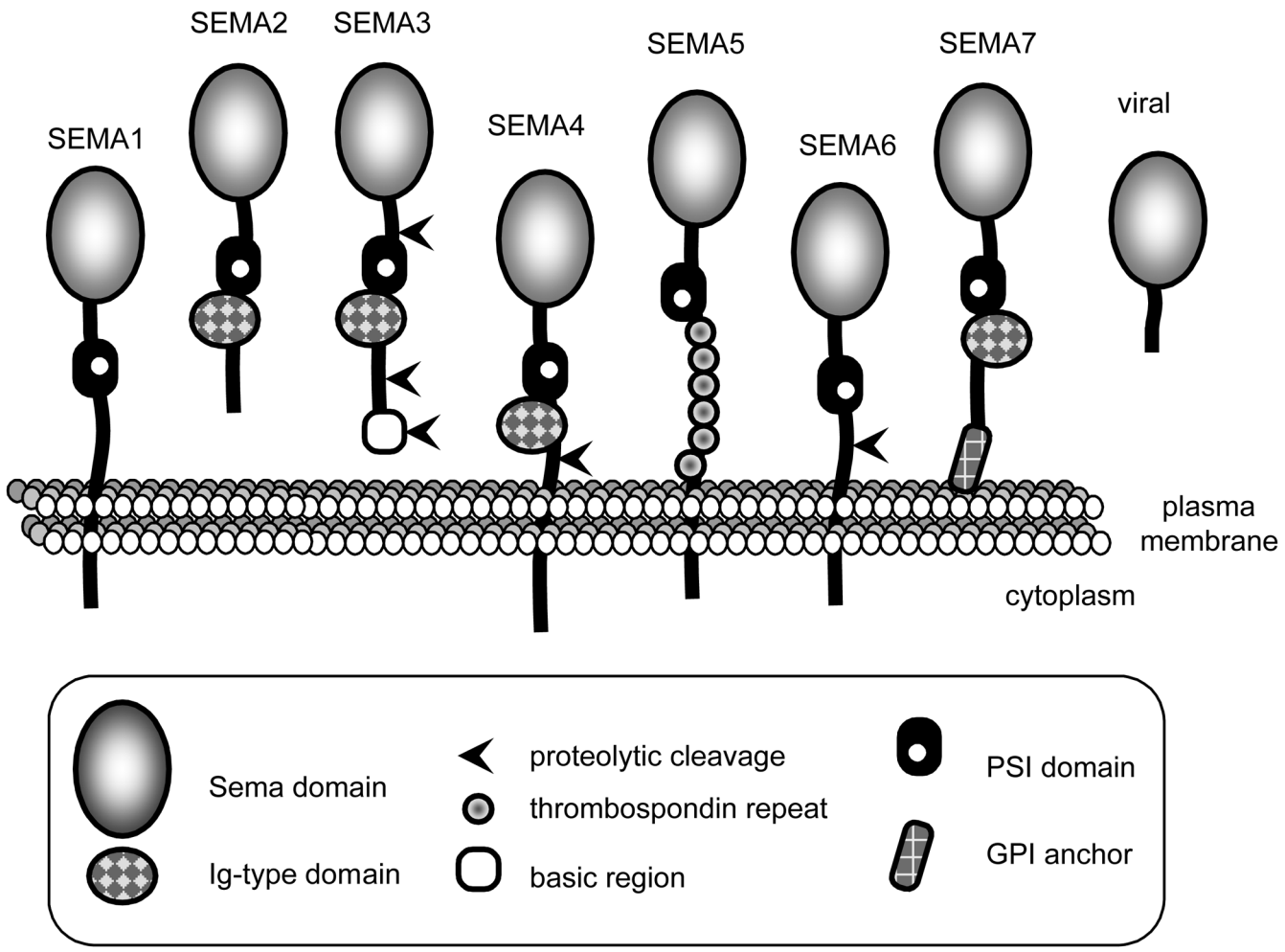


Fig. 2. The semaphorin family structure

Semaphorins are either secreted, membranous or associated to the membrane. GPI: glycosyl phosphatidyl inositol. Ig: immunoglobulin-like domain. PSI: plexin-semaphorin-integrin.

