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Slit/Robo pathway: a promising therapeutic target for cancer

Rishi K. Gara[†], Sonam Kumari[†], Aditya Ganju, Murali M. Yallapu, Meena Jaggi^{*}, and Subhash C. Chauhan^{*}

Department of Pharmaceutical Sciences and Center for Cancer Research, College of Pharmacy, University of Tennessee Health Science Center, Memphis, TN 38163, USA

Abstract

Axon guidance molecules, slit glycoprotein (Slit) and Roundabout receptor (Robo), have implications in the regulation of physiological processes. Recent studies indicate that Slit and Robo also have important roles in tumorigenesis, cancer progression and metastasis. The Slit/Robo pathway can be considered a master regulator for multiple oncogenic signaling pathways. Herein, we provide a comprehensive review on the role of these molecules and their associated signaling pathways in cancer progression and metastasis. Overall, the current available data suggest that the Slit/Robo pathway could be a promising target for development of anticancer drugs.

Keywords

Slit/Robo; cancer progression; cancer metastasis; hormonal-dependent and - independent cancers; drug target

Introduction

The development of the nervous system involves several progressive and regressive events that are mainly driven by axon guidance molecules [1], such as Slit and Roundabout (Robo) [2]. Slit/Robo signaling was first established as an extracellular signature to guide axon path finding, promote axon branching and control neuronal migration. The interaction of Slit and Robo proteins is critically involved in the developmental processes of various vital organs such as breast, lung, liver, kidney, eye and reproductive systems. Slit proteins are highly conserved, secreted glycoproteins that mediate their functions by binding to the transmembrane receptors known as Robo receptors [1]. Slits and Robos are large proteins

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^{*}Corresponding authors: Chauhan, S.C. (schauha1@uthsc.edu); Jaggi, M. (mjaggi@uthsc.edu).

[†]Authors contributed equally to this work.

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Teaser: Recent studies indicate that Slit and Robo play an important part in tumorigenesis, cancer progression and metastasis. This review suggests that the Slit/Robo pathway could be a promising target for the development of anticancer drugs.

Conflicts of interest

The authors have no conflict of interest to declare regarding this work.

involved in several cell signaling pathways including axon guidance, cell proliferation, cell motility and angiogenesis [2–4]. Slit and Robo proteins were first discovered as secreted proteins in *Drosophila* [5–7]. Thereafter, homologs of Slit and Robo proteins have been discovered in rat, mice and humans [8]. Many reports have suggested that, in addition to axon guidance, the Slit/Robo pathway is also involved in the developmental processes and in the regulation of several physiological processes. An aberrant Slit/Robo expression in cells can lead to cancer development, progression and metastasis. Herein, we have reviewed recent advances regarding the roles of the Slit/Robo pathway and proteins in different types of cancer, molecular crosstalk and the modulation of oncogenic signaling pathways.

Structure of Slit and Robo proteins

In humans, Slits are composed of a single peptide of about 1500 amino acids, and there are three members: Slit1, Slit2 and Slit3 [9,10]. The primary structure of Slit contains four domains at the N terminus (D1–D4) with leucine-rich repeats (LRR), six EGF-like sequences (EGF), a laminin- G domain and a C terminus with a cysteine-rich knot (Figure 1) [9,11,12]. All vertebrates have similar Slit family protein structures. The D2 region domain of LRR of Slits is highly conserved and plays an important part in binding to Robo proteins [9].

Robos are considerably large molecules and are composed of 1000 to 1600 amino acids, and a transmembrane receptor protein with a conserved cytoplasmic domain [10,13]. In humans, four Robos have been identified thus far. All Robo proteins are composed of five immunoglobulin (Ig) and three fibronectin (Fn III) motifs in the extracellular domain with the exception of Robo4, which has only two Ig domains as well as the Fn III motifs (Figure 1). The Ig proteins are highly conserved, and the different expressions of the conserved cytoplasmic domains expressed determine the interaction of Slit protein with downstream signaling pathways. The conserved domain of Slits (D2 LRR), IG_[s1]1 and IG2 are crucial for binding of Slit with Robo. Robo4 has only two Ig, and Fn III is not considered a true receptor. Robo4 is expressed in endothelial cells and is involved in angiogenesis. The interaction of Robo4 with Slits is controversial, and could have its function in a Slit-dependent or -independent manner.

Slit/Robo pathway in cancer progression

The first link between Slit/Robo signaling and cancer was reported by Sundaresan *et al.* [14]. Subsequent studies indicated that the exon 2 of Robo1 was deleted in lung and breast tumor cell lines [14,15]. Subsequently, various studies have shown that Slit1–3 and Robo1–4 promoters are hypermethylated (epigenetic inactivation) in several different types of cancers [40–44]. [s2]The activation or suppression of the Slit/Robo pathway modulates several oncogenic signaling pathways that are associated with the development and progression of cancer [16,17]. In most of the tumors, expression of Slit and Robo proteins is either suppressed or undetectable, which is primarily caused by promoter hypermethylation. Very limited studies have suggested that the downregulation or suppression of Slit/Robo is not directly associated with the inhibition of cell death or apoptosis in cancer cells but that it occurs through indirect interaction with another axon guidance receptor: deleted in

colorectal cancer (DCC). In colorectal cancer, Slit/Robo signaling can induce apoptosis by Slit2–Robo4 interaction. Activated Robo4 physically interacts and suppresses the expression of netrin-1, another axon guidance protein [18]. Netrin-1 (a tumor suppressive protein) binds DCC and prevents DCC-mediated apoptosis in cancer cells [19,20]. Thus, binding of Robo4 with netrin-1 leads to disassociation of netrin-1 from its receptor DCC. Alternatively, Slit2 can also bind to netrin-1 and prevents inhibition of DCC by netrin-1. Free DCC activates caspase-3- and caspase-9-dependent apoptosis and cell cycle arrest in cancer cells [21,22].

