Slit/Robo pathway: a promising therapeutic target for cancer

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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.

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 crucially 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 recptors [1]. Slits and Robos are large proteins 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 (Fig. 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 1000–1600 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 (Fig. 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.

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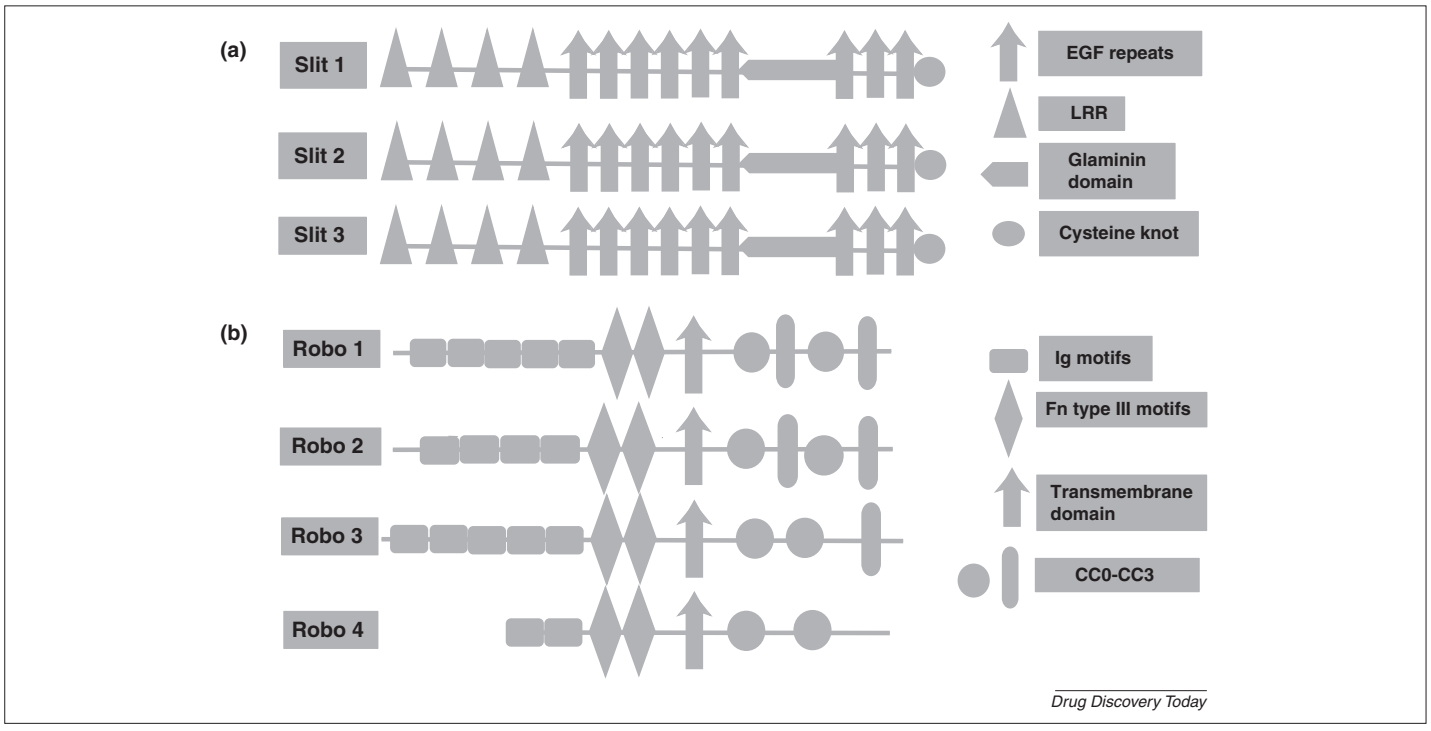

FIGURE 1

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 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) motifs, a transmembrane domain and a conserved cytoplasmic domain (CC0–CC3). Figure drawn considering ref [10].

The conserved domain of Slits (D2 LRR), and Ig1, Ig2 of Robo 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 Robo 1,3 promoters are hypermethylated (epigenetic inactivation) in several different types of cancers [9,16–20]. The activation or suppression of the Slit/Robo pathway modulates several oncogenic signaling pathways that are associated with the development and progression of cancer [21,22]. 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 [23]. Netrin-1 (a tumor suppressive protein) binds DCC and prevents DCC-mediated apoptosis in cancer cells [24,25]. 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 [26,27].

Most of the current reports suggest that the Slit/Robo pathway displays its effects in the late stages of cancer [26,28]. 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 [29–31], whereas in liver cancer Slit2/Robo1 specifically inhibited hepatocyte growth factor (HGF)-mediated cell migration [30]. 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 [32,33]. Most of the reports have 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) [34]. In most of the cancers, Slit/Robo acts as a tumor suppressor by inhibiting cell invasion and migration [2,29,35–39], except in prostate cancer and colorectal cancer [28,40]. The current data indicate that Slit/Robo pathways differentially modulate invasion and migration (Fig. 2), which varies according to signaling and type of cancers. 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 [29,38,41]. Slit/