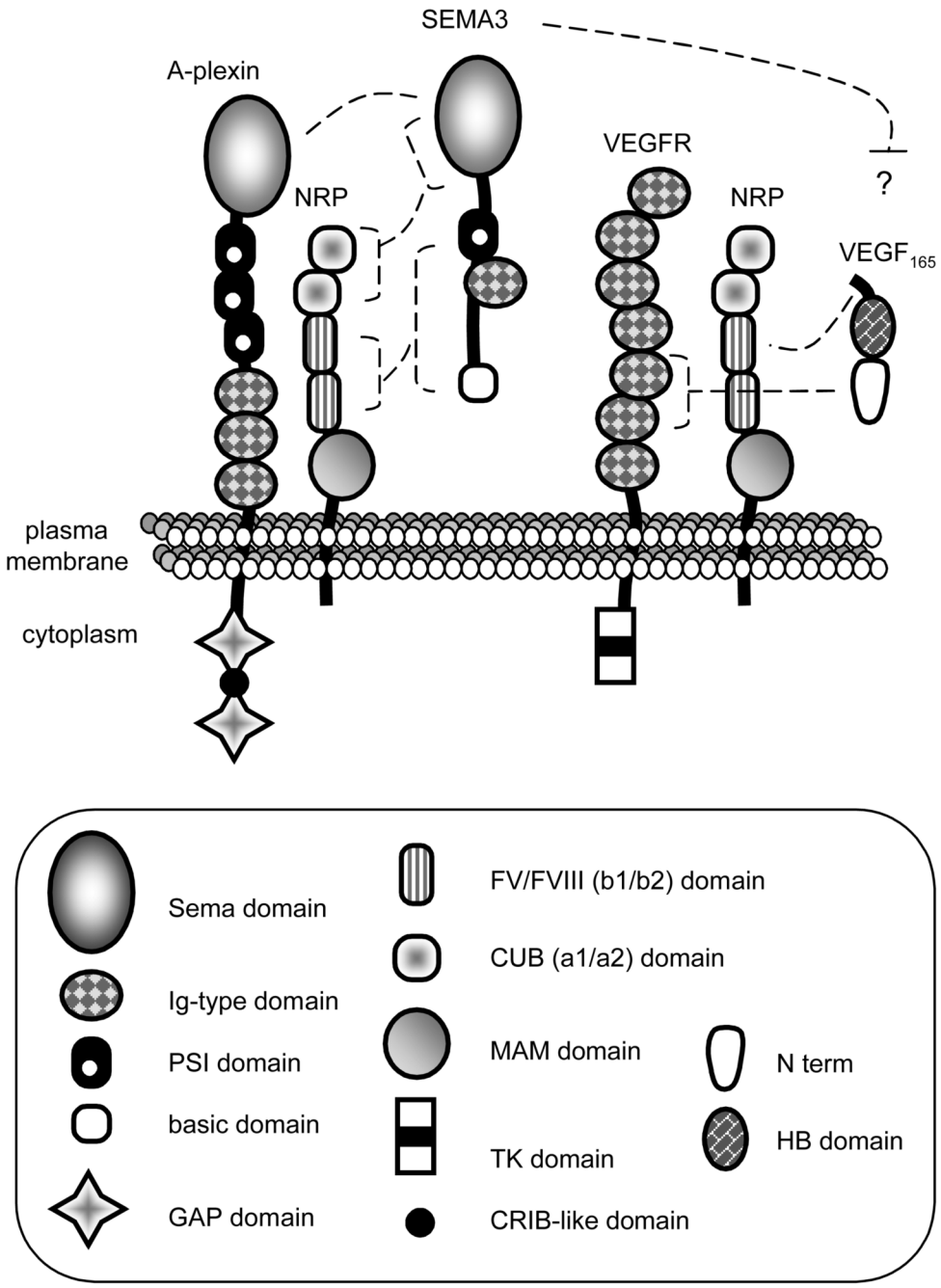