Most of the current reports suggest that the Slit/Robo pathway displays its effects in the late stages of cancer [21,23]. It was reported that the Slit/Robo pathway inhibits cell invasion by interacting with E-cadherin and β -catenin in breast cancer and colorectal cancer [24–26]; whereas in liver cancer Slit2/Robo1 specifically inhibited hepatocyte growth factor (HGF)-mediated cell migration [25]. HGF is a tyrosine kinase receptor that interacts with its ligand, Met, and activation of Met correlates with a metastatic phenotype and poor prognosis in several carcinomas [27,28]. Most of the reports suggested that Slit2 inhibits invasion and migration in cancer cells. However, Denk *et al.* recently demonstrated that Slit3 also inhibited cell migration in melanoma cells through modulation of activator protein-1 (AP-1) [29]. In most of the cancers, Slit/Robo acts as a tumor suppressor by inhibiting cell invasion and migration [2,24,30–34], except in prostate cancer and colorectal cancer [23,35]. The current data indicate that Slit/Robo pathways differentially modulate invasion and migration (Figure 2), which varies according to signaling and type of cancer. It is still not known why the discrepancy occurs in specific types of cancers. One possible reason could be that, out of the three Slits, Slit2 binds more specifically than Slit1 or Slit3 to the Robo1 receptor. Overall, these findings indicate that the Slit/Robo pathway mainly suppresses tumor progression by regulating processes such as invasion, migration and apoptosis.

Regulation of tumor microenvironment

At present, there are no convincing reports that suggest whether the Slit/Robo axis has any role in cancer initiation [24,33,36]. Slit/Robo expression is known to inhibit cancer progression (invasion and migration) by modulating chemokines and growth factors [24,29,37,38]. The Slit/Robo pathway plays an important part in the modulation of tumor microenvironment by inhibiting the migration of leukocyte chemotaxis and inducing a chemorepellent effect in cancer cells [24]. Several reports indicate that the Slit/Robo pathway has an important role in the regulation of immunity in various types of cancers [1,2,39]. The activation of Slit2/Robo1 inhibits chemotaxis and chemoinvasion in breast cancer by inhibiting chemokine receptor CXCR4 expression in breast and lung cancer cells [24,40]. The Slit/Robo axis modulates tumor microenvironment by regulating growth factors such as hepatocyte growth factor (HGF), which supports the motility of tumor cells. In another study, it was suggested that Slit2/Robo1 inhibited HGF-dependent cell migration in breast, ovarian and endometrial cancer cells. Slit2 suppression enhanced HGF-dependent metastatic morphogenesis and cell motility in lung, colorectal and breast cancer cell lines [32], suggesting that the Slit/Robo pathway negatively regulates HGF in cancer cells. The mechanism behind regulation of growth-factor-mediated oncogenic signaling pathways by the Slit/Robo pathway is still unclear [25]. Alternatively, activation of Slit2 inhibits cell motility in *in vitro* studies. Also, addition of recombinant Slit2 decreases colony formation

in ovarian cancer, gliomas [41] and breast and lung carcinoma cells [32]. Colonization of tissuespecific cancer cells occurs in the early stage of metastasis, which supports cancer cells for their settlement, adaptation and further progression to distant organs [42,43].

Cancer metastasis

Tumor microenvironment and tumor metabolism are key regulators of metastasis in cancers [44]. Tumor microenvironment can be either tissue-resident or derived from peripheral reservoirs such as the bone marrow and spleen. The macrophages that are in close proximity or within tumors are called tumor-associated macrophages (TAMs). TAMs could be derived either from circulating monocytes or resident-tissue macrophages [45,46]. The interactions between TAMs and growth factors, cytokines and chemokines are the key determinants for progression of tumor invasion and metastasis [47–49]. In many tumors such as breast, ovarian, glioma and lymphoma, the level of TAM infiltration is associated with poor prognosis [50]. Like neuronal cells, migration of leukocytes also requires the recognition of guidance cues, polarization of the cell and mobilization of the actin cytoskeleton [51]. The secretory protein Slit2 and its receptors Robo1 and Robo4 are considered to regulate mobility and permeability of endothelial cells and other cell types. Slit2 was found to inhibit leukocyte migration in the gradient of monocyte chemotactic protein-1 (MCP-1) in an *in vitro* pancreatic cancer model [52]. Current reports suggest that Slit2 and its receptor Robo1 inhibit metastasis in breast cancer [38], fibrosarcoma and squamous cell carcinoma [53]. In most of the cancers, the Slit–Robo interaction is not directly involved in the progression of metastasis [38,54], but is involved in modulating chemokines, such as stromal-derived factor-1 (SDF-_[s3]1) and MCP-1 [38,55,56]. It is known that CXCL12, a ligand of SDF-1, is constitutively expressed and is an inducible chemokine that modulates several biological and physiological processes, including embryonic development and organ homeostasis [57]. Aberrant expression of SDF-1 has been found in various solid tumor tissues such as ovarian cancer [58], breast cancer [59] and other cancers [60–63]. SDF-1 has been shown to promote growth, invasion and metastasis in breast cancer [59], lung cancer [64], pancreatic cancer [65] and other cancers [22,66]. At present, it is not known if Slit/Robo pathways have any role in tumor metabolism; however, it can definitely be considered that the Slit/Robo pathway inhibits metastasis by abrogating multiple oncogenic signaling pathways in tumors [37,67,68].