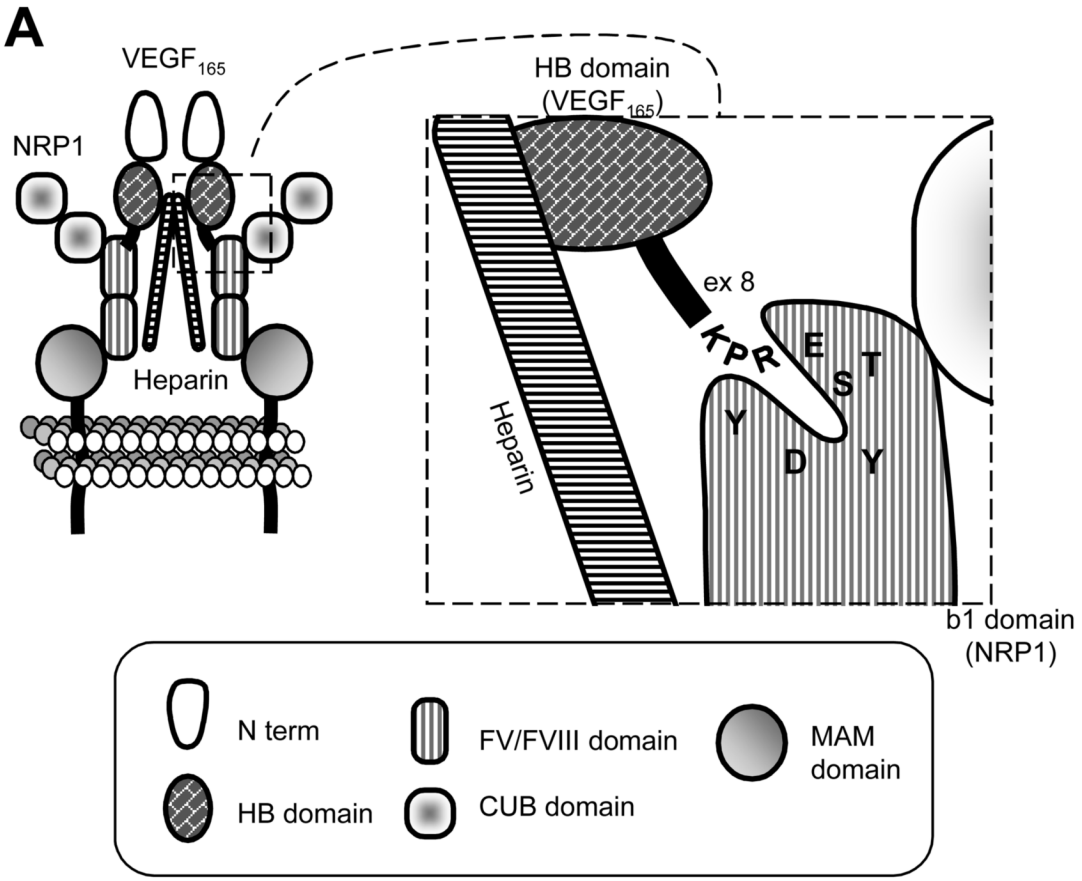


Fig. 3. Receptors of class-3 semaphorins and VEGF

Left: binding of SEMA3 to NRP and A-plexin. The sema domain of SEMA3 binds the a domains (CUB) of NRP. The PSI/Ig/basic region binds the b1b2 domain. Right: binding of $VEGF_{165}$ to NRP and VEGFR2. The HB domain of $VEGF_{165}$ binds the b1 domain of NRP and the N terminus binds the 2d and 3rd Ig domains of VEGFR2. VEGFR2 has a tyrosine kinase activity, but not plexins. CRIB: cdc42/rac interactive binding; GAP: GTPase activating protein; HB: heparin binding; MAM: meprin/A5 antigen/tyrosine protein phosphatase μ; N term: exons 1 to 5; TK: tyrosine kinase.



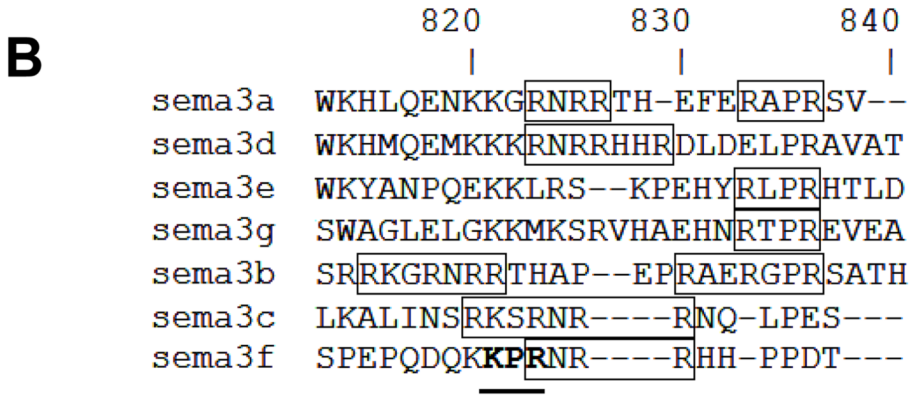


Fig. 4. VEGF binding to neuropilins

(A) A model of VEGF binding to NRPs with involvement of heparin. The KPR motif interacts with amino acids of the NRP b1 domain groove. (B) The amino acid composition of SEMA3s basic C-ter domain. SEMA3s have different amino acids motifs in the basic domain: only SEMA3F possesses the KPR (bold) that overlaps the RxxR furin cleavage motif (squared).