Role of Slit/Robo pathway in different cancers

The tumor suppressor or antitumor activity of the Slit/Robo pathway is not unanimous, and how cancer-specific expression of Slit and Robo proteins governs the different type of cancers is not clearly comprehended. This section summarizes the recent updates on the regulation of oncogenesis by the Slit/Robo pathway in different cancer types.

Hormone-dependent cancers

Hormone-dependent cancers are primarily driven by sex hormones. Slit and Robo proteins have a crucial role in regulating physiological functions in the reproductive organs of humans. The differential expression and modulation of Slit and Robo proteins determine cell death and proliferation in an autocrine and paracrine manner. Estrogen and selective

estrogen receptor modulators (SERMs) have been shown to regulate the expression of Slit1 in bones of ovariectomized rats [2,69]. Therefore, the effect of sex hormones on Slit and Robo proteins in hormone-dependent cancers such as breast, prostate, ovarian, endometrial and kidney cancers is still a major area of research to investigate. Herein, we summarize the current updates on the modulation of metastasis by the Slit/Robo pathway in some hormone-dependent cancers.

Breast cancer

The Slit/Robo pathway has an important role in breast development and morphology by regulating outgrowth of mammary branches, and by inhibiting translocation of cytosolic nuclear β -catenin to the nucleus of the basal myoepithelial cells [70]. In breast cancer, Slit/Robo has antitumor activity. This is evidenced by the fact that most breast tumors have low expression of Slit/Robo and its higher expression is correlated with increased survival rate in cancer patients, whereas low Slit2 expression is associated with poor survival and increased metastasis [22,38,71]. Moreover, downregulation of Slit/Robo is associated with amplification of c-Myc and cyclin D1 gene expression in many breast cancer tumors [72,73]. Recently, Chang *et al.* demonstrated that activation of Slit2/Robo1 inhibits activation of β -catenin by inhibiting Akt, thereby preventing translocation of cytosolic β -catenin to the nucleus of the fibroblast cells [40]. Upregulation of Robo1 and Slit2 expression inhibited translocation of nuclear β -catenin in breast cancer cells. β -catenin is one of the major oncogenic pathways responsible for the motility, invasiveness and triple-negative phenotype of breast cancer cells [74–76]. Most of the breast cancers initially respond to antiestrogen therapy but, during the course of the treatment, resistance to antiestrogen therapy is very common. Antiestrogen therapy can fail as a result of one or more of the possible mechanisms such as activation of phosphoinositide 3-kinase (PI3K)/Akt and chemokines (CXCL12/CXCR4 or CCL2) [67,77–79]. Prasad *et al.* reported for the first time that Slit/Robo also regulates chemokine CXCL12. Addition of recombinant Slit2 suppresses CXCL12-induced breast cancer cell chemotaxis, chemoinvasion and adhesion in MDA-MB-231 breast cancer cells [24]. There was no remarkable effect of Slit2 on the CXCL12-induced internalization process of CXCR4, but it inhibits the CXCL12-mediated RAFTK/Pyk2 signaling pathway [38]. Slit2/Robo1 also inhibits CXCL12-induced PI3K/Akt, p44/42 mitogen-activated protein (MAP) kinase and matrix metalloproteinase (MMP)-2 and -9 activities in breast cancer cells [24,38]. Slit2 expression did not inhibit c-Jun N-terminal kinase (JNK) or p38 MAP kinase activities in breast cancer cells [24]. The Slit/Robo pathway regulates chemokine-mediated cellular responses in breast cancer cells and thus inhibits cellular migration by activation of ubiquitin/proteasome degradation pathways [80]. Combined, these findings suggest that the Slit/Robo pathway might be a key regulator of breast cancer metastasis.

Ovarian cancer

Slit–Robo interaction involves autocrine and paracrine mechanisms, which seem to play an important part in the development of fetal ovaries during organogenesis [81]. In humans, normal ovarian tissues and ovarian cancer tissues widely express three components of Slit and Robo proteins (Slit2/3 and Robo1), suggesting that Slit/Robo has an important role in normal and malignant ovarian tissues [21]. However, expression of Robo4 was much higher