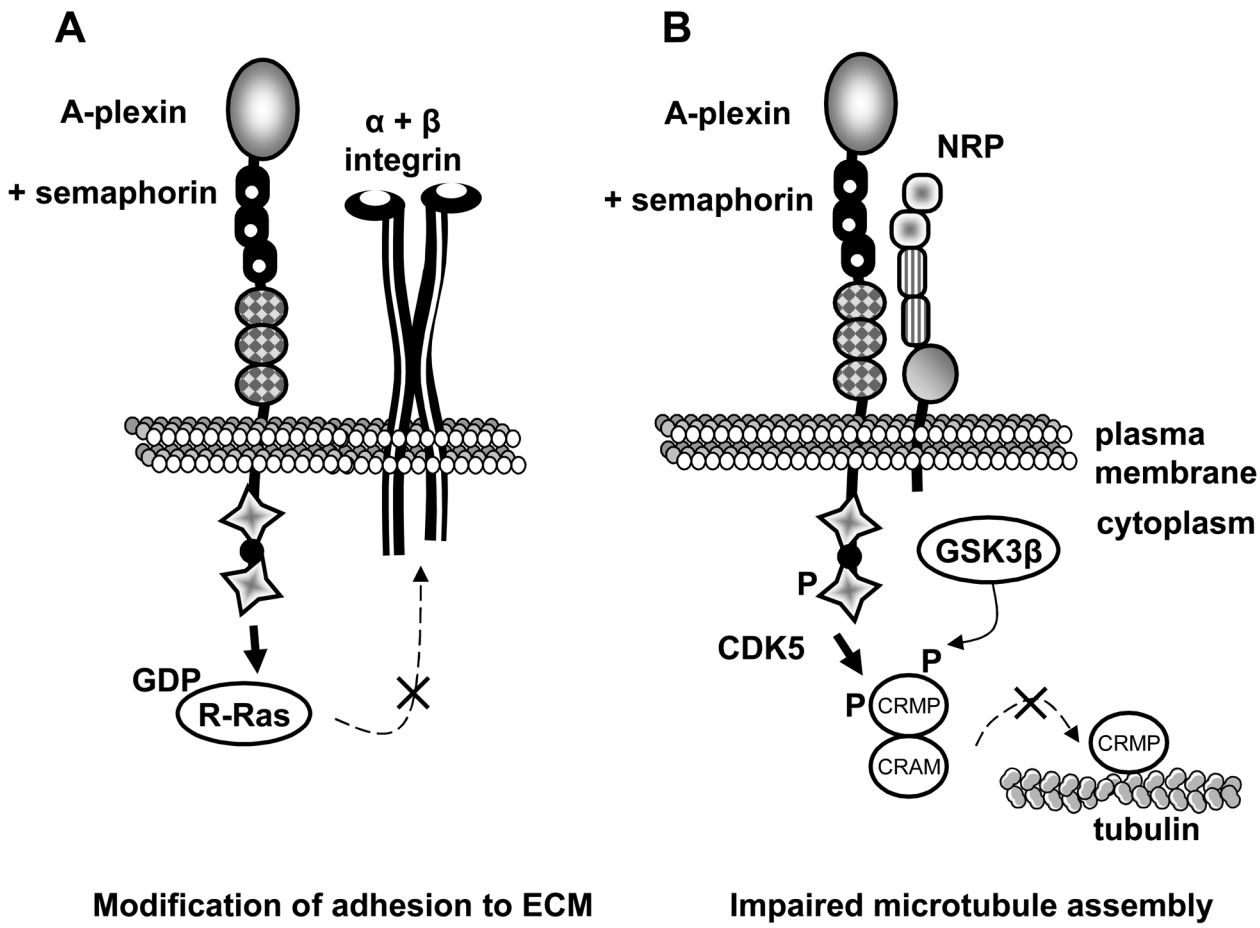


Fig. 5. Intracellular signaling by A-plexin upon semaphorin stimulus

(A) The FARP2/R-Ras/talin pathway. When plexins are inactive, the Rac GEF FARP2 is bound to a KRK motif on their cytoplasmic region. After semaphorin binding, FARP2 is released from plexins and transiently activates Rac1. In turn, Rac1 activates plexins allowing the interaction with Rnd1. Rnd1 recruits R-Ras which is inhibited by the GAP activity of plexins. Also, free FARP2 binds and sequesters PIPKI (PIP Kinase Isoform) which is required in the final step of integrin activation by talin binding. These mechanisms result in a lack of integrin activation and stabilization in focal points. Note that this mechanism is thought to be common to all types of plexins. Thus neuropilin participation might be variable (not represented). (B) The Fes/CRMP2 pathway. When stimulated by a class-3 semaphorin, A-plexin interacting kinases Fes and Fyn phosphorylate CDK5, which subsequently phosphorylates CRMP2. CRMP2 priming phosphorylation enables an additional phosphorylation of CRMP2 by GSK3β. This results in the inability of CRMP2 to interact with tubulin and to promote microtubules assembly, suggesting a role in cell migration.