in ovarian tumor tissues, and Robo1 was also detected in ovarian tumor tissues. Robo4 is known to induce angiogenesis by inhibiting Slit2, however blockage of Robo leads to inhibition of angiogenesis in ovarian cancer *in vitro*. It is not clear whether overexpression of Robo4 has anti-pro-angiogenic effect in ovarian cancer cells [21]. This variation could be the result of the small sample size and the heterogeneous nature of ovarian cancer cells used in this study [21,82]. *In vitro* cell line models suggested that the ovarian cancer cell lines OVCAR-3 and SKOV-3 express Slit2/3 and Robo1/4. However, addition of recombinant human Slit2 did not significantly affect cell proliferation or induce ERK1/2 and Akt1 phosphorylation in these cells [21]. In another study, it was reported that the basal mRNA expression levels of Slit2,3 and Robo1,2,4 was low in primary cultures of ovarian cancer epithelial cells compared with normal ovarian surface epithelial (OSE) cells [83]. The expression of Slit/Robo also depends on the level of differentiation of ovarian cancer cells [21,83]. In all cases, high-grade malignant cells had lower expression of Slits and Robos. When exogenous cortisol was added, there was a reduced expression of Slit2,3 and Robo1,2,4 in normal OSE and PEO-14 cells. Furthermore, blocking Slit/Robo activity reduced apoptosis in well-differentiated PEO-14 and SKOV-3 tumor cells. When glucocorticoid receptor was knocked-down using siRNA, there was an upregulation of Slit/Robo. One of the possible reasons that can account for this effect is that Slits and Robos are regulated at the transcriptional level by glucocorticoid receptors in ovarian cancer cells [83].

Prostate cancer

Limited studies suggest that all Slits (Slit1–3) are overexpressed in prostate tumors as compared with the normal prostate tissues. However, no significant association was observed in the expression of Slit2 and Slit3 in prostate tumors as compared with the normal prostate tissues. The expression of Slit1 was more frequent in clinically localized tumors. Slit1–3 were overexpressed in hormone refractory tumors as compared with benign tumors [23]. In contrast to Slit1, Robo1 was downregulated in prostate tumors when compared with the normal prostate tissues. Robo2 was highly overexpressed in hormone refractory tumors as compared with malignant tumors [23]. The possible reason for the upregulation of the Slit/Robo pathway could be caused by suppression of the DCC receptor, by another axon guidance cue netrin-1, in prostate tumors and prostate cancer cells [18,20]. Netrin-1 is also known as a survival cue because it controls apoptosis in many cancers. The binding of netrin-1 to DCC or Slits might be a determinant for the antitumor activity of Slit and Robo proteins in prostate cancers [19,23].

Nonhormonal cancers

Although the Slit/Robo family members are primarily expressed in the developing nervous system and are involved in organogenesis, they are also widely expressed outside the nervous system and reproductive organs in adult tissues, suggesting possible roles outside the developing embryo [84]. Several studies have suggested that, in most of the cancers, specific Slit and Robo proteins are downregulated, such as in lung cancer [31,32,85], brain cancer [31,35], cervical cancer [86], liver cancer [32,35], colon cancer [85] and others. Table 1 summarizes the recent updates on the expression of Slit/Robo in cancer cells.

Lung cancer

In lung cancer, the expression of Slit2 is suppressed [9,31,32,36,85] with a high expression of β -catenin in 60% of formalin-fixed, paraffin-embedded lung tumors as compared with normal tissue. Suppression of Slit2 was associated with an upward trend of pathological stage and poor survival rate of lung cancer patients. However, downregulation of Slit2 and β TrCP, and upregulation of β -catenin, can be predicted for postoperative disease relapse [87]. β TrCP, one of the components of the β -catenin-ubiquitin-ligase complex, is a negative regulator of the Wnt signaling pathway. *In vitro* studies suggested that the suppression of Slit2 is due to activation of Akt/GSK3 β / β TrCP in non-small-cell lung carcinoma cells. Activation of the PI3K/Akt pathway toward the GSK3 β / β -catenin signaling cascade stimulates cancer cells to begin bone metastasis [88]. Slit2 has an inverse association with lung cancer cells with respect to motility in *in vitro* systems.

Cervical cancer

In cervical cancer, primary tumors and cells (Caski, HeLa, SiHa, C33A, HT-3 and ME-180) have Slit proteins (Slit1–3) and Robo1,3 that become downregulated or completely suppressed [86]. The suppression of Slit and Robo proteins in cervical cancer is due to promoter hypermethylation, which is an early event in tumor progression [86]. Re-expression of Slit/Robo by a demethylating drug (5-Aza-2'deoxyctidine and trichostatin A) in cervical cancer cells is only partially restored and is found in very few cervical cancer cells. It can be assumed that only demethylation of promoters of Slit/Robo pathway genes does not effectively reactivate gene expression in cervical cancer. The only known mechanism that accounts for this effect is promoter hypermethylation of Slit/Robo.

Glioblastoma

It is well established that, in the central nervous system, Slit–Robo interactions play an important part in controlling the migration of neurons and neural crest cells by Slit-regulated Rho-GTPase-activating proteins and by the inhibition of CDC42, a small Rho GTPase [30,89]. The expression of the Slit2 protein gets suppressed in glioblastoma, as observed in several other cancers. The Slit/Robo pathway has antitumorigenic properties as evidenced by the suppressed expression of Slit2 in primary glioma tumors and invasive glioma cells as compared with normal human brain cells and in control cell type astrocytes. The Slit/Robo pathway inhibits cell invasion and motility in glioblastoma in *in vitro* and *in vivo* systems [90]. *In vitro* studies suggested that addition of recombinant Slit2, or ectopic expression of Slit2, inhibited glioma cell invasion and motility by inhibiting CDC42 activity in glioma cells. CDC42 is one of the members of the Rho GTPase family and it controls cell polarity during migration of cancer cells [41,90]. It has been shown that the activity of CDC42 is upregulated in several cancers, including glioblastoma, and is responsible for high invasive metastasis in cancers [91]. *In vivo* studies also suggested that the re-expression of Slit2 in SNB19 glioma cells inhibited infiltration of glioma cells into the brain of mice [90]. Activated Slit2 inhibited cell motility without affecting the expression of *N*-cadherin and β -catenin in glioma cells [90].

Future prospects for Slit/Robo pathway as a drug target

Besides functioning as axon guidance molecules, the Slit/Robo pathway proteins also regulate functions such as morphogenesis of tissue as well as performing many non-neuronal roles such as cell growth, migration and cell survival [92]. Slit/Robo is often downregulated in the advanced stage of most tumors [31,85,86]. *In vitro* studies suggested that expression of Slit can be regulated by stress and optimum function can be achieved by modulating the endoplasmic reticulum (ER), Ca^{2+} homeostasis and actin in β cells [92]. For example, cytokines, thapsigargin and palmitate suppress Slit2 expression and serum deprivation downregulates Slit3 [92]. Cancer cells have the tendency to become aggressive and metastatic under extreme stress (low glucose, hypoxic condition) as compared with normoxic conditions [93]. However, robust induction of ER stress upregulates Slit3 and Slit1 expression [92]. It is not known whether a high grade of tumor, besides promoter hypermethylation, deletion of Slit/Robo gene and other factors such as ER, Ca^{2+} and high glucose, has any role in the modulation of Slit/Robo pathways in cancer cells. Many targeted therapeutic agents inhibit the growth of cancer cells by controlling a specific pathway: HER-2/EGFR/VEGFR/mTOR/CD-20. Overall, the literature suggests that the Slit/Robo pathway is a direct or indirect key regulator of these other pathways (Figure 3). Therefore, we propose that targeting the Slit/Robo pathway using an inhibitor is highly useful and can lead to regulation of a wide variety of oncogenic signaling. Such a strategy could emerge as a new therapeutic modality for cancer. In our opinion, it could be achieved by several methods as outlined below.

- i. Developing specific mimetics that can interfere with Slit/Robo expression. Recently such molecules have been designed and used for semaphorin, an axon guidance protein [94]. This type of mimetic was not developed to target the Slit/Robo pathway so it has not been tested in any cancers.
- ii. The Slit/Robo pathway has antitumor effects and, in most of the tumors, its expression is downregulated or undetectable. Like many antioncogenic genes, promoter hypermethylation is one mechanism by which loss of gene function occurs [31,95]. Slit/Robo expression can be restored by histone deacetylase (HDAC) inhibitors. However, *in vitro* results suggested that very few cell types respond better; rather, their response is poor or they do not respond at all. Even demethylation of promoters of Slit/Robo pathway genes does not effectively reactivate gene expression; thus, the upregulation of Slit/Robo by HDAC inhibitors is not an ideal mechanism [31,86].
- iii. Another approach might be the epigenetic modulation of Slit/Robo along with the modulation of intracytoplasmic concentration of intracellular Ca^{2+} and glucose, or induction of strong ER stress by thapsigargin, or other synthetic or natural compounds. However, these approaches need to be tested in *in vitro* and *in vivo* models for their feasibility and applicability for targeted therapies.

Concluding remarks

The overall data suggest that the axon guidance molecule Slit/Robo has important roles in cancer metastasis, tumorigenesis in many tumors and that several possible tumor-specific expressions need to be explored in future studies. However, additional research is necessary before we can provide a robust, comprehensive mechanism to target the Slit/Robo pathway in the specific cancers. Tumor-specific discrepancy of Slit/Robo by previous authors might simply tempt one to apply neurobiological models to cancer. Also, because tumorigenesis is a complex process and involves a huge array of signaling pathways, it is extremely difficult to determine how Slit/Robo participates in this complex system. Most studies reported are primarily based on basic molecular biology techniques that are totally different to the biological conditions in tumors. Further, local concentration of ligand receptors and intracellular concentration of various molecules might be possible reasons why variations exist in recent studies. Multiple crosstalk signaling needs to be reconsidered before making any final conclusions. There are some major questions in this field concerning how different Slits are able to mediate their disparate actions. Slits have been shown to bind with similar affinity to all of the Robo receptors using evolutionary conserved binding residues. However, in different systems, Slits mediate diverse effects. For instance, in most of the tumors, Slit2 has an antitumor effect, as compared with Slit1 or Slit3. Therefore, it will be important to identify the molecules, such as co-receptors, that mediate such ligand specificities. Another remaining paradox is how increasing or decreasing levels of Robo receptors and/or different combinations of Robo receptors mediate varying effects within a given cell or between neighboring cells. There is no drug molecule that can efficiently target the Slit/Robo pathway. Therefore, vast possibilities exist for carrying out additional basic research in this direction in order to develop efficient targeted anticancer therapies.