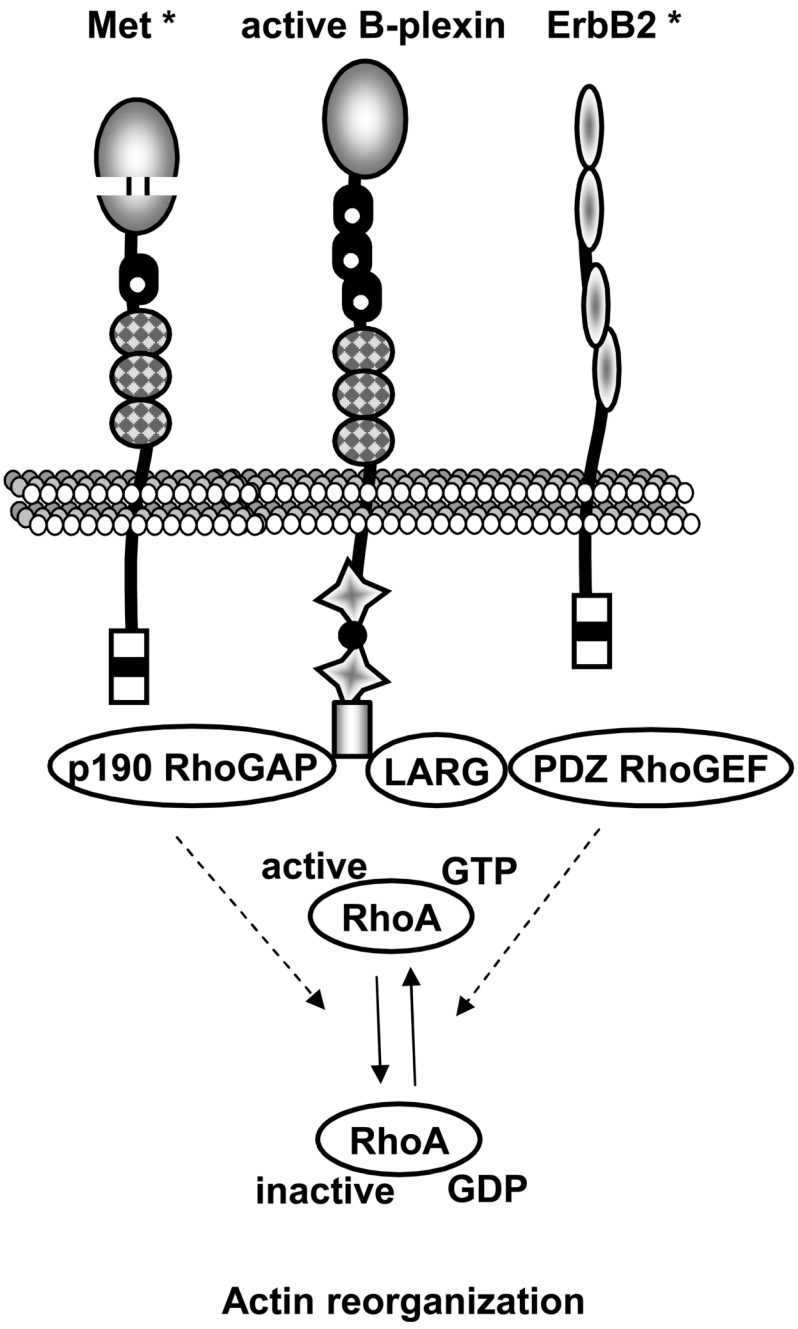


Fig. 6. Regulation of actin dynamics by B-plexins

B-plexins have an additional domain at their cytoplasmic tip that is responsible for interaction with PDZ domain-containing proteins. Stimulation by SEMA4D induces the Rnd1-dependent interaction of Rho regulators such as the GEF LARG and PDZ-RhoGEF, or the GAP p190-RhoGAP, with the PDZ domain of plexins. The association of the plexin with Met would inhibit RhoA by using p190-RhoGAP, and the association of plexin with ErbB2 would promote RhoA activity by using LARG and PDZ-RhoGEF. Asterisks: the domains of these proteins are not detailed here. Note the presence of a sema-like domain in Met. PDZ: PSD-95, DLG, ZO-1.

Table 1

Major genetic characteristics of small (SCLC) and non-small cell (NSCLC) lung cancers.

	SCLC	NSCLC
frequency in lung cancer patients	20%	80%
neuroendocrine properties	100%	rare
FHIT deletion/mutations	80%	40%
p53 deletion/mutations	85%	50%
p16 deletion/mutations	rare	60%
Rb deletion/mutations	90%	20%
LKB1 mutations	rare	30%
PTEN mutations	15–20%	infrequent
E-cadherin loss	?	10%
K-Ras activating mutations	rare	20%
c-myc overexpression	20%	rare
EGFR1 overexpression	rare	60%
HER2/Neu overexpression	rare	20%
Bcl-2 overexpression	85%	20%
autocrine loop	GRP-GRP receptor, SCF-Kit	HGF-Met, TGF-α
PI3K activation	+	+
microsatellite instability	35%	20%
telomerase activity	100%	80%
frequent allelic loss	4p, 4q, 5q, 8p, 10q, 13q, 17p, 22q	6q, 8p, 9p, 13p, 17p, 19q
3p deletion	100%	80%
3q amplification	+	+

Table 2

Chromosomal localization of human semaphorin, neuropilin, and plexin genes.

	semaphorin				
	class 3	**class 4**	**class 5**	**class 6**	**class 7**
A	7p12.1	1q22	5p15.2	5q23.1	15q22.3-q23
B	3p21.3	15q25	3q21.1	19p13.3	
C	7q21-q31	2q11.2		1q21.2	
D	7q21.11	9q22-q31		15q21.1	
E	7q21.11	?			
F	3p21.3	2p13.1			
G	3p21.1	10q24.31			
	plexin				**neuropilin**
	class A	class B	class C	class D	
1	3q21.2	3p21.31	12q23.3	3q21.3	10p12
2	1q32.2	22q13.33			2q33.3
3	Xq28	Xq28			
4	7q32.3				