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Highlights

- Slit and Robo are highly involved in the regulation of physiological processes
- The Slit/Robo pathway has a significant role in tumorigenesis and cancer progression
- The Slit/Robo pathway is a key regulator of many oncogenic pathways
- Molecules targeting the Slit/Robo pathway could be promising anticancer drugs

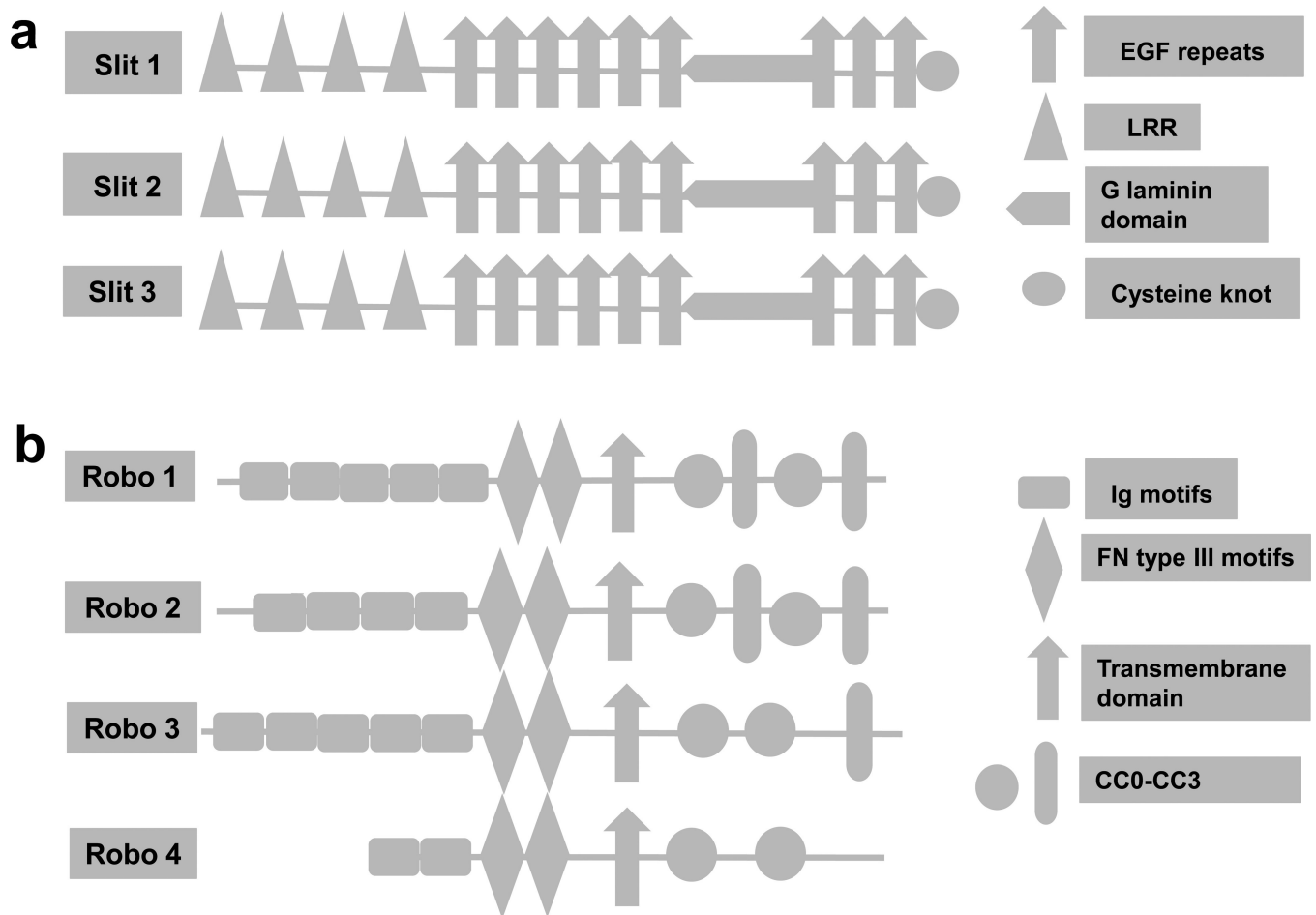


Figure 1. Structure of the Slit/Robo protein family. **(a)** Structure of human Slit protein. It is a large molecular weight glycoprotein, comprising (from N to C terminal): four leucine-rich repeats (LRR), seven to nine EGF_[s4] repeats, laminin G domain and a cysteine-rich knot. **(b)** Structure of human Robo proteins. Robos are the receptors of Slit proteins, which contain immunoglobulin (Ig) motifs, three fibronectin (Fn III_[s5]) motifs, a transmembrane domain and a conserved cytoplasmic domain (CC0–CC3). Figure modified, with permission, _[s6]from [10].

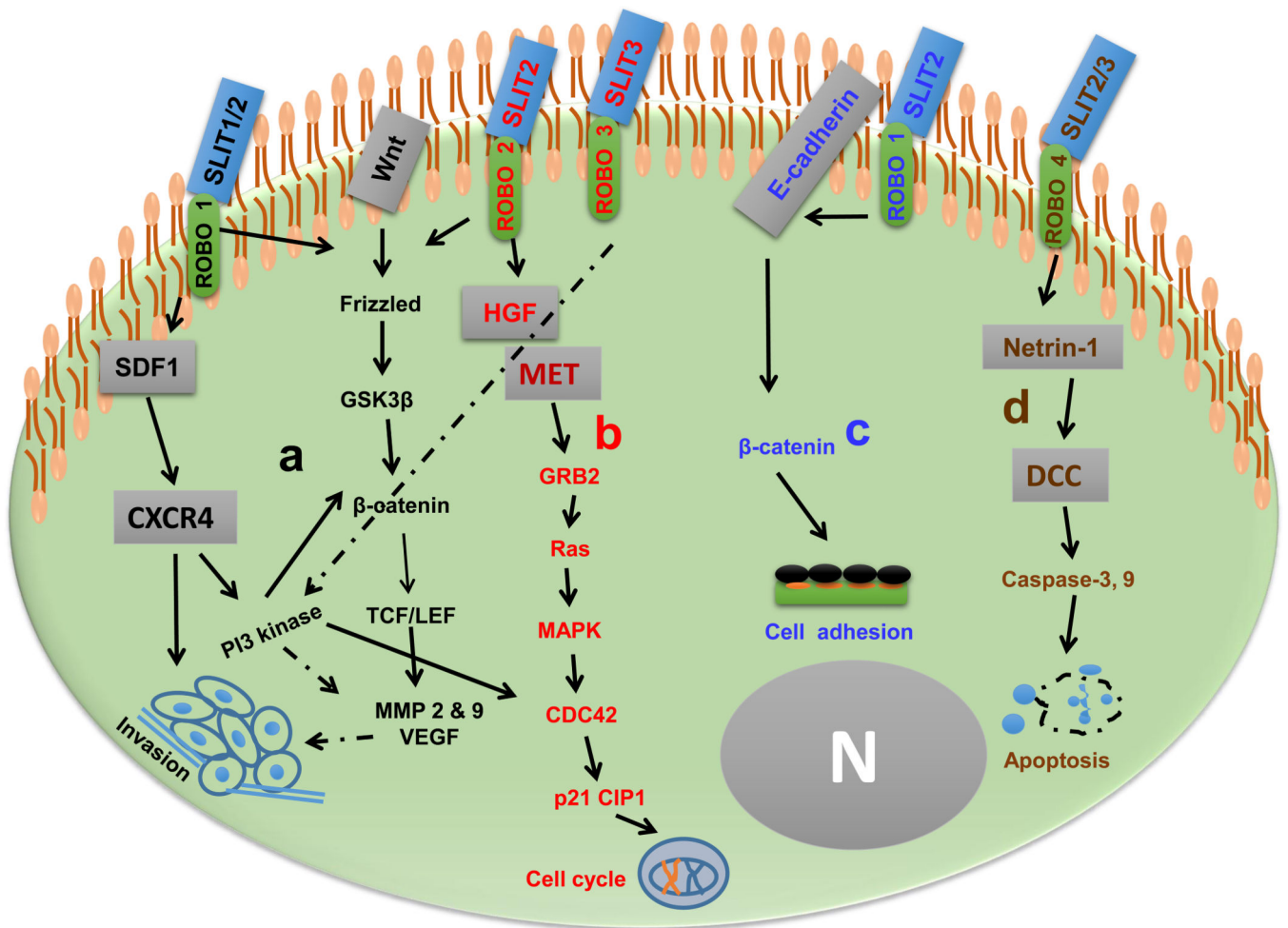


Figure 2.

Slit/Robo interactions and their roles in carcinogenesis. **(a)** Slit1,2/Robo1 can inhibit cell invasion by inhibiting the stromal-derived factor (SDF)-1/CXCR4 axis and can attenuate cell cycle progression by destruction of β -catenin and cell division control protein (CDC)42 expression. **(b)** Alternatively, Slit/Robo interactions suppress hepatocyte growth factor (HGF) expression leading to Met_{[s7][s8]}-dependent inhibition of cell cycle arrest. **(c)** Slit2/Robo1 axis antagonizes E-cadherin/ β -catenin-mediated enhanced cell adhesion. **(d)** Binding of Slit2,3 with Robo4 releases netrin-1, activating DCC_[s9]-regulated activation of caspase-dependent apoptotic cell death. Solid lines represent reported studies and dotted lines represent putative functions. Figure modified _[s10]with permission, from [2]. Abbreviations: GRB, growth factor receptor bound protein; GSK, glycogen synthase kinase; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; N, nucleus; VEGF, vascular endothelial growth factor.