Table 3

Semaphorin receptor complexes. Semaphorins can interact with complexes that include NRPs, plexins, various membrane proteins such as growth factor receptors and cell adhesion molecules (CAMs) which themselves interact. Asterisk: Sema3E is an exception, it cand bind D1-plexin directly.

	receptor complex		
ligand	**transducing unit**	**binding unit**	**additional unit**
SEMA3 *	plexin A	NRP	L1/Nr-CAM, integrin
SEMA4	plexin B + Met/ErbB2	plexin B	CLCP1
SEMA5	plexin B	plexin B	
SEMA6	plexin A	plexin A	VEGFR/Off-Track
SEMA7	plexin C, integrin	plexin C, integrin	
VEGF	VEGFR	VEGFR, NRP	
HGF	Met	NRP, Met	
Galectin	?	NRP	
TGFβ	?	NRP	

Table 4

Semaphorin involvment in diverse cancers. The very recent finding that SEMA3B inhibits tumor growth but fosters a prometastatic environment [95] adds complexity in the classification of SEMA3B as a pro- or anti-tumor semaphorin. exp., experiments

	SEMA	human tumor	*in vitro* exp.	*in vivo* exp.	effect	reference
pro-tumoral	3A	x	≠tumor cell lines	x	inhibits the immune response	135
		pancreas	x	x	correlates with shorter survival	136
	3B	glioblastoma	x	x	co-marker of poorer survival	137
	3C	x	ovarian cancer cells	x	ovarian cancer drug resistance	138
		ovary	x	x	correlates with shorter survival	139
		x	prostate cancer cells	x	invasive cellular growth	140
		x	lung cancer cells	x	overexpression in metastatic cells	109
	3E	breast	breast cancer cells	breast cancer cells	pro-metastatic	17,141
	4B	x	lung cancer cells	x	pro-migratory	57
	4D	leukemia	x	x	pro-proliferative	142
		x	leukemia cells	x	anti-apoptotic	143
		head and neck	h. and n. cancer cells	h. and n. cancer cells	pro-angiogenic	13,77
		x	breast cancer cells	x	invasive growth and pro-migratory	144
	5A	prostate	x	x	correlates with aggressiveness	121
	5B	kidney	x	x	increased expression in tumors	145
	5c	x	x	drosophila model	pro-metastatic	146
	6A	kidney	renal cancer cells	renal cancer cells	correlates with tumor vascularization	78
	6D	stomach	x	x	increased expression in tumors	147
anti-tumoral	3A	x	breast cancer cells	x	anti-migratory	148
		x	mesothelioma cells	x	negative retrocontrol of VEGF	149
		myeloma	myeloma cells	x	negative retrocontrol	104
		x	prostate cancer cells	x	anti-invasive in vitro	140
	3B	lung	x	x	gene deleted in tumors	5
		x	lung cancer cells	x	*in vitro* tumoral suppression	87
		x	lung cancer cells	x	lower expression in metastatic cells	90
		lung	x	x	lower expression in tumors	86
		x	lung cancer cells	x	pro-apoptotic	89
		x	ovarian cancer cells	ovarian cancer cells	*in vivo* tumoral suppression	88
	3F	lung	x	x	gene deleted in tumors	6,7
		lung	≠ cell lines	x	early expression loss	85,98
		lung	x	x	lower expression in tumors	85,98
		x	lung cancer cells	lung cancer cells	anti-angiogenic + tumoral suppression	81,82,101
		x	melanoma cells	melanoma cells	anti-metastatic	79
		x	HEK293 cells	HEK293 cells	anti-angiogenic	80
		x	x	murine fibrosarcoma cells	*in vivo* tumoral suppression	100
		x	breast cancer cells	x	anti-migratory	102,103
	4B	B-cell lymphoma	x	x	expression loss	150
	4D	x	breast cancer cells	x	anti-migratory	63