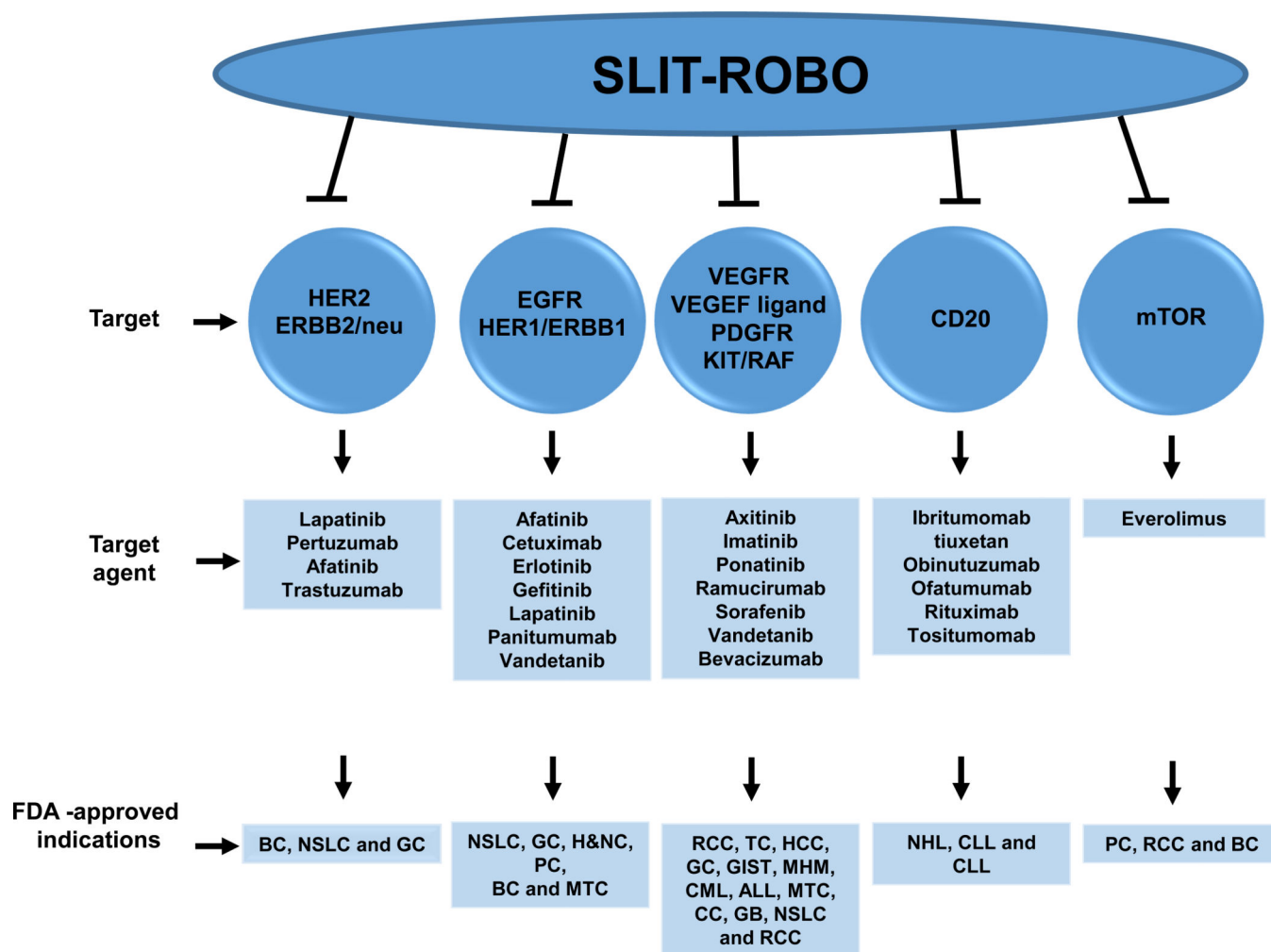


Figure 3.

[s11]. Slit/Robo pathway targets various key signaling pathways in cancer. The list of targeted agents that improve clinical therapeutic outcome of breast cancer (BC), non-small-cell lung cancer (NSLC), gastric cancer (GC), renal cell carcinoma (RCC), head and neck cancer (H&NC), pancreatic cancer (PC), medullary thyroid cancer (MTC), thyroid carcinoma (TC), GI stromal tumor (GIST), multiple hematologic malignancies (MHM), chronic myelogenous leukemia (CML), acute lymphoblastic leukemia (ALL), colorectal cancer (CC), glioblastoma (GB), chronic lymphocytic leukemia (CLL), among others. Inhibitors or agents that regulate the Slit/Robo pathway could be useful therapeutic molecules. Abbreviations: EGFR, [s12]epidermal growth factor receptor; HER2, human epidermal growth factor; HCC, hepatocellular carcinoma; mTOR, mammalian target of rapamycin; NHL, non-Hodgkin lymphoma; PDGFR, platelet-derived growth factor receptor; VEGFR, vascular endothelial growth factor receptor.

Table 1

Expression of Slit/Robo

Cancer types	Cell lines	Proteins	Expression status	Refs
Liver cancer	HuH-7, HepG2	Slit1, Robo1,2	↑	[35]
		Slit3, Robo4	↓	
Lung cancer	A549	Slit1,2,3 Robo1,3	↓	[9,23 _[s13] ,31,32,85–88]
Colon cancer	RKO	Slit2	↑	[85]
		Robo1	↓	
Brain cancer and glioma	GAMG	Slit1,2,3 Robo1	↓	[31,35]
Prostate cancer	LNCaP, PC-3, DU-145	Slit1,2,3 Robo1,3	↑	[9,23,88]
Cervical cancer	CaSki, HT-3, SiHa, SW756, ME-180, HeLa	Slit1,2,3 Robo1,3	↓	[86]
Breast cancer	MCF-7	Slit1,2,3 Robo1,3	↓	[9,32,87,88]
Kidney cancer	ccRCC	Robo1,3	↓	[88]
Acute lymphocytic leukemia	THP-1, Jurkat	Slit2 Robo1	↓	[54]

Slit and Robo proteins are expressed differently depending upon the type of cancer and cancer cell line. In lung, brain, cervical, kidney and acute lymphocytic leukemia these proteins are downregulated; whereas in prostate, liver and colon cancers usually upregulation of Slit1,2/Robo1,2 is found. Prostate cancer cells have shown upregulation of all Slit and Robo proteins.